Supplementary Information

Discovery and SAR study of a sulfonamide hydroxamic acid inhibitor for the botulinum neurotoxin serotype A light chain

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Experimental section

The hydroxylamine Wang Resin and the Fmoc protected amino acids were purchased from Novabiochem. The peptide coupling reagents 6-Chloro-1-Hydroxybenzotriazole was purchased from Ochem Inc and N,N'-Diisopropylcarbodiimide was purchased from Advanced Chem Tech and used without further purification. The sulfonyl chlorides were purchased from Aldrich and all other solvents and reagents were purchased from Fisher Scientific and used without further purification. An Inititator microwave reactor from Biotage was used in the synthesis of the library. The small molecules were purified by a Shimadzu prep HPLC with a C18 reverse phase column (VYDAC cat # 218TP101522) using water and acetonitrile as the mobile phase. The BoNT/A LC and SNAPtide substrate using the enzymatic assays were purchased from List Biological Laboratories, Inc.

General synthesis of sulfonamide hydroxamic acids

Hydroxylamine Wang Resin was placed in a syringe and swollen by shaking for 30 minutes in Dichloromethane (DCM). An Fmoc protected amino acid was activated by stirring amino acid (3 equiv), 6-Chloro-1-Hydroxybenzotriazole (6-Cl HOBt) (3 equiv), and N,N'-Diisopropylcarbodiimide (DIC) (3 equiv) in dimethylformamide (DMF) for 20 minutes. The activated amino acid was added to the resin and shaken for 1.5 hours. The resin was then washed 3x DMF. A 25% Piperidine (PIP) in DMF solution was added to the resin twice, once for 10 minutes and then for 15 minutes. The resin was then washed with 3x DMF, 3x MeOH, and 3x DCM. The resin was placed in a microwave tube with 2,6-lutidine (3 equiv), a sulfonyl chloride (3 equiv), and DCM. The tube was placed in a Biotage microwave for 5 minutes at 100˚C. The resin was then transferred to a syringe and washed 3x DMF, 3x MeOH, and 3x DCM. The small molecule was cleaved off the resin by adding 50% Trifluoroacetic acid (TFA) in DCM. The end product was dried using a rotary evaporator. Each compound was purified by a preparatory HPLC (C18 reverse phase VYDAC HPLC column). A two solvent system was used with water (A) and acetonitrile (B). The gradient was as follows: B concentration progresses to 10% from 0.00-5.00 minutes, B concentration progresses to 80% from 5.00-38.00 minutes, B concentration progresses to 90% from 38.00-42.00 minutes, B concentration progresses to 90% from 42.00-

50.00 minutes, B concentration progresses to 10% from 50.00-55.00 minutes, B concentration stays at 10% from 55.00-58.00 minutes. Fractions were collected and lyophilized to obtain solid material.

Fluorescence Resonance Energy Transfer (FRET) Assay

In a 96-well plate, the BoNT/A light chain (50 μM) was incubated for 2 minutes at 37˚C with a small molecule at various concentrations in 50 mM HEPES buffer at pH 7.4. The reactions were initiated by adding SNAPtide substrate (10 μM) to give a final volume of 100 μL. The reactions were monitored by a plate reader at an excitation of 490 nm and emission of 523 nm.[14]

IC_{50} Calculations

The FRET assay was performed for each compound at various concentrations and the rates were calculated at each concentration. The rates at different concentrations were normalized to the rate of the control. The log (inhibitor) vs. normalized response – variable rate model was used to calculate the IC_{50} using Prism 6.0 (Graftpad, San diego, CA).

Compound Characterization

1. *2-(4'-chloro-[1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-3-methylpentanamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.76 (s, 1H), 8.01 (d, J = 9.1 Hz, 1H), 7.84 (s, 4H), 7.78 (d, J = 8.6 Hz, 2H), 7.57 (d, J = 8.6 Hz, 2H), 1.63 – 1.51 (m, 1H), 1.51 – 1.36 (m, 1H), 1.06 – 0.92 (m, 1H), 0.91 – 0.80 (m, 1H), 0.78 – 0.66 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.52, 142.08, 140.70, 137.39, 133.31, 129.04, 128.79, 127.03, 126.96, 58.16, 40.15, 39.99, 39.94, 39.73, 39.64, 39.52, 39.41, 39.31, 39.19, 39.10, 38.89, 36.72, 24.28, 14.95, 10.42. HRMS: calculated for $C_{18}H_{21}ClN_2O_4S$ 419.0808 $(M+Na)^+$, found 419.0805.

2. *2-(4'-chloro-[1,1'-biphenyl]-4-ylsulfonamido)-3-methylpentanamide.*
Rink Amide Resin was placed in a syringe and swollen by shaking for 30 minutes in DCM. A 25% PIP in DMF solution was added to the resin twice, once for 10 minutes and then for 15 minutes. The resin was then washed with 3x DMF, 3x MeOH, and 3x DCM. Fmoc Protected isoleucine was activated by stirring amino acid (3 equiv), 6-Cl HOBt (3 equiv), and DIC (3 equiv) in DMF for 20 minutes. The activated amino acid was added to the resin and shaken for 1.5 hours. The resin was then washed 3x DMF. A 25% PIP in DMF solution was added to the resin twice, once for 10 minutes and then for 15 minutes. The resin was then washed with 3x DMF, 3x MeOH, and 3x DCM. The resin was placed in a microwave tube with 2,6-lutidine (3 equiv), a sulfonyl chloride (3 equiv), and DCM. The tube was placed in a Biotage microwave for 5 minutes at 100˚C. The resin was then transferred to a syringe and washed 3x DMF, 3x MeOH, and 3x DCM. The small molecule was cleaved off the resin by adding 90% TFA in DCM. The end product was dried using a rotary evaporator. Each compound was purified by a preparatory HPLC (C18 reverse phase VYDAC HPLC column). A two solvent system was used with water (A) and acetonitrile (B). The gradient was as follows: B concentration progresses to 10% from 0.00-5.00 minutes, B concentration progresses to 80% from 5.00-38.00 minutes, B concentration progresses to 90% from 38.00-42.00 minutes, B concentration progresses to 90% from 42.00-

50.00 minutes, B concentration progresses to 10% from 50.00-55.00 minutes, B concentration stays at 10% from 55.00-58.00 minutes. Fractions were collected and lyophilized to obtain solid material.
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.85 (s, 4H), 7.82 - 7.71 (m, 3H), 7.62 - 7.51 (m, 2H), 7.26 (d, J = 2.1 Hz, 1H), 6.93 (s, 1H), 3.60 - 3.47 (m, 1H), 1.69 - 1.52 (m, 1H), 1.51 - 1.32 (m, 1H), 1.13 - 0.93 (m, 1H), 0.86 - 0.66 (m, 6H). ^{13}C NMR (DMSO-*d*$_6$, 400 MHz): δ 171.91, 142.10, 140.41, 137.29, 133.34, 129.06, 128.77, 127.30, 126.84, 60.52, 37.00, 24.08, 15.31, 10.83. HRMS: calculated for $C_{18}H_{21}ClN_2O_3S$ 403.0859 $(M+Na)^+$, found 403.0855.

3. *2-(4'-chloro-[1,1'-biphenyl]-4-ylsulfonamido)-3-methylpentanoic acid.*
4-benzyloxybenzyl Alcohol Wang Resin was placed in a syringe and swollen by shaking for 30 minutes in DCM. An amino acid was activated by stirring amino acid (3 equiv) and DIC (3 equiv) in DMF for 20 minutes. The activated amino acid was added to the resin with a hint od 4-dimethylaminopyridine (DMAP) and shaken for 2.5 hours. The resin was then washed 3x DMF. A 25% PIP in DMF solution was added to the resin twice, once for 10 minutes and then for 15 minutes. The resin was then washed with 3x DMF, 3x MeOH, and 3x DCM. The resin was placed in a microwave tube with 2,6-lutidine (3 equiv), a sulfonyl chloride (3 equiv), and DCM. The tube was placed in a Biotage microwave for 5 minutes at 100˚C. The resin was then transferred to a syringe and washed 3x DMF, 3x MeOH, and 3x DCM. The small molecule was cleaved off the resin by adding 95% TFA in DCM. The end product was dried using a rotary evaporator. Each compound was purified by a preparatory HPLC (C18 reverse phase VYDAC HPLC column). A two solvent system was used with water (A) and acetonitrile (B). The gradient was as follows: B concentration progresses to 10% from 0.00-5.00 minutes, B concentration progresses to 80% from 5.00-38.00 minutes, B concentration progresses to 90% from 38.00-42.00 minutes, B concentration progresses to 90% from 42.00-50.00 minutes, B concentration progresses to 10% from 50.00-55.00 minutes, B concentration stays at 10% from 55.00-58.00 minutes. Fractions were collected and lyophilized to obtain solid material.
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.13 (d, J = 9.3 Hz, 2H), 7.87 (d, J = 1.5 Hz, 4H), 7.83 - 7.72 (m, 2H), 7.62 - 7.52 (m, 2H), 3.68 - 3.55 (m, 1H), 1.77 - 1.63 (m, 1H), 1.47 - 1.30 (m, 1H), 1.22 - 1.02 (m, 1H), 0.83 (d, J = 6.7 Hz, 3H), 0.77 (t, J = 7.3 Hz, 3H). ^{13}C NMR (DMSO-*d*$_6$, 400 MHz): δ 172.16, 142.27, 140.30, 137.31, 133.44, 129.11, 128.80, 127.32, 126.99, 60.14, 36.96, 24.44, 15.42, 10.93. HRMS: calculated for $C_{18}H_{20}ClNO_4S$ 404.0699 $(M+Na)^+$,found: 404.0704

4. *1-((4'-chloro-[1,1'-biphenyl]-4-yl)sulfonyl)-N-hydroxypyrrolidine-2-carboxamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.72 (s, 1H), 7.90 (d, J = 29.3 Hz, 4H), 7.77 (t, J = 29.5 Hz, 2H), 7.58 (d, J = 8.5 Hz, 2H), 4.13 – 3.80 (m, 2H), 3.28 – 3.09 (m, 1H), 2.08 (s, 1H), 2.01 – 1.63 (m, 3H), 1.63 – 1.39 (m, 1H). ^{13}C NMR (DMSO-*d*$_6$, 400 MHz): δ 167.87, 143.08, 137.12, 137.10, 133.60, 129.12, 128.93, 128.05, 127.49, 59.37, 49.05, 30.77, 24.25. HRMS: calculated for 403.0495 $(M+Na)^+$, found 403.0485.

5. *2-(4'-chloro-[1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-3-phenylpropanamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.67 (s, 1H), 8.90 (s, 1H), 8.31 (d, J = 8.9 Hz, 1H), 7.87 – 7.67 (m, 4H), 7.67 – 7.50 (m, 4H), 7.30 – 6.97 (m, 5H), 3.92 – 3.78 (m, 1H), 2.88 – 2.75 (m, 1H), 2.72 – 2.57 (m, 1H). ^{13}C NMR (DMSO-*d*$_6$, 400 MHz): δ 166.87, 142.02, 140.39, 137.49, 136.88, 133.31, 129.15, 129.09, 129.07, 128.80,

128.01, 126.97, 126.79, 126.26, 55.57, 38.44. HRMS: calculated for $C_{21}H_{19}ClN_2O_4S$ 453.0652 $(M+Na)^+$, found 453.0659.

6. *2-(4'-chloro-[1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-3-methylbutanamide.* General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.80 (s, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.89 – 7.81 (m, 4H), 7.81 – 7.73 (m, 2H), 7.62 – 7.50 (m, 2H), 1.95 – 1.65 (m, 2H), 0.83 – 0.61 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.59, 142.09, 140.73, 137.36, 133.33, 129.05, 128.80, 127.04, 126.98, 59.72, 30.80, 18.90, 18.58. HRMS: calculated for $C_{17}H_{19}ClN_2O_4S$ 405.0652 $(M+Na)^+$, found: 405.0645

7. *2-(4'-chloro-[1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-4-methylpentanamide.* General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.68 (s, 1H), 8.07 (d, J = 8.5 Hz, 1H), 7.90 – 7.81 (m, 4H), 7.81 – 7.72 (m, 2H), 7.63 – 7.53 (m, 2H), 3.65 – 3.55 (m, 1H), 2.08 (s, 1H), 1.52 – 1.36 (m, 1H), 1.36 – 1.18 (m, 2H), 0.76 (d, J = 6.5 Hz, 3H), 0.65 (d, J = 6.5 Hz, 3H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 167.35, 142.25, 140.52, 137.37, 133.37, 129.07, 128.81, 127.11, 127.06, 52.32, 41.70, 23.79, 22.42, 21.71. HRMS: calculated for $C_{18}H_{21}ClN_2O_4S$ 419.0808 $(M+Na)^+$, found 419.0792.

8. *(R)-2-(4'-chloro-[1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-4-methylpentanamide.* General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.88 (s, 4H), 7.83 - 7.73 (m, 2H), 7.63 - 7.52 (m, 2H), 3.71 - 3.58 (m, 1H), 2.09 (d, J = 1.1 Hz, 2H), 1.46 (hept, J = 6.6 Hz, 1H), 1.39 - 1.19 (m, 1H), 0.78 (d, J = 6.5 Hz, 3H), 0.67 (d, J = 6.5 Hz, 3H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 167.41, 142.30, 140.55, 137.39, 133.40, 129.06, 128.80, 127.15, 127.06, 118.00, 52.38, 41.74, 23.82, 22.43, 21.71. HRMS: calculated for $C_{18}H_{21}ClN_2O_4S$ 419.0808 $(M+Na)^+$, found: 419.0802

9. *(2R,3R)-2-(4'-chloro-[1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-3-methylpentanamide.* General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.78 (s, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.89 (d, J = 39.5 Hz, 4H), 7.78 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H), 2.54 (s, 1H), 1.64 – 1.51 (m, 1H), 1.52 – 1.37 (m, 1H), 1.08 – 0.92 (m, 1H), 0.87 – 0.65 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.54, 142.10, 140.70, 137.40, 133.33, 129.06, 128.81, 127.04, 126.98, 58.17, 36.73, 24.29, 14.97, 10.43. HRMS: calculated for $C_{18}H_{21}ClN_2O_4S$ 419.0808 $(M+Na)^+$, found 419.0808.

10. *(2S,3R)-2-(4'-chloro-[1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-3-methylpentanamide.* General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.76 (s, 1H), 8.01 (d, J = 9.1 Hz, 1H), 7.82 (d, J = 18.3 Hz, 4H), 7.78 (d, J = 8.6 Hz, 2H), 7.57 (d, J = 8.6 Hz, 2H), 1.64 – 1.51 (m, 1H), 1.51 – 1.37 (m, 1H), 1.04 – 0.92 (m, 1H), 0.91 – 0.81 (m, 1H), 0.78 – 0.65 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.52, 142.08, 140.70, 137.39, 133.31, 129.04, 128.79, 127.03, 126.96, 58.16, 36.72, 24.28, 14.95, 10.42. HRMS: calculated for $C_{18}H_{21}ClN_2O_4S$ 419.0808 $(M+Na)^+$, found 419.0807.

11. *(2R,3S)-2-(4'-chloro-[1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-3-methylpentanamide.* General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 8.78 (s, 1H), 7.93 (d, J = 9.1 Hz, 1H), 7.84 (s, 4H), 7.81 – 7.71 (m, 2H), 7.62 – 7.47 (m, 2H), 3.54 – 3.42 (m, 1H), 1.65 – 1.48 (m, 1H), 1.38 – 1.17 (m, 1H), 1.06 – 0.90 (m, 1H), 0.82-

0.63 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 167.02, 142.03, 140.60, 137.36, 133.37, 129.06, 128.81, 127.07, 126.98, 58.10, 37.46, 25.12, 14.77, 11.20. HRMS: calculated for $C_{18}H_{21}ClN_2O_4S$ 419.0808 $(M+Na)^+$, found 419.0820.

12. *N-hydroxy-3-methyl-2-(phenylsulfonamido)pentanamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 7.89 (d, J = 7.5 Hz, 1H), 7.80 – 7.70 (m, 1H), 7.53 (d, J = 9.0 Hz, 1H), 7.42 (t, J = 7.5 Hz, 2H), 7.37 – 7.26 (m, 2H), 4.40 – 4.06 (m, 2H), 3.73 – 3.55 (m, 1H), 2.02 – 1.84 (m, 1H), 0.89 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.8 Hz, 3H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.62, 141.56, 132.13, 128.85, 128.84, 126.30, 58.16, 36.72, 24.25, 14.96, 10.44. HRMS calculated for $C_{12}H_{18}N_2O_4S$ 309.0885 $(M+Na)^+$, found 309.0891

13. *2-(2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamido)-N-hydroxy-3-methylpentanamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H), 7.73 (d, J = 8.9 Hz, 1H), 7.23 (d, J = 1.2 Hz, 2H), 7.21 (d, J = 2.2 Hz, 1H), 6.98 (t, J = 1.1 Hz, 1H), 6.96 (t, J = 1.1 Hz, 1H), 4.33 – 4.23 (m, 4H), 1.53 (d, J = 7.2 Hz, 1H), 1.46 – 1.32 (m, 1H), 1.03 – 0.83 (m, 1H), 0.77 – 0.61 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.47, 146.35, 142.78, 133.94, 119.71, 117.13, 115.56, 99.38, 64.18, 63.84, 57.97, 36.61, 24.17, 14.82, 10.33. HRMS: calculated for $C_{14}H_{20}N_2O_6S$ 367.0940 $(M+Na)^+$, found 367.0951.

14. *2-(4'-chloro-[1,1'-biphenyl]-3-ylsulfonamido)-N-hydroxy-3-methylpentanamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (d, J = 1.4 Hz, 1H), 8.77 (d, J = 1.7 Hz, 1H), 8.10 – 7.99 (m, 2H), 7.92 – 7.84 (m, 1H), 7.81 – 7.71 (m, 2H), 7.67 – 7.53 (m, 3H), 3.39 (d, J = 6.3 Hz, 1H), 2.08 (s, 1H), 1.62 – 1.38 (m, 1H), 1.05 – 0.91 (m, 2H), 0.74 (d, J = 6.8 Hz, 3H), 0.70 (t, J = 7.4 Hz, 3H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 165.32, 141.13, 138.28, 136.57, 131.89, 129.11, 128.49, 127.86, 127.54, 124.32, 123.30, 98.38, 57.06, 35.55, 23.11, 13.85, 9.26.HRMS: calculated for $C_{18}H_{21}ClN_2O_4S$ 419.0808 $(M+Na)^+$, found: 419.0807

15. *2-([1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-3-methylpentanamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.78 (s, 1H), 7.99 (d, J = 9.0 Hz, 1H), 7.83 (s, 3H), 7.79 – 7.71 (m, 2H), 7.54 – 7.47 (m, 2H), 7.47 – 7.39 (m, 1H), 3.42 – 3.37 (m, 2H), 1.57 (d, J = 6.9 Hz, 1H), 1.51 – 1.38 (m, 1H), 1.04 – 0.91 (m, 1H), 0.78 – 0.63 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.58, 143.47, 140.38, 138.58, 129.08, 128.38, 127.00, 126.98, 58.16, 36.74, 24.28, 14.96, 10.43. HRMS: calculated for $C_{18}H_{22}N_2O_4S$ 385.1198 $(M+Na)^+$, found 385.1198.

16. *N-hydroxy-2-(4'-methoxy-[1,1'-biphenyl]-4-ylsulfonamido)-3-methylpentanamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H), 8.73 (s, 1H), 7.87 (d, *J* = 8.9 Hz, 2H), 7.78 – 7.67 (m, 4H), 7.60 (t, *J* = 19.5 Hz, 2H), 6.99 (d, *J* = 8.7 Hz, 2H), 3.74 (s, 3H), 1.58 – 1.44 (m, 1H), 1.44 – 1.29 (m, 1H), 1.02 – 0.83 (m, 1H), 0.73 – 0.51 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.70, 166.69, 159.67, 143.17, 139.60, 130.83, 129.54, 128.22, 127.23, 127.03, 126.30, 126.14, 114.58, 114.56, 58.22, 55.28, 36.82, 24.33, 15.01, 10.49. HRMS: calculated for $C_{19}H_{24}N_2O_5S$ 415.1304 $(M+Na)^+$, found: 415.1299

17. *2-(4'-acetamido-[1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-3-methylpentanamide.*

General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (d, J = 5.7 Hz, 1H), 7.95 (d, J = 9.0 Hz, 1H), 7.82 (d, J = 13.3 Hz, 5H), 7.74 – 7.65 (m, 4H), 7.64 – 7.51 (m, 1H), 7.22 (d, J = 8.4 Hz, 1H), 2.07 (d, J = 6.1 Hz, 3H), 1.64 – 1.51 (m, 1H), 1.50 – 1.35 (m, 1H), 1.09 – 0.90 (m, 1H), 0.81 – 0.60 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 168.49, 166.59, 143.01, 139.82, 139.67, 132.86, 127.31, 126.99, 126.34, 126.08, 125.57, 119.30, 58.17, 36.78, 24.29, 24.08, 14.98, 10.46. HRMS: calculated for $C_{20}H_{25}N_3O_5S$ 442.1413 $(M+Na)^+$, found 442.1401.

18. *2-(4'-fluoro-[1,1'-biphenyl]-4-ylsulfonamido)-N-hydroxy-3-methylpentanamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (d, J = 1.7 Hz, 1H), 8.79 (t, J = 1.2 Hz, 1H), 8.00 (d, J = 8.9 Hz, 1H), 7.91 - 7.74 (m, 6H), 7.41 - 7.26 (m, 2H), 3.41 (t, J = 8.7 Hz, 1H), 1.68 - 1.53 (m, 1H), 1.52 - 1.39 (m, 1H), 1.07 - 0.91 (m, 1H), 0.82 - 0.62 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.59, 163.62, 161.18, 142.42, 140.37, 135.09, 135.06, 129.18, 129.10, 127.03, 126.94, 116.05, 115.84, 58.20, 36.76, 24.31, 14.98, 10.45. HRMS: calculated for $C_{18}H_{21}FN_2O_4S$ 403.1104 $(M+Na)^+$, found 403.1090.

19. *N-hydroxy-3-methyl-2-(4'-methyl-[1,1'-biphenyl]-4-ylsulfonamido)pentanamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H), 8.73 (s, 1H), 7.89 (d, J = 9.0 Hz, 2H), 7.82 – 7.69 (m, 4H), 7.57 (d, J = 8.2 Hz, 2H), 7.32 – 7.16 (m, 2H), 2.29 (s, 3H), 1.58 – 1.44 (m, 1H), 1.47 – 1.21 (m, 1H), 1.02 – 0.79 (m, 1H), 0.74 – 0.44 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.65, 143.41, 140.07, 137.92, 135.69, 129.70, 129.69, 127.00, 126.84, 126.65, 58.20, 36.79, 24.31, 20.72, 14.99, 10.46. HRMS: calculated for $C_{19}H_{24}N_2O_4S$ 399.1354 $(M+Na)^+$, found 399.1356.

20. *(2R,3R)-2-(4'-chloro-[1,1'-biphenyl]-3-ylsulfonamido)-N-hydroxy-3-methylpentanamide.*
General synthesis was used to make this compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 8.78 (s, 1H), 8.09 - 8.00 (m, 2H), 7.92 - 7.86 (m, 1H), 7.82 - 7.72 (m, 3H), 7.64 (t, J = 7.8 Hz, 1H), 7.60 - 7.54 (m, 2H), 3.41 (t, J = 8.7 Hz, 1H), 1.66 - 1.52 (m, 1H), 1.52 - 1.37 (m, 1H), 1.07 - 0.90 (m, 1H), 0.82 - 0.62 (m, 6H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 166.44, 142.31, 139.45, 137.69, 133.06, 130.25, 129.64, 129.00, 128.70, 125.43, 124.45, 58.17, 36.70, 24.27, 14.95, 10.34. HRMS: calculated for $C_{18}H_{21}ClN_2O_4S$ 419.0808 $(M+Na)^+$, found: 419.0807

Molecular Docking

Molecular Docking was performed using AutoDock Vina (The Scripps Research Institute, La Jolla, CA) on a Dell Precision T1650 desktop running Windows 8 with an Intel core i7-3770 CPU. The solved X-ray crystal structure of the BoNT/A LC (pdb# 4EJ5) was used as a static receptor for docking. The crystal structure was prepared for docking by removing all crystallographic waters and merging all non-polar hydrogens into the structure and leaving polar hydrogens. A docking grid box (43 x 49 x 39 Angstrom) was generated and centered around the zinc in active site and used as the search space for docking.
The small molecule ligands were drawn using ChemDraw (Perkin Elmer) and minimized with Chem3D (Perkin Elmer). The docking was set at an exhaustiveness of 100 and nine binding conformation were produced. The best binding energies (kcal/mol) for **1** and **20** in the active site are shown in Figure 3.