The C-terminus of Botulinum A Protease Has Profound and Unanticipated Kinetic Consequences Upon the Catalytic Cleft

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Supporting Information

General. D-chicoric acid was purchased from ChromaDex. 2,4-Dichlorocinnamic hydroxamate was synthetized and described previously.[1] Lomofungin was purchased from Santa Cruz Biotechnology, Inc.

LC/MS Assay for BoNT/A Light Chain Activity with SNAP-25 (141-206) [2]

BoNT/A LC (1-448) at 0.1 nM was assayed at 20 °C, pH 7.4, in 40 mM HEPES in 100 µl volumes with a total DMSO concentration of 2%. At timed intervals, ranging from 20 min to 40 min, 25 µl aliquots were withdrawn and quenched by the addition of 3 µl of 15% aqueous TFA, ^{13}C – labeled internal standard was added to a 1.0 µM final concentration. Sample analysis was done by use of an Agilent 1100 LC/MS system. A 20 µl sample was injected onto a Zorbax 300SB-C8 column (4.6 x 50 mm, 5 µm, Agilent Technologies) subjected to a gradient (A to B where A = 0.1 % formic acid in water and B = 0.1 % formic acid in acetonitrile) of 2.5 % B from 0 to 2.5 min, 2.5 % B to 97.5 % B from 2.5 to 10 min, and 97.5 % B from 10 to 13 min at a constant flow rate of 0.5 ml/min. A column-solvent equilibration time of 4 min was conducted prior to the next sample analysis. Mass spectral acquisition included a solvent front delay of 2.5 min. Operational parameters were: positive single ion monitoring of m/z 460.9 and 462.9 corresponding to the M^{+2} peak of the reaction product and labeled internal standard respectively, nitrogen as a nebulizing and drying gas (20 psi, 3 L/min), HV capillary voltage at 4 kV and the drying gas temperature to 300 °C. Run analysis and quantitation was done by use of Chemstation software (Agilent). Enzyme velocities were determined from a linear fit of product formation versus incubation time. The inhibition constants were determined by a non-linear least squares global fit of the suitable inhibition model to the initial rates of product formation for a matrix of substrate and inhibitor concentrations bracketing K_M(apparent) and K_I(apparent).

Competitive: $v = \frac{V_{max}\mathrm{S}}{K_m\left(1+\frac{\mathrm{I}}{K_{is}}\right)+\mathrm{S}}$

Uncompetitive: $v = \frac{V_{max}\mathrm{S}}{K_m+\mathrm{S}\left(1+\frac{\mathrm{I}}{K_{ii}}\right)}$

Noncompetitive: $v = \frac{V_{max}\mathrm{S}}{K_m\left(1+\frac{\mathrm{I}}{K_{is}}\right)+\mathrm{S}\left(1+\frac{\mathrm{I}}{K_{ii}}\right)}$

Partial Noncompetitive: $v = \frac{V_{max}\mathrm{S}\left(1+\delta\frac{\mathrm{I}}{K_{ii}}\right)}{K_m\left(1+\frac{\mathrm{I}}{K_{is}}\right)+\mathrm{S}\left(1+\frac{\mathrm{I}}{K_{ii}}\right)}$

V_{max}= velocity at saturating substrate concentration

K_m = substrate concentration producing a velocity = 0.5 V_{max}

K_{is} = inhibition constant for the free enzyme

K_{ii} = inhibitor constant for the enzyme-substrate complex

δ = fractional velocity at saturating inhibitor concentration

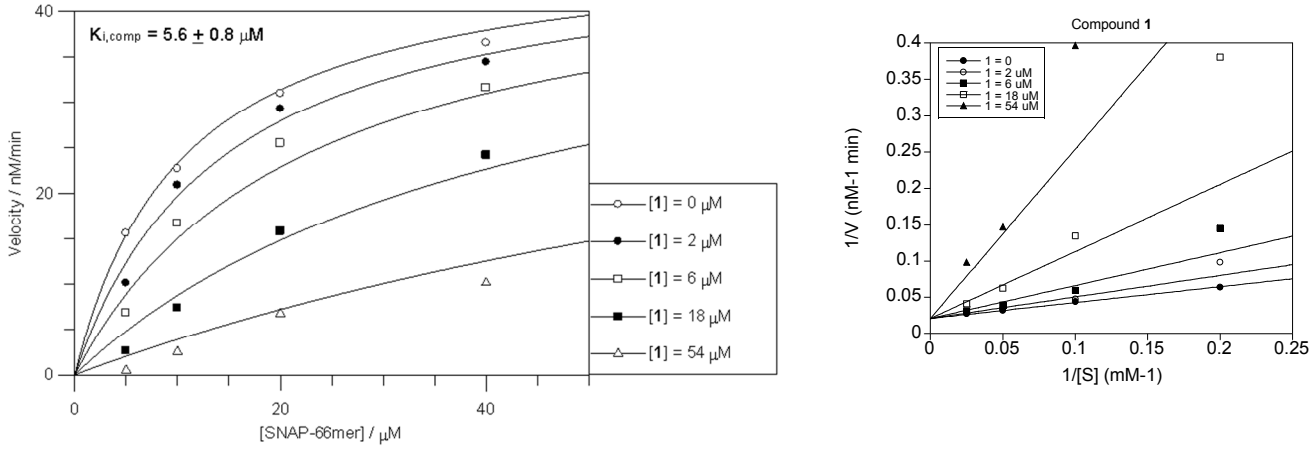

Figure S1. Inhibition profile of 2,4-dichlorocinnamic hydroxamate against BoNT/A LC (1-448).

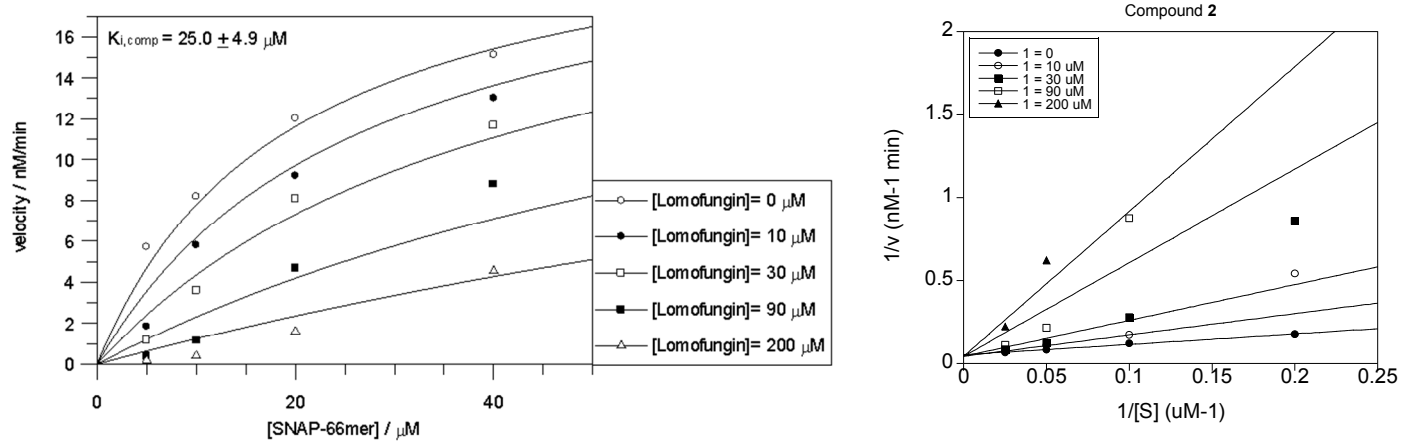

Figure S2. Inhibition profile of Lomofungin against BoNT/A LC (1-448).

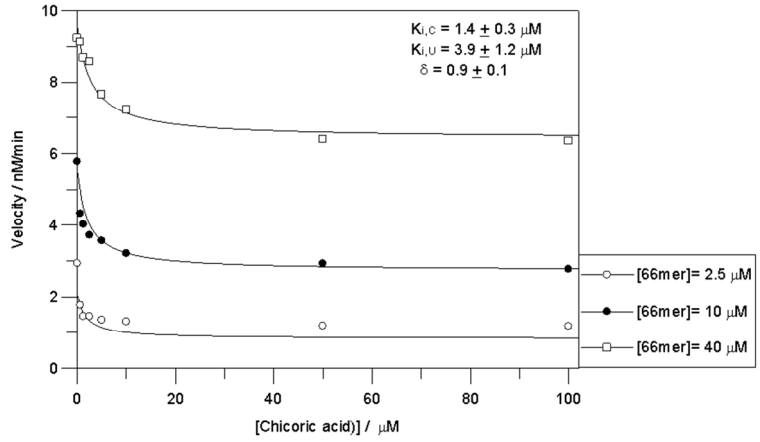

Figure S3. Inhibition profile of chicoric acid against BoNT/A LC (1-448).

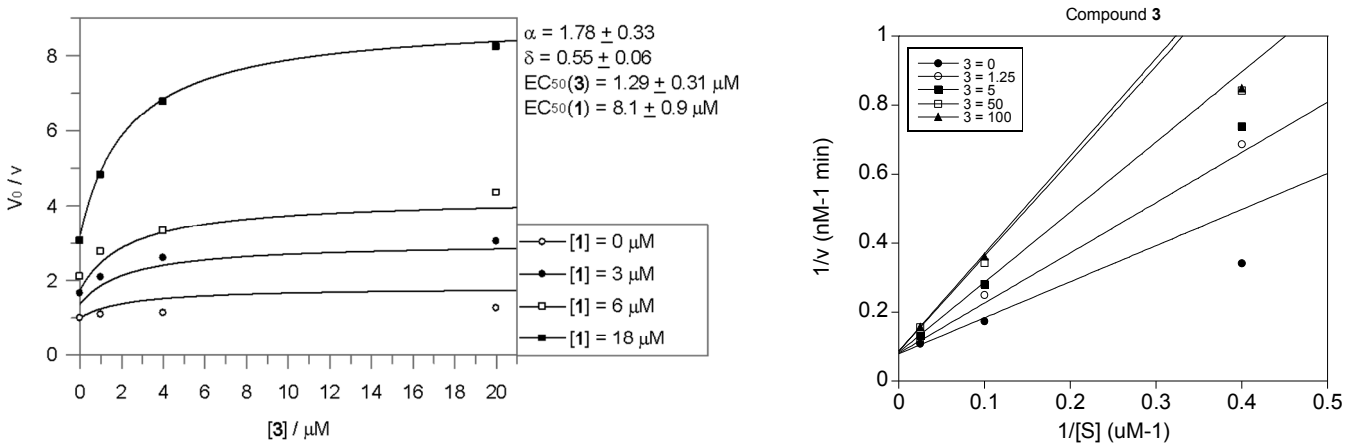

Figure S4. Double inhibition experiment against BoNT/A LC (1-448) – chicoric acid (**3**) in combination with 2,4-dichlorocinnamic hydroxamate (**1**) displaying synergistic inhibition (α – synergistic coefficient, δ – partial inhibition coefficient for chicoric acid, EC_{50} – effective inhibition concentration).

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[1] Boldt, G. E.; Kennedy, J. P.; Janda, K. D. *Org. Lett.* **2006**, *8*, 1729-1732.
[2] Čapková, K., Hixon, M.S., McAllister, L.A.; Janda, K.D. *Chem. Commun,* **2008**, *14*, 3525-3527.