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Synthesis/biological evaluation of hydroxamic acids and their prodrugs as inhibitors for Botulinum neurotoxin A light chain

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ABSTRACT

Botulinum neurotoxin A (BoNT/A) is the most potent toxin known. Unfortunately, it is also a potential bioweapon in terrorism, which is without an approved therapeutic treatment once cellular intoxication takes place. Previously, we reported how hydroxamic acid prodrug carbamates increased cellular uptake, which translated to successful inhibition of this neurotoxin. Building upon this research, we detail BoNT/A protease molecular modeling studies accompanied by the construction of small library of hydroxamic acids based on 2,4-dichlorocinnamic hydroxamic acid scaffold and their carbamate prodrug derivatization along with the evaluation of these molecules in both enzymatic and cellular models.

1. Introduction

Botulinum neurotoxin, a protein produced by *Clostridium botulinum*, is the most potent toxin known to date.[1] Although the toxin has been historically recognized in food poisoning, its application to medical maladies such as chronic pain and migraines; as well as cosmetic improvements including facial wrinkles, provides a clinical significance that has led to its commercial production.[2,3] This, however, also implicates the increased potential use of this toxin as a bioweapon in terrorism.[4] Indeed, Botulinum neurotoxin has been classified as a category A agent, the highest class for potential misuse as a biothreat by the Center for Disease Control and Prevention.

Botulinum neurotoxin consists of 100 kDa heavy chain and 50 kDa light chain, which are appended to each other by a disulfide linkage.[5] In brief, the etiology of this neurotoxin consists of the heavy chain acting to deliver the light chain into the cytosol by translocation through target receptors on nerve terminals, and depending on the serotype, the light chain cleaves one of the proteins necessary for SNARE complex formation.[6] As a result, the release of acetylcholine into neuromuscular junction ceases, which can lead to fatal flaccid paralysis. The serotypes A, B, and E are known to cause Botulism in humans. Among the SNARE components, type A and E target SNAP-25 whereas type B cleaves VAMP.[7,8] In addition to the aforementioned human intoxication, its potency (LD_{50} value of 0.001 μg per kg)[9] and the length of duration (up to several months) sets Botulinum neurotoxin A apart from the other serotypes and renders it to be a focal point of many laboratories including our own.[10]

While there are known treatments such as vaccination and antitoxin treatment for serotype A, no therapeutic is currently available once BoNT/A cellular intoxication takes place. Because the BoNT protease is responsible for the pathology seen with all neurotoxins, it is incumbent to develop small molecules to counteract its mechanism of action. To date, several potent light chain protease inhibitors have been reported based on in vitro enzyme assays, including peptidic and non-peptidic molecules.[11,12] However, the development of efficacious inhibitors that can function intracellularly still remains a major hurdle to overcome in the scientific community.

Hydroxamic acids developed in our laboratory as BoNT/A light chain protease inhibitors have suffered from a lack of cellular protection, presumably due to their poor cellular uptake and toxicity.[11] However, we recently discovered that these issues could be resolved and ultimately circumvented through protection of the

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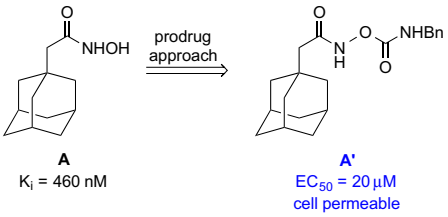


Scheme 1. *O*-Carbamation of the hydroxamic acid moiety as a prodrug approach.

hydroxamic acid moiety as a carbamate (Scheme 1).[13] Thus, the *N*-benzyl carbamate of hydroxamic acid **A** became cell-permeable and now demonstrated inhibition of BoNT/A with the EC_{50} value of 20 μM in human induced pluripotent stem cells (hiPSCs).[14]

With this prodrug approach in mind, we next turned our focus to additional druggable scaffolds (Scheme 2). Previously our laboratory disclosed cinnamic hydroxamic acid **1** as a potent hit from hydroxamic acid library screening,[15,16] and a congener of this molecule, hydroxyethyl hydroxamate **2** as an inhibitor with an additional element of chemical functionality.[17] To further diversify this line of research, we devised two other scaffolds: amides (**3**) and ethers (**4**) as a means to further increase hydrophobic interactions within the active site of enzyme. Herein, we communicate the synthesis/molecular modeling of amides (**3**), ethers (**4**), and β-(2,4-dichlorophenyl)-hydroxamic acid *O*-carbamates along with their inhibitory activity as determined through enzymatic as well as cellular assays.

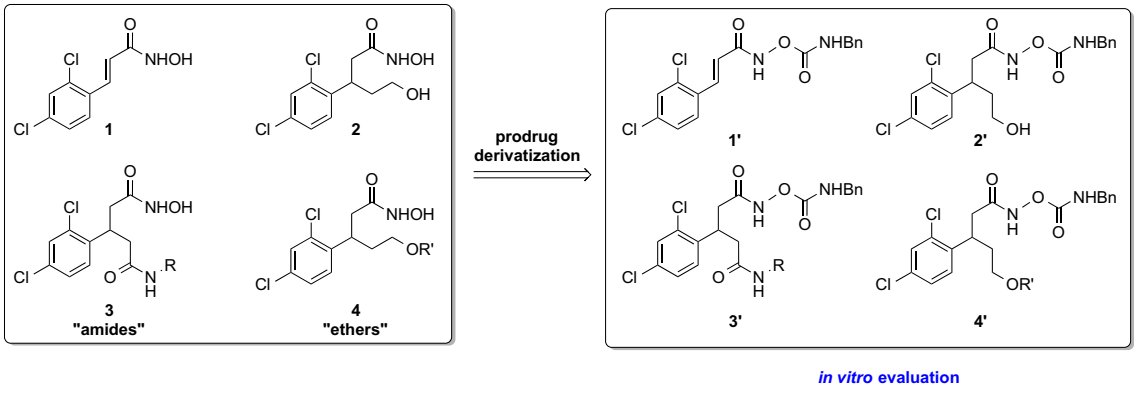
2. Results and discussion

2.1. Computational studies

The program Autocorrelator was utilized to construct computational models predicting and ranking potency of derived structures against BoNT LC/A using **2** as a starting point for synthetically accessible targets, amides **3** and ethers **4**.[18] In brief, a model was developed through Autocorrelator using the 2IMB co-crystal structure of BoNT LC/A with a computational pipeline consisting of Omega v2.4.6 and Fred v2.2.5 ($R^2 = 0.757$).[16,19,20] Autocorrelator was also used to derive a new scoring function, produced by the LARS methodology in R, shown in Eq. 1[21,22]:

$$\begin{aligned} pIC_{50} = &-0.43 + \text{Chemgauss3}^{*} - 0.0714 \\ &+ \text{Chemgauss3.Desolvation}^{*} - 0.142 \\ &+ \text{Chemgauss3.Metal}^{*} - 0.0388 \end{aligned} \quad (1)$$

Thus, computational modeling provided us a series of structures as potential inhibitors with predicted IC_{50} values (Fig. 1). From this computational exercise, aryl groups or planar structures seemed to



Scheme 2. Application of a prodrug approach to β-(2,4-dichlorophenyl)-hydroxamic acids.

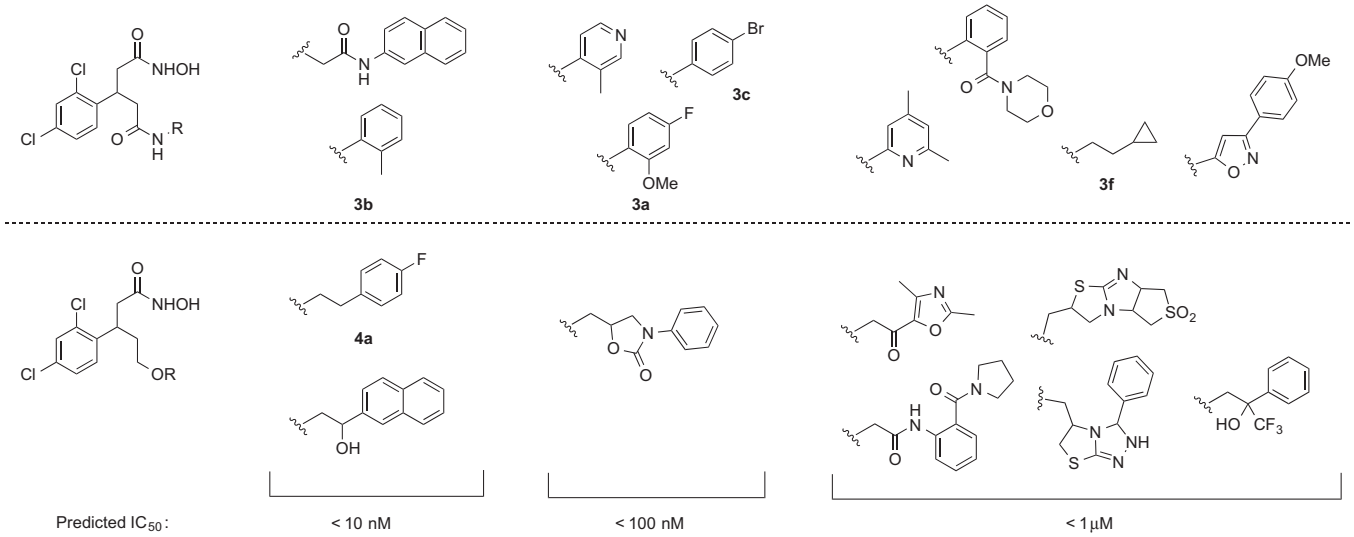


Figure 1. Predicted BoNT/A protease inhibitors and their IC_{50} values.

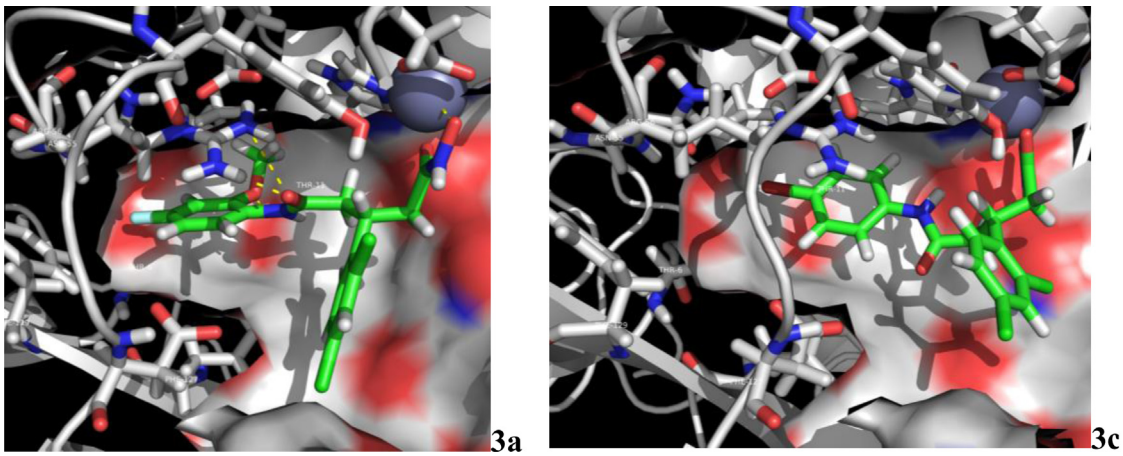


Figure 2. The docking solutions of **3a** and **3c** within the 2IMB co-crystal using the automated pipeline derived by Autocorrelator.

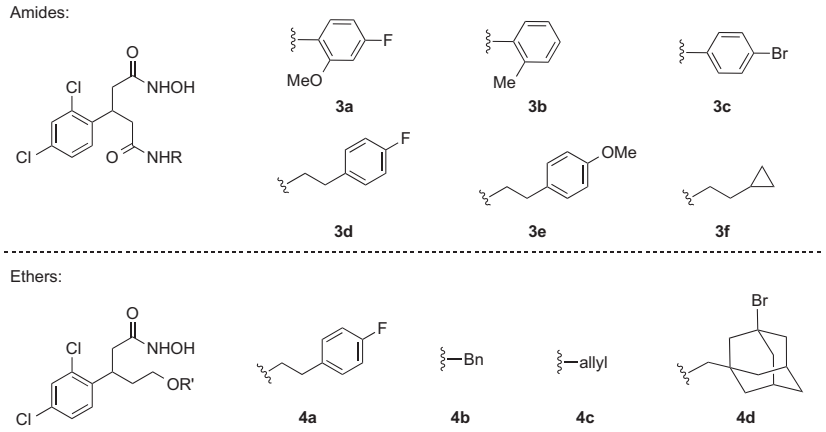


Figure 3. Selected synthetic targets as possible protease inhibitors.

be important for engaging active site interactions. Two docking solutions are shown in Figure 2, and based on these modeling parameters, compounds such as **3a** and **3c** displayed optimized steric and hydrogen-bonding interactions within the active site. The size of the substituents also affected both the orientation and hydrogen-bonding partners of our docked poses. Thus, larger substituents forced molecules to lose interactions with Thr11, Arg56, and Thr6, although this should have only a modest impact on potency, as predicted molecules were able to still maintain the key hydroxamate-zinc interaction. Hence, among the compounds shown in Figure 1, we selected **3a**–**c**, **3f**, and **4a** due to the synthetic accessibility. Lastly, as many of the structures shown in Figure 1 were synthetically challenging, close congeners (**3d**, **3e**, **4b**–**d**, Fig. 3) were prepared for examination.

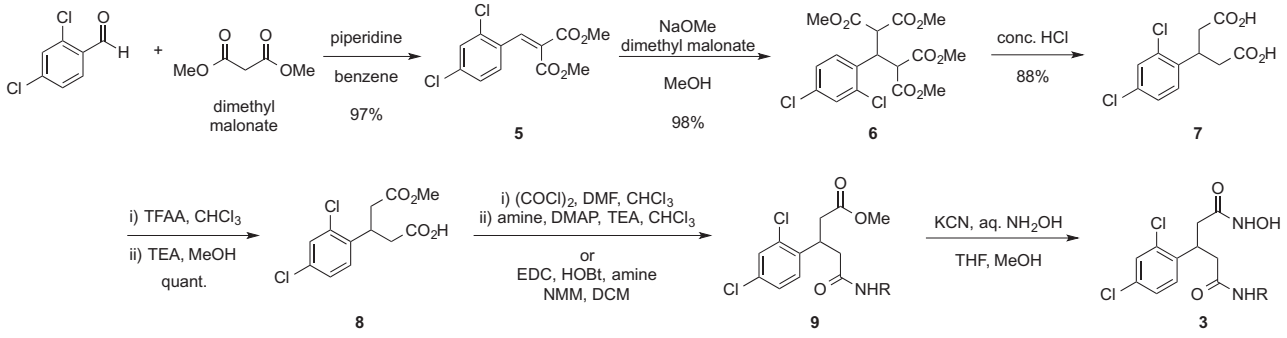
2.2. Synthesis of hydroxamic acids

Synthesis of the target amide molecules was conducted using a similar strategy to our previous report (Scheme 3).[17] Thus, dichlorobenzaldehyde was condensed with dimethyl malonate in the presence of piperidine to provide the α,β-unsaturated diester **5**. Dimethyl malonate underwent a Michael addition with **5** under basic conditions, affording tetraester **6**, which was then subjected to decarboxylation to furnish diacid **7**. This diacid was mono-esterified via the initial formation of an anhydride with TFAA and subsequent nucleophilic opening with MeOH. The resulting acid **8** was converted to various amides **9** under the conventional coupling conditions. Finally, each amide was reacted with hydroxylamine to provide the desired hydroxamic acid **3**.[23]

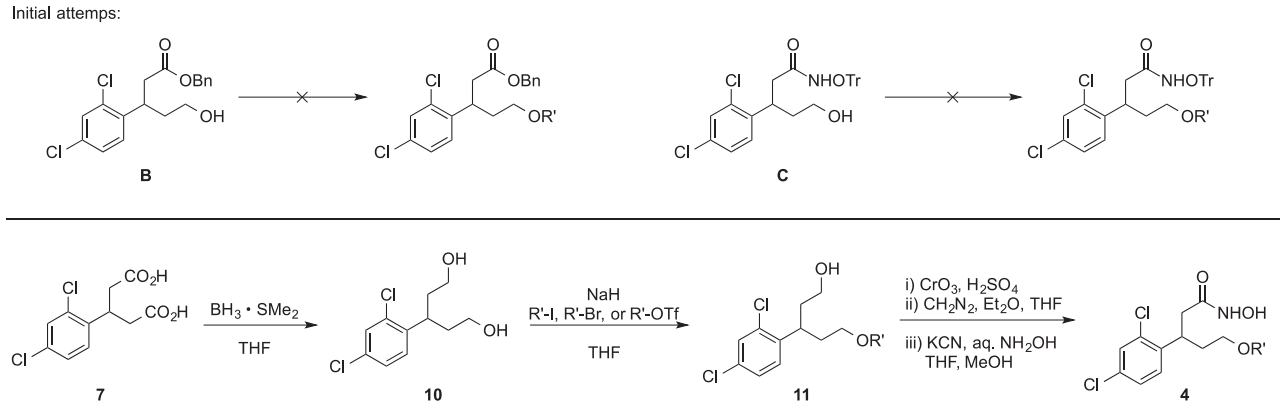
In terms of a similar set of ethers, we initially envisioned the alkylation of alcohol **B** or **C** as a convergent approach (Scheme 4). However, either route was found to be untenable, likely due to the instability of starting materials under basic conditions. Thus, although slightly more cumbersome, an alternative strategy was undertaken. Hence, diacid **7** was reduced to diol **10**, which was then mono-alkylated with several electrophiles to provide alcohols **11**. Each alcohol was subjected to three transformations in one-pot: (1) oxidation of the alcohol to an acid by Jones' reagent; (2) formation of methyl ester using diazomethane; (3) conversion of the ester to hydroxamic acid **4**. We note that using this sequence all attempts to isolate the methyl ester were not successful.

2.3. Screening of selected hydroxamic acids against the BoNT/A light chain 1–425

With desired hydroxamic acids in hand, their inhibitory activity was evaluated in vitro using an established FRET-based assay (Table 1).[24] The compounds were tested against truncated Botulinum neurotoxin A light chain (1–425 residues) in the presence of SNAPtide, a 13mer SNAP-25 pseudosubstrate containing a FITC fluorophore and a DABCYL quencher. Based on the results of the SNAPtide assay, the aryl moiety seems to be important.

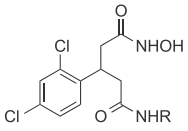


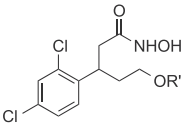
Scheme 3. Synthesis of amides **3**.

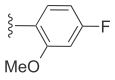
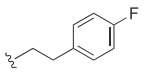
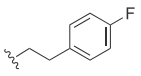
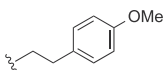
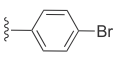
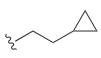
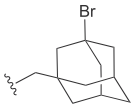


Scheme 4. Synthesis of ethers **4**.

Table 1
In vitro evaluation of amides **3** and ethers **4**





	R	IC_{50} (μM)		R	IC_{50} (μM)		R′	IC_{50} (μM)
3a		1.1 K_i = 1.0	**3d**		2.6	**4a**		0.97 K_i = 2.1
3b		2.0	**3e**		6.7	**4b**		2.9
						4c		5.4
3c		6.4	**3f**		9.2	**4d**		13

This was true with either the amide or ether appendage, whereas simple alkyl chains did not contribute to the inhibition seen. Based on these findings, the most potent amide and ether homologue of **2** were further evaluated in terms of K_i using our 66mer assay, where a cleaved product from 66 residues found within SNAP-25 (141–206 residues) was quantified by LCMS analysis.[25] As anticipated, inhibitors **3a** and **4a** showed competitive inhibition with K_i values of 1.0 and 2.1 μM respectively (Table 1 and Fig. 4).

2.4. Synthesis of BoNT/A protease prodrugs

With in vitro assessment accomplished, several of the hydroxamic acids (**1**, **2**, **3a**–**3d**, **4a**–**b**, **4d**) were converted to the

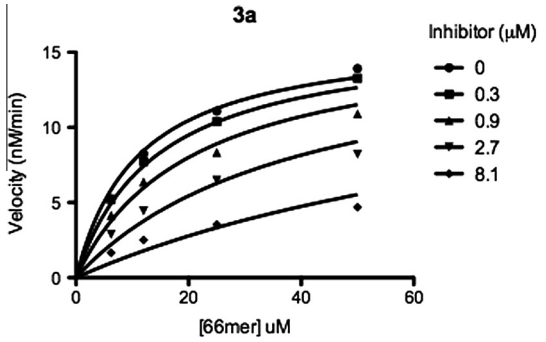
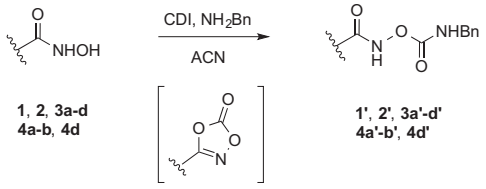


Figure 4. Kinetic analysis of inhibitor **3a**.



Scheme 5. Generic scheme showing synthetic access to the carbamate prodrugs.

Table 2
Prodrug screening using hiPSC-derived neurons

Prodrug	Inhibition
1′	Cytotoxic at 150 μM[a]
2′	Partial inhibition at 300 μM
3a′	EC_{50} > 300 μM
3b′	EC_{50} = 209 μM
3c′	EC_{50} = 198 μM
3d′	EC_{50} > 300 μM
4a′	Cytotoxic at 1 mM[a]
4b′	Cytotoxic at 1 mM[a]
4d′	EC_{50} >1 mM

[a] No inhibition was observed up to the cytotoxic concentration examined.

corresponding benzylcarbamates (**1′**, **2′**, **3a′**-**3d′**, **4a′**-**b′**, **4d′**) as prodrugs via the formation of a carbonate intermediate and subsequent nucleophilic addition of benzylamine (Scheme 5).[26] This protocol achieves a selective *O*-carbamation (vs *N*-carbamation) as shown previously.[14]

2.5. Testing of selected protease inhibitors in a BoNT/A cell based assay

Selected hydroxamic acids and corresponding prodrugs were tested in a hiPSC-derived neuron assay. The importance of this cell line and assay is that it expresses the necessary receptors and substrates for BoNT/A intoxication. The intoxicated cells were incubated with each compound for 8 h, and upon cell lysis, the SNAP-25 cleavage was analyzed by Western blot. The initial screening was conducted at one or two concentrations (typically 0.1 and 1.0 mM). Unfortunately this assay revealed that most of the hydroxamic acids were not effective due to cytotoxicity. Interestingly, **2**, was an exception, as it did not show cytotoxicity, although no inhibition was observed at 200 μM. Next, the carbamate prodrugs were examined in this cellular assay (Table 2). Carbamate prodrugs **1′** and **2′** were found to be ineffective inhibitors due to cytotoxicity and potency respectively, whereas amide prodrugs **3a′**–**3d′** showed reasonable protection of SNAP-25 from the neurotoxin without apparent cytotoxicity. The EC_{50} values were further determined for these compounds with **3c′** being the most potent, 198 μM (Table 2). Disappointingly, prodrug ethers (**4a′**, **4b′** and **4d′**) did not improve their cellular activity. This could partially be due to a lack of solubility, which may also contribute to cytotoxicity seen.

3. Conclusions

In summary, we have applied molecular modeling and a prodrug strategy to our 2,4-dichlorocinnamic hydroxamic acid scaffold, thus preparing a series of amide and ether *O*-carbamate hydroxamic acids. The computational modeling studies granted insights into the BoNT/A protease's active site, which translated to a more effective approach, generating molecules for our screening efforts. In general, these molecules as hydroxamic acids were toxic to the cells, whereas their prodrug *O*-carbamates showed modest inhibitory activity without major cellular toxicity. While we were pleased to see our prodrug approach was translatable to alternate scaffolds, in vitro cellular potency was marginal. We surmise this could be due to the inefficient release of the hydroxamic acid warhead from the corresponding prodrug, presumably enzyme-assisted within the cell. Future research will entail exploration of the enzyme responsible for carbamate hydrolysis as well as an alternative Zn chelator to avoid the inherent toxicity of hydroxamic acids.

4. Experimental section

4.1. Chemistry

4.1.1. Tetramethyl 2-(2,4-dichlorophenyl)propane-1,1,3,3-tetracarboxylate (6)

To a stirred solution of dimethylmalonate (26.2 mmol, 3.46 g, 1.20 equiv) in NaOMe/MeOH solution (0.5 M, 26.2 mmol, 52 mL), dimethyl 2-(2,4-dichlorobenzylidene)malonate (21.9 mmol, 6.32 g) was added dropwise at ambient temperature. After 1 h, the reaction mixture was cooled to 0 °C and quenched by the addition of AcOH (87.4 mmol, 5.24 g, 4.0 equiv). Upon evaporation of volatiles, reaction mixture was re-dissolved in dichloromethane and H_2O. The partitioned organic layer was dried over $MgSO_4$, and concentrated in vacuo. The crude mixture was subjected to column chromatography (hexanes/EtOAc) to afford the titled compound as clear oil (9.02 g, 98%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 (s, 2H), 7.19 (d, J = 8.5 Hz, 1H), 4.79 (s, 1H), 4.37−4.12 (m, 2H), 3.70 (s, 6H), 3.57 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.1, 167.8, 135.9, 134.5, 134.2, 130.0, 129.9, 127.4, 54.0, 53.0, 52.8; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{17}H_{19}Cl_2O_8$: 421.0451, found 421.0450.

4.1.2. 3-(2,4-Dichlorophenyl)pentanedioic acid (7)

Tetraester **6** (10.4 mmol, 4.40 g) was dissolved in aq. HCl (37%, 30 mL) and heated to reflux overnight. The white solid was collected by filtration to provide the titled compound as white solid (2.56 g, 88%).

^{1}H NMR (600 MHz, MeOD-d_4) δ 7.42 (s, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 4.06 (p, J = 6.9 Hz, 1H), 2.70 (m, 4H); ^{13}C NMR (151 MHz, MeOD-d_4) δ 174.9, 140.6, 135.8, 133.9, 130.4, 130.3, 128.4, 39.4, 35.4; HRMS (ESI-TOF) m/e calc'd for $[M+H]^+$ $C_{11}H_{11}Cl_2O_4$: 277.0029, found 277.0033.

4.1.3. 3-(2,4-Dichlorophenyl)-5-methoxy-5-oxopentanoic acid (8)

To a solution of diacid **7** (3.00 mmol, 831 mg, 1.0 equiv) in chloroform (20 mL) at 0 °C, TFAA (6.00 mmol, 1.26 g, 2.0 equiv) was added dropwise. The reaction was stirred at ambient temperature for 2 h and concentrated in vacuo. The resulting acid anhydride

was dissolved in MeOH (40 mL) at 0 °C. TEA (12.0 mmol, 1.21 g, 4.0 equiv) was added to this solution, and the reaction mixture was stirred at ambient temperature overnight. Upon evaporation of solvent, the crude mixture was re-dissolved in EtOAc, washed with aq. HCl (1 M), brine, dried over $MgSO_4$, and concentrated to afford the titled product (900 mg, quant.) as yellow oil, which was used to next reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (s, 1H), 7.24−7.15 (m, 2H), 4.07 (m, 1H), 3.61 (s, 3H), 2.76 (m, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.1, 171.7, 138.1, 134.6, 133.4, 130.0, 128.9, 127.5, 52.0, 38.4, 38.2, 34.0; HRMS (ESI-TOF) *m*/*e* calcd for $[M+H]^+$ $C_{12}H_{13}Cl_2O_4$: 290.0185, found 290.0189.

4.1.4. 3-(2,4-Dichlorophenyl)pentane-1,5-diol (10)

To a solution of diacid **7** (4.00 mmol, 1.11 g, 1.0 equiv) in THF (40 mL), BH_3 (THF solution of dimethyl sulfide complex, 2 M, 12.0 mmol, 6.00 mL, 3.0 equiv) was added at −78 °C. The reaction mixture was warmed to ambient temperature and stirred for 3 h, which was quenched by the addition of aq $NaHCO_3$ at −78 °C. This crude mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting crude product was purified by column chromatography (MeOH/dichloromethane) to afford the titled compound (918 mg, 92%) as clear oil.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.23 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 3.61–3.41 (m, 5H), 1.97 (m, 2H), 1.90–1.70 (m, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 140.7, 135.0, 132.5, 129.5, 129.1, 127.8, 60.6, 38.8, 33.9; HRMS (ESI-TOF) *m*/*e* calcd for $[M+H]^+$ $C_{11}H_{15}Cl_2O_2$: 249.0444, found 249.0448.

4.1.5. General procedure for mono-alkylation of diol 10

To a solution of diol **10** (1.0 equiv) in THF (0.1 M) was added NaH (2.0 equiv) at 0 °C. After stirring for 0.5 h, electrophile (1.0 equiv) was added, and the solution was stirred at ambient temperature overnight. Upon evaporation of solvent, crude mixture was directly subjected to column chromatography (Hexanes/EtOAc) to afford mono-alkylated product as clear oil.

- For the synthesis of **11c**, TBAI (1.0 equiv) was added together with electrophile.
- For the synthesis of **11d**, 18-Crown-6 (1.0 equiv) was added together with electrophile.

4.1.6. 3-(2,4-Dichlorophenyl)-5-(4-fluorophenethoxy)pentan-1-ol (11a)

43.8 mg, 49% ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36 (s, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.13 (d, J = 7.4 Hz, 3H), 6.96 (t, J = 8.2 Hz, 2H), 3.60–3.38 (m, 6H), 3.31 (q, J = 6.5, 5.9 Hz, 1H), 3.23 (q, J = 8.0, 7.5 Hz, 1H), 2.79 (t, J = 6.9 Hz, 2H), 1.96 (tq, J = 13.9, 6.7 Hz, 2H), 1.82 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.6 (J_{C-F} = 243 Hz), 140.7, 135.1, 134.8 (J_{C-F} = 3.02 Hz), 132.4, 130.4 (J_{C-F} = 7.55 Hz), 129.4, 129.1, 127.6, 115.2 (J_{C-F} = 21.1 Hz), 72.0, 68.7, 60.8, 39.0, 35.9, 35.6, 34.4; HRMS (ESI-TOF) *m*/*e* calc'd for $[M+H]^+$ $C_{19}H_{22}Cl_2FO_2$: 371.0975, found 371.0973.

4.1.7. 4-Fluorophenethyl trifluoromethanesulfonate

To a solution of 4-fluorophenethyl alcohol (1.00 mmol, 140 mg, 1.0 equiv) in chloroform (5 mL) were added Tf_2O (1.50 mmol, 423 mg, 1.5 equiv) and 2,6-lutidine (1.70 mmol, 182 mg, 1.7 equiv) at 0 °C. After stirring for 1 h, the reaction was concentrated in vacuo, re-dissolved in hexanes/EtOAc (2:1), washed with aq. citric acid (10%), aq $NaHCO_3$, brine, dried over $MgSO_4$, and again concentrated. Due to the instability of titled compound on SiO_2, the obtained crude product (244 mg, 90%, reddish oil) was used for the synthesis of **11a** without further purification.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.23–7.13 (m, 2H), 7.04 (t, J = 8.2 Hz, 2H), 4.67 (t, J = 6.8 Hz, 2H), 3.11 (t, J = 6.8 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.3 (J_{C-F} = 246 Hz), 130.67, 130.67 (J_{C-F} = 7.55 Hz), 118.7 (q, J_{C-F} = 320 Hz), 115.8 (J_{C-F} = 21.1 Hz), 77.1, 35.1; HRMS (ESI-TOF) *m*/*e* calc'd for $[M+H]^+$ $C_9H_9F_4O_3S$: 273.0209, found none.

4.1.8. 5-(Benzyloxy)-3-(2,4-dichlorophenyl)pentan-1-ol (11b)

47.4 mg, 47% ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39–7.29 (m, 3H), 7.27 (s, 2H), 7.24–7.13 (m, 2H), 4.40 (t, J = 8.2 Hz, 2H), 3.50 (m, 4H), 3.37 (m, 1H), 3.30 (m, 1H), 1.93 (m, 5H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 140.7, 138.4, 135.1, 132.4, 129.5, 128.5, 128.4, 127.8, 127.7, 127.6, 73.2, 68.2, 60.8, 39.1, 35.9, 34.5; HRMS (ESI-TOF) *m*/*e* calcd for $[M+H]^+$ $C_{18}H_{21}Cl_2O_2$: 339.0913, found 339.0908.

4.1.9. 5-(Allyloxy)-3-(2,4-dichlorophenyl)pentan-1-ol (11c)

44.2 mg, 51% ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.23 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H), 5.85 (ddt, J = 15.8, 10.3, 5.6 Hz, 1H), 5.21 (d, J = 17.2 Hz, 1H), 5.13 (d, J = 10.4 Hz, 1H), 3.87 (d, J = 4.5 Hz, 2H), 3.57–3.43 (m, 3H), 3.31 (q, J = 7.1 Hz, 1H), 3.24 (q, J = 8.9, 7.9 Hz, 1H), 1.99 (m, 2H), 1.85 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 140.7, 135.1, 134.8, 132.4, 129.5, 129.1, 127.6, 117.1, 72.1, 68.1, 60.8, 39.1, 36.0, 34.4; HRMS (ESI-TOF) *m*/*e* calcd for $[M+H]^+$ $C_{14}H_{19}Cl_2O_2$: 289.0757, found 289.0754.

4.1.10. ((1*r*,3*r*)-3-Bromoadamantan-1-yl)methyl trifluoromethanesulfonate

To a solution of alcohol (1.10 mmol, 270 mg, 1.0 equiv) in dichloromethane (10 mL) were added Tf_2O (1.32 mmol, 372 mg, 1.2 equiv) and 2,6-lutidine (1.43 mmol, 153 mg, 1.3 equiv) at 0 °C. After stirring for 3 h, the reaction was concentrated in vacuo, re-dissolved in Et_2O, washed with aq. citric acid (10%), aq. $NaHCO_3$, brine, dried over $MgSO_4$, and again concentrated. The obtained crude product (377 mg, 91%, pinkish oil) was used for the synthesis of **11d** without further purification. The alcohol, starting material, was prepared from the corresponding acid according to the literature procedure.[27]

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.11 (s, 2H), 2.35 (d, J = 11.7 Hz, 2H), 2.30–2.21 (m, 4H), 2.18 (s, 2H), 1.78–1.71 (m, 1H), 1.69–1.62 (m, 1H), 1.59 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 118.8 (q, J_{C-F} = 320 Hz), 84.2, 62.7, 49.6, 48.2, 38.5, 36.7, 34.7, 31.5; HRMS (ESI-TOF) *m*/*e* calcd for $[M+H]^+$ $C_{12}H_{17}BrF_3O_3S$: 375.9956, found none.

4.1.11. 5-(((1*r*,3*r*)-3-Bromoadamantan-1-yl)methoxy)-3-(2,4-dichlorophenyl)pentan-1-ol (11d)

98.4 mg, 51% ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.23 (d, J = 8.3 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H), 3.51 (m, 3H), 3.24 (m, 2H), 2.91 (s, 2H), 2.36–2.21 (m, 4H), 2.12 (m, 4H), 2.03–1.91 (m, 2H), 1.91–1.75 (m, 2H), 1.67 (m, 1H), 1.60 (m, 2H), 1.47 (m, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 140.9, 135.0, 132.3, 129.5, 129.1, 127.6, 80.6, 69.4, 66.4, 60.8, 51.3, 48.9, 39.5, 39.3, 37.9, 35.9, 35.3, 34.6, 32.3; HRMS (ESI-TOF) *m*/*e* calcd for $[M+H]^+$ $C_{22}H_{30}BrCl_2O_2$: 475.0801, found 475.0807.

4.1.12. General procedure for amide coupling

4.1.12.1. EDC coupling. To a mixture of acid (1.0 equiv), amine (1.2 equiv), HOBt (2.0 equiv), NMM (2.0 equiv) in DCM (0.1 M) was added EDC (2.0 equiv) at 0 °C. The reaction was warmed to ambient temperature and stirred overnight. Upon evaporation, crude mixture was re-dissolved in EtOAc, washed with aq HCl (1 M), aq $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography (Hexanes/EtOAc) to afford the amide product as white solid.

4.1.12.2. Coupling via acid chloride formation. To a solution of acid **8** (1.0 equiv), DMF (1 drop) in chloroform (0.1 M) at 0 °C was added $(COCl)_2$ (4.0 equiv). The resulting solution was warmed to ambient temperature and stirred for 1 h. Upon evaporation, this acid chloride was dissolved in chloroform (0.1 M). The solution was cooled to 0 °C, followed by the addition of TEA (1.2 equiv), DMAP (1 crystal) and aniline (1.0 equiv), and stirred at ambient temperature overnight. Upon evaporation, the crude mixture was dissolved in EtOAc/H_2O. The partitioned organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The crude product was purified by column chromatography (Hexanes/EtOAc) to provide amide product as white solid.

4.1.13. Methyl 3-(2,4-dichlorophenyl)-5-((4-fluoro-2-methoxyphenyl)amino)-5-oxopentanoate (9a)

The aniline was prepared from the corresponding nitro compound according to the literature procedure.[28]

55.0 mg, 66% ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (t, J = 6.8 Hz, 1H), 7.56 (s, 1H), 7.39 (s, 1H), 7.21 (q, J = 8.2 Hz, 2H), 6.60 (m, 2H), 4.18 (q, J = 6.5 Hz, 1H), 3.83 (s, 3H), 3.60 (s, 3H), 2.90 (m, 1H), 2.84–2.75 (m, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.0, 168.4, 159.3 (J_{C-F} = 243 Hz), 149.1 (J_{C-F} = 9.06 Hz), 138.4, 134.6, 133.3, 130.0, 129.1, 127.5, 123.5 (J_{C-F} = 3.02 Hz), 120.9 (J_{C-F} = 9.06 Hz), 106.8 (J_{C-F} = 22.7 Hz), 98.8 (J_{C-F} = 27.2 Hz), 56.1, 51.9, 41.8, 38.1, 35.0; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{19}H_{19}Cl_2FNO_4$: 414.0670, found 414.0678.

4.1.14. Methyl 3-(2,4-dichlorophenyl)-5-oxo-5-(*o*-tolylamino)pentanoate (9b)

114 mg, 84% ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 (d, J = 8.0 Hz, 1H), 7.41 (s, 1H), 7.23 (m, 2H), 7.17 (m, 2H), 7.07 (m, 2H), 4.21–4.11 (m, 1H), 3.62 (s, 3H), 2.86 (m, 4H), 2.14 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.2, 168.8, 138.3, 135.5, 134.5, 133.5, 130.6, 130.0, 129.2, 129.1, 127.6, 126.9, 125.5, 123.2, 52.0, 41.5, 38.1, 35.4, 17.7; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{19}H_{20}Cl_2NO_3$: 380.0815, found 380.0811.

4.1.15. Methyl 5-((4-bromophenyl)amino)-3-(2,4-dichlorophenyl)-5-oxopentanoate (9c)

159 mg, 71% ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51–7.30 (m, 6H), 7.24–7.16 (m, 2H), 4.19–4.09 (m, 1H), 3.62 (s, 3H), 2.92–2.69 (m, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.4, 168.7, 138.2, 136.8, 134.4, 133.5, 132.1, 130.0, 129.1, 127.6, 121.5, 117.1, 52.1, 41.6, 38.1, 35.0; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{18}H_{17}BrCl_2NO_3$: 443.9763, found 443.9758.

4.1.16. Methyl 3-(2,4-dichlorophenyl)-5-((4-fluorophenethyl)amino)-5-oxopentanoate (9d)

56.7 mg, 80% ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.06 (t, J = 6.2 Hz, 2H), 6.97 (t, J = 8.1 Hz, 2H), 5.46 (s, 1H), 4.03 (p, J = 6.9 Hz, 1H), 3.59 (s, 3H), 3.43 (dh, J = 14.1, 6.4 Hz, 2H), 2.80 (dd, J = 16.1, 6.3 Hz, 1H), 2.70 (q, J = 7.3 Hz, 3H), 2.53 (h, J = 7.6 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.1, 170.3, 161.8 (J_{C-F} = 246 Hz), 138.5, 134.5, 134.4 (J_{C-F} = 3.02 Hz), 133.3, 130.2 (J_{C-F} = 7.55 Hz), 129.9, 129.1, 127.5, 115.6 (J_{C-F} = 21.1 Hz), 51.9, 40.8, 40.7, 38.1, 35.1, 34.9; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{20}H_{21}Cl_2FNO_3$: 412.0877, found 412.0875.

4.1.17. Methyl 3-(2,4-dichlorophenyl)-5-((4-methoxyphenethyl)amino)-5-oxopentanoate (9e)

58.8 mg, 81% ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.01 (d, J = 1.5 Hz, 2H), 6.83 (d, J = 7.8 Hz, 2H), 5.45 (s, 1H), 4.03 (p, J = 7.4 Hz, 1H), 3.79 (s, 3H), 3.59 (s, 3H), 3.49–3.36 (m, 2H), 2.81 (dd, J = 15.9, 6.6 Hz, 1H), 2.75–2.62 (m, 3H), 2.52 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.1, 170.3, 158.4, 138.5, 134.5, 133.2, 130.7, 129.9, 129.7, 129.1, 127.5, 114.2, 55.4, 51.9, 40.8, 40.8, 38.1, 35.1, 34.7; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{21}H_{24}Cl_2NO_4$: 424.1077, found 424.1080.

4.1.18. Methyl 5-((2-cyclopropylethyl)amino)-3-(2,4-dichlorophenyl)-5-oxopentanoate (9f)

60.6 mg, 85% ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.19 (s, 2H), 5.56 (s, 1H), 4.07 (pd, J = 7.2, 1.9 Hz, 1H), 3.60 (s, 3H), 3.25 (q, J = 6.5 Hz, 2H), 2.86 (dd, J = 15.9, 6.7 Hz, 1H), 2.75 (dd, J = 15.9, 7.5 Hz, 1H), 2.57 (h, J = 7.4 Hz, 2H), 1.30 (q, J = 6.9 Hz, 2H), 0.58–0.49 (m, 1H), 0.42 (d, J = 7.8 Hz, 2H), 0.01 (d, J = 4.9 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.1, 170.2, 138.5, 134.5, 133.3, 129.9, 129.2, 127.5, 51.9, 40.9, 39.9, 38.1, 35.2, 34.4, 8.6, 4.3, 4.3; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{17}H_{22}Cl_2NO_3$: 358.0971, found 358.0976.

4.1.19. General procedure for the synthesis of hydroxamic acid[23]

To a solution of methyl ester **9** (1.0 equiv) and KCN (3.0 equiv) in THF/MeOH (1 mL/1 mL) was added aq NH_2OH (50%, 0.5 mL). This solution was stirred overnight at ambient temperature. Upon evaporation of solvent, crude mixture was purified by preparative TLC (MeOH/dichloromethane) to afford hydroxamic acid **3**.

4.1.20. 3-(2,4-Dichlorophenyl)-N^1-(4-fluoro-2-methoxyphenyl)-N^5-hydroxypentanediamide (3a)

39.4 mg, 45% ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.39 (s, 1H), 9.12 (s, 1H), 8.71 (s, 1H), 7.68 (t, J = 7.7 Hz, 1H), 7.53 (s, 1H), 7.41 (q, J = 8.9 Hz, 2H), 6.93 (d, J = 10.8 Hz, 1H), 6.68 (t, J = 9.0 Hz, 1H), 4.03 (p, J = 7.9 Hz, 1H), 3.79 (d, J = 2.0 Hz, 3H), 2.72 (d, J = 7.5 Hz, 2H), 2.40 (dd, J = 14.9, 7.7 Hz, 1H), 2.32 (dd, J = 14.8, 6.8 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.0, 166.8, 159.1 (J_{C-F} = 242 Hz), 151.5, 139.9, 139.2, 134.1, 131.4, 128.7, 128.0, 127.2, 124.9, 105.8 (J_{C-F} = 21.1 Hz), 99.6 (J_{C-F} = 27.2 Hz), 56.1, 40.4, 36.7, 34.4; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{18}H_{18}Cl_2N_2O_4$: 415.0622, found 415.0616.

4.1.21. 3-(2,4-Dichlorophenyl)-N^1-hydroxy-N^5-(*o*-tolyl) pentanediamide (3b)

The titled compound was purified by preparative HPLC (acetonitrile/water) due to its limited solubility in organic media.

80.4 mg, 70% ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.31 (s, 1H), 9.16 (s, 1H), 8.62 (s, 1H), 7.40 (s, 1H), 7.32–7.24 (m, 2H), 7.07 (d, J = 7.6 Hz, 1H), 7.01 (d, J = 7.2 Hz, 1H), 6.97 (t, J = 7.4 Hz, 1H), 6.90 (t, J = 7.2 Hz, 1H), 3.97–3.85 (m, 1H), 2.56 (m, 2H), 2.32–2.17 (m, 2H), 1.86 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.9, 167.0, 139.8, 136.2, 134.2, 131.9, 131.6, 130.3, 129.9, 128.9, 127.3, 126.0, 125.4, 125.3, 40.3, 37.1, 34.6, 17.7; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{18}H_{19}Cl_2N_2O_4$: 381.0767, found 381.0763.

4.1.22. N^1-(4-Bromophenyl)-3-(2,4-dichlorophenyl)-N^5-hydroxypentanediamide (3c)

The titled compound was purified by recrystallization from MeOH.

36 mg, 80% ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.39 (s, 1H), 10.06 (s, 1H), 8.71 (s, 1H), 7.53 (s, 1H), 7.50–7.41 (m, 4H), 7.38 (s, 2H), 4.13–3.96 (m, 1H), 2.70 (d, J = 7.1 Hz, 2H), 2.40 (dd, J = 14.4, 8.1 Hz, 1H), 2.34 (dd, J = 14.5, 6.9 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.1, 166.7, 139.7, 138.3, 134.0, 131.5, 129.6, 128.8, 127.2, 124.9, 121.0, 114.6, 40.7, 36.6, 34.1; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{17}H_{16}BrCl_2N_2O_3$: 444.9716, found 444.9717.

4.1.23. 3-(2,4-Dichlorophenyl)-N^1-(4-fluorophenethyl)-N^5-hydroxypentanediamide (3d)

28.1 mg, 60% ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.69 (s, 1H), 7.88 (s, 1H), 7.52 (s, 1H), 7.39–7.26 (m, 2H), 7.12 (t, J = 6.1 Hz, 2H), 7.06 (t, J = 8.2 Hz, 2H), 3.93 (p, J = 7.7 Hz, 1H), 3.16 (d, J = 5.9 Hz, 2H), 2.57 (t, J = 6.9 Hz, 2H), 2.37 (m, 3H), 2.24 (dd, J = 14.5, 6.6 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.7, 166.8, 160.8 (J_{C-F} = 240 Hz), 139.9, 135.6 (J_{C-F} = 3.02 Hz), 134.0, 131.3, 130.3 (J_{C-F} = 7.55 Hz), 129.6, 128.7, 127.1, 114.9 (J_{C-F} = 21.1 - Hz), 40.1, 40.0, 36.6, 34.2, 34.1; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{19}H_{20}Cl_2FN_2O_3$: 413.0829, found 413.0823.

4.1.24. 3-(2,4-Dichlorophenyl)-N^1-hydroxy-N^5-(4-methoxyphenethyl)pentanediamide (3e)

22.7 mg, 46% ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.69 (s, 1H), 7.87 (s, 1H), 7.53 (s, 1H), 7.39–7.28 (m, 2H), 7.01 (d, J = 7.4 Hz, 2H), 6.81 (d, J = 7.3 Hz, 2H), 4.00–3.90 (m, 1H), 3.71 (s, 3H), 3.13 (d, J = 6.0 Hz, 2H), 2.55–2.47 (m, 2H), 2.45–2.30 (m, 3H), 2.25 (dd, J = 14.3, 5.6 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.6, 166.8, 157.6, 139.9, 134.0, 131.3, 131.3, 129.6, 129.5, 128.7, 127.1, 113.7, 55.0, 40.3, 40.01, 36.6, 34.19, 34.17; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{20}H_{23}Cl_2N_2O_4$: 425.1029, found 425.1023.

4.1.25. N^1-(2-Cyclopropylethyl)-3-(2,4-dichlorophenyl)-N^5-hydroxypentanediamide (3f)

14.3 mg, 40% ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.37 (s, 1H), 8.68 (s, 1H), 7.79 (s, 1H), 7.51 (s, 1H), 7.39–7.29 (m, 2H), 4.03–3.87 (m, 1H), 2.99 (s, 2H), 2.37 (m, 3H), 2.28 (dd, J = 14.8, 6.8 Hz, 1H), 1.16 (d, J = 6.8 Hz, 2H), 0.51 (s, 1H), 0.32 (d, J = 7.3 Hz, 2H), −0.04 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.5, 166.9, 139.9, 134.0, 131.3, 129.6, 128.7, 127.1, 40.1, 38.6, 36.6, 34.3, 34.0, 8.4, 4.1; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{16}H_{21}Cl_2N_2O_3$: 359.0924, found 359.0926.

4.1.26. General procedure to synthesize hydroxamic acid from alcohol 11

To a solution of alcohol **11** (1.0 equiv) in acetone (0.1 M) was added Jones' reagent (2.0 M solution, 2.0 equiv) at 0 °C.[29] The reaction was stirred at ambient temperature for 1 h, and quenched by the addition of IPA (a few drops). The organic layer was washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting acid was dissolved in THF (0.1 M), and diazomethane (excess) in Et_2O was added at 0 °C until the yellow color stays in the solution. After stirring overnight, upon evaporation, crude methyl ester was subjected to the conditions above to form hydroxamic acid as reddish oil.

4.1.27. 3-(2,4-dichlorophenyl)-5-(4-fluorophenethoxy)-N-hydroxypentanamide (4a)

20.0 mg, 42% ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.70 (s, 1H), 7.53 (d, J = 2.3 Hz, 1H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.32 (d, J = 8.5 Hz, 1H), 7.21 (dd, J = 8.5, 5.7 Hz, 2H), 7.07 (t, J = 8.9 Hz, 2H), 3.66 (p, J = 7.6 Hz, 1H), 3.49–3.39 (m, 2H), 3.23 (dt, J = 12.5, 6.3 Hz, 1H), 3.21–3.13 (m, 1H), 2.72 (t, J = 6.8 Hz, 2H), 2.29 (qd, J = 14.5, 7.6 Hz, 2H), 1.84 (dq, J = 13.0, 6.6 Hz, 1H), 1.75 (dt, J = 14.8, 7.3 Hz, 1H); ^{13}C NMR (151 MHz, DMSO) δ 167.0, 160.8 (J_{C-F} = 242 - Hz), 140.5, 135.2 (J_{C-F} = 3.02 Hz), 134.1, 131.3, 130.5 (J_{C-F} = 9.06 - Hz), 129.8, 128.7, 127.4, 114.8 (J_{C-F} = 21.1 Hz), 70.9, 67.7, 40.4, 37.6, 34.6, 34.5; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{19}H_{21}Cl_2$-FNO_3: 400.0877, found 400.0895.

4.1.28. 5-(Benzyloxy)-3-(2,4-dichlorophenyl)-N-hydroxypentanamide (4b)

17 mg, 36% ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.69 (s, 1H), 7.52 (d, J = 1.8 Hz, 1H), 7.38–7.32 (m, 2H), 7.30 (t, J = 7.3 Hz, 2H), 7.23 (m, 3H), 4.38–4.29 (m, 2H), 3.72 (p, J = 7.7 Hz, 1H), 3.28 (m, 1H), 3.23 (m, 1H), 2.29 (hept, J = 7.6 Hz, 2H), 1.96–1.86 (m, 1H), 1.86–1.77 (m, 1H); ^{13}C NMR (151 MHz, DMSO) δ 167.0, 140.4, 138.4, 134.2, 131.3, 129.8, 128.7, 128.1, 127.42, 127.35, 127.3, 71.8, 67.3, 40.4, 37.7, 34.4; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{18}H_{20}Cl_2NO_3$: 368.0815, found 368.0813.

4.1.29. 5-(Allyloxy)-3-(2,4-dichlorophenyl)-N-hydroxypentanamide (4c)

11.2 mg, 27% ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.69 (s, 1H), 7.53 (s, 1H), 7.37 (d, J = 13.4 Hz, 2H), 5.80 (td, J = 10.9, 5.3 Hz, 1H), 5.16 (d, J = 17.2 Hz, 1H), 5.07 (d, J = 10.3 Hz, 1H), 3.81 (s, 2H), 3.75–3.61 (m, 1H), 3.20 (dt, J = 28.2, 8.2 Hz, 2H), 2.38–2.20 (m, 2H), 1.87 (dd, J = 13.7, 6.8 Hz, 1H), 1.78 (p, J = 7.1 Hz, 1H); ^{13}C NMR (151 MHz, DMSO) δ 167.0, 140.5, 135.2, 134.1, 131.3, 129.7, 128.7, 127.4, 116.1, 70.8, 67.1, 37.7, 34.5, 34.2; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{14}H_{18}Cl_2NO_3$: 318.0658, found 318.0658.

4.1.30. 5-((3-Bromoadamantan-1-yl)methoxy)-3-(2,4-dichlorophenyl)-N-hydroxypentanamide (4d)

53.5 mg, 55%^{1}H NMR (600 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.69 (s, 1H), 7.50 (s, 1H), 7.35 (q, J = 8.6 Hz, 2H), 3.68 (s, 1H), 3.17 (m, 2H), 2.92–2.77 (m, 2H), 2.23 (d, J = 12.2 Hz, 4H), 2.16 (d, J = 11.2 Hz, 2H), 2.02 (m, 4H), 1.84 (s, 1H), 1.77 (s, 1H), 1.62 (d, J = 12.4 Hz, 1H), 1.52 (d, J = 12.0 Hz, 1H), 1.40 (d, J = 11.9 Hz, 2H), 1.34 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 140.6, 134.1, 131.2, 129.8, 128.7, 127.3, 79.4, 68.6, 67.9, 50.8, 48.4, 39.0, 38.0, 37.0, 34.5, 34.3, 31.7, 29.1; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{22}H_{29}BrCl_2NO_3$: 504.0702, found 504.0697.

4.1.31. General procedure to synthesize carbamate prodrug[23,26]

To a solution of hydroxamic acid (1.0 equiv) in acetonitrile (0.05 M) was added CDI (1.0 equiv) at 0 °C. After 0.5 h, $BnNH_2$ was added to the solution, which was stirred overnight at ambient temperature. Upon evaporation, crude mixture was subjected to column chromatography (Hexanes/EtOAc) to provide carbamate prodrug.

Hydroxamic acid **1** and **2** were synthesized based on the literature procedure.[17,30]

4.1.32. (*E*)-*N*-((Benzylcarbamoyl)oxy)-3-(2,4-dichlorophenyl)acrylamide (1′)

200 mg, 71% ^{1}H NMR (500 MHz, D_2O) δ 11.89 (s, 1H), 8.39 (s, 1H), 7.86–7.71 (m, 3H), 7.51 (dd, J = 8.5, 2.0 Hz, 1H), 7.40–7.22 (m, 5H), 6.63 (d, J = 15.8 Hz, 1H), 4.26 (d, J = 6.1 Hz, 2H); ^{13}C NMR (151 MHz, D_2O) δ 162.7, 155.2, 139.0, 135.0, 134.6, 134.3, 131.3, 129.5, 129.2, 128.4, 128.1, 127.1, 127.0, 121.5, 44.2; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{17}H_{15}Cl_2N_2O_3$: 365.0454, found 365.0450.

4.1.33. *N*-((Benzylcarbamoyl)oxy)-3-(2,4-dichlorophenyl)-5-hydroxypentanamide (2′)

33 mg, 34% ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (s, 1H), 8.19 (t, J = 6.1 Hz, 1H), 7.53 (d, J = 1.5 Hz, 1H), 7.37 (s, 2H), 7.36–7.29 (m, 2H), 7.25 (d, J = 8.0 Hz, 3H), 4.42 (t, J = 5.0 Hz, 1H), 4.21 (d, J = 6.1 Hz, 2H), 3.65 (s, 1H), 3.25 (s, 2H), 2.43 (d, J = 7.8 Hz, 2H), 1.78 (d, J = 33.4 Hz, 2H); ^{13}C NMR (151 MHz, D_2O) δ 168.1, 155.2, 140.5, 139.0, 134.0, 131.2, 129.8, 128.7, 128.3, 127.4, 127.03, 126.98, 58.4, 44.1, 37.4, 37.2, 33.9; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{19}H_{21}Cl_2N_2O_4$: 411.0873, found 411.0865.

4.1.34. N^1-((Benzylcarbamoyl)oxy)-3-(2,4-dichlorophenyl)-N^5-(4-fluoro-2-methoxyphenyl)pentanediamide (3a′)

7.1 mg, 43% ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.20 (s, 1H), 8.07 (dd, J = 8.9, 6.3 Hz, 1H), 7.61 (s, 1H), 7.41 (s, 1H), 7.34 (t, J = 7.5 Hz, 2H),

7.29 (dt, J = 6.6, 3.3 Hz, 3H), 7.15 (s, 2H), 6.64 (td, J = 8.4, 2.4 Hz, 1H), 6.58 (dd, J = 10.1, 2.6 Hz, 1H), 5.65 (s, 1H), 4.41 (d, J = 5.9 Hz, 2H), 4.12 (m, 1H), 3.76 (s, 3H), 3.05 (m, 1H), 2.96–2.82 (m, 2H), 2.67–2.48 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.3, 169.9, 159.9 (J_{C-F} = 246 Hz), 155.4, 149.8, 138.0, 137.3, 134.1, 133.5, 129.8, 129.4, 128.9, 128.0, 127.7, 127.5, 122.7, 122.3, 107.0 (J_{C-F} = 21.1 Hz), 99.0 (J_{C-F} = 27.2 Hz), 56.1, 45.7, 39.9, 35.8, 35.6; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{26}H_{25}Cl_2FN_3O_5$: 548.1150, found 548.1138.

4.1.35. N^1-((benzylcarbamoyl)oxy)-3-(2,4-dichlorophenyl)-N^5-(*o*-tolyl)pentanediamide (3b′)

16.4 mg, 83% ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.46 (s, 1H), 9.29 (s, 1H), 8.23 (s, 1H), 7.55 (s, 1H), 7.46 (d, J = 7.7 Hz, 1H), 7.41 (d, J = 8.3 Hz, 1H), 7.33 (m, 2H), 7.23 (m, 4H), 7.14 (d, J = 7.1 Hz, 1H), 7.09 (t, J = 7.3 Hz, 1H), 7.03 (t, J = 6.7 Hz, 1H), 4.22 (s, 2H), 4.06 (s, 1H), 2.78 (d, J = 8.3 Hz, 2H), 2.50 (m, 2H), 1.99 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.7, 167.7, 155.2, 139.5, 139.0, 136.1, 134.0, 131.8, 131.6, 130.2, 129.7, 128.8, 128.5, 128.3, 127.0, 127.0, 125.8, 125.2, 125.2, 44.1, 40.1, 36.7, 34.3, 17.6; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{26}H_{26}Cl_2N_3O_4$: 514.1295, found 514.1294.

4.1.36. N^1-((Benzylcarbamoyl)oxy)-N^5-(4-bromophenyl)-3-(2,4-dichlorophenyl)pentanediamide (3c′)

18.6 mg, 87% ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.47 (s, 1H), 10.07 (s, 1H), 8.22 (s, 1H), 7.54 (s, 1H), 7.48–7.35 (m, 6H), 7.32 (t, J = 7.2 Hz, 2H), 7.24 (d, J = 6.5 Hz, 3H), 4.21 (s, 2H), 4.04 (ddt, J = 15.1, 9.0, 4.5 Hz, 1H), 2.77 (s, 2H), 2.50 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.0, 167.7, 155.2, 139.5, 139.0, 138.3, 134.0, 131.5, 131.5, 129.6, 128.8, 128.3, 127.3, 127.0, 127.0, 121.0, 114.7, 44.1, 40.3, 36.5, 33.9; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{25}H_{23}BrCl_2N_3O_4$: 578.0243, found 578.0240.

4.1.37. N^1-((Benzylcarbamoyl)oxy)-3-(2,4-dichlorophenyl)-N^5-(4-fluorophenethyl)pentanediamide (3d′)

9.4 mg, 77% ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.22 (s, 1H), 7.40–7.27 (m, 5H), 7.16 (d, J = 6.6 Hz, 2H), 7.08–7.00 (m, 2H), 6.97 (d, J = 8.8 Hz, 2H), 5.68 (d, J = 10.7 Hz, 2H), 4.40 (d, J = 6.1 Hz, 2H), 3.94 (t, J = 7.3 Hz, 1H), 3.41 (dt, J = 17.7, 6.8 Hz, 2H), 2.91–2.59 (m, 5H), 2.59–2.41 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.8, 169.9, 161.8 (J_{C-F} = 245 Hz), 155.3, 138.2, 137.3, 134.1, 134.1, 133.5, 130.2 (J_{C-F} = 7.55 Hz), 129.8, 129.3, 128.9, 128.0, 127.6, 127.4, 115.6 (J_{C-F} = 21.1 Hz), 45.7, 40.7, 38.9, 35.9, 35.5, 34.7; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{27}H_{27}Cl_2FN_3O_4$: 546.1357, found 546.1361.

4.1.38. *N*-((Benzylcarbamoyl)oxy)-3-(2,4-dichlorophenyl)-5-(4-fluorophenethoxy)pentanamide(4a′)

9.4 mg, 77% ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.20 (s, 1H), 7.39–7.26 (m, 6H), 7.16 (m, 3H), 7.03 (d, J = 8.4 Hz, 1H), 6.97 (t, J = 8.6 Hz, 2H), 5.55 (s, 1H), 4.39 (d, J = 5.6 Hz, 2H), 3.76 (m, 1H), 3.65–3.51 (m, 2H), 3.34 (s, 2H), 2.83 (t, J = 6.4 Hz, 2H), 2.61 (dd, J = 14.1, 8.1 Hz, 1H), 2.49 (dd, J = 13.2, 4.7 Hz, 1H), 2.06 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.0, 161.7 (J_{C-F} = 245 Hz), 155.0, 139.0, 137.3, 134.7, 134.6, 132.9, 130.3 (J_{C-F} = 7.55 Hz), 129.7, 129.4, 128.9, 128.0, 127.7, 127.3, 115.3 (J_{C-F} = 21.1 Hz), 72.0, 68.9, 45.6, 37.9, 35.8, 35.5, 32.8; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{27}H_{28}Cl_2FN_2O_4$: 533.1405, found 533.1399.

4.1.39. *N*-((Benzylcarbamoyl)oxy)-5-(benzyloxy)-3-(2,4-dichlorophenyl)pentanamide (4b′)

9.5 mg, 46% ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.36 (s, 1H), 7.36–7.30 (m, 5H), 7.28 (d, J = 8.9 Hz, 6H), 7.14 (dd, J = 8.4, 2.2 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 5.68 (s, 1H), 4.49–4.39 (m, 2H), 4.36 (d, J = 5.9 Hz, 2H), 3.84 (p, J = 6.3 Hz, 1H), 3.40 (m, 2H), 2.64 (dd, J = 14.3, 8.0 Hz, 1H), 2.52 (m, 1H), 2.07 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.8, 155.2, 139.0, 138.0, 137.4, 134.7, 132.9, 129.7, 129.4, 128.9, 128.6, 128.0, 127.90, 127.88, 127.6, 127.4, 73.3, 68.0, 45.5, 37.9, 35.6, 33.3; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{26}H_{27}Cl_2N_2O_4$: 501.1342, found 501.1339.

4.1.40. *N*-((Benzylcarbamoyl)oxy)-5-(((1*r*,3*r*)-3-bromoadamantan-1-yl)methoxy)-3-(2,4-dichlorophenyl)pentanamide (4d′)

5.0 mg, 50% ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.05 (s, 1H), 7.35 (dd, J = 16.2, 9.0 Hz, 3H), 7.29 (dd, J = 13.0, 7.2 Hz, 3H), 7.20 (q, J = 8.2 Hz, 2H), 5.46 (s, 1H), 4.40 (d, J = 4.8 Hz, 2H), 3.87–3.77 (m, 1H), 3.40–3.23 (m, 2H), 2.95 (q, J = 9.0 Hz, 2H), 2.68–2.52 (m, 2H), 2.30 (d, J = 11.8 Hz, 2H), 2.25 (d, J = 11.8 Hz, 2H), 2.12 (m, 4H), 1.68 (d, J = 12.7 Hz, 1H), 1.65–1.42 (m, 7H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.0, 155.7, 139.8, 137.9, 135.3, 133.6, 130.5, 130.2, 129.7, 128.7, 128.4, 128.2, 81.5, 70.2, 61.3, 52.0, 49.5, 46.4, 40.1, 39.2, 38.6, 35.9, 34.1, 33.0, 30.6; HRMS (ESI-TOF) m/e calcd for $[M+H]^+$ $C_{30}H_{36}BrCl_2N_2O_4$: 637.1230, found 637.1232.

4.2. Biology

4.2.1. SNAPtide assay and 66mer assay

Recombinant Botulinum neurotoxin light chain A (1-425) was prepared as previously described.[31] SNAPtide assay was conducted according to the literature procedure with the enzyme concentration of 37 nM.[31] 66mer assay was conducted according to the literature procedure with the enzyme concentration of 0.5 nM. Prism 5 was used for K_i determination.[25]

4.2.2. Cell assay

Pure Botulinum neurotoxin (BoNT) A was prepared from *C. botulinum* strains Hall A hyper as previously described.[32] The toxin was dissolved in phosphate buffered saline, pH 7.4 and 40% glycerol, and stored at −20 °C until use. Activity of the BoNT/A preparation was determined by the mouse bioassay,[33,34] and specific toxicity was about 1.25×10^8 mouse LD_{50} Units/mg. The inhibitors were dissolved in 100% DMSO to 100 mM and stored at 4 °C.

The hiPSC derived neurons and culture medium were purchased from Cellular Dynamics International (Madison, WI), and cultured in 96-well plates as described for 5 days prior to the assay.[35] For the inhibition assay, 200 LD_{50} units of BoNT/A1 was added to the cells in 50 µl stimulation medium (modified neurobasal containing 2.2 mM $CaCl_2$ and 56 mM KCl (Invitrogen) and supplemented with B27 and glutamax), and the cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 7.5 min. The toxin was removed and cells were washed 3 times in 200 µl of culture medium (provided by Cellular Dynamics), and the inhibitors were added in culture medium, 1% DMSO at the indicated concentrations. Cells were incubated for 8 h at 37 °C, 5% CO_2 to allow for SNAP-25 cleavage, and the inhibitor mixtures were aspirated and cells lysed in 50 µl of 1x LDS lysis buffer (Invitrogen). The samples were analyzed by Western blot using a monoclonal anti-SNAP-25 antibody (Synaptic Systems, Germany) as described previously,[36,37] except that Phosphaglo chemiluminescent reagent (KPL) was used and the bands visualized on a Fotoanalyst FX (Fotodyne) equipped with a CCD camera and GraphQuant software (Fotodyne) for densitometry. GrahPad Prism 6 software was used for graph creation and data analysis.

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