SUPPORTING INFORMATION

Development of a chimeric c-Src kinase and HDAC inhibitor

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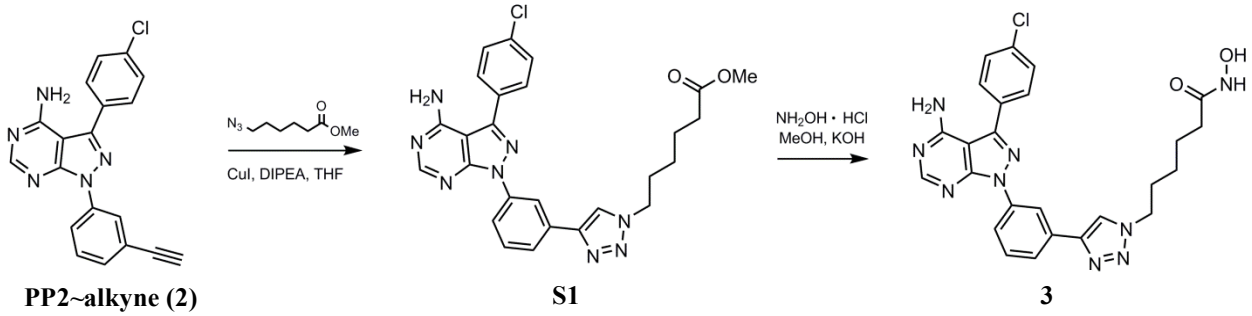
I. GENERAL SYNTHETIC METHODS.

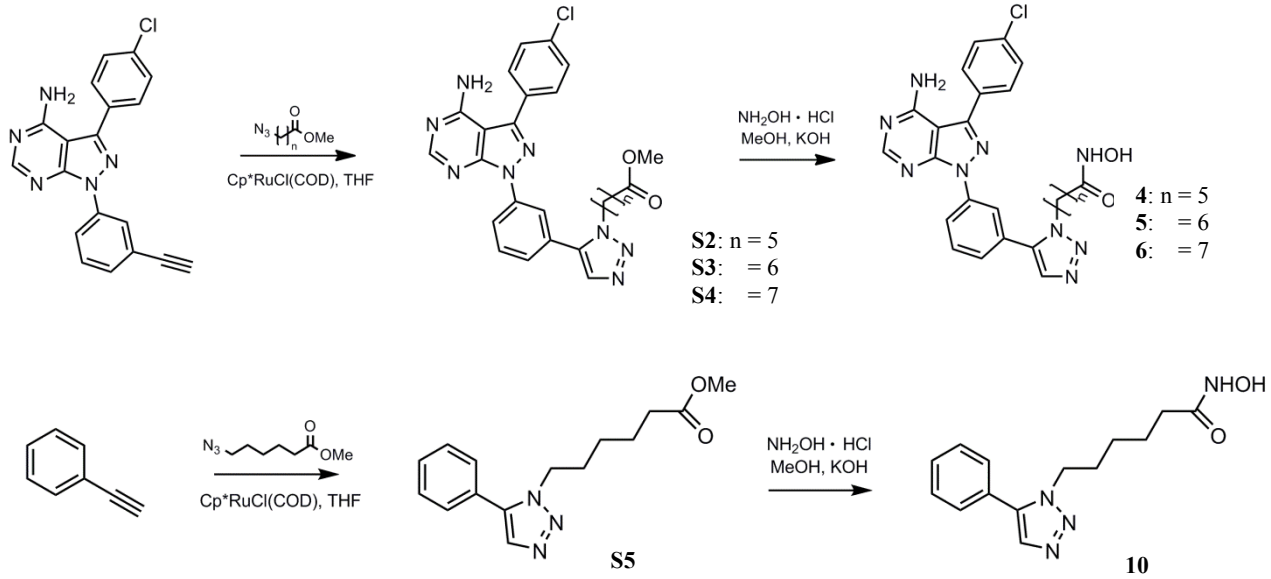
Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. All ^{1}H and ^{13}C NMR spectra were measured with a Varian MR400 and Inova 500 spectrometer. Mass spectrometry (HRMS) was carried out at the University of Michigan Ann Arbor Mass Spectrometry Facility (J. Windak, Director). Azido alkyl esters of 5–7 methylene length were synthesized adapting literature procedure.[1] 3-(4-chlorophenyl)-1-(4-ethynylphenyl)-1H-pyrazolo [3,4-d]pyrimidin-4-amine (PP2~alkyne) was prepared as described previously.[2] (E)-ethyl 3-(4-(azidomethyl)phenyl)acrylate was synthesized by adapting literature protocol.[2,3] Flash column chromatography was performed using a Biotage Isolera 1 Flash Purification System using KP-Sil SNAP cartridges. In all cases, ethyl acetate was used to transfer the crude reaction material onto the silica gel samplet. A gradient elution using hexane and ethyl acetate was performed, based on the recommendation from the Biotage TLC Wizard.

II. SYNTHESIS OF COMPOUNDS 3-10



Scheme S1. Synthesis of Compound 3

Synthesis of S1: PP2~alkyne (0.14 mmol) and methyl 6-azidohexanoate (0.318 mmol) were dissolved in THF (1 mL) and stirred under nitrogen at room temperature. Copper (I) iodide (0.011 mmol) and Hunig's base (0.038 mL) were added to the reaction mixture which was stirred under nitrogen overnight. The reaction mixture was diluted with dichloromethane (8 mL) and washed with 1:4 NH_4OH/saturated NH_4Cl (3x 12 mL) and saturated NH_4Cl (12 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by Biotage Isolera 1 Flash Purification System to give 20 mg (27% yield) of **S1** as a white solid. **Spectral Data.** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.65 (s, 1 H), 8.52 (s, 1 H), 8.26-8.24 (m, 1H), 7.89 (s, 2 H), 7.77 (d, J = 10.0 Hz, 2 H), 7.62-7.56 (m, 3 H), 5.59 (s, 2H), 4.44 (t, J = 8.0 Hz, 2 H), 3.67 (s, 3 H), 2.34 (t, J = 8 Hz, 2 H), 2.02-1.97 (m, 2 H), 1.72-1.68 (m, 2 H), 1.43-1.39 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.77, 157.97, 156.53, 154.68, 147.14, 144.41, 139.23, 135.59, 131.73, 131.15, 129.90, 129.62, 123.79, 121.12, 120.0, 118.76, 99.69, 51.56, 50.12, 33.62, 29.99, 25.91, 24.17; HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{26}H_{25}ClN_8O_2$, 517.1862; found, 517.1863.



Scheme S2. Synthesis of Compound 4-6, and 10

Procedure of Cp*RuCl(COD) catalyzed cycloaddition reaction.

Synthesis of S2: PP2~alkyne (50 mg, 0.14 mmol) and Cp*RuCl(COD) (5.3 mg, 0.014 mmol) were added into a flame-dried round bottom flask and subsequently purged with nitrogen gas for 5 min. THF (1mL) and methyl 6-azidohexanoate (50 mL, 0.43 mmol) were then added. The reaction was allowed to stir under nitrogen at room temperature overnight. The reaction mixture was diluted with ethyl acetate (10mL) and washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by Biotage Isolera 1 Flash Purification System to give a 28 mg (37% yield) of compound **S2** as a yellow solid. **Spectral Data.** ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.47 (s, 1 H), 8.42 (s, 2 H), 7.77 (s, 1 H), 7.72 (s, *J* = 7.2 Hz, 2 H), 7.63 (t, *J* = 8.0 Hz, 1 H), 7.56 (d, *J* = 8.4 Hz, 2 H), 7.33 (d, *J* = 8.0 Hz, 1 H), 5.67 (s, 1H), 4.44 (t, *J* = 8 Hz, 2 H), 3.59 (s, 3 H), 2.21 (t, *J* = 8.0 Hz, 2 H), 1.94-1.86 (m, 2 H), 1.61-1.53 (m, 2 H), 1.35-1.27 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.71, 157.94, 156.72, 154.98, 144.89, 139.52, 137.09, 135.92, 133.26, 130.96, 129.99, 129.61,128.11, 126.49, 121.90, 121.19, 99.93, 51.49, 48.27, 33.64, 29.82, 25.99, 24.23; HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{26}H_{25}ClN_8O_2$, 517.1862; found, 517.1861.

Synthesis of S3: Reaction of PP2~alkyne (50 mg, 0.14 mmol) and methyl 7-azidohexanoate (40 mL, 0.28 mmol) was prepared as described for the synthesis of **S2**. The crude product was purified by Biotage Isolera 1 Flash Purification System to give a 36 mg (47% yield) of compound **S3** as a yellow solid. **Spectral Data.** ^{1}H NMR (500 MHz, $CDCl_3$): 8.48 (s, 1H), 8.43 (s, 2H), 7.77 (s, 1H), 7.72 (d, *J* = 6.8, 2H), 7.64 (t, *J* = 8 Hz, 1H), 7.58-7.53 (m, 2H), 7.34 (d, *J* = 8.0 Hz, 1H), 4.44 (t, *J* = 7.2 Hz, 2H), 3.61 (s, 3H), 2.19 (t, *J* = 7.6 Hz, 2H), 1.92-1.85 (m, 2H), 1.56-1.48 (m, 2H), 1.28-1.23 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 173.93, 158.07, 156.63, 154.94, 144.92, 139.50, 137.07, 135.86, 133.20, 130.95, 129.83, 128.12, 126.46, 121.86, 121.17,

99.88, 51.43, 48.37, 33.76, 29.96,28.42, 26.14, 24.57 HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{27}ClN_8O_2$, 531.2018; found, 531.2022.

Synthesis of S4: Reaction of PP2~alkyne (50 mg, 0.14 mmol) and methyl 8-azidohexanoate (40 mL, 0.28 mmol) was prepared as described for the synthesis of **S2**. The crude product was purified by Biotage Isolera 1 Flash Purification System to give a 36 mg (45% yield) of compound **S4** as a yellow solid. **Spectral Data.** ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.52 (s, 1 H), 8.46 (s, 2 H), 7.81 (s, 1 H), 7.77-7.73 (m, 2 H), 7.70-7.64 (m, 1 H), 7.61-7.58 (m, 2 H), 7.37 (d, *J* = 7.5 Hz, 1 H), 5.52 (s, 2H), 4.46 (t, *J* = 10 Hz, 2 H), 3.65 (s, 3 H), 2.24 (t, *J* = 7.5 Hz, 2 H), 1.94-1.88 (m, 2 H), 1.57-1.51 (m, 2 H), 1.32-1.23 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): 174.08, 158.03, 156.65, 154.94, 144.91, 139.49, 137.06, 135.87, 133.22, 130.95, 129.84, 128.17,126.49, 121.86, 121.21, 99.89, 51.44, 48.44, 33.88, 30.08, 28.8, 28.58, 26.27, 24.69; HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{28}H_{29}ClN_8O_2$, 545.2180; found, 545.2180.

Synthesis of S5: Reaction of phenylacetylene (54 mL, 0.49 mmol) and methyl 6-azidohexanoate (69 mL, 0.59 mmol) was prepared as described for the synthesis of **S2**. The crude product was purified by Biotage Isolera 1 Flash Purification System to give 70 mg (52% yield) of compound **S5** as light brown oil. **Spectral Data.** ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.69 (s, 1 H), 7.51-7.49 (m, 3 H), 7.39-7.38 (m, 2 H), 4.36 (t, *J* = 10 Hz, 2 H), 3.65 (s, 3 H), 2.29-2.22 (m, 2 H), 1.85 (m, 2 H), 1.59-1.55 (m, 2 H), 1.32-1.26 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.68, 137.64, 132.98, 129.41, 129.07, 128.66, 127.14, 51.45, 47.96, 33.57, 29.64, 25.84, 24.12; HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{15}H_{19}N_3O_2$, 274.2550; found, 274.1553.

Procedure for conversion of methyl esters to hydroxamic acid

Synthesis of 3: A solution of hydroxylamine hydrochloride (271 mg, 3.9 mmol) in 10 mL of MeOH, KOH (219 mg, 3.9 mmol) was added and stirred at 40 ºC for 10 min. The reaction mixture was cooled to 0 ºC and filtered. Compound **S1** (10 mg, 0.02 mmol) was added to the filtrate followed by KOH (0.04 mmol) at room temperature for 3 hours. The reaction mixture was extracted with EtOAc. The organic layer was washed with saturated NH_4Cl solution and brine, and dried over $MgSO_4$, filtered and concentrated. The residue was purified by reverse-phase preparative HPLC (linear gradient of 5→ 95% acetonitrile and water) to give 7.3 mg of compound **3** (73%) as a white powder. **Spectral Data.** ^{1}H NMR (500 MHz, CD_3OD): δ 8.64 (s, 1H), 8.47 (s, 2H), 8.16 (d, *J* = 5 Hz, 1H), 7.92-7.86 (m, 1H), 7.86-7.79 (m, 2H), 7.67-7.63 (m, 3H), 4.49 (t, *J* = 7 Hz, 2H), 2.12 (t, *J* = 7.5 Hz, 2H), 2.03-1.99 (m, 2H), 1.74-1.68 (m, 2H), 1.44-1.34 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 169.32, 157.08, 155.06, 146.32, 145.06, 139.62, 134.36, 132.31, 131.47, 130.75, 130.20, 129.64, 123.52, 122.22, 120.81, 117.85, 99.19, 49.89, 32.47, 29.77, 25.89, 24.93; HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{25}H_{24}ClN_9O_2$, 518.1814; found, 518.1822.

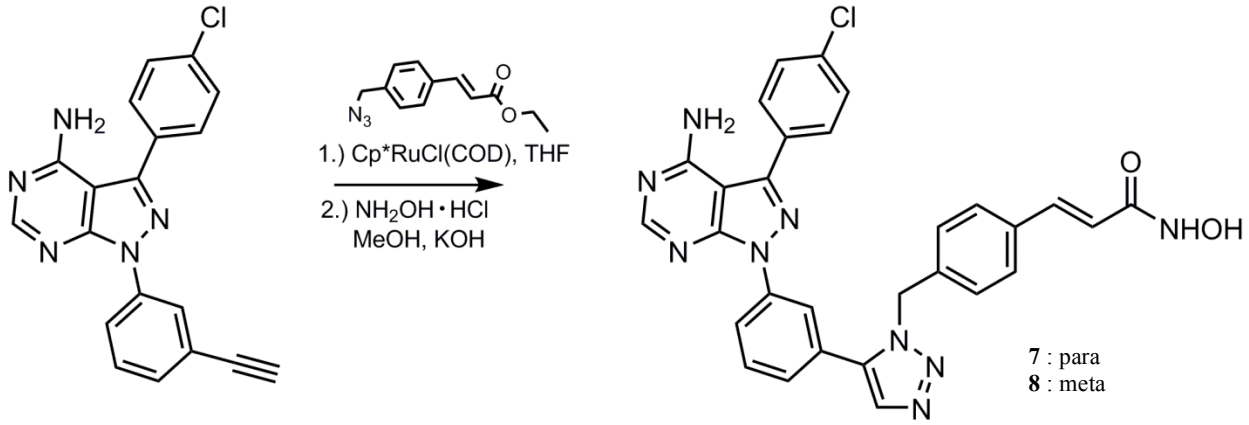
Synthesis of 4: Compound **S2** (8.9 mg, 0.02 mmol) was added to the hydroxylamine hydrochloride solution as described for the synthesis of **3** to give 7.2 mg of compound **4** (82%) as a white powder. **Spectral Data.** ^{1}H NMR (500 MHz, CD_3OD): δ 8.49 – 8.43 (m, 2H), 8.34 (d, *J* = 10 Hz, 1 H), 7.92 (s, 1 H), 7.83-7.80 (m, 2 H), 7.77 (t, *J* = 8.0 Hz, 1 H), 7.65-7.63 (m, 2 H), 7.59 (d, *J* = 8.0 Hz, 1 H), 4.57 (t, *J* = 7.0 Hz, 2 H), 2.01 (t, *J* = 7.0 Hz, 2 H), 1.93-1.87 (m, 2 H), 1.60-1.54 (m, 2 H), 1.35-1.28 (m, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.26, 157.77, 155.76, 154.74, 145.69, 139.26, 137.05, 134.58, 133.46, 131.07, 130.74, 129.69, 128.16, 127.04,

122.16, 121.26, 99.27, 48.35, 32.44, 29.62, 25.96, 24.94; HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{25}H_{24}ClN_9O_2$, 518.1814; found, 518.1811.

Synthesis of 5: Compound **S3** (36 mg, 0.07 mmol) was added to the hydroxylamine hydrochloride solution as described for the synthesis of **3** to give 19 mg of compound **5** (53%) as a white powder. **Spectral Data.** ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.42 (s, 2 H), 8.34 (d, *J* = 7.4 Hz, 1 H), 7.98 (d, *J* = 0.9 Hz, 1 H), 7.81 – 7.77 (m, 2 H), 7.74 (t, *J* = 8.0 Hz, 1 H), 7.65 (d, *J* = 8.5 Hz, 2 H), 7.56 (d, *J* = 7.8 Hz, 1 H), 4.46 (t, *J* = 7.2 Hz, 2 H), 1.85 (t, *J* = 7.4 Hz, 2 H), 1.79-1.75 (m, 2 H), 1.41-1.37 (m, 2 H), 1.20-1.16 (m, 4 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.59, 158.63, 156.05, 154.86, 145.39, 139.33, 137.06, 134.52, 133.44, 131.13, 130.71, 129.67, 128.16, 126.92, 122.05, 121.11, 99.31, 48.43, 32.51, 29.77, 28.34, 26.00, 25.29; HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{26}H_{26}ClN_9O_2$, 532.1971; found, 532.1978.

Synthesis of 6: Compound **S4** (35 mg, 0.06 mmol) was added to the hydroxylamine hydrochloride solution as described for the synthesis of **3** to give 5.4 mg of compound **6** (15%) as a white powder. **Spectral Data.** ^{1}H NMR (500 MHz, CD_3OD): δ 8.49 (s, 1 H), 8.43 (s, 1 H), 8.36 (d, *J* = 7.5 Hz, 1 H), 7.92 (s, 1 H), 7.84-7.80 (m, 2 H), 7.78 (t, *J* = 8.0 Hz, 1 H), 7.67-7.63 (m, 2 H), 7.60 (d, *J* = 7.7 Hz, 1 H), 4.56 (t, *J* = 7.5 Hz, 2 H), 1.99 (t, *J* = 7.3 Hz, 2 H), 1.89-1.84 (m, 2H), 1.51-1.46 (m, 2H), 1.29-1.20 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.43, 158.21, 156.29, 154.90, 145.56, 139.36, 137.06, 134.52, 133.41, 131.16, 130.72, 129.67, 128.19, 126.92, 122.03, 121.08, 99.30, 48.34, 32.57, 29.83, 28.76, 28.43, 26.14, 25.37; HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{27}H_{28}ClN_9O_2$, 546.2127; found, 546.2129.

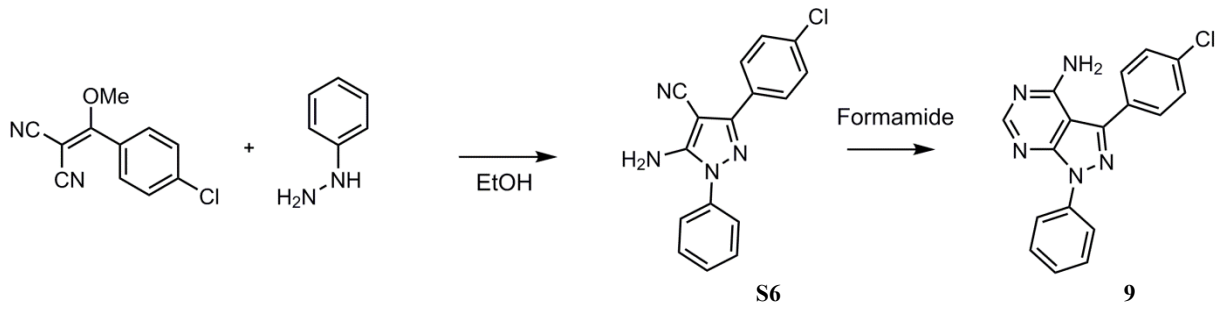
Synthesis of 10: Compound **S5** (30 mg, 0.11 mmol) was added to the hydroxylamine hydrochloride solution as described for the synthesis of **3** to give 15 mg of compound **10** (50%) as an oil. **Spectral Data.** ^{1}H NMR (500 MHz, CD_3OD): δ 7.79 (s, 1 H), 7.58-7.50 (m, 5 H), 4.49 (t, *J* = 7.0 Hz, 2 H), 2.01 (t, *J* = 7.4 Hz, 2 H), 1.84-1.78 (m, 2 H), 1.57-1.51 (m, 2 H), 1.27-1.20 (m, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.28, 137.69, 133.70, 129.75, 129.55, 128.97, 127.30, 48.13, 32.39, 29.40, 25.85, 24.85; HRMS-ESI (*m/z*): $[M + H]^+$ calcd for $C_{14}H_{18}N_4O_2$, 275.1503; found, 275.1508.



Scheme S3. Synthesis of Compound 7 and 8

Synthesis of 7: Reaction of PP2~alkyne (20 mg, 0.06 mmol) and (E)-ethyl 3-(4-(azidomethyl)phenyl)acrylate (16 mg, 0.07 mmol) was prepared as described for the synthesis of **S2**. The crude product was carried on without further purification and was added to the hydroxylamine hydrochloride solution as described for the synthesis of **3** to give 1.3 mg of compound **7** (20%) as a white powder. **Spectral Data.** ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.35 (s, 3 H), 8.10 (s, 1 H), 7.75-7.63 (m, 5 H), 7.47 (t, *J* = 8.1 Hz, 3 H), 7.37 (d, *J* = 15.72 Hz, 1 H), 7.09 (d, *J* = 7.5 Hz, 2 H), 6.40 (d, *J* = 15.84 Hz, 1 H), 5.79 (s, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6): 158.79, 157.05, 155.12, 145.33, 139.43, 138.16, 137.69, 137.15, 135.64, 134.44, 133.83, 131.30, 130.71, 130.54, 129.79, 129.65, 128.39, 127.76, 126.84, 126.02, 122.08, 121, 120.02, 99.23, 51.51; HRMS-ESI (*m/z*): [M - H]$^-$ calcd for $C_{29}H_{22}ClN_9O_2$, 562.1512; found, 562.1502.

Synthesis of 8: Reaction of PP2~alkyne (10 mg, 0.03 mmol) and (E)-ethyl 3-(4-(azidomethyl)phenyl)acrylate (8 mg, 0.04 mmol) was prepared as described for the synthesis of **S2**. The crude product was carried on withour further purification and was added to the hydroxylamine hydrochloride solution as described for the synthesis of **3** to give 1.4 mg of compound **8** (74%) as a white powder. **Spectral Data.** ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.38 – 8.28 (m, 2 H), 8.09 (s, 1 H), 7.74 - 7.64 (m, 6 H), 7.49 (d, *J* = 7.75 Hz, 1 H), 7.42 (d, *J* =7.9 Hz, 1 H), 7.37 – 7.24 (m, 3 H), 7.05 (d, *J* = 7.8 Hz, 1 H), 6.35 (d, *J* = 15.8 Hz, 1 H), 5.78 (s, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.80, 157.06, 145.34, 139.44, 138.18,137.69, 137.16, 134.45, 133.84, 131.31, 130.71, 130.55, 129.79, 129.66, 128.40, 127.77, 126.85,126.04, 122.09, 120.03, 99.23, 51.51; HRMS-ESI (*m/z*): [M - H]$^-$ calcd for $C_{29}H_{22}ClN_9O_2$, 562.1512; found, 562.1496.



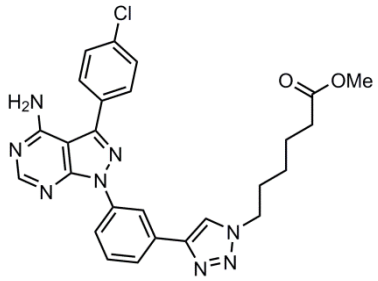
S6 **9**

Scheme S3. Synthesis of Compound 9

Synthesis of S6: To an oven-dried round bottom flask was added 2-((4-chlorophenyl)(methoxy)methylene)malononitrile[2] (500 mg, 2.29 mmol). Ethanol (11.4 mL) was added, followed by phenylhydrazine (247 mg, 2.29 mmol). The reaction mixture was then heated to 85 ˚C for 1 hour. The reaction was then allowed to cool to room temperature. During the cooling process visible precipitation began to occur. After sufficient cooling the reaction mixture was filtered to provide the product **S6** as a fluffy light pink solid (290 mg, 43% yield). **Spectral data.** ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.89-7.85 (m, 2 H), 7.60-7.53 (m, 2 H), 7.49-7.44 (m, 1 H), 6.88 (s, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.44, 149.50, 137.70, 134.18, 130.49, 129.95, 129.36, 128.56, 128.05, 124.79, 115.83; HRMS-APCI (*m/z*): $[M + H]^+$ calcd for $C_{79}H_{95}ClN_{20}O_{22}$, 295.0746; found 295.0746.

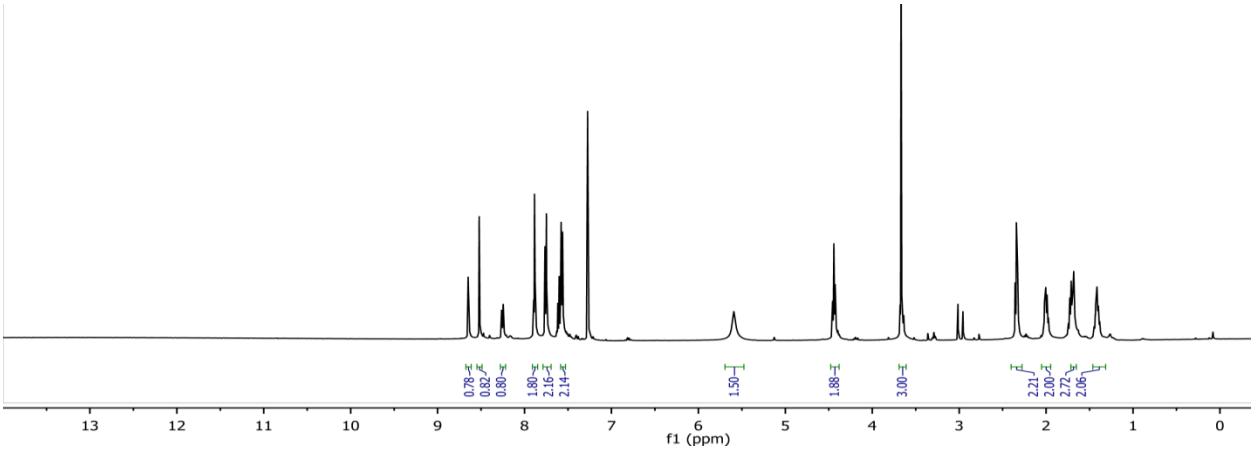
Synthesis of 9: To an oven-dried round bottom flask was added **S6** (205 mg, 0.7 mmol). Formamide (2 mL) was then added. The reaction mixture was heated to 220 ˚C for 5 hours. The reaction was then allowed to cool to room temperature. After sufficient cooling, water (6 mL) was added to precipitate the reaction. The reaction was then filtered, and the resulting solid was rinsed with water (2 mL x 3). After drying the product **9** was obtained as a light brown solid (190 mg, 85% yield). **Spectral data.** ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.38 (s, 1 H), 8.23-8.20 (m, 2 H), 7.79-7.76 (m, 2 H), 7.66-7.62 (m, 2 H), 7.59-7.54 (m, 2 H), 7.39-7.34 (m, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.84, 157.02, 154.91, 144.88, 139.03, 134.31, 131.53, 130.70, 129.64, 129.59, 129.83, 121.55, 99.13; HRMS-APCI (*m/z*): $[M + H]^+$ calcd for $C_{79}H_{95}ClN_{20}O_{22}$, 322.0854; found 322.0864.

III. SPECTRAL DATA FOR COMPOUNDS 3-10



S1

Compound **S1** ^{1}H:



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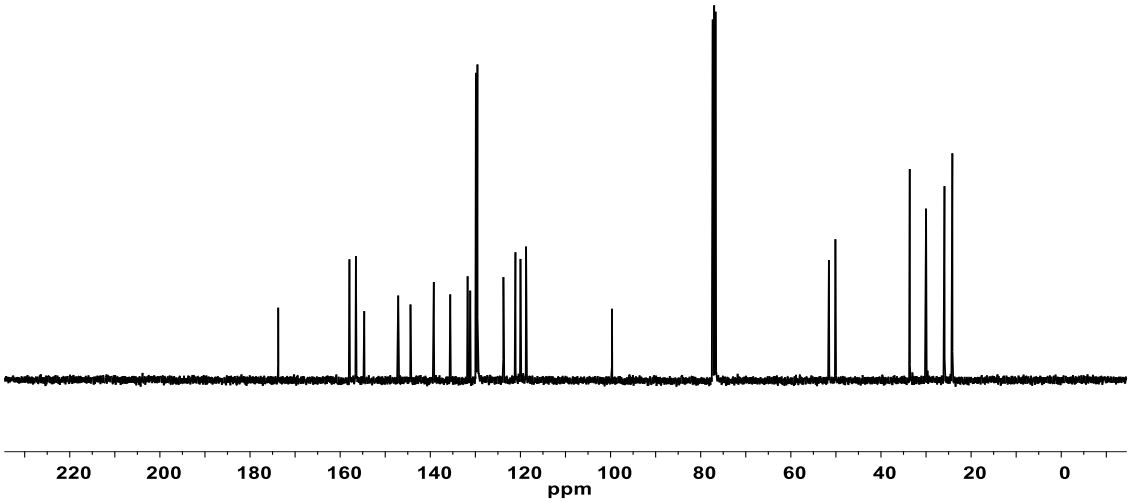
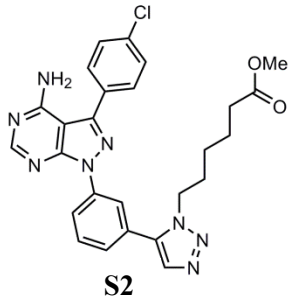
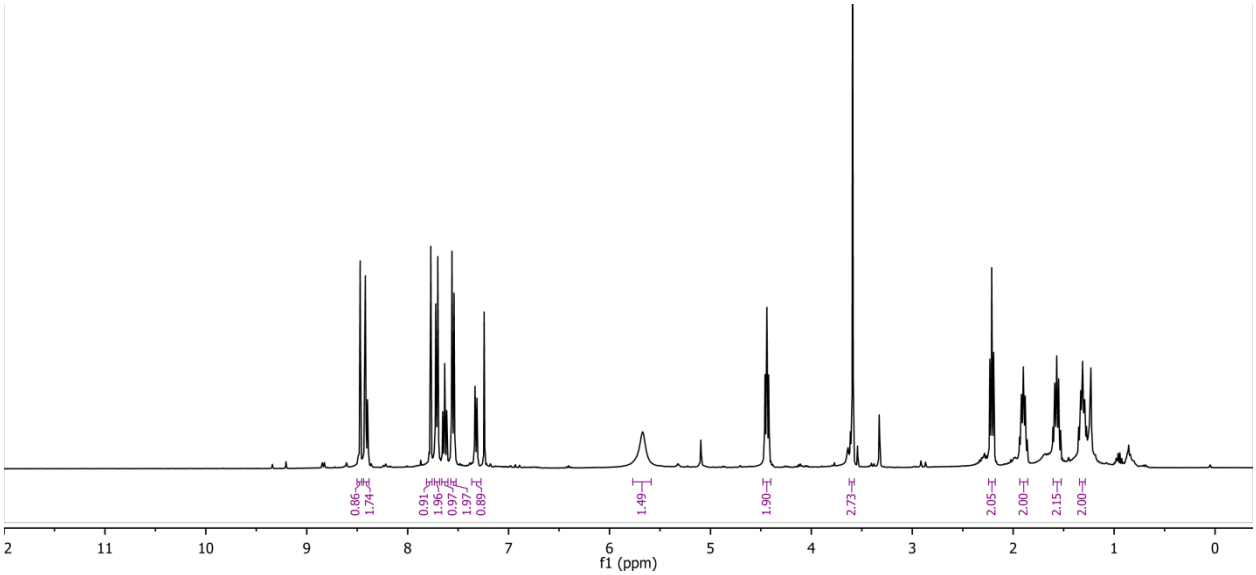




S2

Compound **S2** ^{1}H:



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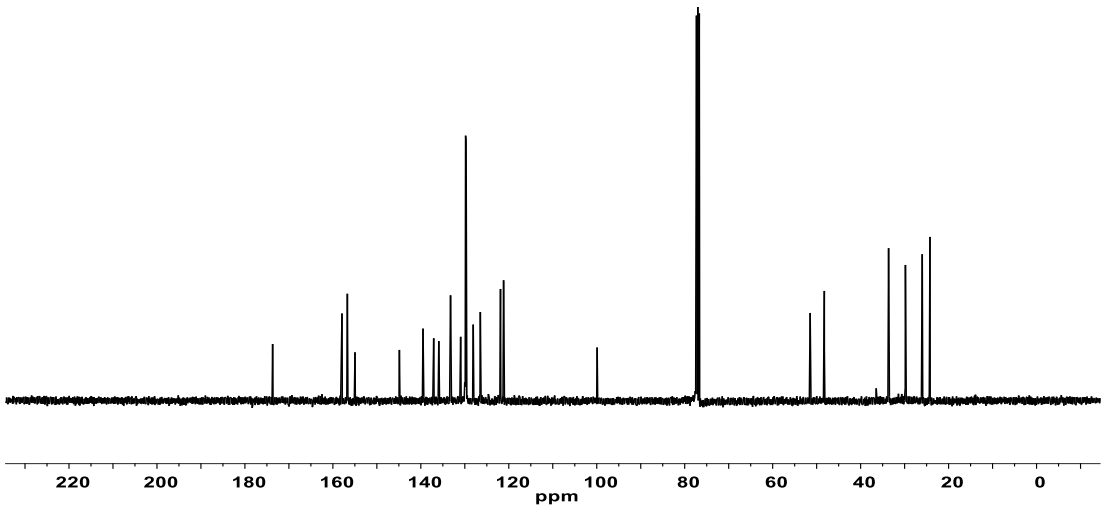
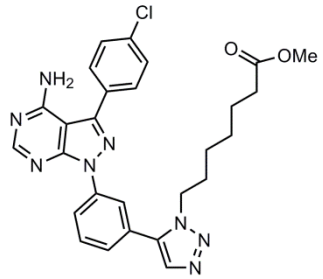
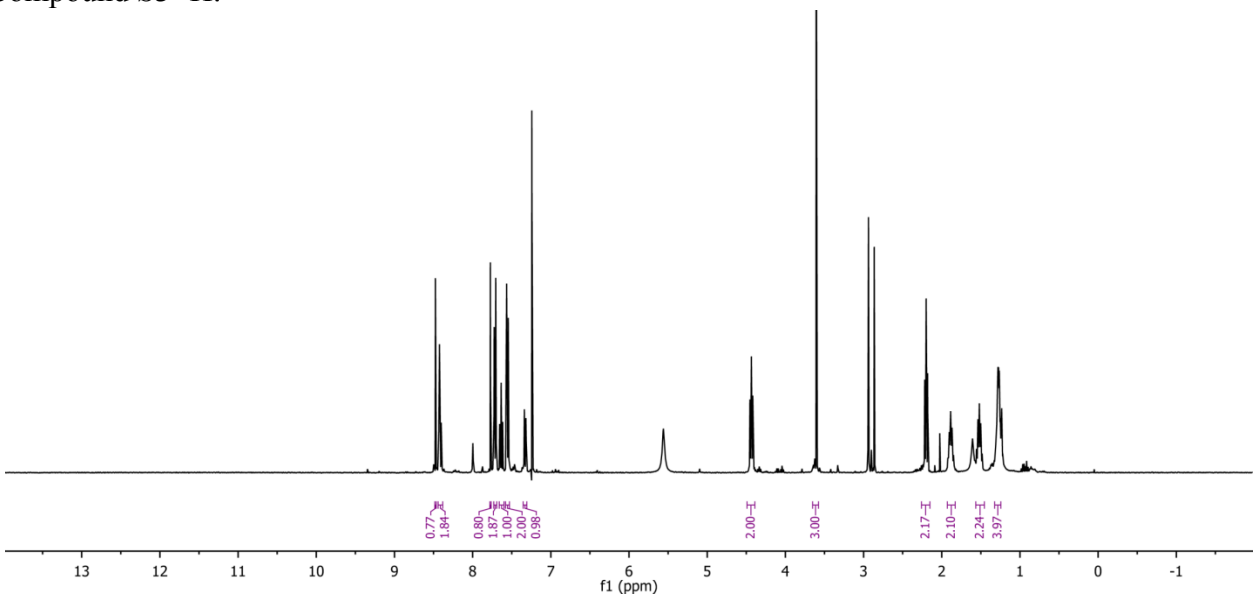


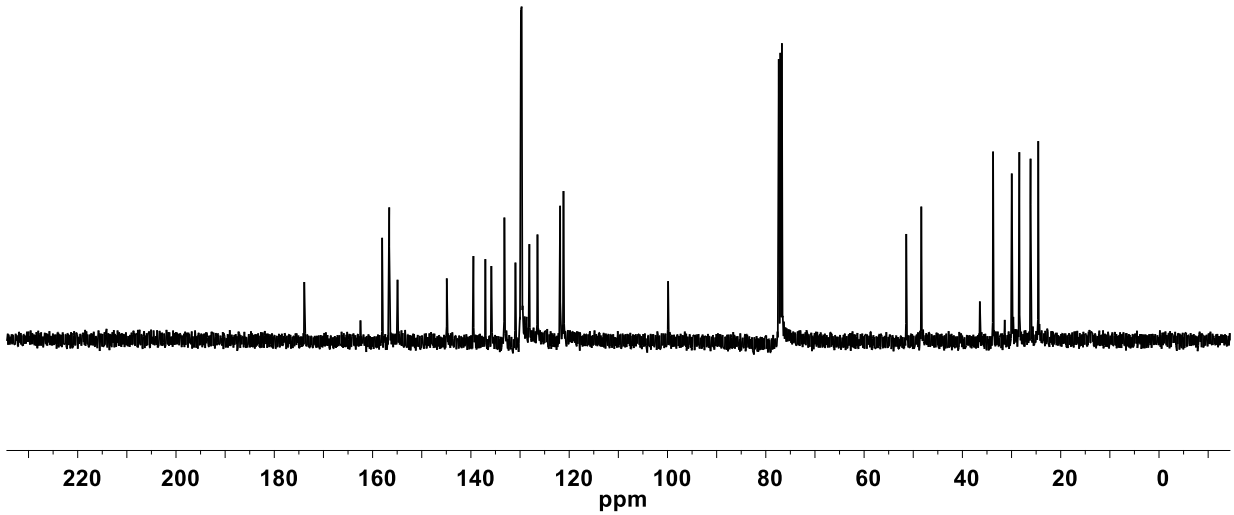


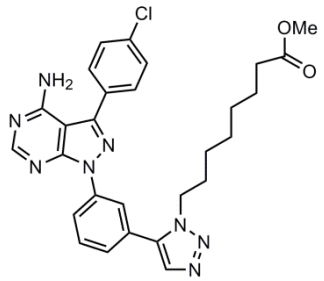
S3

Compound **S3** ^{1}H:



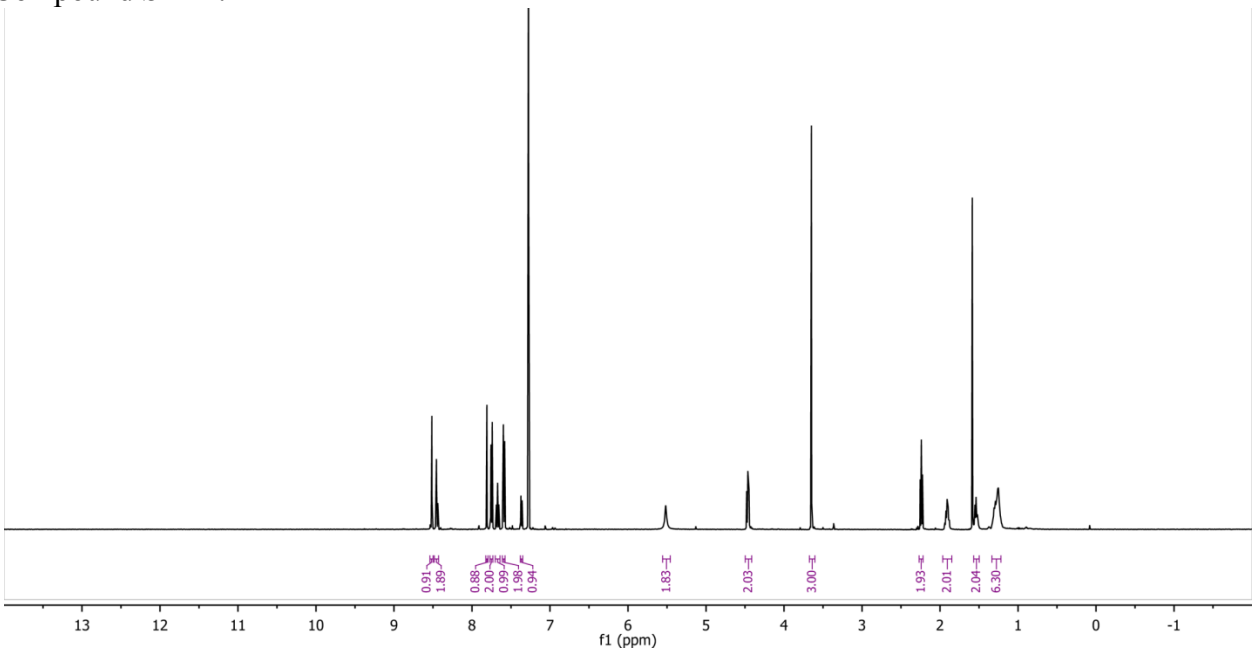
Compound **S3** ^{13}C:



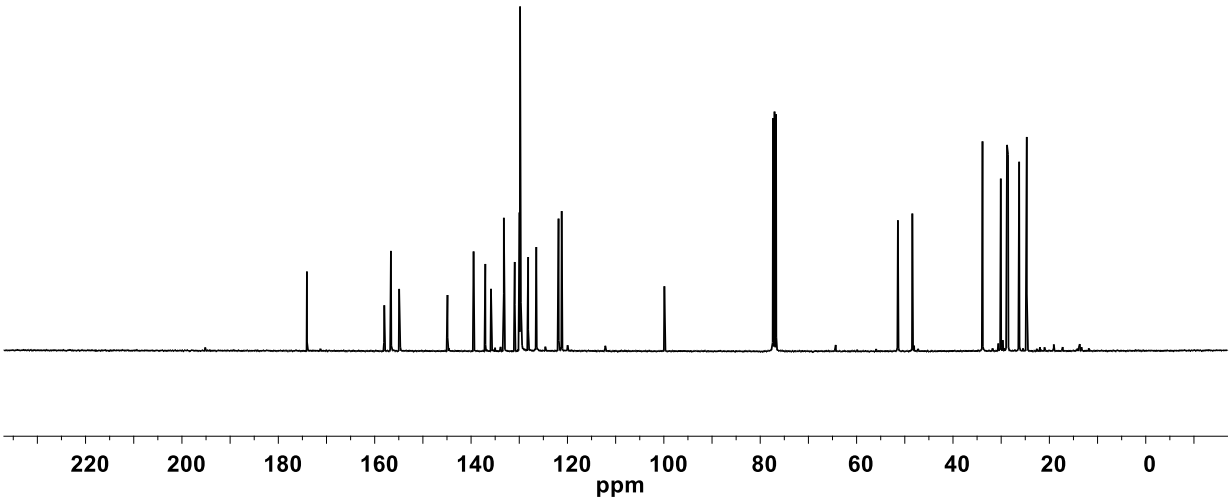


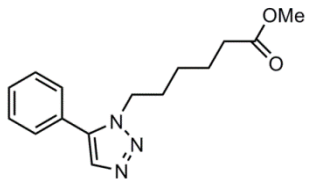
S4

Compound **S4** ^{1}H:



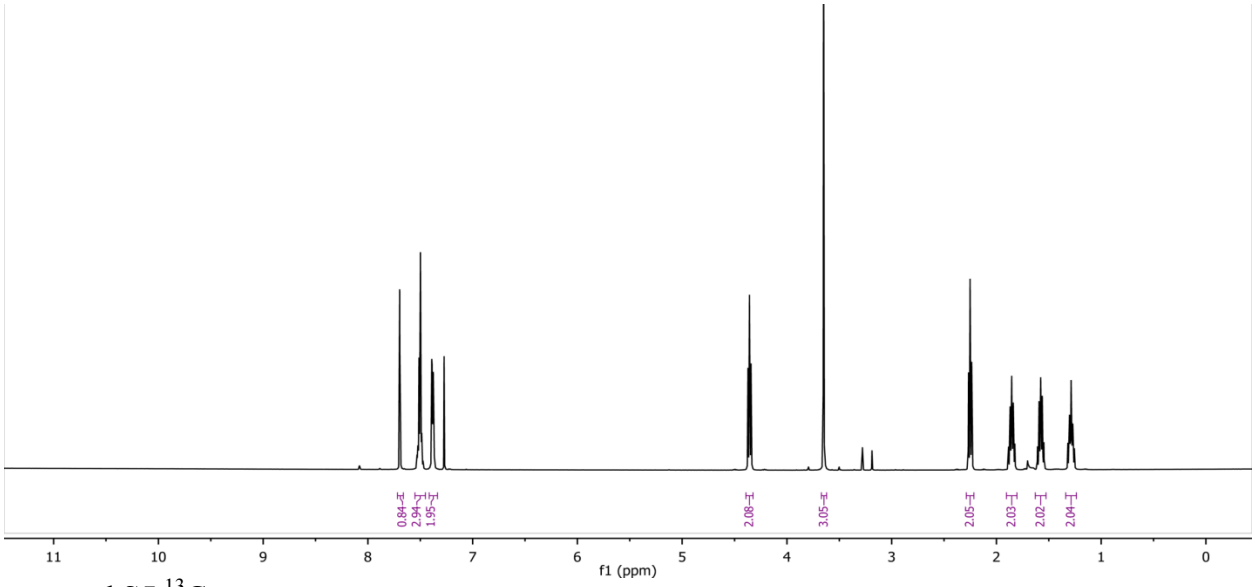
Compound **S4** ^{13}C:



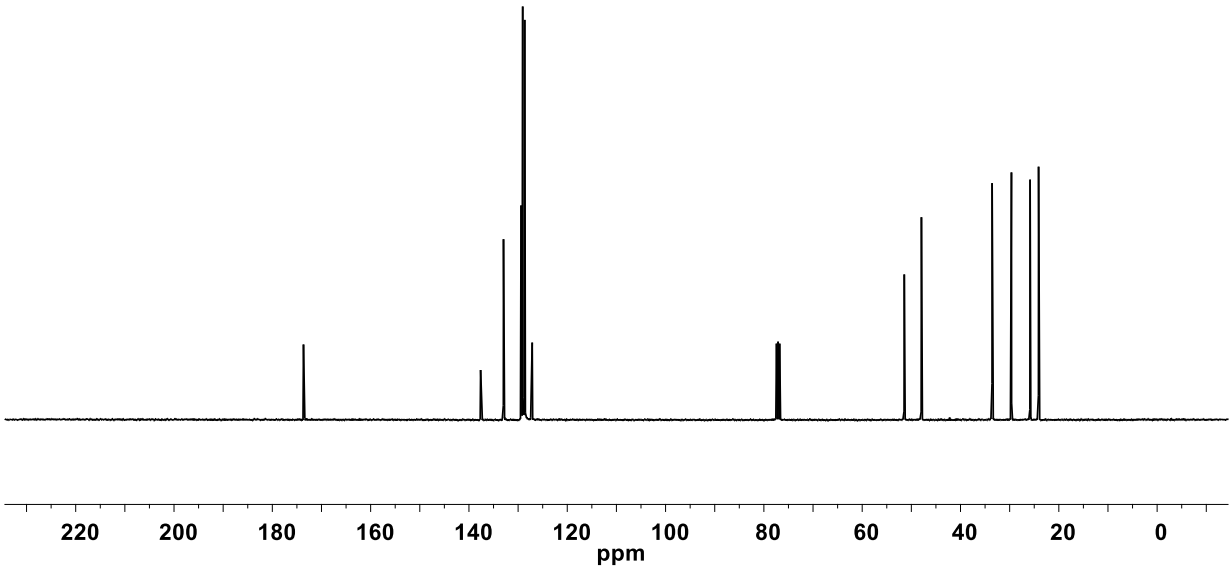


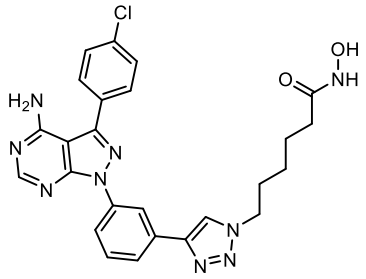
S5

Compound **S5** ^{1}H:



Compound **S5** ^{13}C:





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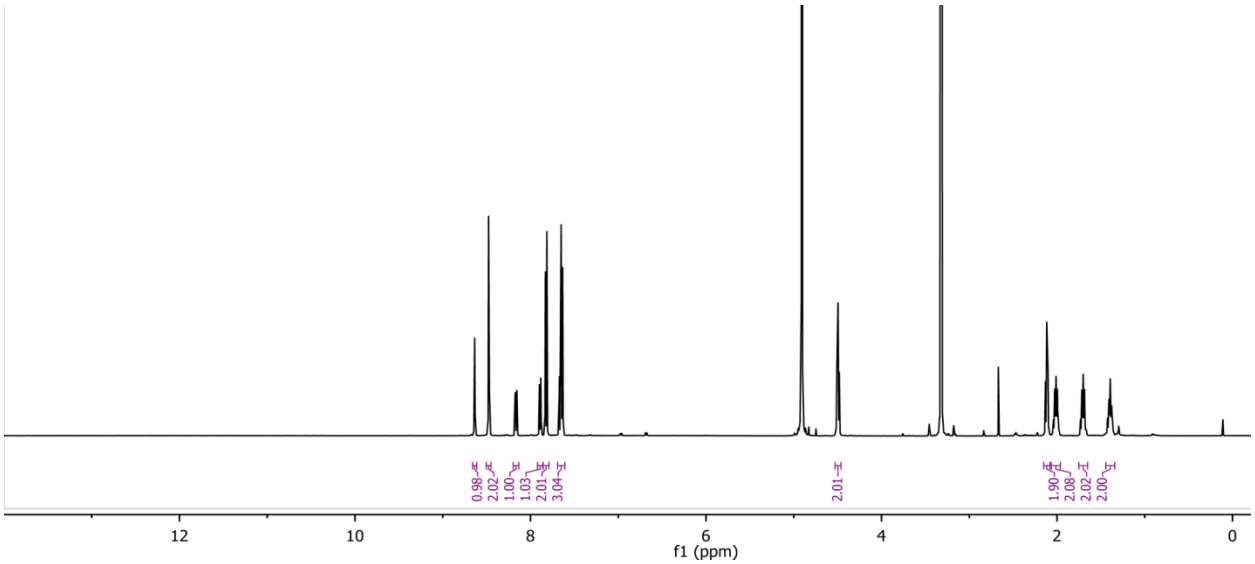
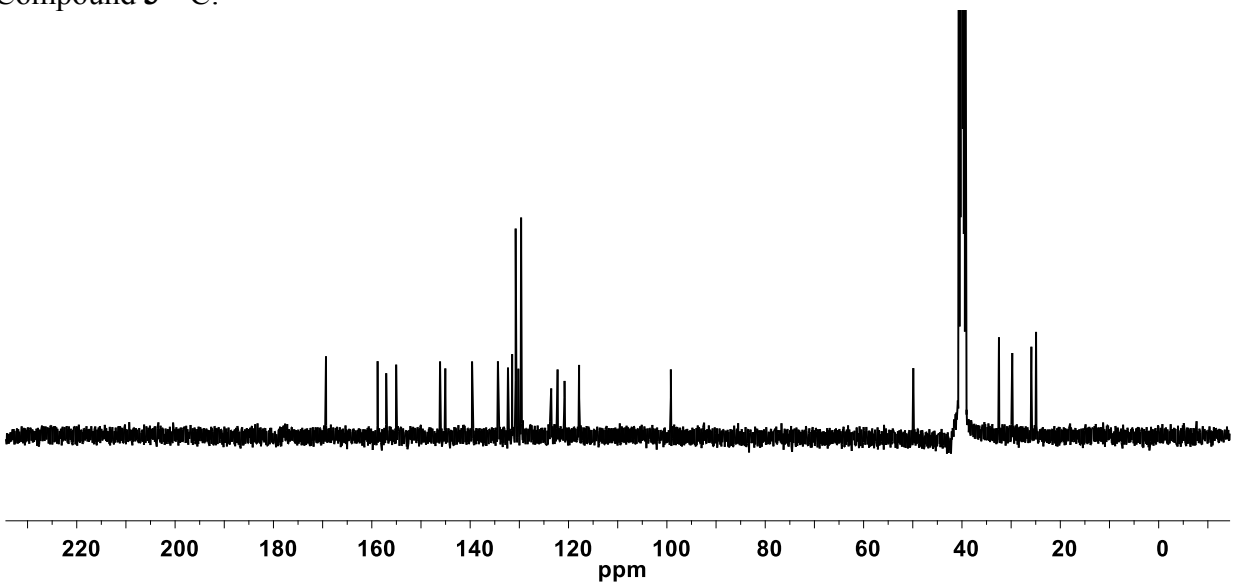
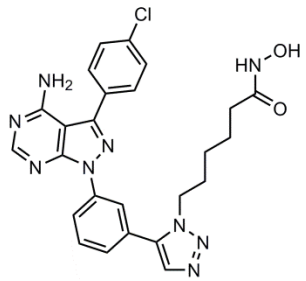


Compound **3** ^{13}C:





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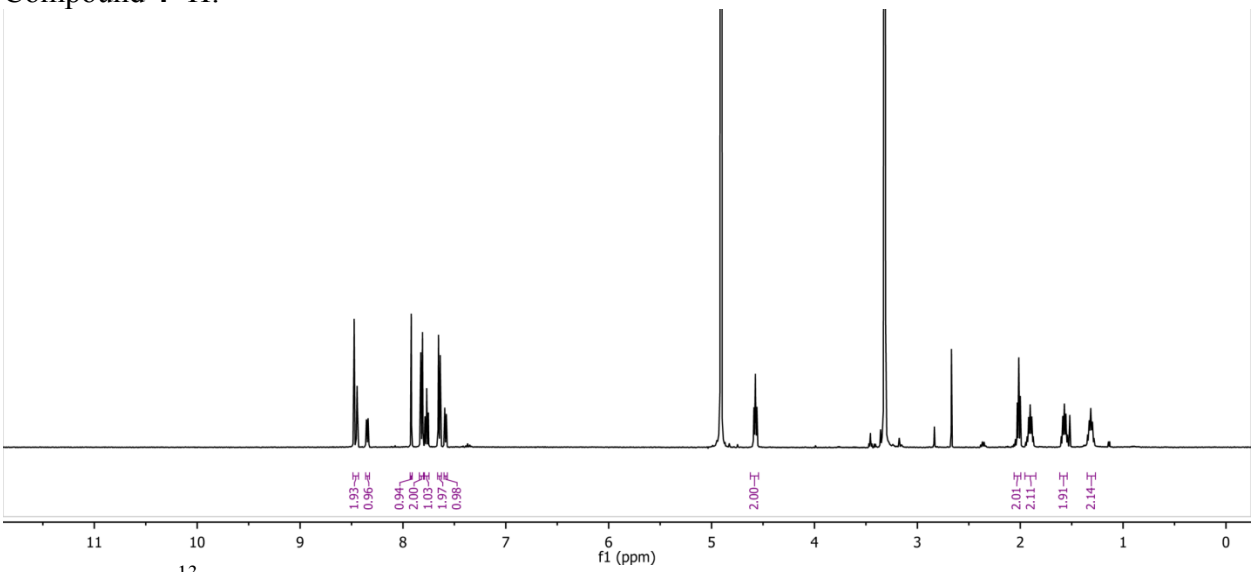
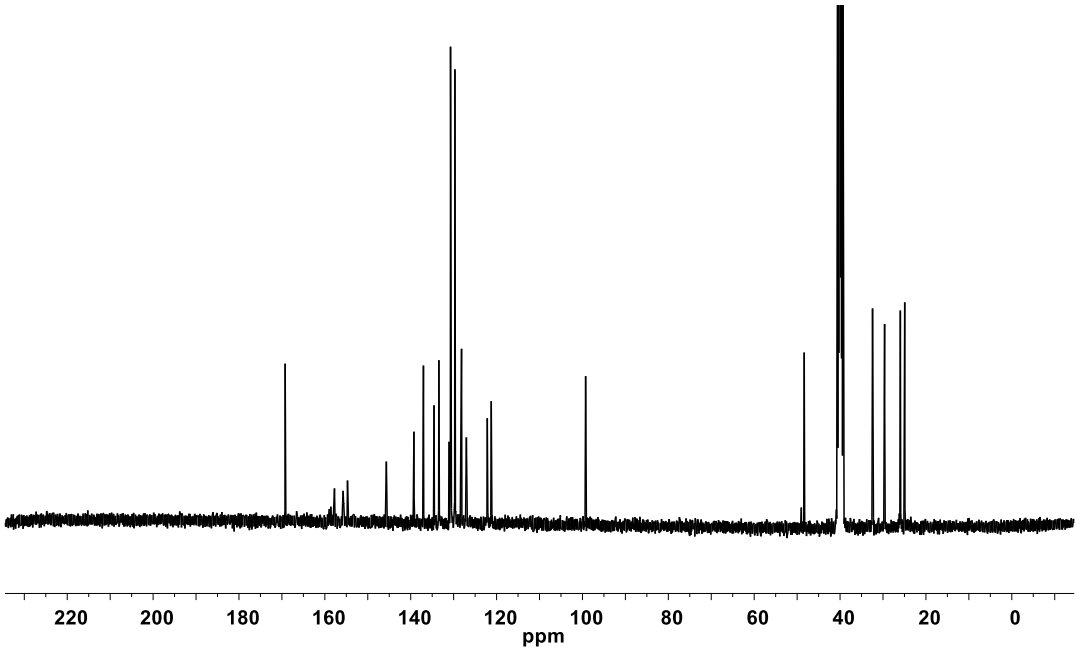
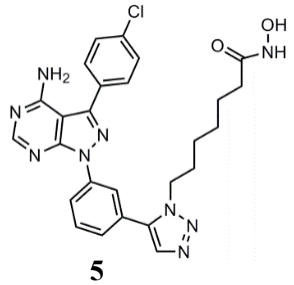


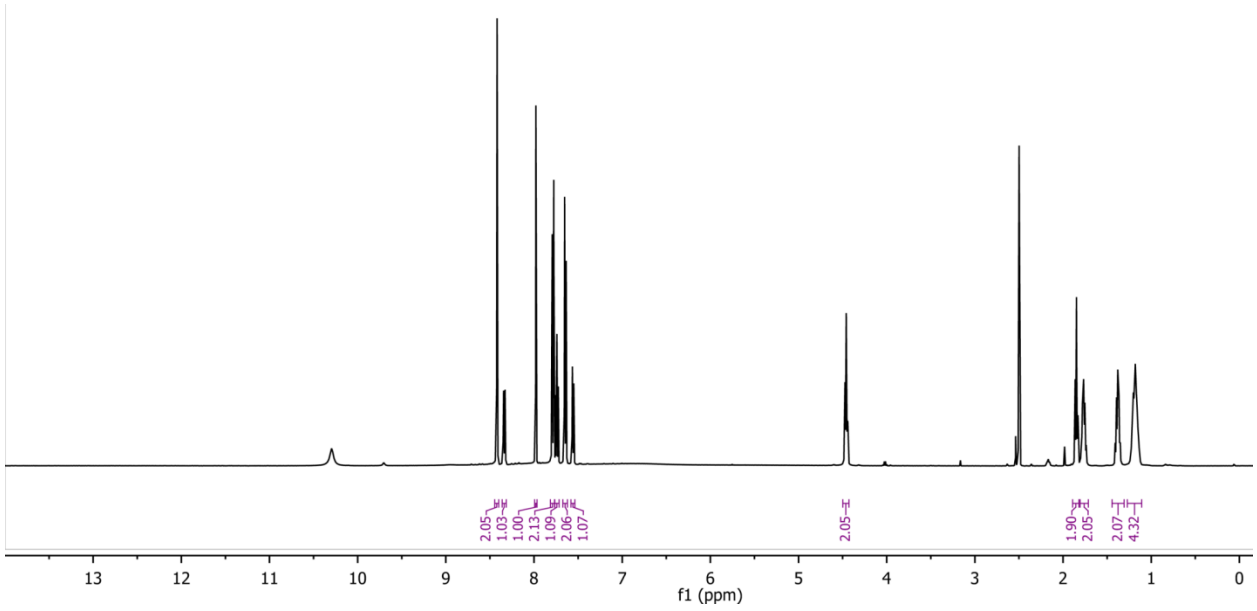
Compound **4** ^{13}C:



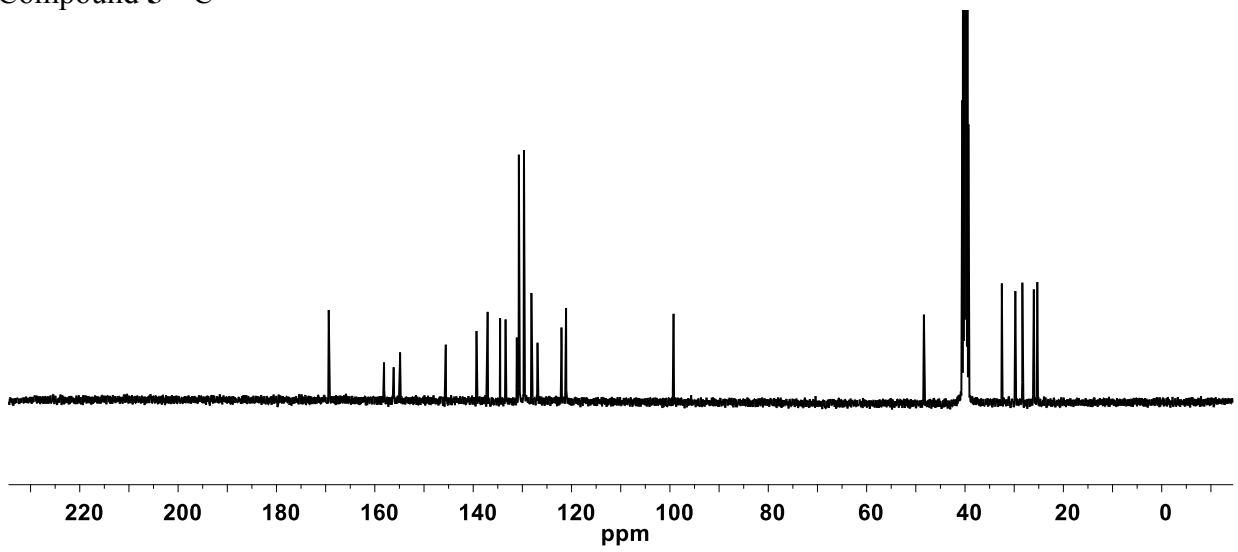


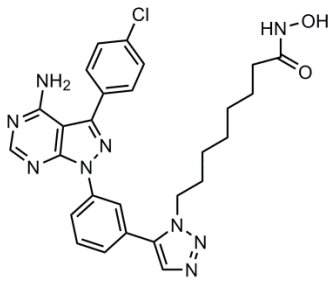
5

Compound **5** ^{1}H:



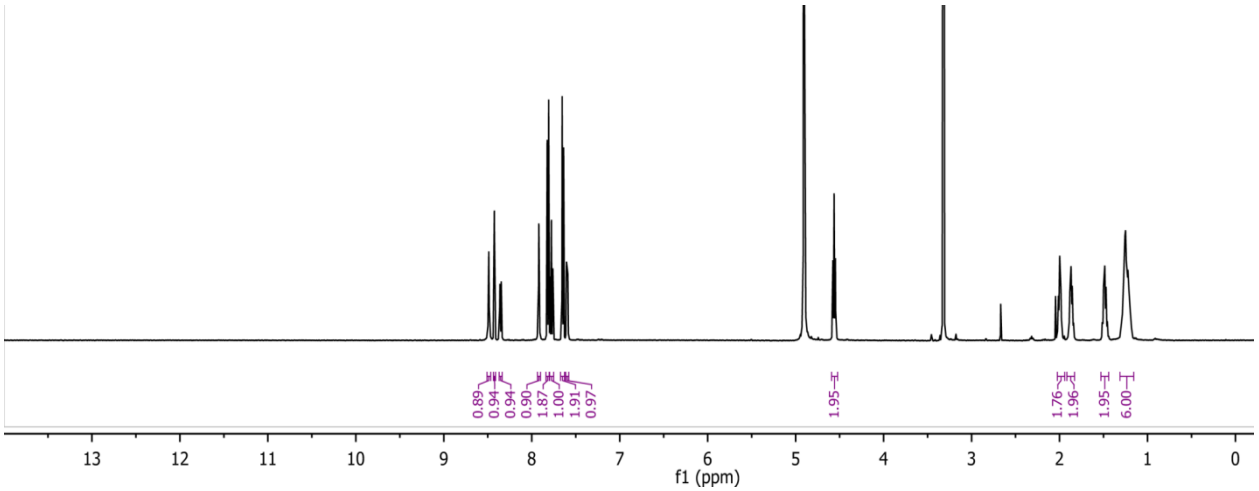
Compound **5** ^{13}C



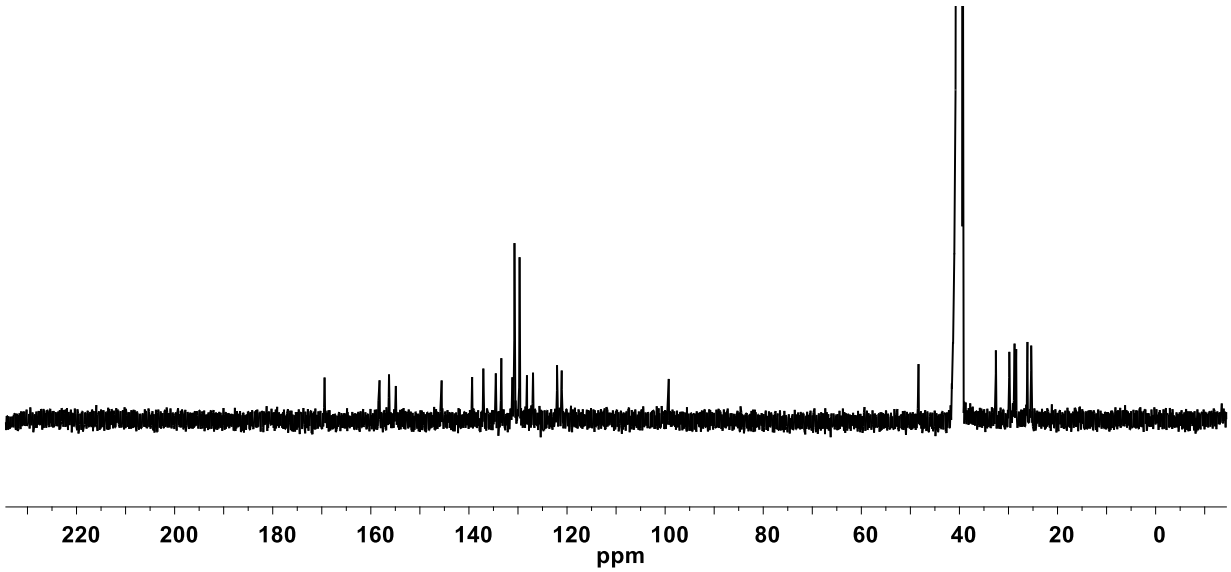


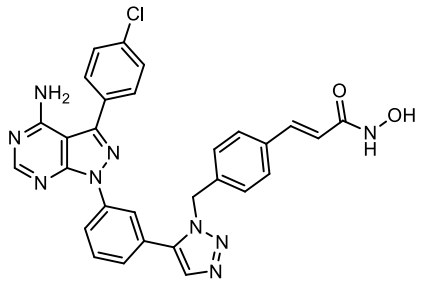
6

Compound **6** ^{1}H:



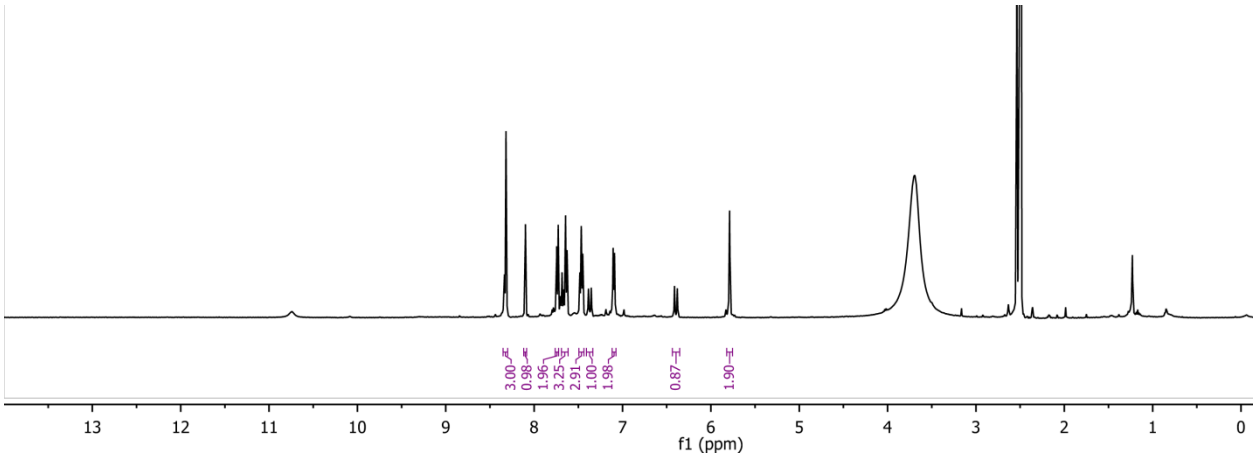
Compound **6** ^{13}C



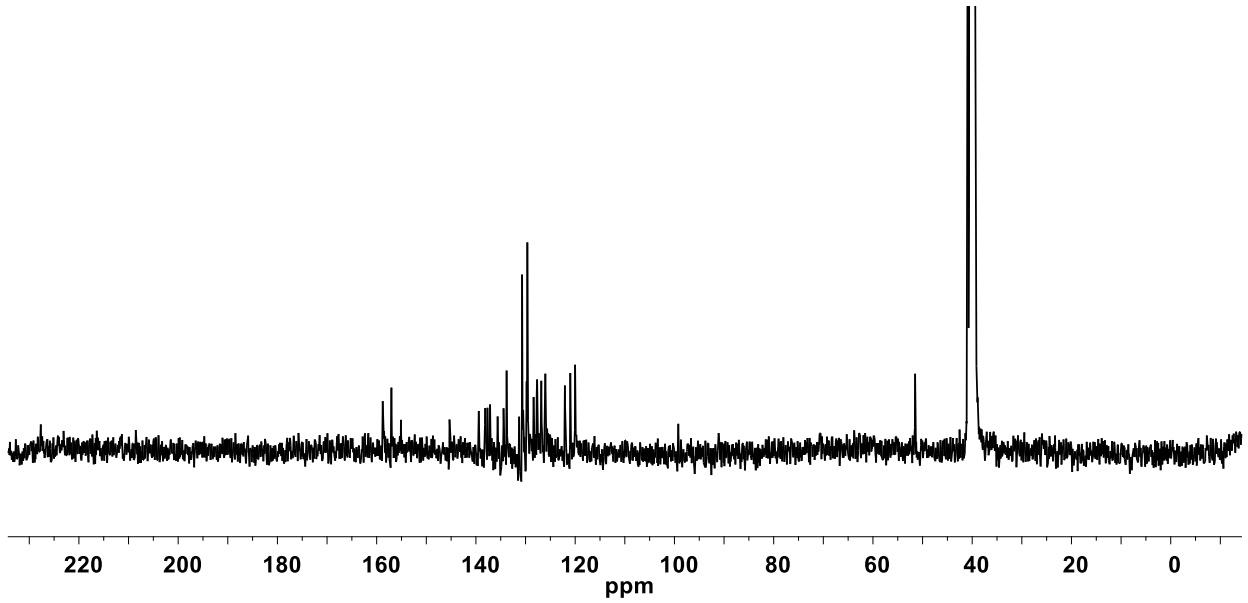


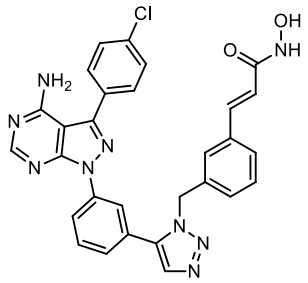
7

Compound 7 ^{1}H:



Compound **7** ^{13}C:





8

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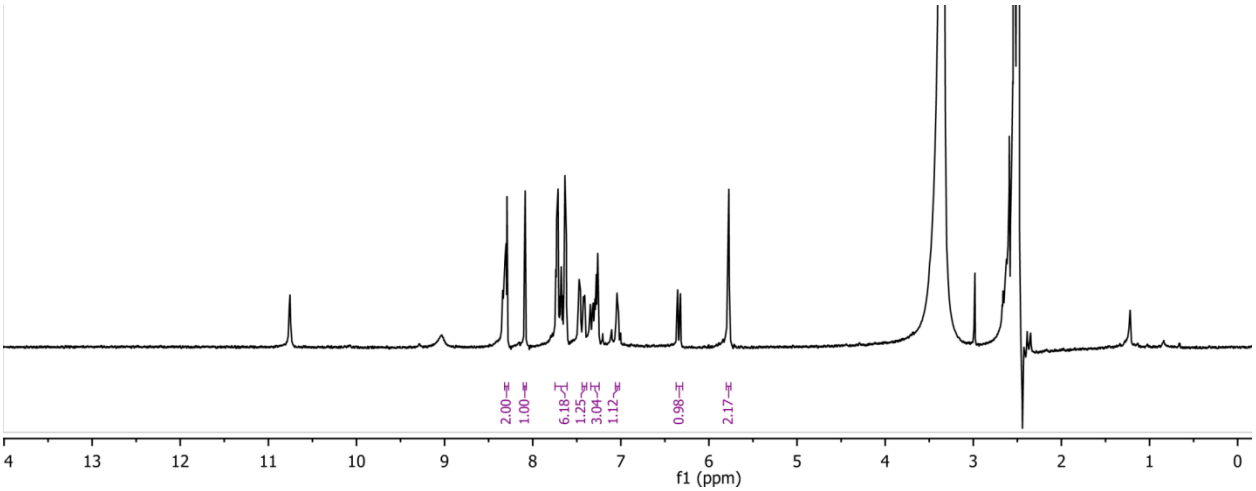

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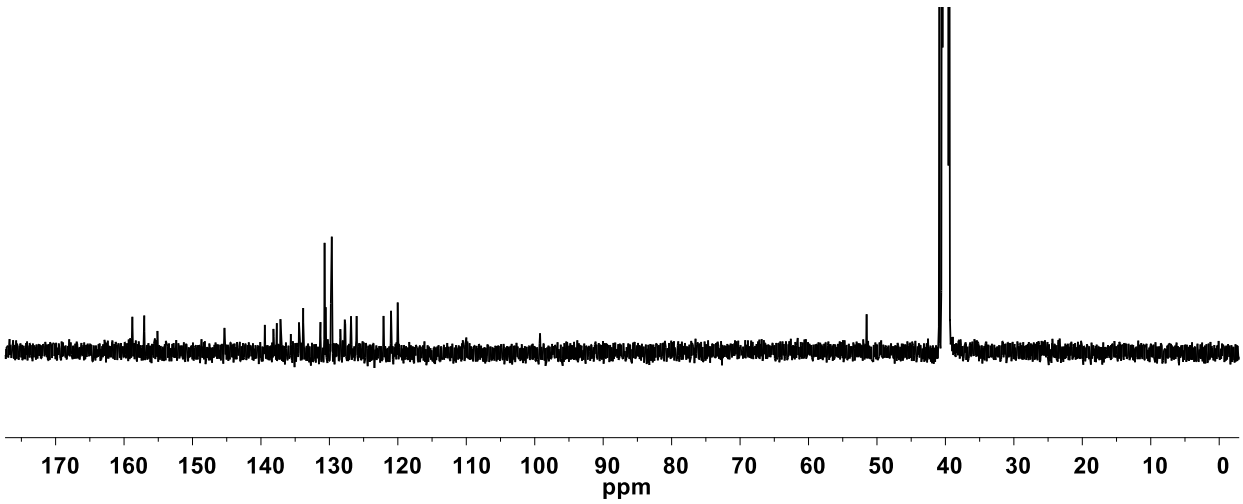
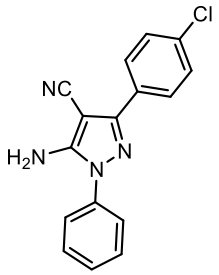
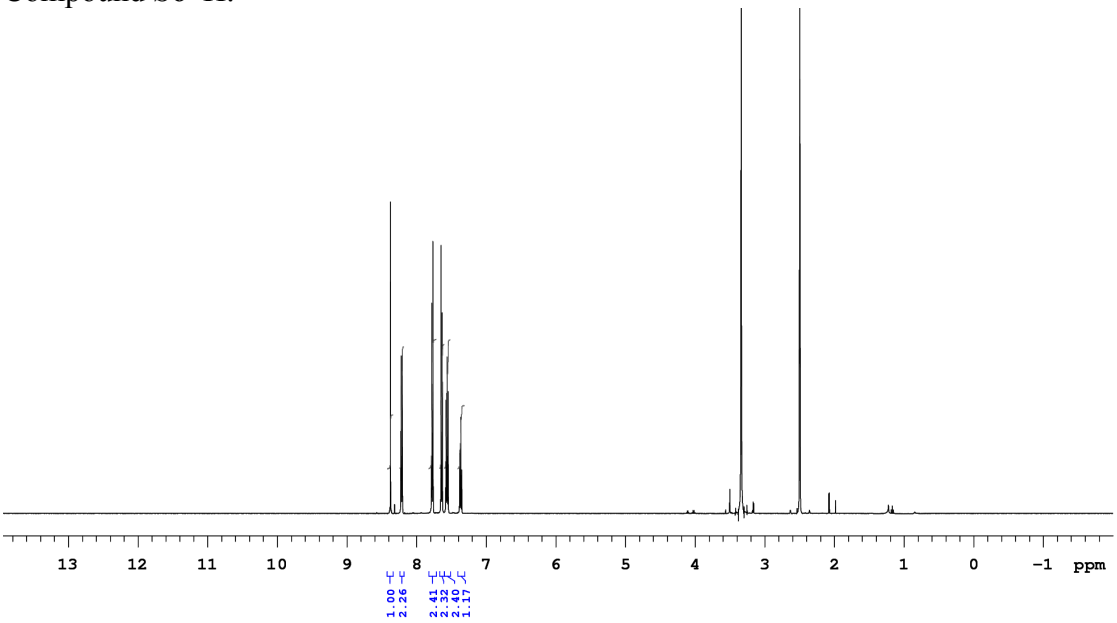


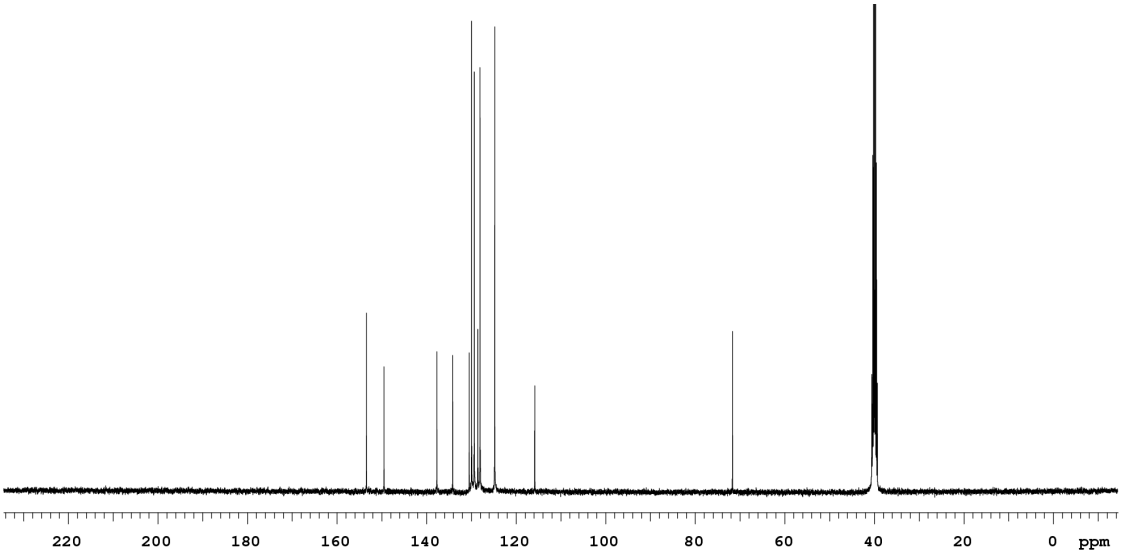


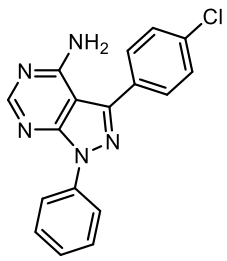
S6

Compound **S6** ^{1}H:



Compound **S6** ^{13}C:





9

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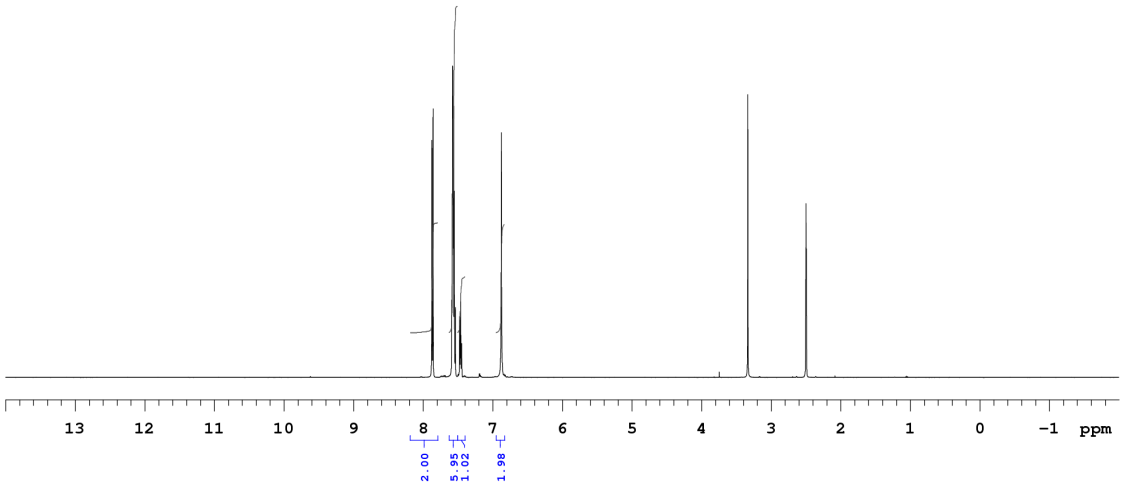
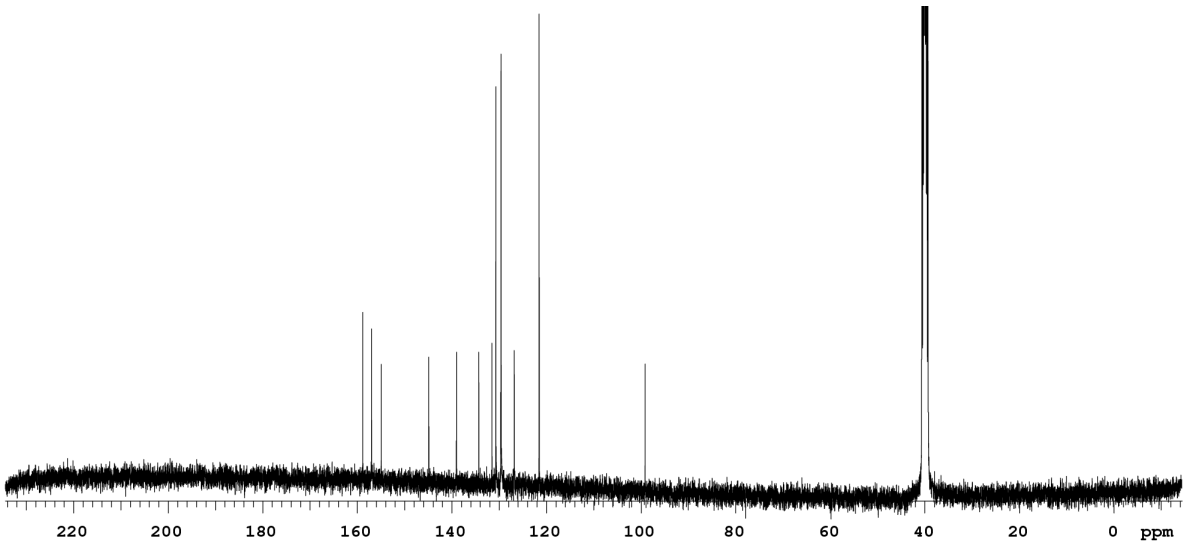
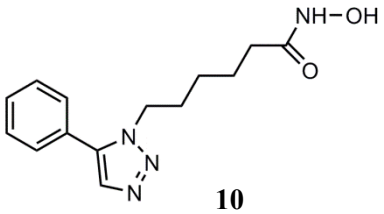


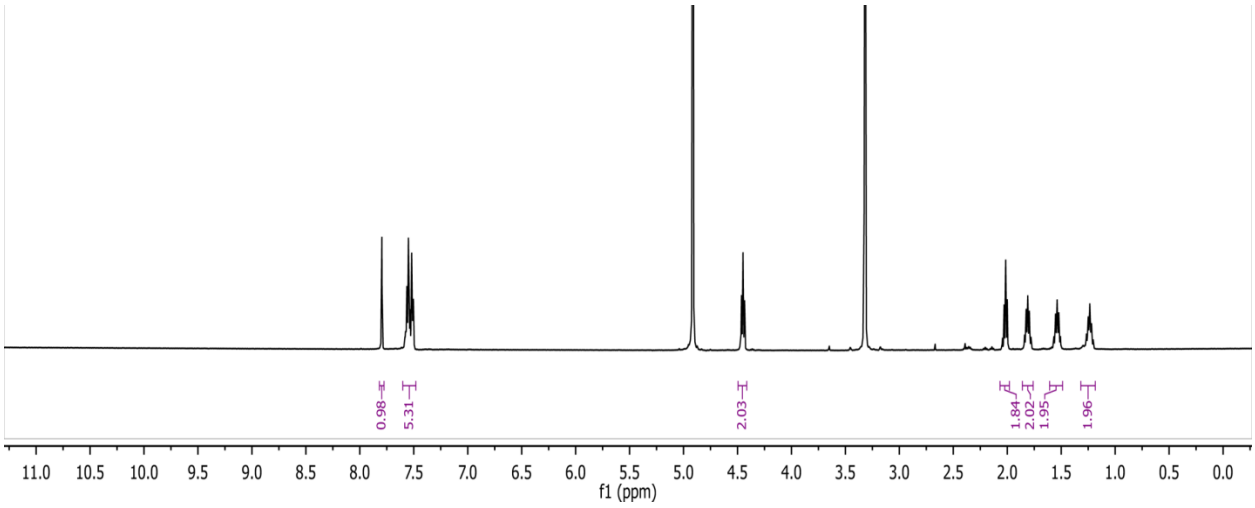
Compound **9** ^{13}C:



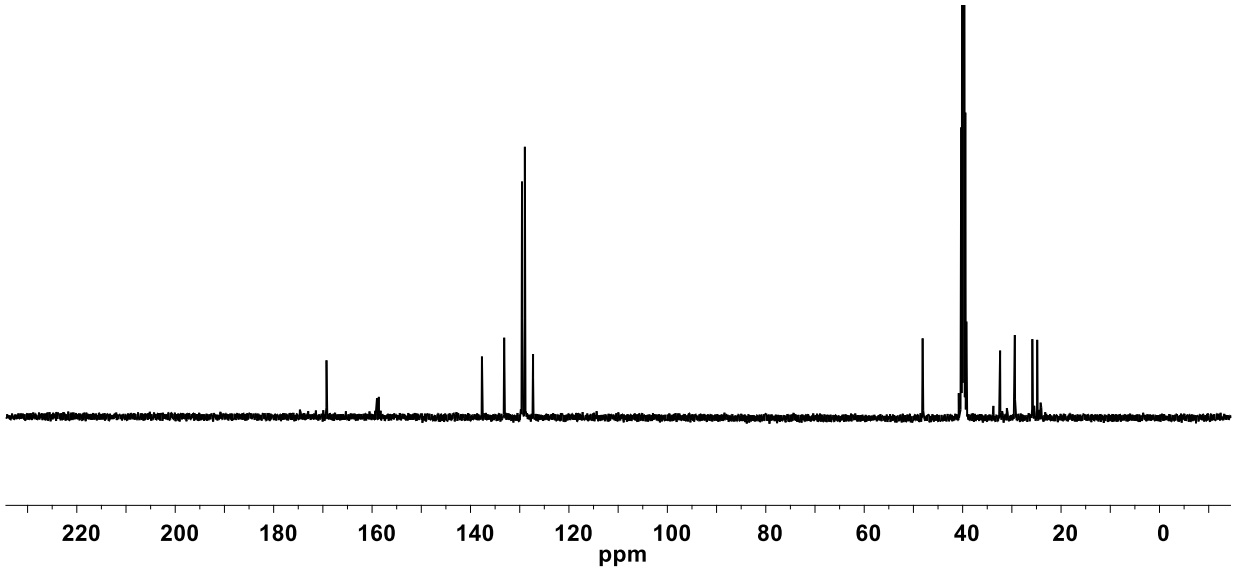


10

Compound **10** ^{1}H:



Compound **10** ^{13}C:



IV. BIOCHEMICAL CHARACTERIZATION

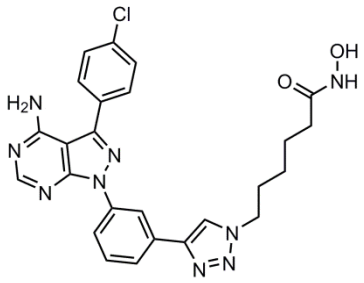
Recombinant, human histone deacteylase 1 (HDAC1) was obtained from Cayman Chemicals (Ann Arbor, MI). Trypsin was purchased from Sigma-Aldrich. Black, opaque-bottom 96 well plates were purchased from Nunc. c-Src, c-Abl, and c-Hck were expressed in *E. coli* using previously published procedures.[9]

General procedure for determination of inhibitor K_i for c-Src: c-Src inhibition assay was performed using a continuous, fluorimetric assay as previously described.[4] Reaction volumes of 100 µL were used in 96-well plates. To each well was added 85 µL of buffer + enzyme. 2.5 µL of varying concentrations of inhibitor was then added (typically 10000, 2500, 625, 156, 39, 10, 2.4, 0.61, 0.15, 0 µM in DMSO). 2.5 µL of peptide substrate ("compound 3" as described in Wang et. al.)[4] solution (1.8 mM in DMSO) was added. 10 µL of ATP (1 mM in water) was added to initiate the reaction and was immediately monitored at 405 nm (ex. 340 nm) for 10 minutes. Final concentrations in the reaction are 30 nM enzyme, 45 µM peptide substrate, 100 µM ATP, 100 µM Na_3VO_4, 100 mM Tris buffer (pH 8), 10 mM $MgCl_2$, 0.01% Triton X-100. The initial rate of the reaction was used to determine K_i values. For K_i determination, the kinetic values were obtained directly from nonlinear regression of substrate-velocity curves in the presence of various concentrations of the inhibitor. The equation Y = Bottom + (Top – Bottom)/1 + 10^{x} – $LogIC_{50}$), X = log(concentration) and Y = % activity; was used in the nonlinear regression.

General procedure for determination of inhibitor K_i for HDAC1: HDAC1 assay was performed in a fluorescence assay in 96-well plates with a reaction volume of 100 µL as was previously described.[5] To each well was added buffer (75 µL), trypsin (10 µL), and HDAC1 enzyme (10 µL). 2.5 µL of varying concentrations of inhibitor was then added (typically 781, 195, 49, 12, 3, 0.76, 0.19, 0.05, 0.01, 0.003, 0 nM in DMSO). 2.5 µL of peptide substrate (Ac-Leu-Gly-Lys(Ac)AMCA) solution (2 mM in DMSO) was added to initiate the reaction and was monitored at 370 nm (ex. 455 nm) for 30 min. after a 30 min. lag phase. Final concentrations in the reaction are 400 pM HDAC 1, 1 mM trypsin, 50 mM peptide substrate (K_M = 39.5. µM), 15 mM Tris buffer (pH 8.1), 250 mM EDTA, 250 mM NaCl, 10% glycerol, and 0.01% Triton X-100. The initial rate of the reaction was used to determine K_i values. For K_i determination, the kinetic values were obtained directly from nonlinear regression of substrate-velocity curves in the presence of various concentrations of the inhibitor. The equation Y = Bottom + (Top – Bottom)/1 + 10^{x} – $LogIC_{50}$), X = log(concentration) and Y = % activity; was used in the nonlinear regression.

Analytical data for K_i determination. Each inhibitor K_i value was determined using at least 3 independent measurements. An example curve is provided for each inhibitor along with average K_i ± standard deviation.

c-Src Assay Data:



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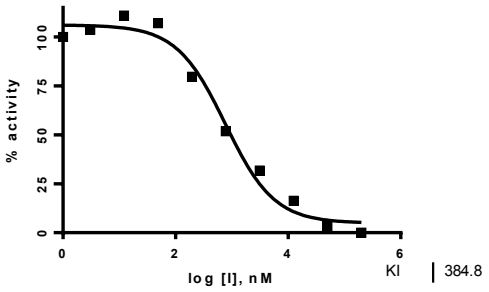
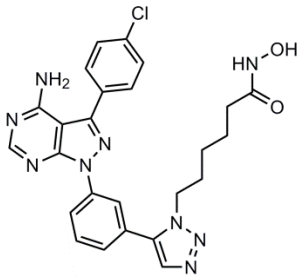


Avg K_i = 371 ± 12.0 nM



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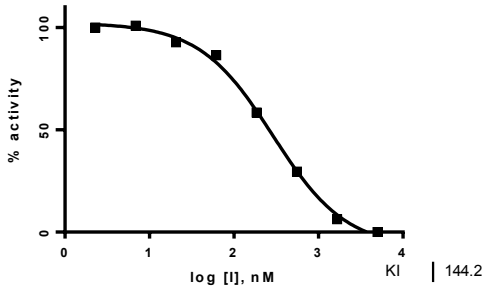
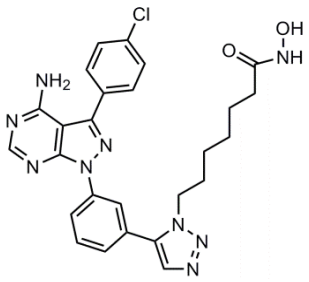


Avg K_i = 138 ± 14.6 nM



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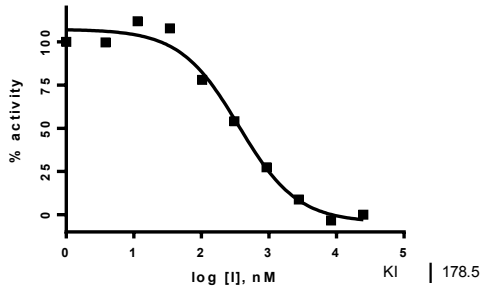
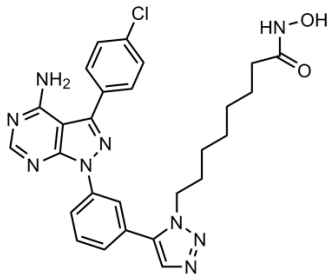


Avg K_i = 190 ± 23.0 nM



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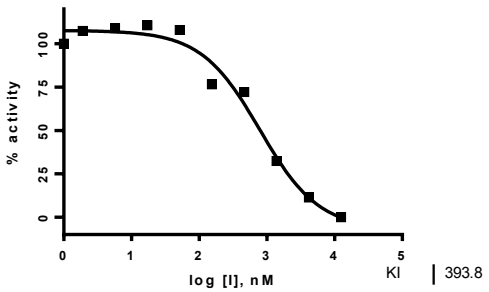
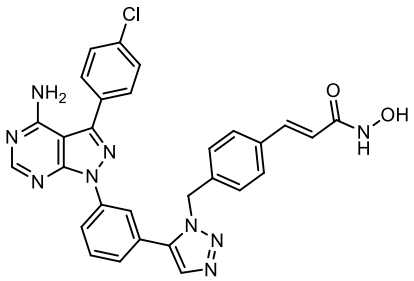


Avg K_i = 407 ± 46.0 nM



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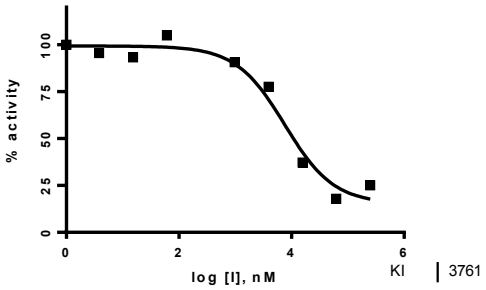
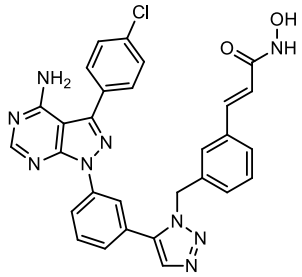


Avg K_i = 4305 ± 444 nM



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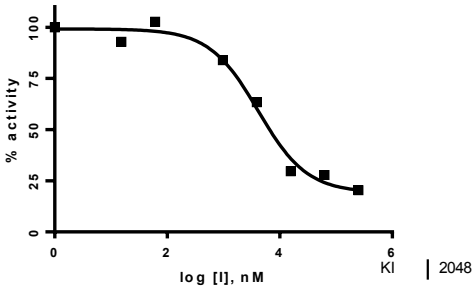
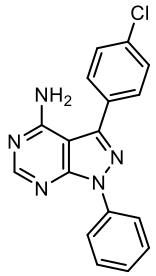


Avg K_i = 2136 ± 393 nM



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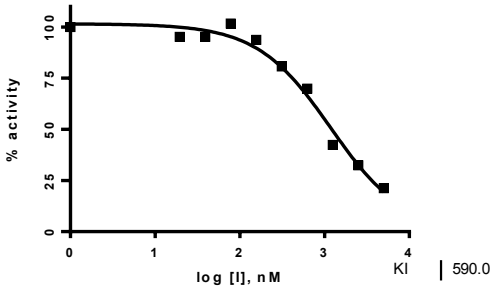
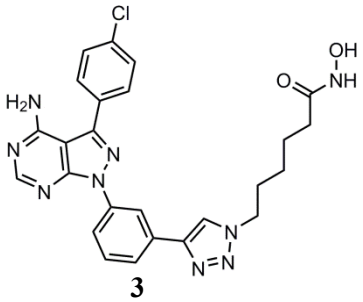


Avg K_i = 605 ± 90 nM

HDAC1 Assay Data:



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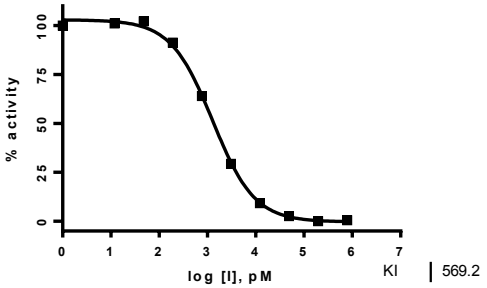
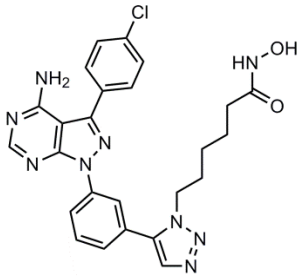


Avg K_i = 621 ± 46 pM



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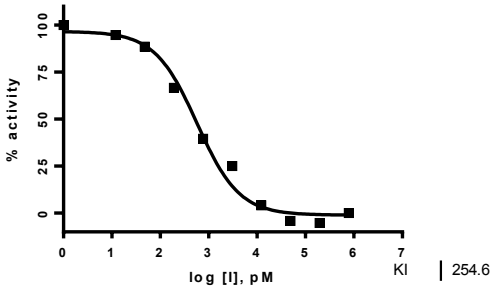
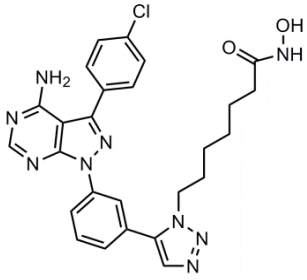
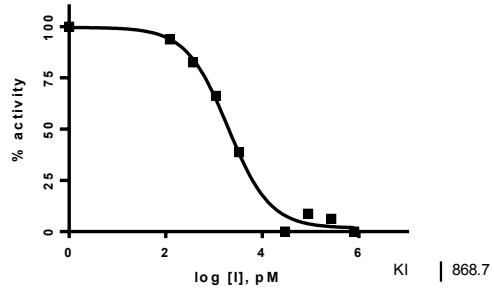


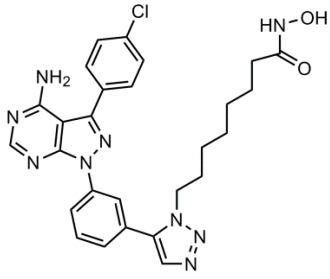
Avg K_i = 256 ± 22 pM



5



Avg K_i = 820 ± 285 pM



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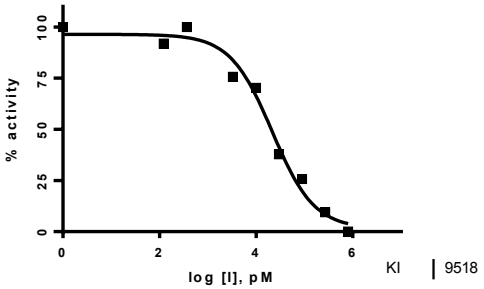
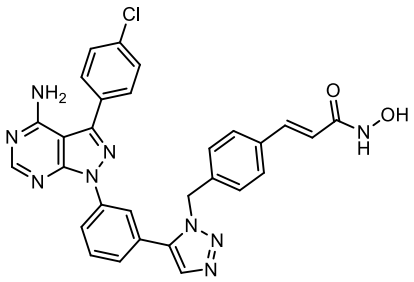


Avg K_i = 9800 ± 804 pM



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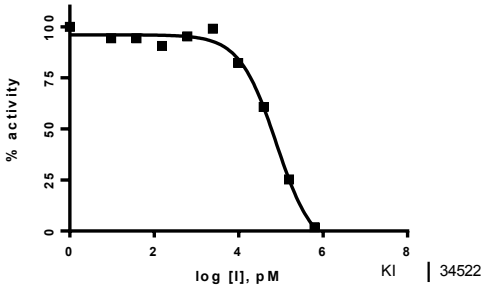
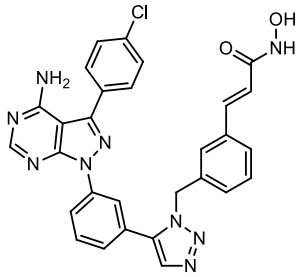


Avg K_i = 35 ± 0.6 nM



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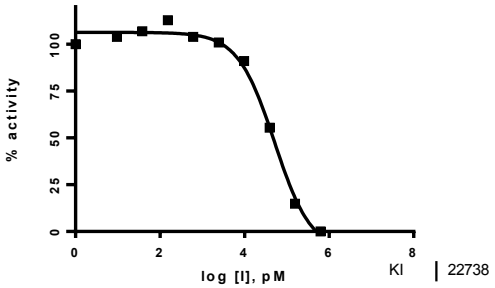
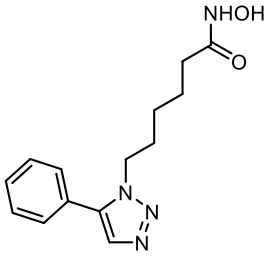


Avg K_i = 23 ± 1.5 nM



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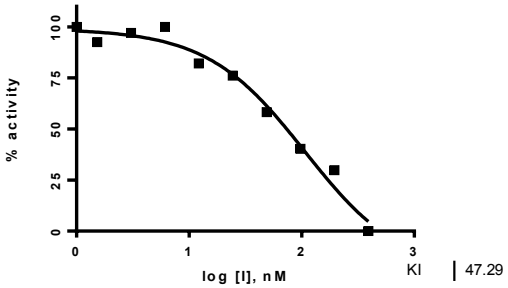
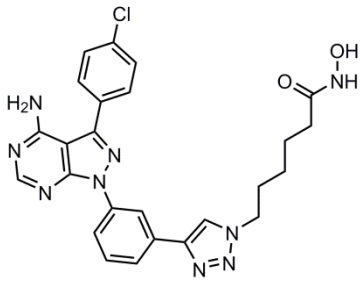


Avg K_i = 45 ± 1.6 nM

c-Abl Assay Data:



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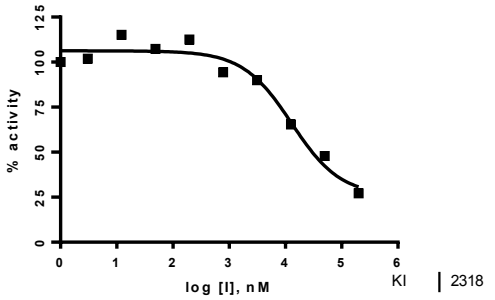
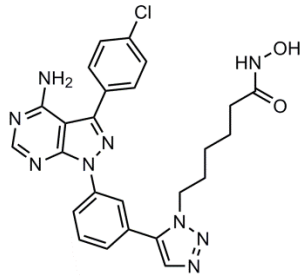


Avg K_i = 2929 ± 1190 nM



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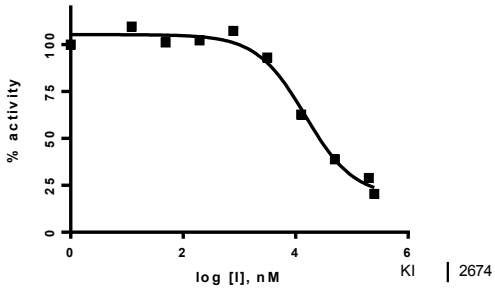
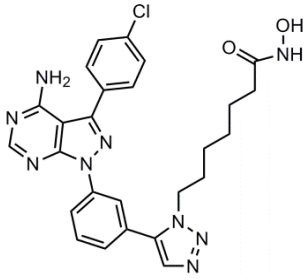


Avg K_i = 2347 ± 249 nM



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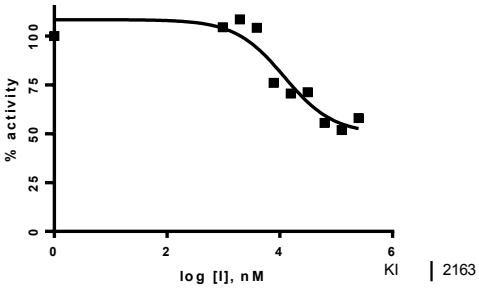
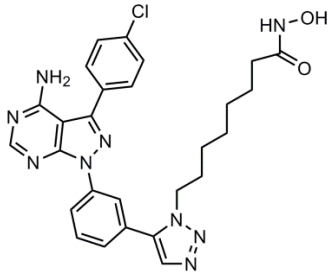


Avg K_i = 1866 ± 147 nM



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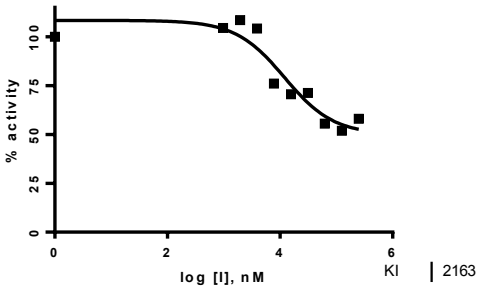
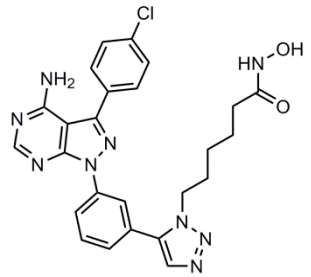


Avg K_i = 1659 ± 336 nM

c-Hck Assay Data:



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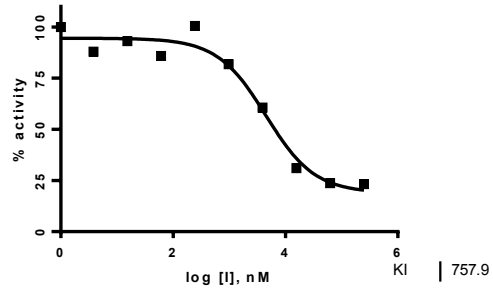
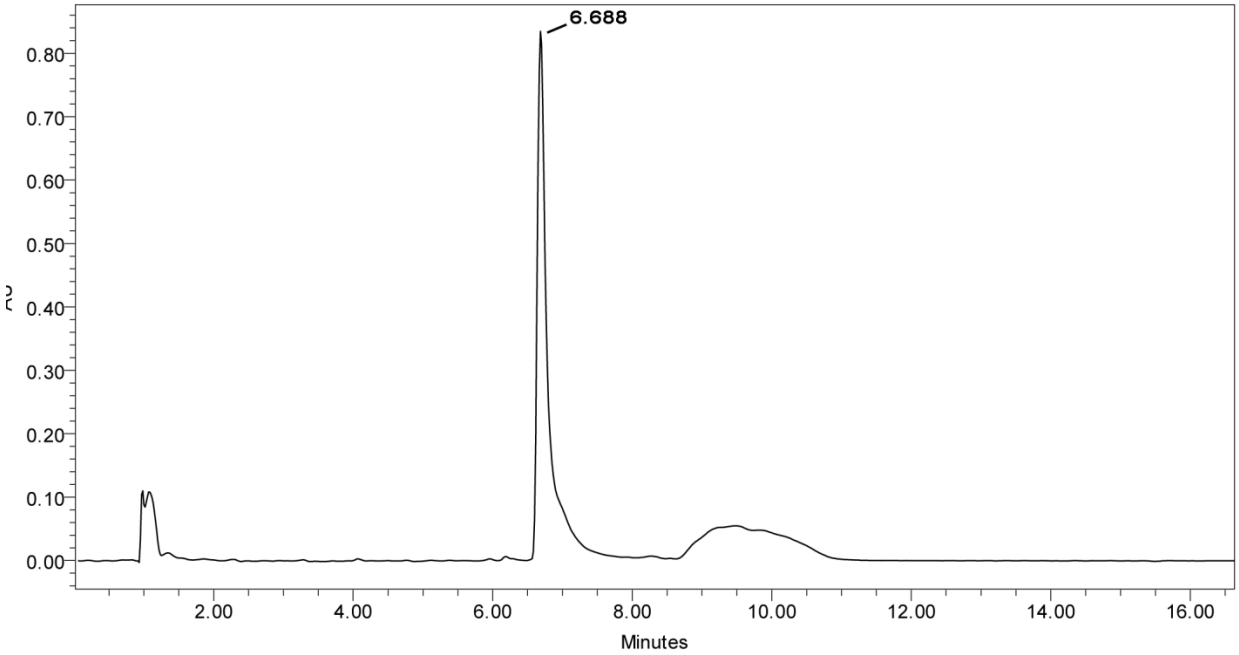


1Avg K_i = 504 ± 172 nM

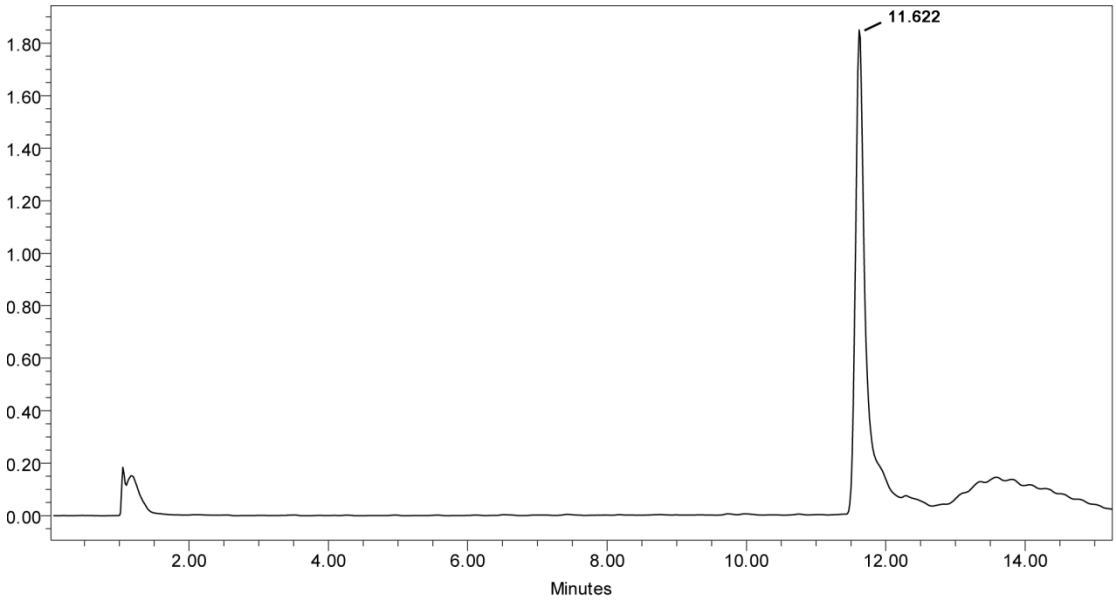
V. Analytical HPLC trace for Compounds 3-10

Compounds were dissolved in 100% DMSO to 1 mM final concentration. An aliquot (10 μL) was injected into a Waters© Xbridge C18 column (2.1 x 100 mm) and eluted using both a linear gradient of CH_3CN (5-95%) in H_2O over 15 min or CH_3OH (5-95%) in H_2O over 15 min at a flow rate of 0.5 mL/min and monitored at 254 nm unless otherwise stated. The peak eluted before 2 min. is the DMSO injection peak.

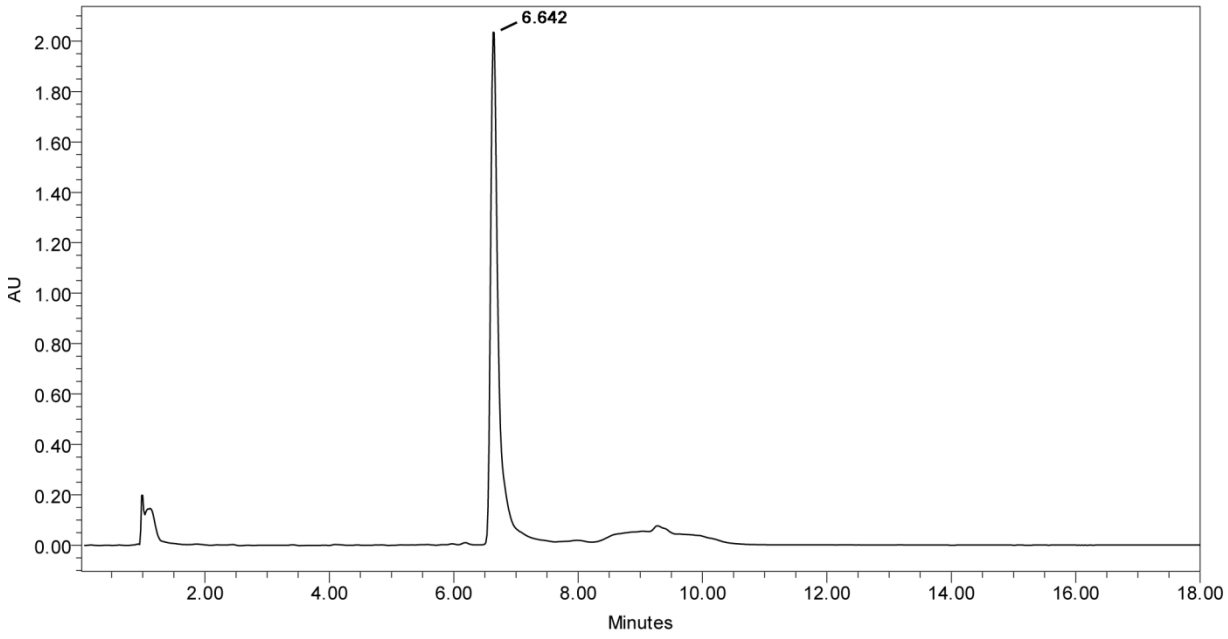
Compound **3** 5-95% CH_3CN/H_2O gradient:



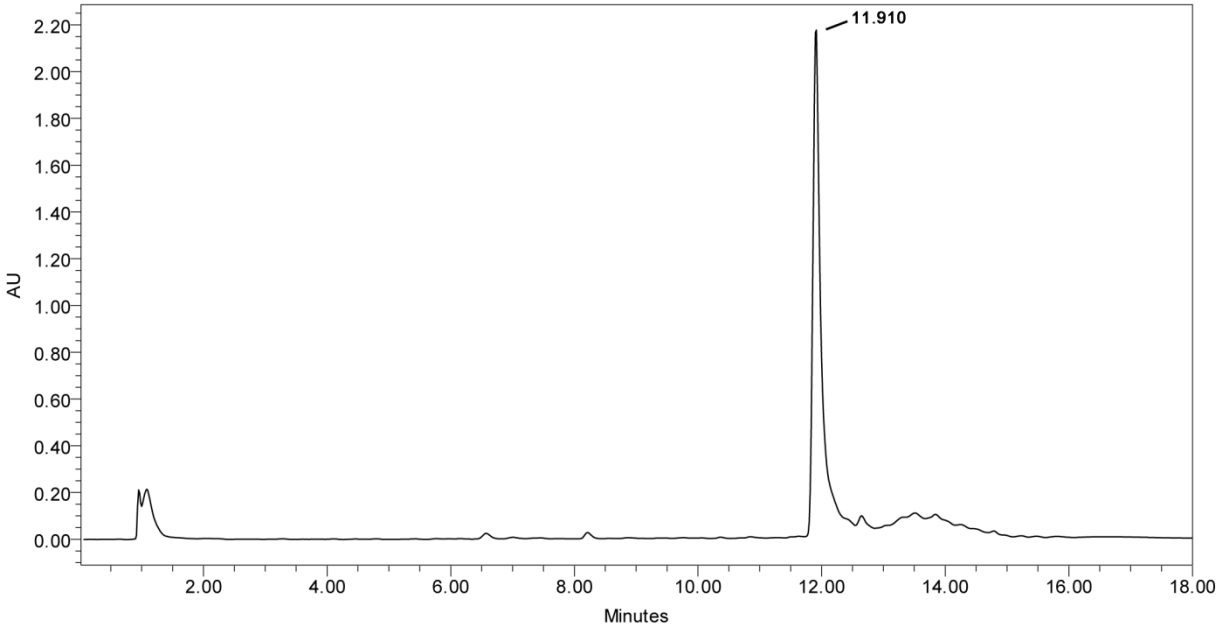
Compound **3** 5-95% CH_3OH/H_2O gradient:



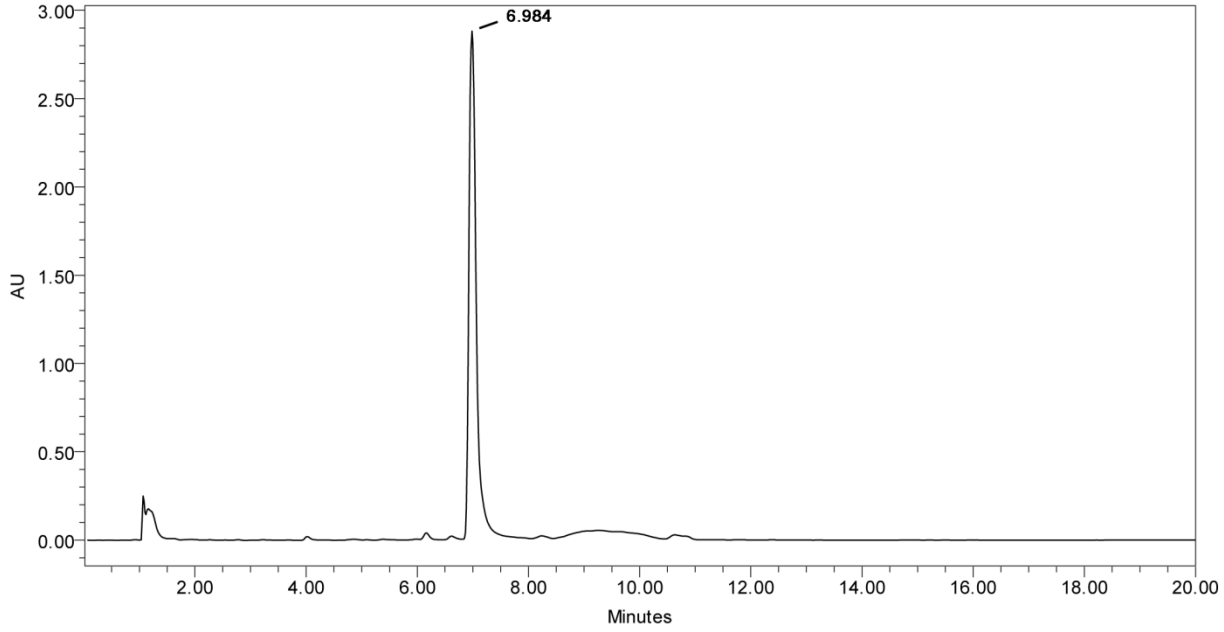
Compound **4** 5-95% CH_3CN/H_2O gradient:



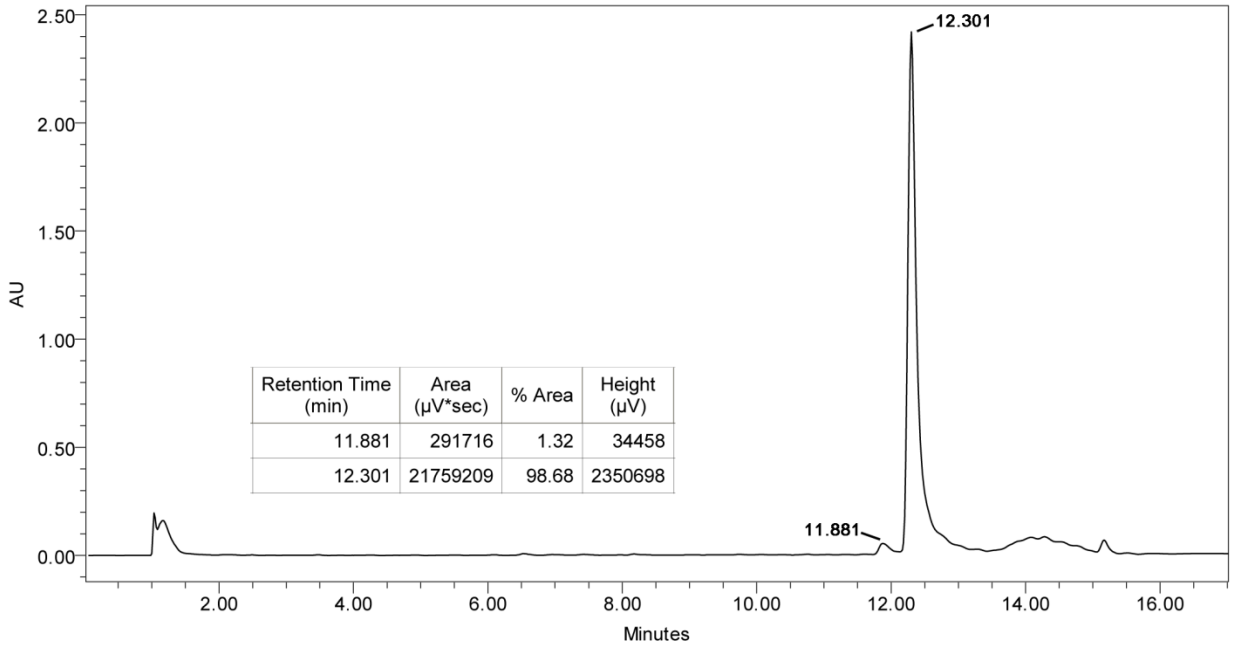
Compound **4** 5-95% CH_3OH/H_2O gradient



Compound **5** 5-95% CH_3CN/H_2O gradient:

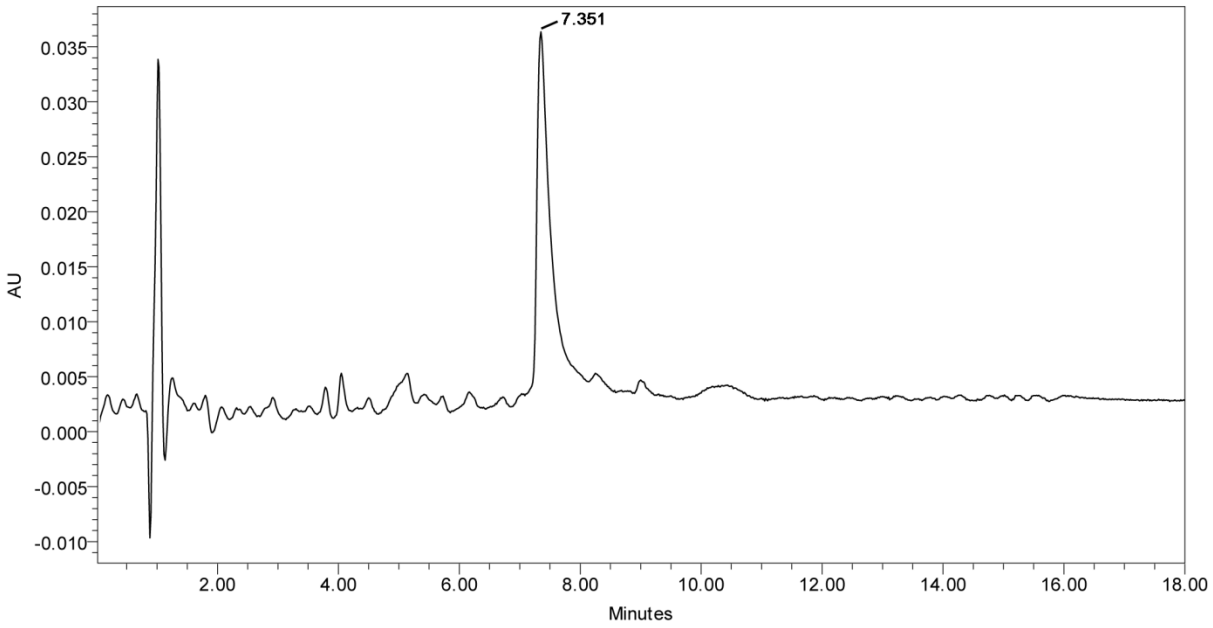


Compound **5** 5-95% CH_3OH/H_2O gradient:

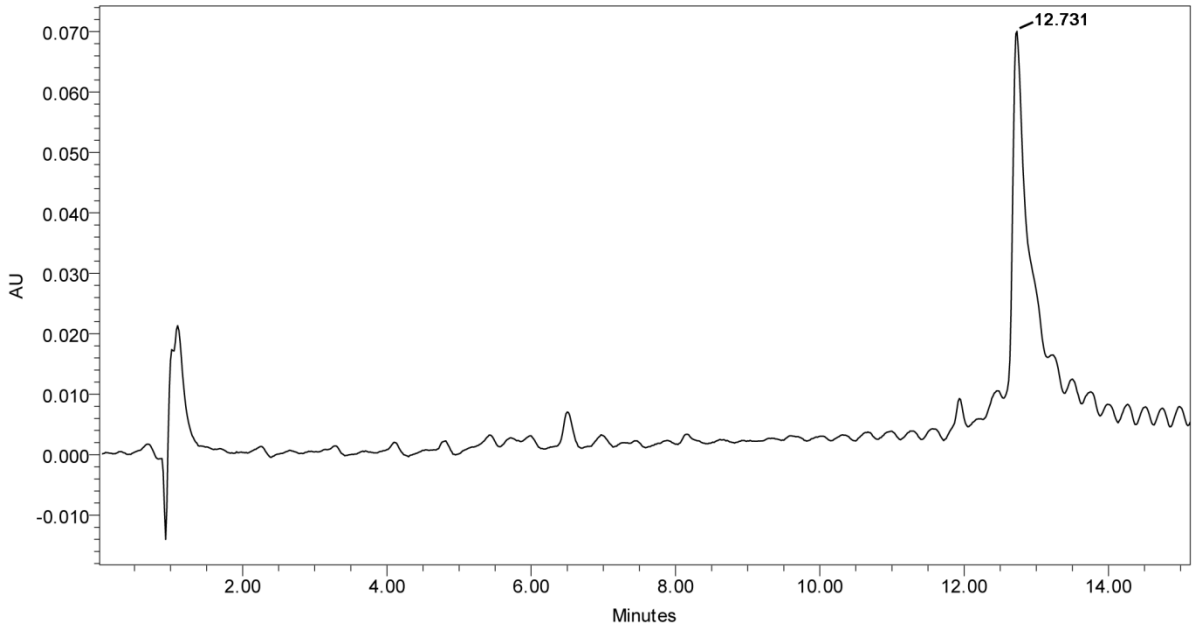


Retention Time (min)	Area (μV*sec)	% Area	Height (μV)
11.881	291716	1.32	34458
12.301	21759209	98.68	2350698

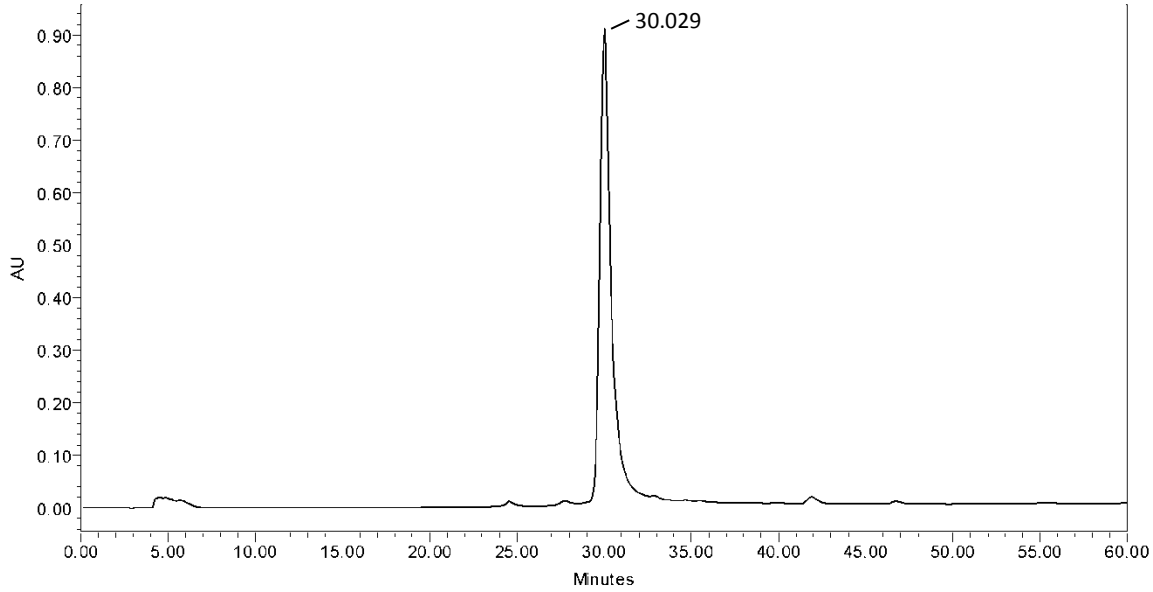
Compound **6** 5-95% CH_3CN/H_2O gradient:



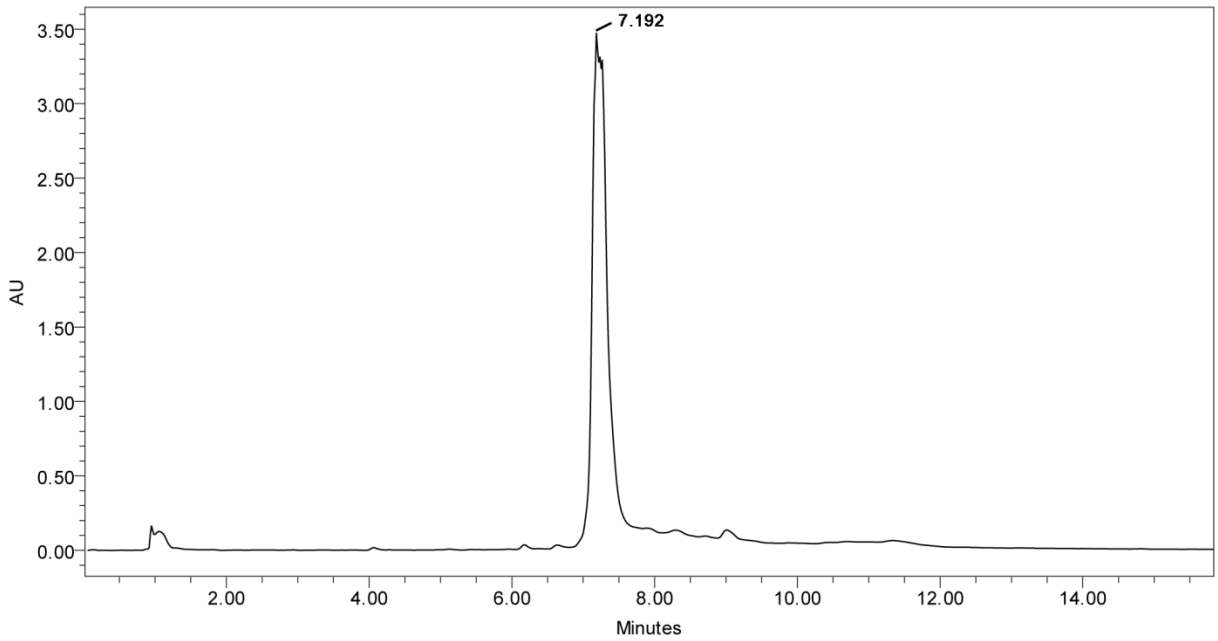
Compound **6** 5-95% CH_3OH/H_2O gradient:



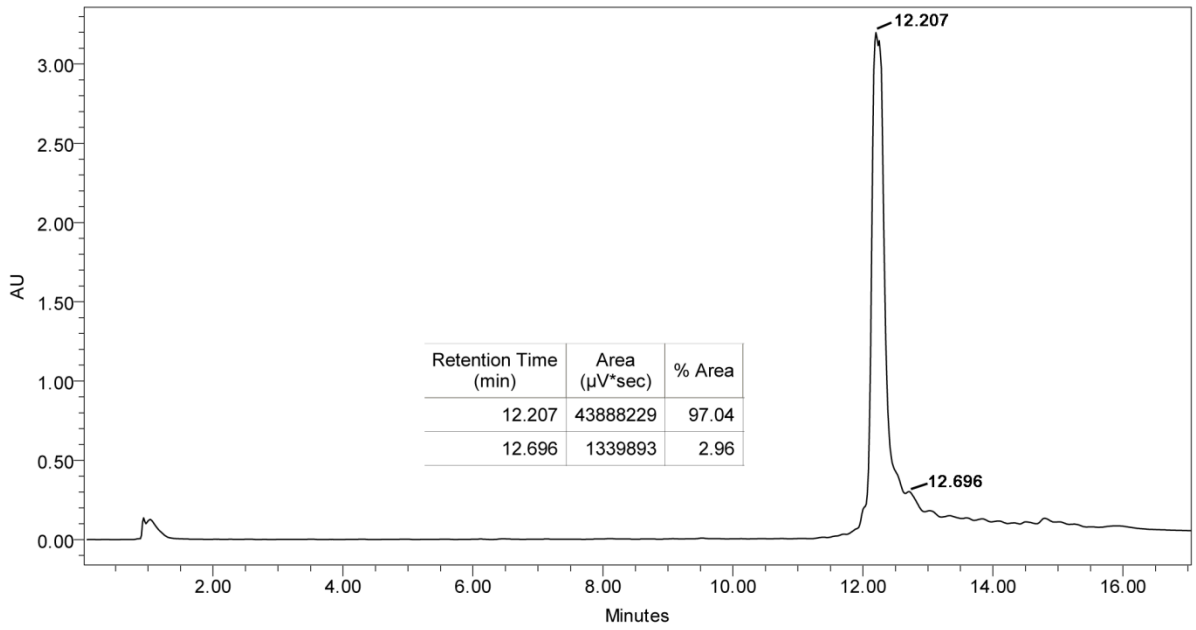
Compound **7** 5-95% CH_3OH/H_2O gradient:



Compound **8** 5-95% CH_3CN/H_2O gradient:

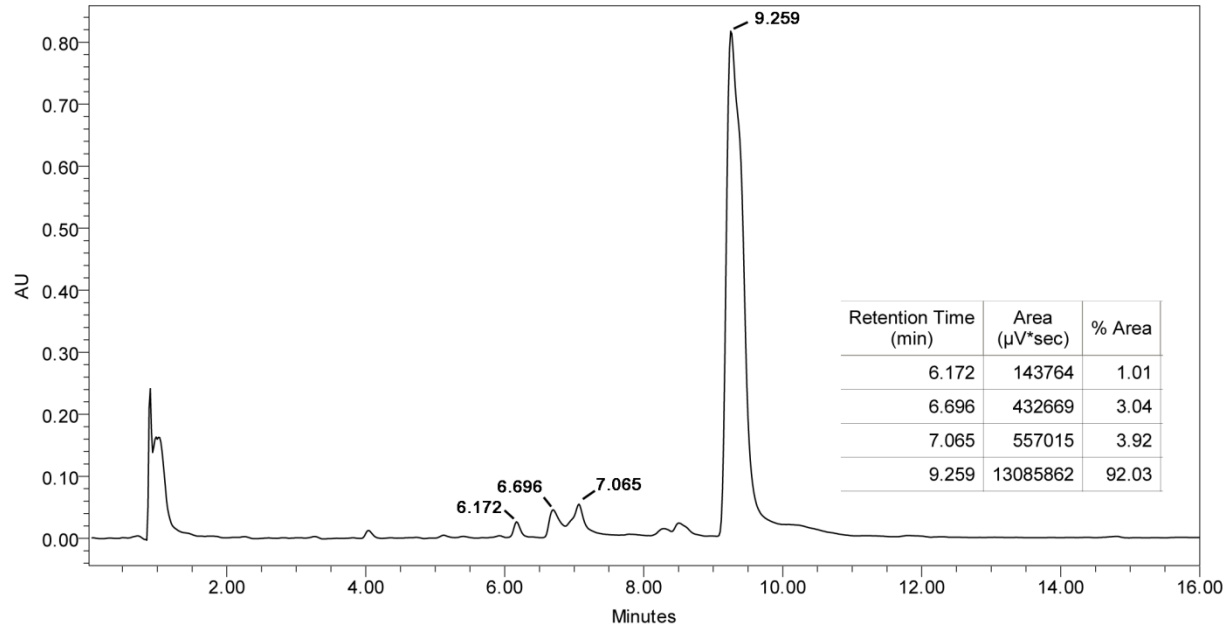


Compound **8** 5-95% CH_3OH/H_2O gradient:



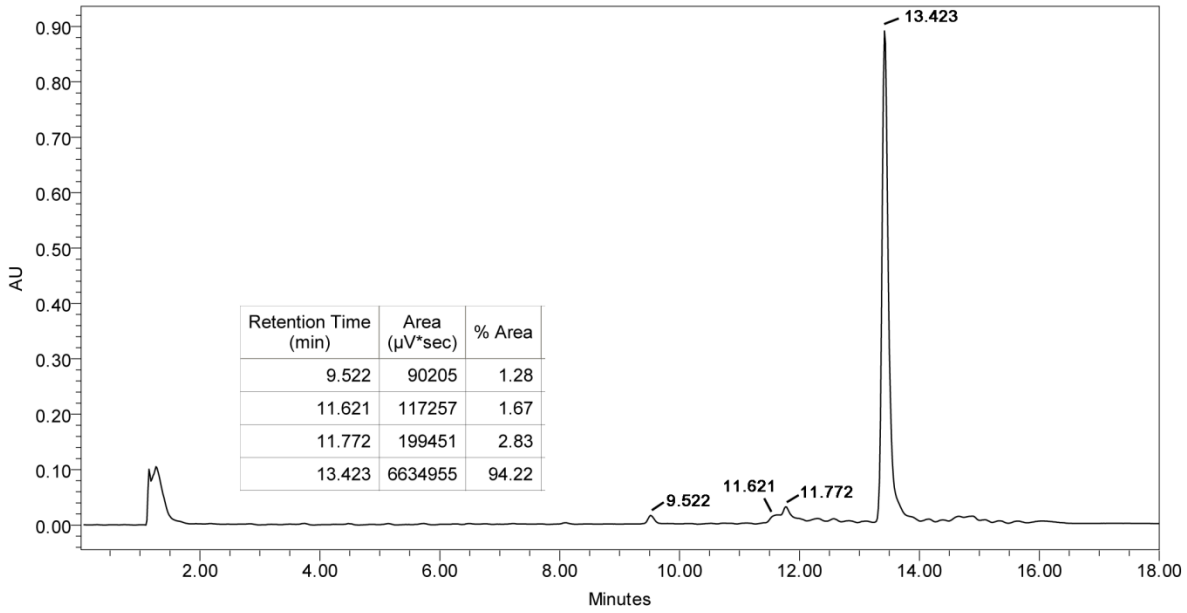
Retention Time (min)	Area (μV*sec)	% Area
12.207	43888229	97.04
12.696	1339893	2.96

Compound **9** 5-95% CH_3CN/H_2O gradient:



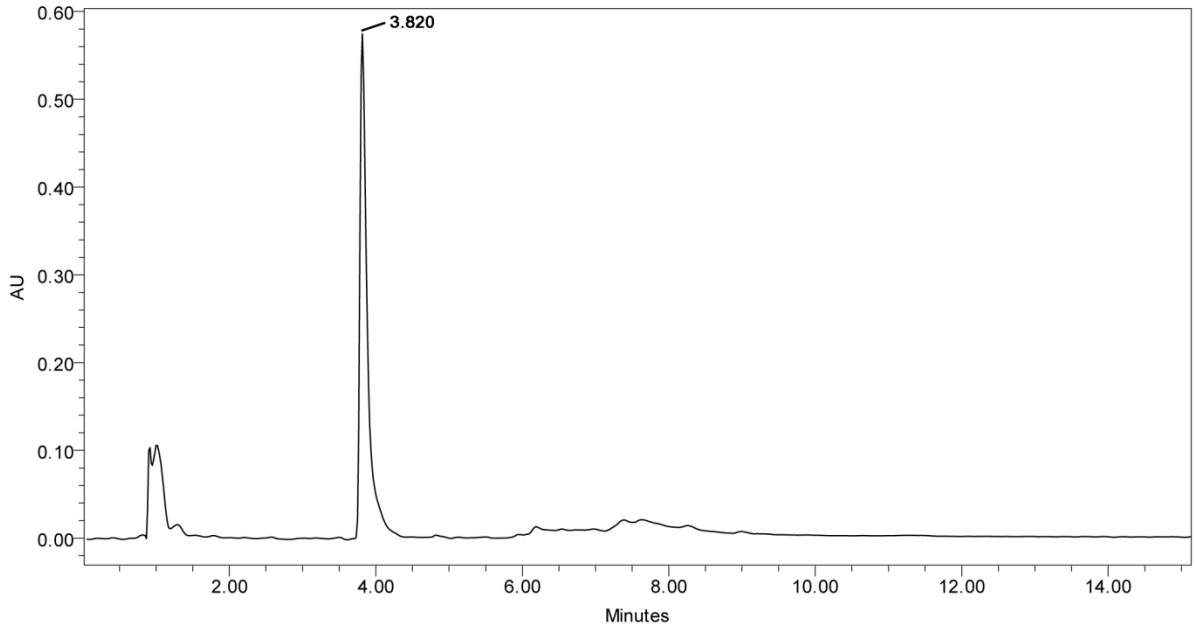
Retention Time (min)	Area (μV*sec)	% Area
6.172	143764	1.01
6.696	432669	3.04
7.065	557015	3.92
9.259	13085862	92.03

Compound **9** 5-95% CH_3OH/H_2O gradient:

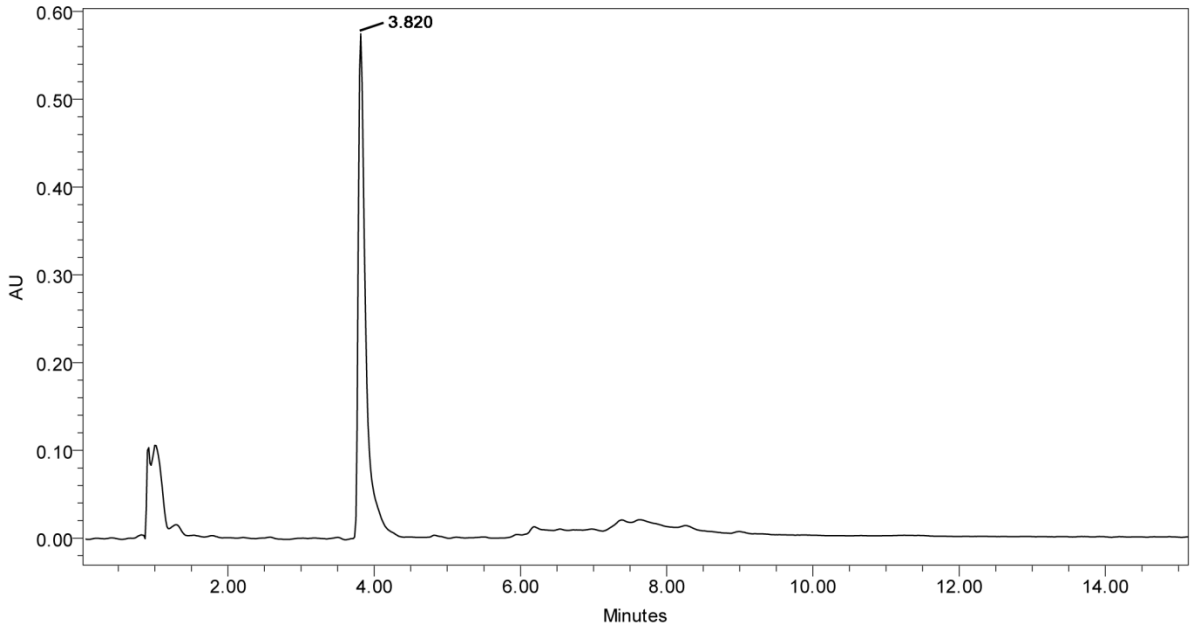


Retention Time (min)	Area (μV*sec)	% Area
9.522	90205	1.28
11.621	117257	1.67
11.772	199451	2.83
13.423	6634955	94.22

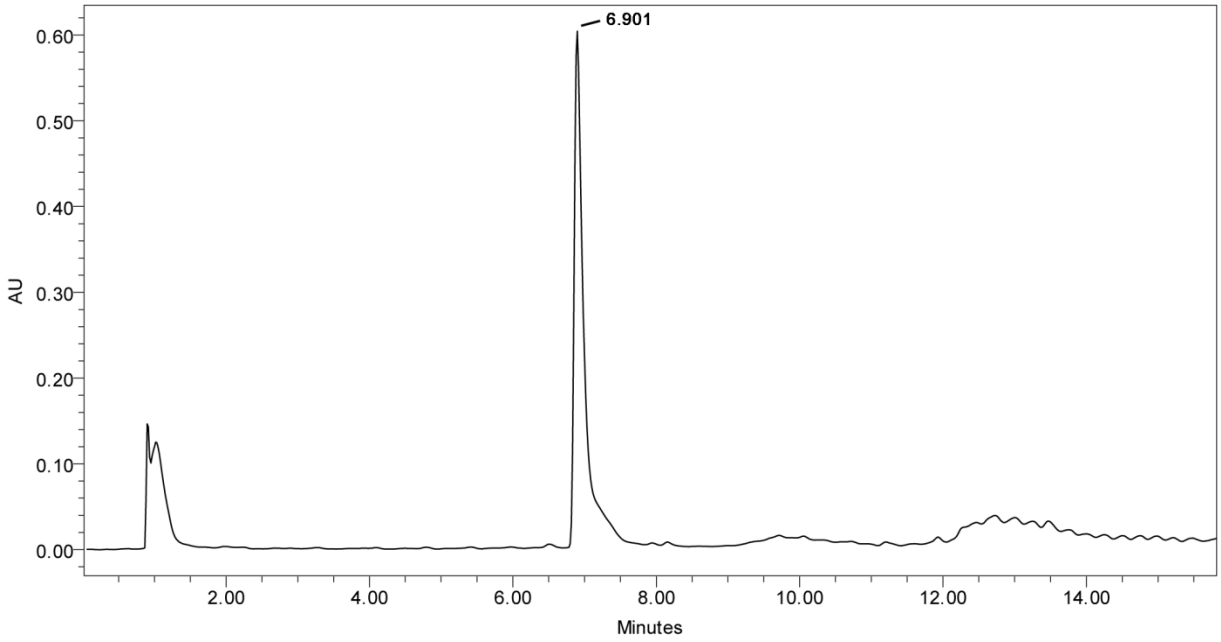
Compound **10** 5-95% CH_3CN/H_2O gradient:



Compound **10** 5-95% CH_3CN/H_2O gradient:



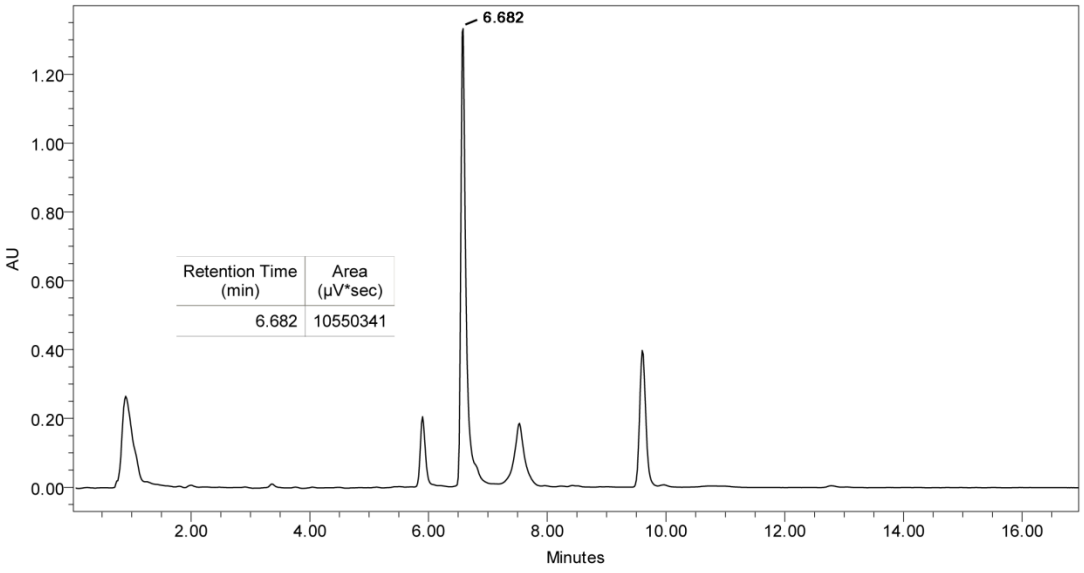
Compound **10** 5-95% CH_3OH/H_2O gradient:



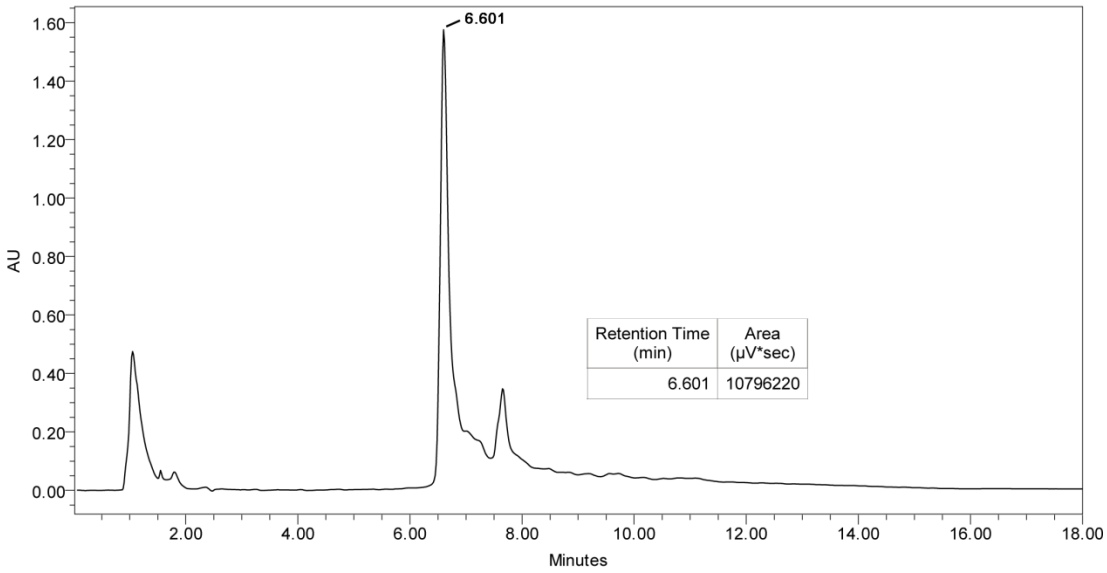
VI. Analytical HPLC trace of compound 4 stability in cell lysate

Compound 4 was incubated with SK-BR-3 cell lysate at 500 μM for 24 hours at 37 ˚C. An aliquot (10 μL) was injected at 0 min and 24 h into a Waters© Xbridge C18 column (2.1 x 100 mm) and eluted using a linear gradient of CH_3CN (5-95%) in H_2O over 15 min. at a flow rate of 0.5 mL/min.

Time = 0 min



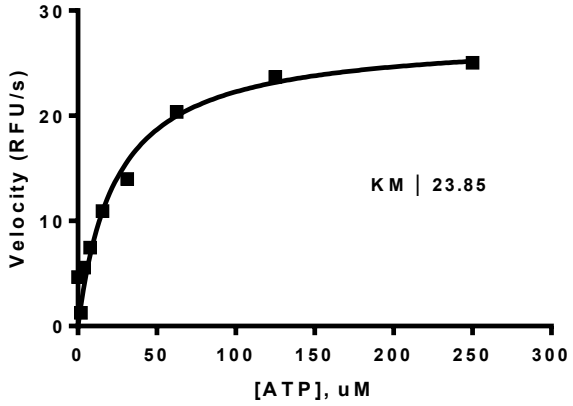
Time = 24 hours



VII. ATP K_M Curves

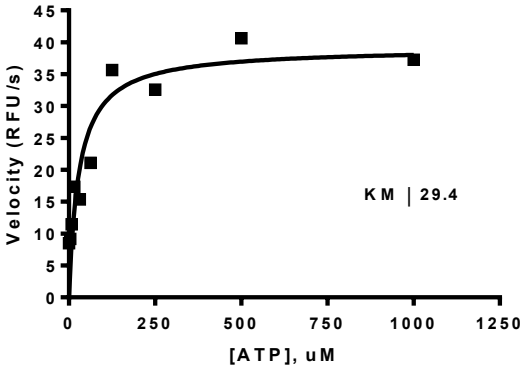
General procedure for ATP K_M determination. The previously described fluorescence assay[4] was used to determine K_M values. Reaction volumes of 100 μL were used in 96-well plates. 85 μL of enzyme in buffer was added to each well. 2.5 μL of DMSO was then added followed by 2.5 μL of a substrate peptide ("compound 3" as described in Wang et al)[4] solution (1.8 mM in DMSO). The reaction was initiated with 10 μL of the appropriate ATP dilution (typically 1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 2.0 μM in H_2O) and reaction progress was immediately monitored at 405 nm (ex. 340 nm) for 10 minutes. Reactions had final concentrations of 30 nM enzyme, 45 μM peptide substrate, 100 μM Na_3VO_4, 100 mM Tris buffer (pH 8), 10 mM MgCl2, 0.01% Triton X-100. The initial rate data collected was used for determination of K_M values. For K_m determination, the kinetic values were obtained directly from nonlinear regression of substrate-velocity curves in the presence of varying concentrations of ATP. The equation Y = (Vmax * X)/(Km + X), X = substrate concentration (μM) and Y = enzyme velocity (RFU/s); was used in the nonlinear regression. Each ATP K_M value was determined using at least three independent experiments. K_M values were used to determine K_i values using the Cheng-Prusoff equation for competitive inhibition.[6] A representative K_M curve is shown. The K_M for c-Src that was used here is 98 μM and was previously determined by our group.[7]

A. ATP K_M Curve with KD c-Hck enzyme:



K_M = 20 ± 1.6 μM
V_{max} = 23 ± 2.9 μM (RFU/s)

B. ATP K_M Curve with KD c-Abl enzyme:

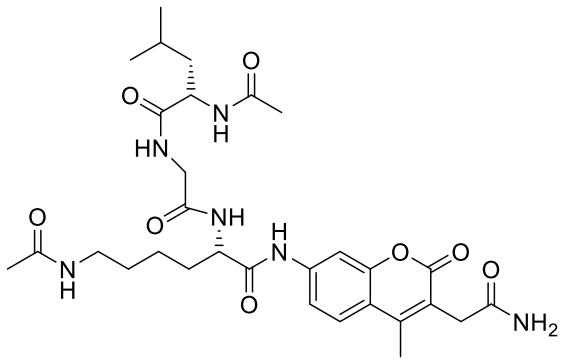


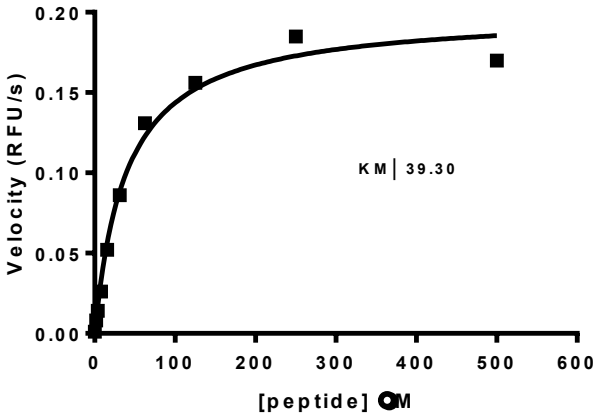
K_M = 22 ± 7.0 μM
V_{max} = 45 ± 4.2 μM (RFU/s)

VIII. K_M Curve for peptide substrate of HDAC1

The K_M value for Ac(Lys) substrated used in our Fluor de Lys-based assay was determined to enable conversion of IC_{50} values to K_i values.

Acetyl-Leu-Gly-Lys(Acetyl)-AMCA



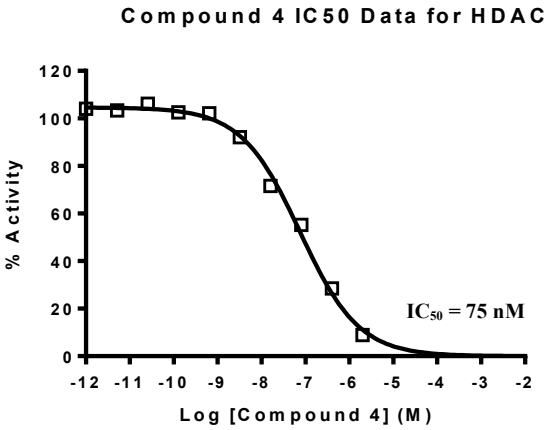


$K_m = 39.6 \pm 0.78$ μM
$V_{max} = 0.2 \pm 0.002$ μM (RFU/s)

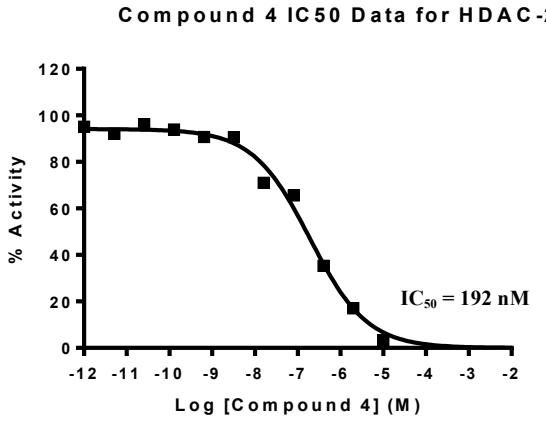
IX. HDAC PROFILING

HDAC profiling was performed by Reaction Biology (Malvern, PA). Compound 4 was tested in 10-dose response format in duplicate with 5-fold serial dilution starting at 10 μM against HDAC-1, -2, -3, -6, -8, and -10 and -11. Compound 4 was tested in 10-dose IC_{50} mode in duplicate with 5-fold serial dilution starting at 100 μM against HDAC-4, -5, -7 and -9. General substrate used for HDAC-1, -2, -3, -6, -10, and -11 is a fluorogenic peptide from p53 residues 379-382 (RHKK(Ac)). Substrate for HDAC-8 used is a fluorogenic peptide from p53 residues 379-382 (RHK(Ac)K(Ac)). Fluorogenic HDAC Class IIa Substrate (Boc-Lys(trifluoroacetyl)-AMC) was used for HDAC-4,-5,-7, and -9.

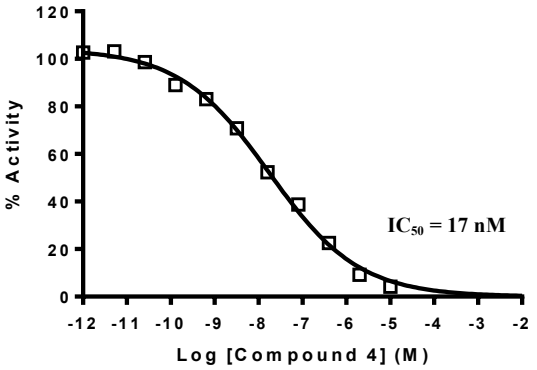
Class I HDAC



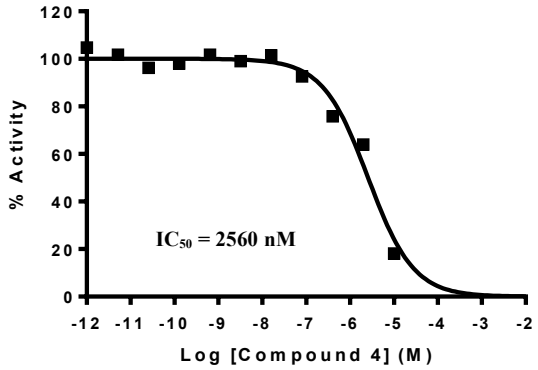
Avg IC_{50} = 86 ± 11.0 nM



Avg IC_{50} = 231 ± 39 nM

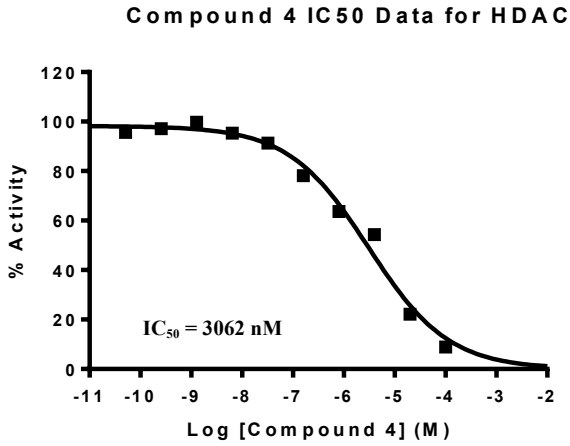


Avg IC_{50} = 19 ± 1.4 nM

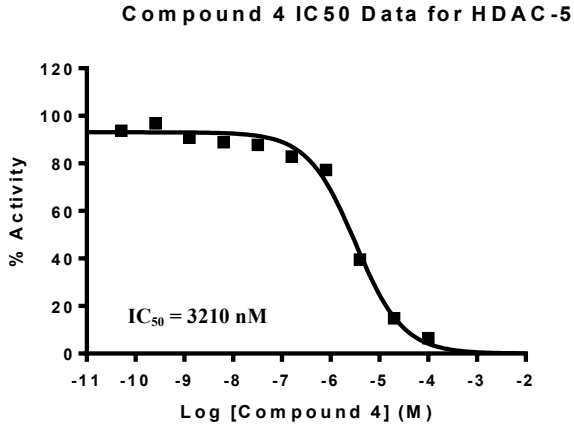


Avg IC_{50} = 2311 ± 251 nM

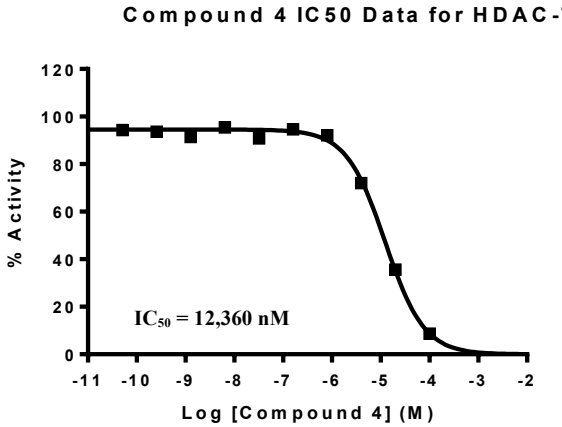
Class IIa



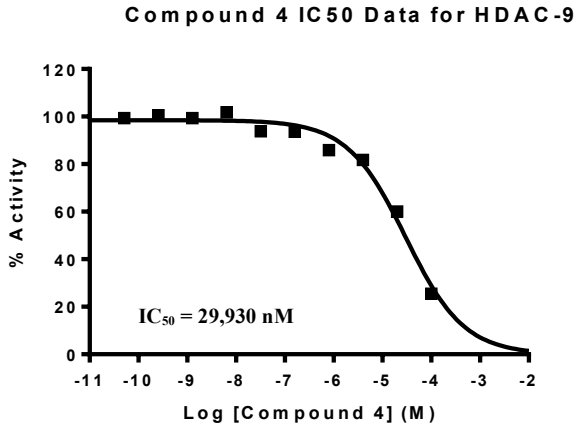
Avg IC_{50} = 3982 ± 920 nM



Avg IC_{50} = 3891 ± 681 nM

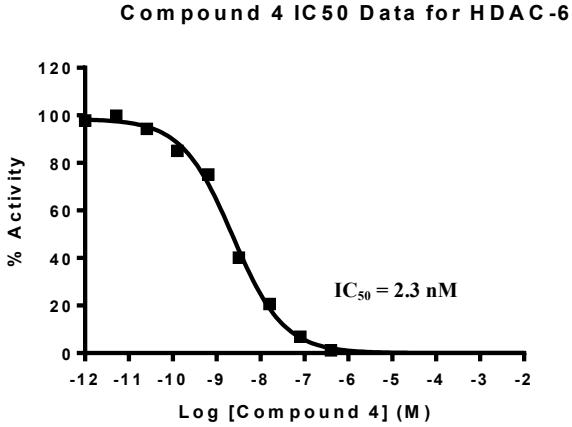


Avg IC_{50} = 13,220 ± 860 nM

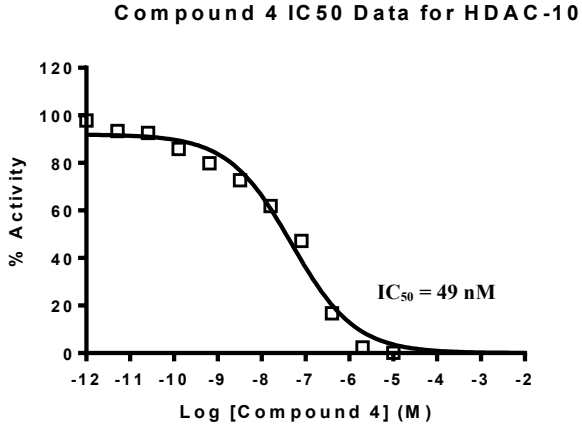


Avg IC_{50} = 28,020 ± 1910 nM

Class IIb

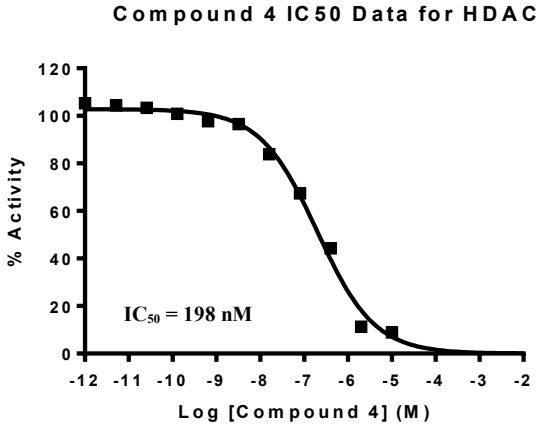


Avg IC_{50} = 2.7 ± 0.5 nM



Avg IC_{50} = 51 ± 3.2 nM

Class IV



Avg IC_{50} = 224 ± 26 nM

X. CELLULAR CHARACTERIZATION

A. Cell growth inhibition assays.

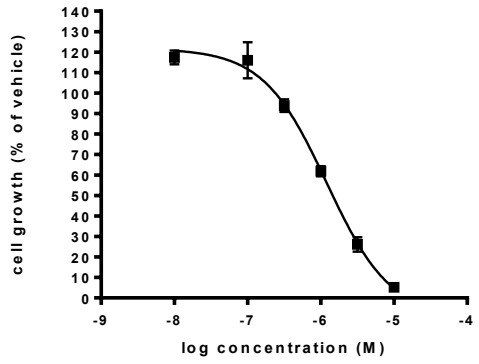
WST-1 reagent was obtained from Roche Applied Science. The cell proliferation colorimetric assay using WST-1 was performed according to manufacturer's procedure (https://cssportal.roche.com/LFR_PublicDocs/ras/11644807001_en_11.pdf).

Cell Culture and Seeding Procedure: Cells were dispersed from flasks and collected by centrifugation (200xg for 5 minutes at room temperature). An aliquot of the resuspended cells was mixed with trypan blue solution and the cell number was quantified using a hemacytometer. In general, depending on the growth rate of the untreated cells, the cells were plated at 5.0 – 7.5 x 10^3 cells per well. The cells were plated into sterile, clear bottom 96 well plates and cultured under normal growth conditions overnight prior to dosing with compound.

Dosing: 100% DMSO compound stocks were prepared to 100X the final concentration desired in the assay. 3 μL of the DMSO stock solution was then added to 297 μL of the cell growth media to give a DMSO concentration of 1%. The cell media was removed by aspiration for adherent cells and replaced with 100 μL per well of the cell growth media containing the compound. In general, each compound concentration was dosed in triplicate wells. Assay: After the dosing period (72 hours) was complete, the plates were removed from the incubator and 10 μL per well of WST-1 reagent was added. The plates were returned to the incubator and incubated for 1 h, followed by shaking on a plate shaker for 60 seconds prior to the absorbance read (450 nm) on a BioTek Synergy 4 multimode plate reader.

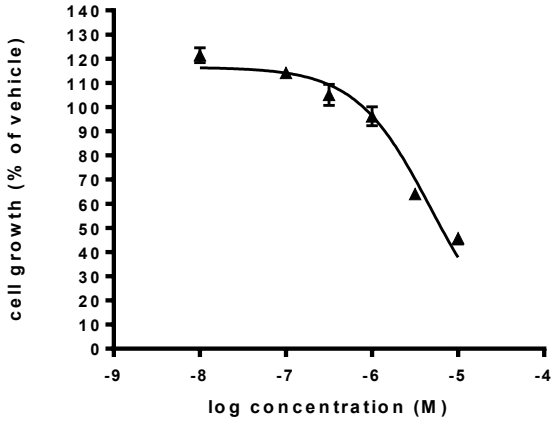
Data Analysis: The reference absorbance reading was subtracted from the formazan absorbance at 690 nm (background control well no compound added, 1% DMSO) and the data was plotted as a percentage of the vehicle (1% DMSO alone). Data analysis and curve fitting was performed using Graphpad Prism. For each cell line, there were n = 3 data points for each concentration. Each dose response curve was performed at least twice, providing n ≥ 6 for each data point.

A. SK-BR-3 with Vorinostat:



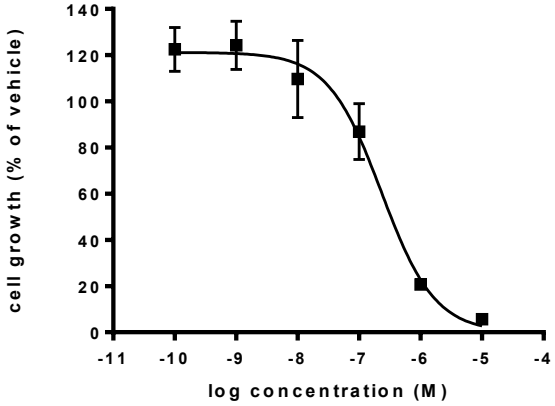
GI_{50} = 1.21 ± 0.09 μM

B. SK-BR-3 with Compound 1:



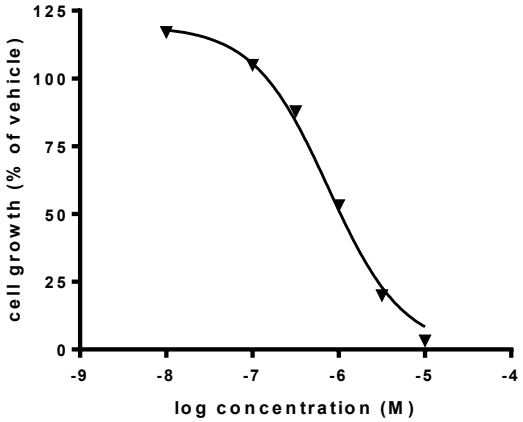
$GI_{50} = 4.80 \pm 0.22$ μM

C. SK-BR-3 with Compound 4:



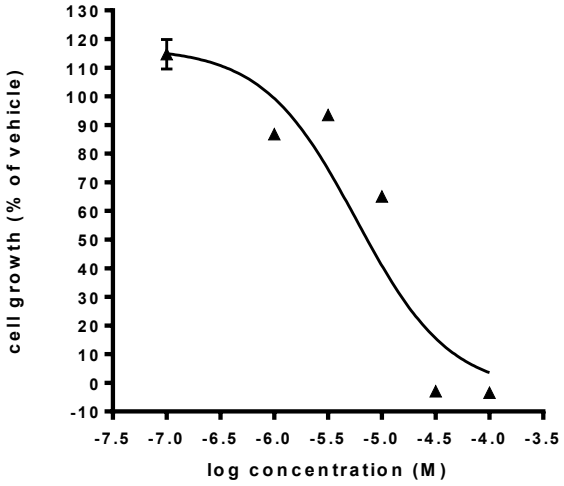
$GI_{50} = 0.23 \pm 0.03$ μM

D. SK-BR-3 with 1:1 combination of Vorinostat + Compound 1:



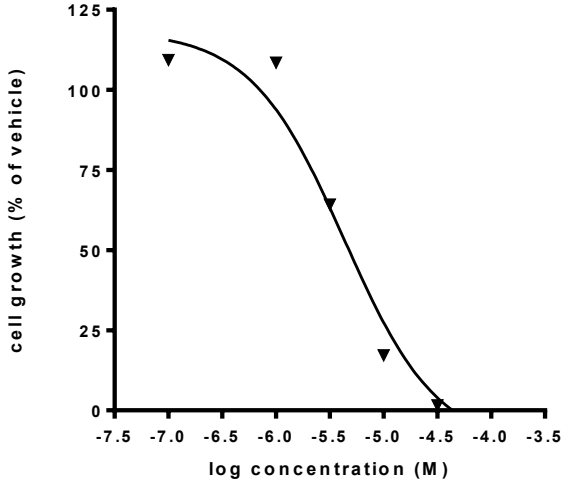
$GI_{50} = 0.75 \pm 0.03$ μM

E. HMEC with Vorinostat:



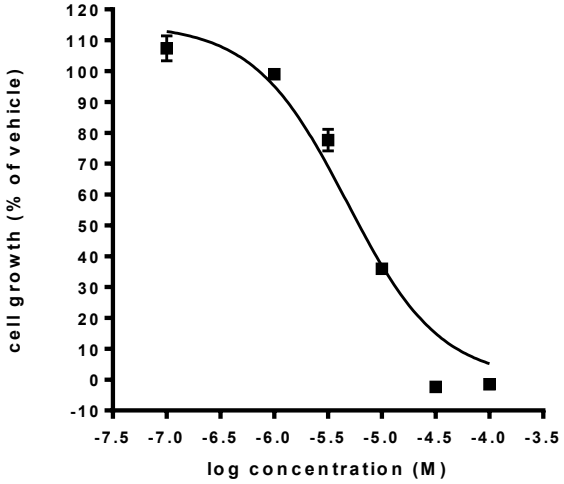
$GI_{50} = 5.79 \pm 0.24$ μM

F. HMEC with Compound 1:



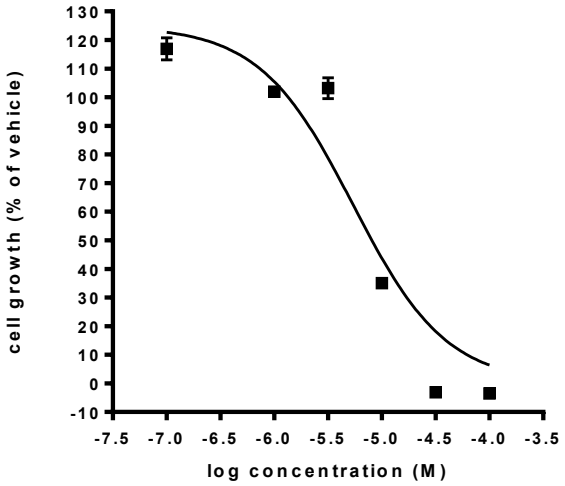
$GI_{50} = 4.29 \pm 0.40$ μM

G. HMEC with Compound 4:



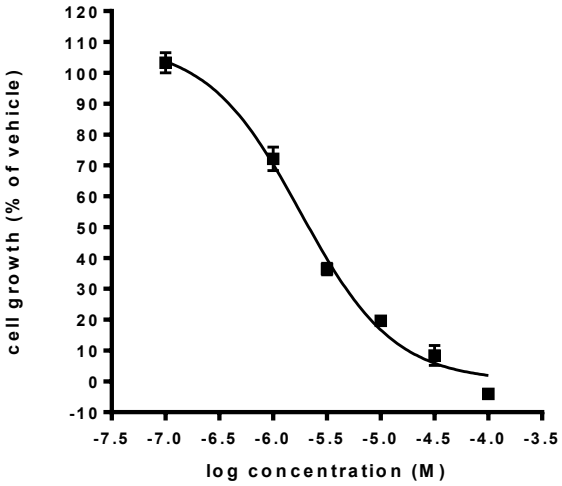
$GI_{50} = 4.73 \pm 0.33$ μM

H. HMEC with 1:1 combination of Vorinostat + Compound 1 :



GI_{50} = 5.40 ± 0.19 μM

I. HMEC with Dasatinib



GI_{50} = 1.80 ± 0.33 μM

XI. NCI Cancer Cell Profiling for Compound 4

The protocol for NCI-60 profiling is provided by the NCI Developmental Therapeutics Program[8] and is as follows:

The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37°C, 5 % CO2, 95 % air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:
[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz
[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50 % (GI50) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning)

indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

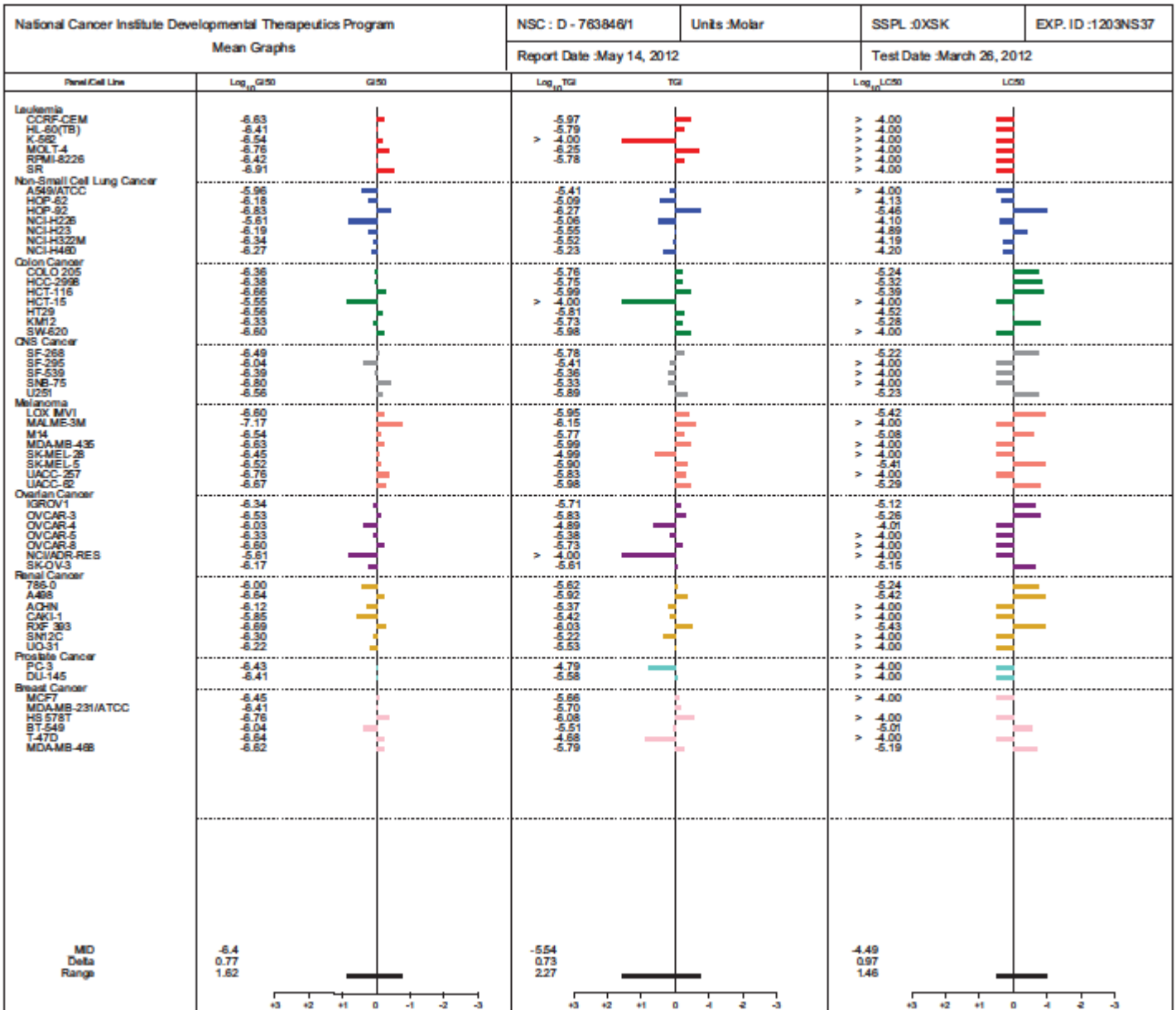
NCI-60 profile for compound **4**:

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 763846 / 1	Experiment ID : 1203NS37	Test Type : 08	Units : Molar
Report Date : May 14, 2012	Test Date : March 26, 2012	QNS :	MC :
COMI : KK-137 (115692)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0XSK	

		Log10 Concentration												
	Time	Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.709	2.464	2.480	2.098	0.729	0.423	0.621	101	79	1	-40	-12	2.36E-7	1.06E-6	> 1.00E-4
HL-60(TB)	0.902	2.582	2.672	2.669	1.104	0.484	0.637	105	105	12	-46	-29	3.91E-7	1.61E-6	> 1.00E-4
K-562	0.273	1.696	1.680	1.466	0.408	0.316	0.389	99	84	9	3	8	2.85E-7	> 1.00E-4	> 1.00E-4
MOLT-4	0.728	2.328	2.261	1.912	0.551	0.396	0.639	96	74	-24	-46	-12	1.75E-7	5.66E-7	> 1.00E-4
RPMI-8226	0.911	2.351	2.418	2.452	1.045	0.613	0.755	105	107	9	-33	-17	3.83E-7	1.66E-6	> 1.00E-4
SR	0.453	1.459	1.421	0.999	0.543	0.336	0.524	96	54	9	-26	7	1.24E-7	.	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.256	1.124	1.115	1.119	0.722	0.161	0.159	99	99	54	-37	-38	1.10E-6	3.90E-6	> 1.00E-4
HOP-62	0.332	0.975	0.983	0.926	0.596	0.319	0.144	101	92	41	-4	-57	6.68E-7	8.12E-6	7.44E-5
HOP-92	1.339	1.669	1.635	1.553	1.027	0.369	0.438	89	65	-23	-72	-67	1.47E-7	5.43E-7	3.49E-6
NCI-H226	0.563	1.107	1.097	1.078	1.031	0.530	0.253	98	95	86	-6	-55	2.46E-6	8.62E-6	7.89E-5
NCI-H23	0.598	1.786	1.755	1.680	1.080	0.305	0.252	97	91	41	-49	-58	6.51E-7	2.84E-6	1.30E-5
NCI-H322M	0.849	1.539	1.502	1.471	1.054	0.572	0.389	95	90	30	-33	-54	4.61E-7	2.99E-6	6.39E-5
NCI-H460	0.255	1.993	2.053	2.007	0.802	0.232	0.101	103	101	31	-9	-60	5.40E-7	5.93E-6	6.27E-5
Colon Cancer															
COLO 205	0.321	1.125	1.197	1.101	0.509	0.085	0.124	109	97	23	-74	-62	4.35E-7	1.74E-6	5.70E-6
HCC-2998	0.364	1.120	1.121	0.994	0.588	0.047	0.098	100	83	30	-87	-73	4.17E-7	1.79E-6	4.81E-6
HCT-116	0.227	1.625	1.594	1.283	0.241	0.039	0.043	98	76	1	-83	-81	2.20E-7	1.03E-6	4.06E-6
HCT-15	0.311	1.825	1.717	1.704	1.445	0.604	0.538	93	92	75	19	15	2.80E-6	> 1.00E-4	> 1.00E-4
HT29	0.223	1.183	1.106	1.000	0.323	0.128	0.095	92	81	10	-43	-58	2.74E-7	1.57E-6	3.05E-5
KM12	0.443	1.959	1.934	1.793	0.914	0.080	0.087	98	89	31	-82	-80	4.71E-7	1.88E-6	5.21E-6
SW-620	0.288	1.659	1.684	1.425	0.295	0.223	0.214	102	83	1	-23	-26	2.51E-7	1.05E-6	> 1.00E-4
CNS Cancer															
SF-268	0.605	1.761	1.693	1.552	0.827	0.184	0.102	94	82	19	-70	-83	3.22E-7	1.64E-6	6.01E-6
SF-295	0.963	2.290	2.230	2.252	1.598	0.642	0.633	96	97	48	-33	-34	9.04E-7	3.88E-6	> 1.00E-4
SF-539	0.954	2.372	2.289	2.135	1.362	0.797	0.633	94	83	29	-17	-34	4.08E-7	4.32E-6	> 1.00E-4
SNB-75	0.687	1.396	1.276	1.094	0.834	0.616	0.502	83	57	21	-10	-27	1.58E-7	4.62E-6	> 1.00E-4
U251	0.326	1.385	1.326	1.195	0.417	0.106	0.140	94	82	9	-67	-57	2.73E-7	1.30E-6	5.89E-6
Melanoma															
LOX IMVI	0.300	1.906	1.838	1.578	0.381	0.029	0.096	96	80	5	-91	-68	2.49E-7	1.13E-6	3.77E-6
MALME-3M	0.686	1.167	1.094	0.894	0.632	0.565	0.351	85	43	-8	-18	-49	6.84E-8	7.01E-7	> 1.00E-4
M14	0.454	1.436	1.422	1.229	0.617	0.200	0.072	99	79	17	-56	-84	2.91E-7	1.69E-6	8.25E-6
MDA-MB-435	0.461	1.788	1.716	1.516	0.467	0.242	0.244	95	79	.	-48	-47	2.36E-7	1.02E-6	> 1.00E-4
SK-MEL-28	0.371	0.950	0.991	0.845	0.507	0.374	0.241	107	82	23	1	-35	3.51E-7	1.03E-5	> 1.00E-4
SK-MEL-5	0.681	2.262	2.202	2.040	0.852	0.050	0.044	96	86	11	-93	-94	3.01E-7	1.27E-6	3.87E-6
UACC-257	0.558	1.205	1.167	0.967	0.618	0.313	0.306	94	63	9	-44	-45	1.76E-7	1.49E-6	> 1.00E-4
UACC-62	0.917	2.102	2.114	1.788	0.939	0.262	0.422	101	73	2	-71	-54	2.13E-7	1.06E-6	5.10E-6
Ovarian Cancer															
IGROV1	0.652	1.692	1.669	1.697	0.901	0.262	0.274	98	100	24	-60	-58	4.56E-7	1.93E-6	7.62E-6
OVCAR-3	0.521	1.470	1.451	1.289	0.659	0.142	0.130	98	81	14	-73	-75	2.92E-7	1.47E-6	5.49E-6
OVCAR-4	0.418	0.816	0.790	0.782	0.612	0.442	0.207	93	91	49	6	-51	9.28E-7	1.28E-5	9.76E-5
OVCAR-5	0.453	1.278	1.213	1.191	0.703	0.368	0.358	92	89	30	-19	-21	4.64E-7	4.14E-6	> 1.00E-4
OVCAR-8	0.282	1.144	1.124	0.947	0.359	0.214	0.206	98	77	9	-24	-27	2.50E-7	1.86E-6	> 1.00E-4
NCI/ADR-RES	0.393	1.428	1.428	1.369	1.105	0.610	0.544	100	94	69	21	15	2.47E-6	> 1.00E-4	> 1.00E-4
SK-OV-3	0.531	1.096	1.125	1.028	0.770	0.177	0.118	105	88	42	-67	-78	6.77E-7	2.44E-6	7.03E-6
Renal Cancer															
786-0	0.774	2.313	2.285	2.138	1.547	0.144	0.090	98	89	50	-81	-88	1.00E-6	2.41E-6	5.77E-6
A498	1.111	1.671	1.589	1.524	1.158	0.090	0.105	85	74	8	-92	-91	2.30E-7	1.21E-6	3.82E-6
ACHN	0.344	1.448	1.381	1.340	0.833	0.255	0.216	94	90	44	-26	-37	7.50E-7	4.28E-6	> 1.00E-4
CAKI-1	0.809	2.162	2.130	2.121	1.731	0.411	0.432	98	97	68	-49	-47	1.43E-6	3.81E-6	> 1.00E-4
RXF 393	0.682	1.085	1.037	0.979	0.667	0.099	0.051	88	74	-2	-85	-93	2.06E-7	9.36E-7	3.75E-6
SN12C	0.545	1.762	1.707	1.545	0.984	0.489	0.473	95	82	36	-10	-13	4.98E-7	5.98E-6	> 1.00E-4
UO-31	0.897	1.955	1.822	1.757	1.330	0.479	0.501	87	81	41	-47	-44	5.96E-7	2.93E-6	> 1.00E-4
Prostate Cancer															
PC-3	0.466	1.720	1.689	1.500	0.781	0.522	0.389	97	82	25	4	-17	3.68E-7	1.62E-5	> 1.00E-4
DU-145	0.365	1.324	1.309	1.229	0.579	0.251	0.232	98	90	22	-31	-37	3.90E-7	2.60E-6	> 1.00E-4
Breast Cancer															
MCF7	0.330	1.665	1.535	1.501	0.592	0.205	0.204	90	88	20	-38	-38	3.57E-7	2.19E-6	> 1.00E-4
MDA-MB-231/ATCC	0.599	1.147	1.173	1.098	0.720	0.290	0.320	105	91	22	-52	-47	3.92E-7	1.99E-6	.
HS 578T	0.853	1.499	1.453	1.292	0.805	0.649	0.704	93	68	-6	-24	-17	1.75E-7	8.37E-7	> 1.00E-4
BT-549	0.908	1.722	1.703	1.671	1.302	0.444	0.405	98	94	48	-51	-55	9.18E-7	3.06E-6	9.75E-6
T-47D	0.558	1.262	1.230	1.064	0.639	0.596	0.494	95	72	11	5	-11	2.30E-7	2.09E-5	> 1.00E-4
MDA-MB-468	0.551	1.211	1.125	1.016	0.665	0.190	0.106	87	70	17	-66	-81	2.42E-7	1.62E-6	6.49E-6

Mean growth graphs for compound **4**:



NCI Cancer Cell Profiling for Vorinostat (Zolinza), NSC 701852

Data provided by the DTP NCI/NIH website, NCI 60 cell line screen dose response data from 08/2012.[8]

Concentration Unit	CellPanelName	CellLineName	logValue, GI50	logValue, TGI50	logValue, LC50
log10(M)	Leukemia	CCRF-CEM	-6.133	-5.082	-4
log10(M)	Leukemia	HL-60(TB)	-5.901	-4.855	-4
log10(M)	Leukemia	K-562	-6.318	-4.926	-4.17
log10(M)	Leukemia	MOLT-4	-6.433	-4.957	-4
log10(M)	Leukemia	RPMI-8226	-6.515	-5.427	-4.151
log10(M)	Leukemia	SR	-6.403	-4.471	-4
log10(M)	Non-Small Cell Lung	A549/ATCC	-5.766	-4.655	-4.081
log10(M)	Non-Small Cell Lung	EKVX	-5.848	-4.277	-4
log10(M)	Non-Small Cell Lung	HOP-62	-5.805	-4.112	-4
log10(M)	Non-Small Cell Lung	HOP-92	-5.534	-4.488	-4
log10(M)	Non-Small Cell Lung	NCI-H226	-5.375	-4.192	-4
log10(M)	Non-Small Cell Lung	NCI-H23	-5.953	-5.009	-4.233
log10(M)	Non-Small Cell Lung	NCI-H322M	-6.072	-4.886	-4.159
log10(M)	Non-Small Cell Lung	NCI-H460	-6.111	-4.146	-4
log10(M)	Non-Small Cell Lung	NCI-H522	-6.331	-5.161	-4.051
log10(M)	Colon	COLO 205	-6.051	-5.581	-5.084
log10(M)	Colon	HCC-2998	-5.733	-4.819	-4.154
log10(M)	Colon	HCT-116	-6.411	-5.231	-4.633
log10(M)	Colon	HCT-15	-5.562	-4.071	-4
log10(M)	Colon	HT29	-6.127	-4.807	-4.054
log10(M)	Colon	KM12	-5.732	-4.64	-4.058
log10(M)	Colon	SW-620	-6.205	-5.051	-4.165
log10(M)	Central Nervous System	SF-268	-5.775	-4.477	-4.044
log10(M)	Central Nervous System	SF-295	-5.88	-5.046	-4.166
log10(M)	Central Nervous System	SF-539	-5.723	-4.316	-4
log10(M)	Central Nervous System	SNB-19	-5.681	-4.748	-4.037
log10(M)	Central Nervous System	SNB-75	-6.1	-4.258	-4
log10(M)	Central Nervous System	U251	-5.805	-4.766	-4.328
log10(M)	Melanoma	LOX IMVI	-5.939	-4.99	-4.474
log10(M)	Melanoma	MALME-3M	-6.576	-5.417	-4
log10(M)	Melanoma	M14	-5.886	-4.695	-4.067
log10(M)	Melanoma	MDA-MB-435	-6.294	-5.228	-4.043
log10(M)	Melanoma	MDA-N	-6.271	-5.495	-4.458
log10(M)	Melanoma	SK-MEL-2	-5.889	-4.767	-4.033

log10(M)	Melanoma	SK-MEL-28	-5.926	-5.07	-4.227
log10(M)	Melanoma	SK-MEL-5	-6.183	-5.574	-5.056
log10(M)	Melanoma	UACC-257	-6.308	-5.028	-4.049
log10(M)	Melanoma	UACC-62	-6.351	-5.561	-4.826
log10(M)	Ovarian	IGROV1	-5.963	-5.089	-4.372
log10(M)	Ovarian	OVCAR-3	-5.867	-5.01	-4.226
log10(M)	Ovarian	OVCAR-4	-5.38	-4.011	-4
log10(M)	Ovarian	OVCAR-5	-6.091	-4.944	-4.111
log10(M)	Ovarian	OVCAR-8	-6.286	-4.649	-4
log10(M)	Ovarian	NCI/ADR-RES	-6.806	-5.568	-4.375
log10(M)	Ovarian	SK-OV-3	-5.955	-4.881	-4.134
log10(M)	Renal	786-0	-5.52	-4.258	-4.03
log10(M)	Renal	A498	-5.865	-5.174	-4.628
log10(M)	Renal	ACHN	-5.855	-5.124	-4.305
log10(M)	Renal	CAKI-1	-5.921	-5.403	-4.721
log10(M)	Renal	RXF 393	-5.9	-5.266	-4.334
log10(M)	Renal	SN12C	-5.645	-4.515	-4.238
log10(M)	Renal	TK-10	-6.194	-5.095	-4.188
log10(M)	Renal	UO-31	-6.262	-5.199	-4.159
log10(M)	Prostate	PC-3	-5.683	-4.174	-4
log10(M)	Prostate	DU-145	-5.89	-4.747	-4
log10(M)	Breast	MCF7	-5.644	-4.385	-4
log10(M)	Breast	MDA-MB-231/ATCC	-5.607	-4	-4
log10(M)	Breast	HS 578T	-5.449	-4.155	-4
log10(M)	Breast	BT-549	-5.77	-4.629	-4.016
log10(M)	Breast	T-47D	-6.278	-5.36	-4
log10(M)	Breast	MDA-MB-468	-6.046	-4.965	-4.067

NCI Cancer Cell Profiling for Dasatinib (Sprycel), NSC 723517

Data provided by the DTP NCI/NIH website, NCI 60 cell line screen dose response data from 08/2012.[8]

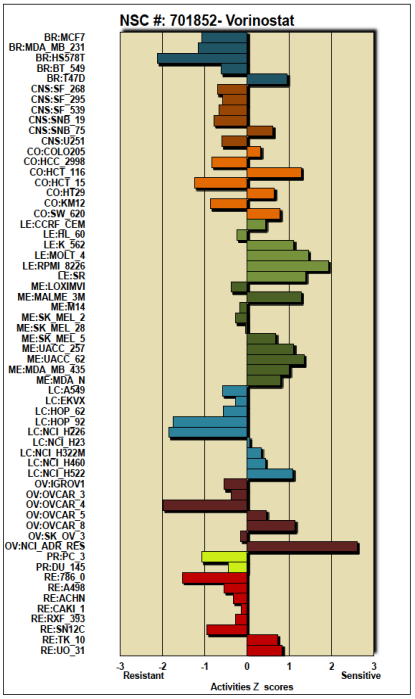
Concentration Unit	Cell Panel Name	Cell Line Name	logValue, GI50	logValue, TGI	logValue, LC50
log10(M)	Leukemia	CCRF-CEM	-5.135	-4.699	-4.699
log10(M)	Leukemia	HL-60(TB)	-5.111	-4.699	-4.699
log10(M)	Leukemia	K-562	-8.699	-4.699	-4.699
log10(M)	Leukemia	MOLT-4	-5.271	-4.699	-4.699
log10(M)	Leukemia	RPMI-8226	-5.132	-4.699	-4.699
log10(M)	Leukemia	SR	-5.199	-4.699	-4.699
log10(M)	Non-Small Cell Lung	A549/ATCC	-7.378	-5.484	-4.699
log10(M)	Non-Small Cell Lung	EKVX	-5.374	-4.699	-4.699
log10(M)	Non-Small Cell Lung	HOP-62	-7.439	-4.699	-4.699
log10(M)	Non-Small Cell Lung	NCI-H226	-7.334	-4.699	-4.699
log10(M)	Non-Small Cell Lung	NCI-H23	-5.282	-4.699	-4.699
log10(M)	Non-Small Cell Lung	NCI-H322M	-6.757	-4.699	-4.699
log10(M)	Non-Small Cell Lung	NCI-H460	-5.054	-4.699	-4.699
log10(M)	Non-Small Cell Lung	NCI-H522	-6.939	-4.825	-4.699
log10(M)	Colon	COLO 205	-7.431	-4.699	-4.699
log10(M)	Colon	HCC-2998	-4.887	-4.699	-4.699
log10(M)	Colon	HCT-116	-5.431	-4.699	-4.699
log10(M)	Colon	HCT-15	-6.101	-4.699	-4.699
log10(M)	Colon	HT29	-7.883	-4.699	-4.699
log10(M)	Colon	KM12	-5.128	-4.699	-4.699
log10(M)	Colon	SW-620	-5.074	-4.699	-4.699
log10(M)	Central Nervous System	SF-268	-6.95	-4.782	-4.699
log10(M)	Central Nervous System	SF-295	-5.479	-4.865	-4.699
log10(M)	Central Nervous System	SF-539	-7.273	-4.699	-4.699
log10(M)	Central Nervous System	SNB-19	-5.264	-7.25	-4.699
log10(M)	Central Nervous System	SNB-75	-8.329	-4.699	-4.699
log10(M)	Central Nervous System	U251	-5.551	-6.435	-4.699
log10(M)	Melanoma	LOX IMVI	-8.017	-4.699	-5.226
log10(M)	Melanoma	MALME-3M	-5.18	-4.699	-4.699
log10(M)	Melanoma	M14	-5.309	-4.699	-4.699
log10(M)	Melanoma	MDA-MB-435	-5.221	-4.699	-4.699
log10(M)	Melanoma	SK-MEL-2	-5.11	-4.699	-4.699
log10(M)	Melanoma	SK-MEL-28	-5.038	-4.699	-4.699
log10(M)	Melanoma	SK-MEL-5	-5.171	-4.76	-4.699
log10(M)	Melanoma	UACC-257	-5.571	-4.699	-4.699

log10(M)	Melanoma	UACC-62	-5.245	-4.699	-4.699
log10(M)	Ovarian	IGROV1	-7.599	-4.699	-4.699
log10(M)	Ovarian	OVCAR-3	-6.761	-4.699	-4.699
log10(M)	Ovarian	OVCAR-4	-5.151	-4.699	-4.699
log10(M)	Ovarian	OVCAR-5	-7.302	-6.294	-4.699
log10(M)	Ovarian	OVCAR-8	-7.307	-4.699	-4.699
log10(M)	Ovarian	NCI/ADR-RES	-5.363	-5.476	-4.699
log10(M)	Ovarian	SK-OV-3	-6.649	-4.699	-4.699
log10(M)	Renal	786-0	-6.909	-6.728	-4.699
log10(M)	Renal	A498	-7.65	-4.699	-4.983
log10(M)	Renal	ACHN	-7.736	-6.059	-4.699
log10(M)	Renal	CAKI-1	-7.725	-7.014	-4.699
log10(M)	Renal	RXF 393	-8.045	-4.699	-5.003
log10(M)	Renal	SN12C	-7.545	-7.14	-4.699
log10(M)	Renal	TK-10	-8.074	-4.769	-4.699
log10(M)	Renal	UO-31	-7.705	-4.699	-4.699
log10(M)	Prostate	PC-3	-5.646	-4.699	-4.699
log10(M)	Prostate	DU-145	-6.801	-4.699	-4.699
log10(M)	Breast	MCF7	-5.08	-5.42	-4.699
log10(M)	Breast	MDA-MB-231/ATCC	-7.809	-4.699	-4.729
log10(M)	Breast	HS 578T	-7.601	-4.699	-4.699
log10(M)	Breast	BT-549	-5.117	-4.699	-4.699
log10(M)	Breast	T-47D	-6.387	-5.902	-4.699
log10(M)	Breast	MDA-MB-468	-7.065		-4.699

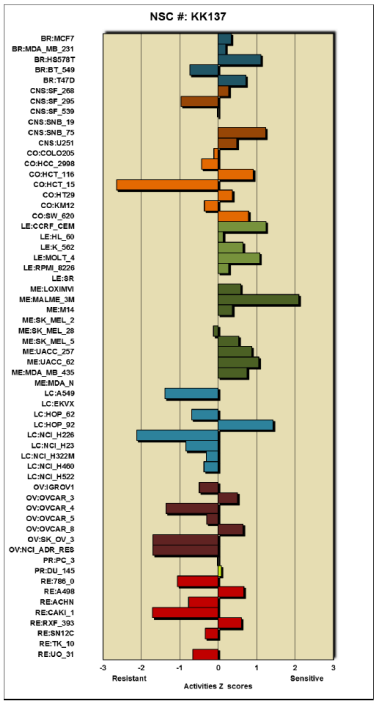
XII. CELLMINER PROFILE DATA

CellMiner[10] (http://discover.nci.nih.gov/cellminer/) was used to generate sensitivity plots for vorinostat, chimera **4**, and dasatinib. Visualization the CellMiner[10] output further demonstrates that chimera **4** has a unique cellular activity profile relative to vorinostat and dasatinib.

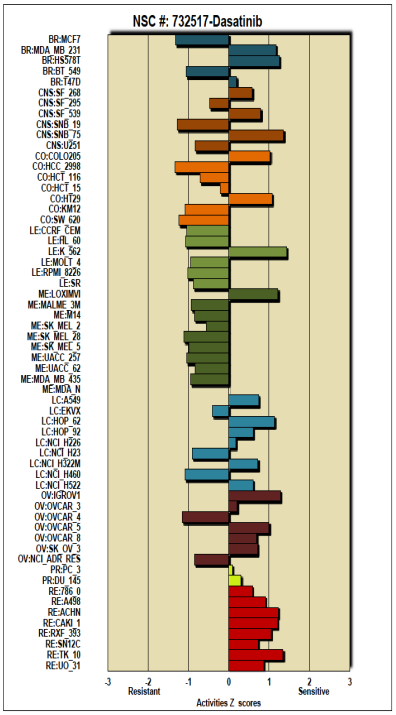
vorinostat:



chimera 4:



dasatinib:



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