Supporting Information for

Kinetically Selective Inhibitors of Histone Deacetylase 2 (HDAC2) as Cognition Enhancers

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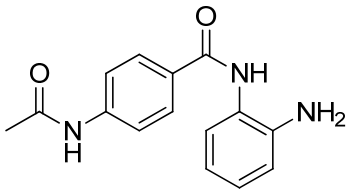
1. Compound Synthesis

1.1. General details.

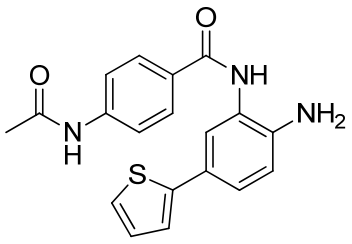
All reagents and solvents were purchased from commercial vendors and used as received. All final compounds were confirmed to be of >95% purity based on HPLC LC-MS analysis (Alliance 2795, Waters, Milford, MA). Purity was measured by UV absorbance at 210 nm. Identity was determined on a SQ mass spectrometer by positive and negative electrospray ionization. Mobile phase A consisted of 0.01% formic acid in water, while mobile phase B consisted of 0.01% formic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 1.75 minutes at 1.75 mL/min. An Agilent Poroshell 120 EC-C18, 2.7 µm, 3.0x30 mm column was used with column temperature maintained at 40 oC. 2.1 µL of sample solution were injected. All reagents and solvents were purchased from commercial vendors and used as received. 1H and 13C NMR spectra were recorded on a Bruker 300 MHz or Varian UNITY INOVA 500 MHz spectrometer as indicated. Proton and carbon chemical shifts (δ) are reported in ppm relative to tetramethylsilane (δ 0 for both 1H and 13C) and d6-DMSO (1H δ 2.50, 13C δ 39.5). NMR data are reported as follows: δ, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, m = multiplet); coupling constants in Hz; integration. NMR data were collected at 25°C. Flash chromatography was performed using 40-60 µm Silica Gel (60 Å mesh) on a Teledyne Isco Combiflash Rf system.

1.2. Characterization of known compounds

The analytical data collected for all known compounds matched the published literature.



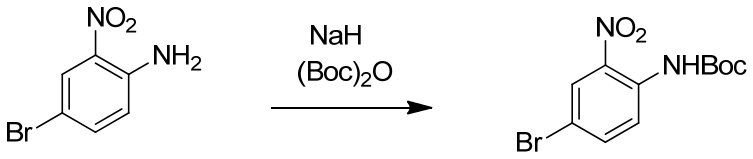
4-acetamido-*N*-(2-aminophenyl)benzamide (**CI-994**):
ESI+ MS: m/z 270 ($[M+H]^+$); ^{1}HNMR (500 MHz, d^6-DMSO): δ 10.18 (s, 1H), 9.54 (s, 1H), 7.93 (d, *J* = 8.5Hz, 2H), 7.69 (d, *J* = 8.5Hz, 2H), 7.16 (d, *J* = 7.5Hz, 1H), 6.96 (t, *J* = 7.5Hz, 1H), 6.78 (d, *J*=7.5Hz, 1H), 6.60 (t, *J*=7.5Hz, 1H), 4.87 (s, 2H), 2.09 (s, 3H); HPLC purity: >99%.



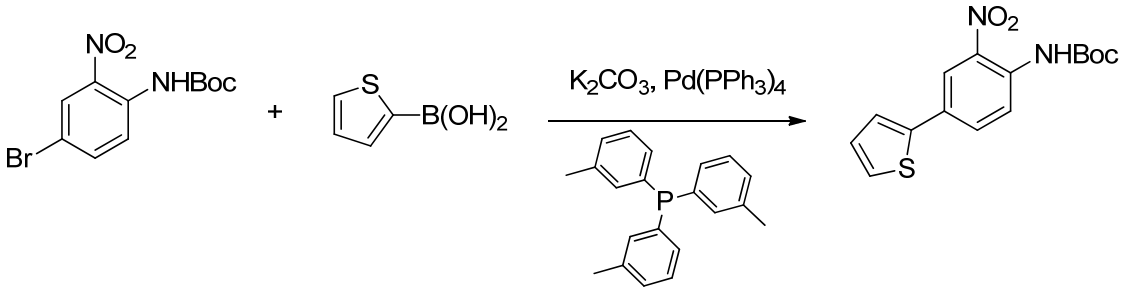
4-acetamido-*N*-(2-amino-5-(thiophen-2-yl)phenyl)benzamide (**1**)
ESI+ MS: m/z 352 ($[M+H]^+$); ^{1}HNMR (500 MHz, d^6-DMSO): δ 10.18 (s, 1H), 9.61 (s, 1H), 7.95 (d, *J* = 8.5Hz, 2H), 7.69 (d, *J* = 9.0Hz, 2H), 7.46 (d, *J* = 1.5Hz, 1H), 7.34 (dd, *J* = 5.5Hz, *J* = 1.0Hz, 1H), 7.28 (dd, *J* = 8.5Hz, *J* = 2.5Hz, 1H), 7.23 (dd, *J* = 3.5Hz, *J* = 1.0Hz, 1H), 7.04 (dd, *J* = 5.0Hz, *J* = 4.0Hz, 1H), 6.80 (d, *J* = 8.5Hz, 1H), 5.11 (s, 2H), 2.08 (s, 3H); HPLC purity: 98%.

1.3. Characterization of newly synthesized compounds

1.3.1. Key Intermediates Syntheses

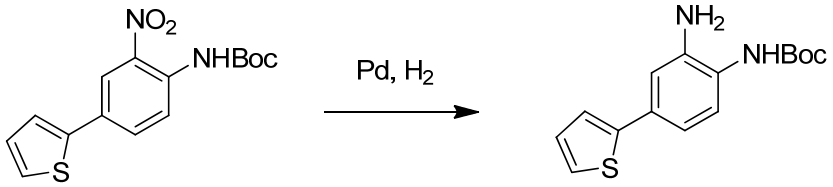


tert-Butyl (4-bromo-2-nitrophenyl)carbamate. To a solution of 4-bromo-2-nitroaniline (1, 10.0 g, 46.1 mmol, 1.0 eq.) in DMF (50 mL) at 0°C was added sodium hydride (1.8 g, 73.7 mmol, 1.6 eq.) slowly. After 30 minutes, a solution of di-*tert*-butyl dicarbonate (12.1 g, 55.3 mmol, 1.2 eq.) in DMF (50 mL) was added dropwise. The reaction mixture was then stirred for 16 h at room temperature. The reaction was quenched with water. The product was extracted with methyl tertiary butyl ether. The organic layer was washed with water and brine. The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The product was purified by column chromatography (silica gel, 0-20 % EtOAc/hexanes) to give *tert*-butyl (4-bromo-2-nitrophenyl)carbamate (7.5 g, 51% yield) as yellow solid.

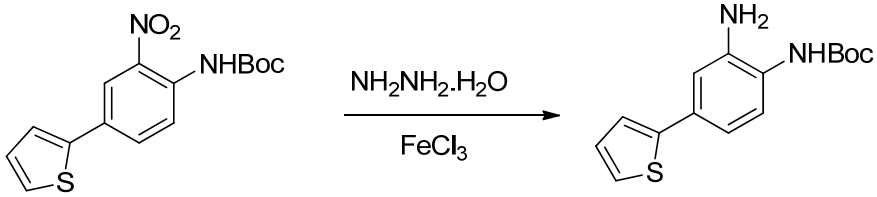


tert-Butyl (2-nitro-4-(thiophen-2-yl)phenyl)carbamate. A mixture of tert-butyl (4-bromo-2-nitrophenyl)carbamate (6.0 g, 18.92 mmol, 1.0 eq.), thiophen-2-ylboronic acid (3.2 g, 24.6 mmol, 1.3 eq.), potassium carbonate (7.84 g, 56.8 mmol, 3.0 eq.) and tetrakis(triphenylphosphine)palladium(0) (1.53 g, 1.32 mmol, 0.07 eq.), tri-tolylphosphine in DME/H2O (105 mL) was first degassed then heated to 90°C for 20 h. The reaction was then filtered through Celite. The product was extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The crude product

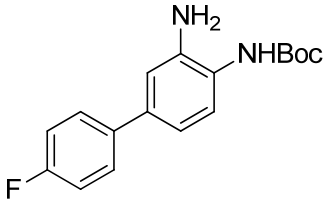
was purified by flash column chromatography (silica gel, 2% EtOAc/hexanes) to obtain pure tert-butyl (2-nitro-4-(thiophen-2-yl)phenyl)carbamate (4.42 g, 73 % yield).



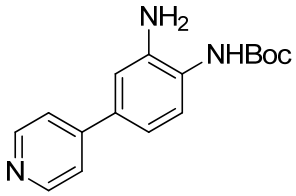
tert-Butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate. To a solution of *tert*-butyl (2-nitro-4-(thiophen-2-yl)phenyl)carbamate (2 g, 6.2 mmol, 1.0 eq.) in ethanol (20 mL) and methanol (20 mL) was added 10 % Pd/C (0.66 g, 0.1 eq.). The reaction mixture was stirred 12h under a hydrogen atmosphere. The reaction was filtered and the filtrate was concentrated under reduced pressure to give *tert*-butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate (1.25 g, 69 % yield) as an off-white solid.



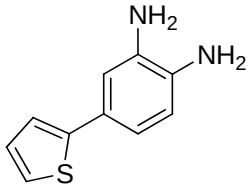
tert-Butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate. To a solution of *tert*-butyl (2-nitro-4-(thiophen-2-yl)phenyl)carbamate (1.6 g, 4.99 mmol, 1 eq.) in methanol (20 mL) was added hydrazine hydrate (14 mL) and ferric chloride (0.05 g, 0.3 mmol, 0.06 eq.). The resulting mixture was warmed to 60°C and stirred for 2 h. The reaction was then filtered through Celite, the solids were washed with MeOH. The filtrate was concentrated under reduced pressure. Water was added to the residue and the suspension was stirred for 1 h. The obtained solid was filtered, washed with hexanes then dried to yield *tert*-butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate (1.2 g, 83 % yield). ESI+ MS: m/z 313 ($[M+Na]^+$).



tert-Butyl (3-amino-4'-fluoro-[1,1'-biphenyl]-4-yl)carbamate was prepared using the same procedure with 4-fluorophenylboronic acid. ESI+ MS: m/z 303 ($[M+H]^+$).

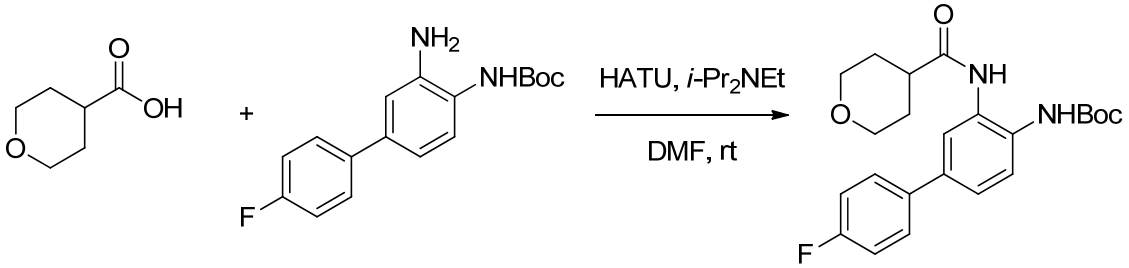


tert-Butyl (2-amino-4-(pyridin-4-yl)phenyl)carbamate was prepared using the same procedure with 4-pyridylboronic acid. ESI+ MS: m/z 286 ($[M+H]^+$).

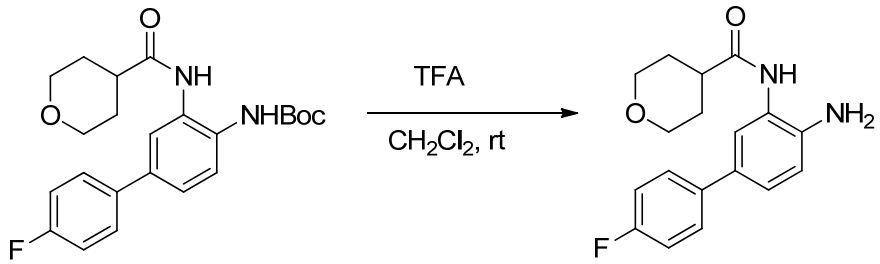


4-(Thiophen-2-yl)benzene-1,2-diamine (**4**). A solution of *tert*-butyl 2-amino-4-(thiophen-2-yl)phenylcarbamate (70 mg, 0.24 mmol, 1.0 eq.) in dichloromethane (3 mL) was treated with TFA (15 eq.). The resulting mixture was stirred at room temperature for 20h. The crude reaction was concentrated under reduced pressure. A saturated solution of sodium bicarbonate was added. The product was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated to afford 4-(thiophen-2-yl)benzene-1,2-diamine (**4**) (42 mg, 92% yield). ESI+ MS: m/z 191 ($[M+H]^+$); ^{1}H NMR (500 MHz, d^6-DMSO): δ 7.26 (d, *J* = 5.0 Hz s, 1H), 7.09 (d, *J* = 3.5Hz, 1H), 6.99 (t, *J* = 3.5Hz, 1H), 6.79 (d, *J* = 2.0 Hz, 1H), 6.69 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 6.49 (d, *J* = 8.5 Hz, 1H), 4.62 (bs, 4H); HR ESI MS calcd. for C10H11N2S *m/z* $[M]^+$ 190.0565, found 190.0564; HPLC purity: 90%.

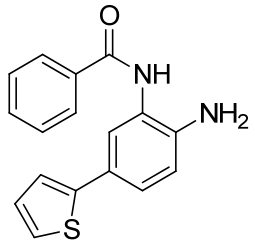
1.3.2. Representative synthesis of amide analogs using carboxylic acids:



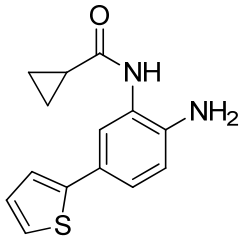
A solution of *tert*-butyl (2-amino-4-(4-fluorophenyl)phenyl)carbamate (12.1 g, 40.1 mmol, 0.9 eq.), tetrahydro-2*H*-pyran-4-carboxylic acid (6.0 g, 46.1 mmol, 1.0 eq.), HATU (21.0 g, 55.3 mmol, 1.2 eq.) and Hünigs base (16.1 mL, 92.3 mmol, 2.0 eq.) in DMF (60 mL) was stirred at room temperature. After completion, the reaction mixture was diluted with water. The solid was isolated by filtration and washed with pentane to afford *tert*-butyl (2-(tetrahydro-2*H*-pyran-4-carboxamido)-4-(thiophen-2-yl)phenyl) carbamate (15.1 g, 79 % yield).



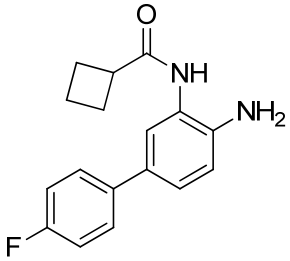
To a stirred solution of *tert*-butyl (2-(tetrahydro-2*H*-pyran-4-carboxamido)-4-(4-fluorophenyl)phenyl)carbamate (15.0g, 36.2 mmol, 1.0 eq.) in dichloromethane (120 mL) was added trifluoroacetic acid (40 mL, 519 mmol, 15.0 eq.) at 0^{o}C. The reaction mixture was stirred at room temperature for 2 h. The solvents were then removed under reduced pressure. A saturated aqueous solution of sodium bicarbonate was added. The product was extracted with EtOAc, washed with water and brine, dried, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, MeOH/CH_2Cl_2) to afford *N*-(2-amino-5-(4-fluorophenyl)phenyl)tetrahydro-2*H*-pyran-4-carboxamide (**BRD4884**) as a beige solid (7.96 g, 70% yield). ESI+ MS: m/z 331 ($[M+H]^+$), ^{1}H NMR (500 MHz, d^6-DMSO): δ 9.11 (s, 1H), 7.55-7.48 (m, 3H), 7.25-7.15 (m, 3H), 6.78 (d, *J* = 8.0 Hz, 1H), 4.99 (s, 2H), 3.90 (broad d, *J* = 9.5 Hz, 2H), 3.40-3.31 (m, 2H), 2.70-2.60 (m, 1H), 1.79-1.62 (m, 4H). HR ESI MS calcd. for C18H20FN2O2 *m/z* $[M + H]^+$ 315.1503, found 315.1508; HPLC purity: >99%.



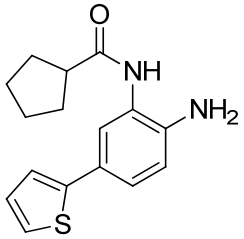
N-(2-amino-5-(thiophen-2-yl)phenyl)benzamide (**2**) was prepared by substituting tetrahydro-2*H*-pyran-4-carboxylic acid with benzoic acid and by substituting *tert*-butyl (3-amino-4'-fluoro-[1,1'-biphenyl]-4-yl)carbamate with *tert*-butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate. ESI+ MS: m/z 295 ($[M+H]^+$), ^{1}HNMR (500 MHz, d^6-DMSO): 9.73 (s, 1H), 8.01 (d, *J* = 8.0 Hz, 2H), 7.62-7.56 (m, 1H), 7.55-7.46 (m, 3H), 7.35 (d, *J* = 4.5 Hz, 1H), 7.31-7.29 (m, 1H), 7.24 (d, *J* = 3.5 Hz, 1H), 7.05 (t, *J* = 5.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 5.14 (s, 2H). HR ESI MS calcd. for C17H15N2OS *m/z* [M + H]+ 295.0900, found 295.0901; HPLC purity: 95%.



N-(2-amino-5-(thiophen-2-yl)phenyl)cyclopropanecarboxamide (**7**) was prepared by substituting tetrahydro-2*H*-pyran-4-carboxylic acid with cyclopropanecarboxylic acid and by substituting *tert*-butyl (3-amino-4'-fluoro-[1,1'-biphenyl]-4-yl)carbamate with *tert*-butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate. ESI+ MS: m/z 259 ($[M+H]^+$), ^{1}H NMR (500 MHz, d^6-DMSO): δ 9.46 (s, 1H), 7.56 (s, 1H), 7.34 (d, *J* = 4.5 Hz, 1H), 7.25-7.16 (m, 2H), 7.04 (t, *J* = 3.5 Hz, 1H), 6.75 (d, *J* = 9.0 Hz, 1H), 5.09 (s, 2H), 1.86-1.82 (m, 1H), 0.82-0.77 (m, 4H). HR ESI MS calcd. for C14H15N2OS *m/z* [M + H]+ 259.0900, found 259.0905; HPLC purity: >99%.

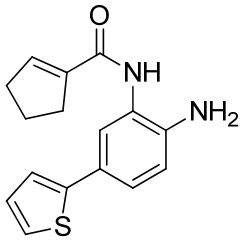


N-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)cyclobutanecarboxamide (**8**) was prepared by substituting tetrahydro-2*H*-pyran-4-carboxylic acid with cyclobutanecarboxylic acid. ESI+ MS: m/z 308 ($[M+Na]^+$), ^{1}HNMR (500 MHz, d^6-DMSO): δ 8.99 (s, 1H), 7.54-7.51 (m, 3H), 7.21-7.18 (m, 3H), 6.79 (d, *J* = 8.5Hz, 1H), 4.98 (s, 2H), 3.32-3.22 (m,1H), 2.26-2.21 (m, 2H), 2.20-2.09 (m, 2H), 1.99-1.91 (m, 1H), 1.90-1.75 (m, 1H); HR ESI MS calcd. for C17H18FN2O *m/z* [M + H]+ 285.1398, found 285.1410; HPLC purity: 98%.

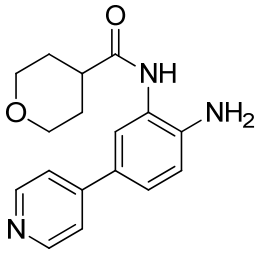


N-(2-amino-5-(thiophen-2-yl)phenyl)cyclopentanecarboxamide (**9**) was prepared by substituting tetrahydro-2*H*-pyran-4-carboxylic acid with cyclopentanecarboxylic acid and by substituting *tert*-butyl (3-amino-4'-fluoro-[1,1'-biphenyl]-4-yl)carbamate with *tert*-butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate. ESI+ MS: m/z 287 ($[M+H]^+$), ^{1}H NMR (500 MHz, d^6-DMSO): δ 9.15 (s, 1H), 7.52 (s, 1H), 7.34 (d, *J* = 4.0 Hz, 1H), 7.26-7.18 (m, 2H), 7.04 (t, *J* = 4.0 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 5.06 (bs, 2H), 2.84 (q, *J* =

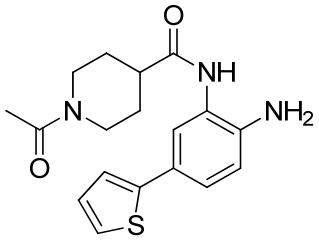
7.5 Hz, 1H), 1.95-1.75 (m, 2H), 1.75-1.60 (m, 4H), 1.60-1.50 (m, 2H). HR ESI MS calcd. for C16H19N2OS *m/z* $[M + H]^+$ 287.1213, found 287.1224; HPLC purity: >99%.



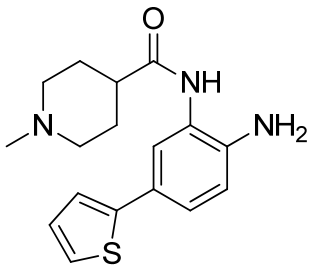
N-(2-amino-5-(thiophen-2-yl)phenyl)cyclopent-1-enecarboxamide (**BRD2283**) was prepared by substituting tetrahydro-2*H*-pyran-4-carboxylic acid with cyclopent-1-enecarboxylic acid and by substituting *tert*-butyl (3-amino-4'-fluoro-[1,1'-biphenyl]-4-yl)carbamate with *tert*-butyl (2-amino-4-(thiophen-2-yl)phenyl) carbamate. ESI+ MS: m/z 285 ($[M+H]^+$), ^{1}H NMR (500 MHz, d^6-DMSO): δ 9.12 (s, 1H), 7.40 (d, *J* = 2.0 Hz, 1H), 7.35 (dd, *J* = 5.0, 1.0 Hz, 1H), 7.26 (dd, *J* = 8.0 ; 2.0 Hz, 2H), 7.22 (dd, *J* = 4.0 Hz; 1.5 Hz, 1H), 7.04 (dd, *J* = 5.0; 4.5 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 6.73-6.68 (m, 1H), 5.06 (s, 2H), 2.61-2.57 (m, 2H), 2.51-2.48 (m, 2H), 1.93-1.90 (m, 2H); HR ESI MS calcd. for C16H17N2OS *m/z* $[M + H]^+$ 285.1056, found 285.1051; HPLC purity: >99%.



N-(2-amino-5-(pyridin-4-yl)phenyl)tetrahydro-2*H*-pyran-4-carboxamide (**12**) was prepared by substituting *tert*-butyl (3-amino-4'-fluoro-[1,1'-biphenyl]-4-yl)carbamate with *tert*-butyl (2-amino-4-(pyridin-4-yl)phenyl)carbamate. ESI+ MS: m/z 298 ($[M+H]^+$); ^{1}HNMR (500 MHz, d6-DMSO): δ 9.13(s, 1H), 8.50 (d, *J* = 5.0 Hz, 2H), 7.71 (d, *J* = 1.5 Hz, 1H), 7.53 (d, *J* = 5.5 Hz, 2H), 7.42 (dd, *J* = 8.5, 8.5 Hz, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 5.27 (bs, 2H), 3.91 (brd, *J* = 9.0 Hz, 2H), 3.41-3.30 (m, 2H), 2.70-2.61 (m, 1H), 1.79-1.65 (m, 4H); HR ESI MS calcd. for C17H19N3O2 *m/z* $[M + H]^+$ 298.1550, found 298.1565; HPLC purity: >99%.

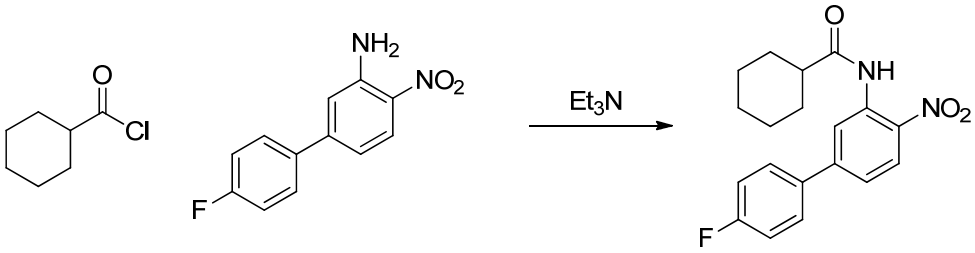


1-acetyl-*N*-(2-amino-5-(thiophen-2-yl)phenyl)piperidine-4-carboxamide (**BRD3349**) was prepared by substituting tetrahydro-2*H*-pyran-4-carboxylic acid with 1-acetylpiperidine-4-carboxylic acid and by substituting *tert*-butyl (3-amino-4'-fluoro-[1,1'-biphenyl]-4-yl)carbamate with *tert*-butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate. ESI+ MS: m/z 316 ($[M+H]^+$), ^{1}H NMR (500 MHz, d^6-DMSO): δ 9.19 (s, 1H), 7.50 (d, *J* = 1.5 Hz, 1H), 7.34 (d, *J* = 4.5 Hz, 1H), 7.24-7.18 (m, 2H), 7.04 (dd, *J* = 4.0, 5.5 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 5.06 (s, 2H), 4.35 (d, *J* = 13.0 Hz, 1H), 3.79 (d, *J* = 13.5 Hz, 2H), 3.01 (t, *J* = 11.5 Hz, 1H), 2.68-2.58 (m, 2H), 2.00 (s, 3H), 1.88-1.82 (m, 2H), 1.64-1.58 (m, 1H), 1.48-1.43 (m, 1H). HR ESI MS calcd. for C18H22N3O2S *m/z* $[M + H]^+$ 344.1427, found 344.1438; HPLC purity: >99%.



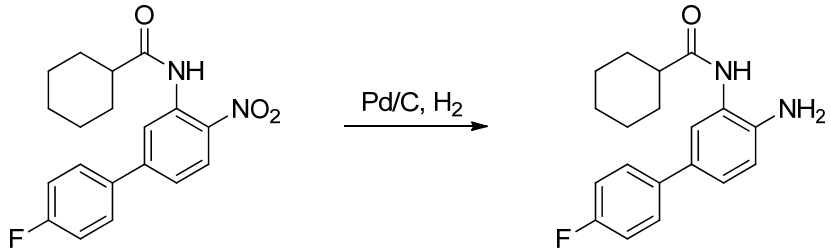
N-(2-amino-5-(thiophen-2-yl)phenyl)-1-methylpiperidine-4-carboxamide (**11**) was prepared by substituting tetrahydro-2*H*-pyran-4-carboxylic acid with 1-methylpiperidine-4-carboxylic acid and by substituting *tert*-butyl (3-amino-4'-fluoro-[1,1'-biphenyl]-4-yl)carbamate with *tert*-butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate. ESI+ MS: m/z 316 ($[M+H]^+$), ^{1}H NMR (500 MHz, d^6-DMSO): δ 9.12 (s, 1H), 7.51 (s, 1H), 7.34 (d, *J* = 5.0 Hz, 1H), 7.24-7.18 (m, 2H), 7.04 (t, *J* = 4.0 Hz, 1H), 6.75 (t, *J* = 8.5 Hz, 1H), 5.06 (bs, 2H), 2.82 (d, *J* = 11.0 Hz, 2H), 2.37-2.25 (m, 1H), 2.16 (s, 3H), 1.90-1.60 (m, 6H). HR ESI MS calcd. for C17H22N3OS *m/z* $[M+H]^+$ 316.1478, found 316.1487; HPLC purity: >99%.

1.3.3. Representative synthesis of amide analogs using acid chlorides:

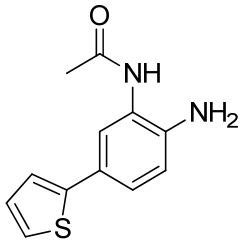


N-(4'-fluoro-4-nitro-[1,1'-biphenyl]-3-yl)cyclohexanecarboxamide. To a solution of 4'-fluoro-4-nitro-[1,1'-biphenyl]-3-amine (100mg, 0.43 mmol, 1.0 eq.) in DCM (10 mL) was added triethylamine (0.18 mL, 1.29 mmol, 3.0 eq.) followed by cyclohexanecarbonyl chloride (76 mg, 0.52 mmol, 1.2 eq.) and the reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with DCM and washed

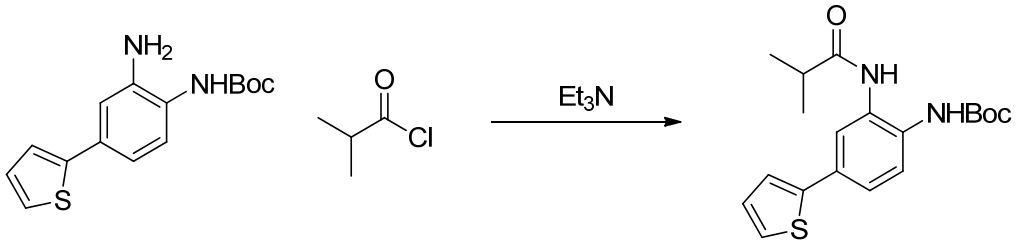
successively with a citric acid solution, water, and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel using a gradient of ethyl acetate in hexanes to afford and off-white solid (100mg, 68% yield). ESI- MS: m/z 341 ($[M-H]^-$).



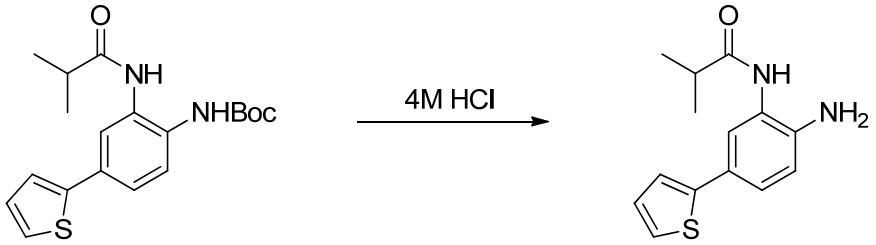
N-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)cyclohexanecarboxamide (**10**). To a solution of *N*-(4'-fluoro-4-nitro-[1,1'-biphenyl]-3-yl)cyclohexanecarboxamide (50 mg, 0.15 mmol, 1.0 eq.) in methanol (10 mL) was added 10 % Pd/C (10 mg, 1.0 eq.). The reaction mixture was stirred 2h under a hydrogen atmosphere. The reaction was filtered on celite and washed with methanol. The filtrate was concentrated under reduced pressure and washed with diethyl ether to afford the product (10 mg, 22% yield) as an off-white solid. ESI+ MS : m/z 313 ($[M+H]^+$), ^{1}HNMR (500 MHz, d^6-DMSO) : δ 9.05 (s, 1H), 7.60 - 7.50 (m, 3H), 7.25 - 7.18 (m, 3H), 6.79 (d, *J* = 8.5 Hz, 1H), 4.97 (s, 2H), 2.44-2.37 (m, 1H), 1.90-1.10 (m, 10H). HR ESI MS calcd. for C19H22FN2O *m/z* $[M + H]^+$ 313.1711, found 313.1718; HPLC purity: 96%.



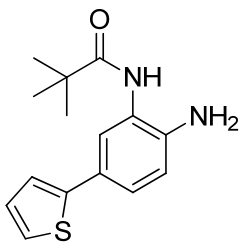
N-(2-amino-5-(thiophen-2-yl)phenyl)acetamide (**3**) was prepared by substituting isobutyryl chloride with acetyl chloride and by substituting 4'-fluoro-4-nitro-[1,1'-biphenyl]-3-amine with 2-nitro-5-(thiophen-2-yl)aniline. ESI+ MS: m/z 233 ($[M+H]^+$), ^{1}HNMR (500 MHz, d^6-DMSO): δ 9.16 (s, 1H), 7.48 (d, *J* = 1.5 Hz, 1H), 7.33 (d, *J* = 5.0 Hz, 1H), 7.23-7.17 (m, 2H), 7.03 (dd, *J* = 4.0, 5.0 Hz, 1H), 6.73 (d, *J* = 8.5 Hz, 1H), 5.10 (bs, 2H), 2.06 (s, 3H). HR ESI MS calcd. for C12H13N2OS *m/z* $[M + H]^+$ 233.0743, found 233.0738; HPLC purity: >99%.



To a solution of *tert*-butyl 2-amino-4-(thiophen-2-yl)phenylcarbamate (200mg, 0.69 mmol, 1.0 eq.) in DCM (4 mL) were added successively triethylamine (0.23 mL, 1.72 mmol, 3.0 eq.) and isobutyryl chloride (80 μL, 0.76 mmol, 1.2 eq.) and the reaction mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with DCM and washed successively with water, and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel using a gradient of ethyl acetate in hexanes to afford and off-white solid (200mg, 81% yield). ESI- MS: m/z 262 ([M+H-Boc]$^+$).

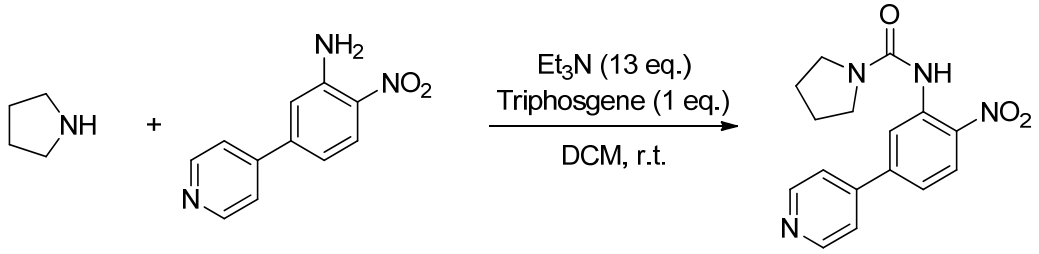


<u>*N*-(2-amino-5-(thiophen-2-yl)phenyl)isobutyramide (**5**)</u>: *tert*-butyl 2-isobutyramido-4-(thiophen-2-yl)phenylcarbamate (190mg, 0.53 mmol, 1.0 eq.) was diluted in methanol (4 mL) and cooled to 0 ^oC. A solution of HCl in dioxane (4M, 2mL) was added and the reaction was stirred at room temperature for 2 hours. Solvents were evaporated under reduced pressure and the resulting solid was neutralized with aq. NaHCO3, filtered and dried. The crude material was purified by column chromatography on silica gel using a gradient of methanol in DCM to afford and off-white solid (50mg, 36% yield).
ESI+ MS: m/z 261 ([M+H]$^+$), ^{1}H NMR (500 MHz, d^6-DMSO): δ 9.10 (s, 1H), 7.50 (s, 1H), 7.34 (d, *J* = 4.5 Hz, 1H), 7.25-7.18 (m, 2H), 7.04 (t, *J* = 3.5 Hz, 1H), 6.75 (d, *J* = 9.0 Hz, 1H), 5.03 (s, 2H), 2.70-2.60 (m, 1H), 1.13 (s, 3H), 1.12 (s, 3H); HR ESI MS calcd. for C14H16N2OS *m/z* [M+H]$^+$ 261.1056, found 261.1067. ; HPLC purity: 98%.

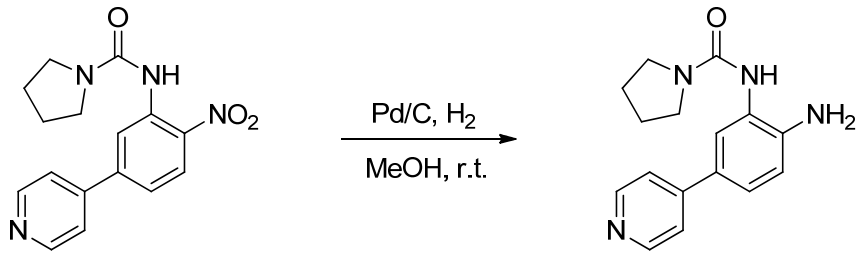


N-(2-amino-5-(thiophen-2-yl)phenyl)pivalamide (**6**):
ESI+ MS: m/z 275 ($[M+H]^+$), ^{1}H NMR (500 MHz, d^6-DMSO): δ 8.84 (s, 1H), 7.40-7.20 (m, 4H), 7.04 (s, 1H), 6.80-6.75 (m, 1H), 4.89 (bs, 2H), 1.25 (s, 9H); HR ESI MS calcd. for C15H18N2OS *m/z* $[M+H]^+$ 275.1213, found 275.1215; HPLC purity: >99%.

1.3.4. Representative synthesis of urea and carbamate analogs:

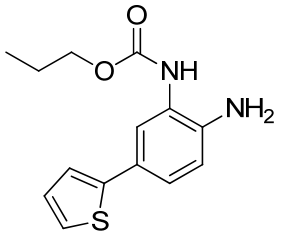


N-(2-nitro-5-(pyridin-4-yl)phenyl)pyrrolidine-1-carboxamide. To a stirred solution of 2-nitro-5-(pyridin-4-yl)aniline (7.0g, 32.5 mmol, 1.0 eq.) in DCM (100 mL) were added triphosgene (9.65g, 32.5 mmol, 1.0 eq.) and triethylamine (42.8g, 423 mmol, 13.0 eq.) at 0^{o}C. The solution was stirred at room temperature for 2 hours. Then, the reaction mixture was cooled to 0^{o}C, pyrrolidine (3.46g, 48.8 mmol, 1.5 eq.) was added and the mixture was slowly brought back to room temperature. After completion, the reaction mixture was quenched with sat. citric acid solution and extracted with DCM. The organic layer was washed with water, dried over Na_2SO_4 and concentrated. The crude mixture was purified by silica gel chromatography using 2% methanol in DCM to afford an off-white solid (5.0g, 50% yield). ESI+ MS:m/z 313 ($[M+H]^+$).

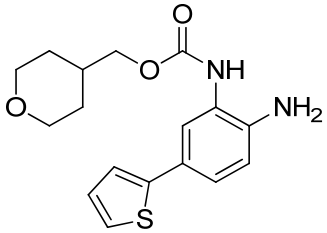


N-(2-amino-5-(pyridin-4-yl)phenyl)pyrrolidine-1-carboxamide (**BRD6688**). To a stirred solution of *N*-(2-nitro-5-(pyridin-4-yl)phenyl)pyrrolidine-1-carboxamide (7.0g, 22.41 mmol, 1.0 eq.) in methanol (350 mL) was added 10% palladium on activated carbon (3.0g, 1.0 eq.) and the solution was stirred at room temperature under hydrogen atmosphere for 4 hours. After completion, the reaction mixture was filtered through celite and washed with methanol. The filtrate was concentrated under reduced pressure and purified by washing with pentane to afford an off-white solid (4.5g, 71% yield). ESI+ MS:m/z 283 ($[M+H]^+$), ^{1}HNMR (500 MHz, d^6-DMSO): δ 8.49 (d, *J* = 5.5 Hz, 2H), 7.56-7.52 (m, 3H), 7.50 (s, 1H), 7.39 (dd, *J* = 1.5, 8.0 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 5.23

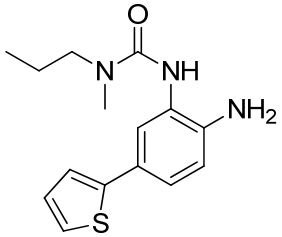
(s, 2H), 3.39-3.27 (m, 4H), 1.90-1.75 (m, 4H). HR ESI MS calcd. for C16H19N4O *m/z* [M + H]+ 283.1553, found 283.1562; HPLC purity: 95%.



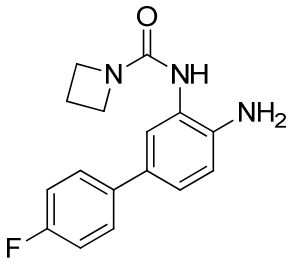
Propyl (2-amino-5-(thiophen-2-yl)phenyl)carbamate (**13**) was prepared by substituting pyrrolidine with propanol. ESI+ MS: m/z 277 ($[M+H]^+$), 1H NMR (500 MHz, d^6-DMSO): δ 8.60 (bs, 1H), 7.52 (bs, 1H), 7.34 (d, *J* = 5.0 Hz, 1H), 7.21-7.16 (m, 2H), 7.05-7.02 (m, 1H), 6.71 (d, *J* = 8.5 Hz, 1H), 5.11 (s, 2H), 4.02 (t, *J* = 7.0 Hz, 2H), 1.68-1.60 (m, 2H), 0.94 (t, *J* = 7.5 Hz, 3H); HR ESI MS calcd. for C14H17N2O2S *m/z* [M + H]+ 277.1005, found 277.1012; HPLC purity: 98%.



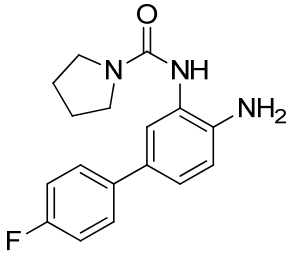
(Tetrahydro-2H-pyran-4-yl)methyl (2-amino-5-(thiophen-2-yl)phenyl)carbamate (**14**) was prepared by substituting pyrrolidine with (tetrahydro-2H-pyran-4-yl)methanol. ESI+ MS: m/z 333 ($[M+H]^+$), ^{1}H NMR (500 MHz, d^6-DMSO): δ 8.61 (bs, 1H), 7.52 (bs, 1H), 7.34 (d, *J* = 5.0 Hz, 1H), 7.20-7.16 (m, 2H), 7.03 (dd, *J* = 3.5, 5.0 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 5.12 (s, 2H), 3.94 (d, *J* = 7.0 Hz, 2H), 3.90-3.82 (m, 2H), 3.35-3.28 (m, 2H), 1.95-1.85 (m, 1H), 1.64-1.56 (m, 2H), 1.34-1.20 (m, 2H). HR ESI MS calcd. for C17H21N2O3S *m/z* [M + H]+ 333.1267, found 333.1275; HPLC purity: >99%.



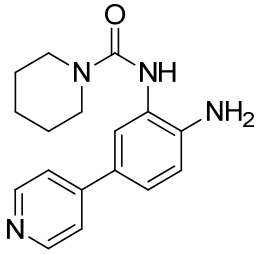
3-(2-amino-5-(thiophen-2-yl)phenyl)-1-methyl-1-propylurea (**16**) was prepared by substituting pyrrolidine with *N*-methylpropan-1-amine. ESI+ MS: m/z 290 ($[M+H]^+$), ^{1}HNMR (500 MHz, d^6-DMSO): δ 7.69 (bs, 1H), 7.34 (d, *J* = 5.0 Hz, 1H), 7.29 (bs, 1H), 7.22-7.16 (m, 2H), 7.05-7.02 (m, 1H), 6.74 (d, *J* = 8.5 Hz, 1H), 4.94 (bs, 2H), 3.26 (t, *J* = 7.5 Hz, 2H), 2.94 (s, 3H), 1.60-1.48 (m, 2H), 0.87 (t, *J* = 7.0 Hz, 3H). HR ESI MS calcd. for C15H19N3OS *m/z* [M + H]+ 290.1322, found 290.1328; HPLC purity: 96%.



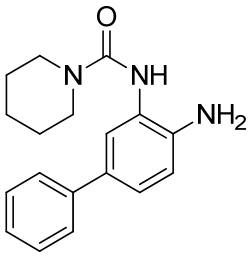
N-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)azetidine-1-carboxamide (**BRD3321**) can be prepared by substituting pyrrolidine with azetidine and by substituting 2-nitro-5-(pyridin-4-yl)aniline with 4'-fluoro-4-nitro-[1,1'-biphenyl]-3-amine. ESI+ MS: m/z 286 ($[M+H]^+$), ^{1}HNMR (500MHz, d^6-DMSO): δ 7.62 (s,1H), 7.52 (dd, *J* = 8.5, 5.5 Hz, 2H), 7.36 (d, *J* = 2.0 Hz, 1H), 7.23-7.12 (m, 3H), 6.76 (d, *J* = 8.0 Hz, 1H), 4.98 (s, 2H), 3.94 (t, *J* = 7.5Hz, 4H), 2.18 (qt, *J* = 7.5Hz, 2H); HR ESI MS calcd. for C16H17FN3O *m/z* [M + H]+ 286.1350, found 286.1354; HPLC purity: 94%.



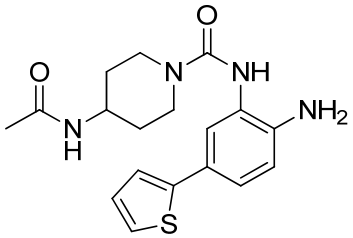
N-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)pyrrolidine-1-carboxamide (**17**) was prepared by substituting 2-nitro-5-(pyridin-4-yl)aniline with 4'-fluoro-4-nitro-[1,1'-biphenyl]-3-amine. ESI+ MS: m/z 300 ($[M+H]^+$), 1H NMR (500 MHz, d^6-DMSO): δ 7.58-7.50 (m, 2H), 7.48 (s, 1H), 7.38 (d, *J* = 2.5 Hz, 1H), 7.24-7.14 (m, 3H), 6.78 (d, *J* = 8.5 Hz, 1H), 4.97 (s, 2H), 3.38 (t, *J* = 6.5 Hz, 4H), 1.86 (t, *J* = 6.5 Hz, 4H). HR ESI MS calcd. for C17H19FN3O *m/z* [M + H]+ 300.1507, found 300.1512; HPLC purity: 98%.



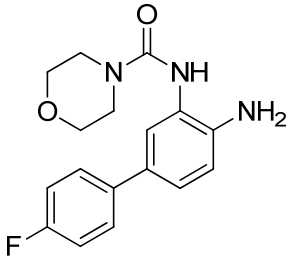
N-(2-amino-5-(pyridin-4-yl)phenyl)piperidine-1-carboxamide (**18**) can be prepared by substituting pyrrolidine with piperidine. ESI+ MS: m/z 297 ($[M+H]^+$), ^{1}HNMR (500 MHz, d^6-DMSO): δ 8.48 (d, *J* = 6.0 Hz, 2H), 7.86 (s, 1H), 7.53 (d, *J* = 6.0Hz, 2H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.39-7.37 (m, 1H), 6.80 (d, *J* = 8.5 Hz, 1H), 5.12 (s, 2H), 3.42-3.39 (m, 4H), 1.64-1.48 (m, 6H). HR ESI MS calcd. for C17H21N4O *m/z* $[M + H]^+$ 297.1710, found 297.1718; HPLC purity: 98%.



N-(4-amino-[1,1'-biphenyl]-3-yl)piperidine-1-carboxamide (**19**) can be prepared by substituting pyrrolidine with piperidine and by substituting 2-nitro-5-(pyridin-4-yl)aniline with 4-nitro-[1,1'-biphenyl]-3-amine. ESI+ MS: m/z 296 ($[M+H]^+$), ^{1}HNMR (400 MHz, d^6-DMSO): δ 7.86 (s, 1H), 7.52 (d, *J* = 9.0 Hz, 2H), 7.40-7.35 (m,2H), 7.32 (d, *J* = 2.5 Hz, 1H), 7.24-7.18 (m, 2H), 6.78 (d, *J* = 10.5Hz, 1H), 4.87 (s, 2H), 3.45-3.30 (m, 4H), 1.62-1.55 (m, 2H), 1.55-1.45 (m, 4H). HR ESI MS calcd. for C18H22N3O *m/z* [M + H]+ 296.1757, found 296.1757; HPLC purity: 95%.



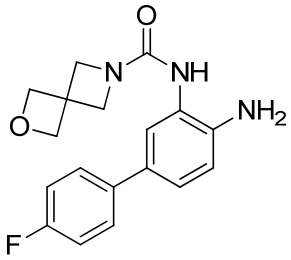
4-acetamido-*N*-(2-amino-5-(thiophen-2-yl)phenyl)piperidine-1-carboxamide (**BRD3227**) was prepared by substituting pyrrolidine with *N*-(piperidin-4-yl)acetamide and by substituting 2-nitro-5-(pyridin-4-yl)aniline with 2-nitro-5-(thiophen-2-yl)aniline. ESI+ MS: m/z 359 ($[M+H]^+$), 1H NMR (500 MHz, d^6-DMSO): δ 7.98 (s, 1H), 7.86 (d, *J* = 9.0 Hz, 1H), 7.35 (dd, *J* = 1.0, 6.0 Hz, 1H), 7.27 (d, *J* = 3.0 Hz, 1H), 7.23-7.18 (m, 2H), 7.05 (dd, *J* = 3.0, 4.0 Hz, 1H), 6.75 (d, *J* = 9.0 Hz, 1H), 4.95 (s, 2H), 4.08-3.95 (m, 2H), 3.85-3.70 (m, 1H), 3.00-2.86 (m, 2H), 1.81 (s, 3H), 1.81-1.70 (m, 2H), 1.40-1.20 (m, 2H). HR ESI MS calcd. for C18H23N4O2S *m/z* $[M + H]^+$ 359.1536, found 359.1537; HPLC purity: >99%.



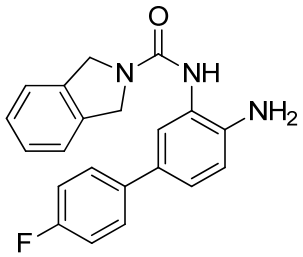
10.

N-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)morpholine-4-carboxamide (**20**) can be prepared by substituting pyrrolidine with morpholine and by substituting 2-nitro-5-(pyridin-4-yl)aniline with 4'-fluoro-4-nitro-[1,1'-biphenyl]-3-amine. ESI+ MS: m/z 316 ($[M+H]^+$), ^{1}HNMR (400 MHz, d^6-DMSO): δ 8.10 (s, 1H), 7.58-7.55 (m, 2H), 7.36 (d, *J* =

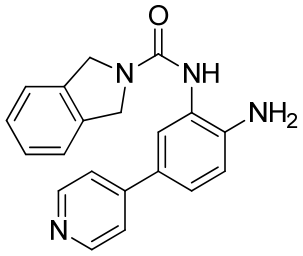
2.4Hz, 1H), 7.27-7.19 (m, 3H), 6.90 (d, J = 8.4Hz, 1H), 6.02 (bs, 2H), 3.63 (t, J = 4.6Hz, 4H), 3.44 (t, J = 4.6Hz, 4H). HR ESI MS calcd. for C17H19FN3O2 *m/z* $[M + H]^+$ 316.1456, found 316.1459; HPLC purity: >99%.



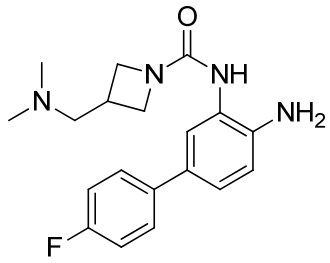
N-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)-2-oxa-6-azaspiro[3.3]heptane-6-carboxamide (**BRD3386**) can be prepared by substituting pyrrolidine with 2-oxa-6-azaspiro[3.3]heptanes and by substituting 2-nitro-5-(pyridin-4-yl)aniline with 4'-fluoro-4-nitro-[1,1'-biphenyl]-3-amine. ESI+ MS: m/z 328 ($[M+H]^+$), ^{1}HNMR (500 MHz, d^6-DMSO): δ 7.73 (s, 1H), 7.51 (dd, J = 5.5, 8.5 Hz, 2H), 7.32 (d, J = 1.5 Hz, 1H), 7.21 - 7.15 (m, 3H), 6.76 (d, J = 8.5 Hz, 1H), 4.98 (s, 2H), 4.68 (s, 4H), 4.10 (s, 4H). HR ESI MS calcd. for C18H19FN3O2 m/z [M + H]+ 328.1456, found 328.1457; HPLC purity: 98%.



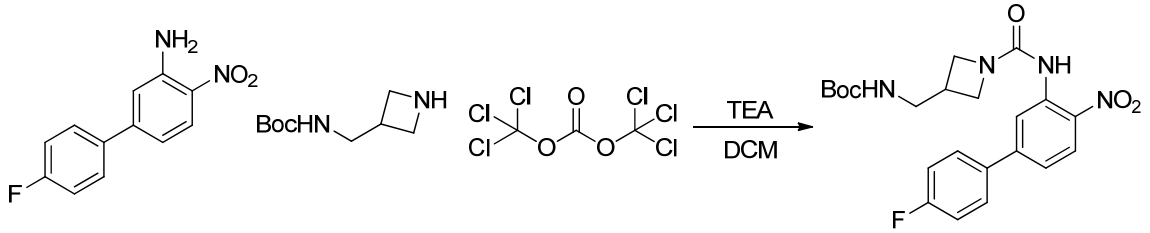
N-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)isoindoline-2-carboxamide (**BRD8951**) was prepared by substituting pyrrolidine with isoindoline and by substituting 2-nitro-5-(pyridin-4-yl)aniline with 4'-fluoro-4-nitro-[1,1'-biphenyl]-3-amine. ESI+ MS: m/z 348 ($[M+H]^+$), ^{1}HNMR (400 MHz, d^6-DMSO): δ 7.70 (s, 1H), 7.57-7.54 (m, 2H), 7.44 (d, J = 2.0 Hz, 1H), 7.38-7.35 (m, 2H), 7.33-7.30 (m, 2H), 7.23-7.18 (m, 3H), 6.81 (d, J = 8.4 Hz, 1H), 5.06 (s, 2H), 4.79 (s, 4H). HR ESI MS calcd. for C21H19FN3O *m/z* $[M + H]^+$ 348.1507, found 348.1519; HPLC purity: >99%.



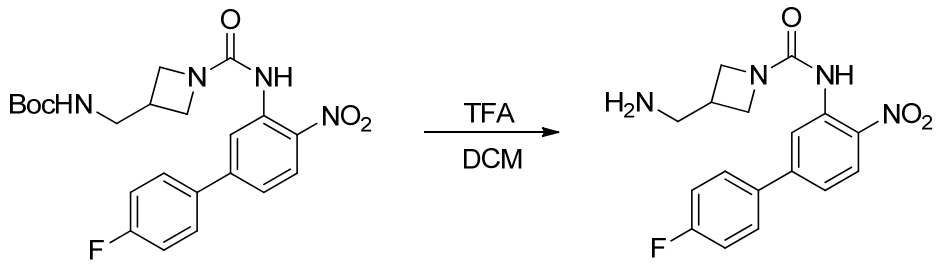
N-(2-amino-5-(pyridin-4-yl)phenyl)isoindoline-2-carboxamide (**BRD4161**) was prepared by substituting pyrrolidine with isoindoline. ESI+ MS: m/z 331 ($[M+H]^+$), ^{1}HNMR (500 MHz, d^6-DMSO): δ 8.52 (d, *J* = 4.5 Hz, 2H), 7.74 (s, 1H), 7.65-7.60 (m, 3H), 7.47 (d, *J* = 8.5 Hz, 1H), 7.40-7.35 (m, 2H), 7.35-7.30 (m, 2H), 6.84 (d, *J* = 8.5Hz, 1H), 4.79 (s, 4H); HR ESI MS calcd. for C20H19N4O *m/z* [M + H]+ 331.1553, found 331.1566; HPLC purity: 97%.



Synthesis of N-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)-3-((dimethylamino)methyl) azetidine-1-carboxamide (**BRD0302**)

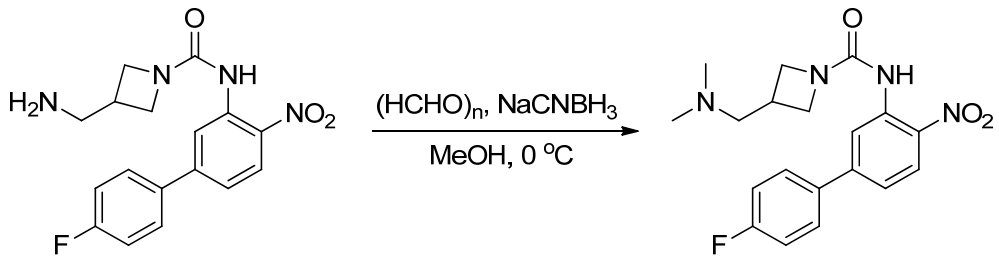


To a solution of 4'-fluoro-4-nitro-[1,1'-biphenyl]-3-amine (1.5g, 6.46 mmol) in DCM (50 mL) were added triphosgene (1.917g, 6.46 mmol) and TEA (8.5g, 84 mmol) at 0^{o}C. The reaction mixture was stirred at room temperature for two hours. *tert*-butyl azetidin-3-ylmethylcarbamate (1.203g, 6.46 mmol) was then added at 0^{o}C and the reaction mixture was stirred at room temperature for four hours. The reaction crude was diluted with saturated citric acid solution and extracted with DCM. The organic layer was washed with water, brine, dried over Na_2SO_4, filtered and concentrated to yield crude residue which was purified by column chromatography eluting with 30% EtOAc in Hexane to afford *tert*-butyl ((1-((4'-fluoro-4-nitro-[1,1'-biphenyl]-3-yl)carbamoyl)azetidin-3-yl)methyl)carbamate (2g, 69.7% yield).

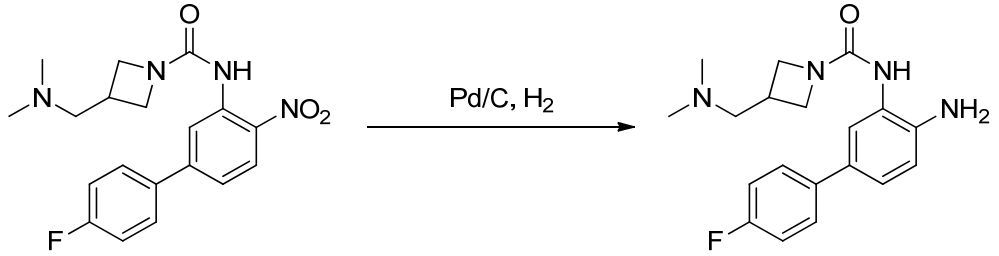


To a solution of tert-butyl (1-(4'-fluoro-4-nitrobiphenyl-3-ylcarbamoyl)azetidin-3-yl)methylcarbamate (2.0 g, 4.5 mmol) in DCM (60 mL) was added TFA (8 mL, 104

mmol) at 0°C. The reaction was warmed to room temperature and stirred for 2 h. The reaction was then concentrated under reduced pressure. The residue was basified with a saturated aqueous solution of $NaHCO_3$ and extracted with a 15% MeOH in DCM. The organic layer was dried over Na_2SO_4 and concentrated to afford 3-(aminomethyl)-N-(4'-fluoro-4-nitro-[1,1'-biphenyl]-3-yl)azetidine-1-carboxamide (1.6g, 100% yield) as yellow syrup which is used in the next step as such without any further purification.

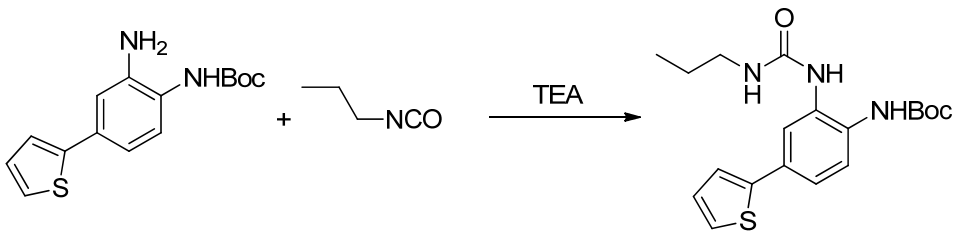


To a solution of 3-(aminomethyl)-N-(4'-fluoro-4-nitro-[1,1'-biphenyl]-3-yl)azetidine-1-carboxamide (2g, 5.81 mmol) in MeOH (50 mL) were added formaldehyde (1.74g, 58.1 mmol) and acetic acid (1.74g, 29.0 mmol) at room temperature and stirred for 1h. $NaCNBH_3$ (1.46 g, 23.23 mmol) was then added at 0 °C. The reaction mixture was stirred at room temperature for two hours. The reaction was quenched with saturated $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with water, brine and dried over Na_2SO_4. The organic layer was concentrated under vacuum to afford crude residue which was purified by column chromatography eluting with 2% MeOH in DCM to yield 3-((dimethylamino)methyl)-N-(4'-fluoro-4-nitro-[1,1'-biphenyl]-3-yl)azetidine-1-carboxamide (1.69g, 78% yield).

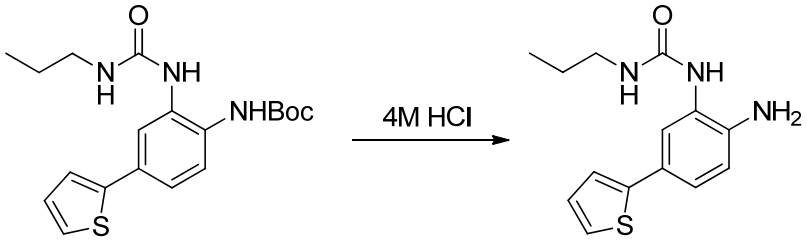


To a solution of 3-((dimethylamino)methyl)-N-(4'-fluoro-4-nitro-[1,1'-biphenyl]-3-yl)azetidine-1-carboxamide (1.7 g, 4.57 mmol, 1.0 eq.) in methanol (100 mL) and was added 10 % Pd/C (1.0 g, 0.94 mmol). The reaction mixture was stirred 2h under a hydrogen atmosphere. The reaction was then filtered and the filtrate was concentrated under reduced pressure to give N-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)-3-((dimethylamino)methyl)azetidine-1-carboxamide (35 mg, 3 % yield) as an off-white solid. ESI+ MS: m/z 343 ($[M+H]^+$), ^{1}HNMR (500 MHz, d^6-DMSO): δ 7.61 (s, 1H), 7.53-7.51 (m, 2H), 7.36 (d, *J* = 2.0 Hz, 1H), 7.21-7.15 (m, 3H), 6.76 (d, *J* = 8.5 Hz, 1H), 4.98 (s, 2H), 3.99 (t, *J* = 8.0 Hz, 2H), 3.58 (dd, *J* = 6.0, 8.0 Hz, 2H), 2.72-2.69 (m, 1H), 2.45-2.44 (m, 2H), 2.13 (s, 6H). HR ESI MS calcd. for C19H24FN4O *m/z* $[M + H]^+$ 343.1929, found 343.1935; HPLC purity: 96%.

1.3.5. Representative synthesis of ureas analogs using isocyanates:



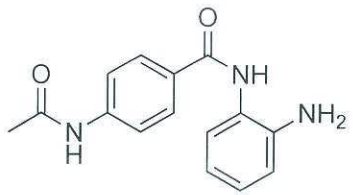
To a solution of *tert*-butyl (2-amino-4-(thiophen-2-yl)phenyl)carbamate (0.25 g, 0.86 mmol, 1.0 equiv.) in dichloromethane (5 mL) was added *n*-propyl isocyanate (0.088 mg, 1.03 mmol, 1.2 equiv.) and TEA (0.24 mL, 1.72 mmol) at 0^{o}C. The reaction was stirred at room temperature for 16 h. The reaction was then diluted with dichloromethane and water. The organic layer was separated, washed with water and brine, dried over magnesium sulfate, then concentrated under reduced pressure. The crude material was purified by column chromatography (silica gel, 20% EtOAc/hexanes) to give *tert*-butyl (2-(3-propylureido)-4-(thiophen-2-yl)phenyl)carbamate (0.25 g, 77 % yield).



1-(2-Amino-5-(thiophen-2-yl)phenyl)-3-propylurea (**17**). A 4M solution of HCl in dioxane (2 mL) was added to a stirred solution of *tert*-butyl (2-(3-propylureido)-4-(thiophen-2-yl)phenyl)carbamate (0.16 g, 0.43 mmol, 1 equiv.) in methanol (4 mL) at 0 ^{o}C. The reaction was warmed to room temperature and stirred for 2h. The reaction was then concentrated under reduced pressure. A saturated aqueous solution of sodium bicarbonate was added. The obtained solid was filtered, washed with water and dried to yield 1-(2-Amino-5-(thiophen-2-yl)phenyl)-3-propylurea (**17**) (0.035 g, 30% yield). ESI+ MS: m/z 276 ([M+H]$^{+}$), ^{1}HNMR (500 MHz, d^{6}-DMSO): δ 7.65 (s, 1H), 7.56 (s, 1H), 7.33 (d, *J* = 5.0 Hz, 1H), 7.18 (d, *J* = 3.0 Hz, 1H), 7.10 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.03 (t, *J* = 4.5 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 6.21 (t, *J* = 5.5 Hz, 1H), 4.90 (s, 2H), 3.05 (t, *J* = 6.5 Hz, 2H), 1.45 (sext, *J* = 6.5 Hz, 2H), 0.89 (t, *J* = 6.5 Hz, 3H). HR ESI MS calcd. for C14H18N3OS *m/z* [M + H]+ 276.1165, found 276.1170; HPLC purity: >99%.

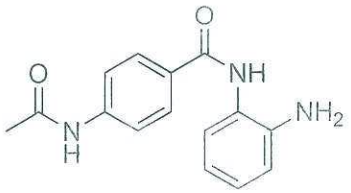
2. ^{1}HNMR and ^{13}CNMR spectra and HPLC/UPLC traces of synthesized compounds

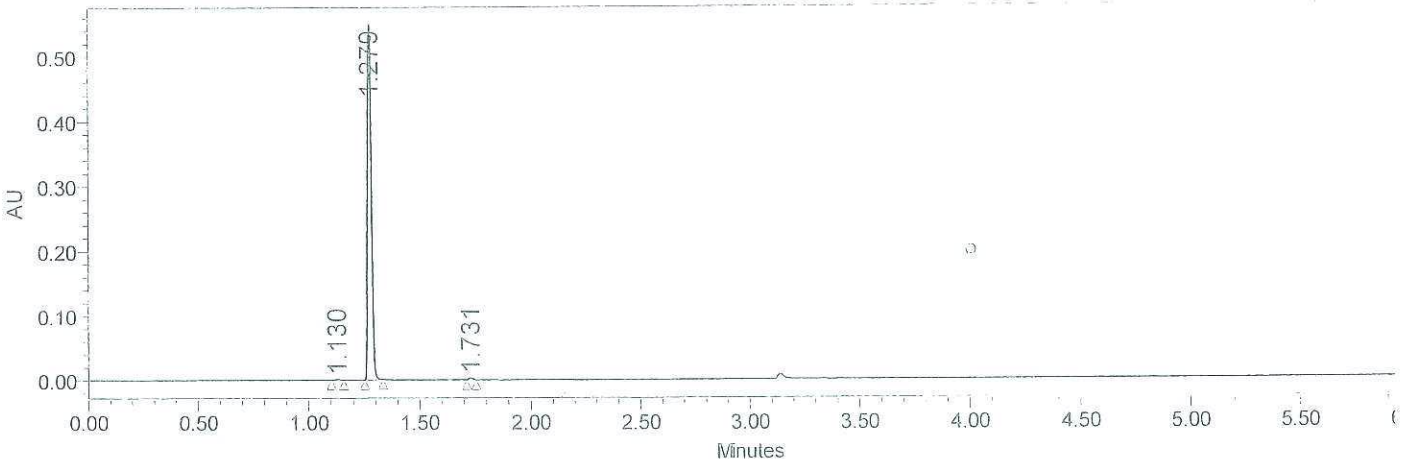
SBF-MA1003-015 in DMSO-d6
File No.B180310A59



Sample Name:	**SBF-MA1003-015 # IP10030940**	Acquired By:	System
Vial:	2:C,6	Acq. Method Set:	PDA _METH_2A
Injection Volume:	0.20 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	3/18/2010 6:33:44 PM IST
Project Name: SAPL_UNIT-1_AMCL_ALC-13_MAR-10			

Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25°C
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0/90,0.5/90,3/10,6/10
Diluent : ACN:Water (1:1)





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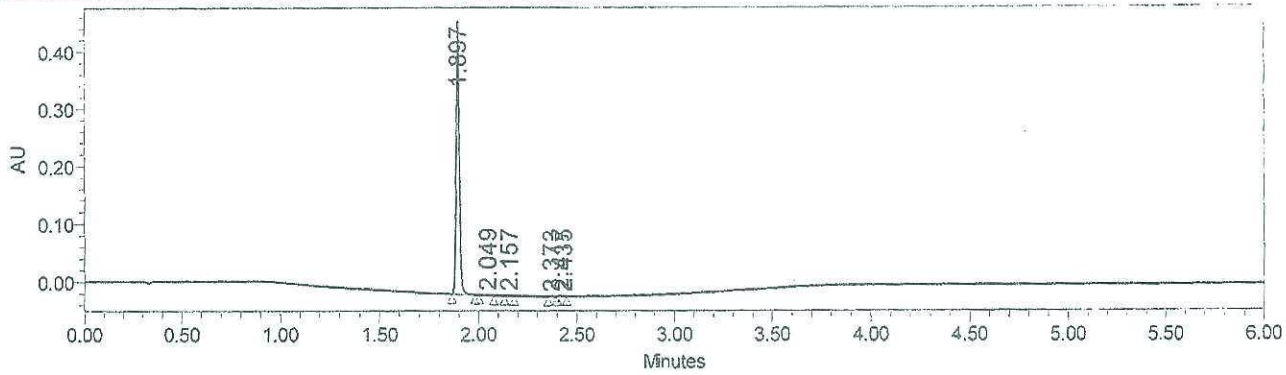
3	1.73	3862	0.58

SBF-8002-069 in DMSO-D6
File.No.8050109A61

SAMPLE INFORMATION

Sample Name:	**SBF-8002-069#IP0901034**	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	020109_02
Vial:	2:D,5	Acq. Method Set:	PDA_METH_2A
Injection #:	1	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Injection Volume:	0.50 ul	Date Acquired:	1/2/2009 7:04:24 PM IST
Run Time:	6.0 Minutes	Date Processed:	1/2/2009 7:14:37 PM IST
Project Name:	JAN-09		

Column: Acquity UPLC BEH C18{2.1 x50mm,1.7u}
Column Temp:25°C
Mobile Phase:A: Acetonitrile
Mobile Phase:B:0.025%Aq TFA
Flow:0.5ml/min
Gradient programe:
T/%B:0.01/90,0.5/90,3/10,6/10
Diluent: Water:ACN(1:1)



	RT	Area	% Area
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3	2.16	1534	0.29
4	2.37	525	0.10
5	2.42	1668	0.32
6	2.43	513	0.10

SBF-HA9003-026 In DMSO-d6
File No.B140809A74

Varian 500MHz Unity INOVA
Date & Time:Fri Aug 14 19:51:34 IST 2009
Analyst: Anji Karun

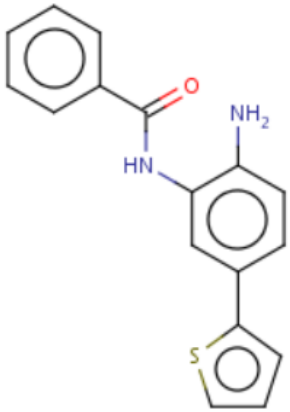
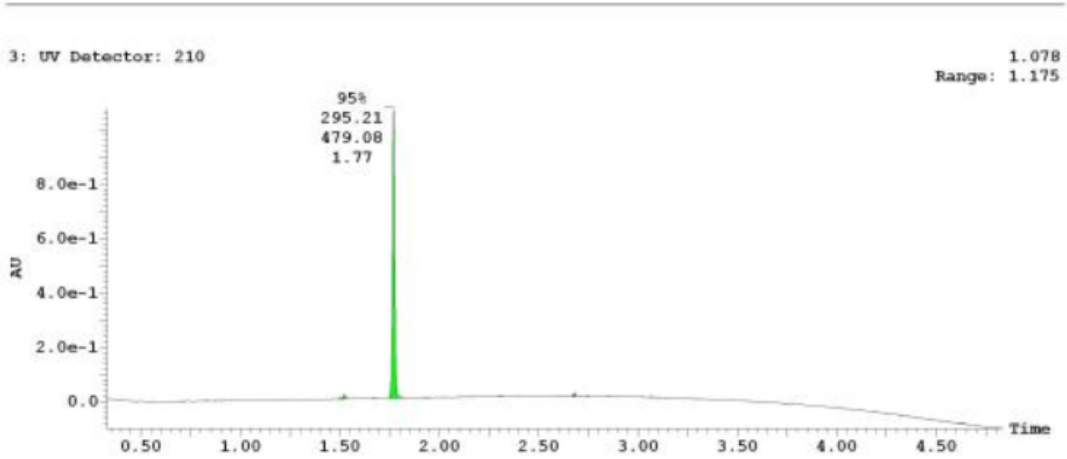


Plate: GR00010798
Well: E09
Sample ID: SA76173
Broad ID:
SMILES: Nc1ccc(cc1NC(=O)c2cc ...
Source: 1087726290
Row: 27



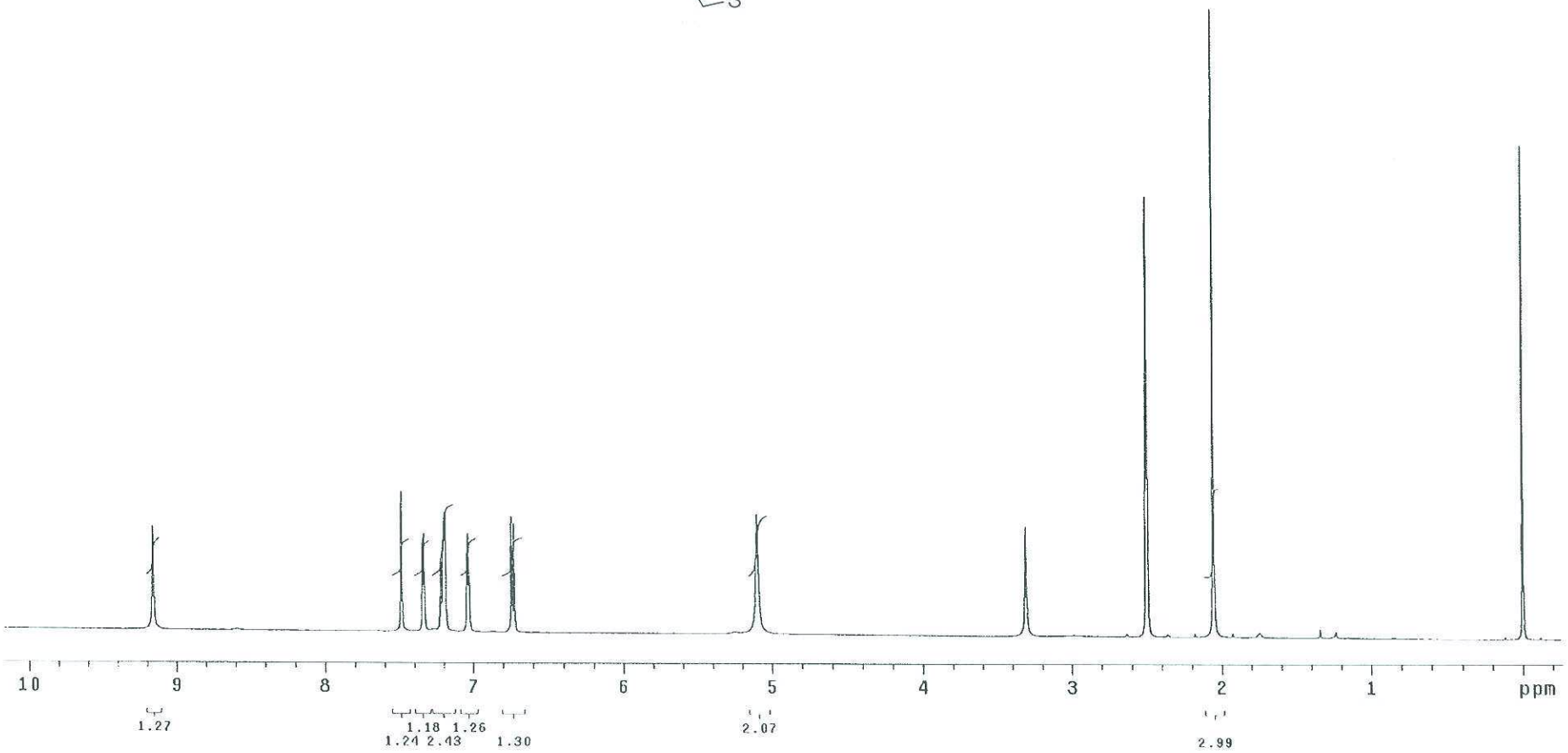
Target Mass: 294.08
with Salt: 294.08
Run: # 1 on 2014/01/30 10:45 PM
Integration: # 1 on 2014/01/30 10:45 PM
Method: acidic

SpectrixX

OpenLynx

SBF-HB9002-090 in DHSO-d6
FILE.NO.B170709A13

Varian 500MHz Unity INOVA
Date & Time:Fri Jul 17 12:45:36 IST 2009
Analyst: padma



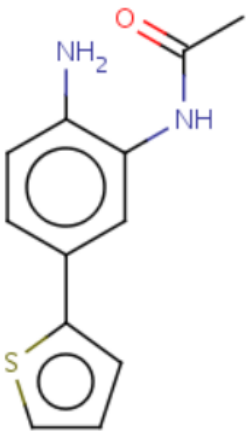


Plate: GR00006567
Well: F05
Sample ID: SA441056
Broad ID:
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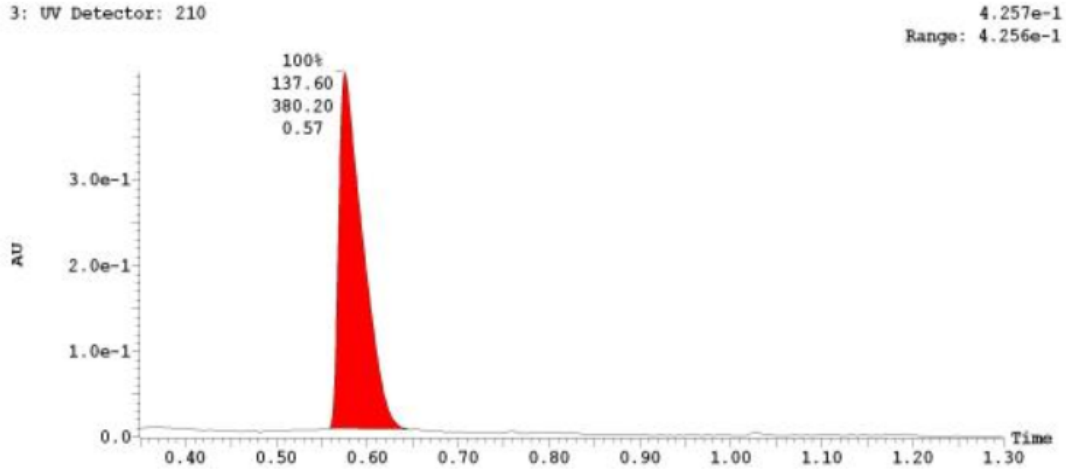
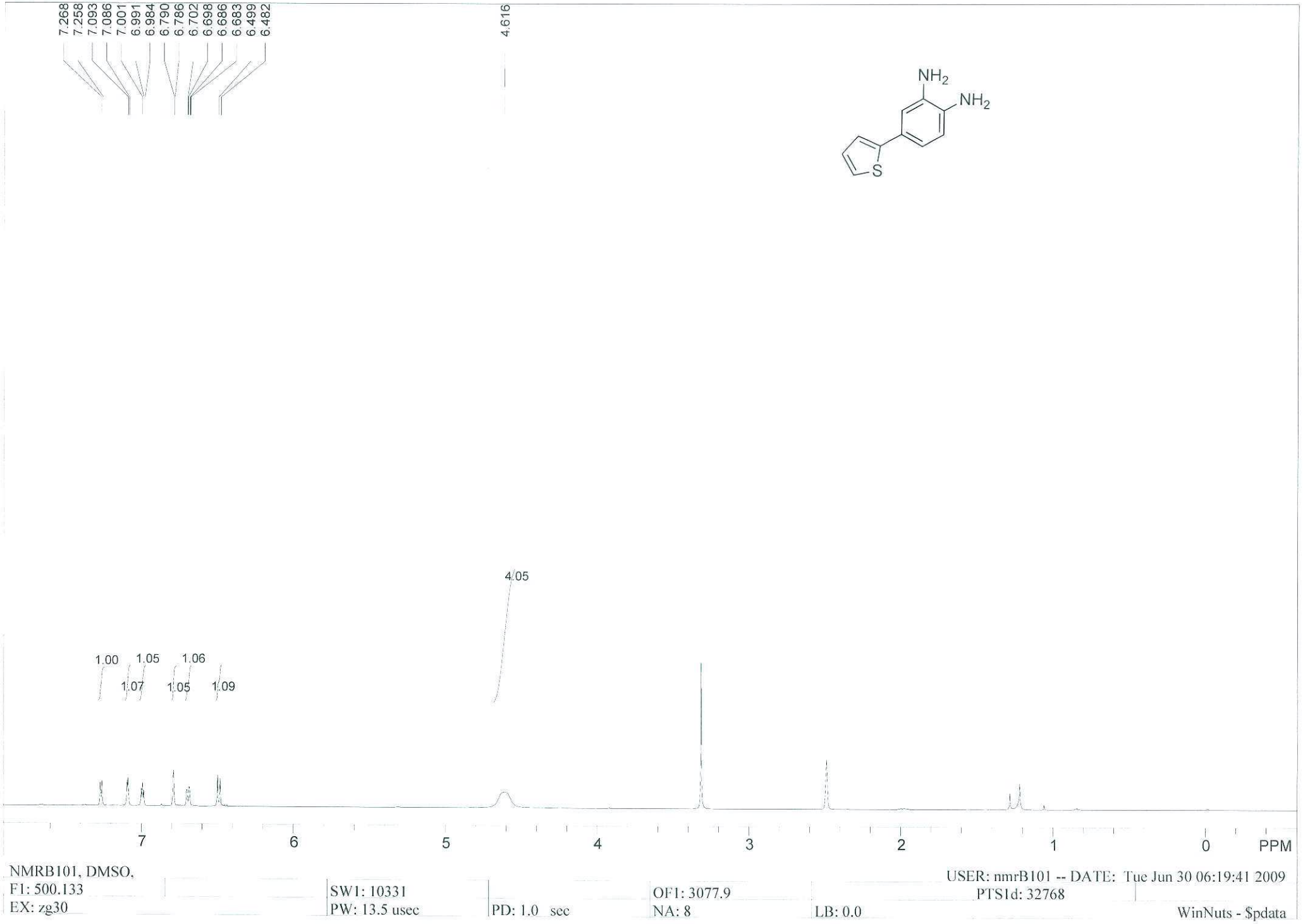
Source: TV002739
Row: 30



Target Mass: 232.07
with Salt: 232.07
Run: # 1 on 2009/07/09 09:10 PM
Integration: # 1 on 2009/07/09 09:10 PM
Method: acidic

SpectrixX
OpenLynx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7.258
7.093
7.086
7.001
6.991
6.984
6.790
6.786
6.702
6.698
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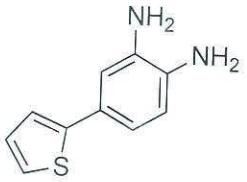
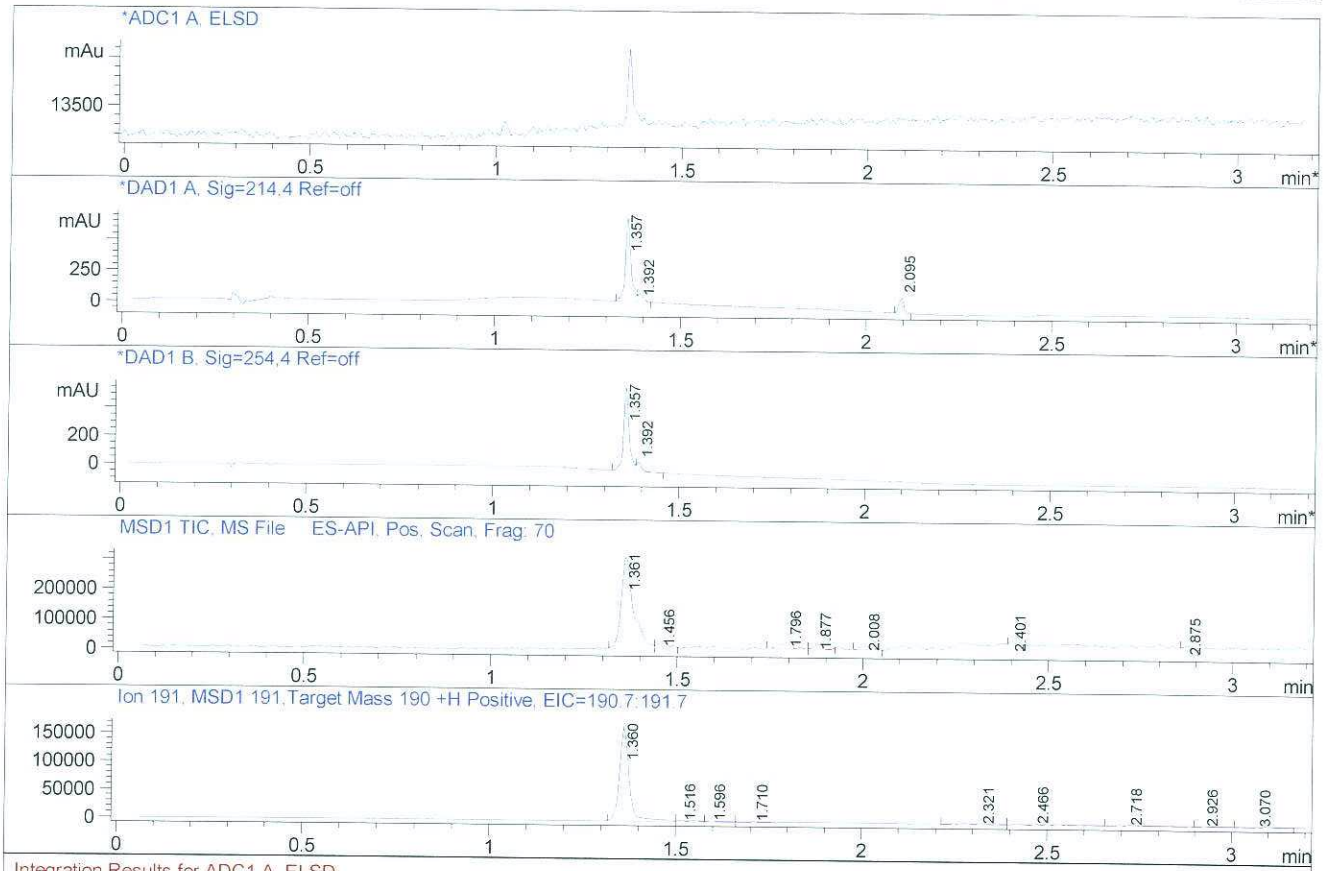
6.683
6.499
6.482
4.616
NH2
NH2
S
4.05
1.00
1.05
1.06
1.07
1.05
1.09
7
6
5
4
3
2
1
0
PPM
NMRB101, DMSO,
F1: 500.133
EX: zg30
SW1: 10331
PW: 13.5 usec
PD: 1.0 sec
OF1: 3077.9
NA: 8
LB: 0.0
USER: nmrB101 -- DATE: Tue Jun 30 06:19:41 2009
PTS1d: 32768
WinNuts - $pdata

MS Report

File D:\EZXDATA\T46\06-09\CP-0003507-032-00182.D
Injection Date : 30 Jun 09 1:01 pm +0800
Sample Name : CP-0003507-032
Acq. Operator : LCMSA102
Spec. Reported : MS Integration
Acq. Method : C:\Chem32\1\METHODS\1-T46.M
Analysis Method : C:\Chem32\1\METHODS\1-T46.M
Sample Info : 190.00
Method Info : Mobile Phase: A: water(0.05%TFA) B: ACN(0.05%TFA)
Gradient: 5%-95% B in 1.4min
Flow Rate: 2.2ml/min
Column: SunFire C18,4.6*50mm,3.5um
Oven Temperature: 50 C

Tgt Mass (EZX): 190.00
Seq. Line : 0
Location : P2-C-04
Inj : 1
Inj Volume : 2 ul





Integration Results for ADC1 A, ELSD

RetTim	Width	Area	Height	Area%

Integration Results for DAD1 A, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
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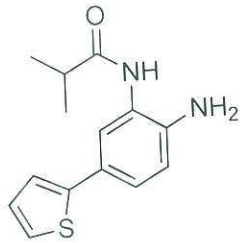
1.39	0.01	66.13	69.71	7.40
2.09	0.01	109.75	120.48	12.29

Integration Results for DAD1 B, Sig=254,4 Ref=off

RetTim	Width	Area	Height	Area%
1.36	0.02	626.17	590.69	90.01
1.39	0.01	69.51	69.18	9.99

SBF-MA9002-015 in DMSO-d6
FILE.NO.8090709A27

Varian 500MHz Unity INOVA
Date & Time:Thu Jul 9 16:15:31 IST 2009
Analyst: padma

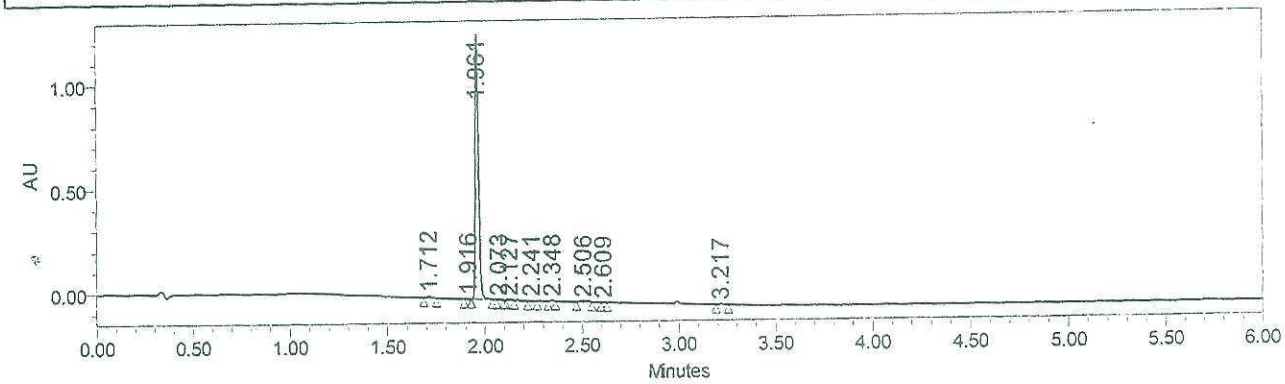


SAMPLE INFORMATION

Sample Name: **SBF-MA9002-015 # IP0907279**
Sample Type: Unknown
Vial: 1:A,7
Injection #: 1
Injection Volume: 0.50 ul
Run Time: 6.0 Minutes
Project Name: SAPL_UNIT-I_AMCL_JULY-09

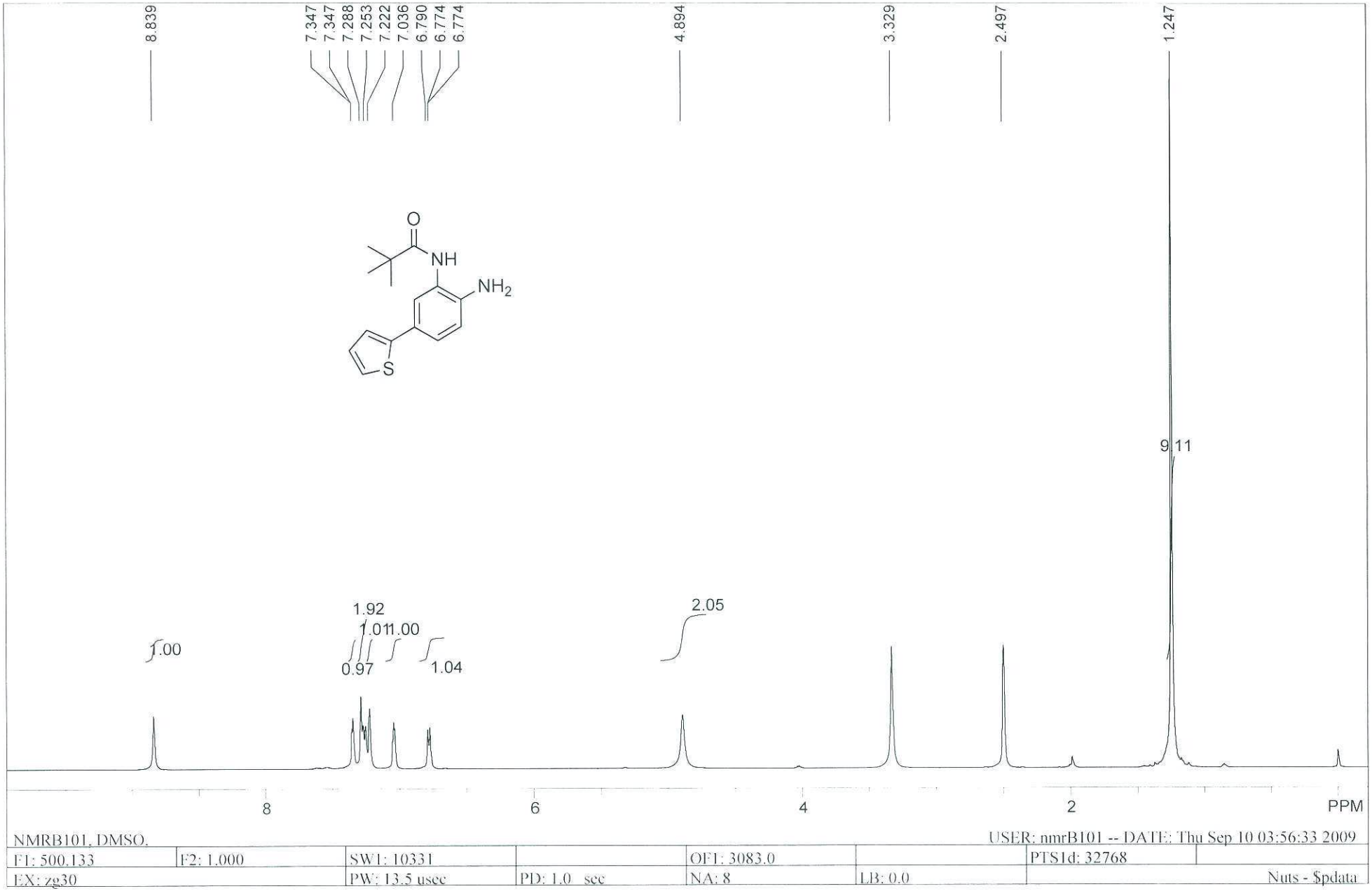
Acquired By: System
Sample Set Name: 080709_05
Acq. Method Set: PDA_METH_2A
Proc. Chnl. Descr.: **PDA 210.0 nm**
Date Acquired: 7/9/2009 10:25:15 AM IST
Date Processed: 7/9/2009 11:02:24 AM IST

Column: Acquity UPLC BEH C8{2.1 x50mm,1.7u}
Column Temp:25°C
Mobile Phase:A: Acetonitrile
Mobile Phase:B:0.025%Aq TFA
Flow:0.5ml/min
Gradient programe:
T/%B:0.01/90,0.5/90,3/10,6/10
Diluent: ACN:Water(1:1)



	RT	Area	% Area
1	1.71	8338	0.56
2	1.92	1144	0.08
3	1.96	1449835	98.05
4	2.07	667	0.05
5	2.13	2656	0.18
6	2.24	1390	0.09
7	2.35	3569	0.24
8	2.51	8225	0.56
9	2.61	695	0.05

	RT	Area	% Area
10	3.22	2161	0.15



NMRB101, DMSO.

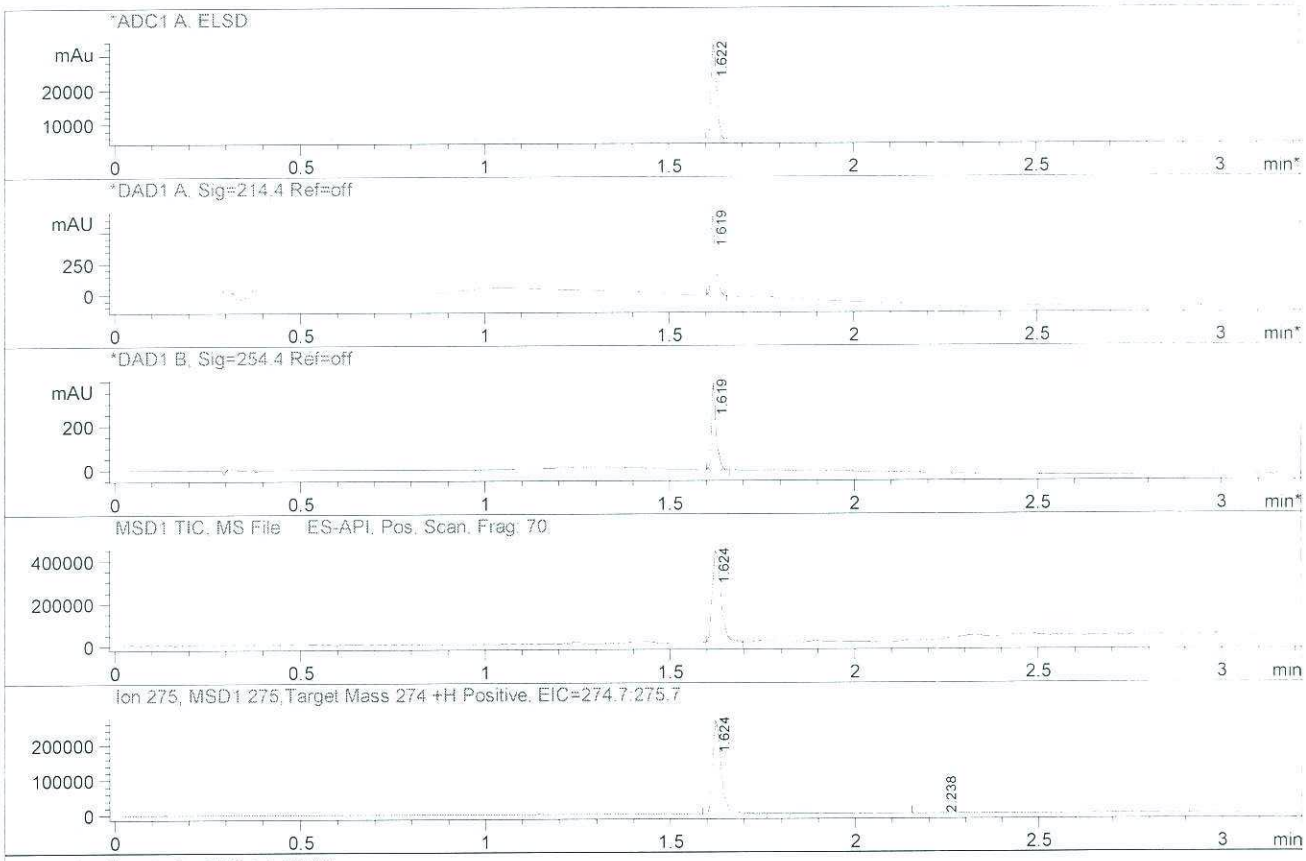
USER: nmrB101 -- DATE: Thu Sep 10 03:56:33 2009

F1: 500.133	F2: 1.000	SW1: 10331		OF1: 3083.0		PTS1d: 32768
EX: zg30		PW: 13.5 usec	PD: 1.0 sec	NA: 8	LB: 0.0	Nuts - $pdata

MS Report

File D:\EZXDATA\T46\09-09\CP-3819-081-08341.D
Injection Date : 9 Sep 09 11:17 am +0800
Sample Name : CP-3819-081
Acq. Operator : LCMSA102
Spec. Reported : MS Integration
Acq. Method : C:\Chem32\1\METHODS\1-T46.M
Analysis Method : C:\Chem32\1\METHODS\1-T46.M
Sample Info : 274.00
Method Info : Mobile Phase: A: water(0.05%TFA) B: ACN(0.05%TFA)
Gradient: 5%-95% B in 1.4min
Flow Rate: 2.2ml/min
Column: SunFire C18,4.6*50mm,3.5um
Oven Temperature: 50 C

Tgt Mass (EZX): 274.00
Seq. Line : 0
Location : P2-D-04
Inj : 1
Inj Volume : 1 ul



Integration Results for ADC1 A, ELSD

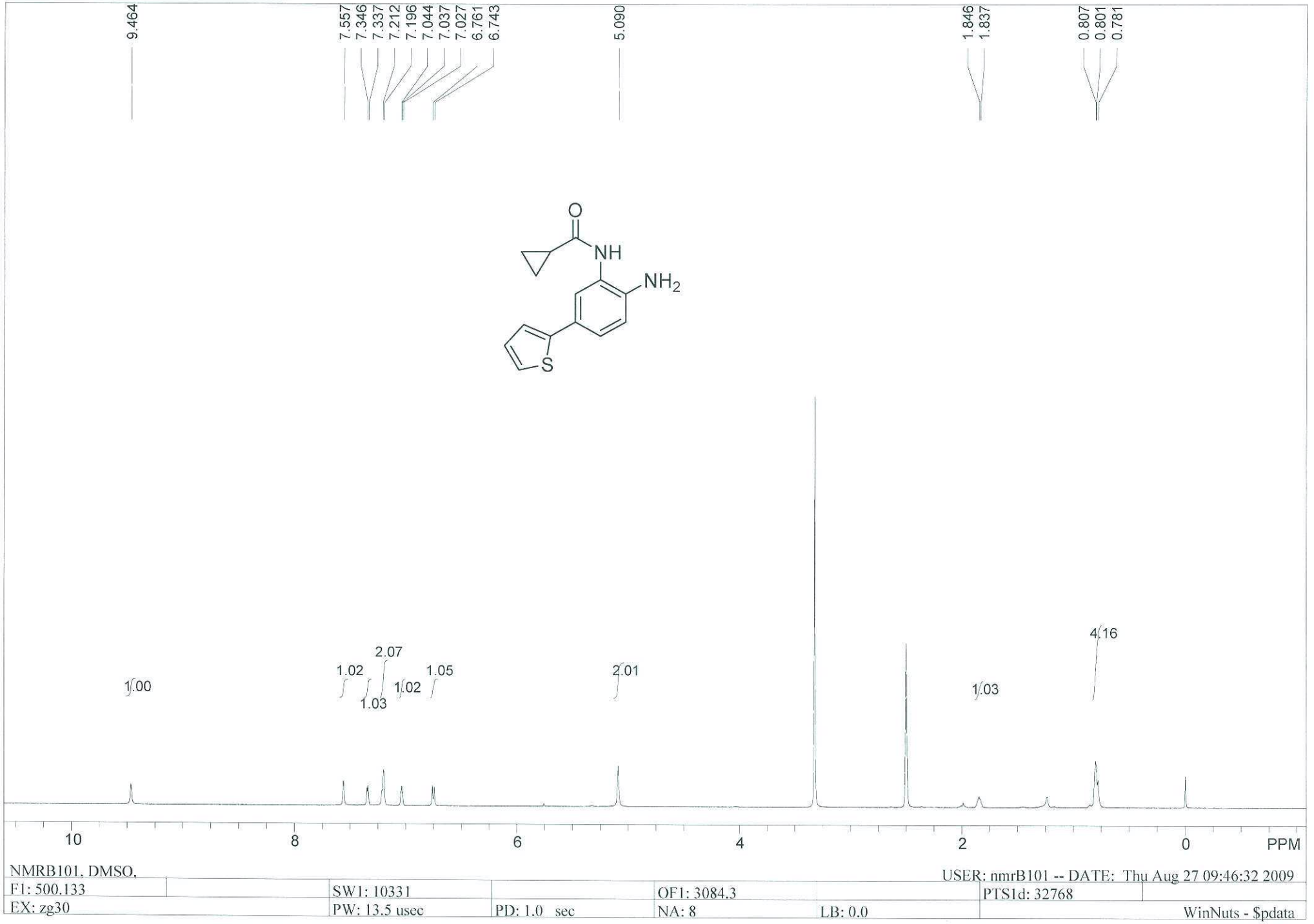
RetTim	Width	Area	Height	Area%
1.62	0.02	28461.57	27050.88	100.00

Integration Results for DAD1 A, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
1.62	0.01	554.05	652.18	100.00

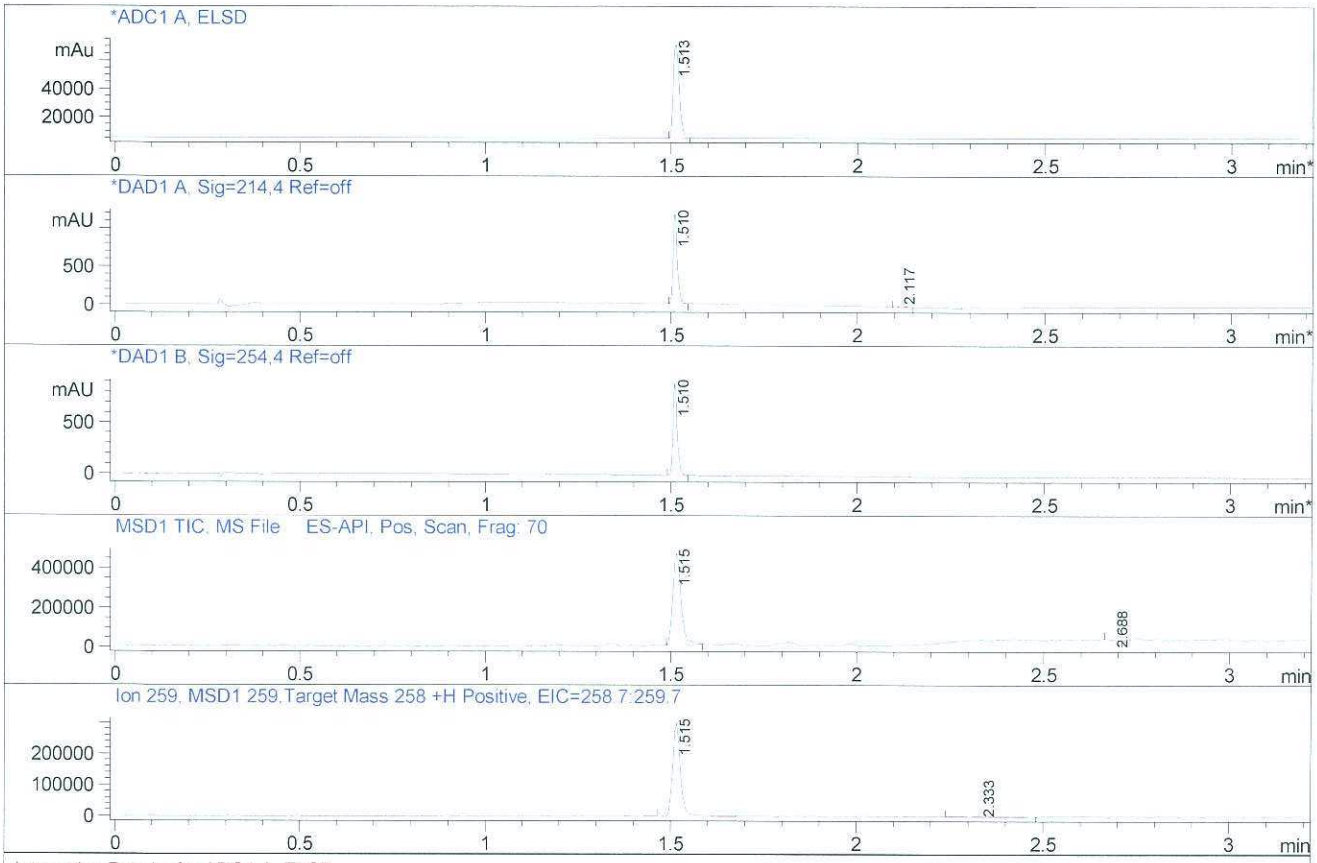
Integration Results for DAD1 B, Sig=254,4 Ref=off

RetTim	Width	Area	Height	Area%
1.62	0.01	333.64	390.67	100.00


9.464
7.557
7.346
7.337
7.212
7.196
7.044
7.037
7.027
6.761
6.743
5.090
1.846
1.837
0.807
0.801
0.781
O
NH
NH2
S
1.00
1.02
1.03
2.07
1.02
1.05
2.01
1.03
4.16
10
8
6
4
2
0
PPM
NMRB101, DMSO,
USER: nmrB101 -- DATE: Thu Aug 27 09:46:32 2009
F1: 500.133
SW1: 10331
OF1: 3084.3
PTS1d: 32768
EX: zg30
PW: 13.5 usec
PD: 1.0 sec
NA: 8
LB: 0.0
WinNuts - $pdata

MS Report

```
File D:\EZXDATA\T46\08-09\CP-0003507-109-06866.D   Tgt Mass (EZX): 258.00
Injection Date    : 27 Aug 09   7:36 pm +0800          Seq. Line : 0
Sample Name       : CP-0003507-109                  Location  : P2-E-04
Acq. Operator     : LCMSA102                            Inj : 1
Spec. Reported    : MS Integration                  Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\1-T46.M
Analysis Method   : C:\Chem32\1\METHODS\1-T46.M
Sample Info : 258.00
Method Info : Mobile Phase:  A: water(0.05%TFA)   B: ACN(0.05%TFA)
              Gradient: 5%-95% B in 1.4min
              Flow Rate: 2.2ml/min
              Column: SunFire C18,4.6*50mm,3.5um
              Oven Temperature: 50 C
```



Integration Results for ADC1 A, ELSD

RetTim	Width	Area	Height	Area%
1.51	0.02	73958.31	65311.74	100.00

Integration Results for DAD1 A, Sig=214,4 Ref=off

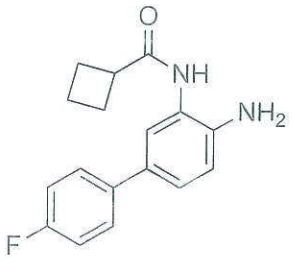
RetTim	Width	Area	Height	Area%
1.51	0.01	914.60	1171.83	97.82
2.12	0.02	20.39	14.20	2.18

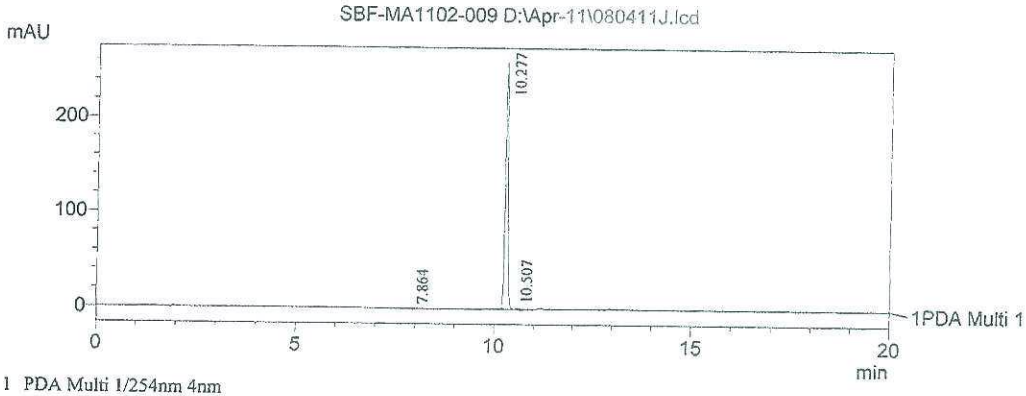
Integration Results for DAD1 B, Sig=254,4 Ref=off

RetTim	Width	Area	Height	Area%
1.51	0.01	698.92	894.47	100.00

SBF-MA1102-009 in DMSO-d6
File.No.B080411A60

D:\Apr-11\080411J.lcd
Sample Name : SBF-MA1102-009
Vial No. : 24
Sample ID : IP 11040132
Injection Volume : 5 uL
Data File Name : 080411J.lcd
Method File Name : sai.lcm
Data Acquired : 4/8/2011 6:52:34 PM
Column : Eclipse XDB C18 (150 x 4.6mm,5um)
Mobile phase A : ACN
Mobile phase B : 5mM NH4OAc
Programme : T/B % : 0.01/90,3/90,10/10,20/10.
Flow : 1.00ml
Diluent : ACN:WATER.



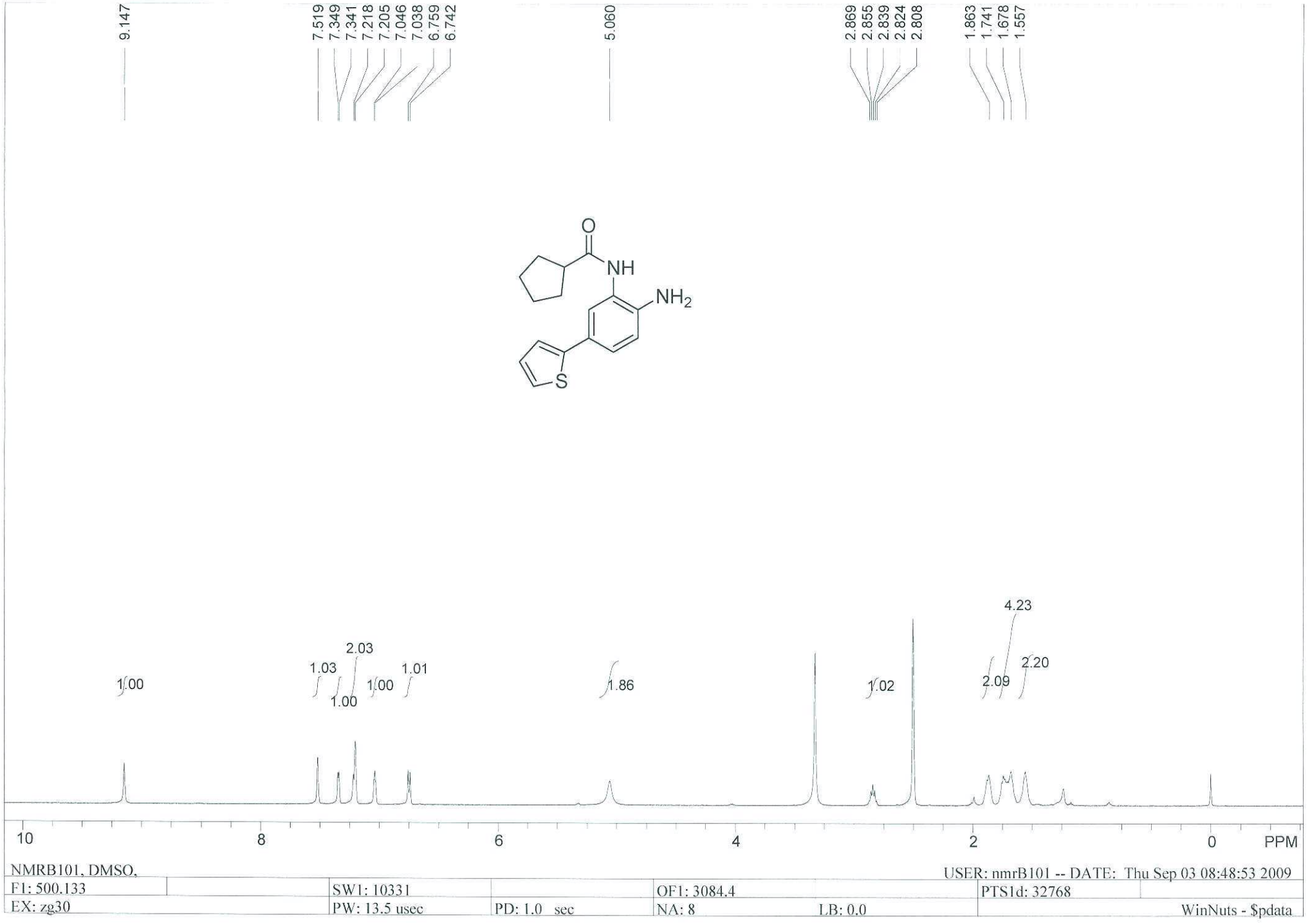


1 PDA Multi 1/254nm 4nm

PeakTable

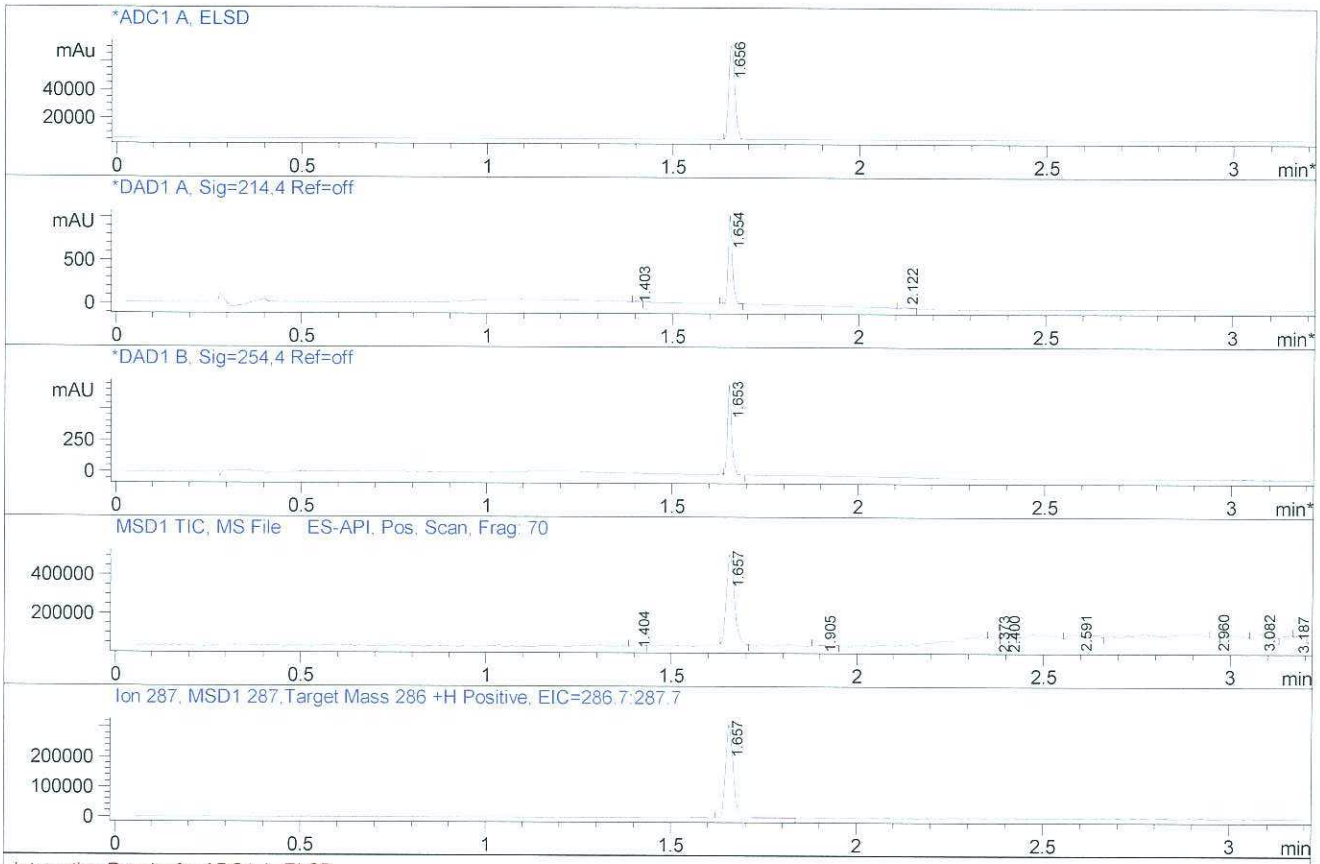
PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	7.864	2622	507	0.214
2	10.277	1208690	260499	98.764
3	10.507	12500	2407	1.021
Total		1223812	263414	100.000


9.147
7.519
7.349
7.341
7.218
7.205
7.046
7.038
6.759
6.742
5.060
2.869
2.855
2.839
2.824
2.808
1.863
1.741
1.678
1.557
O
NH
NH2
S
1.00
1.03
1.00
2.03
1.00
1.01
1.86
1.02
4.23
2.09
2.20
10
8
6
4
2
0
PPM
NMRB101, DMSO,
USER: nmrB101 -- DATE: Thu Sep 03 08:48:53 2009
F1: 500.133
SW1: 10331
OF1: 3084.4
PTS1d: 32768
EX: zg30
PW: 13.5 usec
PD: 1.0 sec
NA: 8
LB: 0.0
WinNuts - $pdata

MS Report

```
File D:\EZXDATA\T46\09-09\CP-0003507-116-07564.D   Tgt Mass (EZX): 286.00
Injection Date    :  2 Sep 09   8:02 pm +0800          Seq. Line : 0
Sample Name       : CP-0003507-116                 Location  : P1-F-07
Acq. Operator     : LCMSA102                             Inj : 1
Spec. Reported    : MS Integration                   Inj Volume : 5 ul
Acq. Method       : C:\Chem32\1\METHODS\1-T46.M
Analysis Method   : C:\Chem32\1\METHODS\1-T46.M
Sample Info : 286.00
Method Info : Mobile Phase:  A: water(0.05%TFA)   B: ACN(0.05%TFA)
              Gradient: 5%-95% B in 1.4min
              Flow Rate: 2.2ml/min
              Column: SunFire C18,4.6*50mm,3.5um
              Oven Temperature: 50 C
```



Integration Results for ADC1 A, ELSD

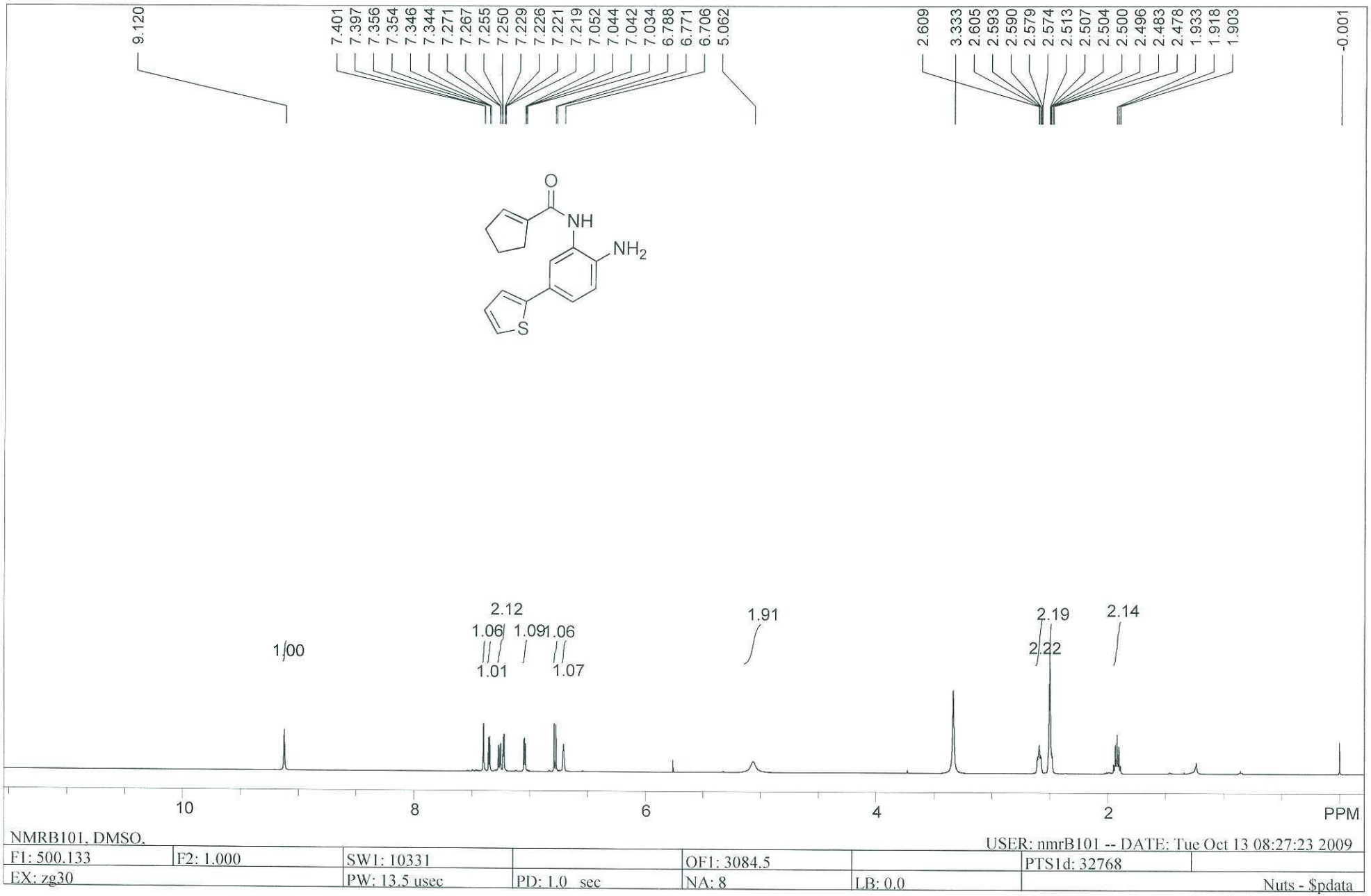
RetTim	Width	Area	Height	Area%
1.66	0.02	75511.81	65010.40	100.00

Integration Results for DAD1 A, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
1.40	0.01	9.19	13.63	1.10
1.65	0.01	808.79	1016.66	96.62
2.12	0.02	19.11	13.30	2.28

Integration Results for DAD1 B, Sig=254,4 Ref=off

RetTim	Width	Area	Height	Area%
1.65	0.01	566.52	714.49	100.00



NMRB101, DMSO,

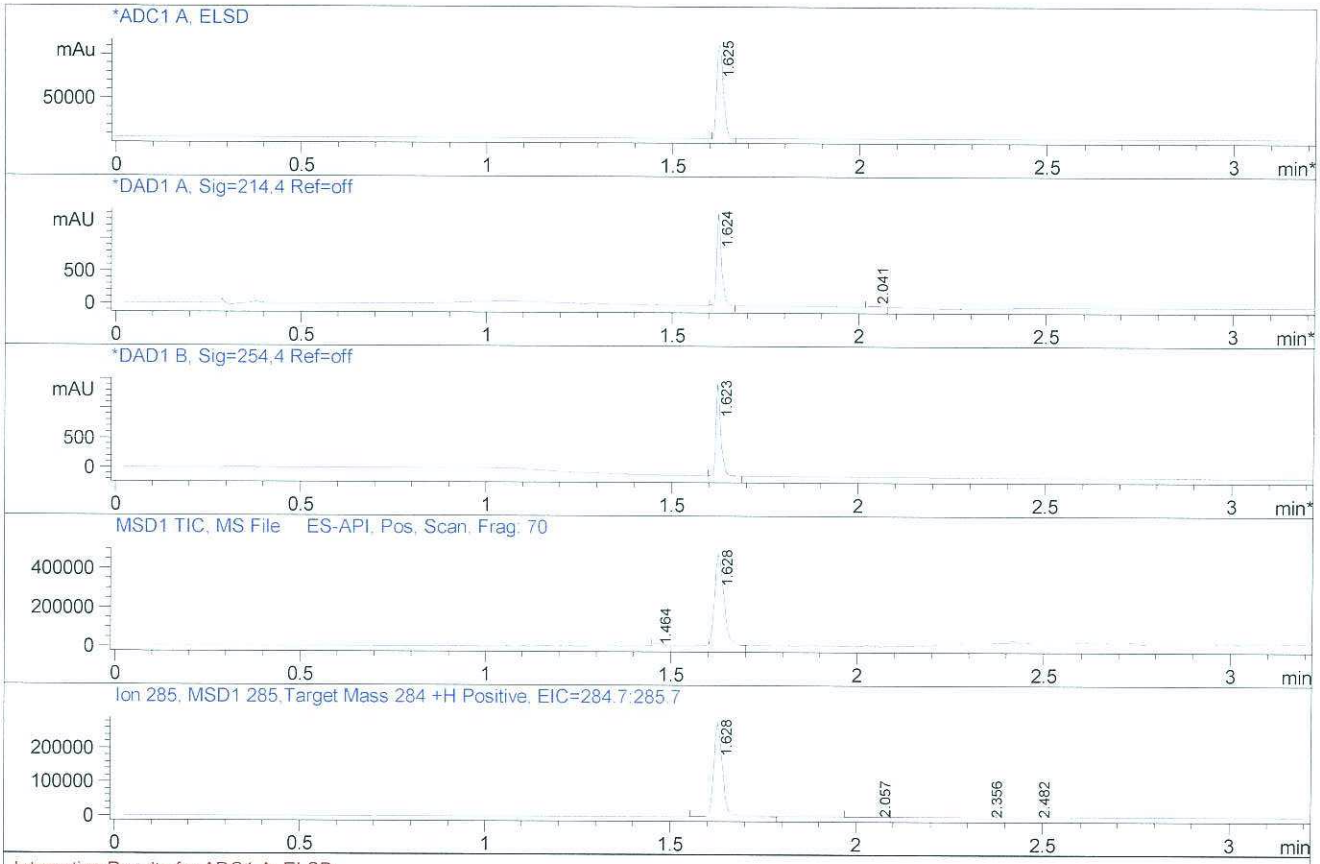
USER: nmrB101 -- DATE: Tue Oct 13 08:27:23 2009

F1: 500.133	F2: 1.000	SW1: 10331		OF1: 3084.5		PTS1d: 32768	
EX: zg30		PW: 13.5 usec	PD: 1.0 sec	NA: 8	LB: 0.0		Nuts - $pdata

MS Report

File D:\EZXDATA\T46\10-09\CP-3819-115-11380.D
Injection Date : 13 Oct 09 11:23 am +0800
Sample Name : CP-3819-115
Acq. Operator : LCMSA102
Spec. Reported : MS Integration
Acq. Method : C:\Chem32\1\METHODS\1-T46.M
Analysis Method : C:\Chem32\1\METHODS\1-T46.M
Sample Info : 284.00
Method Info : Mobile Phase: A: water(0.05%TFA) B: ACN(0.05%TFA)
Gradient: 5%-95% B in 1.4min
Flow Rate: 2.2ml/min
Column: SunFire C18,4.6*50mm,3.5um
Oven Temperature: 50 C

Tgt Mass (EZX): 284.00
Seq. Line : 0
Location : P1-F-02
Inj : 1
Inj Volume : 2 ul



Integration Results for ADC1 A, ELSD

RetTim	Width	Area	Height	Area%
1.62	0.02	128663.87	105955.95	100.00

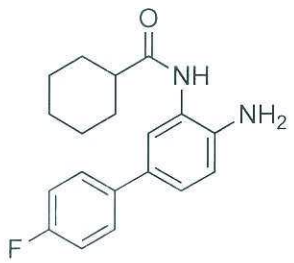
Integration Results for DAD1 A, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
1.62	0.01	1266.79	1407.42	97.68
2.04	0.02	30.05	19.26	2.32

Integration Results for DAD1 B, Sig=254,4 Ref=off

RetTim	Width	Area	Height	Area%
1.62	0.01	1400.78	1554.51	100.00

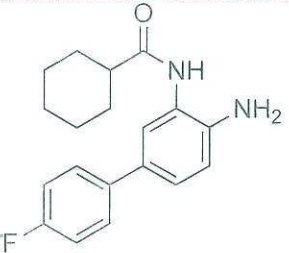
SBF-MA1010-005 in DMSO-d6
File.No.B030910A46

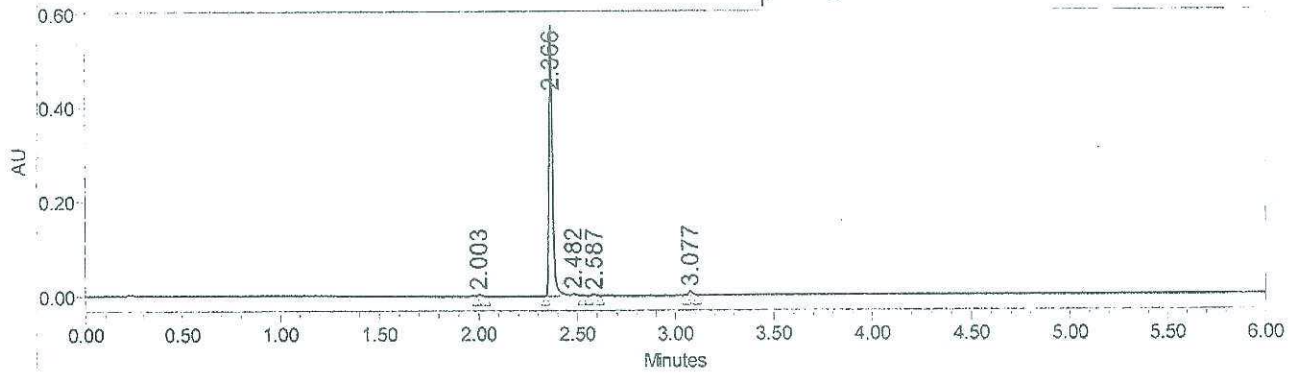


SAMPLE INFORMATION

Sample Name:	**SBF-MA1010-005 # IP10090032**	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	010910_02
Vial:	1:A,7	Acq. Method Set:	PDA_METH_2A
Injection #:	1	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Injection Volume:	2.50 ul	Date Acquired:	9/1/2010 5:56:13 PM IST
Run Time:	6.0 Minutes	Date Processed:	9/1/2010 6:21:12 PM IST
Project Name:	SAPL_UNIT-1_AMCL_ALC-10_SEP-10		

Column: Acquity UPLC BEH C-18 {2.1 x50mm,1.7u}
Column Temp:25°C
Mobile Phase:A: Acetonitrile
Mobile Phase:B:0.025% Aq TFA
Flow:0.5ml/min
Gradient programe:
T/%B:0.01/90,0.5/90,3/10,6/10
Diluent: ACN:Water:(1:1)



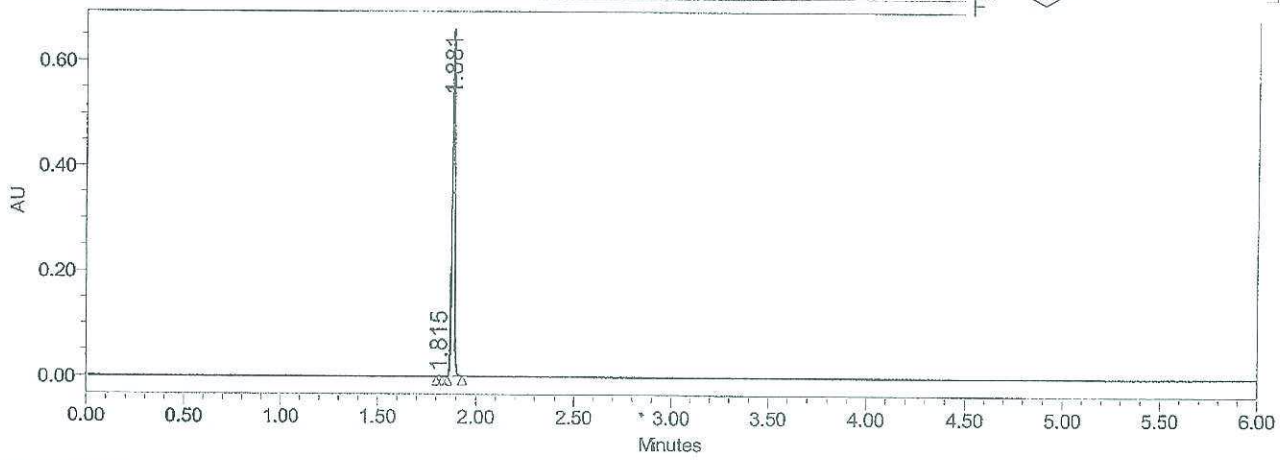


	RT	Area	% Area
1	2.00	4111	0.56
2	2.37	707761	96.12
3	2.48	8042	1.09
4	2.59	4423	0.60
5	3.08	11982	1.63

SBF-MA1201-097 in DMSO-d6
File No.B020712B96

Sample Name:	**SBF-MA1201-097 # IP12070057**	Acquired By:	System
Vial:	2:E,3	Acq. Method Set:	PDA _METH_2A
Injection Volume:	0.50 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	7/2/2012 6:40:43 PM IST
Project Name: SAPL_UNIT-2_AMCL_ALC-13_JUL-12			

Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25℃
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0.01/90,0.5/90,3/10,6/10.
Diluent : ACN : ACN-WATER (1:1)



	RT	Area	% Area
1	1.82	1386	0.23
2	1.88	589771	99.77

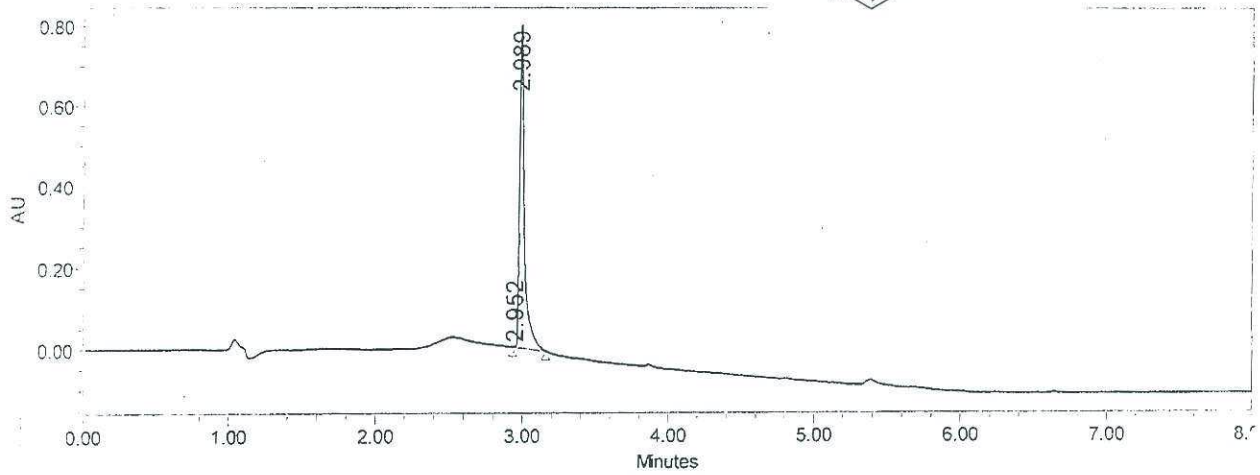
SBF-MA1007-055 in DMSO-d6
File No.B300810A91

SAMPLE INFORMATION

Sample Name:	**SBF-MA1007-055 # IP10081111**	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	082610
Vial:	2:B,8	Acq. Method Set:	PDA METH_T3
Injection #:	1	Proc. Chnl. Descr.:	**PDA 210.0 nm**
Injection Volume:	1.50 ul	Date Acquired:	8/27/2010 11:03:04 PM IST
Run Time:	8.0 Minutes	Date Processed:	8/27/2010 11:18:26 PM IST

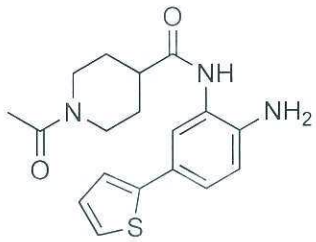
Project Name: SAPL_UNIT-1_AMCL_ALC-10_AUG-10

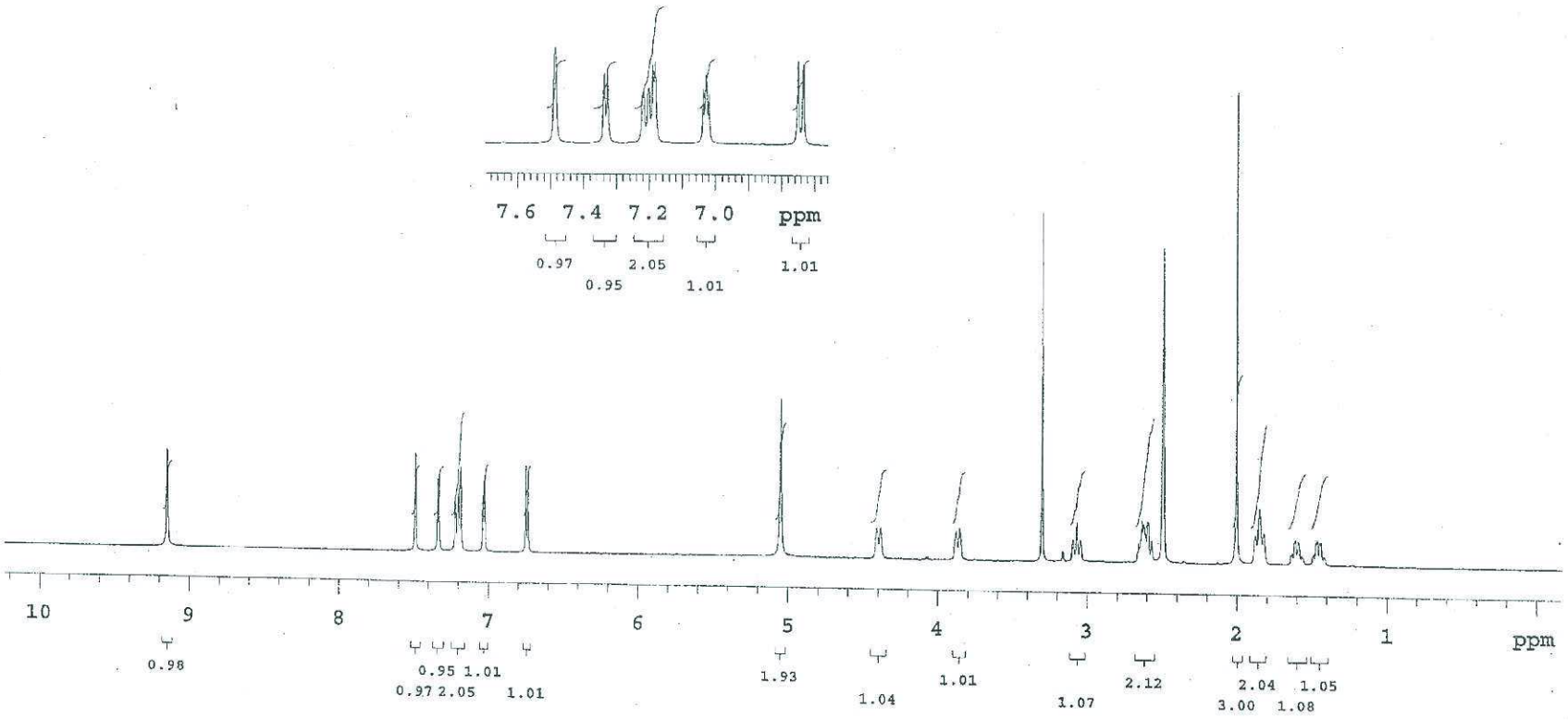
Column:Acquity UPLC HSS T3{2.1x100mm,1.7u}
Column Temp:25°C
Mobile Phase:A: Acetonitrile
MobilePhase:B:0.025% Aqueous TFA
Flow:0.3ml/min
Gradient programe:
T/%B:0.01/95,1/95,5/10,8/10.
Diluent: ACN Water (1:1)



	RT	Area	% Area
1	2.952	2921	0.16
2	2.989	1785791	99.84

SBF-MA1107-095 in DMSO-d6
File No B120312B43

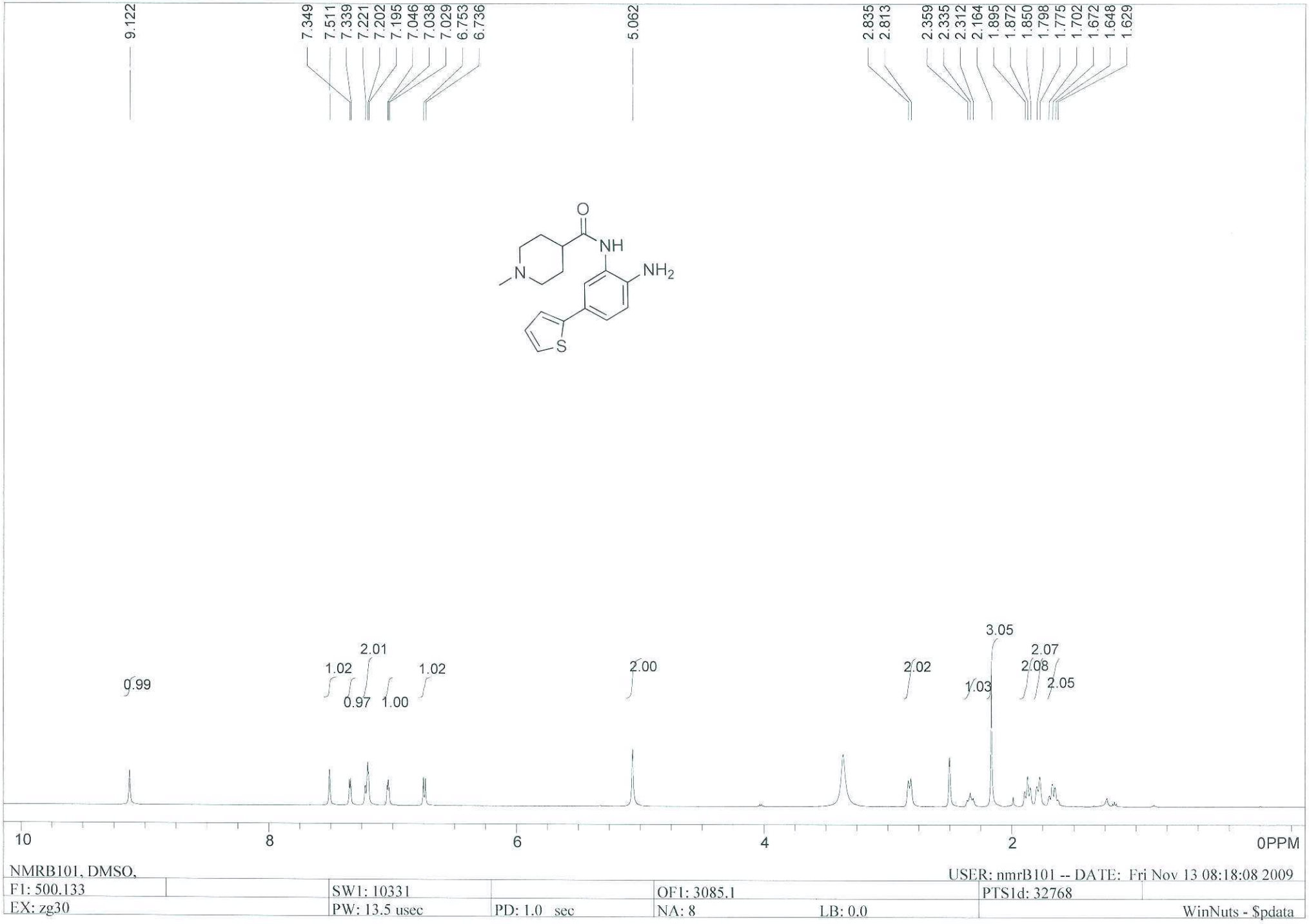




Sample Name:	**SBF-MA1107-095 # IP12030411**	Acquired By:	System
Vial:	2:B,1	Acq. Method Set:	PDA _METH_2A
Injection Volume:	1.00 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	3/12/2012 4:01:31 PM IST
Project Name:	SAPL_UNIT-2_AMCL_ALC-13_MAR-12		

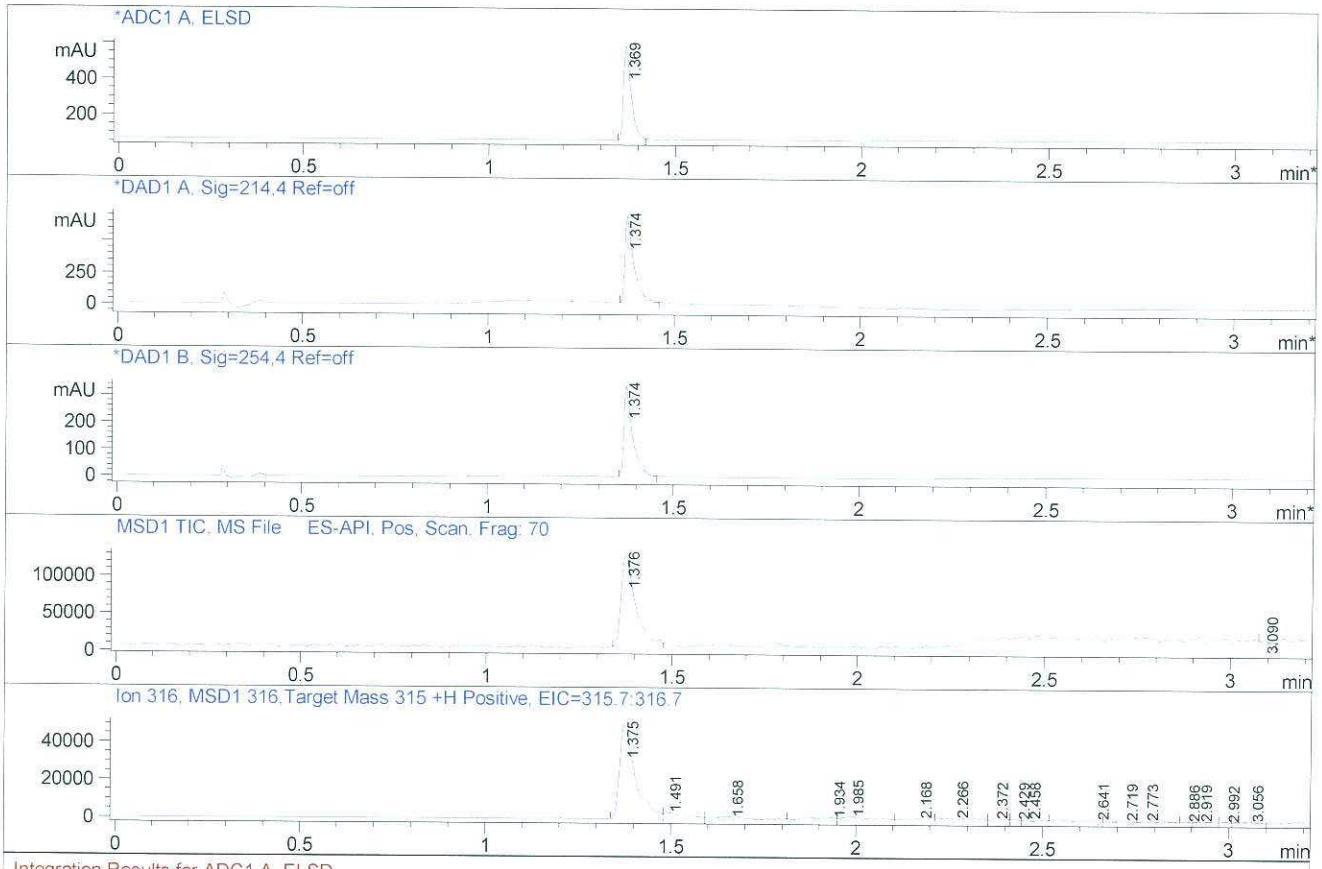
Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25℃
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0/90,0.5/90,3/10,6/10
Diluent : ACN:Water

	RT	Area	% Area
1	1.64	2314	0.21
2	1.68	1750	0.16
3	1.74	1098372	99.63


9.122
7.349
7.511
7.339
7.221
7.202
7.195
7.046
7.038
7.029
6.753
6.736
5.062
2.835
2.813
2.359
2.335
2.312
2.164
1.895
1.872
1.850
1.798
1.775
1.702
1.672
1.648
1.629
O
NH
N
NH2
S
0.99
1.02
2.01
0.97
1.00
1.02
2.00
2.02
1.03
3.05
2.08
2.07
2.05
10
8
6
4
2
0PPM
NMRB101, DMSO,
USER: nmrB101 -- DATE: Fri Nov 13 08:18:08 2009
F1: 500.133
SW1: 10331
OF1: 3085.1
PTS1d: 32768
EX: zg30
PW: 13.5 usec
PD: 1.0 sec
NA: 8
LB: 0.0
WinNuts - $pdata

MS Report

File D:\EZXDATA\T46\11-09\CP-0003507-184-03923.D Tgt Mass (EZX): 315.00
Injection Date : 13 Nov 09 2:56 pm +0800 Seq. Line : 0
Sample Name : CP-0003507-184 Location : P1-B-02
Acq. Operator : LCMSA101 Inj : 1
Spec. Reported : MS Integration Inj Volume : 3 ul
Acq. Method : C:\Chem32\1\METHODS\1-T46.M
Analysis Method : C:\Chem32\1\METHODS\1-T46.M
Sample Info : 315.00
Method Info : Mobile Phase: A: water(0.05%TFA) B: ACN(0.05%TFA)
Gradient: 5%-95% B in 1.4min
Flow Rate: 2.3 ml/min
Column: SunFire C18,4.6*50mm,3.5um
Oven Temperature: 50 C



Integration Results for ADC1 A, ELSD

RetTim	Width	Area	Height	Area%
1.37	0.02	745.87	518.96	100.00

Integration Results for DAD1 A, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
1.37	0.02	1098.71	678.53	100.00

Integration Results for DAD1 B, Sig=254,4 Ref=off

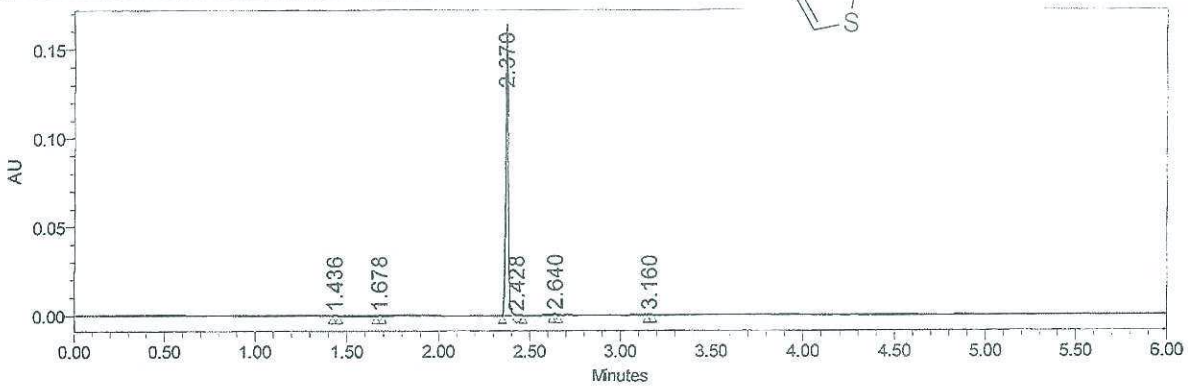
RetTim	Width	Area	Height	Area%
1.37	0.02	538.81	334.76	100.00

SBF-HA9002-087 in DMSO-d6
File.No.B091009A23

Varian 500MHz Unity INOVA
Date & Time:Fri Oct 9 12:56:50 IST 2009
Analyst: padma

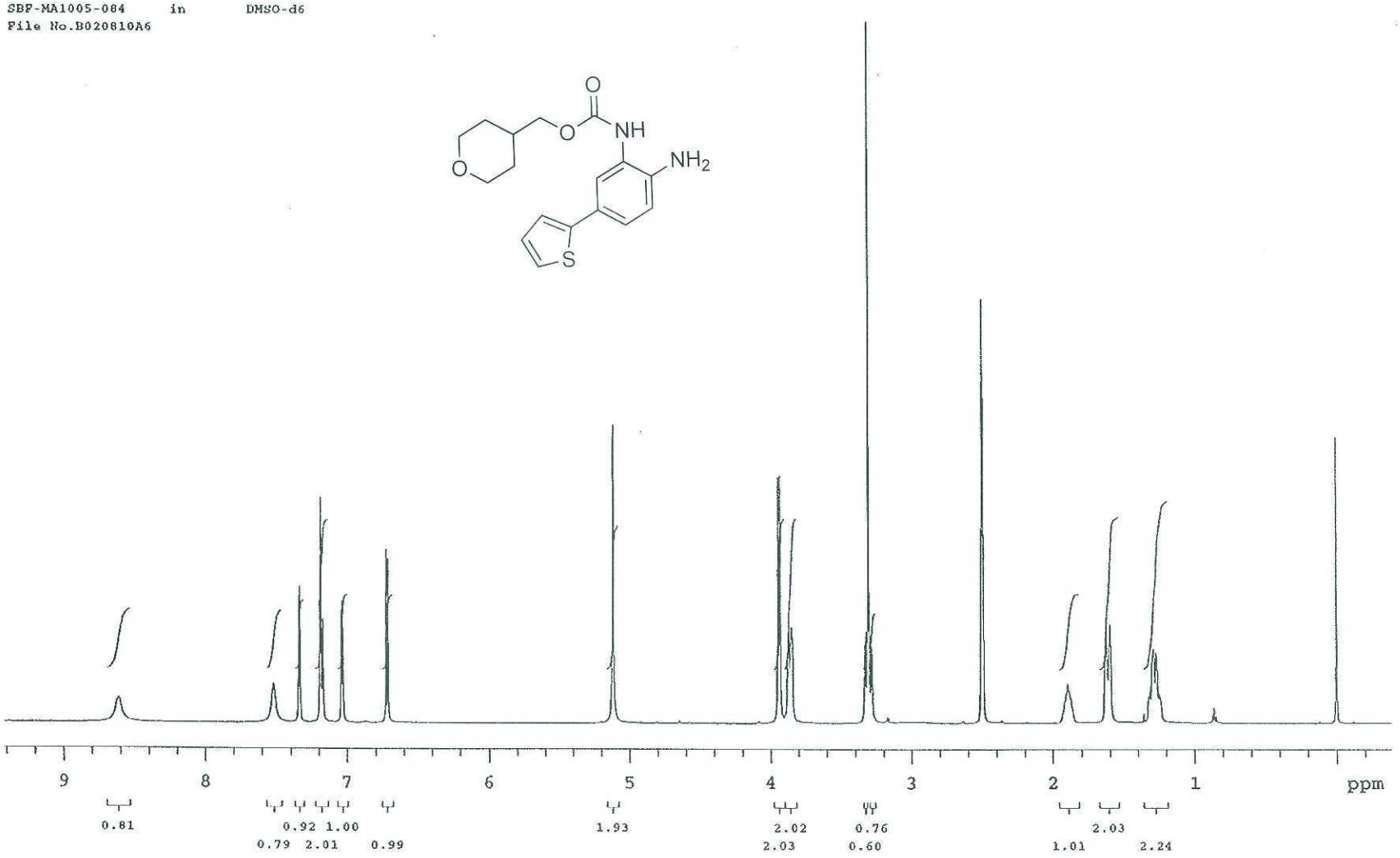
Sample Name:	**SBF-MA9002-087 # IP0910248**	Acquired By:	System
Vial:	1:A,7	Acq. Method Set:	PDA _METH_2A
Injection Volume:	0.30 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	10/9/2009 11:25:26 AM IST
Project Name: SAPL_UNIT-1_AMCL_OCT-09			

Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25°C
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0/90,0.5/90,3/10,6/10
Diluent : ACN:Water (1:1)



	RT	Area	% Area
1	1.44	184	0.12
2	1.68	217	0.14
3	2.37	155357	98.98
4	2.43	400	0.25
5	2.64	571	0.36
6	3.16	223	0.14

SBF-MA1005-084 in DMSO-d6
File No.B020810A6

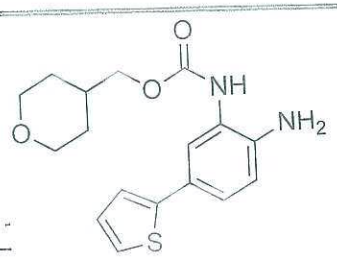


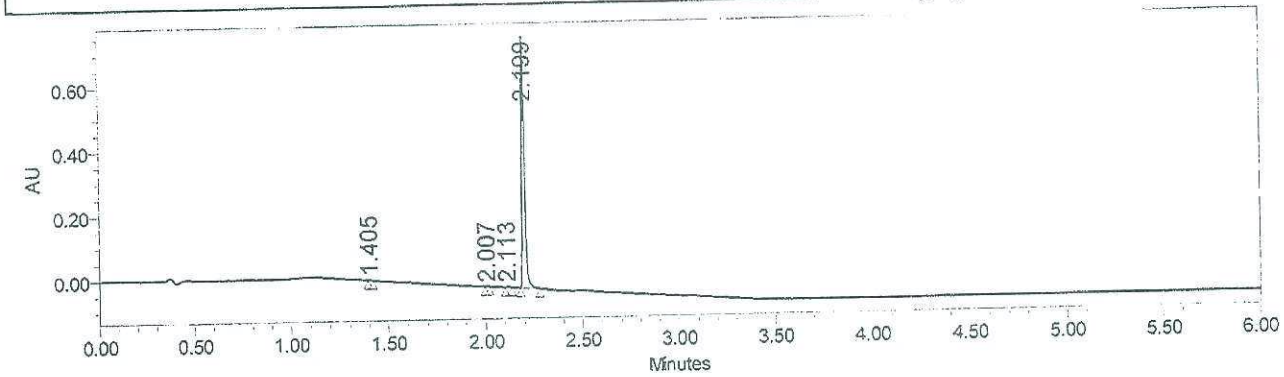
SAMPLE INFORMATION

Sample Name: **SBF-MA1005-084 # IP10080005**
Sample Type: Unknown
Vial: 1:A,6
Injection #: 1
Injection Volume: 0.50 ul
Run Time: 6.0 Minutes
Project Name: SAPL_UNIT-1_AMCL_ALC-10_AUG-10

Acquired By: System
Sample Set Name: 080110_1
Acq. Method Set: PDA_METH_2A
Proc. Chnl. Descr.: **PDA 210.0 nm**
Date Acquired: 8/2/2010 11:17:26 AM IST
Date Processed: 8/2/2010 11:38:44 AM IST

Column: Acquity UPLC BEH C-18 {2.1 x50mm,1.7u}
Column Temp:25°C
Mobile Phase:A: Acetonitrile
Mobile Phase:B:0.025% Aq TFA
Flow:0.5ml/min
Gradient programe:
T/%B:0.01/90,0.5/90,3/10,6/10
Diluent: ACN:Water:(1:1)





	RT	Area	% Area
1	1.40	1282	0.17
2	2.01	1358	0.18
3	2.11	3583	0.49
4	2.20	732233	99.16

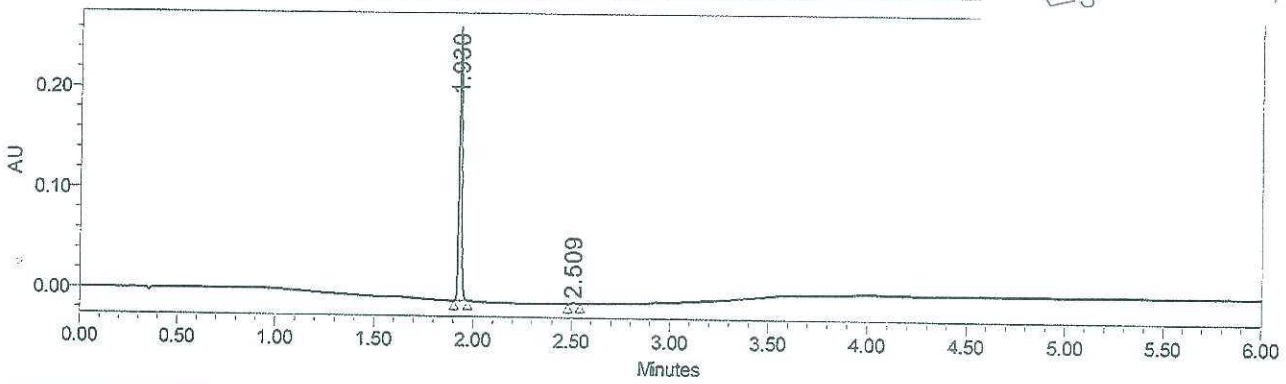
SBF-MB9002-081 in DMSO-d6
File No.B080709A34

Varian 500MHz Unity INOVA
Date & Time:Wed Jul 8 18:09:39 IST 2009
Analyst: Anji Karun

SAMPLE INFORMATION

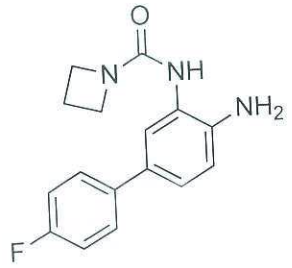
Sample Name:	**SBF-MB9002-081 # IP0907262**	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	080709_03
Vial:	2:D,4	Acq. Method Set:	PDA_METH_2A
Injection #:	1	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Injection Volume:	0.50 ul	Date Acquired:	7/8/2009 6:00:55 PM IST
Run Time:	6.0 Minutes	Date Processed:	7/8/2009 6:11:12 PM IST
Project Name:	SAPL_UNIT-I_AMCL_JULY-09		

Column: Acquity UPLC BEH C8{2.1 x50mm,1.7u}
Column Temp:25°C
Mobile Phase:A: Acetonitrile
Mobile Phase:B:0.025%Aq TFA
Flow:0.5ml/min
Gradient programe:
T/%B:0.01/90,0.5/90,3/10,6/10
Diluent: ACN:Water(1:1)



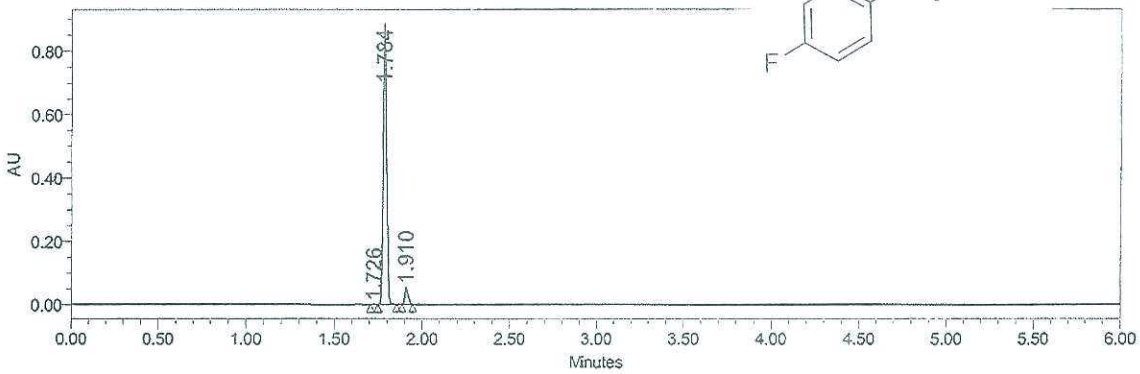
	RT	Area	% Area
1	1.93	256238	99.47
2	2.51	1357	0.53

SBF-MA1013-079 in DMSO-d6
File No:B060411A39



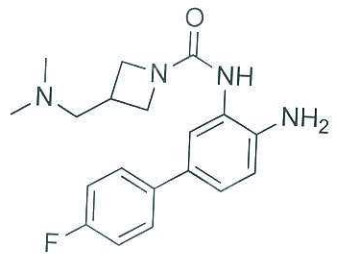
Sample Name:	**SBF-MA1013-079 # IP11040085**	Acquired By:	System
Vial:	2:A,6	Acq. Method Set:	PDA _METH_2A
Injection Volume:	0.80 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	4/7/2011 10:45:30 AM IST
Project Name: SAPL_UNIT-1_AMCL_ALC-13_APR-11			

Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25℃
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0.01/90,0.5/90,3/10,6/10.
Diluent :ACN:WATER (1:1)



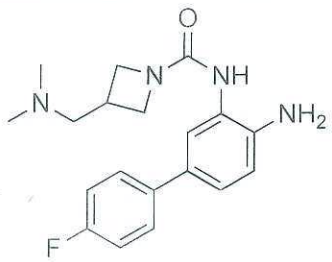
	RT	Area	% Area
1	1.73	887	0.07
2	1.78	1141728	94.35
3	1.91	67546	5.58

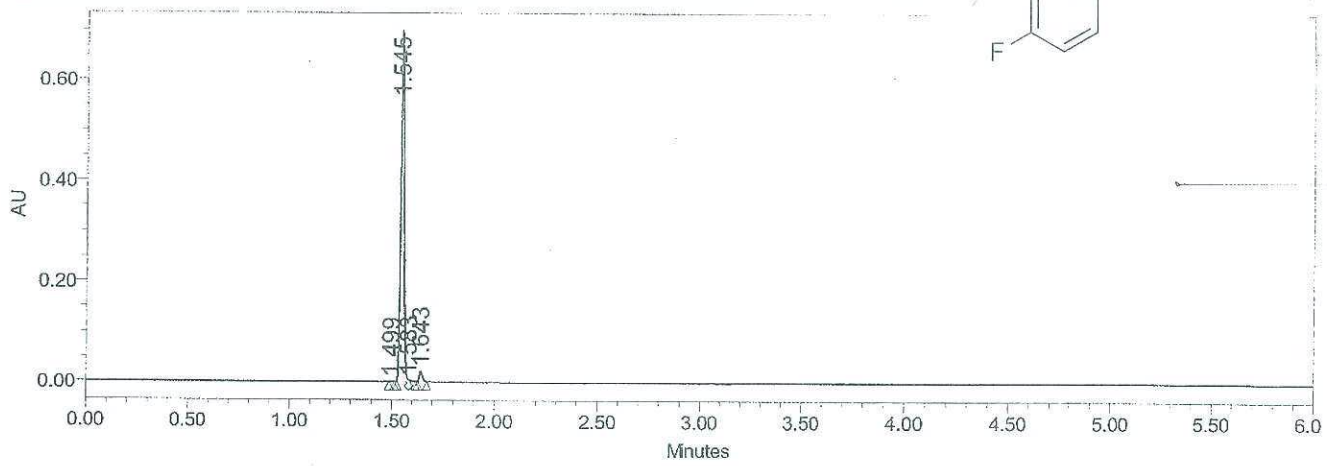
SBF-MA1201-040 in DMSO-d6
File No B300312B77



Sample Name:	**SBF-MA1201-040 # IP12031054**	Acquired By:	System
Vial:	2:C,2	Acq. Method Set:	PDA _METH_2A
Injection Volume:	0.50 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	3/30/2012 4:25:02 PM IST
Project Name:	SAPL_UNIT-2_AMCL_ALC-13_MAR-12		

Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25℃
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0/90,0.5/90,3/10,6/10
Diluent : ACN:Water



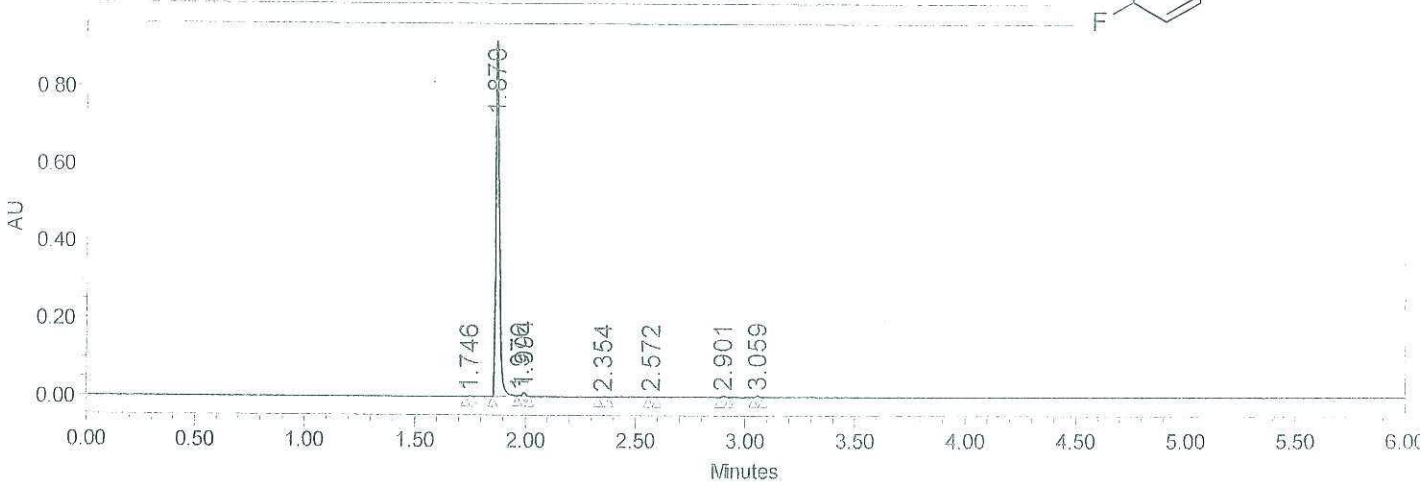


	RT	Area	% Area
1	1.50	891	0.11
2	1.55	774857	96.92
3	1.58	2949	0.37
4	1.64	20752	2.60

SBF-MA1005-094 in DMSO-d6
File No.B180810A16

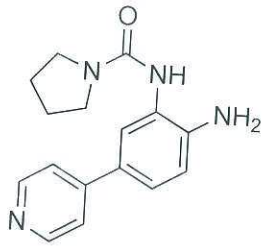
Sample Name:	**SBF-MA1005-094 # IP10080671**	Acquired By:	System
Vial:	2:A,7	Acq. Method Set:	PDA _METH_2A
Injection Volume:	0.70 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	8/18/2010 11:56:50 AM IST
Project Name:	SAPL_UNIT-1_AMCL_ALC-13_AUG-10		

Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25°C
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0.01/90,0.5/90,3/10,6/10.
Diluent :ACN:WATER (1:1)



	RT	Area	% Area
1	1.75	1084	0.10
2	1.87	1039928	98.04
3	1.98	1002	0.09
4	1.99	8698	0.82
5	2.35	1028	0.10
6	2.57	1098	0.10
7	2.90	3671	0.35
8	3.06	4173	0.39

SBF-MA1202-075 in DMSO-d6
D2O EXG
File..NO.300712B40



LCMS REPORT

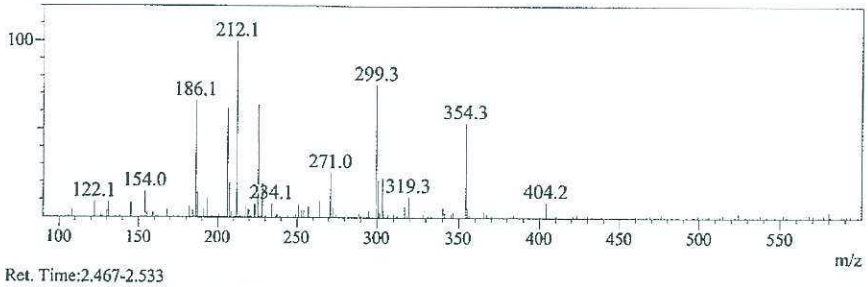
Sample Information

Sample Name : SBF-MA1202-075
Date Acquired : 7/31/2012 9:55:32 PM
Data File : 310712L 59.lcd
Method File : lcms.lcm
Tuning File :D:\Data\Tuning Files\Tuning-ESI-210209-01.lct
[Description] : Column X-Bridge C18 (50 X3.0mm,3.5um)
Mobile Phase : A :5mM NH4OAc;B: ACN
T/B% :0.01/10,0.5/10,4/90,8/90
Flow : 0.8ml / min.(Gradient)

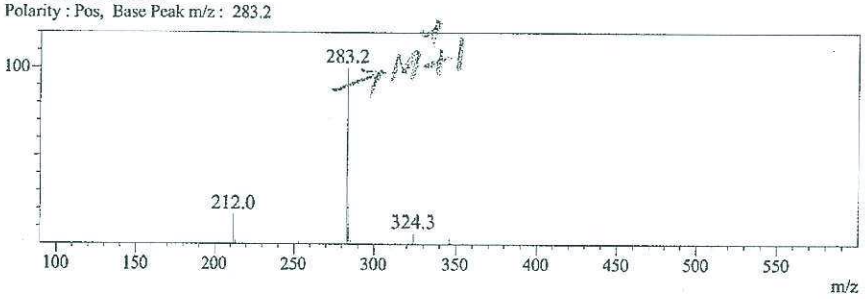
1 PDA Multi 1 / 210nm 4nm

MS Spectrum Graph

Ret. Time:2.233-2.333
Polarity : Pos, Base Peak m/z : 212.1



Ret. Time:2.467-2.533
Polarity : Pos, Base Peak m/z : 283.2



PeakTable

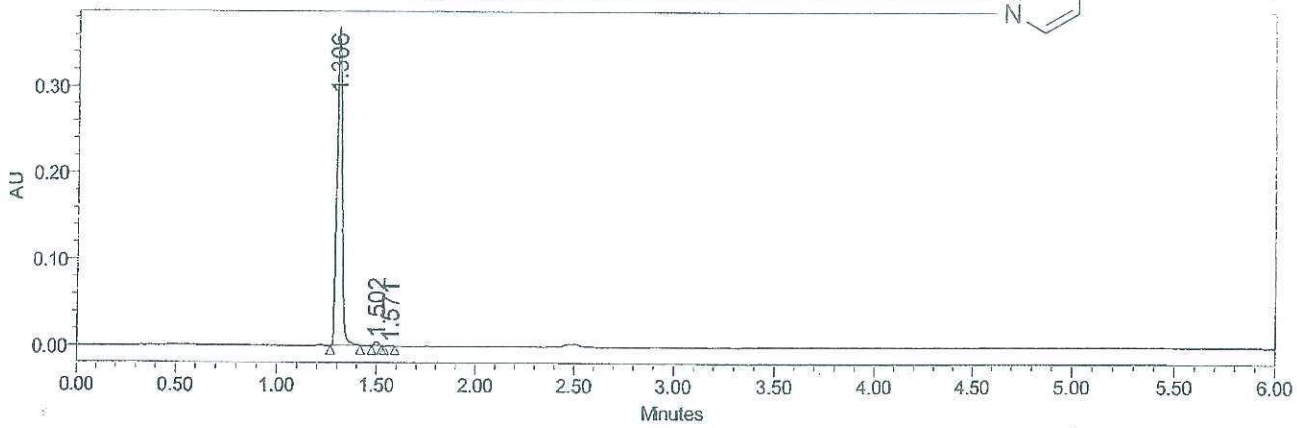
PDA Ch1 210nm 4nm

Peak#	Ret. Time	Area	Area %
1	1.926	363146	1.567
2	2.262	413241	1.783
3	2.496	22153800	95.608
4	2.916	241373	1.042
Total		23171560	100.000

SBF-MA1013-092 in DMSO-D6
File No:B060511B36

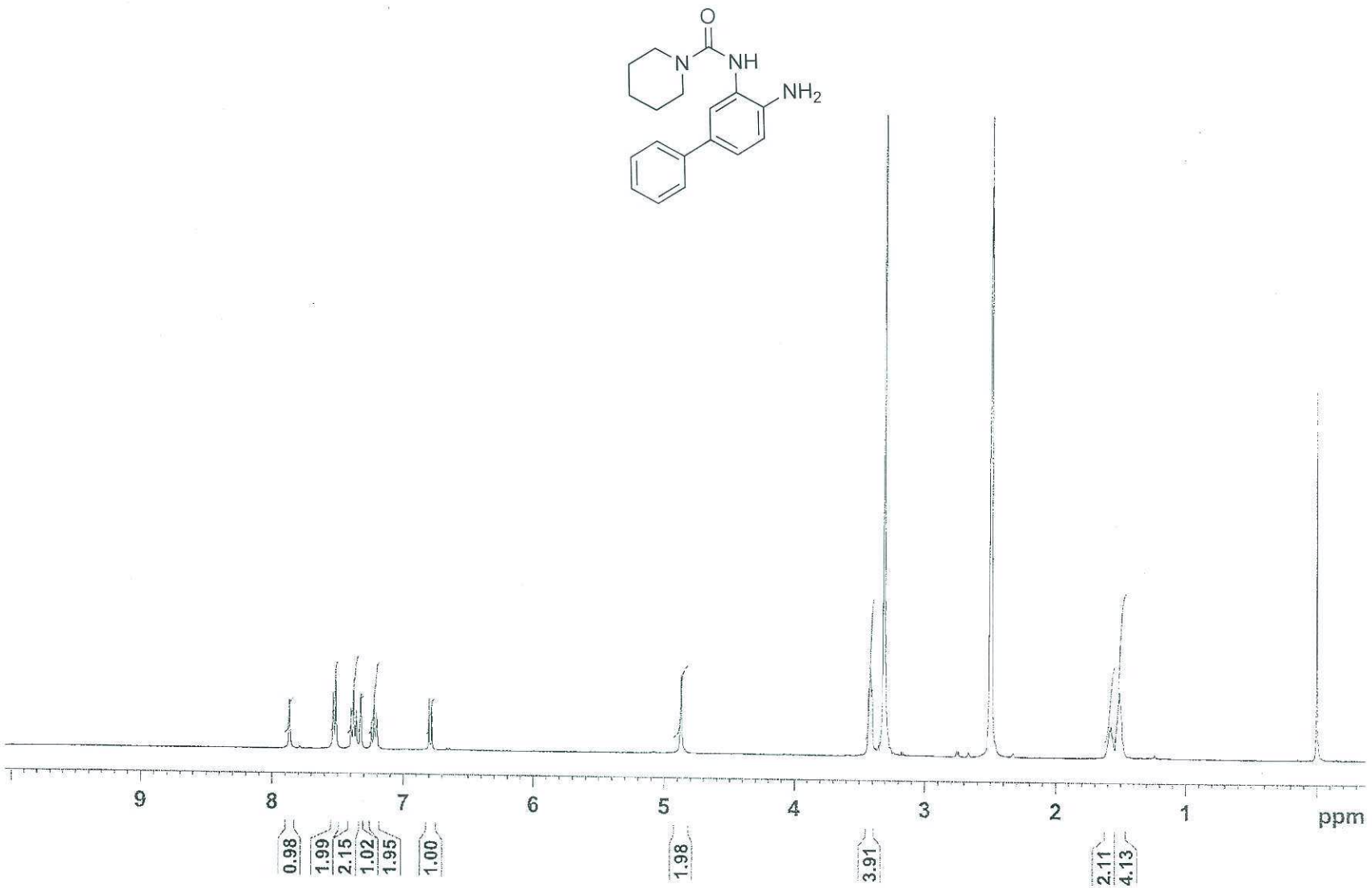
Sample Name:	**SBF-MA1013-092 # IP11050136**	Acquired By:	System
Vial:	1:D,2	Acq. Method Set:	PDA _METH_2A
Injection Volume:	2.00 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	5/5/2011 6:07:24 PM IST
Project Name: SAPL_UNIT-2_AMCL_ALC-13_MAY-11			

Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25°C
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0.01/90,0.5/90,3/10,6/10.
Diluent :ACN:WATER (1:1)



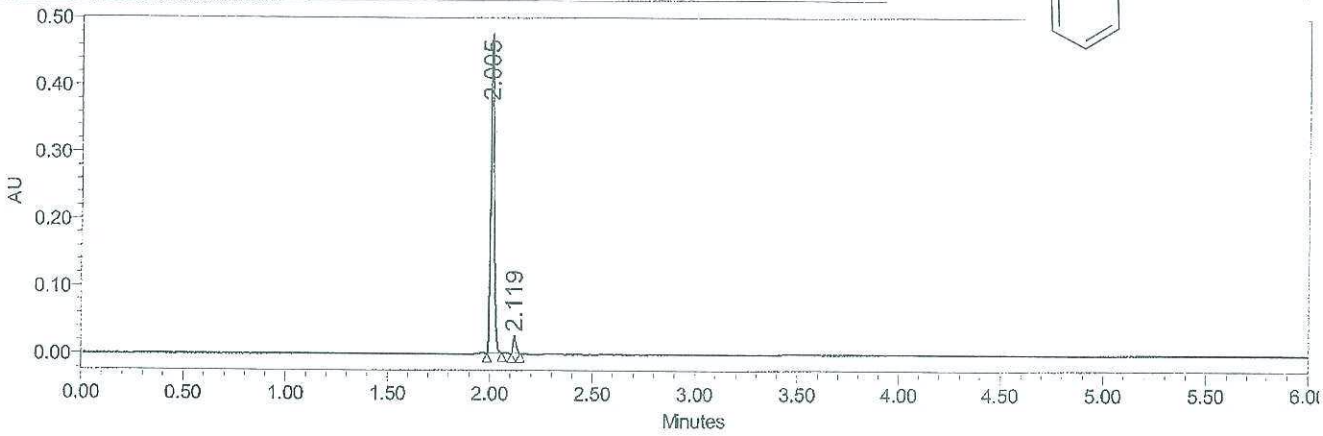
	RT	Area	% Area
1	1.31	660335	98.89
2	1.50	6674	1.00
3	1.57	742	0.11

SBF-MA1105-065 in DMSO-d6
File.No.160811B50



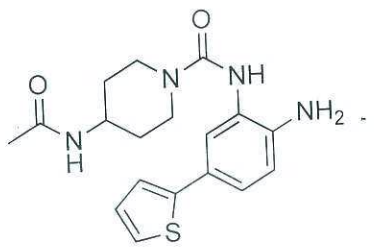
Sample Name:	**SBF-MA1105-065 # IP11080462**	Acquired By:	System
Vial:	2:D,3	Acq. Method Set:	PDA _METH_2A
Injection Volume:	1.00 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	8/16/2011 4:41:25 PM IST
Project Name:	SAPL-UNIT-2_AMCL_ALC-13_AUG-11		

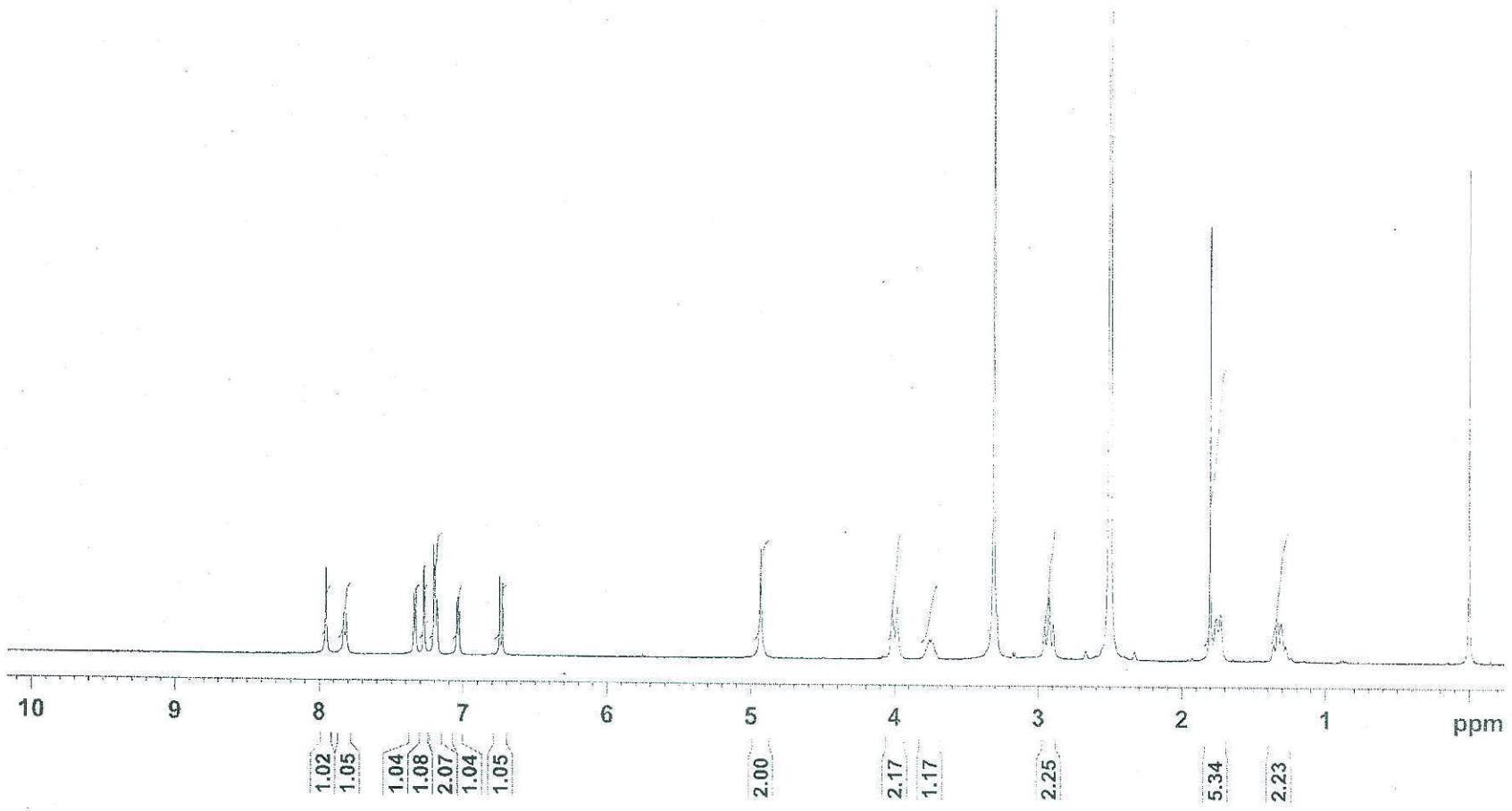
Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 28 ℃
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0.01/90,0.5/90,3/10,6/10.
Diluent : ACN : Water



	RT	Area	% Area
1	2.00	537122	95.49
2	2.12	25365	4.51

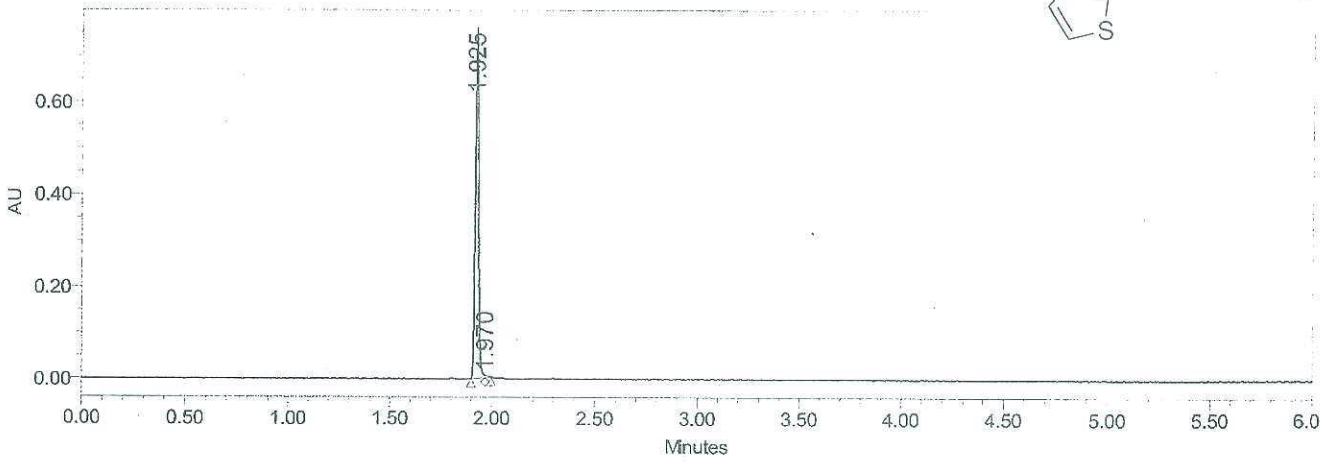
SBF-MA1108-070 in DMSO-d6
File.No.010212B118





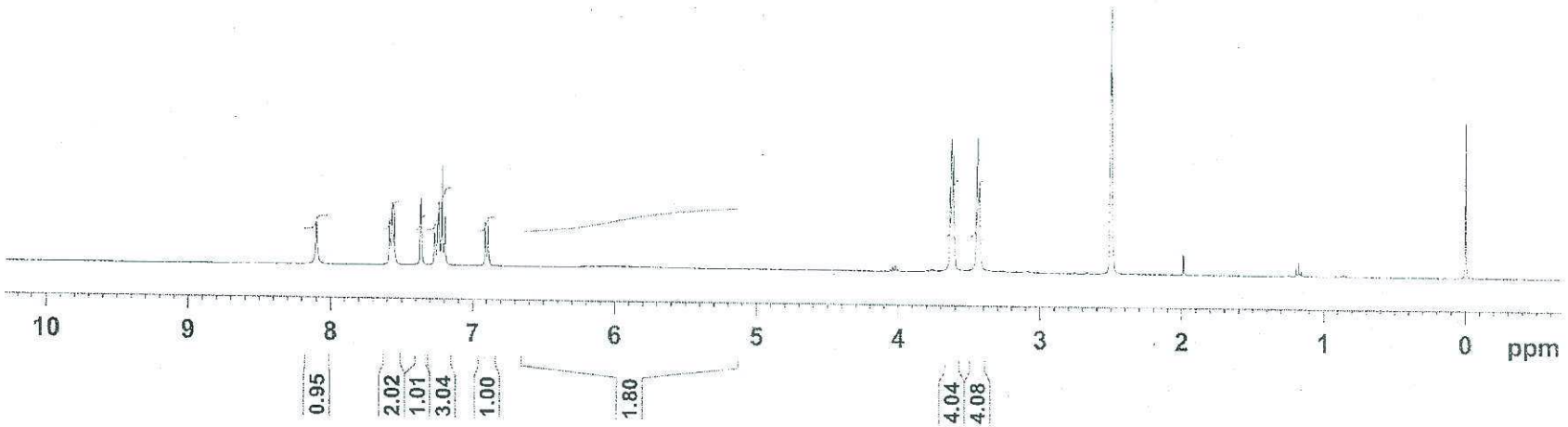
Sample Name:	**SBF-MA1108-070 # IP12020022**	Acquired By:	System
Vial:	1:B,6	Acq. Method Set:	PDA _METH_2A
Injection Volume:	0.50 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	2/1/2012 2:51:09 PM IST
Project Name: SAPL_UNIT-2_AMCL_ALC-13_FEB-12			

Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25℃
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0/90,0.5/90,3/10,6/10
Diluent : ACN:Water



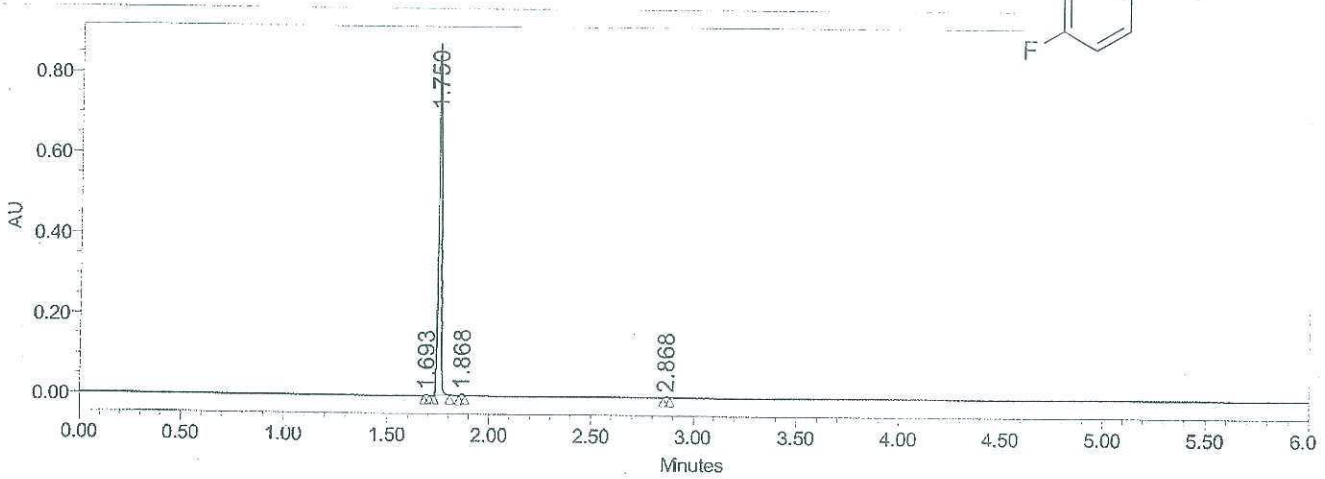
	RT	Area	% Area
1	1.93	844374	99.43
2	1.97	4874	0.57

SBF-MA1201-020 in DMSO-d6
File.No.230212B30



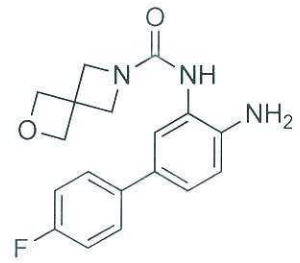
Sample Name:	**SBF-MA1201-020 # IP12020802**	Acquired By:	System
Vial:	1:C,2	Acq. Method Set:	PDA _METH_2A
Injection Volume:	0.80 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	2/23/2012 2:12:31 PM IST
Project Name:	SAPL_UNIT-2_AMCL_ALC-13_FEB-12		

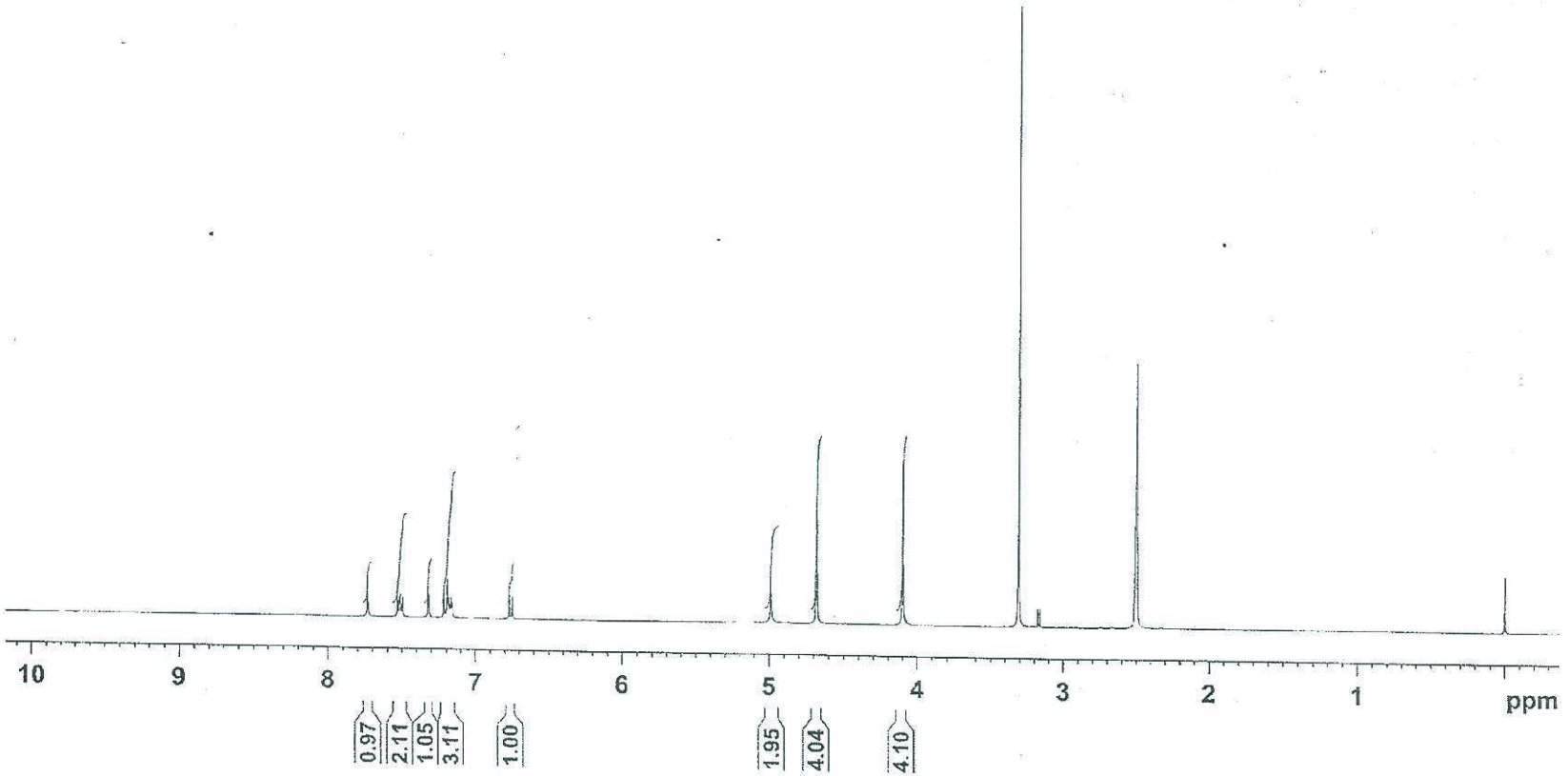
Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25℃
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0/90,0.5/90,3/10,6/10
Diluent : ACN:Water



	RT	Area	% Area
1	1.69	2550	0.30
2	1.75	843031	99.05
3	1.87	4020	0.47
4	2.87	1551	0.18

SBF-MA1109-088 in DMSO-d6
File.No.310312B34

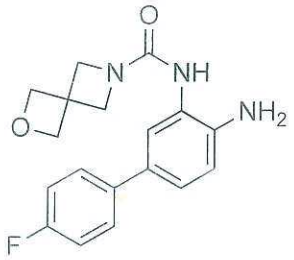


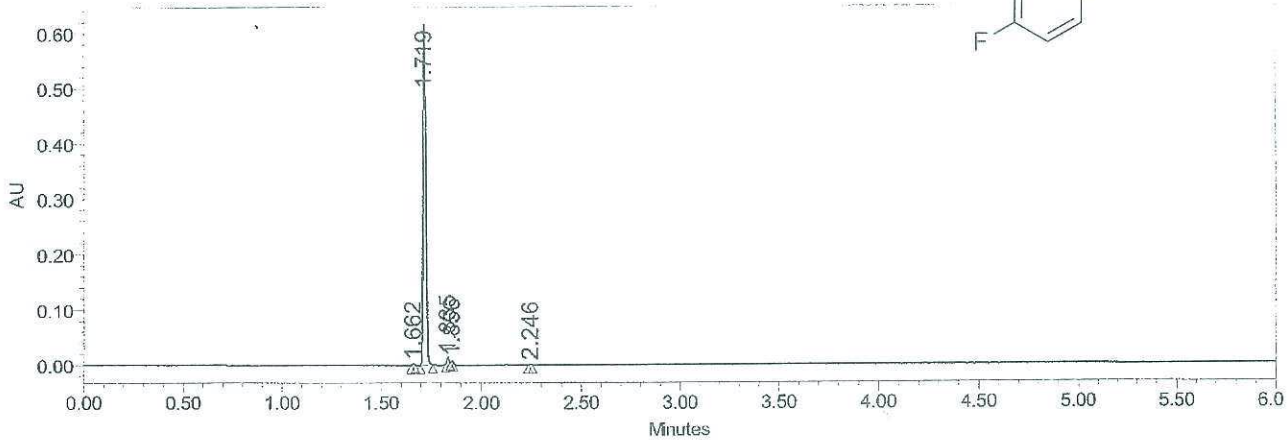


Sample Name:	**SBF-MA1109-088 # IP12031078**	Acquired By:	System
Vial:	1:B,1	Acq. Method Set:	PDA _METH_2A
Injection Volume:	0.80 ul	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Run Time:	6.0 Minutes	Date Acquired:	3/31/2012 11:05:03 AM IST

Project Name: SAPL_UNIT-2_AMCL_ALC-13_MAR-12

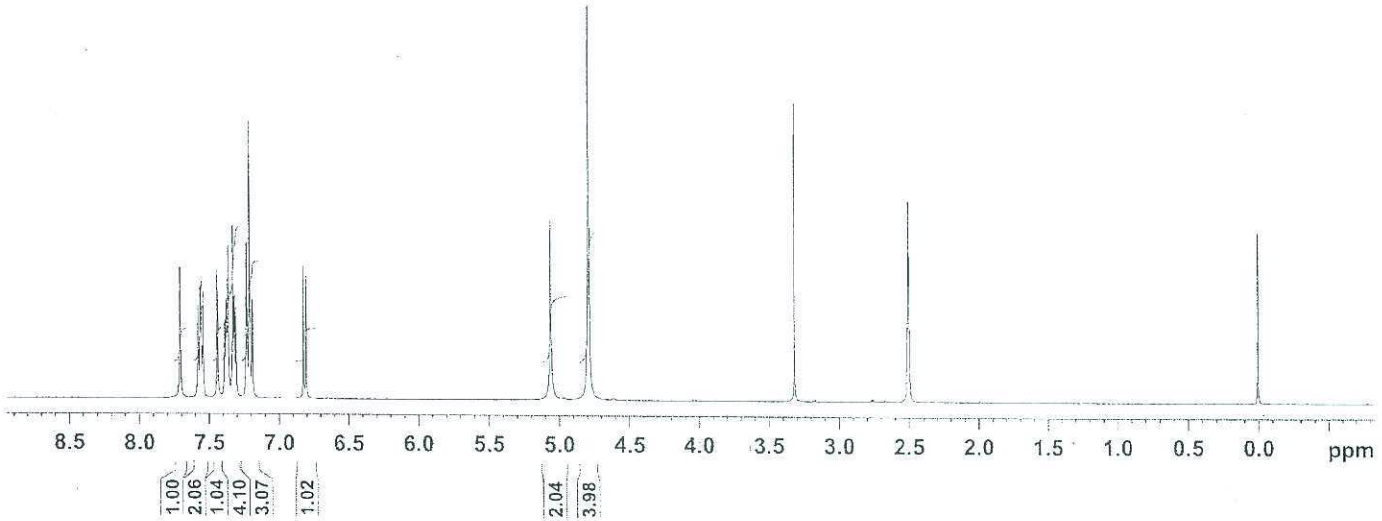
Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 25℃
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0/90,0.5/90,3/10,6/10
Diluent : ACN:Water





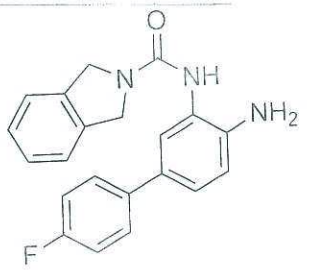
	RT	Area	% Area
1	1.66	1617	0.28
2	1.72	567565	98.04
3	1.84	6712	1.16
4	1.86	2076	0.36
5	2.25	945	0.16

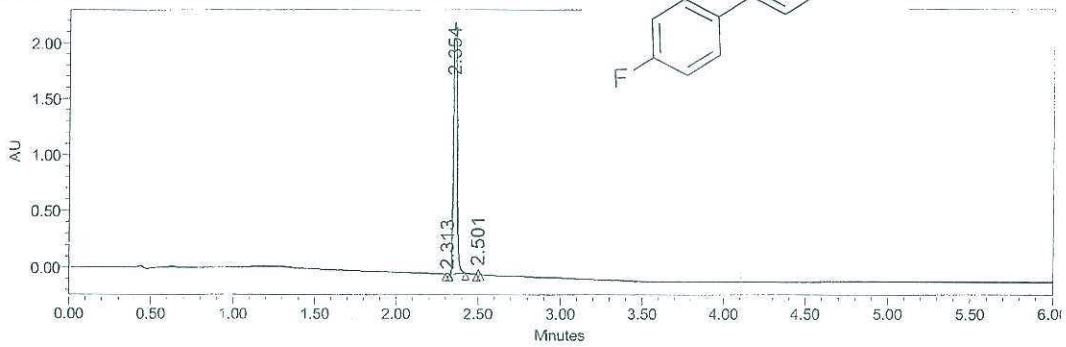
SBF-MA1106-036RC in DMSO-d6
File.No.141111B14



Sample Name:	**SBF-MA1106-036RC # IP11110554**	Acq. Method Set:	PDA _METH_2A
Vial:	1:A,6	Proc. Chnl. Descr.:	**PDA 210.0 nm**
Injection Volume:	0.50 ul	Date Acquired:	11/14/2011 1:33:18 PM IST
Run Time:	6.0 Minutes		
Project Name: SAPL_UNIT-2_AMCL_ALC-13_NOV-11			

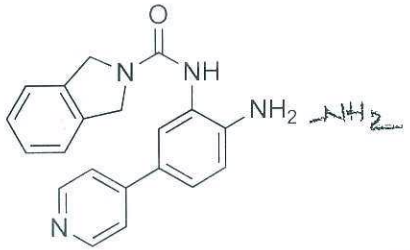
Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 28 ℃
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0.01/90,0.5/90,3/10,6/10.
Diluent : ACN : Water

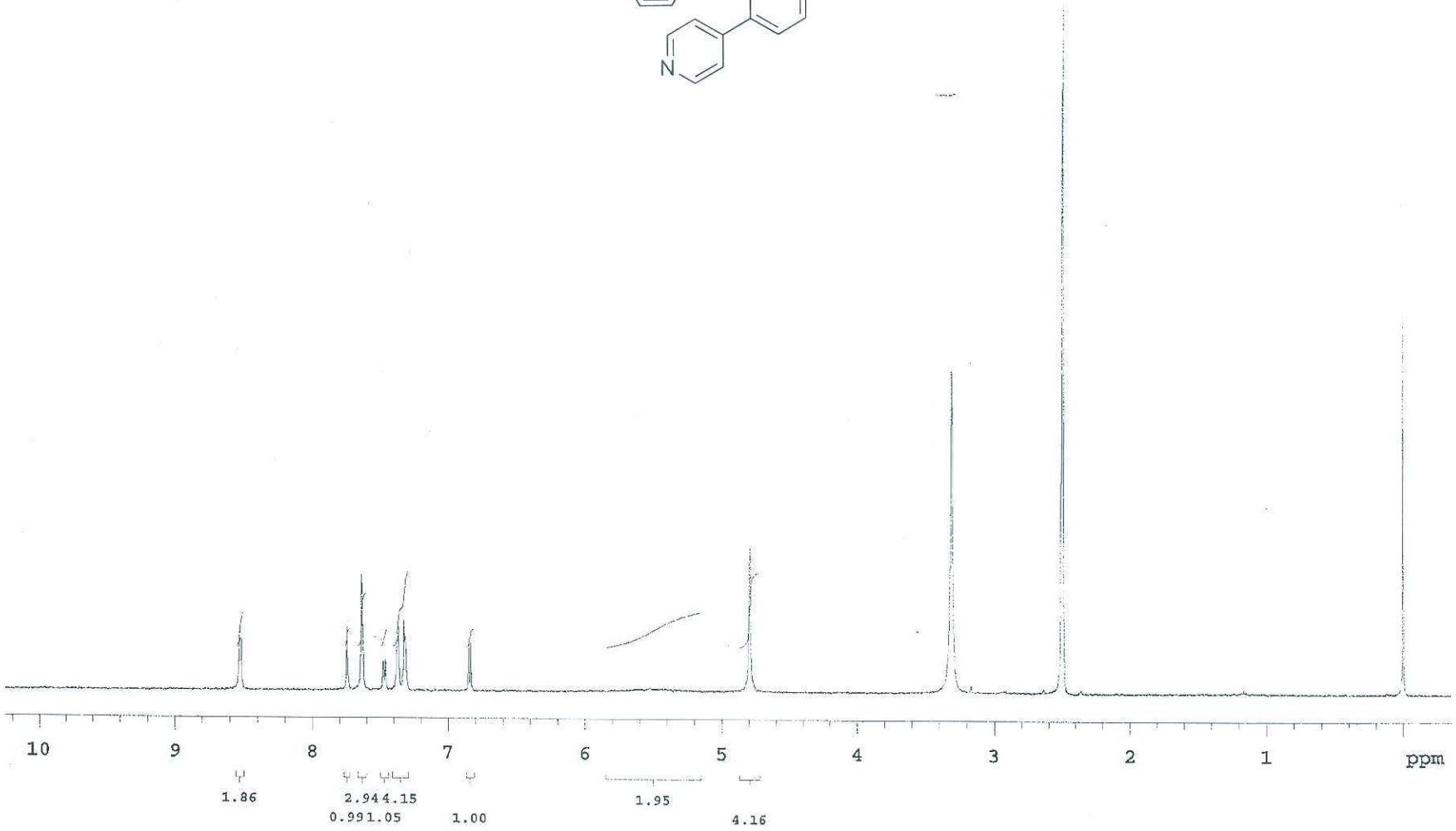




	RT	Area	% Area
1	2.31	4062	0.11
2	2.35	3540431	99.06
3	2.50	29408	0.82

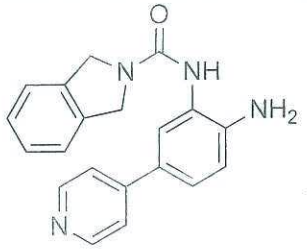
SBF-MA1109-001 in DMSO-d6
File No. B011111B108

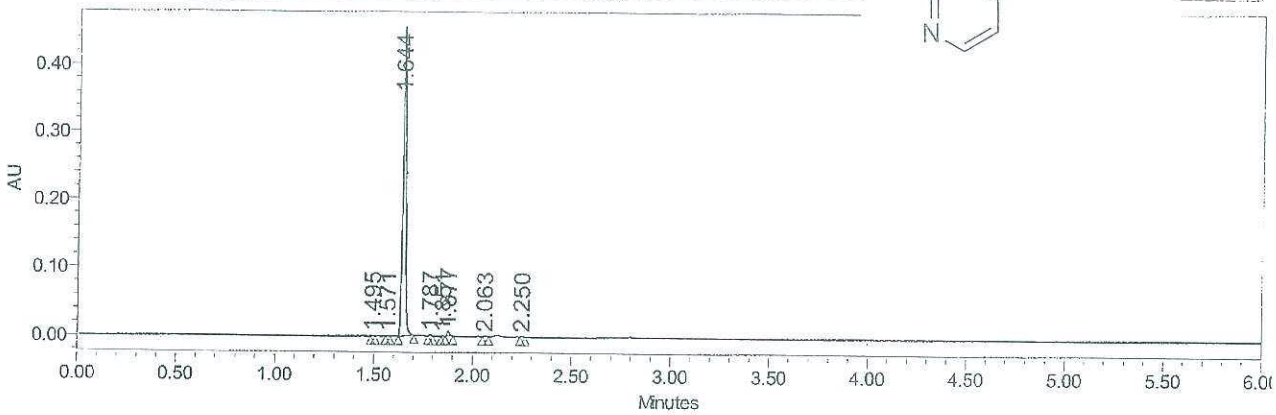




Sample Name:	**SBF-MA1109-001 # IP11110037**	Acq. Method Set:	PDA _METH_2A
Vial:	2:D,1	Proc. Chnl. Descr.:	**PDA 254.0 nm**
Injection Volume:	0.70 ul	Date Acquired:	11/1/2011 3:55:13 PM IST
Run Time:	6.0 Minutes		
Project Name: SAPL_UNIT-2_AMCL_ALC-13_NOV-11			

Column : Acquity BEH C-18 (50 x2.1mm,1.7µ,)
Column Temp : 28 ℃
Mobile Phase A : Acetonitrile
Mobile Phase B : 0.025% TFA (Aq)
Flow Rate : 0.50 mL/Min
Time/% B : 0.01/90,0.5/90,3/10,6/10.
Diluent : ACN : Water





	RT	Area	% Area
1	1.50	824	0.17
2	1.57	813	0.17
3	1.64	479697	97.66
4	1.79	1501	0.31
5	1.85	528	0.11
6	1.88	6525	1.33
7	2.06	709	0.14
8	2.25	609	0.12

3. Protocol for HDAC inhibition assay (microfluidic lab-on-a-chip assay)

All HDACs were purchased from BPS Bioscience. The substrates, Broad Substrate A, and Broad Substrate B, were synthesized in house. All the other reagents purchased from Sigma. Caliper EZ reader II system was used to collect all data.

HDAC inhibition assays:

Compounds were tested in duplicate in a 12-point dose curve with 3-fold serial dilution starting from 33.33 μM.

Purified HDACs were incubated with 2 μM carboxyfluorescein (FAM)-labeled acetylated or trifluoroacetylated peptide substrate (Broad Substrate A and B respectively) and test compound for 60 min at room temperature, in HDAC assay buffer that contained 50 mM HEPES (pH 7.4), 100 mM KCl, 0.01% BSA and 0.001% Tween-20. Reactions were terminated by the addition of the known pan HDAC inhibitor LBH -589 (panobinostat) with a final concentration of 1.5μM. Substrate and product were separated electrophoretically and fluorescence intensity in the substrate and product peaks was determined and analyzed by Labchip EZ Reader. The reactions were performed in duplicate for each sample. IC_{50} values were automatically calculated by Origion8 using 4 Parameter Logistic Model. The percent inhibition was plotted against the compound concentration, and the IC50 value was determined from the logistic dose-response curve fitting by Origin 8.0 software. (Madan Katragadda, Paola Magotti, Georgia Sfyroera, and John D. Lambris, *J. Med. Chem.* **2006,** *49,* 4616-4622).

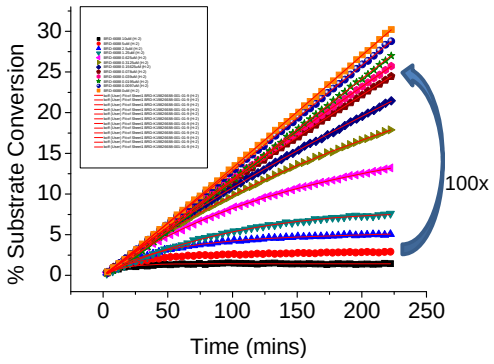
Binding Kinetic Measurements.

Slow, tight-binding kinetics of BRD6688 and BRD4884 with HDACs 1, 2, and 3 were evaluated by the progress curves in inhibition and dilution experiments. To determine the mechanism and associated kinetic values, a series of progress curves of HDACs 1, 2 or 3 inhibition were generated in the presence of BRD6688 or BRD4884 at different concentrations. Kinetic reactions were assembled in microtiter plate wells by adding HDAC enzymes into separate reaction mixtures containing fluorophore conjugated Broad Substrate A and the inhibitors. Plates were immediately placed into a LabChip EZ Reader whereby the wells were sampled periodically throughout a 3-hour reaction period. The fluorescent product and substrate were separated and monitored on the Caliper microfluidic instrument. The conversion of substrate was calculated with Caliper software. The progress curves in the presence of increasing inhibitor concentrations were analyzed with a nonlinear regression program in Origin 8.5 to the integrated rate equation for slow-binding inhibitors (Eq.1). The on-rates were derived from linear fitting of first-order rate constant k_{obs} against the inhibitor concentrations (Eq.2).
The off-rates of BRD6688 and BRD4884 were determined from a dilution experiment. HDACs were incubated with BRD6688 or BRD4884 at saturated concentration (>10 fold IC50) for 1 hour to reach fully inhibition. The reaction was initiated by diluting the enzyme-inhibitor complex (100 fold) into reaction buffer. HDAC-diluted samples were

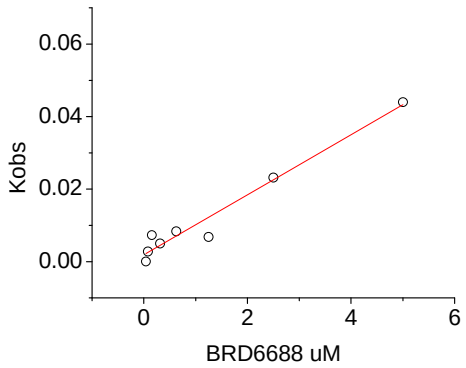
measured continuously over a 4-hour period. The recovery of activity of the diluted samples reflects the dissociation of inhibitor to generate the active enzyme species. The HDAC inhibitor off-rates of the dissociation reaction were calculated by measuring HDAC activity of the inhibitor-preincubated HDAC compared to the non-preincubated control using eq.1 and eq2. The reciprocal of the rate constant for dissociation of the inhibitor-HDAC complex (k_{off}) is residence time, which can be used to quantify the lifetime of the drug–target interaction.

$$[F] = v_s t + (v_0 - v_s)(1 - \exp(-k_{obs} t))/k_{obs} \qquad \text{(Eq. 1)}$$

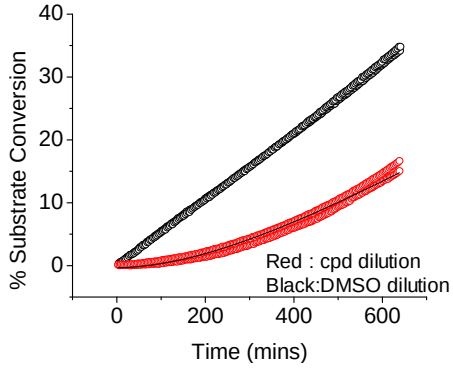
$$k_{obs} = k_{-1} + k_1[I]/(1 + [S]/K_m) \qquad \text{(Eq. 2)}$$



Progression curves for HDAC2-catalyzed deacetylation in the presence of increasing concentrations of BRD6688.

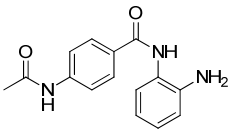
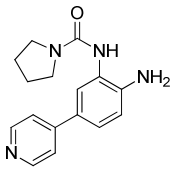
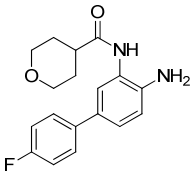


First-order rate constant k_{obs} as a function of the BRD6688 concentration. The k_{obs} values were calculated from fitting the progress curves to eq 1.



Dissociation of BRD6688 from HDAC2-BRD6688 complex upon dilution of the enzyme-inhibitor complex into buffer(100-fold). The recovery of activity reflects the dissociation of inhibitor to generate the active enzyme.

4. *Supplemental Table 1: HDAC inhibitors' IC50s for HDACs 1-9*

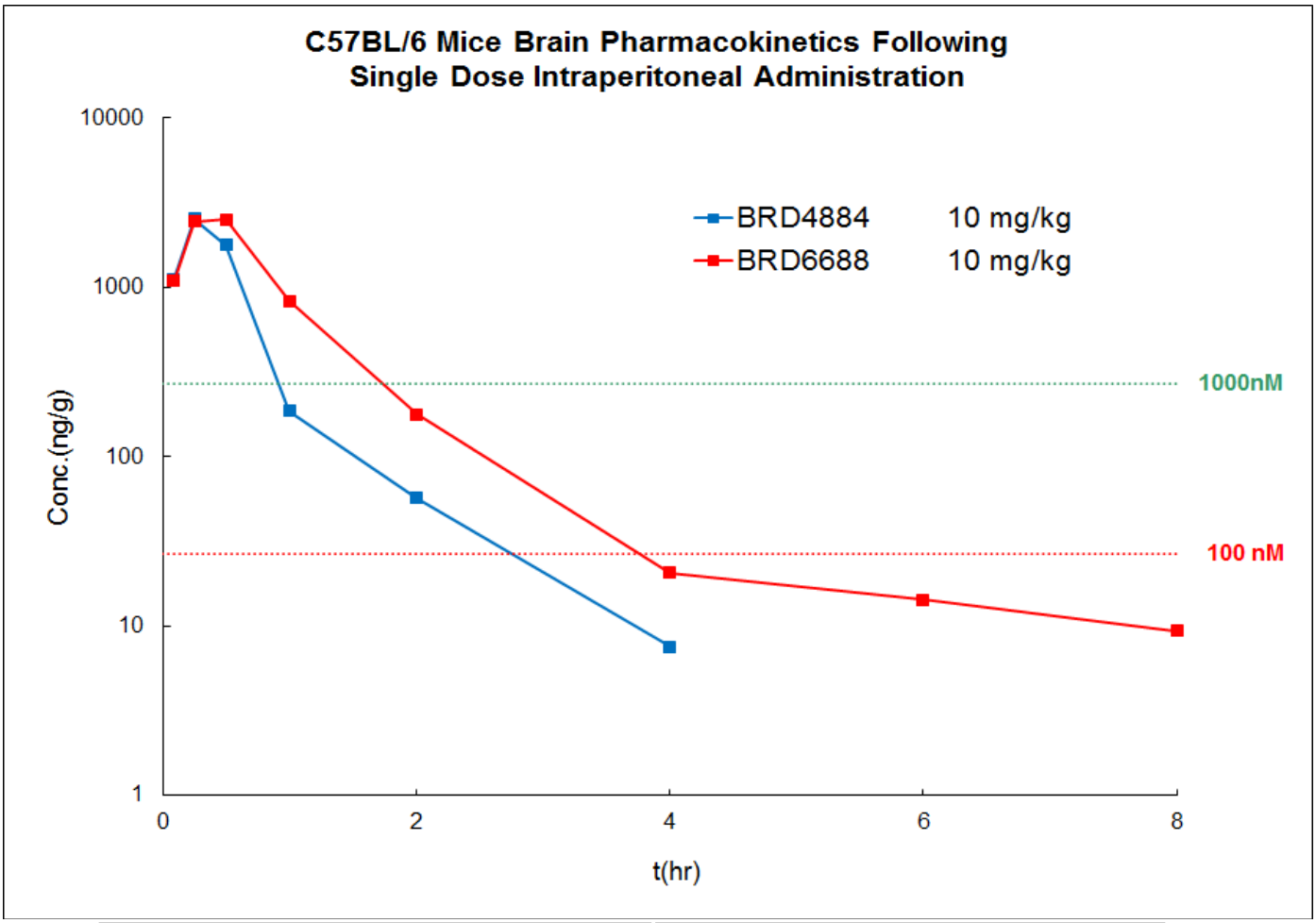
		HDAC Isoform Inhibition IC50 (μM)[a]								
Compound		**HDAC 1**	**HDAC 2**	**HDAC3**	**HDAC4**	**HDAC5**	**HDAC6**	**HDAC7**	**HDAC8**	**HDAC9**
LBH		0.001 ± 0.000005	0.002 ± 0.0001	0.002 ± 0.0002	0.373 ± 0.037	0.092 ± 0.021	0.002 ± 0.0001	2.83 ± 0.135	0.231 ± 0.003	2.68 ± 0.364
SAHA		0.004 ± 0.0001	0.011 ± 0.0005	0.003 ± 0.0005	>33.33	8.75 ± 1.80	0.002 ± 0.0002	>33.33	1.02 ± 0.187	>33.33
CI-994		0.041 ± 0.012	0.147 ± 0.066	0.046 ± 0.018	>33.33	>33.33	>33.33	>33.33	>33.33	>33.33
BRD6688		0.021 ± 0.013	0.100 ± 0.048	11.48 ± 2.54	>33.33	>33.33	>33.33	>33.33	>33.33	>33.33
BRD4884		0.029 ± 0.012	0.062 ± 0.031	1.09 ± 0.38	>33.33	>33.33	>33.33	>33.33	>33.33	>33.33

[a]Values are the mean of two experiments. Data are shown as IC50 values in μM ± standard deviation. Compounds were tested in duplicate in a 12-point dose curve with 3-fold serial dilution starting from 33.33 μM.

5. *Supplemental Table 2: Kinetic parameters for BRD4884 and BRD6688*

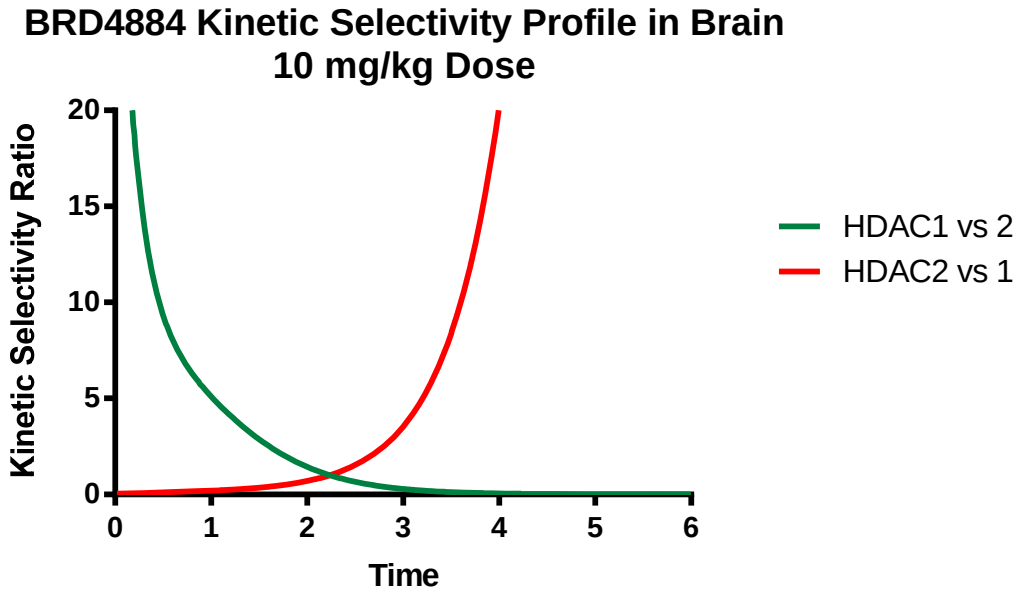
Kinetic Parameters Summary	HDAC1				HDAC2				HDAC3			
	K_{on} ($min^{-1}\mu M^{-1}$)	K_{off} (min^{-1})	$T_{1/2}$ (min)	K_i (μM)	K_{on} ($min^{-1}\mu M^{-1}$)	K_{off} (min^{-1})	$T_{1/2}$ (min)	K_i (μM)	K_{on} ($min^{-1}\mu M^{-1}$)	K_{off} (min^{-1})	$T_{1/2}$ (min)	K_i (μM)
BRD6688	**0.185**	**0.01065**	**65**	**0.058**	**0.00895**	**0.00182**	**381**	**0.203**	**0.00028**	**0.0025**	**280**	**8.93**
BRD4884	**0.41**	**0.0345**	**20**	**0.084**	**0.014**	**0.00485**	**143**	**0.346**	**0.0013**	**0.0027**	**260**	**2.08**

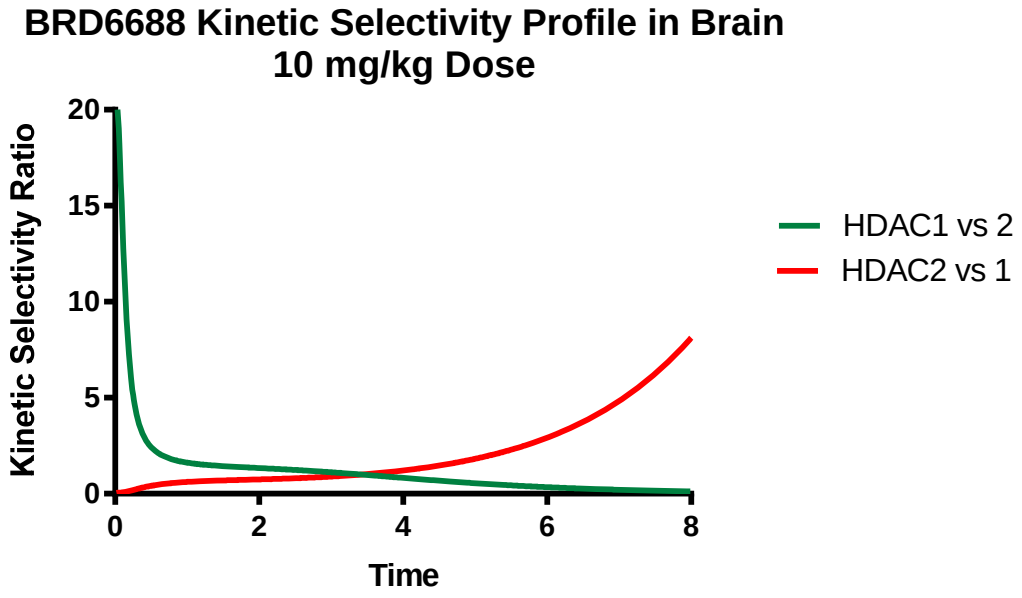
6. ***<u>Supplemental Figure 1:</u> C57BL/6 Mice Brain Pharmacokinetics following single dose of BRD4884 (10 mg/kg) or BRD6688 (10 mg/kg) by intraperitoneal administration.***



		Plasma					Brain						
		AUC Plasma	$T_{1/2}$ Plasma	Cmax Plasma	V_z/F	CL/F	AUC Brain	$T_{1/2}$ Brain	Cmax Brain	V_z/F	CL/F	Brain/Plasma	Brain/Plasma
		µmol/L.hr	hr	µmol/L	mg/(µmol/L)/kg	mg/(hr.µmol/L)/kg	µmol/L.hr	hr	µmol/L	mg/(µmol/L)/kg	mg/(hr.µmol/L)/kg	Cmax	AUC
BRD4884	10 mg/kg	3.90	0.87	5.06	3.23	2.57	5.03	0.65	8.13	1.88	1.99	1.61	1.29
BRD6688	10 mg/kg	33.65	0.74	33.15	0.32	0.30	8.95	0.57	8.95	0.92	1.12	0.27	0.27

7. ***Supplemental Figure 2. Kinetic selectivity for HDAC2 (BRD4884, BRD6688)***





8. ***Supplemental Table 3.*** *In vitro pharmacology: binding and enzyme assays; hERG.*

	Target	% Inhibition at 10 μM*
BRD6688	Dopamine transporter (*h*) antagonist radioligand	21%
	5-HT_6 (*h*) agonist radioligand	16%
	CB_1 (*h*) agonist radioligand	15%
	Norepinphrine transporter	15%
	5-HT_{2B} (*h*) agonist radioligand	14%
	hERG (automated patch-clamp)	8.3%
BRD4884	hERG (automated patch-clamp)	-0.40%

* No hits >25% at 10 μM. Top 5 hits shown. Screen included transmembrane and soluble receptors, ion channels and monoamine transporters (dopaminergic, kinases, GPCR, etc…).

In vitro pharmacology, binding assay list:

A1(*h)* (agonist radioligand)
A2A(*h)* (agonist radioligand)
A3(*h)* (agonist radioligand)
alpha1 (antagonist radioligand)
alpha2 (antagonist radioligand)
beta2(*h)* (agonist radioligand)
beta1(*h)* (agonist radioligand)
AT1(*h)* (antagonist radioligand)
BZD (central) (agonist radioligand)
B2(*h)* (agonist radioligand)
CB1(*h)* (agonist radioligand)
CCK1(*h)* (CCKA) (agonist radioligand)
D1(*h)* (antagonist radioligand)
D2S(*h)* (agonist radioligand)

ETA(*h)* (agonist radioligand)
GABA(non-selective) (agonist radioligand)
GAL2(*h)* (agonist radioligand)
CXCR2 (IL-8B) (*h)* (agonist radioligand)
CCR1(*h)* (agonist radioligand)
H1(*h)* (antagonist radioligand)
H2(*h)* (antagonist radioligand)
MC4(*h)* (agonist radioligand)
MT1 (ML1A) (*h)* (agonist radioligand)
M1(*h)* (antagonist radioligand)
M2(*h)* (antagonist radioligand)
M3(*h)* (antagonist radioligand)
NK2(*h)* (agonist radioligand)
NK3(*h)* (antagonist radioligand)
Y1(*h)* (agonist radioligand)
Y2(*h)* (agonist radioligand)
NTS1 (NT1) (*h)* (agonist radioligand)
delta2 (DOP) (*h)* (agonist radioligand)
kappa (KOP) (*h)* (agonist radioligand)
mu (MOP) (*h)* (agonist radioligand)
NOP (ORL1) (*h)* (agonist radioligand)
EP4(*h)* (agonist radioligand)
5-HT1A(*h)* (agonist radioligand)
5-HT1B(*h)* (antagonist radioligand)
5-HT2A(*h)* (antagonist radioligand)
5-HT2B(*h)* (agonist radioligand)
5-HT3(*h)* (antagonist radioligand)
5-HT5a(*h)* (agonist radioligand)
5-HT6(*h)* (agonist radioligand)
5-HT7(*h)* (agonist radioligand)
sst (non-selective) (*h)* (agonist radioligand)
VPAC1 (VIP1) (*h)* (agonist radioligand)
V1a(*h)* (agonist radioligand)
Ca2+- L (verapamil site) (phenylalkylamine) (antagonist radioligand)
Kv channel (antagonist radioligand)
SKCa channel (antagonist radioligand)
Na+ channel Na+- site 2 (antagonist radioligand)
Cl- channel (GABA-gated) (antagonist radioligand)
norepinephrine transporter(*h)* (antagonist radioligand)
dopamine transporter(*h)* (antagonist radioligand)
5-HT transporter (antagonist radioligand)

9. Pharmacokinetic studies

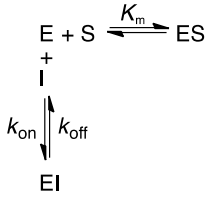
Twelve male C57BL/6 mice were weighed and administered intraperitoneally with a dose of test compound solution formulation. The dosing volume administered for the intraperitoneal route was at 10 mL/kg. Blood samples (approximately 60 µL) were collected from retro-orbital plexus of each mouse under light isoflurane anesthesia at 0.08, 0.5, 1, 2, 4, and 8 hr. Following the collection of blood, plasma was also harvested by centrifugation and stored at -70ºC until analysis. After collection of plasma, the animals were euthanized and brain and CSF samples were isolated at 0.08, 0.5, 1, 2, 4, and 8 hr. Tissue samples (brain) were homogenized using ice-cold phosphate buffer saline (pH 7.4) and homogenates were stored below -70ºC until analysis. Total homogenate volume was three times the tissue weight. Concentrations of test compound in mouse plasma and brain samples were determined by LC-MS/MS method.

Identical extraction procedures were used for the plasma/brain homogenate study samples and the spiked plasma calibration standards: A 25 µL sample of either study sample (plasma/brain) or spiked calibration standard was added to individual pre-labeled micro-centrifuge tubes. A volume of 100 µL of IS (antipyrine, 500 ng/mL) prepared in acetonitrile was then added to the micro-centrifuge tubes, except in a sample used as a negative control where only acetonitrile was added and vortexed for 5 minutes. Samples were centrifuged for 20 minutes at the speed of 4000 rpm at 4^{o}C. Following centrifugation, 100 µL of the supernatant was sampled from each centrifuge tube and transferred into insert vials. These vials remained within the auto-sampler for the LC/MS/MS analysis. Standards used for calibration were prepared by spiking 10 µL of the test compound in 190 µL of control (used as a negative control) mouse plasma/ brain homogenate.

The plasma and brain concentration-time data of test compound was provided for data analysis. The plasma and brain concentration-time data was then used for the pharmacokinetic analysis. Non-Compartmental-Analysis module in Phoenix® WinNonlin® (Version 6.3) was used to assess the pharmacokinetic parameters. Peak plasma concentrations (Cmax) and time for the peak plasma concentrations (Tmax) were the observed values. The areas under the concentration time curve (AUClast and AUCinf) were calculated by linear trapezoidal rule. The terminal elimination rate constant, ke was determined by regression analysis of the linear terminal portion of the log plasma concentration-time curve.

10. Target Engagement Simulations

Simulated pharmacokinetic profiles of the brain-specific, free inhibitor concentration were prepared based on the parameters obtained using non-compartmental pharmacokinetic modeling (See Supplemental Figure 1) and visual inspection to ensure goodness of fit to the experimental data. The density of brain homogenate was assumed to be 1 to facilitate conversion of brain concentrations to umol/L. Intracellular concentrations were assumed to in rapid equilibrium with and equal to the unbound brain homogenate fraction. The simulated pharmacokinetic profiles were then used to calculate the intracellular enzyme species according to Scheme I, through a system of differential equations (Eq 1-4). The system was assumed to be in rapid equilibrium between E and ES, while the inhibition complex EI was driven by the rate constants k_{on} and k_{off}, respectively (Eq 4). Total enzyme concentration (E_0) was fixed over the course of all simulations and the intracellular enzyme concentration was estimated using isoform–specific relative protein amounts determined in brain tissue (Wang *et al.*, 2013) and a total protein concentration of 300 mg/mL (Albe *et al.*, 1990) ([HDAC1] = 0.57 µM, [HDAC2] = 0.74 µM, [HDAC3] = 2.0 µM). Since the specific substrate concentrations and K_m values for each isoform are undefined, these values were assumed to be equivalent ([S]/K_m = 1). The kinetic parameters k_{on}, k_{off} were obtained from *in vitro* activity assessments (Supplemental Table 2). Target engagement was determined as the fraction of the inhibited species relative to the total enzyme concentration (Eq 5) and plotted as a function of time.



Scheme I.

$$[E_0] = [E]_{RE} + [EI] \quad (1)$$

$$\frac{dES}{dt} = [E]_{RE} \cdot \frac{{}^{[S]}/_{K_m}}{\left(1 + {}^{[S]}/_{K_m}\right)} \quad (2)$$

$$\frac{dE}{dt} = [E]_{RE} - [ES] \quad (3)$$

$$\frac{dEI}{dt} = k_{on} \cdot [E] \cdot [I] - k_{off} \cdot [EI] \quad (4)$$

$$\%TE = \frac{[EI]}{[E_0]} \cdot 100 \quad (5)$$

where:

$[E]_{RE}$ = Concentration of free enzyme in rapid equilibrium with substrate
$[E_0]$ = Total enzyme concentration
[EI] = Slow binding inhibitor:enzyme complex
[ES] = Enzyme:Substrate complex, in rapid equilibrium with free enzyme
[I] = Inhibitor concentration
[S] = Concentration of substrate
K_m = Michaelis constant of substrate binding

k_{on} = Inhibitor binding rate constant
k_{off} = Enzyme:inhibitor slow binding complex dissociation rate constant
TE = Target engagement level

Parameter	**BRD4884**			**BRD6688**		
Biochemical Parameters						
	HDAC1	HDAC2	HDAC3	HDAC1	HDAC2	HDAC3
[S] (μM)	50	50	50	50	50	50
K_m (μM)	50	50	50	50	50	50
$[E]_0$ (μM)[a]	0.56	0.74	2.0	0.56	0.74	2.0
k_{on} (μM^{-1} h^{-1})[b]	24.6	0.84	0.078	11.1	0.537	0.017
k_{off} (h^{-1})[b]	2.07	0.291	0.162	0.021	0.100	11.5
Pharmacokinetic Parameters (10mg/kg i.p.)						
fu	0.06			0.54		
k_a (absorption rate, h^{-1})[c]	6.0			6.0		
Clearance $t_{1/2}$ (h)[c]	0.213			0.462		
V_d (L/kg)[c]	1.9			2.5		
F	1.0			1.0		

[a]Calculated using total HDAC isoform protein concentrations in brain (Wang et al, *Epigenetics*, (2013), *8*, 756-764) and total cellular protein concentration of 300mg/mL (Albe et al, *J. Theoretical Biol*., (1990), *143*, 163-95). [b]From Supplemental Table 2. [c]Calculated from fitting data in Supplemental Figure 1.

11. Molecular Modeling and Docking

In order to determine the pose of compound 3, subunit A of the crystal structure deposited to the PDB by Bressi et al. (accession code 3MAX) was used as a starting point to construct a docking grid. The structure was protonated with histidines 145 and 146 protonated at the delta position and histidine 183 protonated at the epsilon position. The grid was prepared using Schrodinger Glide 6.0 with default parameters (25Å3 bounding box, 10 Å3 center of mass box, no constraints) and centered on the crystal ligand (LXX). Neutral hydroxamic acid, compound 3, was then docked using Glide XP with enhanced planarity for conjugated pi groups and post-docking minimization. A reasonable pose was returned with a docking score of -9.6 with Zn-N and Zn-O distances of 2.40 and 2.21Å respectively. Hydrogen bonding geometries of the dashed hydrogen bonds in Figure 1 of the text are: NHis145 – HNligand,NH_2 d= 1.95Å Cγ-Nε-HNH_2=137° NNH_2-HNH_2-Nε=136° , NHis146 - HNligand,NH_2 d=2.42 Å Cγ-Nε-HNH_2=133° NNH_2-HNH_2-Nε =127°, OGly154-HNligand,amide d= 1.8 Å CGly54-OGly54-HNligand,amide=138°, Nligand,amide-Hligand,amide-OGly154=153°. Tyr308 did not have an ideal hydrogen bonding geometry with an amide carbonyl to Tyr OH (Cligand,amide-Oligand,amide-HTyr308OH) angle of 79°. Electrostatic maps were produced using the pdb2pqr and APBS tools web server (http://nbcr-222.ucsd.edu/pdb2pqr_1.8/) with the PARSE force field and visualized in PyMol using the APBS plugin 2.1.

Dolinsky TJ, Czodrowski P, Li H, Nielsen JE, Jensen JH, Klebe G, Baker NA. PDB2PQR: expanding and upgrading automated preparation of biomolecular structures for molecular simulations. Nucleic Acids Research 2007; 35 (suppl 2): W522-W525.

Dolinsky TJ, Nielsen JE, McCammon JA, Baker NA. PDB2PQR: an automated pipeline for the setup, execution, and analysis of Poisson-Boltzmann electrostatics calculations. Nucleic Acids Research 2004; 32:W665-W667.

Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. Electrostatics of nanosystems: application to microtubules and the ribosome. Proceedings of the National Academy of Sciences USA 2001; 98:10037-10041.

MG Lerner and HA Carlson. APBS plugin for PyMOL, 2006, University of Michigan, Ann Arbor.

Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz, DT. Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes, Journal of Medicinal Chemistry 2006; 49: 6177–6196

Small-Molecule Drug Discovery Suite 2013-2: Glide, version 6.0, Schrödinger, LLC, New York, NY, 2013.

12. Neuronal Histone Acetylation Assays

Measurements of increases in neuronal histone acetylation in mouse forebrain primary neuronal cultures induced by HDAC inhibitor compounds was performed exactly as described in Fass et al., (2013). On the 13th day after generating the cultures, cells were treated for 24 hours with compounds at 10 μM. Cells were fixed with formaldehyde, stained with antibodies to acetyl-histone H3, lysine 9 (AcH3K9), or acetyl-histone H4, lysine 12 (AcH4K12), and green fluorescent secondary antibodies, and cellular fluorescence signals were quantitated with an Acumen microcytometer. To determine the efficacy of HDAC inhibitor compounds, we calculated the percentage of compound-treated cells with a fluorescence signal above a baseline threshold established in vehicle (DMSO)-treated cells.

References:

Fass D.M., Reis S.A., Ghosh B., Hennig K.M., Joseph N.F., Zhao W.N., Nieland T.J., Guan J.S., Kuhnle C.E., Tang W., Barker D.D., Mazitschek R., Schreiber S.L., Tsai L.H., Haggarty S.J. Crebinostat: a novel cognitive enhancer that inhibits histone deacetylase activity and modulates chromatin-mediated neuroplasticity. *Neuropharmacology* 2013; 64:81-96.

13. Fear conditioning tests

a. CK-p25 Induction

Mice at 3 months of age, CK-p25 male mice were induced for 6 weeks to obtain forebrain-specific expression of p25 (Cruz et al., 2003). Littermates lacking p25 were used as controls. All mice were heterozygous for their respective genes.

b. Administration of HDAC inhibitors

BRD6688 and BRD4884 were dissolved in DMSO (5% of the total resultant solution) and then diluted in 30% Cremophor/65% in physiological saline (ddH_2O containing 0.9% NaCl (Sigma)), for a final dosage solution of 1 mg/kg and 10 mg/kg, respectively. Vehicle solutions consisted of the abovementioned solution without the compounds. Solutions were prepared immediately before injection and administered daily via intraperitoneal injection for a period of 10 days prior to behaviour.

c. Context-dependent fear conditioning

Training consisted of habituating the mice to the conditioning box (TSE Systems) for a period of 3 mins, which was followed by a foot shock (2 s; 0.8 mA; constant current). The shock was repeated 30 s later and the mice were allowed to remain in the box for an additional 15 s. To assess associative learning, a long-term memory test was performed 24 h later by re-exposing the mice for 3 mins to the conditioning context, while measuring freezing behaviour[1].

d. Immunohistochemistry:

Immunohistochemistry was performed essentially as described in previous reports (Graff et al, 2012; Graff et al. 2014). Coronal brain slices (40 μm thickness) were permeabilized with 0.1% Triton X-100, blocked and incubated overnight with 0.3% Triton X-100/10% fetal bovine serum in 1x PBS containing AcH2K12 (Abcam) and visualized with a fluorescently conjugated secondary antibody (Molecular Probes). Neuronal nuclei were stained with Hoechst 33342 (Invitrogen). Images were acquired using a confocal microscope (LSM 510, Zeiss) at identical settings at the highest intensity for each of the conditions. Using the Hoechst signal channel, 20-40 representative non- apoptotic cells were chosen per experimental condition, and the mean AcH2K12 signal intensity was measured. Images were quantified using ImageJ 1.42q by an experimenter blind to treatment groups.

e. Statistics:

Statistical analyses were performed using GraphPad Prism 5. One-way ANOVAs followed by Tukey's posthoc analyses or one-tailed Student's t tests were used unless

indicated otherwise. All data are represented as mean ± SEM. Statistical significance was set at $p = 0.05$.

References:

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