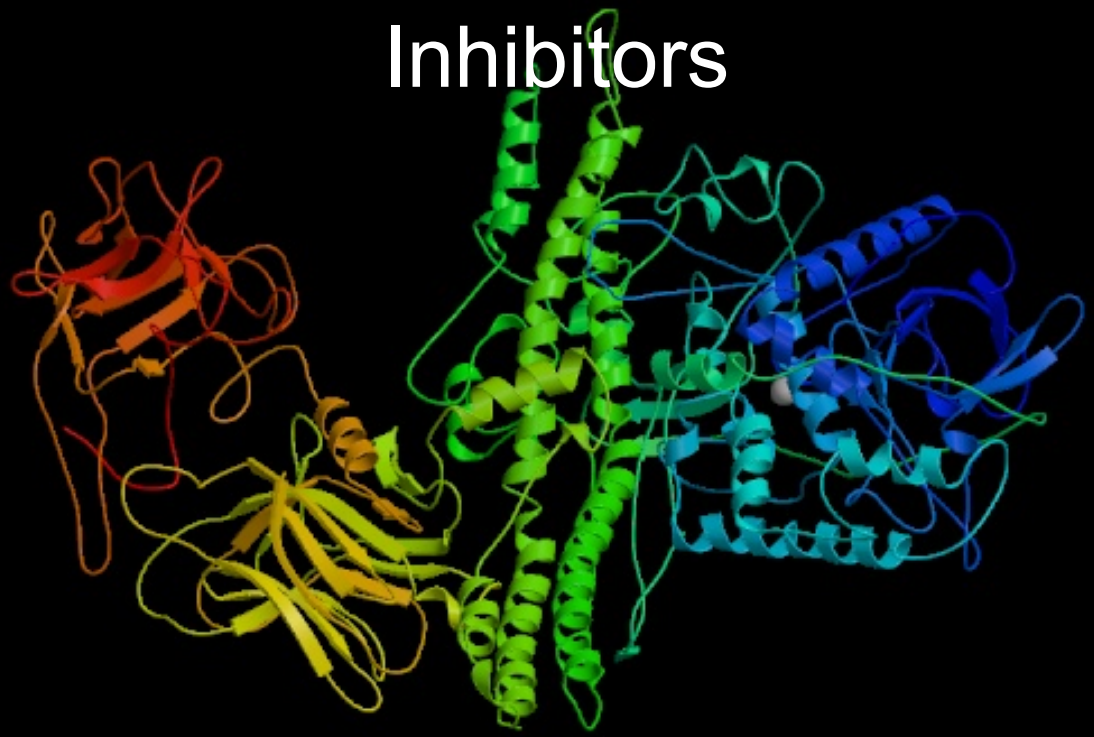
Pharmacophore Development and the Rational Design of Botulinum Neurotoxin Serotype A Inhibitors



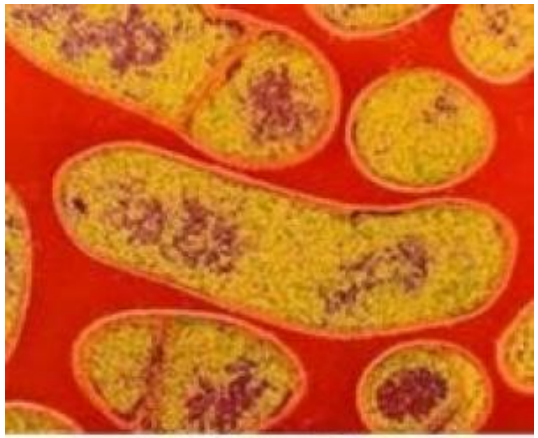
Nolan Griggs Ph.D.

Wipf Group Research Seminar
February 20th, 2010

Topics of Presentation

1. Overview of Botulinum Neurotoxin (BotNT)
2. History of BotNT/A LC Pharmacophore Development
3. History of Inhibitor Development in the Wipf Lab
4. Current Developments and the Synthesis of New Inhibitors
5. Future Directions

Overview of the Botulinum Neurotoxins (BotNT)



Clostridium botulinum

• BotNTs are produced by the bacteria *Clostridium botulinum*

• Responsible for the deadly food poisoning – Botulism which in Latin means “sausage poisoning.”

• Poisoning leads to flaccid paralysis which initially causes difficulty seeing, speaking, and swallowing followed by full muscle failure leading to death by asphyxiation.

“The most toxic substance known to man.” – *Current Medicinal Chemistry* **2005**, 12(6), 667

• Today, seven different unique serotypes of BotNT have been characterized (BotNT/A-G)

• Only serotypes A, B, E, and F are responsible for human toxicity.

• Of these, BotNT/A is the most potent with an LD_{50} = ~1 ng/kg of body mass – 100 billion times more toxic than cyanide.

Uses of the Botulinum Neurotoxins (BotNT)

"Poisons can be employed as a means for the destruction of life or as agents for the treatment of the sick." – Claude Bernard

As a Biological Weapon:

- Can be produced on large scale under fermentation conditions by germinating spores into vegetative bacteria.
- A single gram of crystalline BotNT/A properly dispersed under ideal weather conditions could kill over 1 million people.
- After the gulf war, Iraq admitted to UN weapons inspectors the production of 19k liters of concentrated toxin, of which 10k were loaded into weapons. This is approximately 3 times the amount needed to kill the entire human population.
- In Iraq in the 90s, BotNT is the most popular biological toxin used.

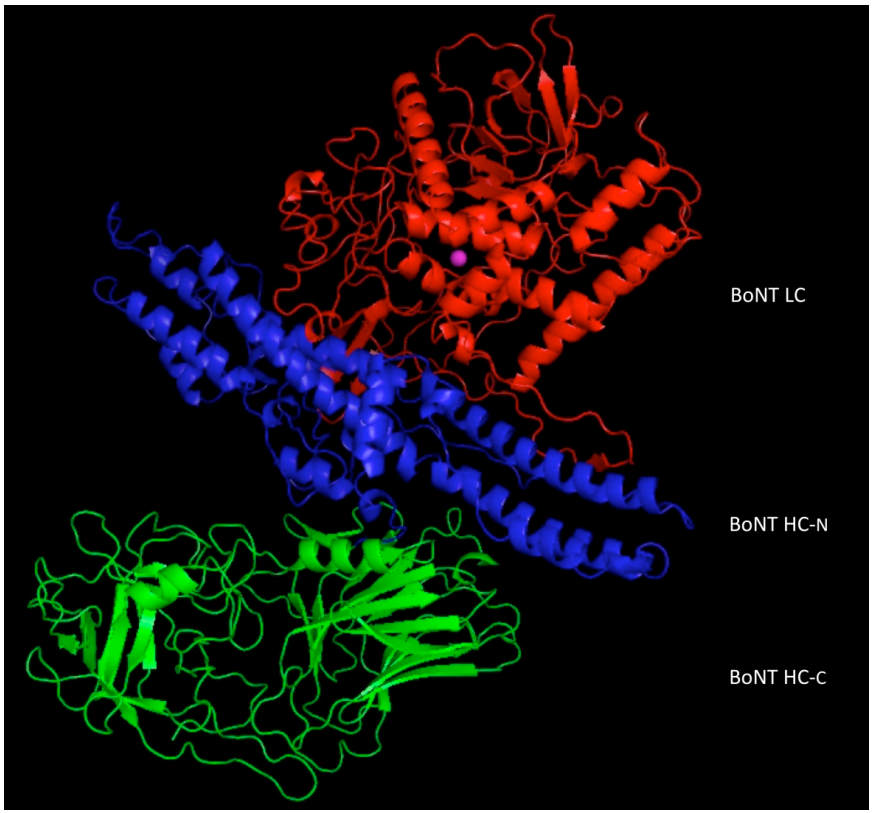
J. Am. Med. Assoc. **2001**, 285(8), 1059.

Medicinal Uses of BotNT:

- Local injections of BotNT has been federally approved to decrease muscle activity, and it has been used for therapeutic and cosmetic reasons (BOTOX®).
- There are increasing concerns, however, of over exposure, containment, and treatment in the case of accidental overdose.

Ann. Rev. Microbiol. **1999**, 53, 551.

Structure of Botulinum Neurotoxin A (BotNT/A)



Light Chain – Zinc atom shown in pink

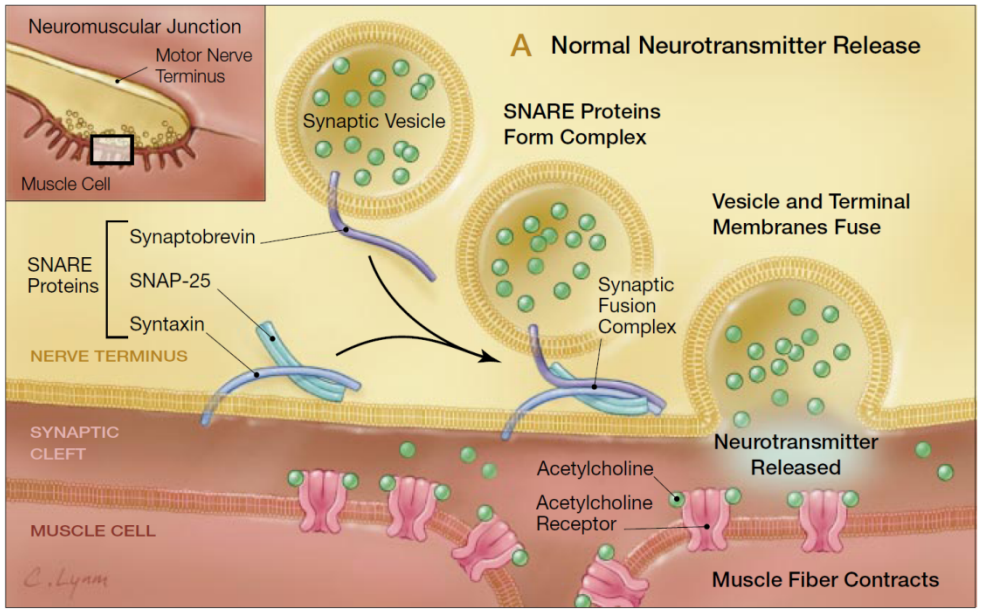
Heavy Chain – Middle domain

Heavy Chain – C-terminal domain

Nat. Struct. Biol. **1998**, 5, 898.

- BotNT/A is a protein consisting of 3 domains:
    i. C-terminal domain – responsible for binding to the synaptic cleft
    ii. Middle domain – mediates translocation into the nerve
    iii. N-terminal domain – a zinc-dependent protease
- The C-terminal and middle domain make up the 100kD Heavy Chain (HC), and the N-terminal domain makes up the 50kD Light Chain (LC).
- The HC and LC are connected by a disulfide bridge.

Mechanism of Action (BotNT/A)



Normal Synaptic function:

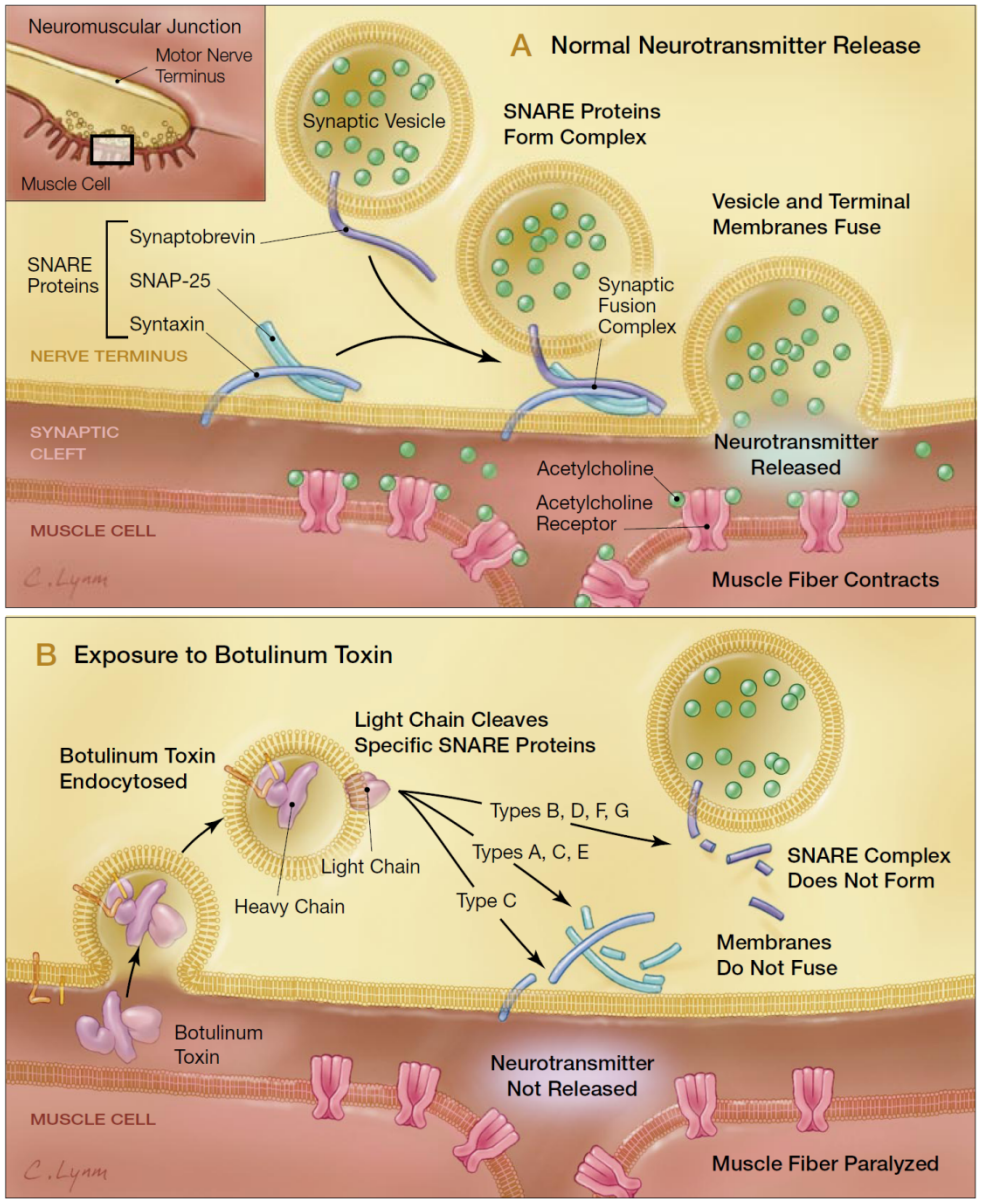
• SNARE (soluble N-ethylmaleimide-sensitive factor attachment protien receptors) Proteins form a complex with the synaptic vesicle and trigger membrane fusion, releasing acetylcholine into the synaptic cleft

• Released acetylcholine then binds to specific receptors on the muscle cell, triggering contraction of the muscle.

• This process is essential for all muscle activity in the body.

J. Am. Med. Assoc. **2001**, 285(8), 1059.

Mechanism of Action (BotNT/A)

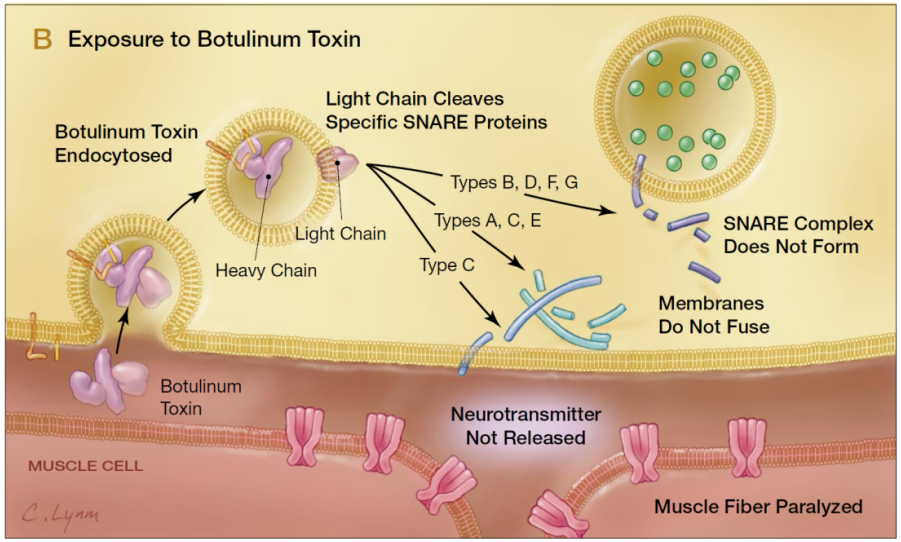


Upon Exposure to BotNT:

- BotNT-HC first binds to the presynaptic membrane.
- Once bound, the toxin is endocytosed with the aid of the Middle domain on the HC.
- Once in a vesicle, the disulfide bond between HC and LC is cleaved, and the LC is translocated into the cytosol.
- BotNT/A-LC then irreversibly cleaves the SNARE protein (SNAP-25), preventing formation of the fusion complex.
- Consequently, acetylcholine is not released, and the muscle loses function.

J. Am. Med. Assoc. **2001**, 285(8), 1059.

Approaches Toward Inhibition (BotNT/A)



- Inhibition could occur at any point prior to SNARE cleavage.

- Antibody treatments focus on inhibiting binding of the toxin, and are less effective post-exposure.[1]

- While several inhibitors of HC have been reported, most efforts have been focused on LC inhibition.[2]

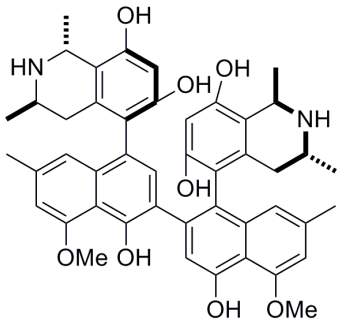
- Inhibition of BotNT/A-LC has been shown to be effective by disrupting the cleavage of the SNAP-25, which has been shown to involve Zn-mediated cleavage of Gln 197 and Arg 198 of SNAP-25. This has been studied employing two strategies:

1. Using peptide mimics of the active site domain of SNAP-25. – Eventually led to the discovery of several peptides with potencies in the low nanomolar range, highlighted by Schmidt and co-workers.[3]

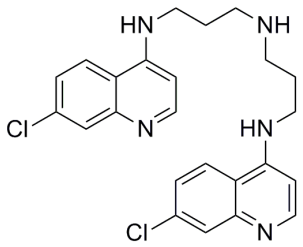
2. Using Zn-complexing small molecules to bind to LC. – Several hydroxamic acids were shown to be active inhibitors; o,p-dichlorocinnamic hydroxamate (IC_{50} < 0.5 μM).[4]

1. *Nature Rev. Microbiology* **2004**, 2, 721
2. *Biochemistry* **2004**, 43, 526 and references therin
3. *FEBS Lett.* **2002, 532, 423.**
4. *Org Lett*. **2006,** 8, 1729.

A New Pharmacophore for Inhibition of BotNT/A L



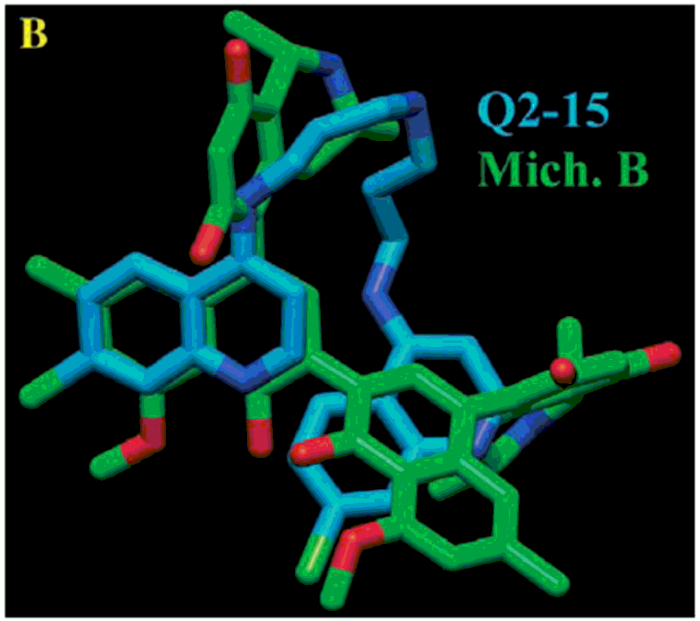
Michellamine B
(62% inhibition @ 20 μM)

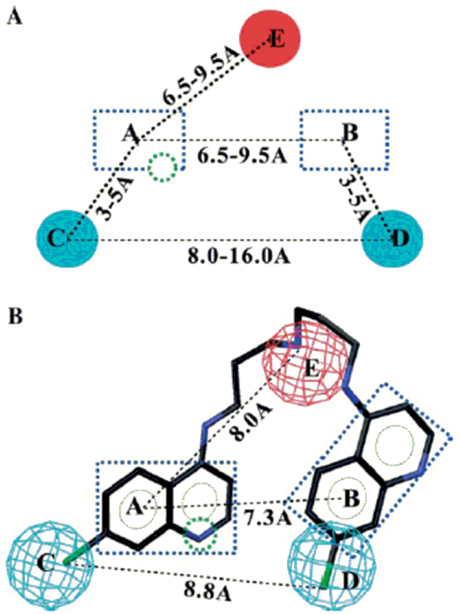


Q2-15
(60% inhibition @ 20 μM)

• In 2003, Rick Gussio, Sina Bavari, and co-workers, through high-throughput screening techniques, identified several small molecule non-peptidic inhibitors (SNPIs) of BotNT/A.

• Molecular docking studies into the active site of BotNT/A LC showed common structural components, and based on the collected data, a crude 2 zone pharmacophore for BotNT/A SNPIs was proposed.



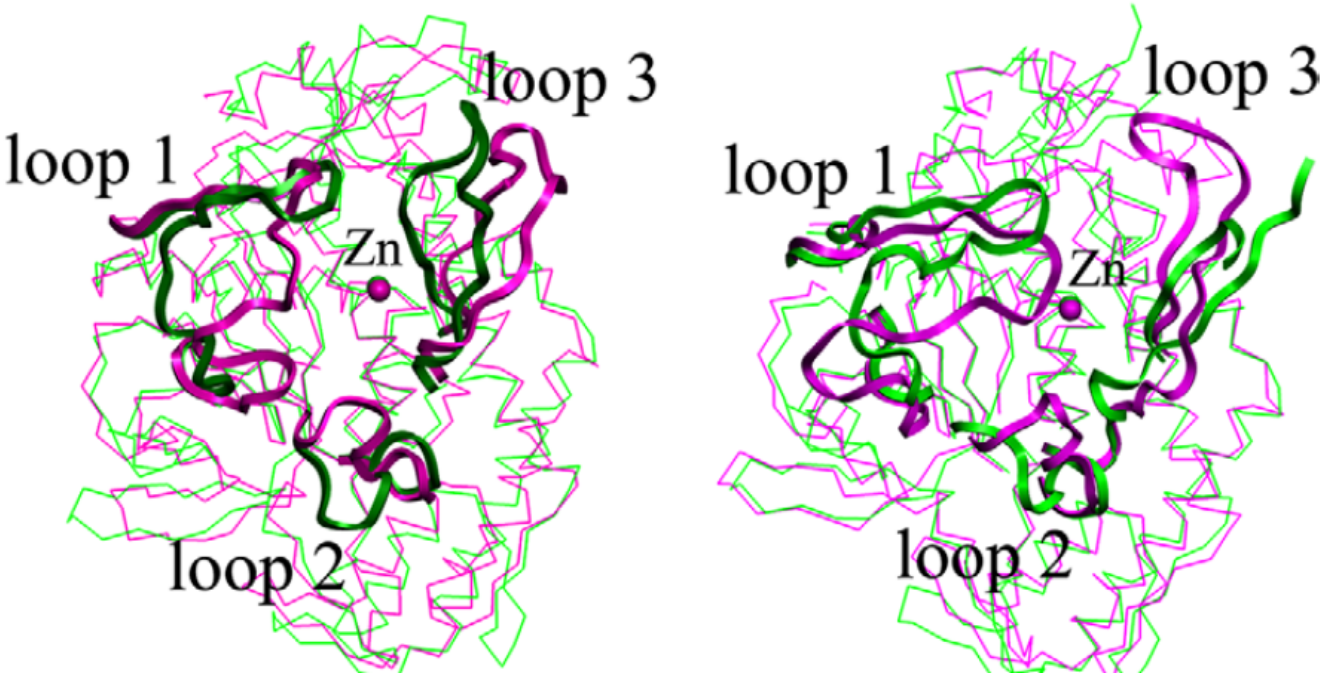


• Tentative requirements for activity were proposed as: 1.) Zones A and B should be planar, with a heteroatom occupying A; 2.) C and D are hydrophobic components capable of π–π or cation-π interactions with SMNPIs; 3.) E should be a positive ionizable component.

Biochem, Biophys. Res. Comm. **2003**, 310, 84.

Modification of the Pharmacophore

• In 2005, Rick Gussio, Sina Bavari, and co-workers expanded their studies using molecular dynamics to explore different conformations of the surface loops BotNT/A LC.



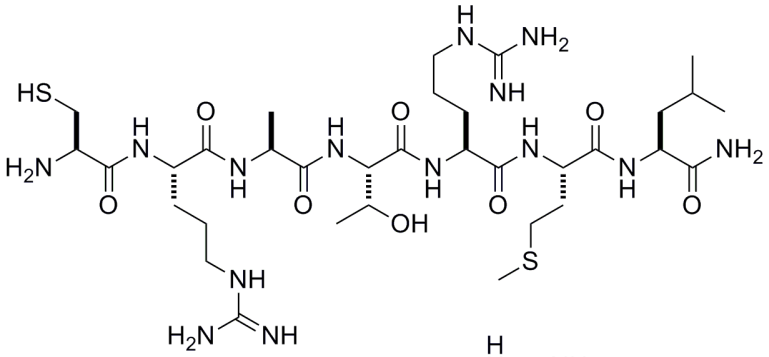
• Findings included that while the majority of the binding cleft stays conformationally defined, 3 surface loops on the periphery of BotNT/A LC are somewhat flexible, and can adopt different conformations that, in some cases, could impact inhibitor binding to the cleft.

• This flexibility was proposed to account for more possible inhibitor contacts.

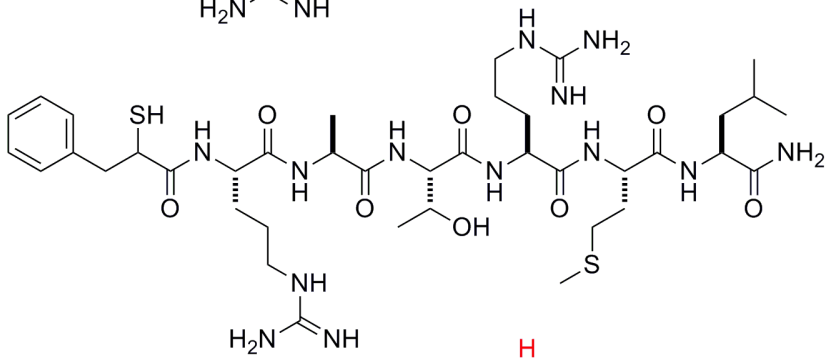
Bioorg. Med. Chem. **2005**, 13, 333.

Comparison with Peptidic Inhibitors

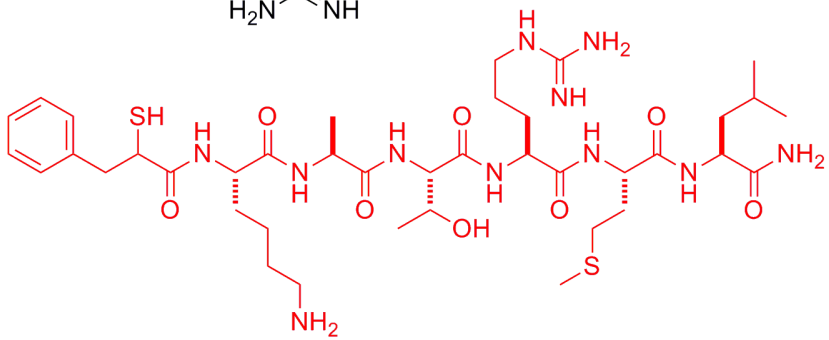
• As previously noted, in 2002, Schmidt and co-workers identified several potent pseudo-peptide inhibitors of BotNT/A with Ki values in the nanomolar range. These peptides mimicked the SNAP-25 residue 194-200 region (the active site domain):



CRATKML (K_i = 2 μM)



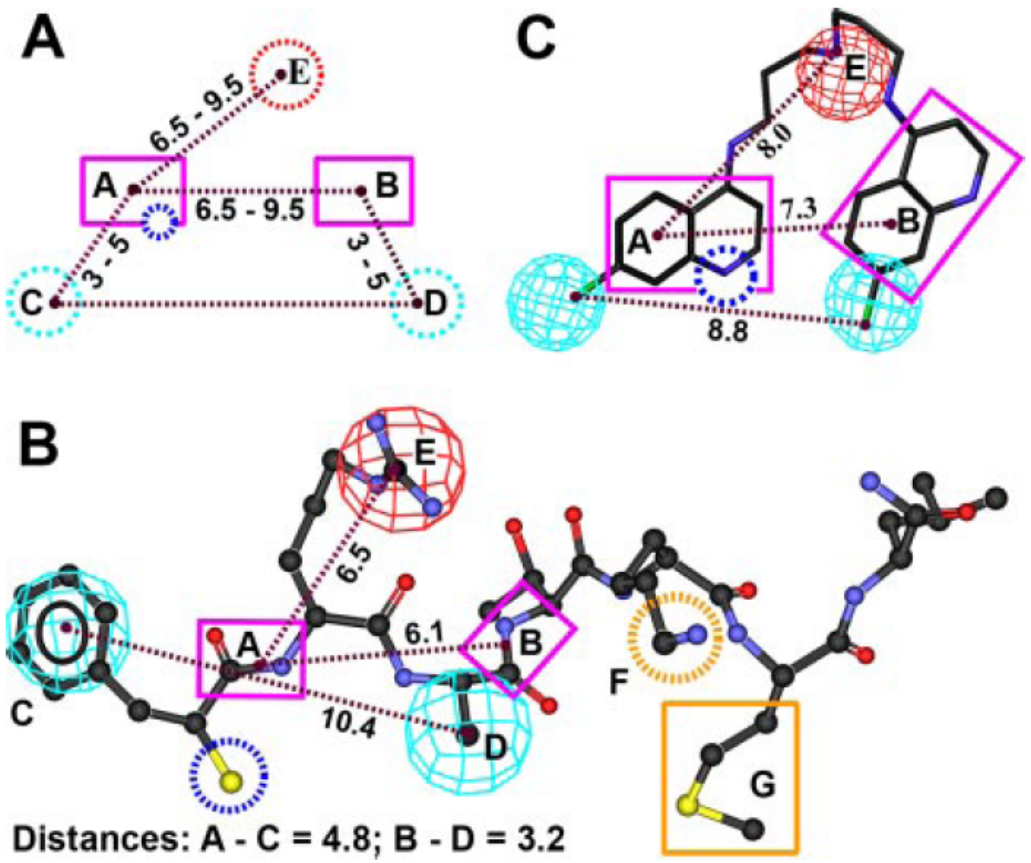
mpp-RATKML (K_i = 330 nM)



mpp-KATKML (K_i > 330 μM)

FEBS Lett. **2002, 532, 423.**

Comparison with Peptidic Inhibitors



• In 2007, Rick Gussio, Sina Bavari, and co-workers modeled the binding mode of the peptide inhibitors discovered by Schmidt into the active site on BotNT/A as previously employed with the discovered SMNPIs.

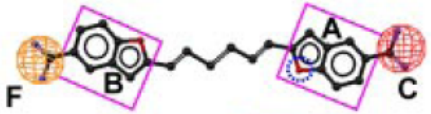
• A precise match between the docked conformation of mppRAT and the existing pharmacophore was observed, suggesting that the SMNPIs were interaction with similar residues in the binding cleft.

• It was hypothesized that by using more information from the peptide interactions, more potent SMNPIs could be discovered.

• The addition of two new interactions, F and G, not seen by the initial modeling studies opened the door for further docking studies and database searches for identification of potential SMNPIs.

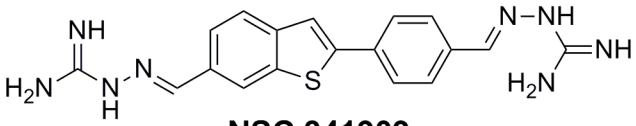
J. Biol. Chem. **2007,** 282(7)**, 5004.**

The Development of a New Lead – NSC 240898

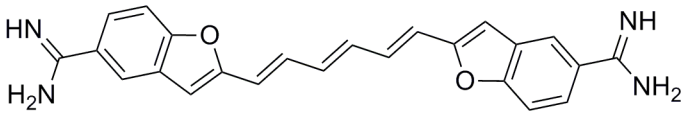
NSC	K_i(μM)	Query Fit	Distances (Ang.) A-B[1]	A-C[1]	A-F[1]	Total[2]
341909	3.0		7.5	4.8	13.5	19.6
308574	6.0		12.8	3.9	16.6	19.9
240898	10.0		9.6	3.9	11.9	17.8
341907	10.0		8.8	4.5	12.8	19.3

1 = Distances taken from planar centroids; 2 = Total length of the compounds

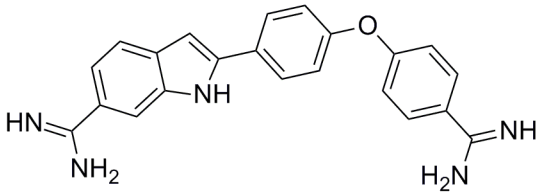
- To test their hypothesis, a database search query was generated searching the NCI open repository. Queries consisting of 4-5 pharmocaphore components in different combinations were run and 4 potent inhibitors were identified.



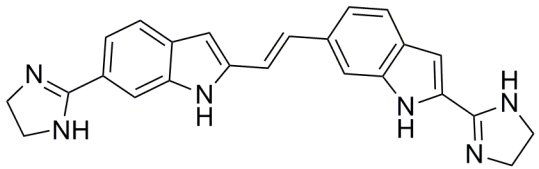
NSC 341909



NSC 308574



NSC 240898



NSC 341907

The Development of a New Lead – NSC 240898

NSC	K_i(μM)	Query Fit	Distances (Ang.) A-B[1]	A-C[1]	A-F[1]	Total[2]
341909	3.0		7.5	4.8	13.5	19.6
308574	6.0		12.8	3.9	16.6	19.9
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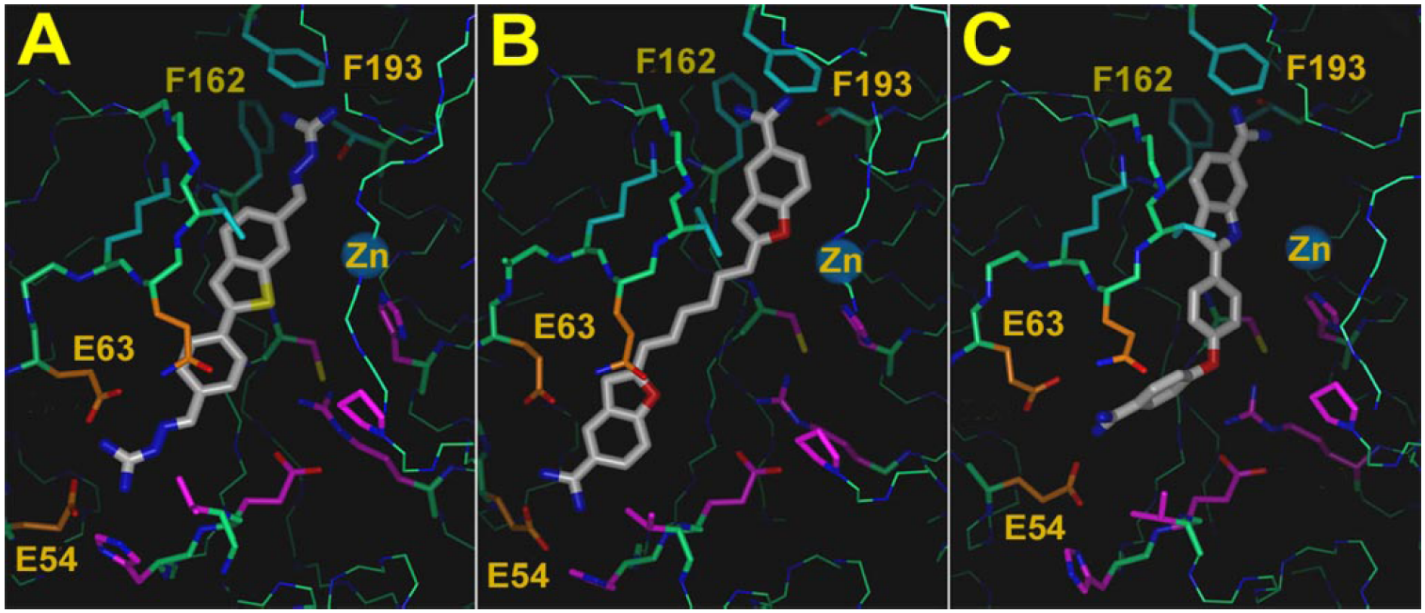
341907	10.0		8.8	4.5	12.8	19.3

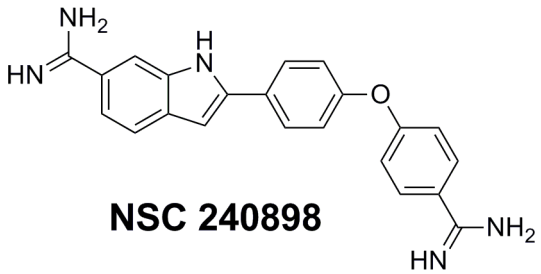
1 = Distances taken from planar centroids; 2 = Total length of the compounds

- To test their hypothesis, a database search query was generated searching the NCI open repository. Queries consisting of 4-5 pharmocaphore components in different combinations were run and 4 potent inhibitors were identified.
- Upon docking NSC 341909 (A), NSC 308574 (B), and NSC 240898 (C), employing identical dynamic conformations of the BOTNT/A LC as with the mpp-RATKML, good steric and chemical complementarities



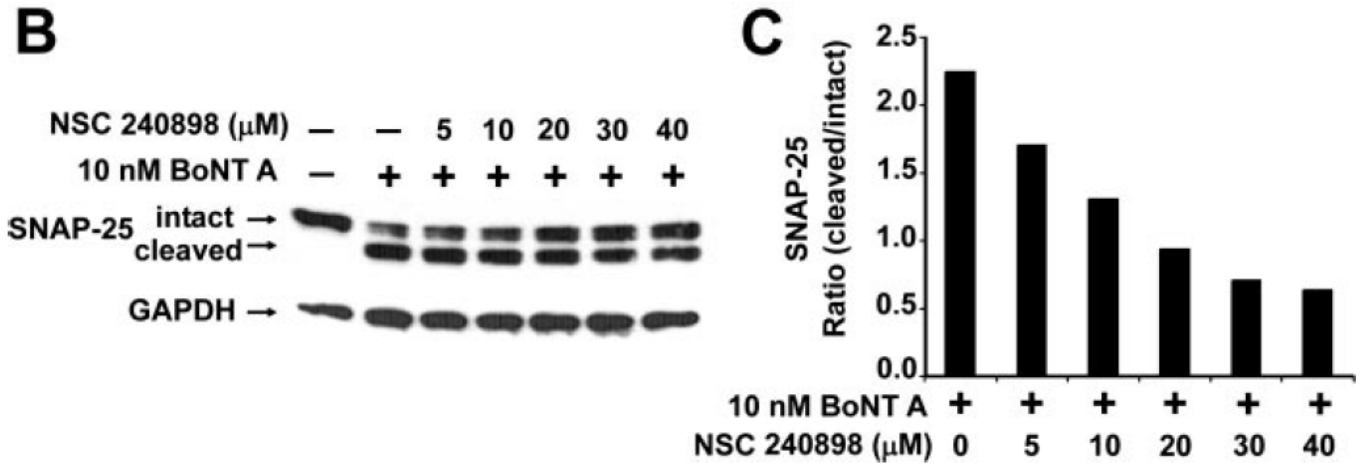
The Development of a New Lead – NSC 240898

• Upon biological testing to the 4 “hits,” NSC 240898 was identified as an impressive lead compound for further study.



NSC 240898

• NSC 240898 was tested for neuronal uptake in chick spinal motor neurons, and was found to be in the cells within 30 min.
• It was also found to exhibit very low toxicity at concentrations as high a 40 μM, and was well tolerated by cells over long periods of time (days).
• Finally, NSC 240898 demonstrated dose-dependent inhibition of SNAP-25 cleavage in Western Blot analysis



Summary

- A 4-Zone Pharmacophore for the identification of SMNPIs of BotNT/A LC has been developed.
- The pharmacophore model has shown efficacy in identifying new lead structures for further study.
- As a result, potent μM inhibitors of BotNT/A LC have been discovered.
- Currently, synthetic work is underway to synthesize more potent SMNPIs, and in vivo studies of previously tested compounds are underway.

Acknowledgments

- Professor Peter Wipf
- Dr. Jonathan Nuss, Dr. Sina Bavari - United States Army Medical Research Institute of Infectious Diseases
- Dr. Rick Gussio, Dr. James Burnett - Target Structure-Based Drug Discovery Group, SAIC-Frederick, Inc.

  National Cancer Institute at Frederick
- Chenbo Wang, Kalyani Patil, Julia Widom, Filip Petronijevic, Jared Hammel – BotNT Contributions
- Wipf Group Members - Past and Present
- NIH/SAIC - funding