Synthesis of novel Small Molecule (Nonpeptidic) Inhibitors (SMNPIs) of the BoNT/A LC metalloprotease

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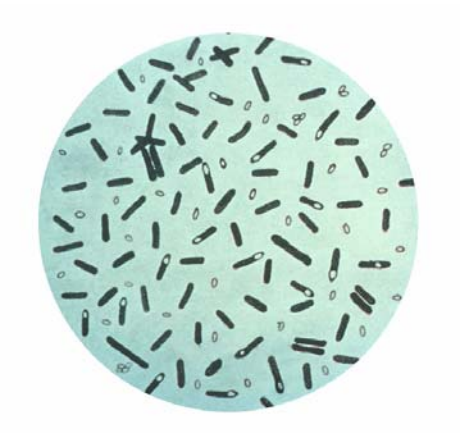
September 24th, 2011

Botulinum Neurotoxin A



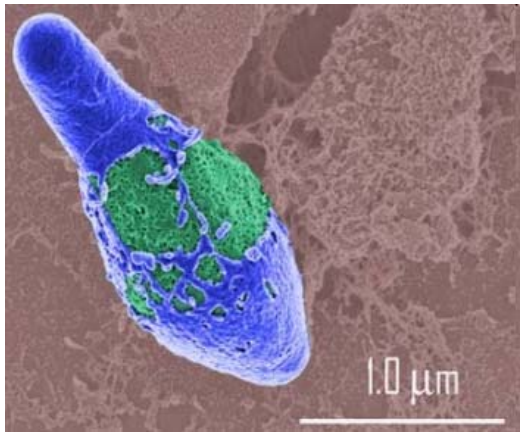
Botulinum neurotoxins (BoNTs), secreted by
rod-shaped, Gram-positive,
anaerobic spore-forming bacterium *Clostridium botulinum*,
are some of the most potent naturally occurring compounds

The lethal dose for humans is 1 ng/kg of body weight

Toxicity 10^6-fold higher than cobra toxin and
10^{11}-fold greater than cyanide

BoNTs are typically associated with:
•food poisoning
•wound infections
•inhalation
•infection in the intestinal tract of infants

SYMPTOMS:
•muscle weakness
•impaired respiratory function
•autonomic dysfunction



1817 J. Kerner - the first clinical documentation of botulism

1897 Emile van Ermengem identified the *Clostridium botulinum* as a producer of BoNT

1944 Edward Schantz purifies BoNT in great quantities

1949. A.S.V. Burgen discover that BoNTs **blocks neurotransmitter (acetylcholine) release**

Death results from a **respiratory failure** that depends on
BoNT-induced **inactivation of neurotransmitter release**

Due to their ease of dissemination, and lethality,
BoNTs are classified as category A,
highest priority biothreat agents by the
Centers for Disease Control and Prevention (CDC)

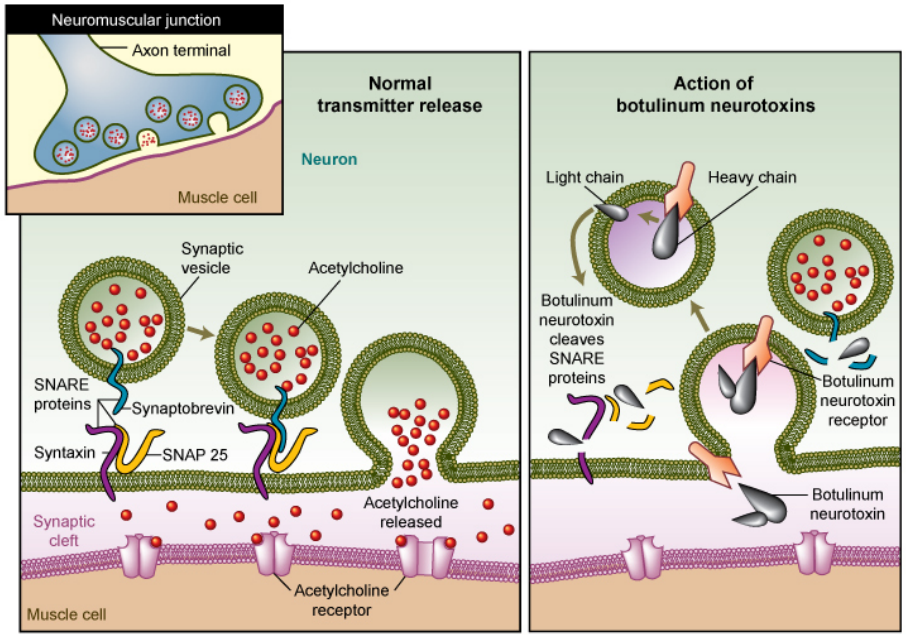
Despite being extremely poisonous, BoNT is a highly effective therapeutic agent

It was observed that injecting BoNT could paralyze individual muscle groups

The most popular BoNT-associated application is **cosmetic**
where the commercial product **Botox** (based on BoNT/A)
is used as an **antiwrinkle agent**

The use of BoNT has also been extended to cover a wide variety of disorders:
•strabismus,
•hemifacial spasm
•excessive sweating
•myofascial pain
•migraine headaches
•multiple sclerosis

Mechanism of Action



Rowland, L. P. *New Engl. J. Med.*, **2002**, *347*, 382
Tobin J. Dickerson, T. J.; Janda, K. D. *ACS Chem. Biol.*, **2006**, 1, 359-369

Each of the 7 serotypes of BoNT cleaves one of the three SNARE proteins:
•synaptobrevin (VAMP)
•SNAP-25
•syntaxin
which are **necessary** for **vesicle fusion** and **acetylcholine release**

There are 7 known BoNT serotypes (A–G)

Proteins have weight of 150 kDa

BoNTs are synthesized as singlepolypeptide chains

Intra- or extracellular proteases converts them into active form - **dimer** composed of a 100-kDa heavy chain (HC) coupled to a 50-kDa light chain (LC) by one or more disulfide bonds.

HC is responsible for recognition of the target cellular surface and translocation out of endosomal vesicles

LC is a Zn-dependent metalloprotease that cleaves specific SNARE proteins within the cell

VAMP is the target for BoNT/B, -D, -F, and -G

The target for BoNT/A and -E is **SNAP-25**,

BoNT/C can cleave both **SNAP-25** and **syntaxin**

Currently, **only** life-saving option available for BoNT intoxication is mechanical ventilation

There is a significant interest in the development of a **drug** (small molecule inhibitors of the LC metalloprotease) that would be effective after BoNT intoxication

Inhibitors for prevention of BoNT Intoxication

- **Potassium Channel Blockers**
- **Antagonists of Toxin Binding to Target Cells**
- **Antagonists of pH-Dependent BoNT Translocation**
- **Inhibition of the BoNT Metalloprotease LC**
- **Therapeutics with an Undefined Mechanism of Action**

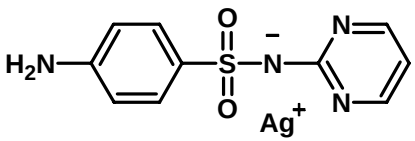
Inhibition of the BoNT Metalloprotease LC

Two approaches for inhibitor development:
- synthesis of small organic molecules that specifically bind to the toxin and inactivate it
- synthesis of peptides with structure similar to native SNARE protein substrate

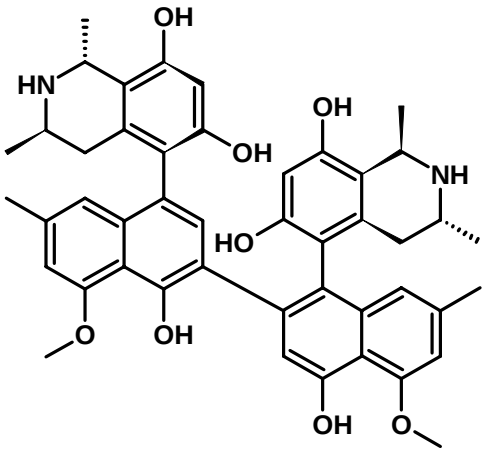
Several effective inhibitors based upon peptide scaffolds have been reported however, but major drawback was short *in vivo* lifetimes

Small molecule (nonpeptidic) inhibitors (SMNPIs) of the BoNT/A LC

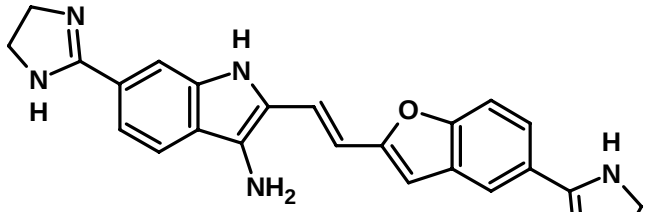
(US Army Medical Research Institute of Infectious Diseases, USAMRIID screened 1990 compounds)



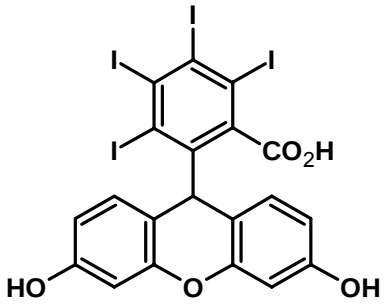
NSC 625324
silver sulfadiazine
(Inhibition: 100%)



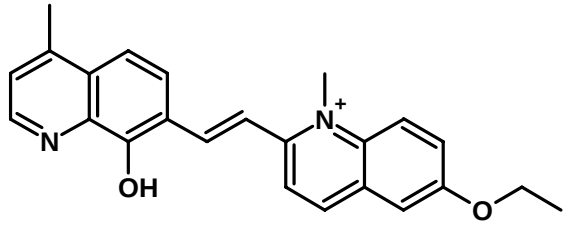
NSC 661755
michellamine B
(Inhibition: 62%)



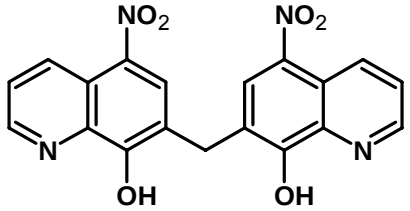
NSC 357756
(Inhibition: 57%)



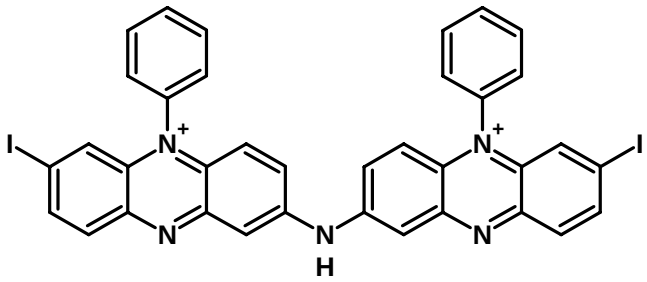
NSC 119889
(Inhibition: 56%)



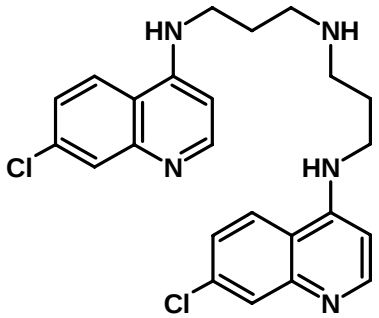
NSC 86372
(Inhibition: 51%)



NSC 130796
(Inhibition: 48%)



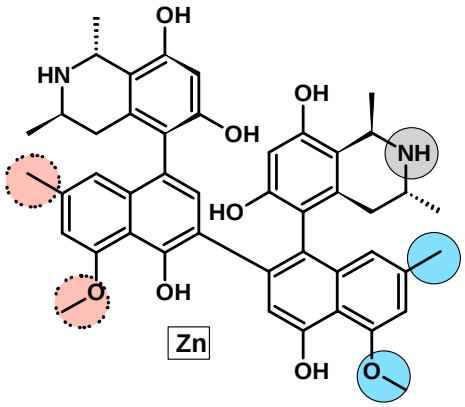
NSC 402959
(Inhibition: 40%)



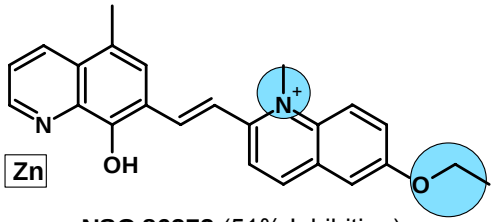
Q2-15
(Inhibition: 60%)

[#]Compounds tested in the HPLC-based assay at 20 μM concentration in the presence of 0.1 mM substrate.

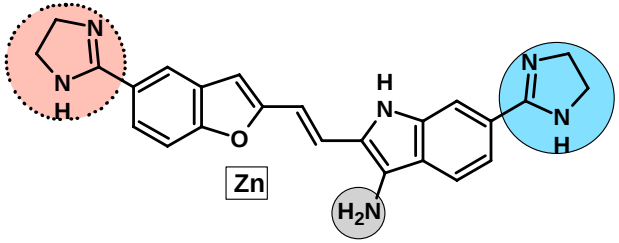
Biochem. Biophys. Res. Commun., **2003,** *310*, 84



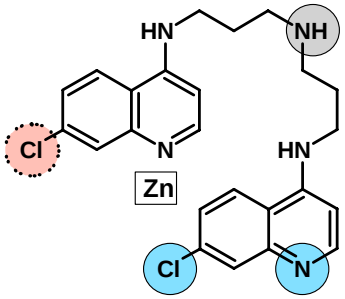
Michellamine B (62% Inhibition)



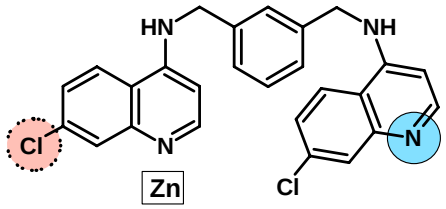
NSC 86372 (51% Inhibition)



NSC 357756 (57% Inhibition)



Q2-15 (60% Inhibition)

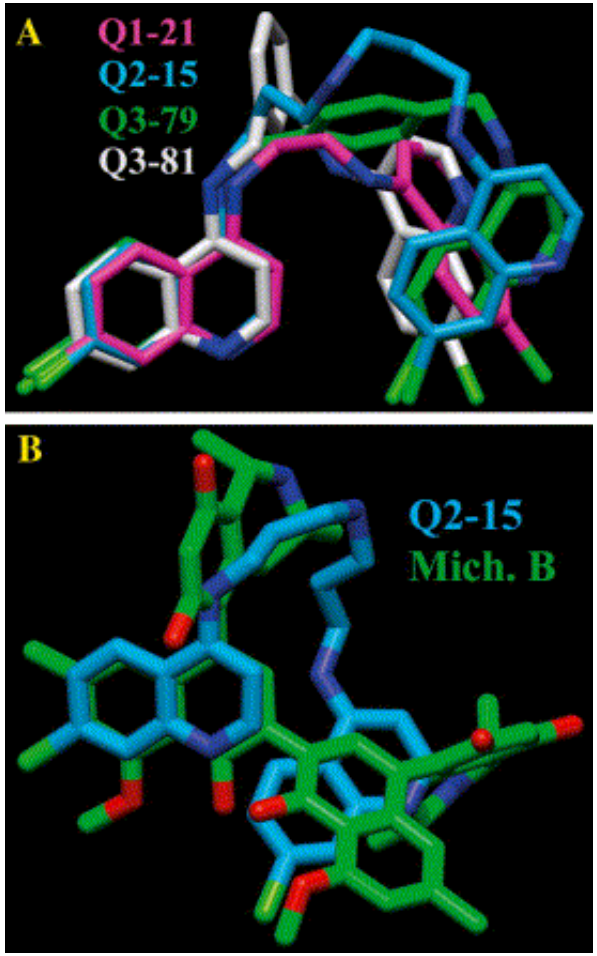


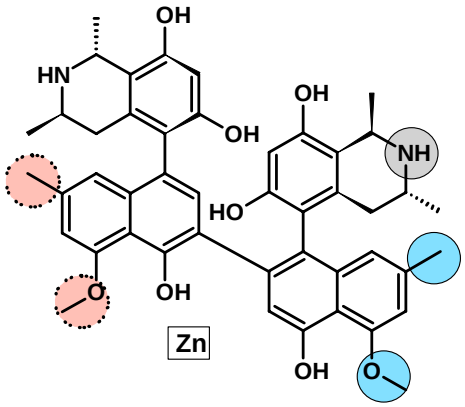
Q3-81 (32% Inhibition)

Binding Subsite 1 Contact

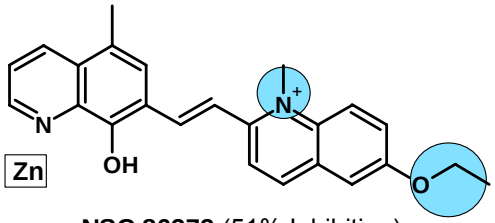
Binding Subsite 2 Contact

Polar Residue Contact

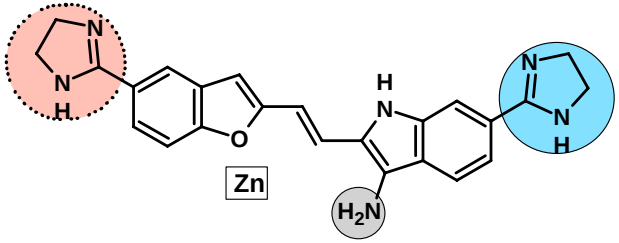




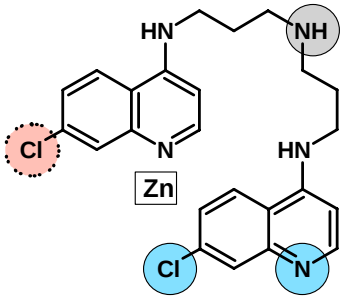
Michellamine B (62% Inhibition)



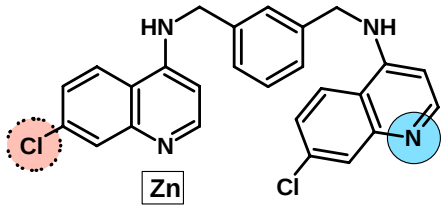
NSC 86372 (51% Inhibition)



NSC 357756 (57% Inhibition)



Q2-15 (60% Inhibition)

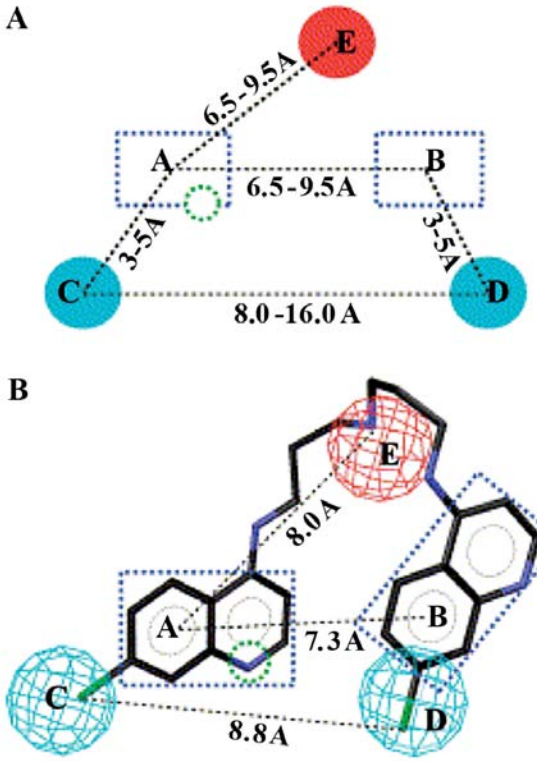


Q3-81 (32% Inhibition)

Binding Subsite 1 Contact

Binding Subsite 2 Contact

Polar Residue Contact



Pharmacophore for BoNT/A LC inhibitors is proposed:

Planar components **A** (presence of heteroatom) and **B**
Hydrophobic components of the pharmacophore (**C** and **D**)
The positive ionizable component of the pharmacophore (**E**)

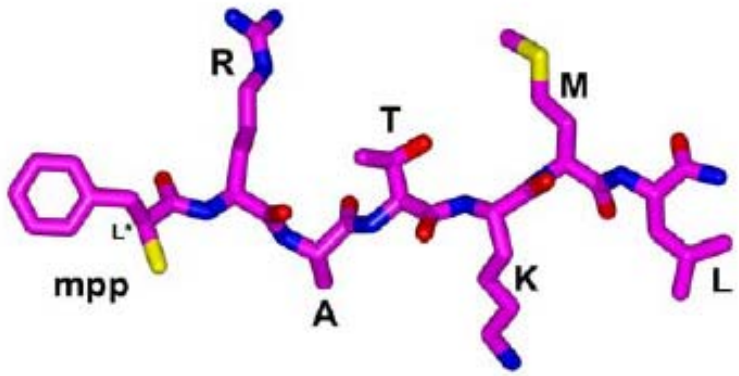
CRATKML K_i = 2 μM
Cys-Arg-Ala-Thr-Lys-Met-Leu

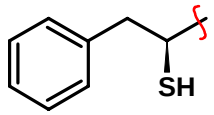
CRATKML peptide displayed competitive kinetics

The major contributors to binding affinity is binding the sulfhydryl group to zink in active site

Peptide (**mpp-RATKML**)
displayed competitive kinetics

The enhance activity of peptide comes from the presence of:
- sulfhydryl group
- phenyl ring
- arginine side chain and
- peptide backbone of the first five amino acids



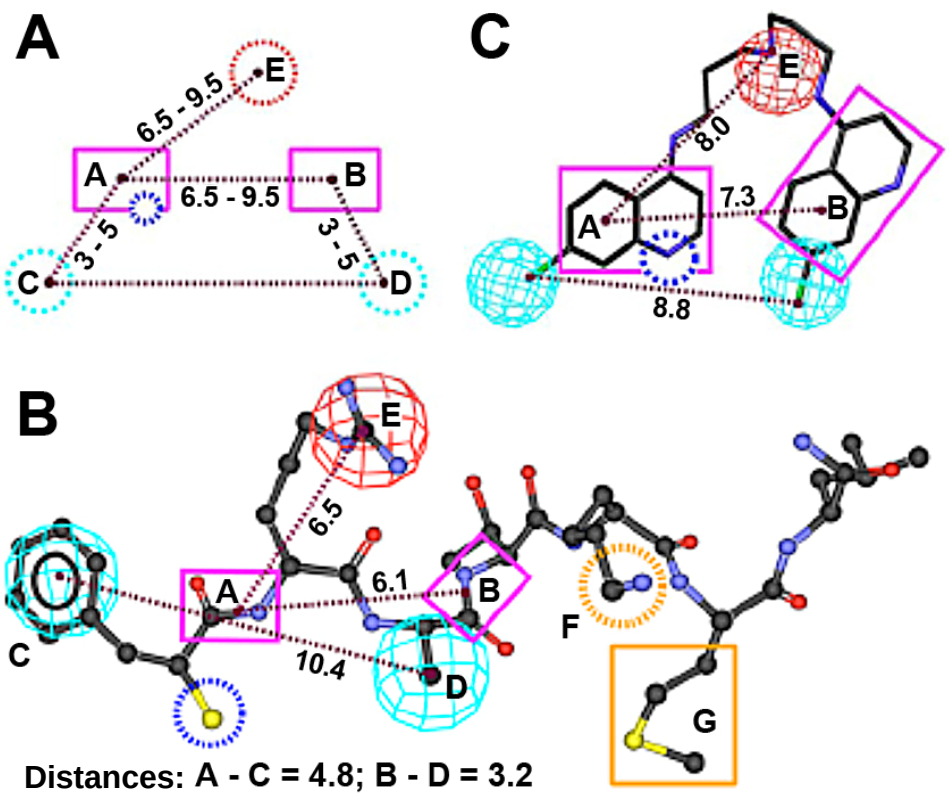


K_i = 0.3 μM

mpp
2-mercapto-3-phenylpropionyl group

FEBS Letters **1998,** *435,* 61
FEBS Letters **2002,** *532,* 423

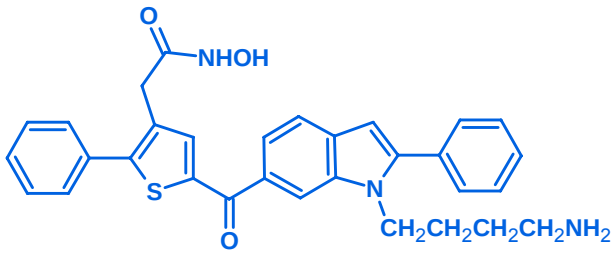
The binding mode of the **mpp-RATKML** peptide fits the previously establish pharmacophore for *SMNPI* BoNT/A LC inhibition



Molecular docking studies showed two additional structural components

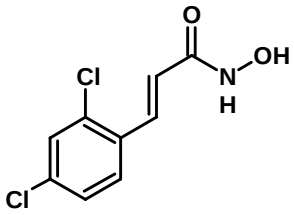
- **Lys residue (component F)**
- **Met residue (component G)**

J. Biol. Chem., **2007**, *282*, 5004



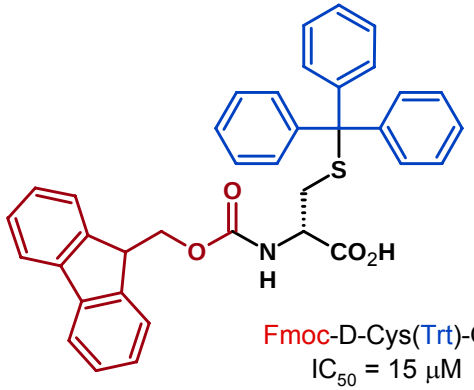
96% Inhibition
K_i = 12 μM

Biorg. Med. Chem., **2006**, *14*, 395



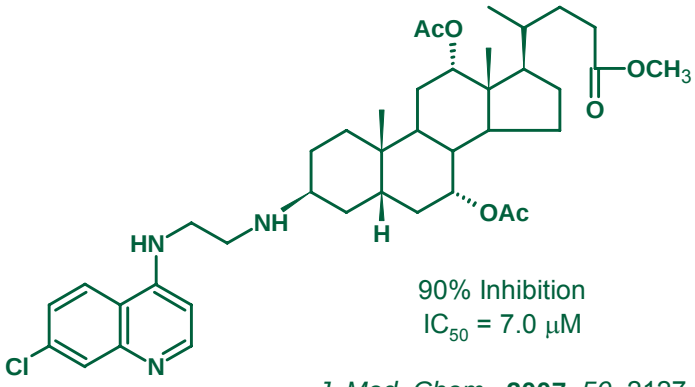
IC_{50} = 0.41 μM
K_i = 0.3 μM

Org. Lett., **2006**, *8*, 1729



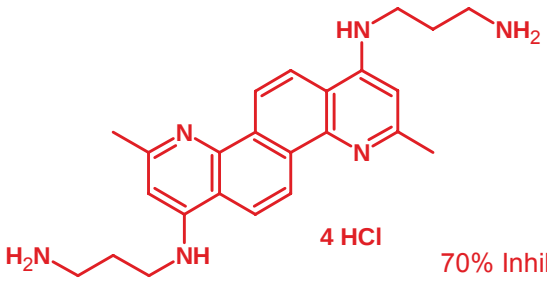
Fmoc-D-Cys(Trt)-OH
IC_{50} = 15 μM
K_i = 18 μM

Chem. Commun., **2006**, 3063



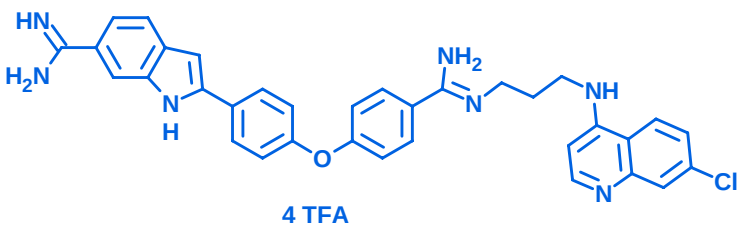
90% Inhibition
IC_{50} = 7.0 μM

J. Med. Chem., **2007**, *50*, 2127

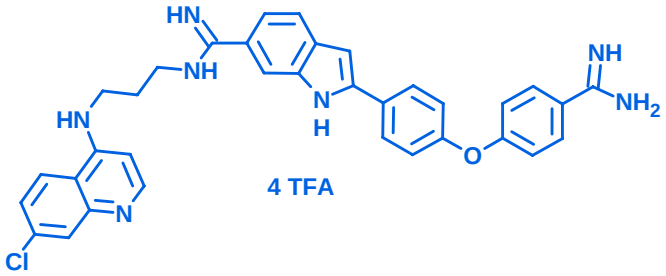


70% Inhibition
K_i = 8.1 μM

J. Med. Chem., **2011**, *54*, 1157



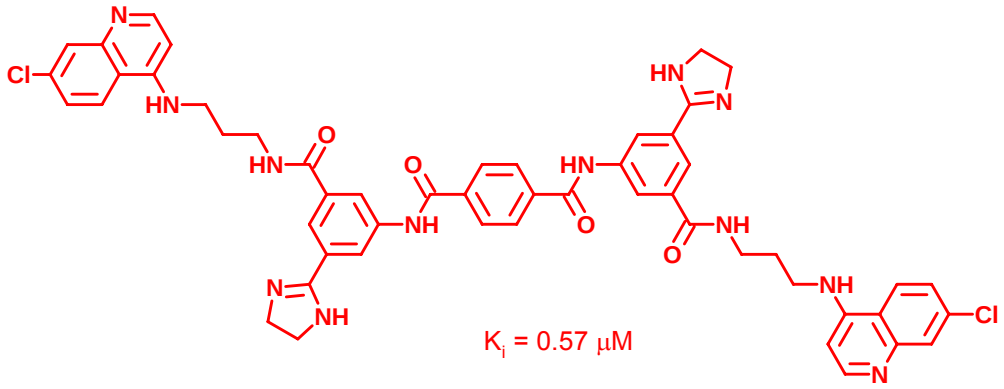
A



B

A/**B**=4/1
95% Inhibition
K_i = 0.60 μM

Biorg. Med. Chem. Lett., **2009**, *19*, 5811

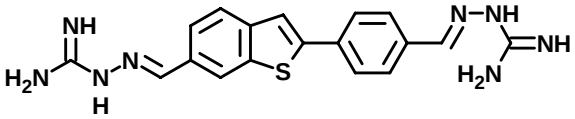


K_i = 0.57 μM

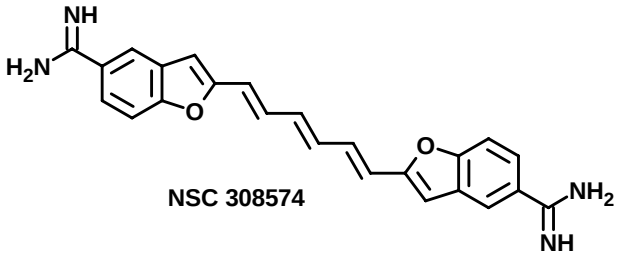
ACS Med. Chem. Lett., **2010**, *1*, 301

Reviews:
Angew. Chem. Int. Ed., **2008**, *47*, 8360
Future Microbiol., **2007**, *2*, 677
ACS Chem. Biol., **2006**, *1*, 359

J. Biol. Chem., **2007**, *282*, 5004



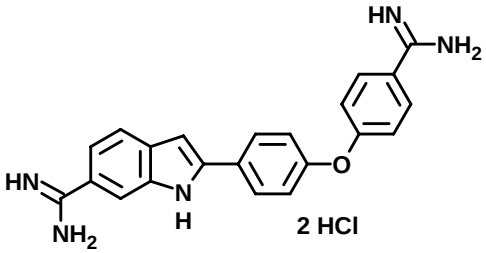
NSC 341909



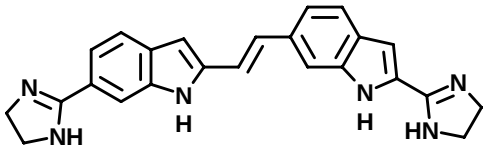
NSC 308574

Data base search queries from the NCI's Open Repository were used to test previously establish pharmacophore model and identified new SMNPI of BoNT/A LC

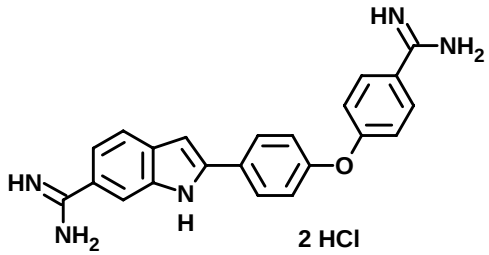
The four potent inhibitors were identified



NSC 240898



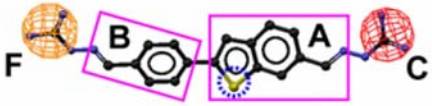
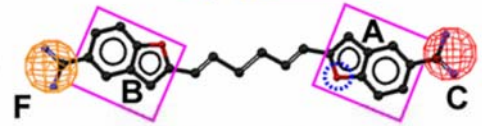
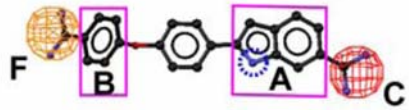
NSC 341907



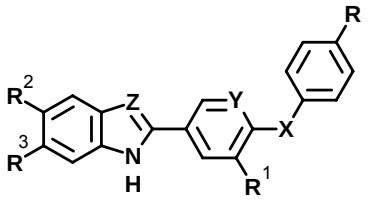
NSC 240898
75% Inhibition at 20 μM

- within 30 min was able to enter in to neuron cells
- exhibit low toxicity (c = 40 μM)
- was well tolerated by cell
- demonstrated a dose-dependent inhibition of SNAP-25 cleavage in Western blot analyses

NCS 240898 was identified as a promising lead SMNPI

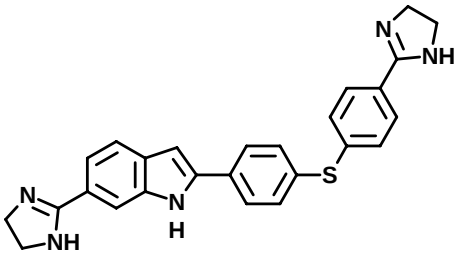
NSC	K_i(μM)	Query Fit	Distances (Ang.) A-B[1]	A-C[1]	A-F[1]	Total[2]
341909	3.0		7.5	4.8	13.5	19.6
308574	6.0		12.8	3.9	16.6	19.9
240898	10.0		9.6	3.9	11.9	17.8
341907	10.0		8.8	4.5	12.8	19.3

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

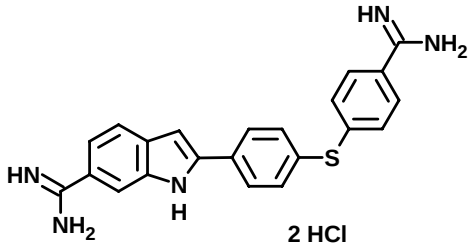


X = CH, N, S
R = Am, Im
R^1 = H, Cl, OMe, Im
R^2 = H, Am, Im
R^3 = H, CF_3, Am, Im

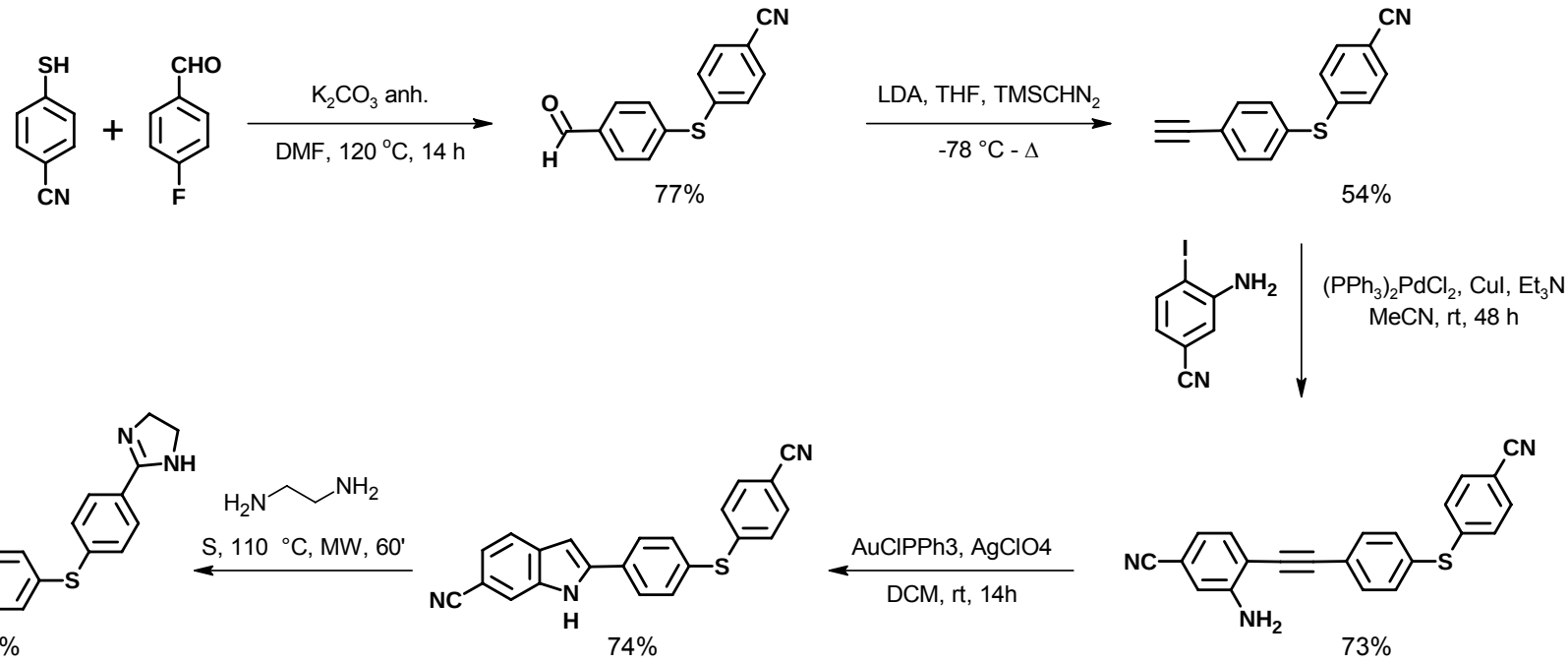
Heterocycles 2009, 79, 487
Chenbo Wang, PhD thesis, 2009, University of Pittsburgh



CWD021
80.3% Inhibition at 20 μM
K_i = 1.34 μM



CWD024
67.3% Inhibition at 20 μM



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