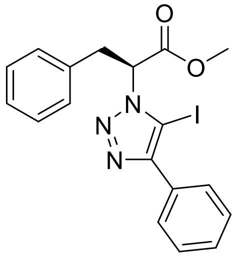
Development of a Potent and Selective HDAC8 Inhibitor

Oscar J. Ingham, Ronald M. Paranal, William B. Smith, Randolph A. Escobar, Han Yueh, Tracy Snyder, John A. Porco, Jr., James E. Bradner, Aaron B. Beeler

General Information: ^{1}H NMR spectra were recorded at 500 MHz at ambient temperature with $CDCl_3$ as a solvent unless otherwise stated. ^{13}C NMR spectra were recorded at 126 MHz at ambient temperature with $CDCl_3$ as a solvent unless otherwise stated. Chemical shifts are reported in parts per million relative to $CDCl_3$ (^{1}H, δ 7.26; ^{13}C, δ 77.0). Data for ^{1}H NMR are reported as follows: chemical shift, multiplicity, integration (app= apparent, par obsc= partially obscure, ovrlp= overlapping, s= singlet, d= doublet, t= triplet, q quartet, m= multiplet) and coupling constants are reported as values in hertz. All ^{13}C NMR spectra were recorded with complete proton decoupling. High-resolution mass spectra were obtained on a Waters QTof API US. Flash chromatography was performed using Silicycle UltraPure silica gel (particle size 40-63μm).

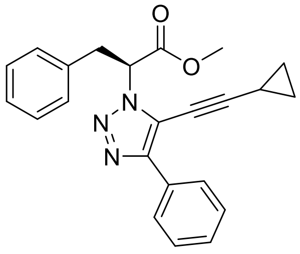
I. Synthesis and Characterization Data

(S)-methyl 2-(5-iodo-4-phenyl-1H-1,2,3-triazol-1-yl)-3-phenylpropanoate (29).



(Iodoethynyl)benzene (2.78 g, 12.2 mmol, 1.5 eq.) (freshly-prepared according to the procedure Fokin and coworkers[1]) was dissolved in 20 mL of anhydrous THF and transferred to a flame-dried flask containing 154 mg (0.81 mmol, 10 mol %) of Copper (I) Iodide. A solution of (S)-methyl 2-azido-3-phenylpropanoate (1.67 g, 8.13 mmol, 1 eq.) in 20 mL of anhydrous THF was added to the flask via syringe. 2.27mL of triethylamine (16.26 mmol, 2 eq.) was added to the flask via syringe and the resulting solution was stirred overnight at room temperature. The yellow solution was filtered through Celite and concentrated *in vacuo*. The crude material was purified by flash chromatography (SiO_2, 20% EtOAc/Hexanes) to afford 3.14 g (89%) of the desired iodide as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) 3.79 (5H, m), 5.49 (1H, dd, *J* = 6.4, 9.3 Hz), 7.10 (2H, dd, *J* = 1.7, 7.6 Hz), 7.19-7.24 (3H, m), 7.39 (1H, t, *J* = 7.3 Hz), 7.45 (2H, t, *J* = 6.8 Hz), 7.90 (2H, d, *J* = 6.8 Hz); ^{13}C NMR (126 MHz, $CDCl_3$) 37.30, 53.14, 64.23, 78.78, 127.19, 127.40, 128.35, 128.49, 128.55, 128.95, 129.91, 135.53, 149.12, 167.73; HRMS calculated for $C_{18}H_{17}IN_3O_2^+$ (M+H): 434.0360, found: 434.0378.

(S)-methyl 2-(5-(cyclopropylethynyl)-4-phenyl-1H-1,2,3-triazol-1-yl)-3-phenylpropanoate (30).



Into a flame-dried flask was added 288 mg (0.41 mmol, 10 mol %) of bis(triphenylphosphine)palladium(II) dichloride and 78 mg (0.41 mmol, 10 mol %) of copper (I) iodide To the flask was added 1.79 g (4.1 mmol, 1 eq.) of iodotriazole (**29**) and anhydrous toluene (40 mL, 0.1 M). Into the solution was added 1.74 mL of cyclopropylacetylene (20.5 mmol, 5 eq.) via syringe, followed by 1.14 mL of triethylamine (8.2 mmol, 2 eq.). The resulting yellow solution was stirred at 80 °C for 5 hours. The solution was filtered through Celite and concentrated *in vacuo*. The black residue was suspended in ethyl acetate and extracted 3 times with water and brine. The organic layer was dried over sodium sulfate and concentrated *in vacuo.* The crude material was purified by flash chromatography (SiO_2, 17% EtOAc/Hexanes) to yield 1.20 g (79 %) of the desired alkyne as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) 0.85 (2H, m), 0.99 (2H, m), 1.55 (1H, m), 3.72 (2H, m), 3.78 (3H, s), 5.49 (1H, dd, *J* = 5.4, 9.8 Hz), 7.10 (2H, dd, *J* = 2.0, 7.8 Hz), 7.19 - 7.24 (3H, m), 7.35 (1H, t, *J* = 7.3 Hz), 7.42 (2H, t, *J* = 7.6 Hz), 8.10 (2H, dd, *J* = 1.0, 8.3 Hz); ^{13}C NMR (106 MHz, $CDCl_3$) 0.42, 9.10, 36.89, 53.03, 61.43, 62.83, 108.08, 118.33, 125.90, 127.11, 128.32, 128.46, 128.56, 128.90, 130.22, 135.68, 146.90, 168.17; HRMS calculated for $C_{23}H_{22}N_3O_2^+$ (M+H): 372.1707, found: 372.1712 .

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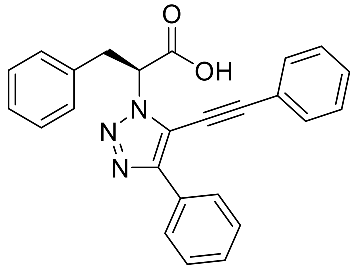
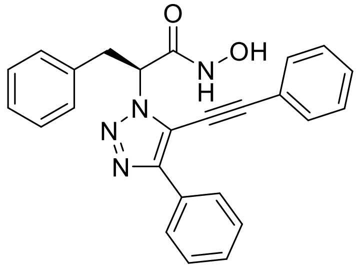
[1] Hein J. E., Tripp J. C., Krasnova L. B., Sharpless K. B., Fokin V. V. *Angew. Chem. Int. Ed.* **2009**, *48*, 8018–8021.

(S)-3-phenyl-2-(4-phenyl-5-(phenylethynyl)-1H-1,2,3-triazol-1-yl)propanoic acid (11).



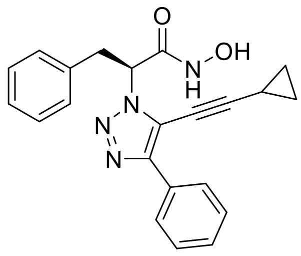
122 mg of ester (0.3 mmol, 1 eq.) was added to a glass vial equipped with a magnetic stir bar and 14 mg (0.6 mmol, 2 eq.) of LiOH was added. The mixture was dissolved in 3.0 mL (0.1 M) of THF/MeOH (4:1) and stirred for 1 hour at room temperature. The solution was acidified with 1N HCl to pH = 2 and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The crude material was purified by flash chromatography (SiO_2, 5 % MeOH in DCM) to afford 110 mg (93%) of the desired compound as a white solid. ^1H NMR (500 MHz, CD_3OD) 3.78 (2H, m), 5.61 (1H, d, *J* = 9.3 Hz), 7.11 (5H, m), 7.37 (6H, m), 7.52 (2H, m), 8.00 (2H, d, *J* = 7.3 Hz); ^{13}C NMR (126 MHz, CD_3OD) 38.53, 76.17, 103.69, 119.87, 122.50, 127.24, 127.99, 129.62, 129.94, 129.97, 130.02, 131.10, 131.26, 132.67, 138.56, 148.44, 173.31 (br, weak); HRMS calculated for $C_{25}H_{20}N_3O_2^+$ (M+H): 394.1556, found: 394.1560.

S)-N-hydroxy-3-phenyl-2-(4-phenyl-5-(phenylethynyl)-1H-1,2,3-triazol-1-yl)propanamide (4).



Into a flask was added 7.4 mg (0.15 mmol, 0.5 eq.) of sodium cyanide and 1.5 mL of methanol. A solution of 122 mg of ester in 1.5 mL THF was added *via* syringe. Into the solution was added 184 µL of 50% aqueous hydroxylamine and the yellow reaction was stirred 16 hours at room temperature. The solution was diluted with water and extracted with EtOAc. The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The resulting residue was purified by flash chromatography (SiO_2, 5 % MeOH in DCM) to afford 76 mg (62 %) of the desired compound as a white solid. ^1H NMR (500 MHz, acetone-D_6) 2.96 (1H, br.), 3.73 - 3.79 (2H, m), 5.74 (1H, t, *J* = 7.5 Hz), 7.15 (1H, t, *J* = 7.1 Hz), 7.21 (2H, t, *J* = 7.3 Hz), 7.26 (2H, d, *J* = 7.3 Hz), 7.39 (1H, t, *J* = 7.3 Hz), 7.47 - 7.51 (5H, m), 7.68 (2H, d, *J* = 7.8 Hz), 8.16 (2H, d, *J* = 7.3 Hz); ^{13}C NMR (126 MHz, acetone-D_6) 37.79, 63.40, 75.90, 103.71, 118.61, 122.27, 126.81, 127.84, 129.30, 129.51, 129.70, 130.14, 130.80, 131.34, 132.57, 137.29, 147.87, 164.97; HRMS calculated for $C_{25}H_{21}N_4O_2^+$ (M+H): 409.1665, found: 409.1657.

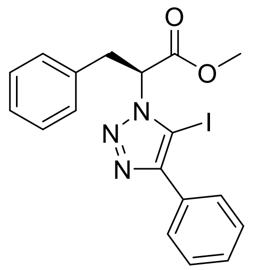
(S)-2-(5-(cyclopropylethynyl)-4-phenyl-1H-1,2,3-triazol-1-yl)-N-hydroxy-3-phenylpropanamide (11).

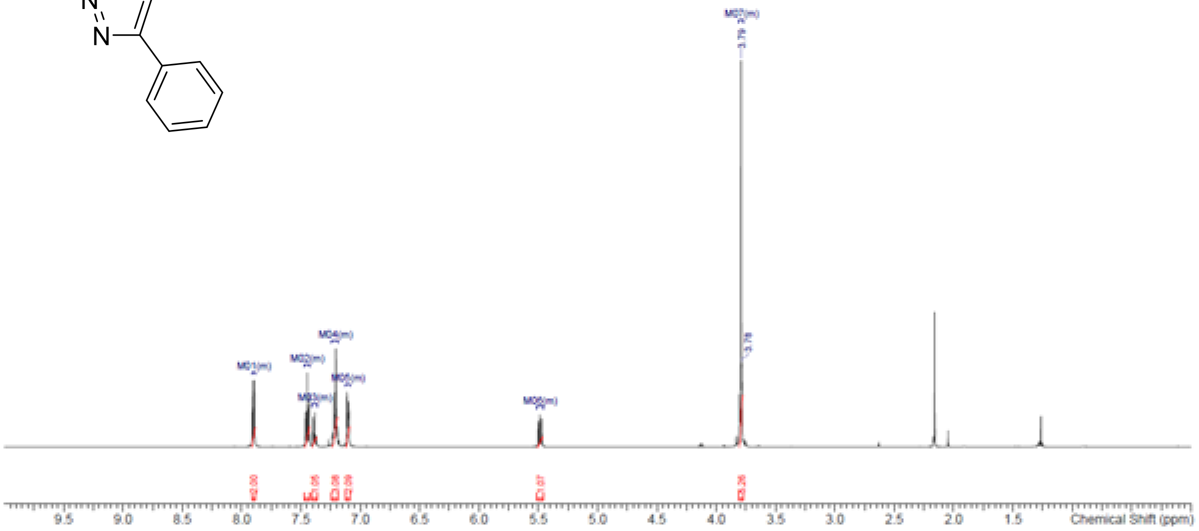


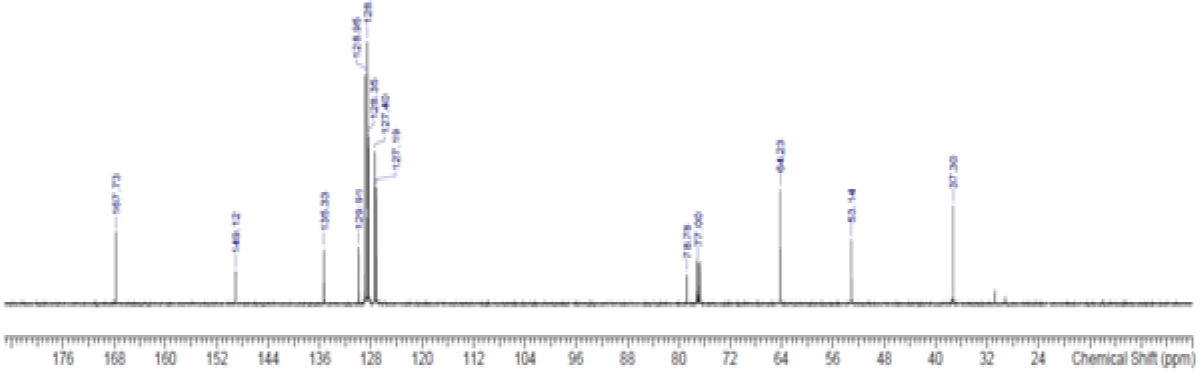
Into a flash was added 18.0 mg (0.36 mmol, 0.5 eq.) of sodium cyanide was and 3.5 mL of MeOH. A solution of 265 mg of the ester in 3.5 mL THF was then added via syringe. To the reaction was added 435 µL of 50% aqueous hydroxylamine and the yellow solution was stirred 16 hours at room temperature. The solution was diluted with water and extracted with EtOAc. The organic layer dried over sodium sulfate and concentrated *in vacuo*. The resulting residue was purified by flash chromatography (SiO_2 5 % MeOH / DCM) to afford 173 mg (65 %) of the desired compound as a white solid. ^1H NMR (500 MHz, d_4-MeOD) 0.80 (2H, m), 0.98 (2H, m), 1.47 (1H, m), 3.51 (1H, dd, *J* = 11.2, 14.2 Hz), 3.71 (1H, dd, *J* = 3.9, 14.2 Hz), 5.49

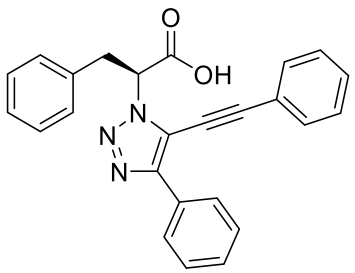
(1H, dd, *J* = 3.9, 11.2 Hz), 6.96 (2H, m), 7.17-7.20 (3H, m), 7.37 (1H, t, *J* = 7.3 Hz), 7.43 (2H, t, *J* = 7.3 Hz), 7.99 (2H, d, *J* = 8.8 Hz); ^{13}C NMR (100 MHz, d$_4$-MeOD) 0.02, 8.55, 37.07, 60.83, 62.59, 109.09, 118.98, 125.9, 127.16, 128.55, 128.65, 128.71, 129.16, 130.07, 136.09, 147.10, 165.20; HRMS calculated for $C_{22}H_{21}N_4O_2^+$ (M+H): 373.1659, found: 373.1665.

II. NMR Spectra





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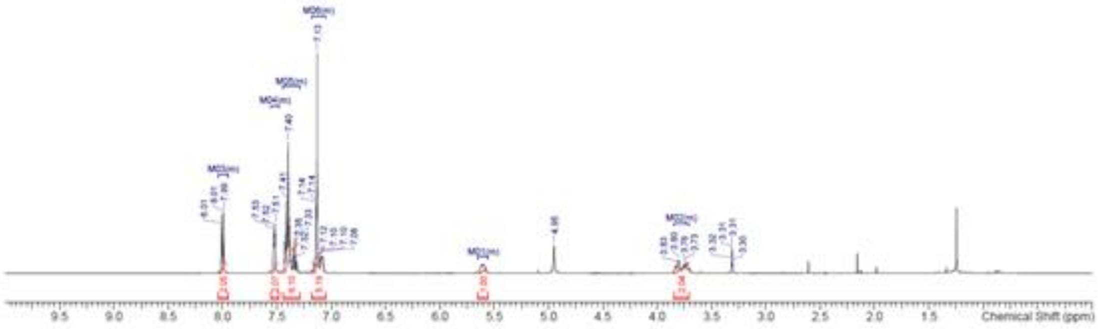
OH
N
N
N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8.01
7.99
M04(m)
7.53
7.52
7.51
7.41
7.40
M05(m)
7.36
7.35
7.33
M06(m)
7.16
7.14
7.12
7.12
7.10
7.10
7.08
M01(m)
4.96
M02(m)
3.83
3.80
3.76
3.73
3.32
3.31
3.31
3.30
9.5
9.0
8.5
8.0
7.5
7.0
6.5
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3.5
3.0
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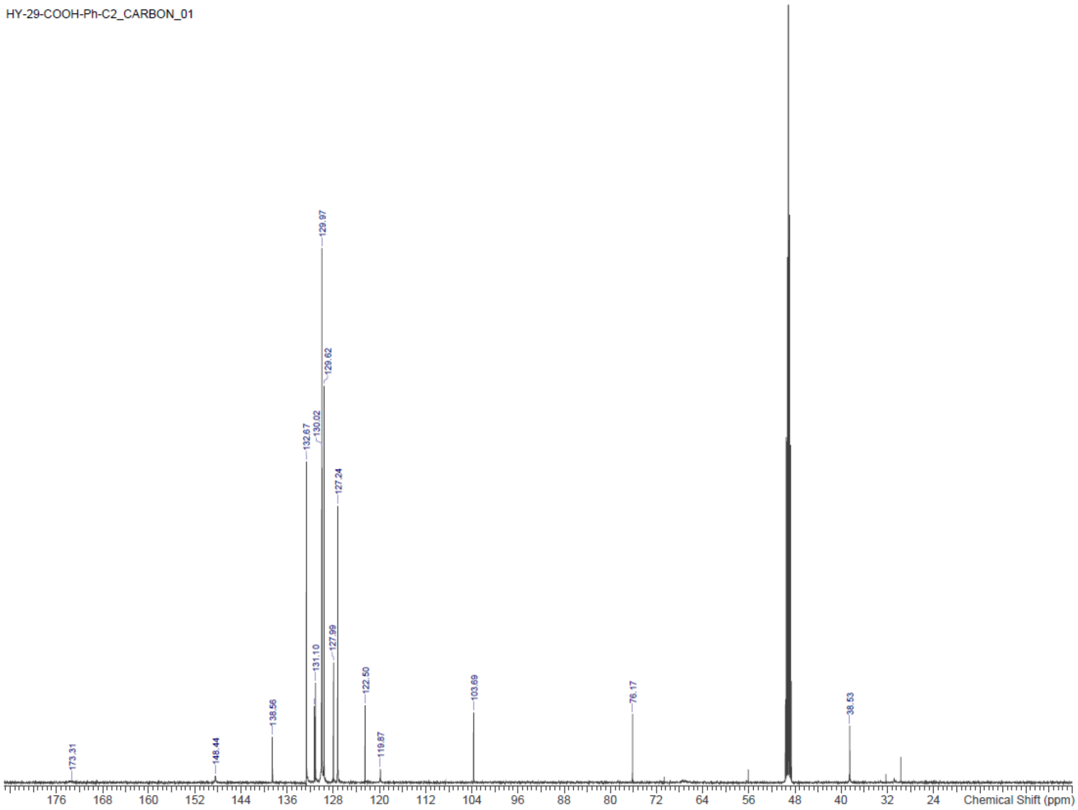
2.0
1.5
Chemical Shift (ppm)


HY-29-COOH-Ph-C2_CARBON_01
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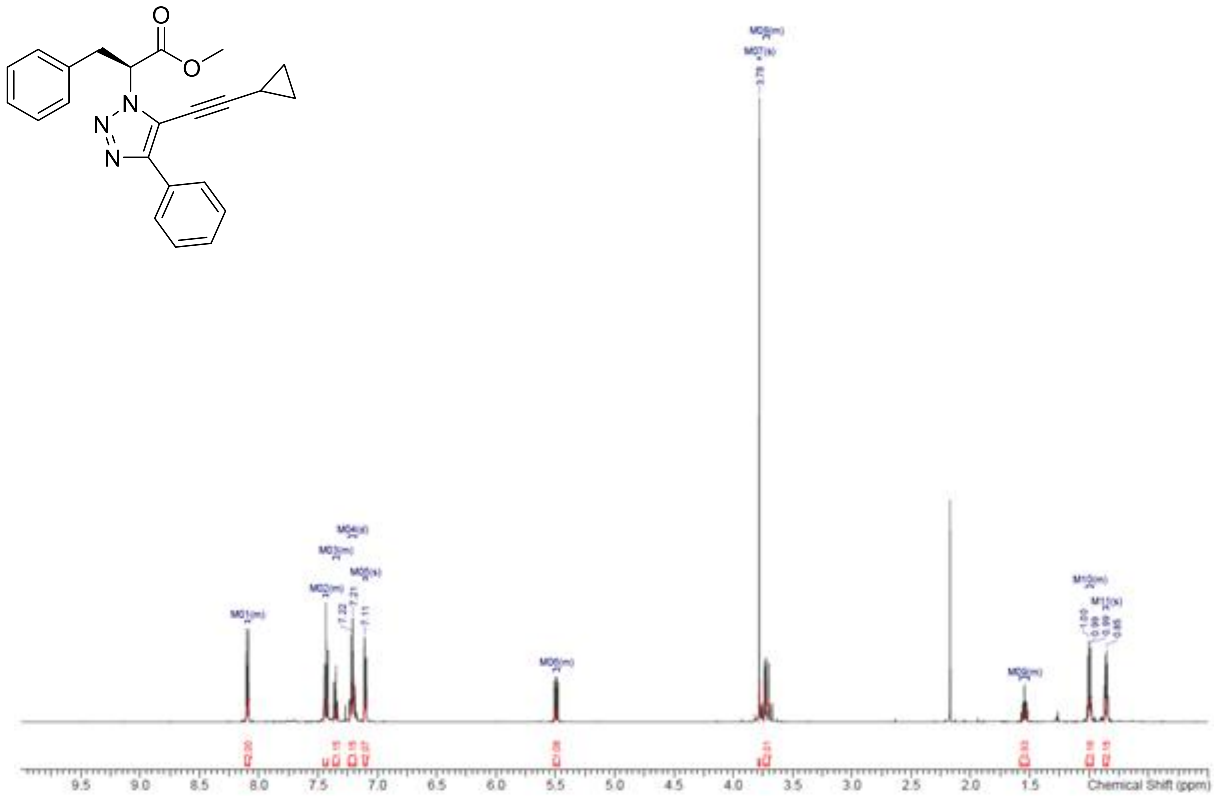
148.44
138.56
132.67
131.10
130.02
129.97
129.62
127.99
127.24
122.50
119.87
103.69
76.17
38.53
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
Chemical Shift (ppm)



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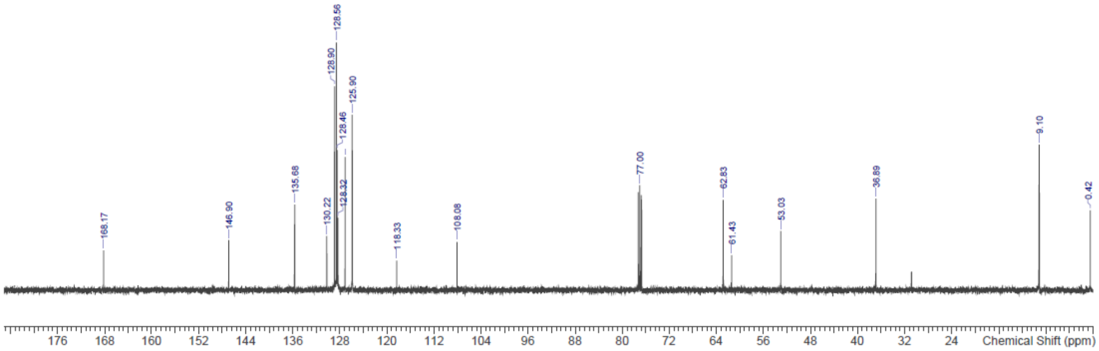

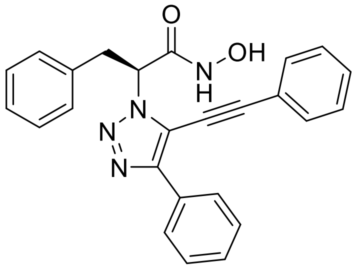

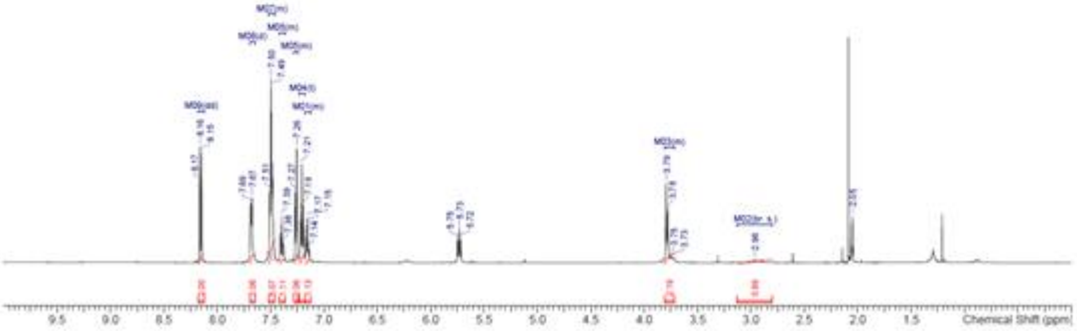

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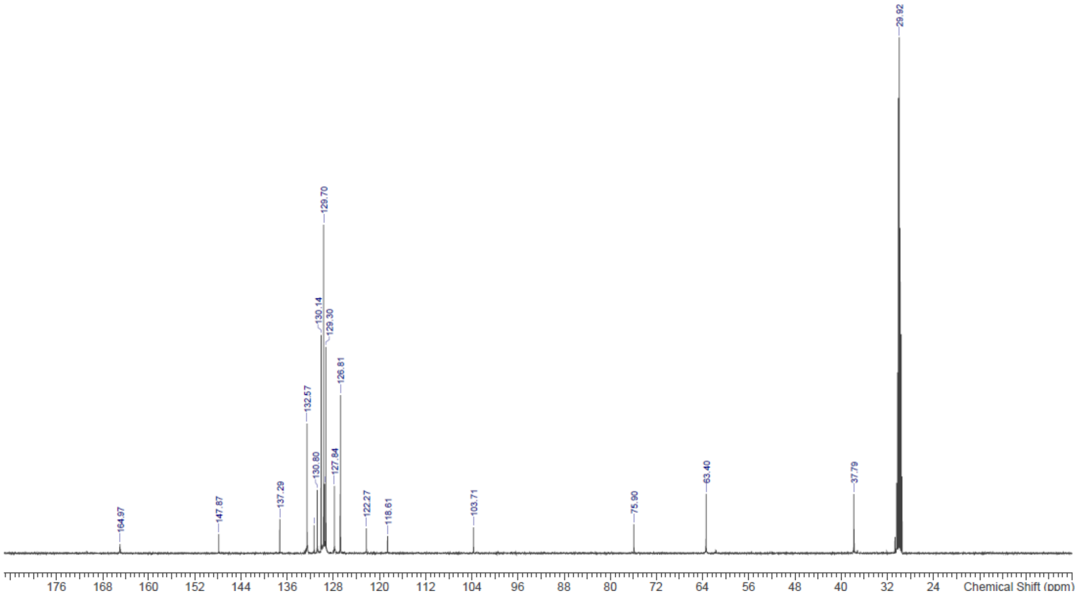

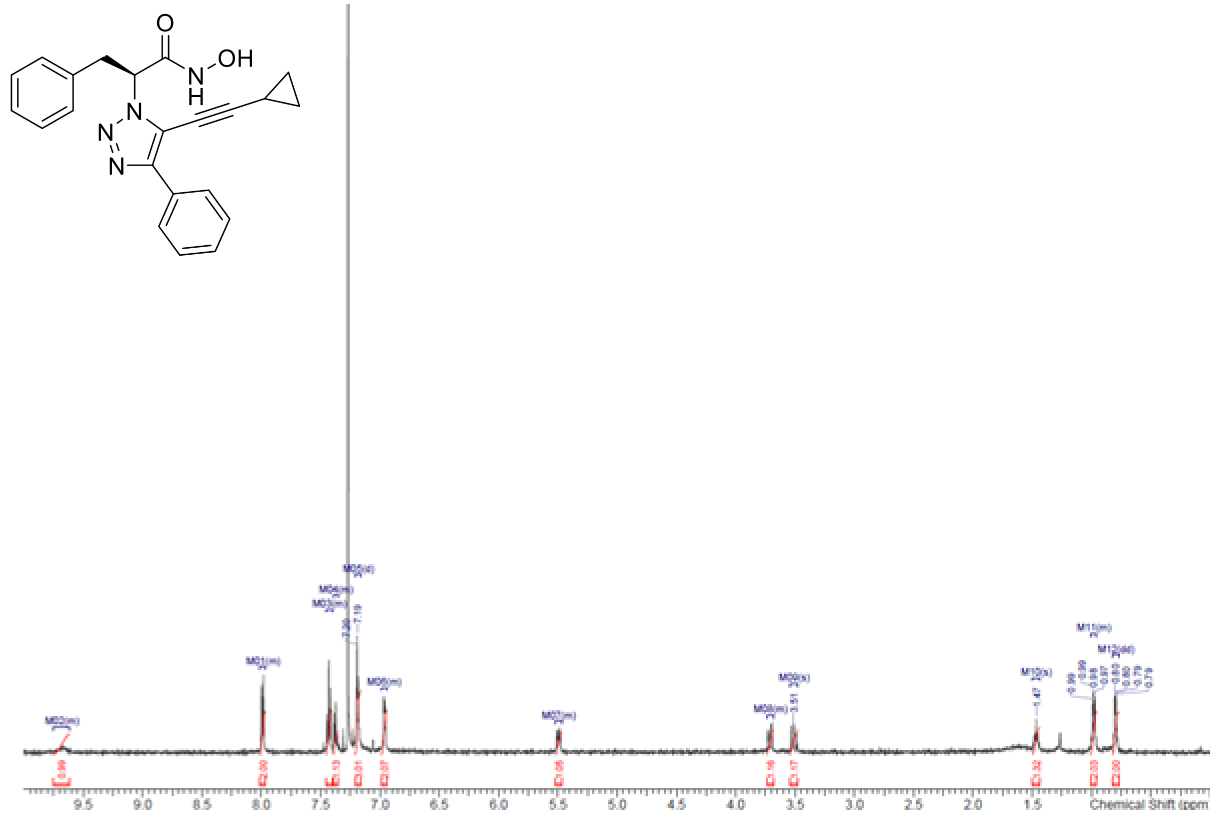
O
N
OH
H
N
N
N
M02(m)
M01(m)
M03(m)
M04(m)
M05(d)
7.20
7.19
M06(m)
M07(m)
M08(m)
M09(s)
3.51
M10(s)
1.47
M11(m)
M12(dd)
0.99
0.99
0.98
0.97
0.83
0.80
0.79
0.79
0.99
2.00
3.13
3.01
2.07
1.05
1.16
1.17
1.32
2.03
2.00
9.5
9.0
8.5
8.0
7.5
7.0
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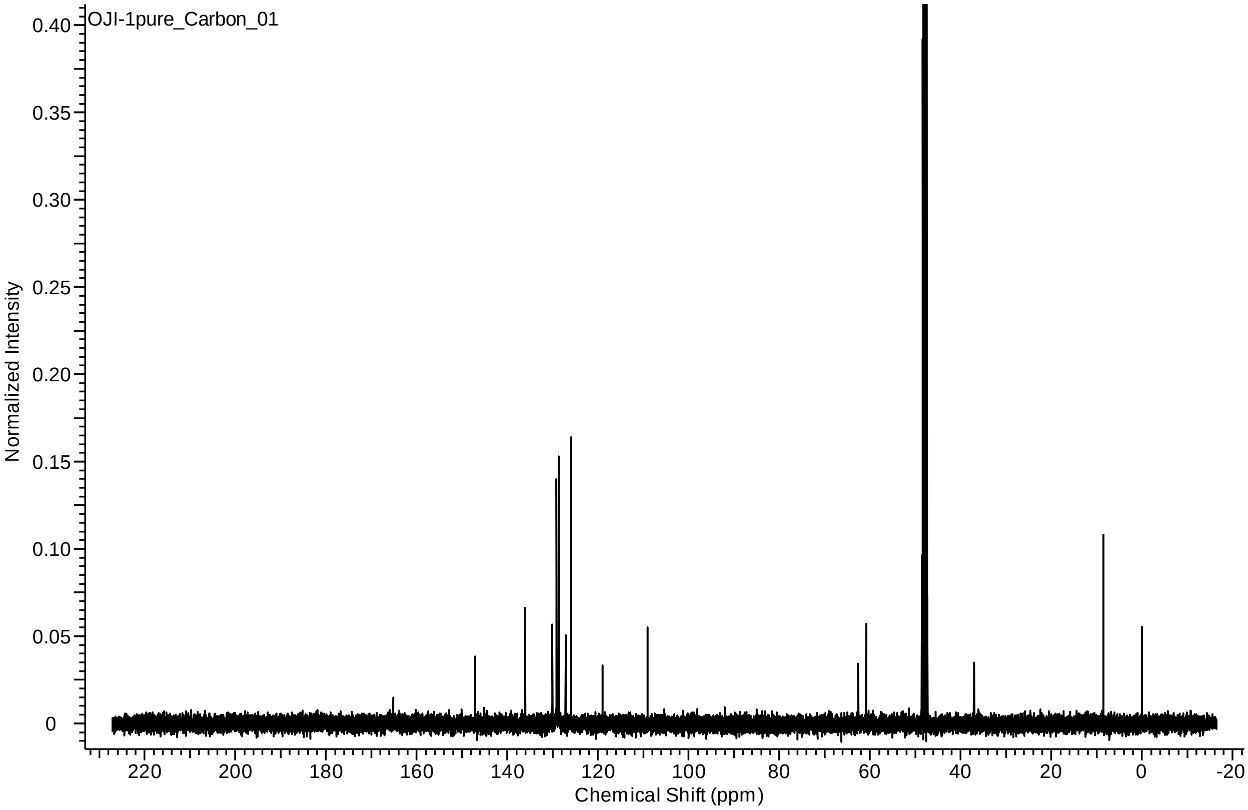
1.5
Chemical Shift (ppm


OJI-1pure_Carbon_01
Normalized Intensity
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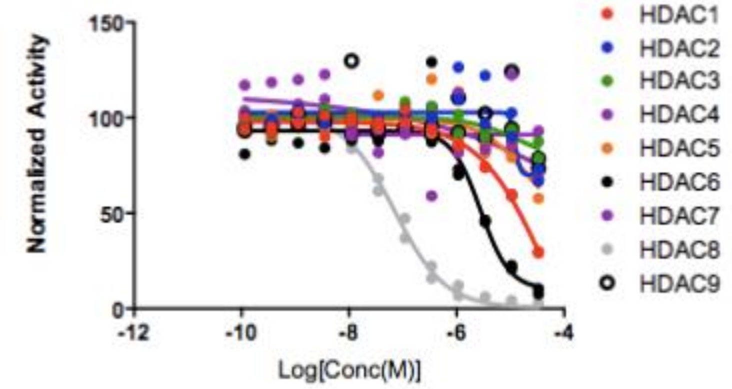
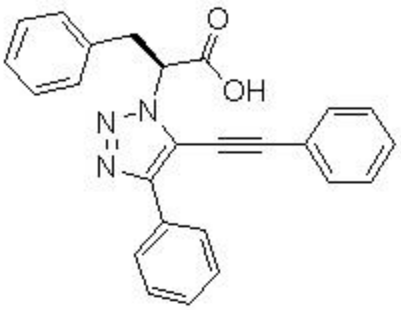
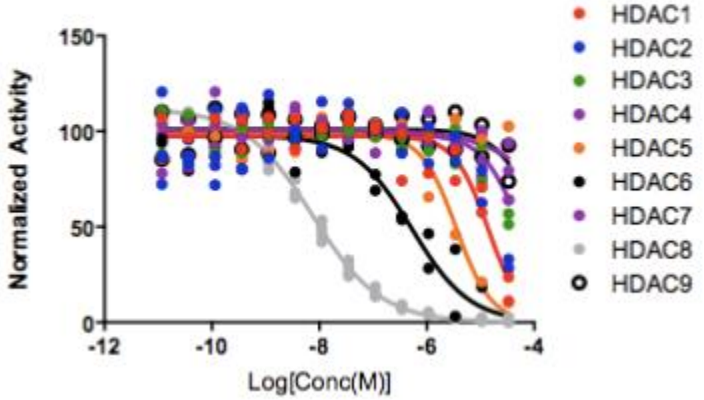
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
220
200
180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)

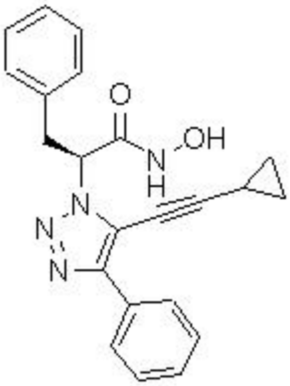
III. HDAC Inhibition Procedure and Data

General Procedure for HDAC Inhibition Assay: HDAC assays were carried out as described previously[2,3]









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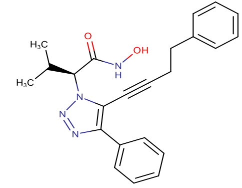
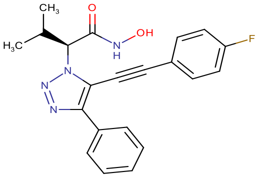
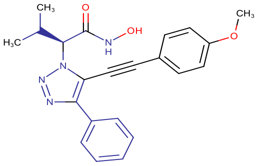
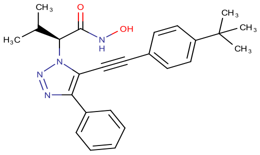
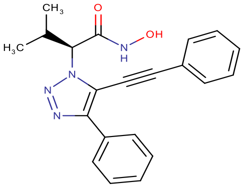
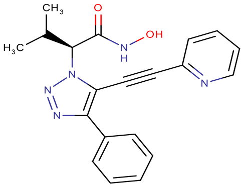
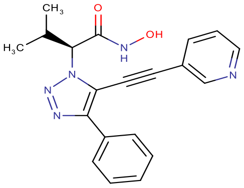
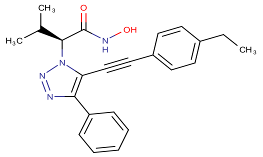
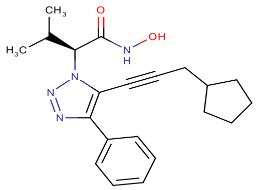
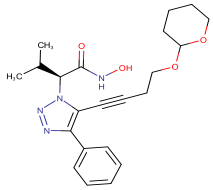
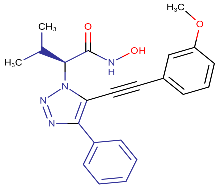
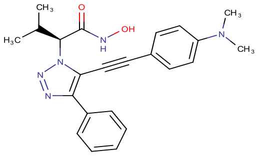
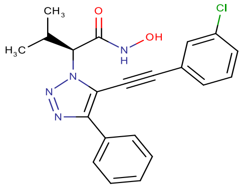
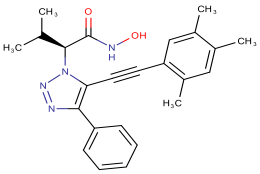
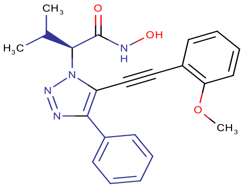
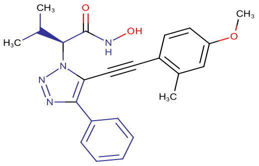
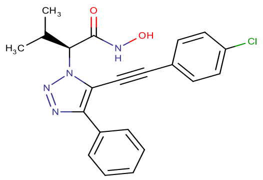
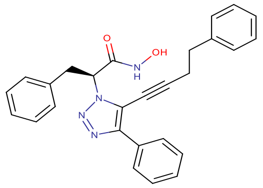
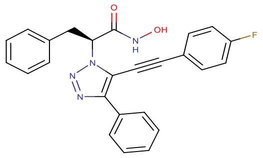
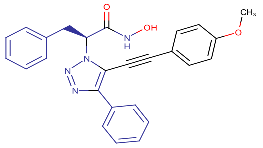
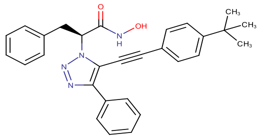
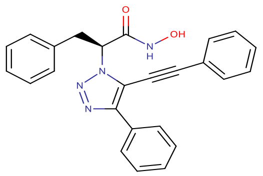
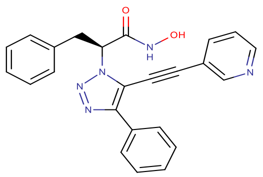
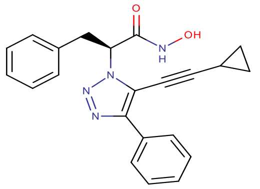
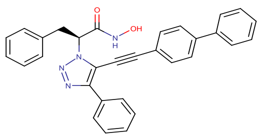
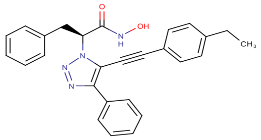
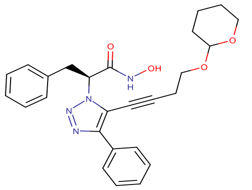
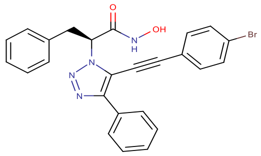
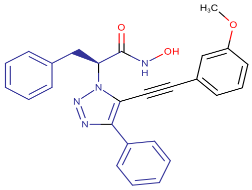
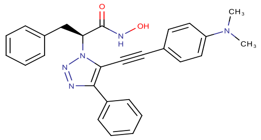
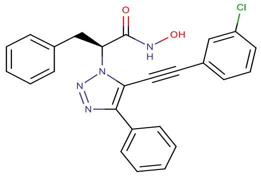
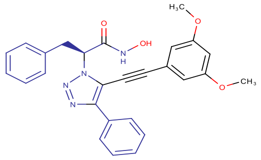
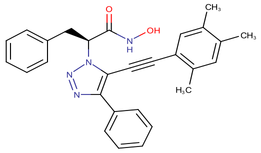
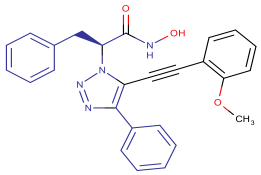
[2] Bowers, A.; West, N.; Taunton, J.; Schreiber, S. L.; Bradner, J. E.; Williams, R. M. *J. Am. Chem. Soc* **2008**, *130*,11219

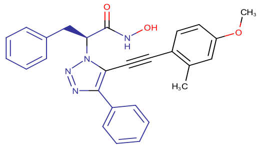
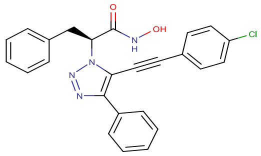
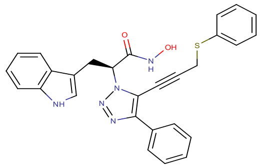
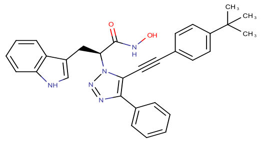
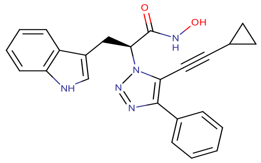
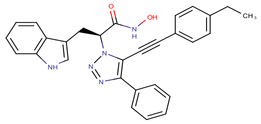
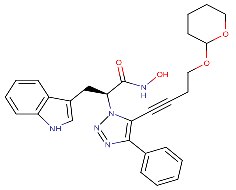
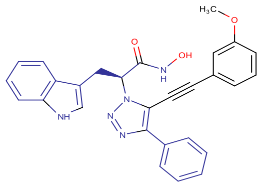
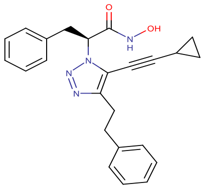
[3] Bowers, A.; Greshock, T.; West, N.; Estiu, G.;Schreiber, S.; Wiest, O.; Williams, R.; Bradner, J. *J. Am. Chem. Soc.* **2009**, 131, 2900

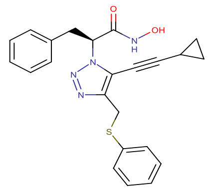
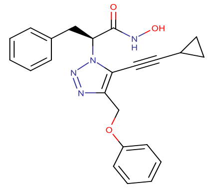
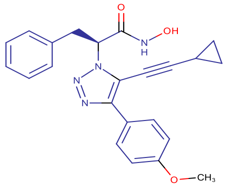
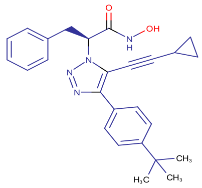
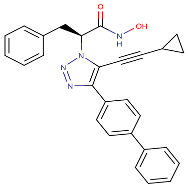
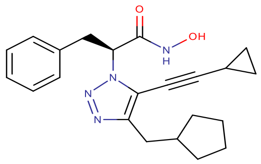
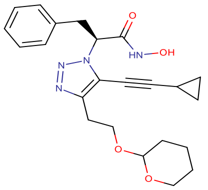
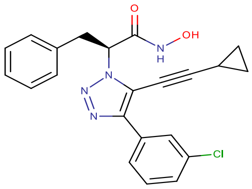
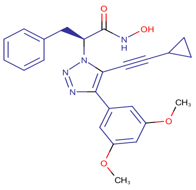
	HDAC1 (IC$_{50}$ μM)	**HDAC2 (IC$_{50}$ μM)**	**HDAC3 (IC$_{50}$ μM)**	**HDAC6 (IC$_{50}$ μM)**	**HDAC8 (IC$_{50}$ μM)**
	>20	>20	>20	1.5	0.5
	>20	>20	>20	2.5	2.6
	>20	>20	>20	3.3	2.4
	>20	>20	>20	>20	4.3
	>20	>20	>20	2.5	1.8
	>20	>20	>20	>20	>20
	>20	>20	>20	>20	>20

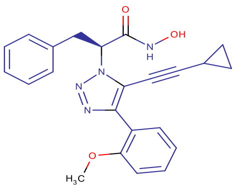
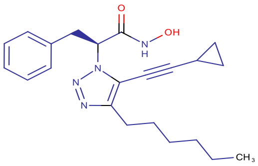
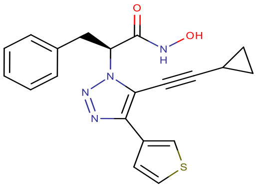
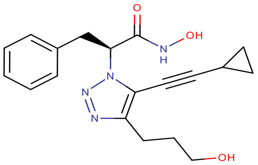
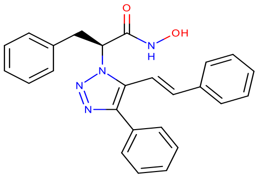
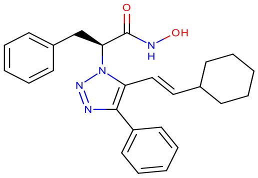
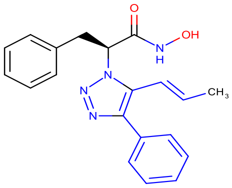
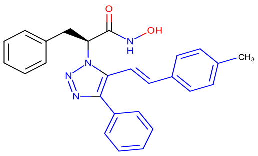
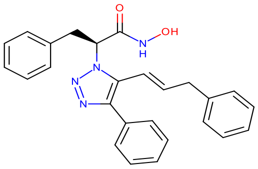
	>20	>20	>20	3.5	1.8
	>20	>20	>20	2.3	7.1
	>20	>20	>20	1.5	>20
	>20	>20	>20	4.5	3.2
	>20	>20	>20	1.5	1.8
	>20	>20	>20	2.6	1.9
	>20	>20	>20	NA	3.3
	>20	>20	>20	3.4	3.1
	>20	>20	>20	3.4	2.6

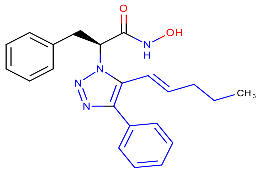
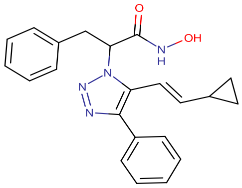
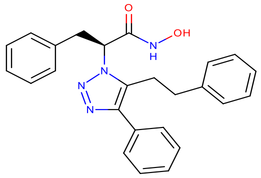
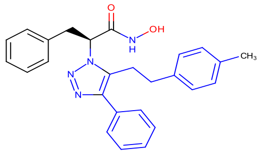
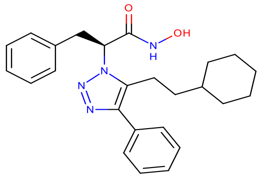
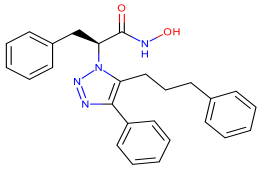
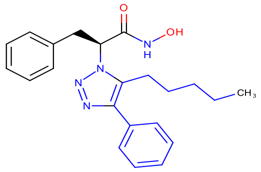
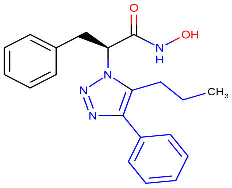
	>20	>20	>20	3.2	3.7
	>20	>20	>20	3.1	0.04
	17	>20	>20	2.6	0.04
	>20	>20	>20	3.5	0.43
	>20	>20	>20	10	5.5
	>20	>20	>20	3.6	0.01
	>20	>20	>20	7.5	0.15
	4.3	>20	>20	1.2	0.0008
	>20	>20	>20	17	11

	>20	>20	>20	5.7	1.7
	>20	>20	>20	4.5	6.9
	>20	>20	>20	>20	2.1
	>20	>20	>20	2.6	0.088
	>20	>20	>20	1.4	2
	>20	>20	>20	2.8	0.51
	>20	>20	>20	2	0.28
	>20	>20	>20	12	0.29
	>20	>20	>20	4.5	0.31

	>20	>20	>20	4.8	0.47
	>20	>20	>20	3.9	0.12
	>20	>20	>20	>20	>20
	>20	>20	>20	>20	>20
	7.7	>20	>20	1.2	0.0069
	>20	>20	>20	11.5	15.7
	>20	>20	>20	0.9	1.7
	>20	>20	>20	2	0.33
	NA	NA	NA	NA	0.353

	NA	NA	NA	NA	0.0078
	NA	NA	NA	NA	0.163
	NA	NA	NA	NA	0.0041
	NA	NA	NA	NA	0.133
	NA	NA	NA	NA	0.262
	NA	NA	NA	NA	0.0041
	NA	NA	NA	NA	0.223
	NA	NA	NA	NA	0.0082
	NA	NA	NA	NA	0.0029

	NA	NA	NA	NA	0.02
	NA	NA	NA	NA	0.38
	NA	NA	NA	NA	0.0041
	NA	NA	NA	NA	0.11
	NA	NA	NA	>20	>20
	NA	NA	NA	>20	>20
	NA	NA	NA	>20	>20
	NA	NA	NA	>20	>20
	NA	NA	NA	>20	>20

	NA	NA	NA	>20	0.464
	NA	NA	NA	>20	>20
	>20	NA	NA	>20	>20
	>20	NA	NA	>20	>20
	>20	NA	NA	>20	>20
	>20	NA	NA	>20	>20
	>20	NA	NA	>20	0.16
	>20	NA	NA	>20	>20