Development of Small Molecule Inhibitors of the Lethal Factor in Anthrax

Brian Englund
Michigan State University
September 13, 2006

Outline

- Introduction
- Proteolysis Mechanism
- Anthrax Inhibitors
  - Panchal and Bavari
  - Pellechia
  - Quinn
  - Merck
- Conclusions
- Acknowledgements

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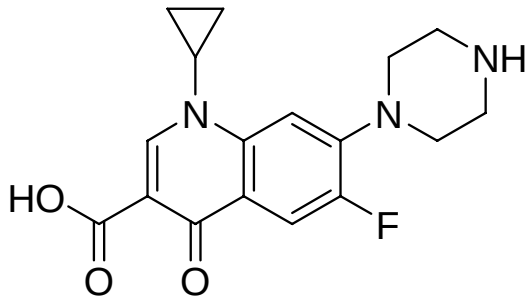
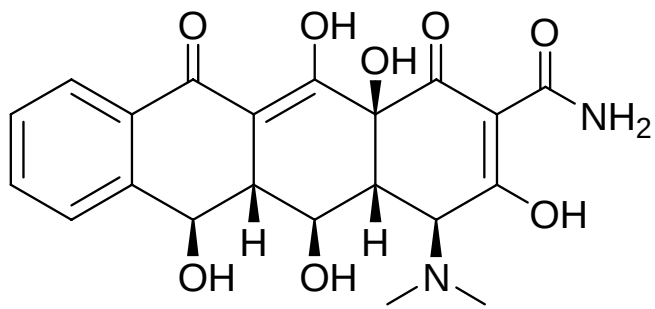
Current Treatment

- Vaccination
  - Only for people at high risk
  - Joint symptoms and gastrointestinal problems
- Antibiotics
  - Ciprofloxacin
  - Doxycycline

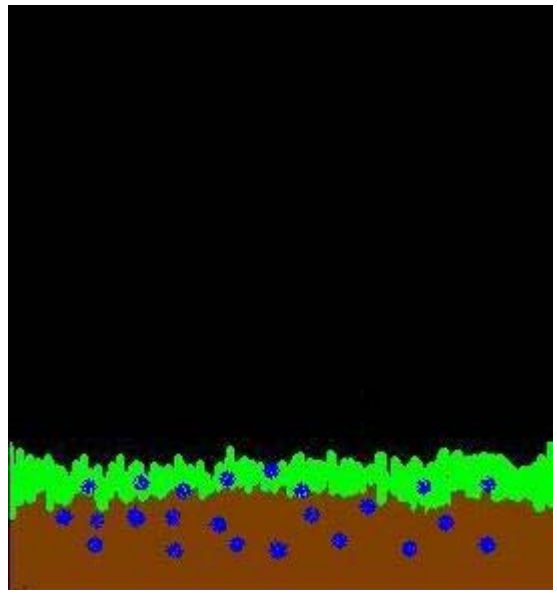




Geier, D.A.; Geier, M.R. *Clin Exp Rheumatol.* **2002,** *20,* 217
Geier, D.A.; Geier, M..R. *Hepatogastroenterology.* **2004,** *51,* 762

Life Cycle of Anthrax

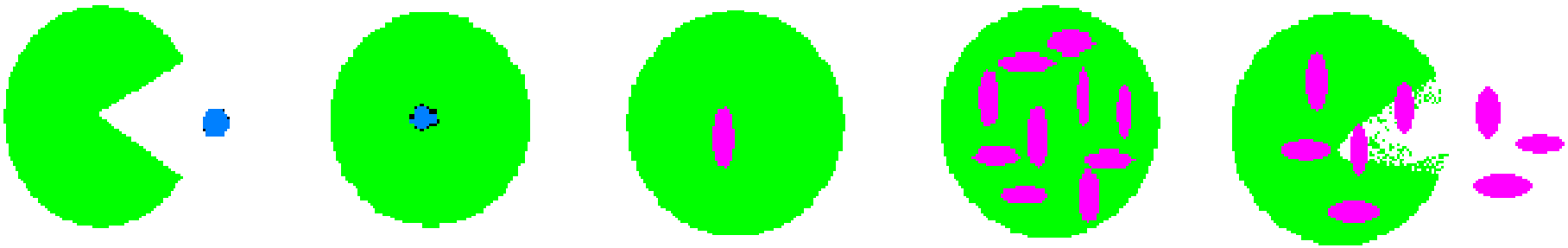
- Caused by *Bacillus anthracis*
- Spores are absorbed by macrophages in the lungs
- Spores are transported to lymph nodes



http://www.teamtornado.co.uk

Abrami, L.; Reig, N.; van der Goot, F. *Trends Microbiol.* **2005,** *13,* 72

How Anthrax Kills

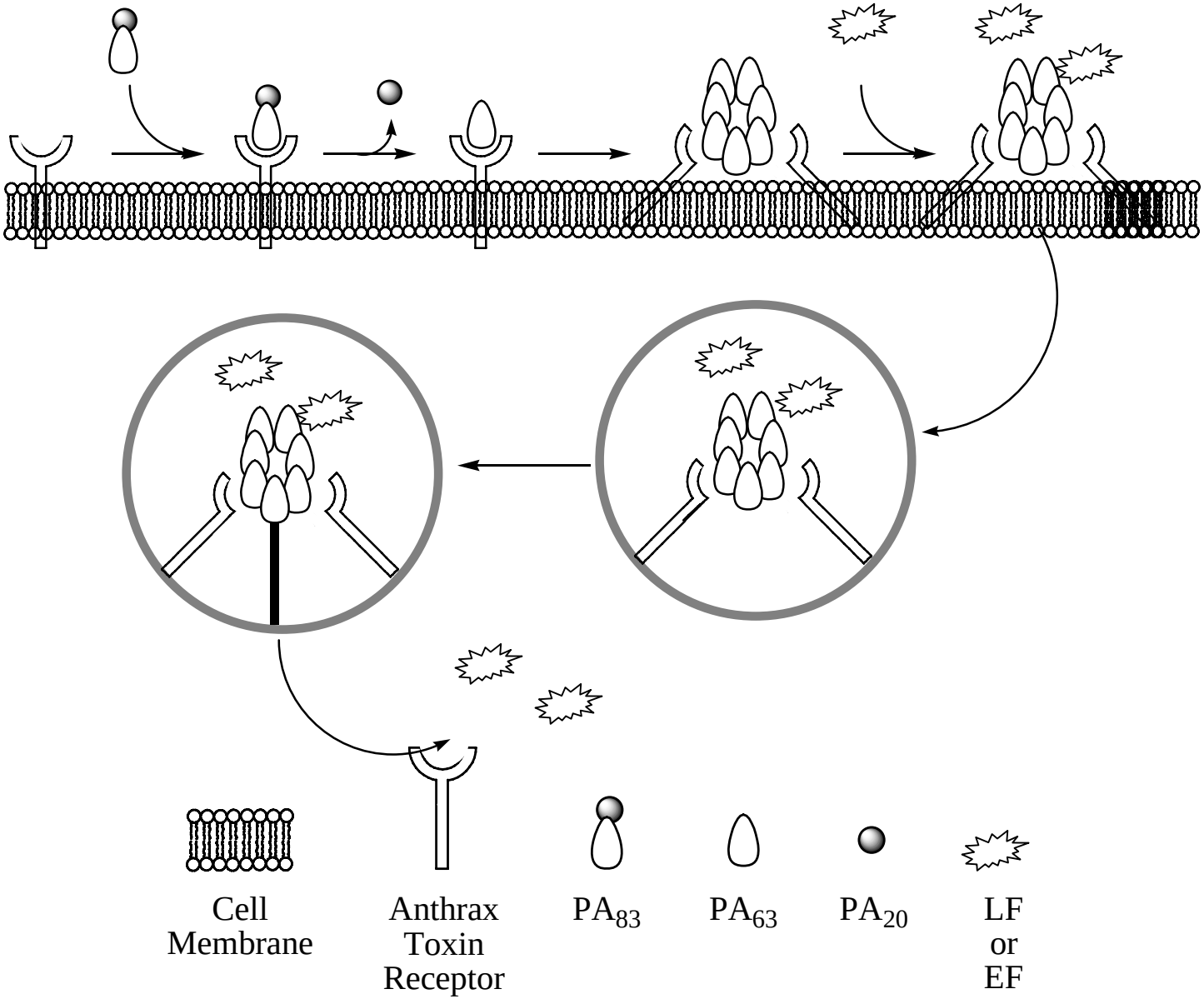


Dixon, T.C. *N. Engl. J. Med.* **1999,** *341*, 815
Fukao T. *Lancet Infectious Diseases* ***2004,*** *4*, 166

The Anthrax Toxin

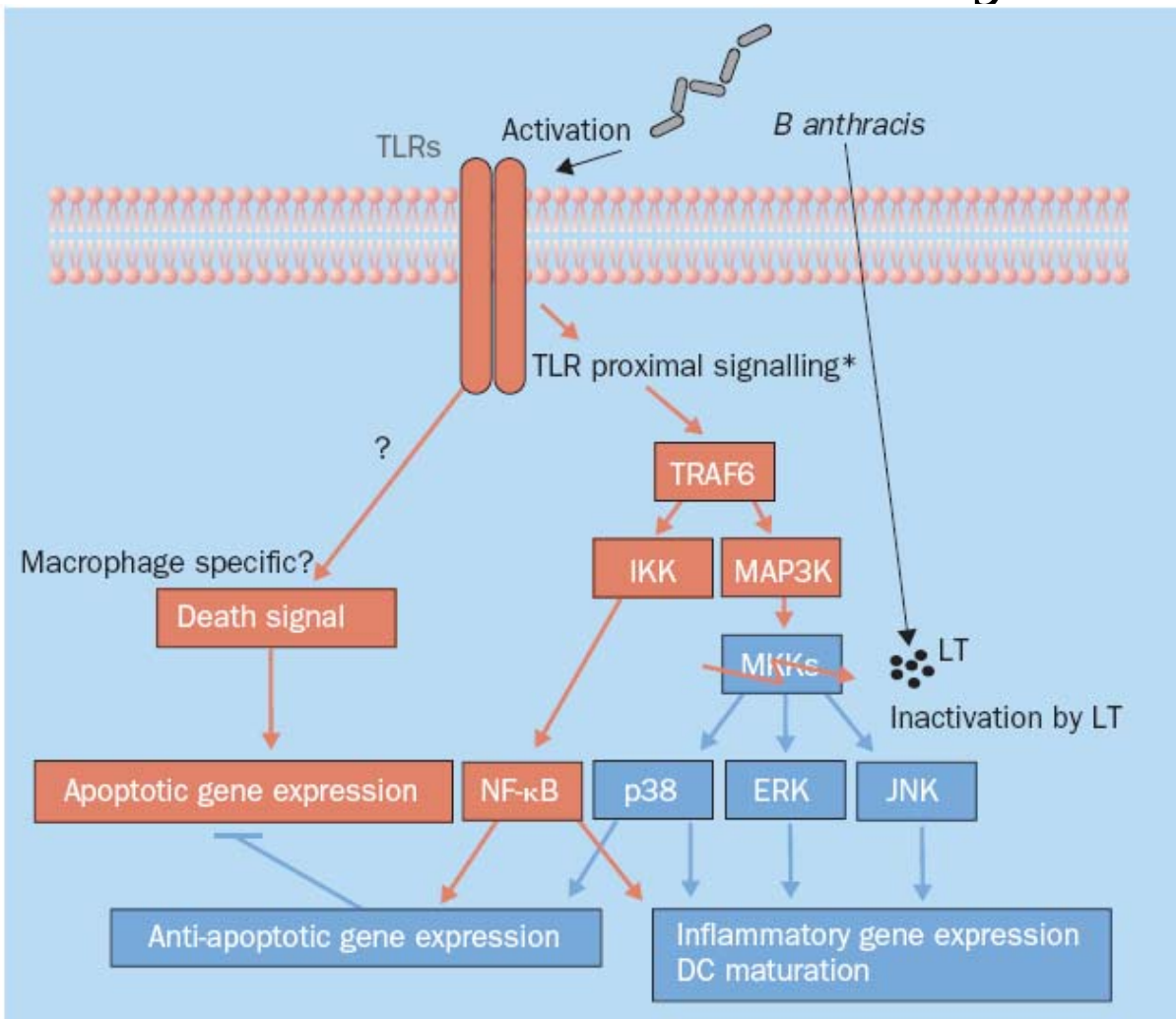
- A-B exotoxin
- Tripartite
  - Protective antigen (PA)
  - Lethal factor (LF)
  - Edema factor (EF)
- Cleaves mitogen activated protein kinase kinase (MAPKK)

Park, J.M. et. al. *Science*. **2002,** *297*, 2048



von Heijne, G. *Science.* **2005,** *309*, 709

MAPKK Pathway



Taken from Fukao T. *Lancet Infectious Diseases* ***2004,*** *4*, 166

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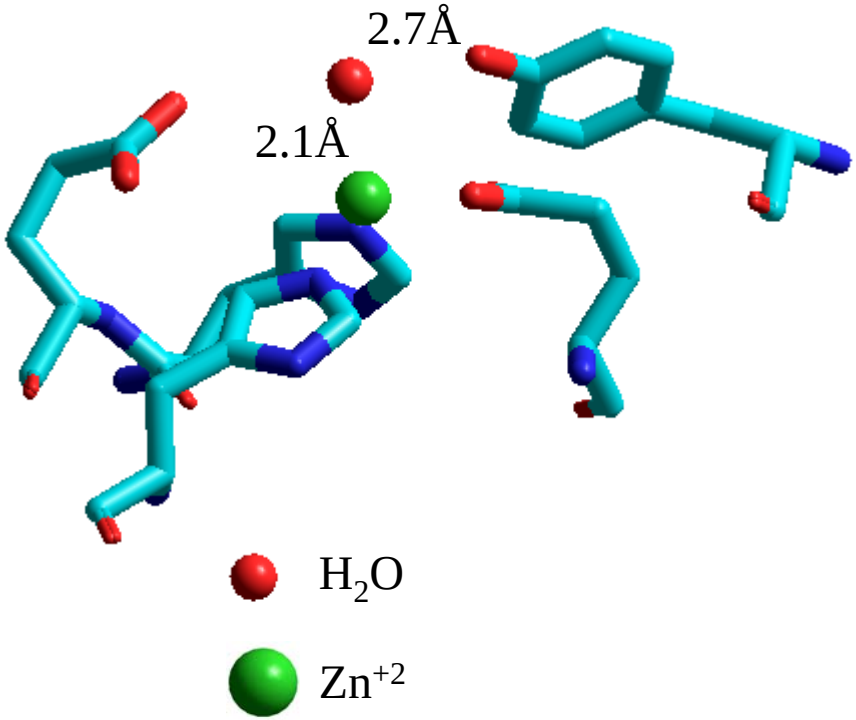
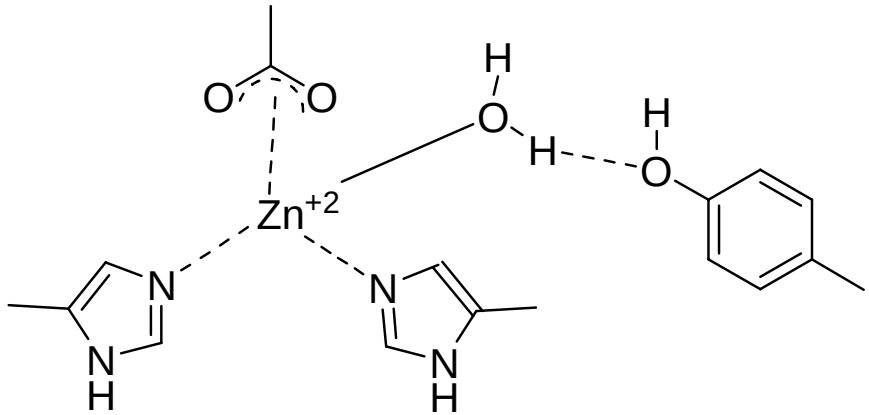
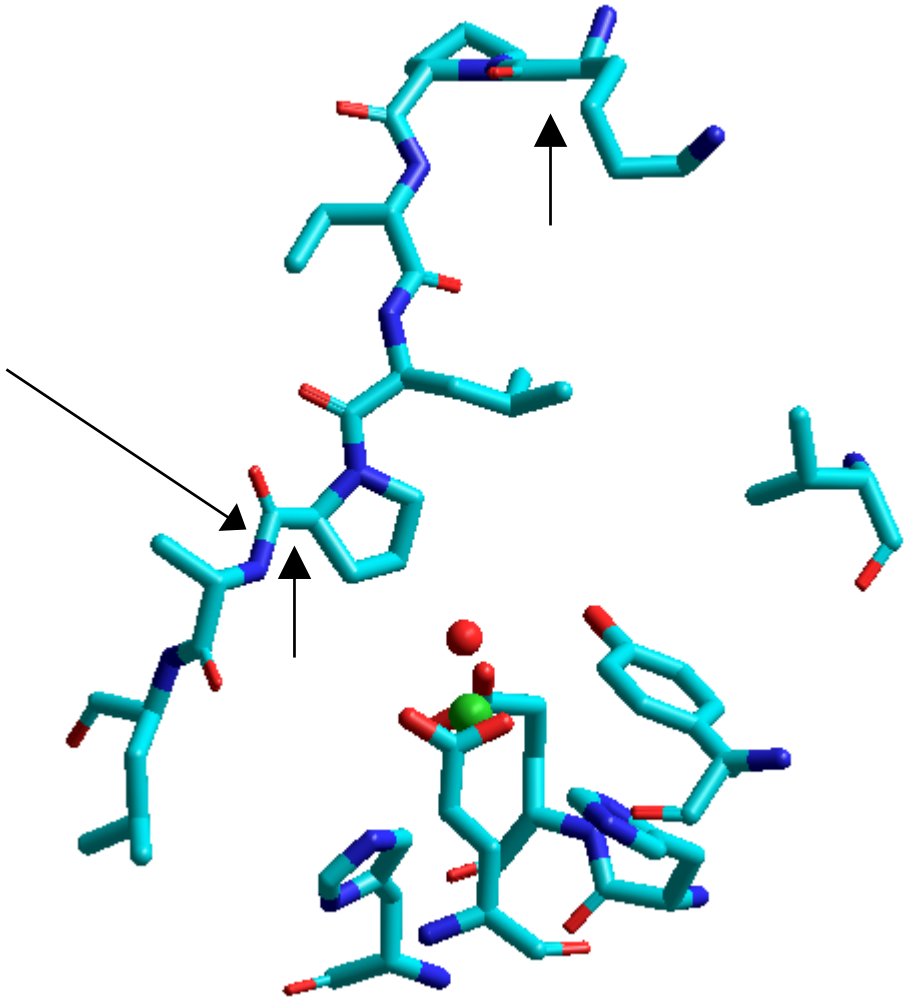
Lethal Factor Crystal Structure





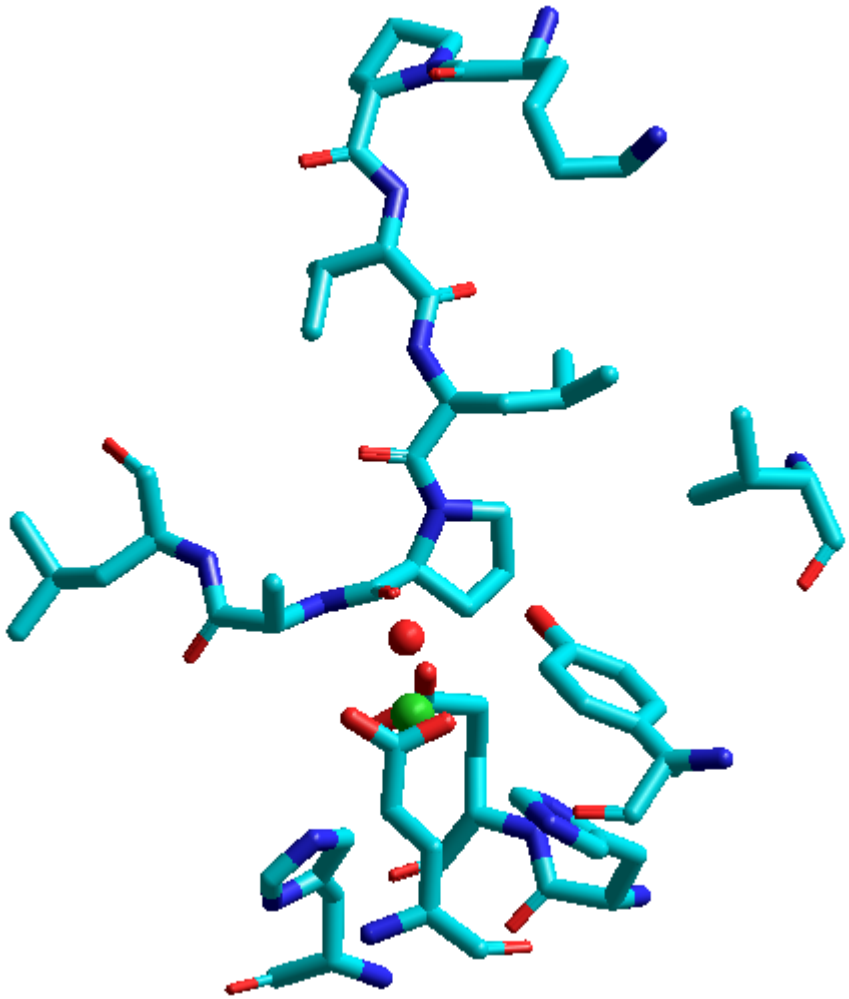
Liddington, R.C. et. al. *Nature*. **2001,** *414*, 229

Lethal Factor and MAPKK



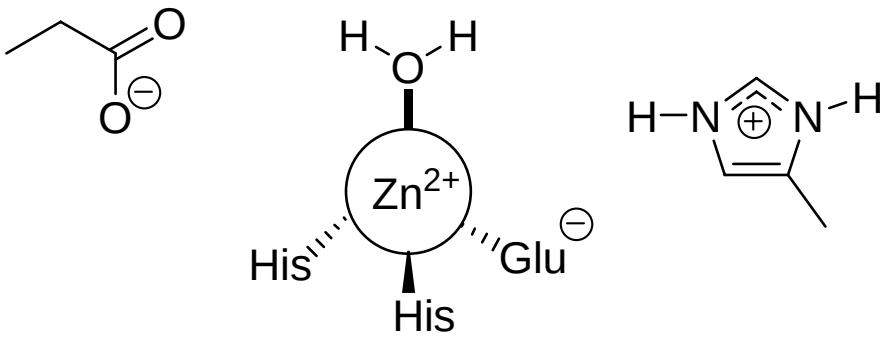
Liddington, R.C. et. al. *Nature*. **2001,** *414*, 229

Lethal factor and MAPKK (cont.)

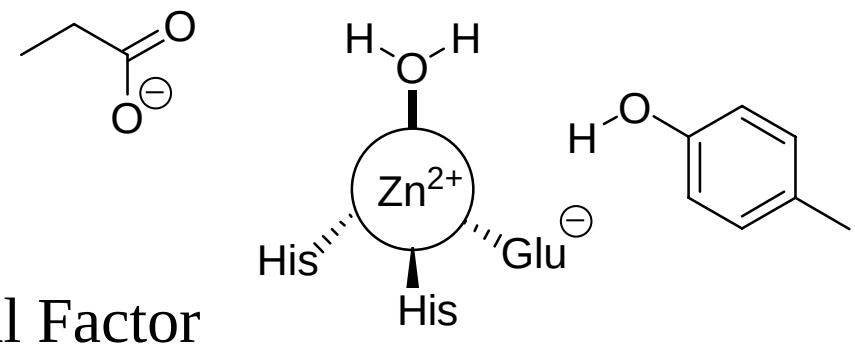


Liddington, R.C. et. al. *Nature*. **2001,** *414*, 229

Thermolysin as a Model

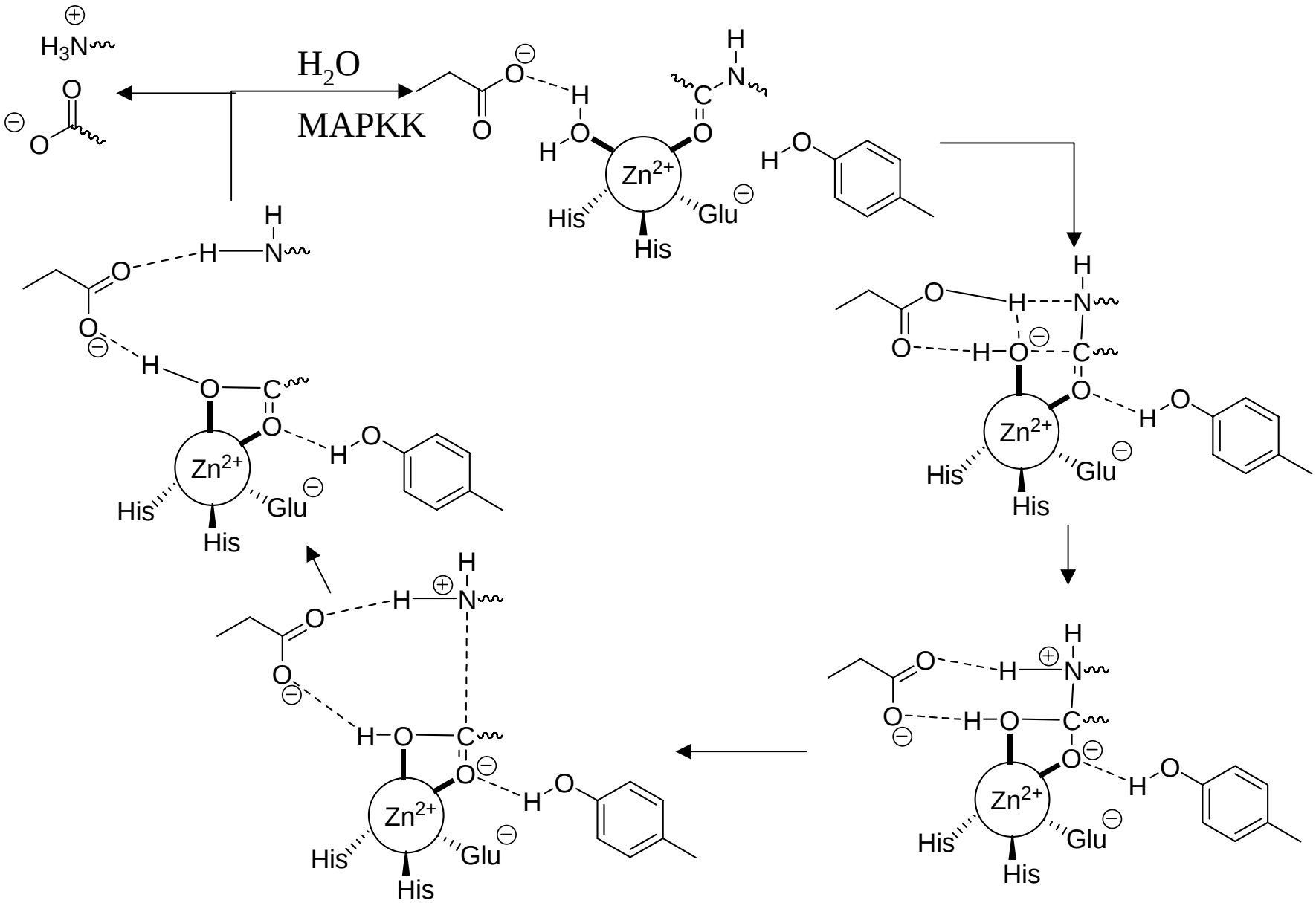


Thermolysin



Lethal Factor

Blomberg, M.R.A.; Pelmenschikov, V. *J. Biol. Inorg. Chem.* **2002,** *7, 284*


H2O
MAPKK
Zn2+
His
Glu
His

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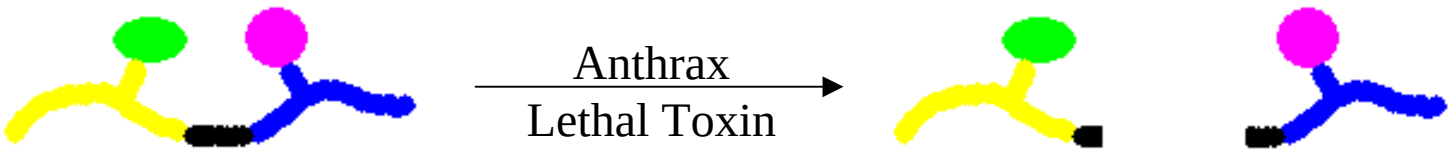
Drug Discovery

- High throughput screening (HTS)
  - Large library
  - Higher molecular weight
  - Fluorescence based screening
- Fragment-based drug design
  - Smaller library
  - Lower molecular weight
  - Simpler molecules
  - Weaker inhibition

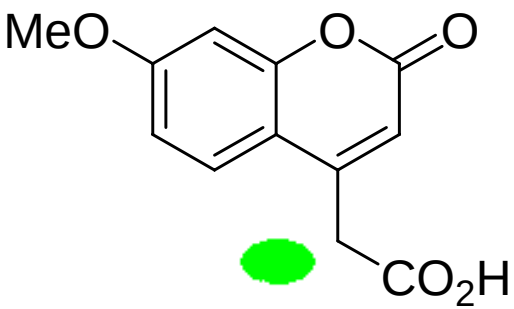
Nestler, H.P. *Curr. Drug Disc. Tech.* **2005,** *2*, 1

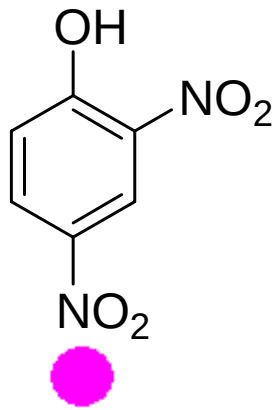
Fluorescence Detection in HTS



No Fluorescence

Fluorescence





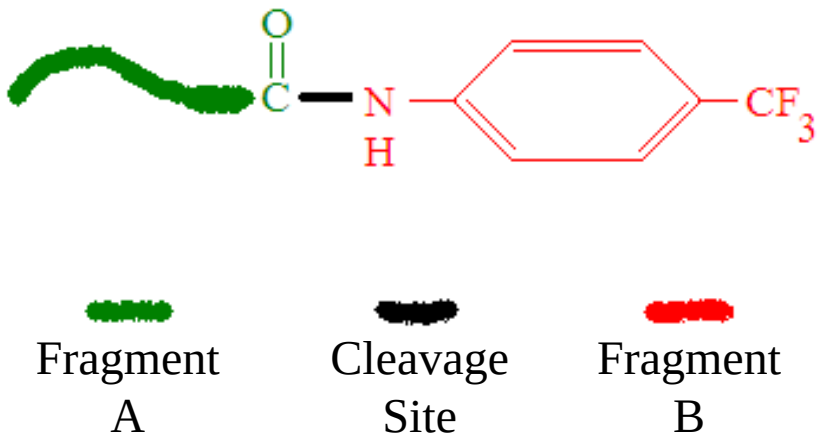
Panchal, R.G. et. al. *Nat. Struct. Mol. Bio.* **2004,** *11,* 67

Fluorescence Experiment

- HEPES pH 7.2
- 5 nM lethal factor
- 20 μM inhibitor
- Fluorescent peptide substrate
- Measure emission every minute for 30 minutes

Panchal, R.G. et. al. *Nat. Struct. Mol. Bio.* **2004,** *11,* 67

^{19}F NMR Detection in Fragment Based Design



Pellechia, M. et. al. *Proc. Natl. Acad. Sci.* **2005,** ***102***, 9499

Verifying Leads With HPLC

- All screening studies verified their promising results with HPLC
- Inhibitors can quench some fluorescence
- Quenching from inhibitor leads to inaccurate IC_{50}

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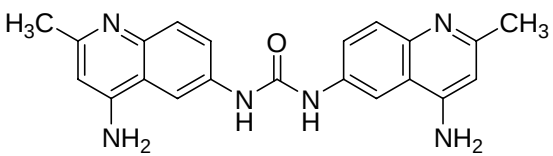
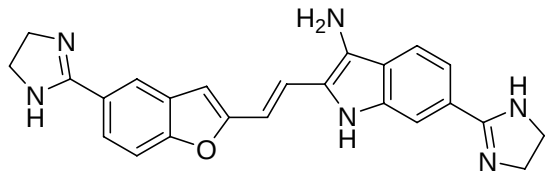
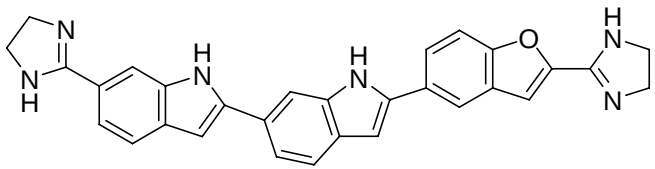
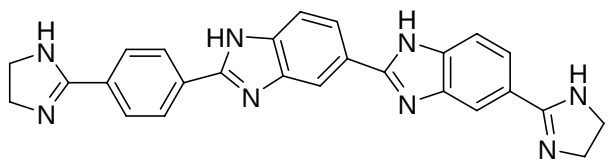
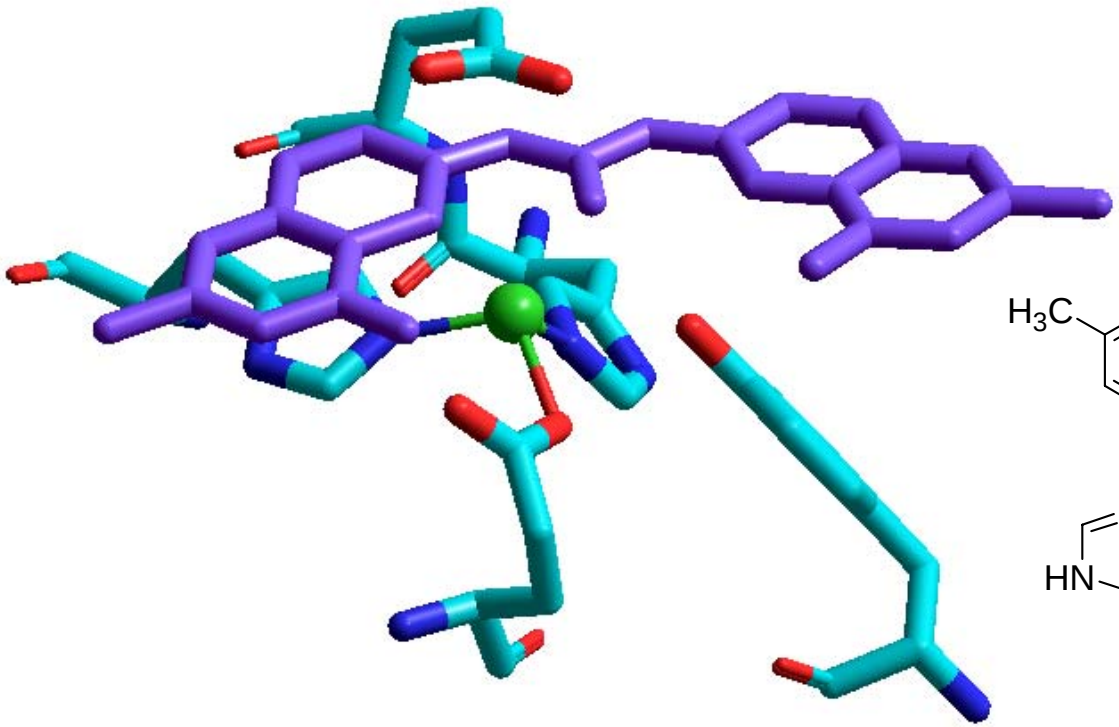
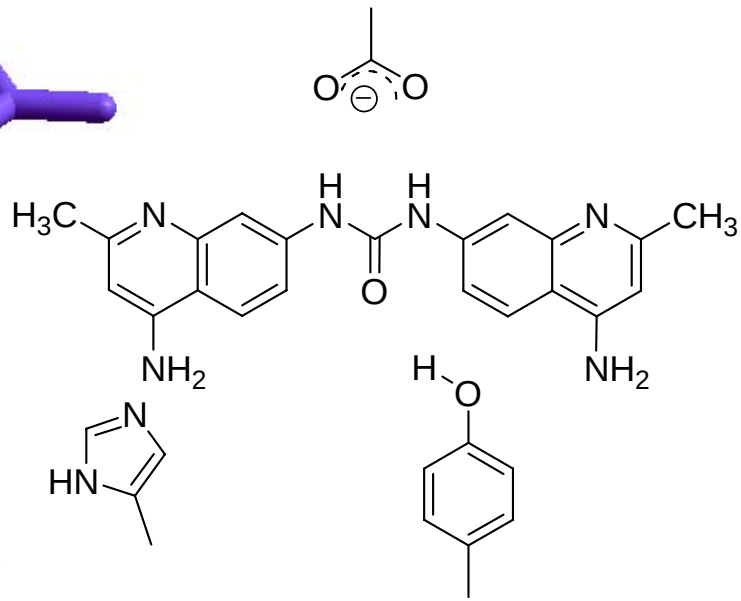
Active Compounds

Structure	% inhibition at 20μM	ID number
	95	PB-1
	90	PB-2
	90	PB-3
	90	PB-4

Panchal, R.G.; Bavari, S. et. al. *Nat. Struct. Mol. Bio.* **2004,** *11,* 67

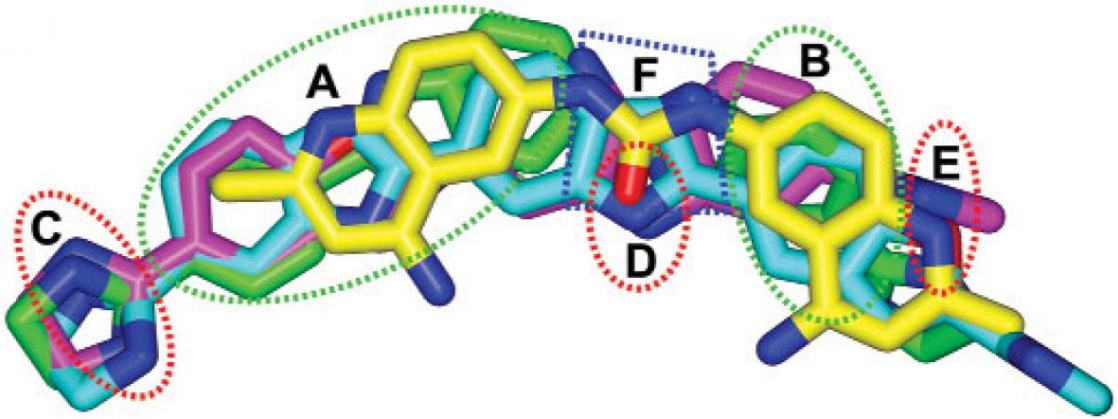
LF Complexed With PB-1



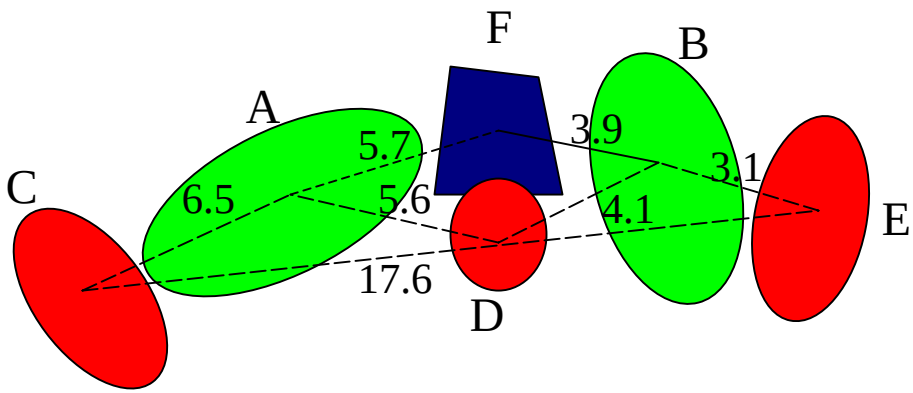


Panchal, R.G.; Bavari, S. et. al. *Nat. Struct. Mol. Bio.* **2004,** *11,* 67

Developing a Pharmacophore

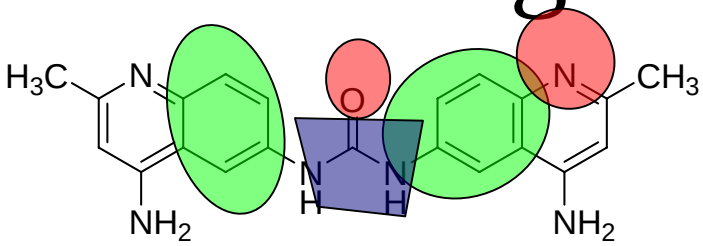


Taken directly from *Nat. Struct. Mol. Bio.* **2004,** *11,* 67



Panchal, R.G.; Bavari, S. et. al. *Nat. Struct. Mol. Bio.* **2004,** *11,* 67

Examining the Pharmacophore



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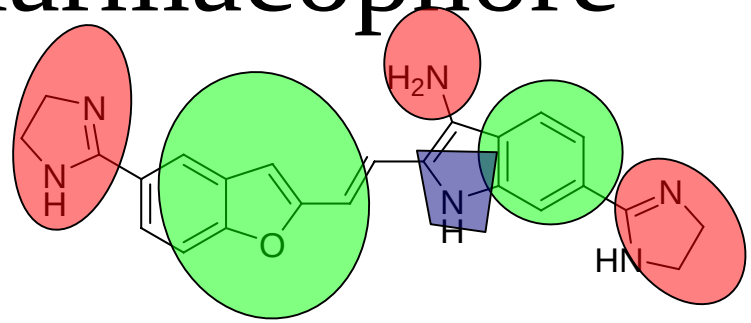

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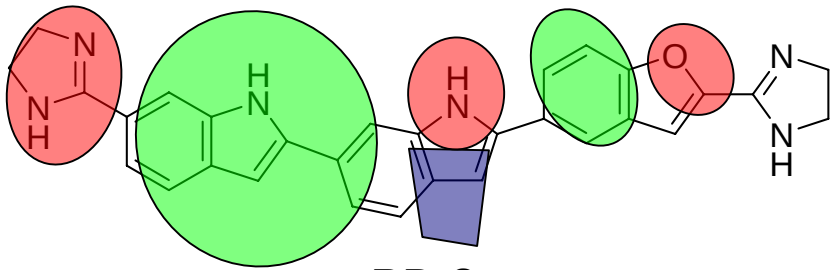

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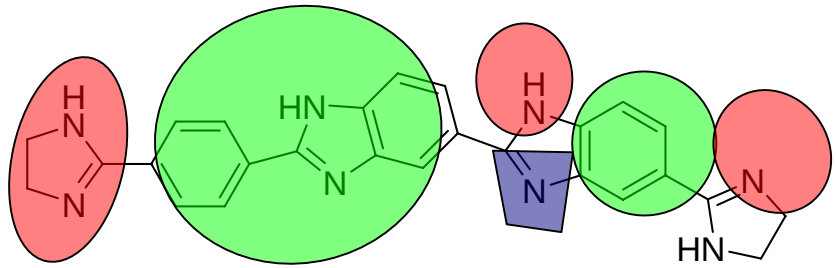

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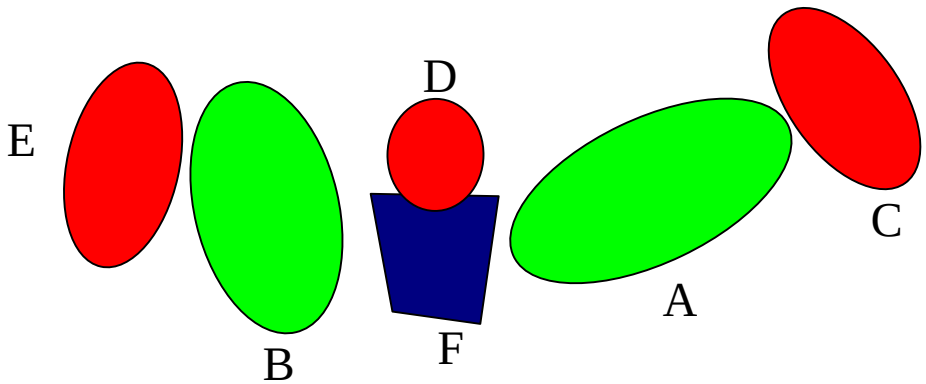

Panchal Summary

- Hydrogen bond acceptor in center
- Aromatic rings on either side of a linker
- If the linker is a hydrogen bond donor, it coordinates to glutamate
- Hydrogen bond acceptors/donors at the end of the aromatic rings

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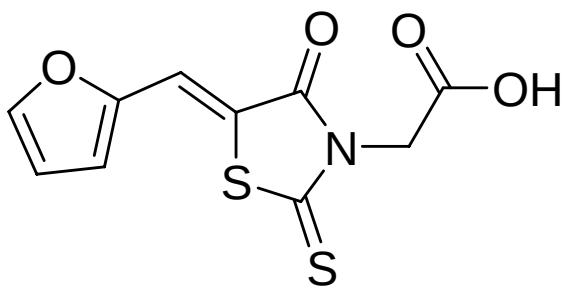
Pellechia's Scaffold

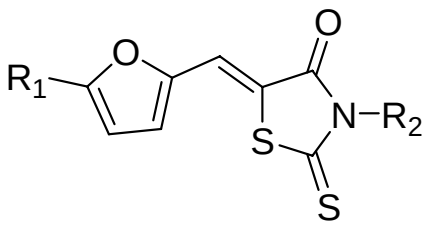
- Fragment-based design

- Hit with an IC_{50} of 140 μM



MP-1

Pellechia, M. et. al. *Proc. Natl. Acad. Sci.* **2005,** ***102****,* 9499



Pellechia's Scaffold

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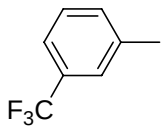
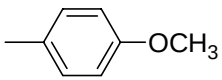
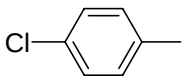
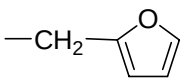
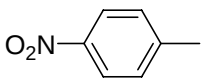
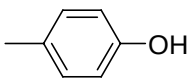
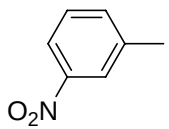
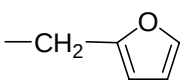
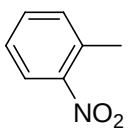
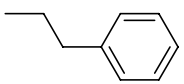
MP-2			300
MP-3			150
MP-4			37.7
MP-5			36.3
MP-6			31.9

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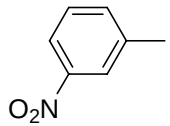
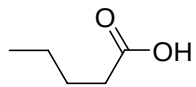
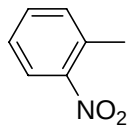
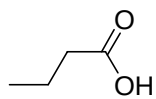
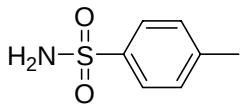
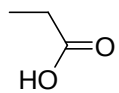
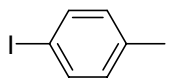
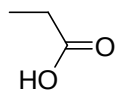
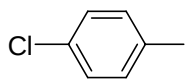
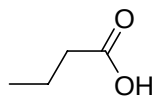
MP-7			20
MP-8			12.8
MP-9			9.1
MP-10			5.5
MP-11			1.7

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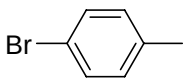
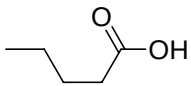
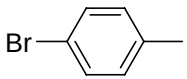
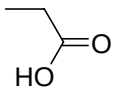
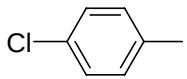
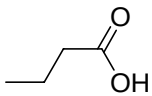
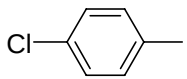
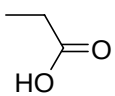
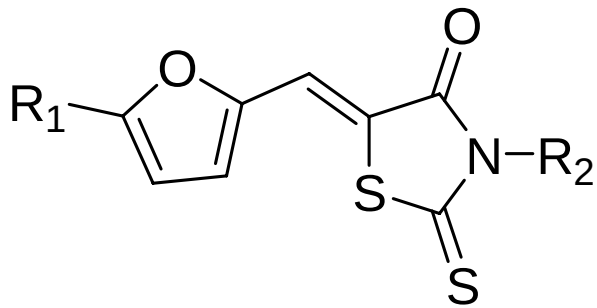
Varying the Acid Side Chain

ID	R_1	R_2	IC_{50} (μM)
MP-12			2.3
MP-13			0.85
MP-11			1.7
MP-14			0.90



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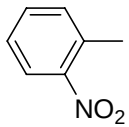
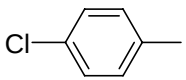
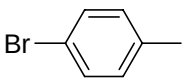
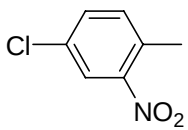
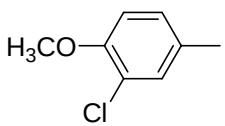
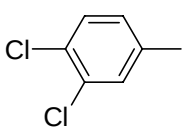
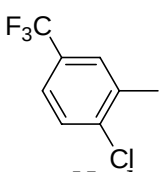
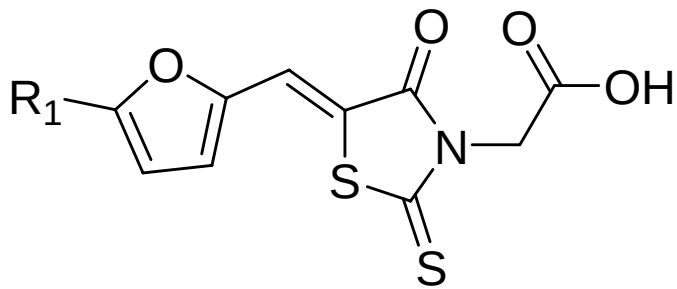
Varying the R_1 Ring

ID	R_1	IC_{50} (μM)
MP-16		3.10
MP-14		0.90
MP-13		0.85
MP-17		0.50
MP-18		0.30
MP-19		0.27
MP-20		0.19



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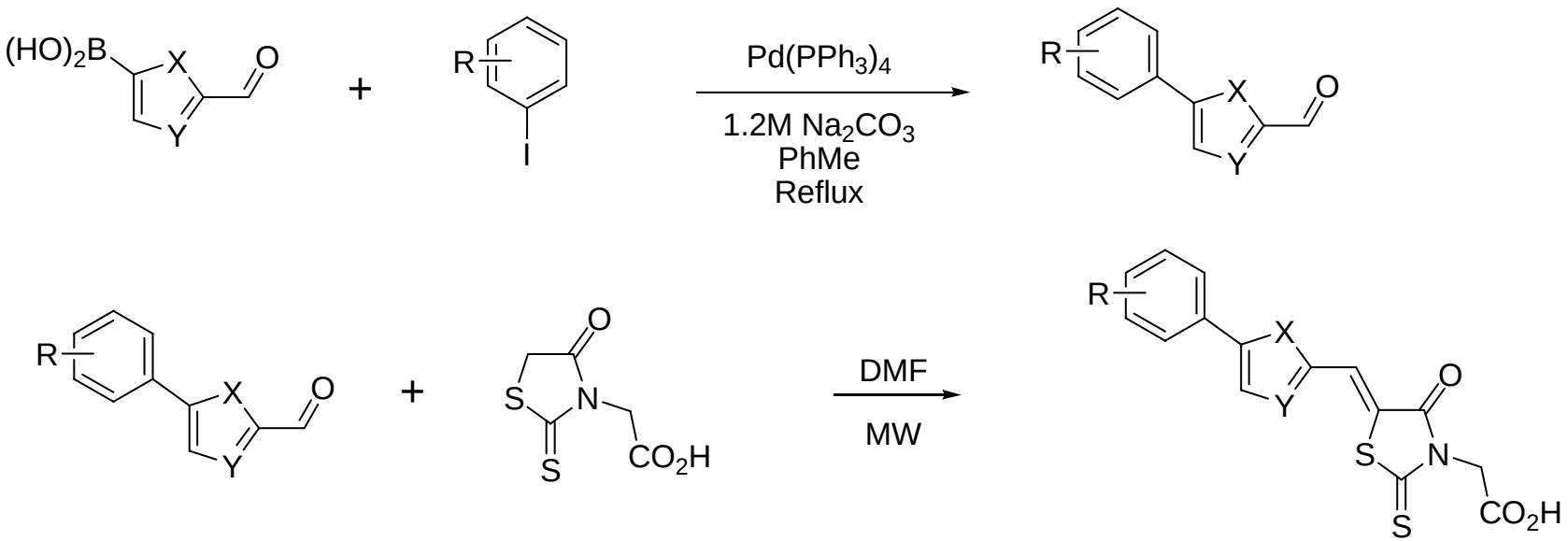
Synthesis of MP-1 Derivatives



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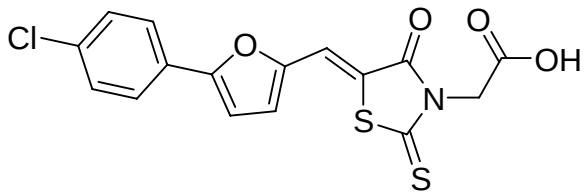
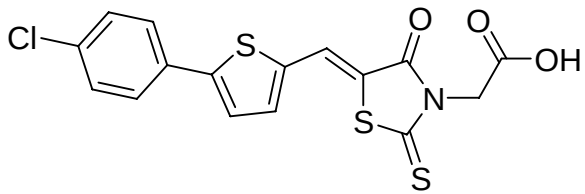
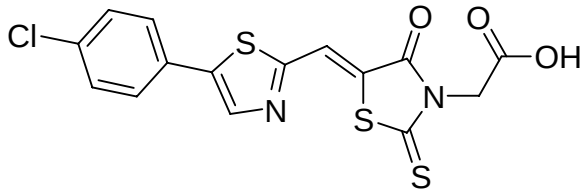
Varying the Furan Ring

ID		IC_{50} (μM)
MP-14		0.9
MP-21		3.2
MP-22		10.0

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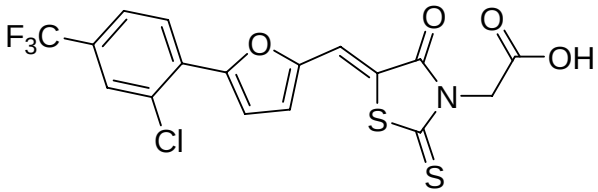
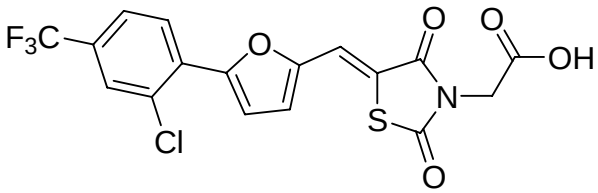
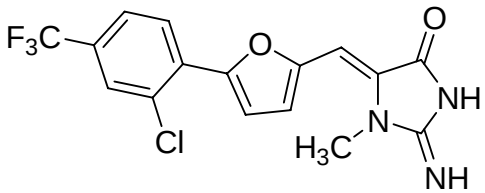
Varying the Thiazolidinone Ring

ID		IC_{50} (μM)
MP-20		0.19
MP-23		5.9
MP-24		100

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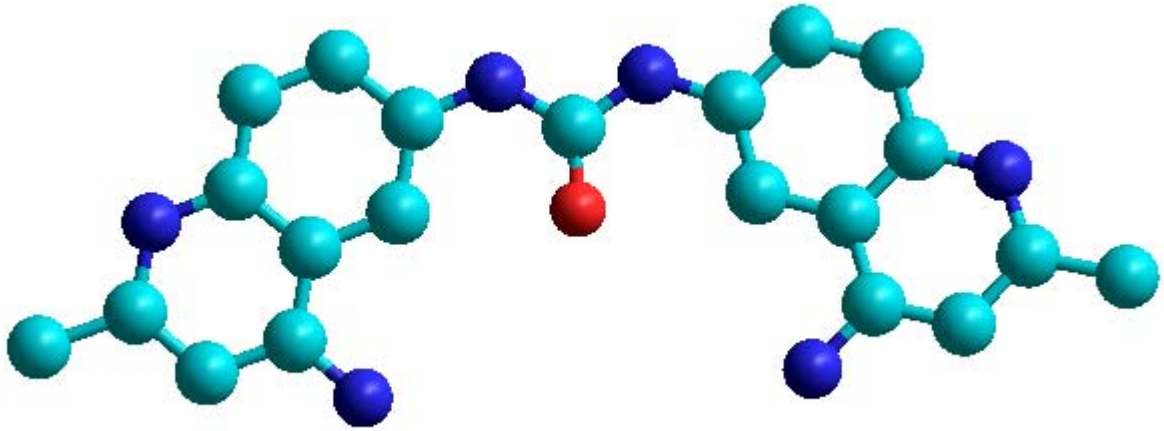
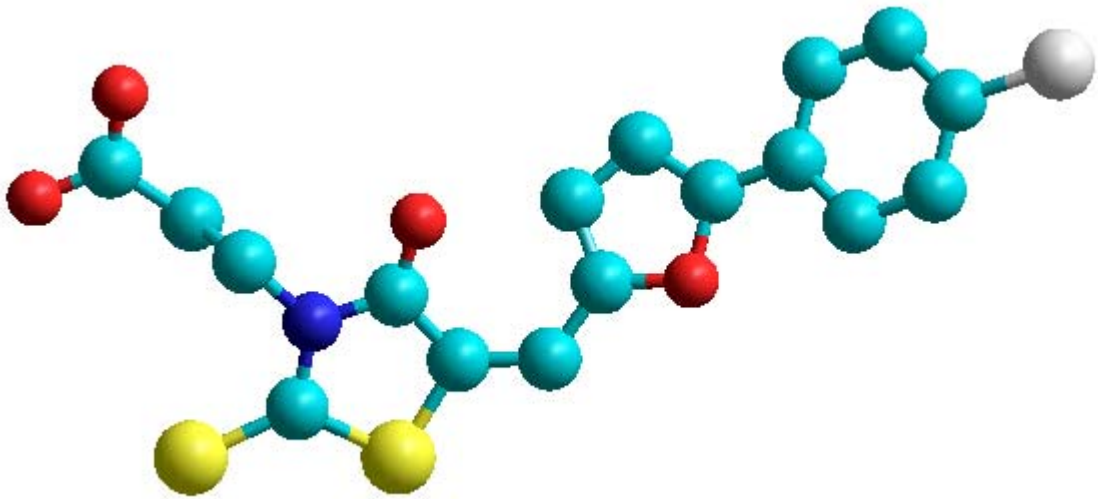
Applying the Pharmacophore

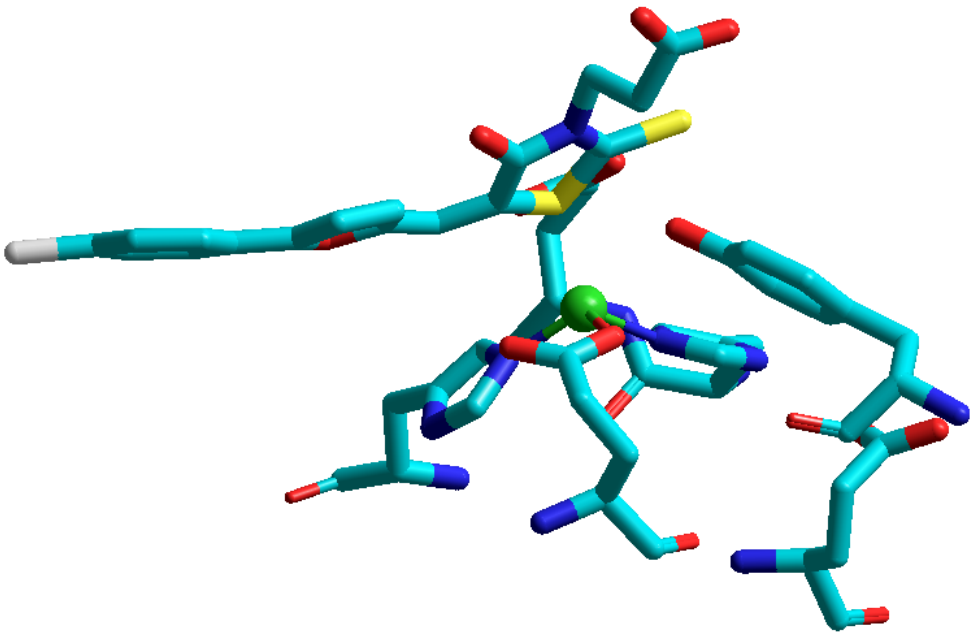
PB-1

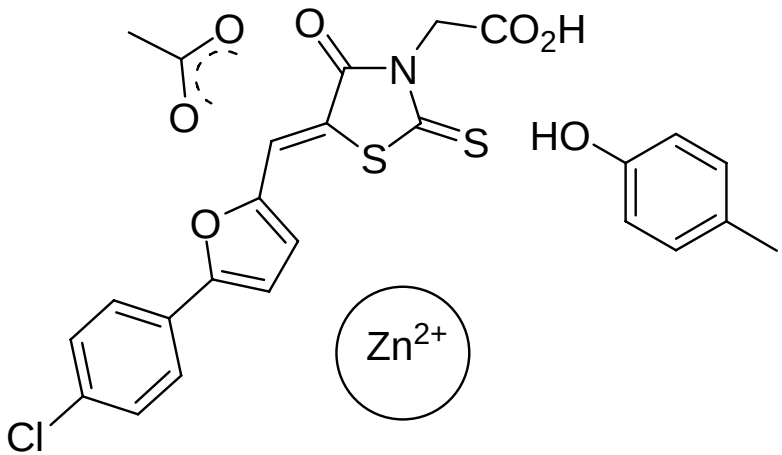


MP-11



LF Complexed With MP-11





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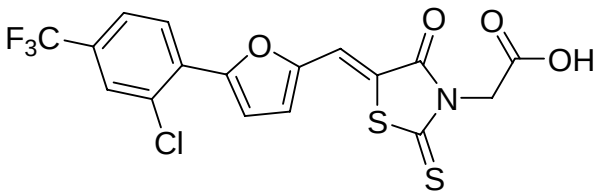
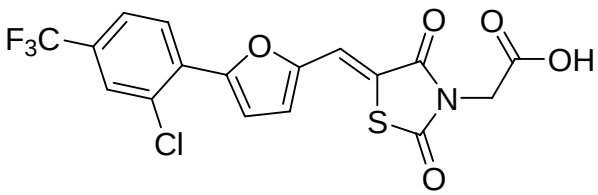
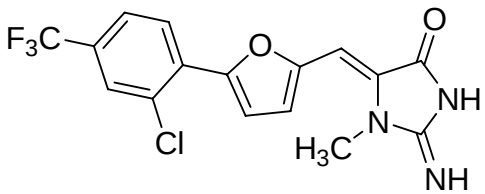
Varying the Thiazolidinone Ring

ID		IC_{50} (μM)
MP-20		0.19
MP-23		5.9
MP-24		100

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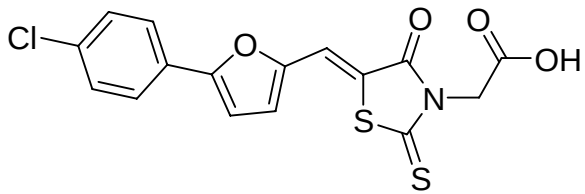
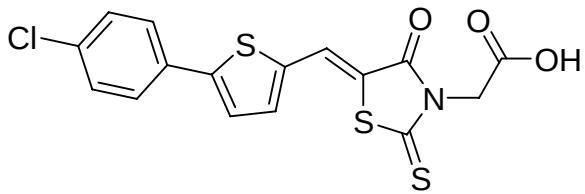
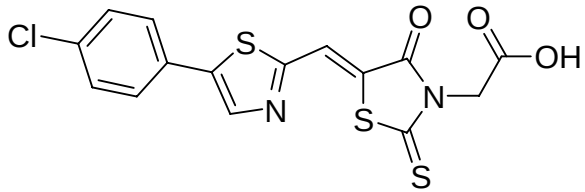
Varying the Furan Ring

ID		IC_{50} (μM)
MP-14		0.9
MP-21		3.2
MP-22		10.0

Pellechia, M. et. al. *J. Med. Chem.* **2006,** *49,* 27

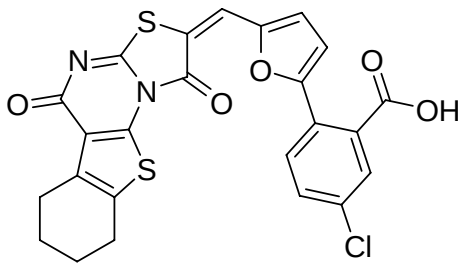
Pellechia Summary

- Sulfur strongly coordinates the zinc
- 2-phenyl furan serves as a core structure
- Carboxylic acid interacts with polar residues
- Confirms the importance of an aromatic ring

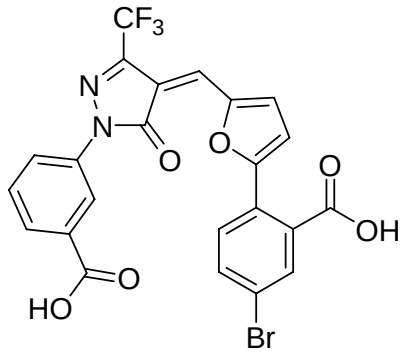
Outline

- Introduction
- Proteolysis Mechanism
- Anthrax Inhibitors
  - Panchal and Bavari
  - Pellechia
  - Quinn
  - Merck
- Conclusions
- Acknowledgements

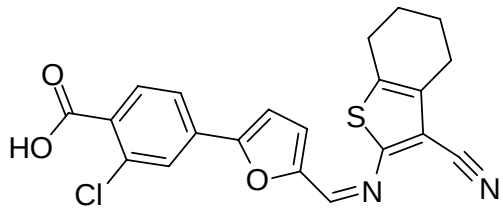
Quinn's Lead Compounds



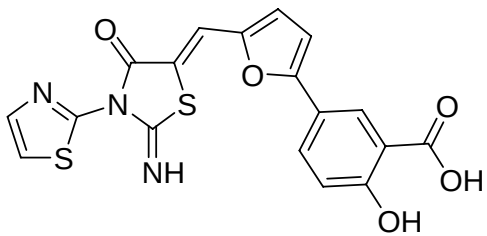
MQ-1
IC_{50} = 1.1 μM



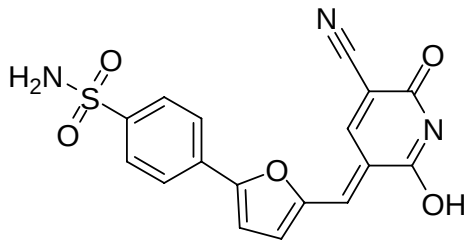
MQ-2
IC_{50} = 1.8 μM



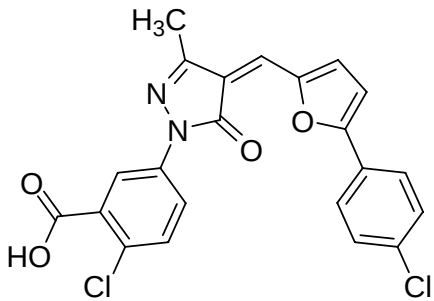
MQ-3
IC_{50} = 5.2 μM



MQ-4
IC_{50} = 4.8 μM



MQ-5
IC_{50} = 8.3 μM



MQ-6
IC_{50} = 10.0 μM

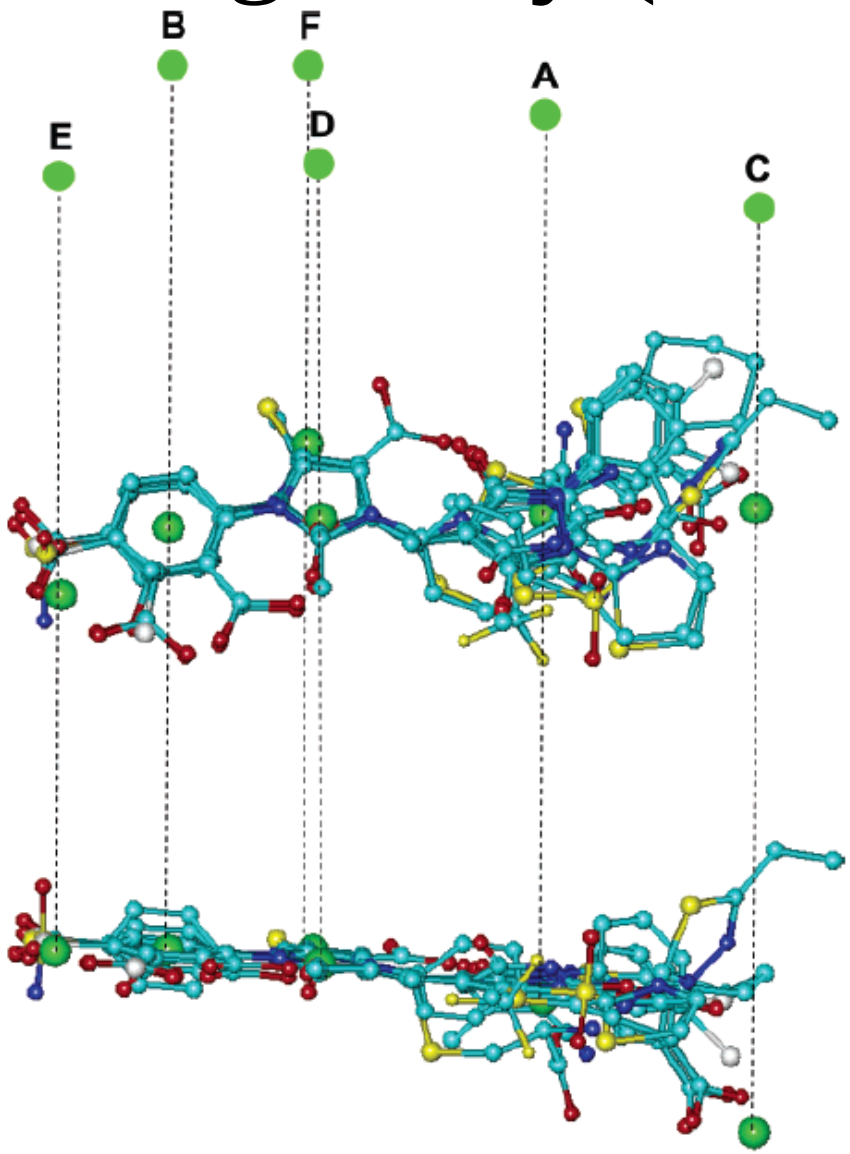
Quinn, M. et. al. *J. Med. Chem.* **2006,** *49,* 5232

Quinn's Modeling Study

- Modeled the compounds
- Scanned all conformations within 6 kcal/mol of global minimum
- Compared to Panchal and Bavari's pharmacophore

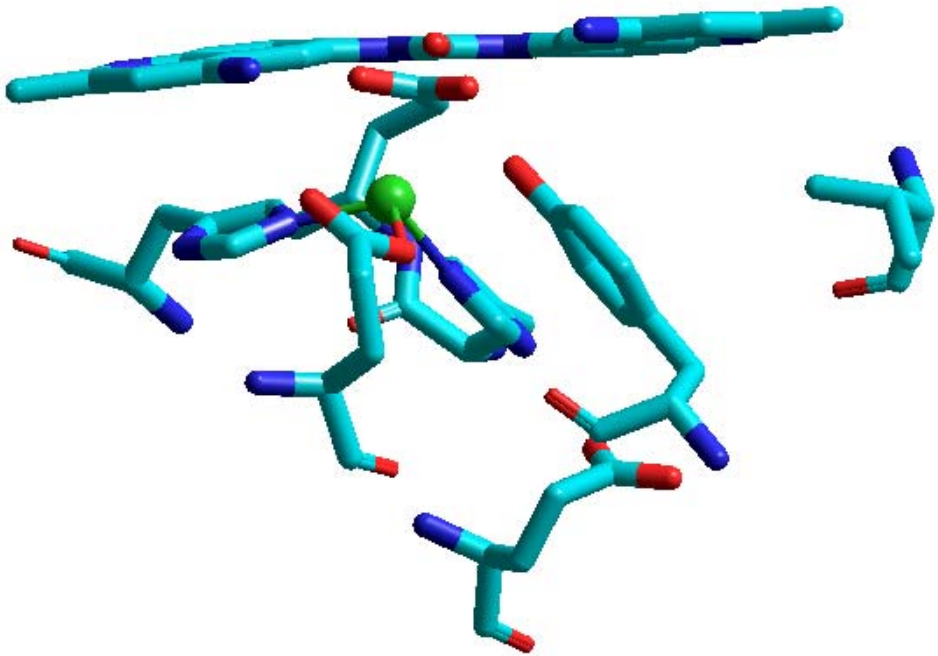
Quinn, M. et. al. *J. Med. Chem.* **2006,** *49,* 5232

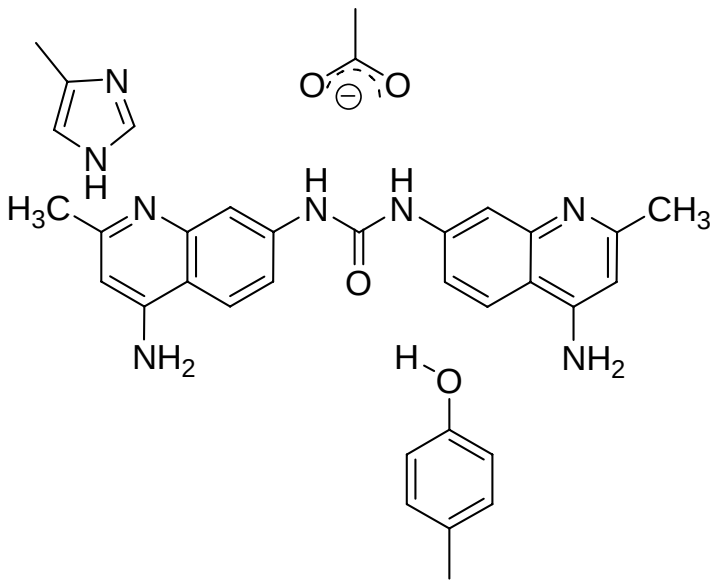
Quinn's Modeling Study (cont.)



Quinn, M. et. al. *J. Med. Chem.* **2006,** *49,* 5232

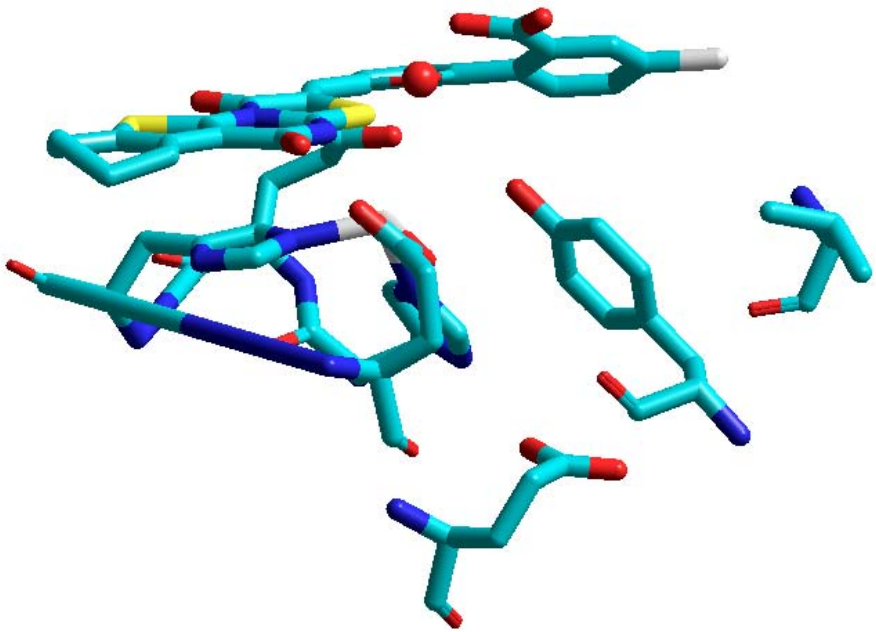
PB-1 Complexed with LF

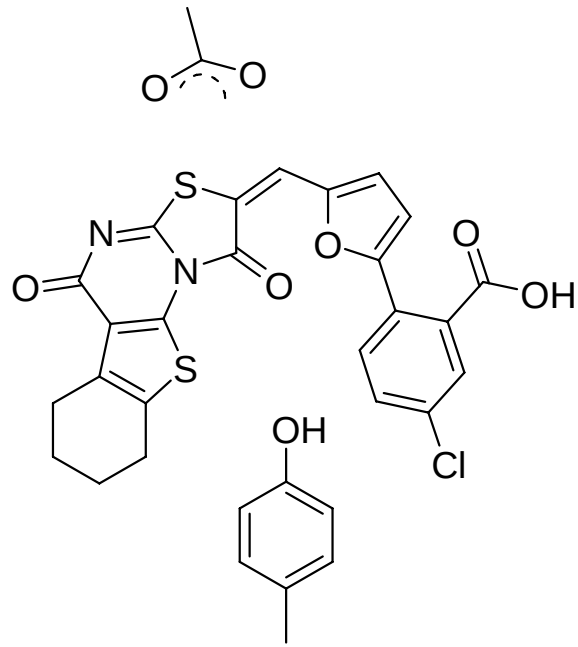




Panchal, R.G.; Bavari, S. et. al. *Nat. Struct. Mol. Bio.* **2004,** *11,* 67

MQ-1 Manually Moved Into the Active Site





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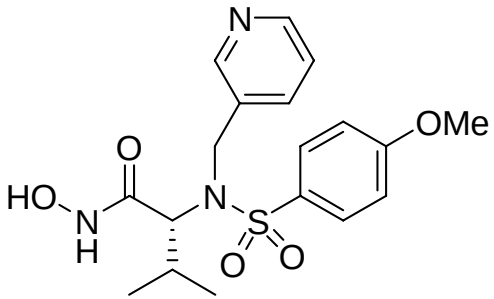
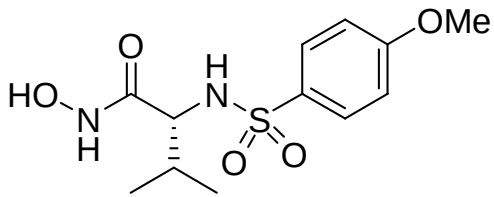
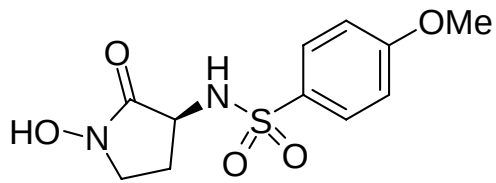
- Acknowledgements

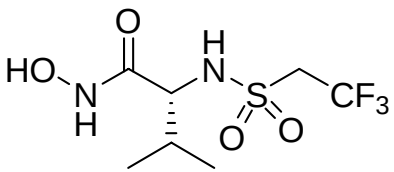
Merck Compounds

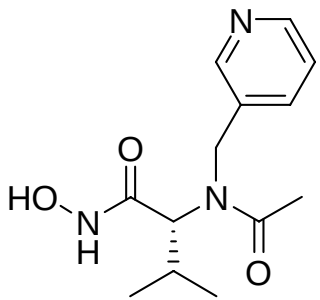


YX-1
IC_{50} = 1.2μM









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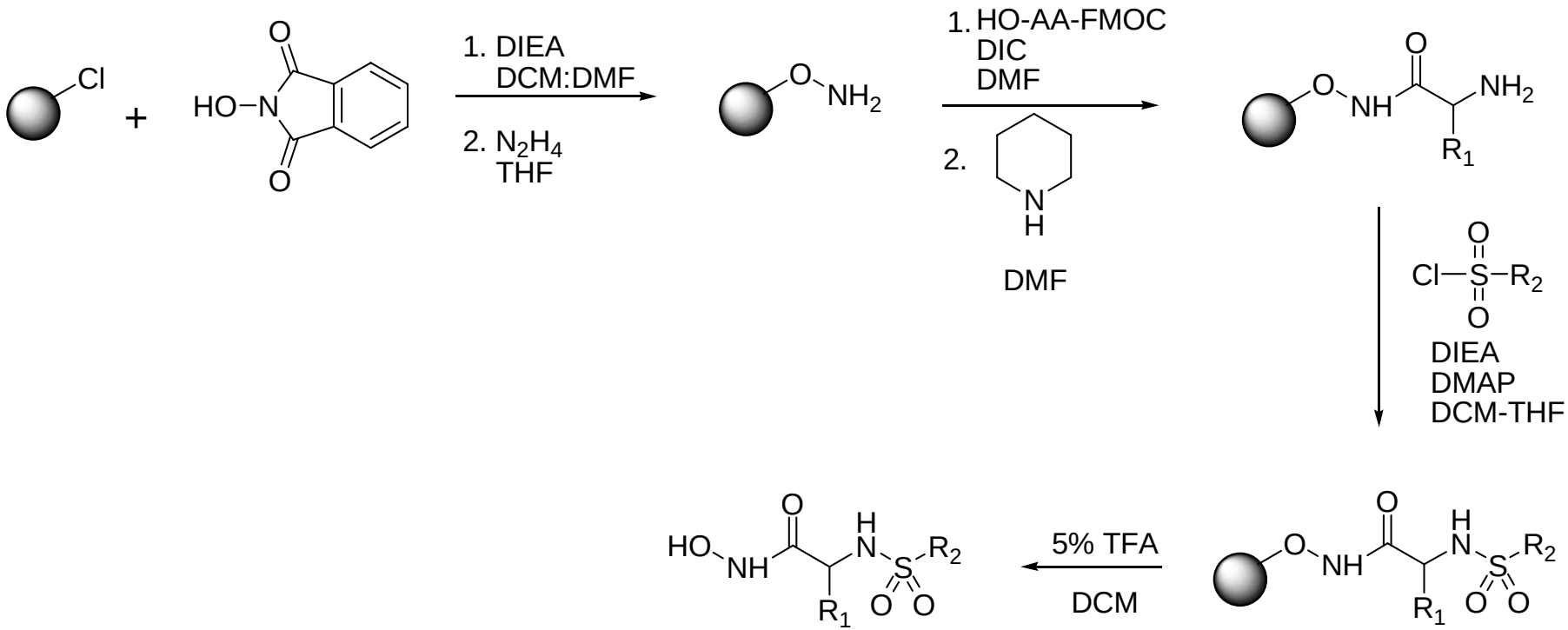
Synthesis of Derivatives



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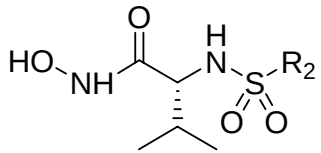
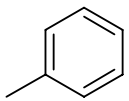
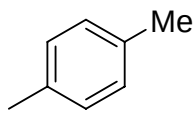
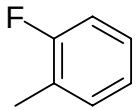
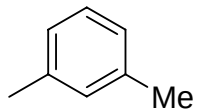
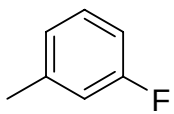
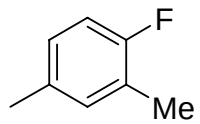
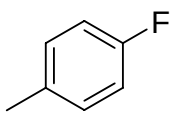
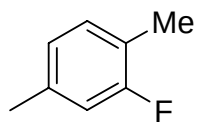
Structure Activity Relationship Study



ID	R	IC_{50} (μM)	ID	R	IC_{50} (μM)
YX-2		1.10	YX-6		3.00
YX-3		6.60	YX-7		0.40
YX-4		2.80	YX-8		0.13
YX-5		0.29	YX-9		5.18

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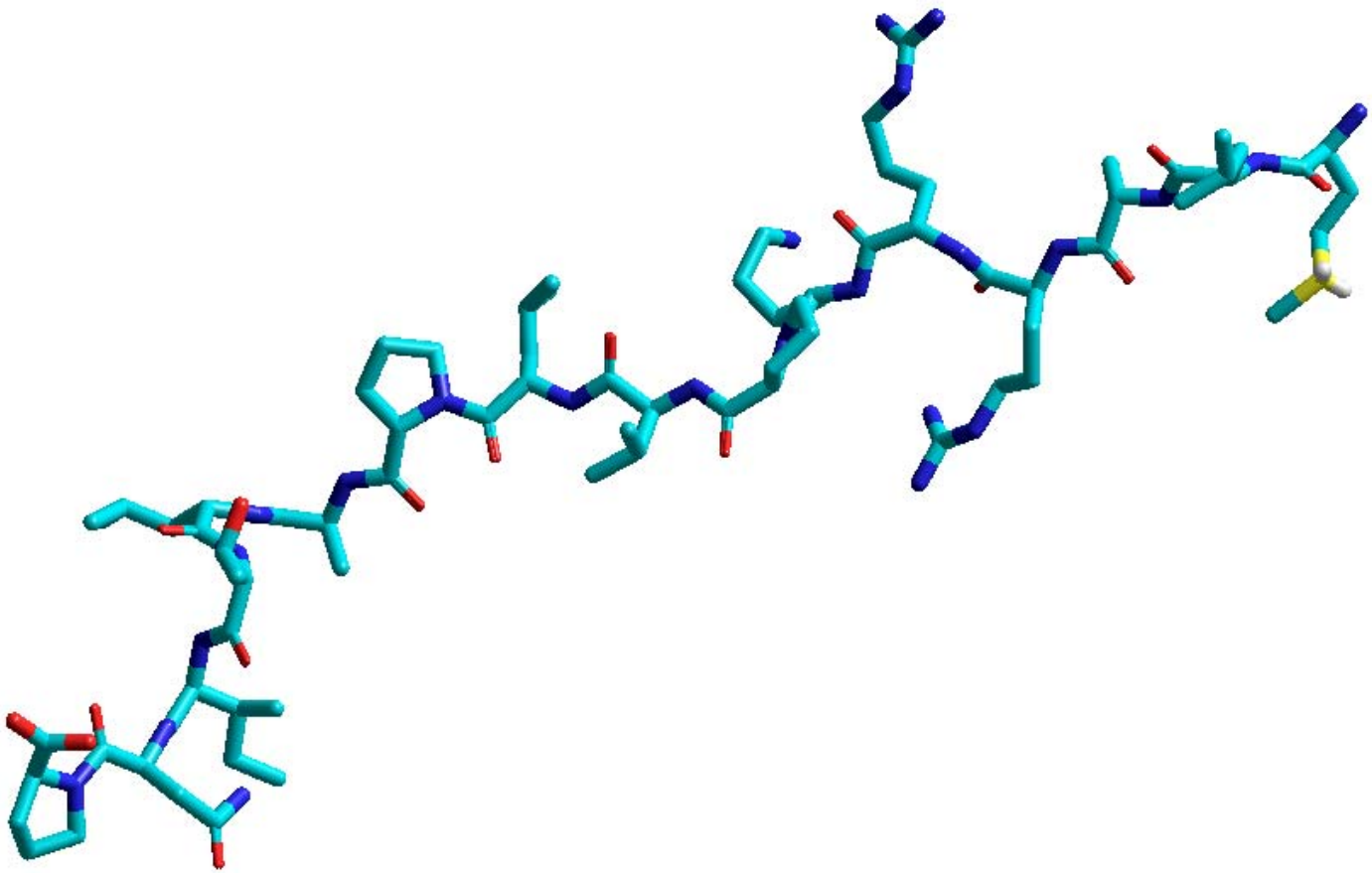
MAPK Kinase in the Active Site



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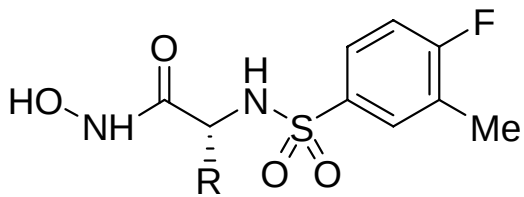
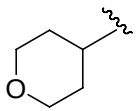
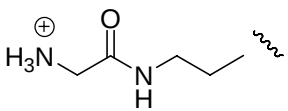
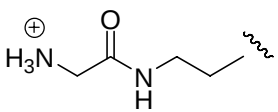
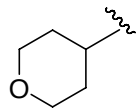
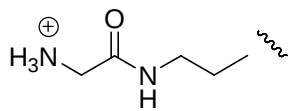
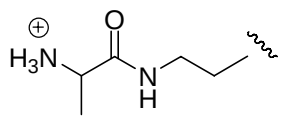
SAR Study (cont.)



Fluorescence

ID	R	IC_{50} (μM)
YX-10	CH_3	0.130
YX-11		0.054
YX-12		0.037
YX-13		0.059

Cell Culture

ID	R	IC_{50} (μM)
YX-10	CH_3	2.10
YX-11		0.19
YX-12		7.40
YX-13		7.47

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Specificity

Protein	Inhibition (μM)
LF	0.054
MMP-1	2.2
MMP-2	2.0
MMP-3	1.4
MMP-9	2.0
MMP-12	6.5

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Outline

- Introduction
- Proteolysis Mechanism
- Anthrax Inhibitors
  - Panchal and Bavari
  - Pellechia
  - Quinn
- Conclusions
- Acknowledgements

Conclusions

- Solving the crystal structure has led to several promising drugs for the inhibition of anthrax
- Effective drugs include
  - Sulfur moiety
  - Free carboxyl group
  - Hydrogen bond acceptor
  - Rigid aromatic rings

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