

Development of Small Molecule Inhibitors of the Lethal Factor in Anthrax

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Outline

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

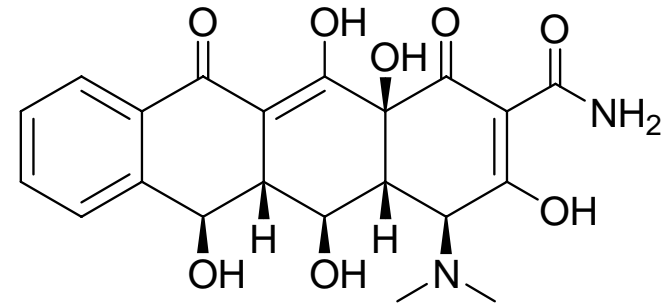
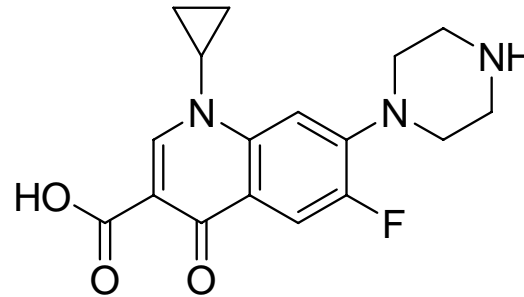
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Current Treatment

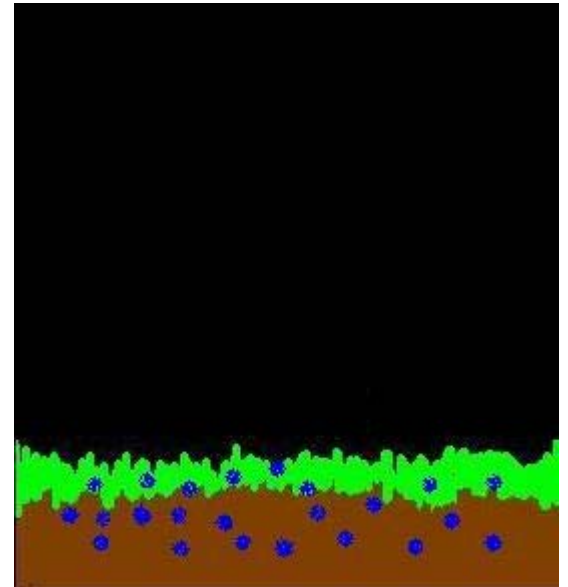
- Vaccination
 - Only for people at high risk
 - Joint symptoms and gastrointestinal problems

- Antibiotics
 - Ciprofloxacin
 - Doxycycline

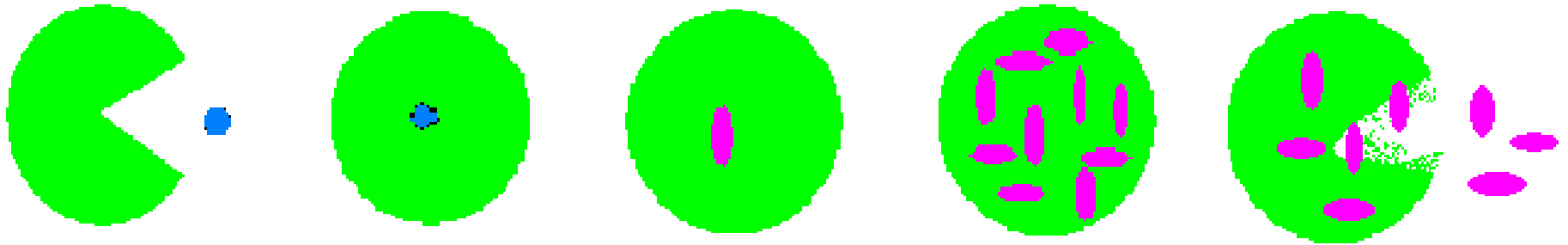


Life Cycle of Anthrax

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

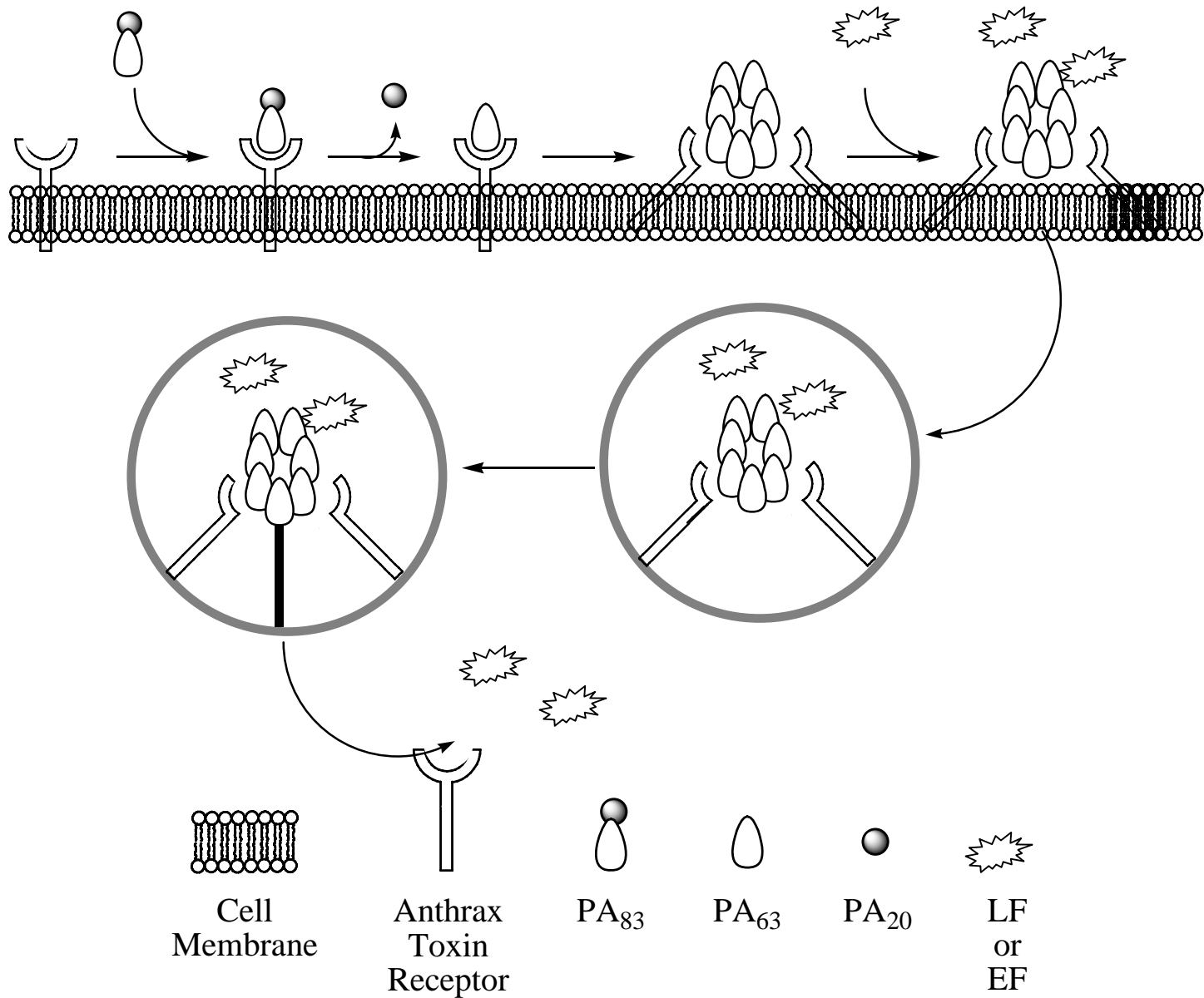


How Anthrax Kills

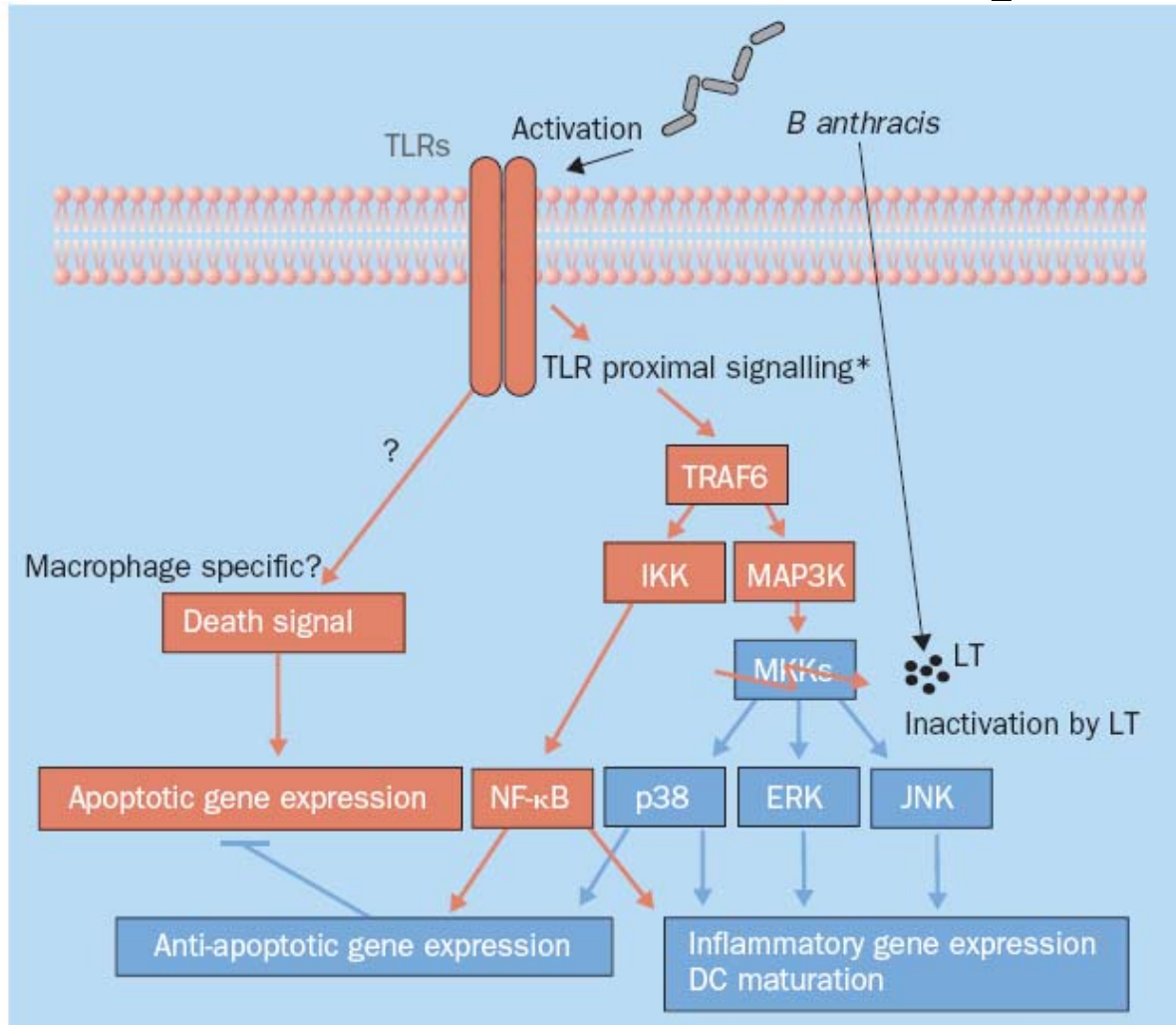


The Anthrax Toxin

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



MAPKK Pathway

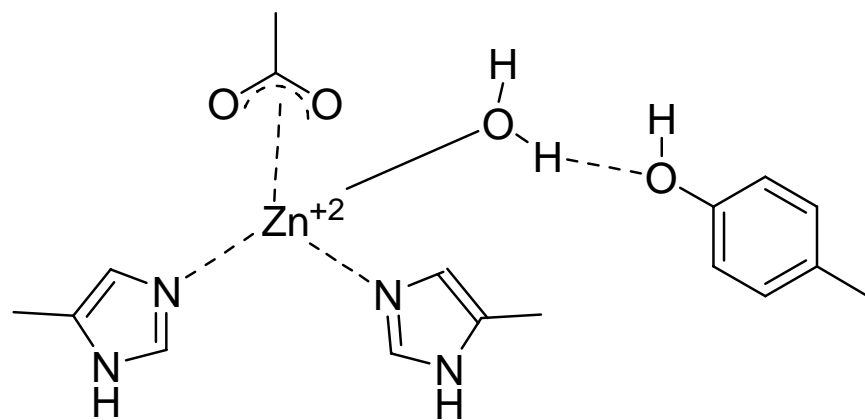
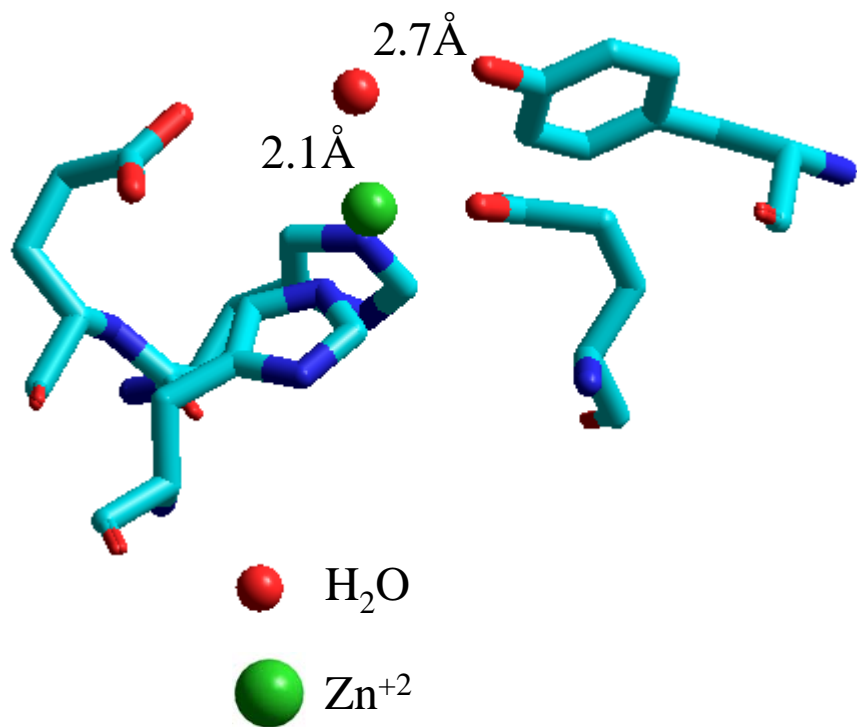


Taken from Fukao T. *Lancet Infectious Diseases* 2004, 4, 166

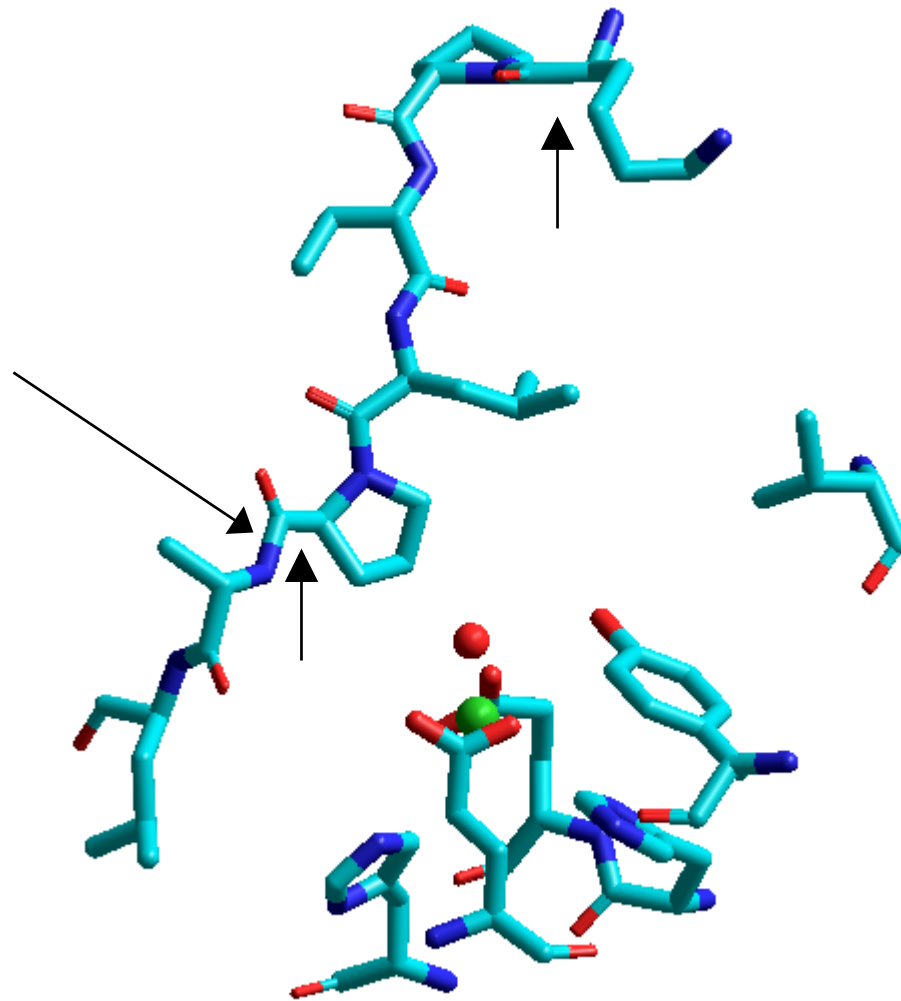
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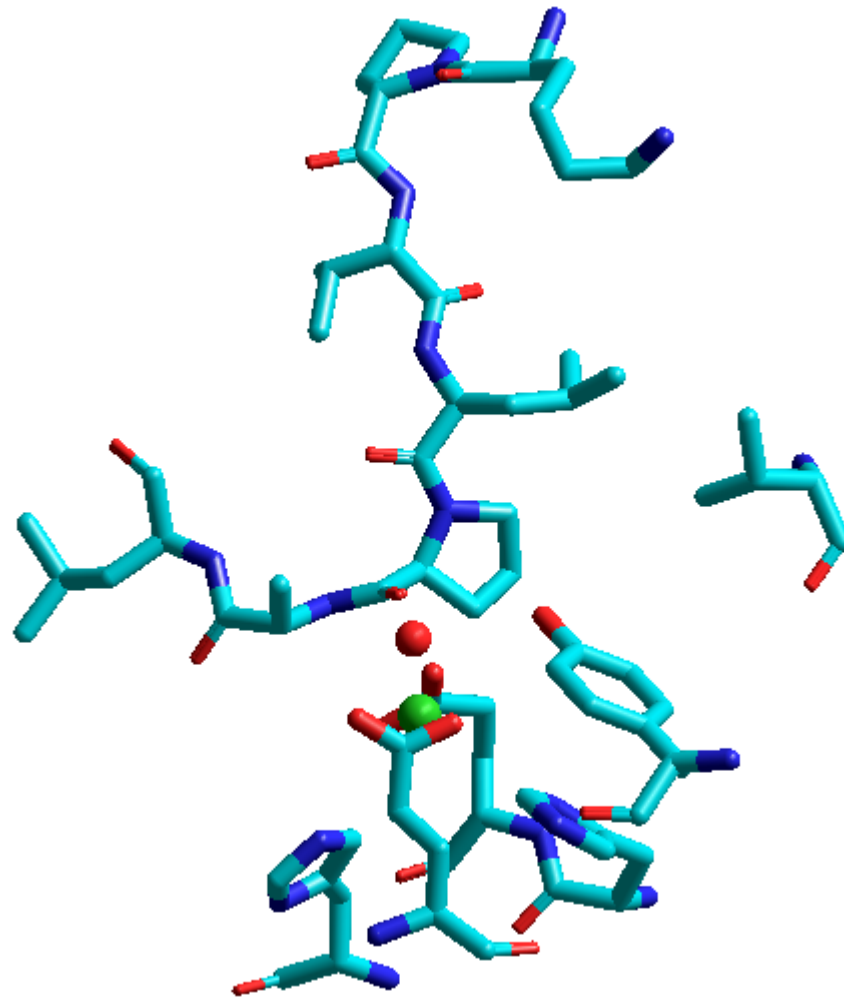
Lethal Factor Crystal Structure



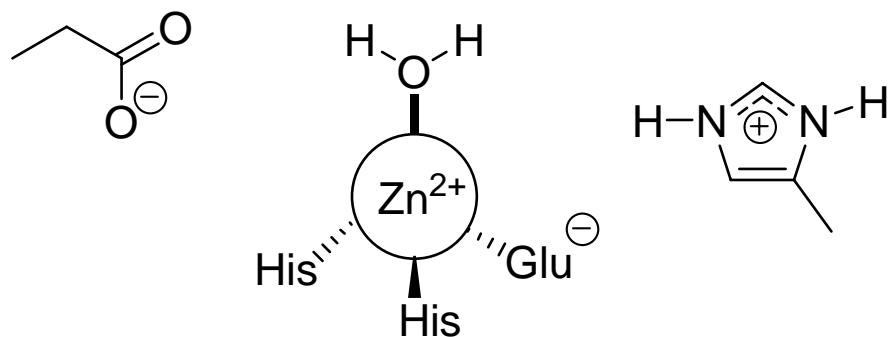
Lethal Factor and MAPKK



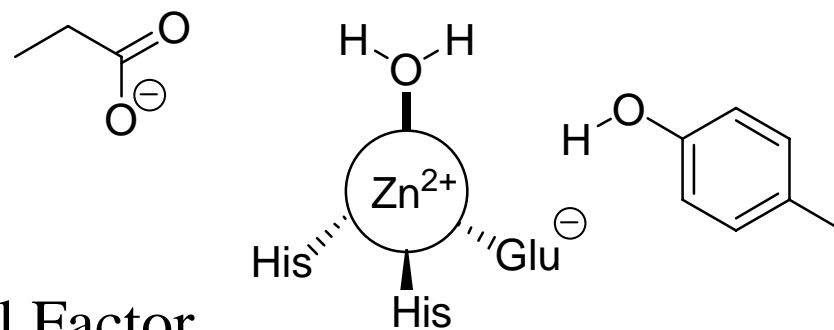
Lethal factor and MAPKK (cont.)



Thermolysin as a Model



Thermolysin



Lethal Factor

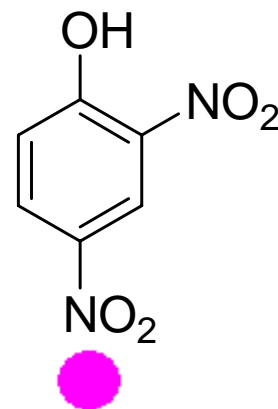
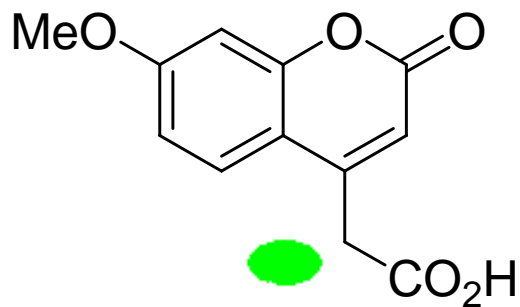
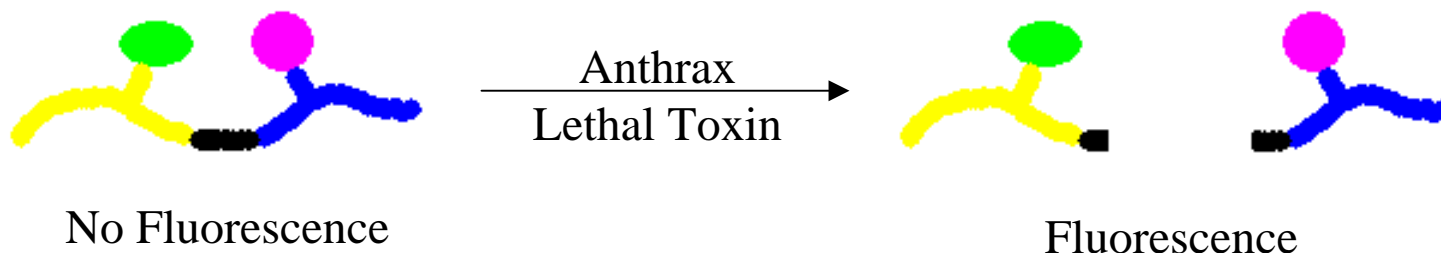
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Drug Discovery

- 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

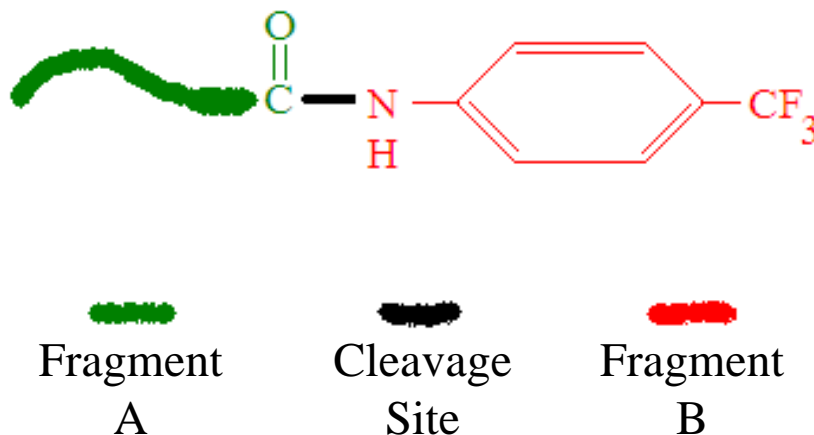
Fluorescence Detection in HTS



Fluorescence Experiment

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

^{19}F NMR Detection in Fragment Based Design



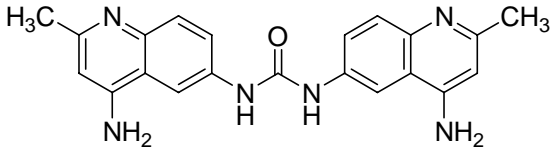
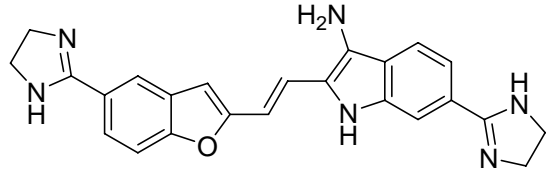
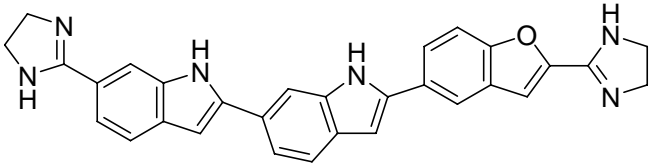
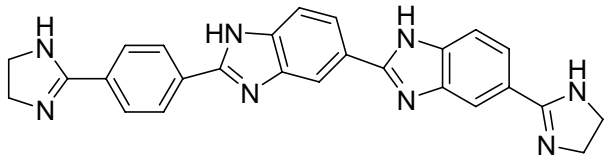
Verifying Leads With HPLC

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

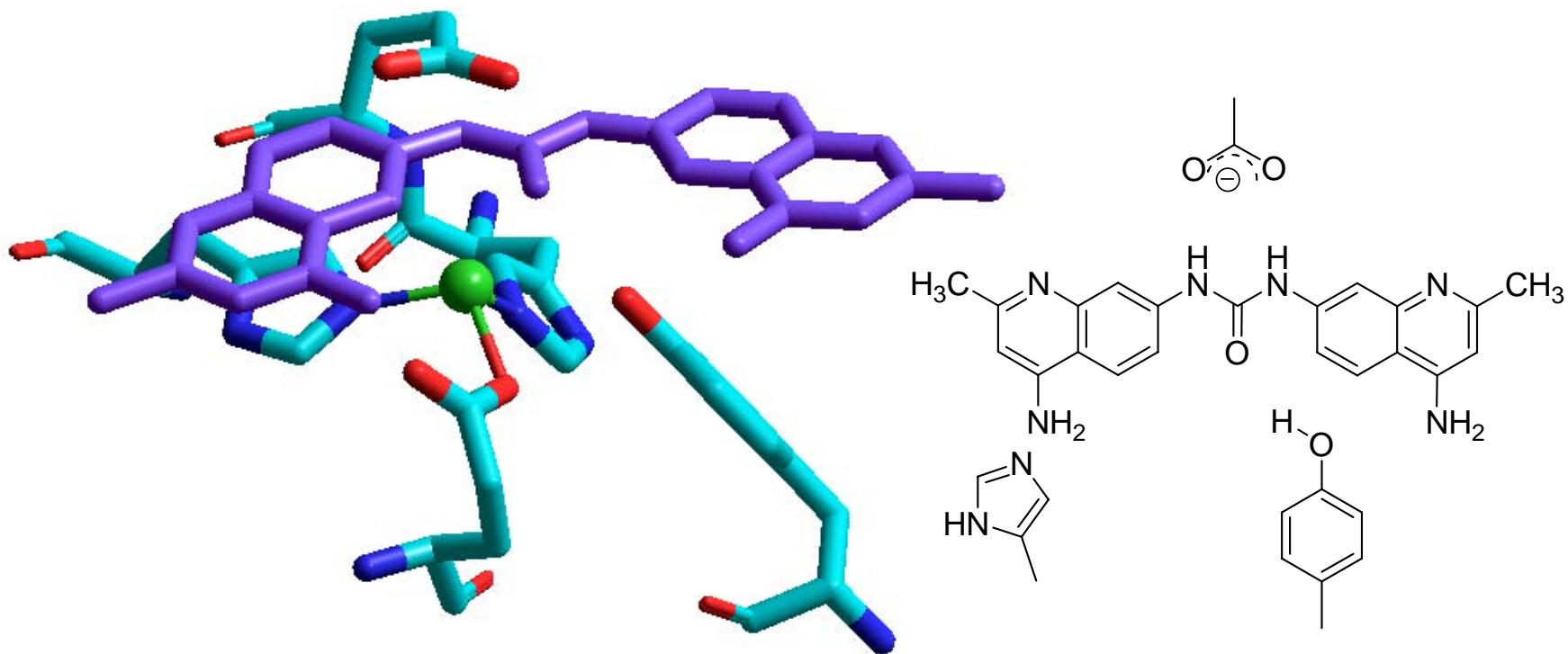
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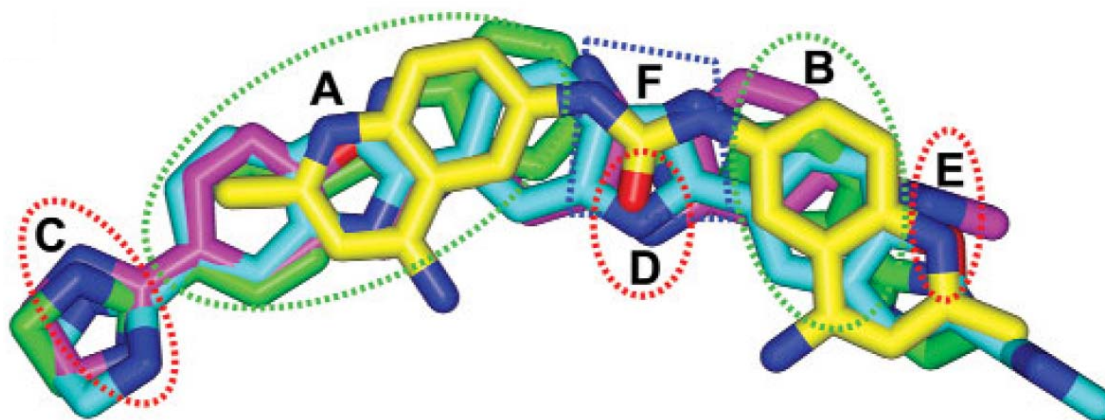
Active Compounds

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

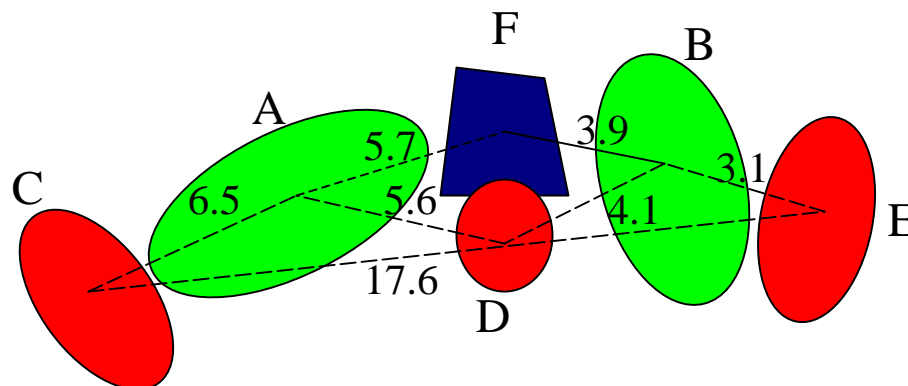
LF Complexed With PB-1



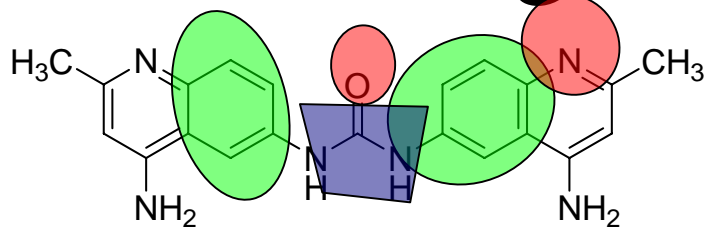
Developing a Pharmacophore



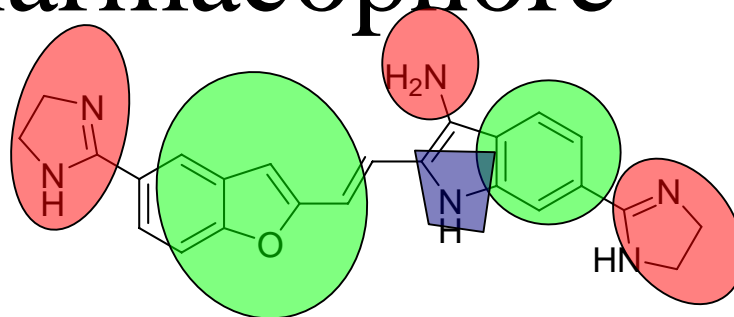
Taken directly from *Nat. Struct. Mol. Bio.* **2004**, *11*, 67



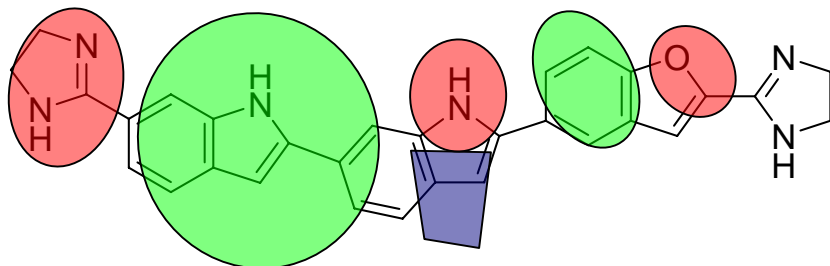
Examining the Pharmacophore



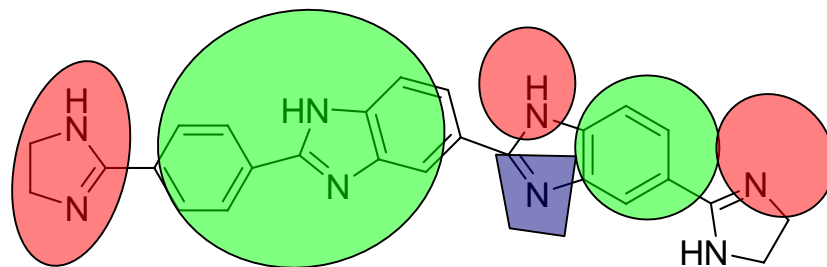
PB-1



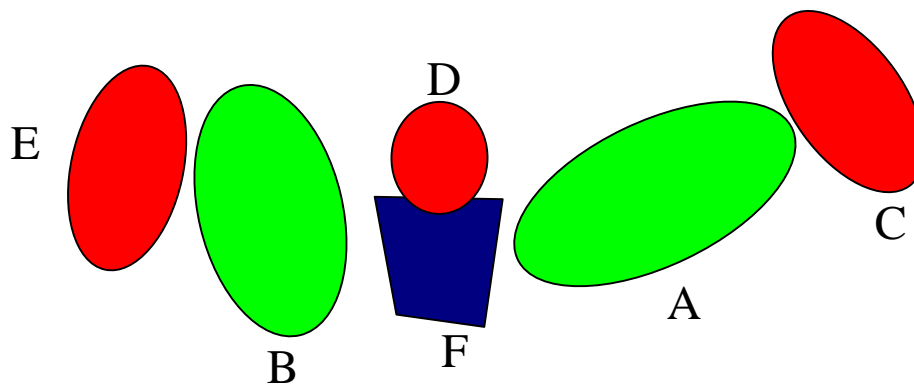
PB-2



PB-3



PB-4



Panchal Summary

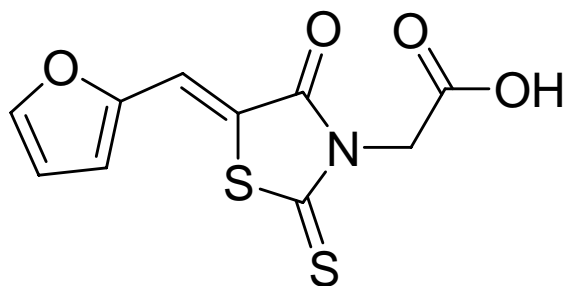
- Hydrogen bond acceptor in center
- Aromatic rings on either side of a linker
- If the linker is a hydrogen bond donor, it coordinates to glutamate
- Hydrogen bond acceptors/donors at the end of the aromatic rings

Outline

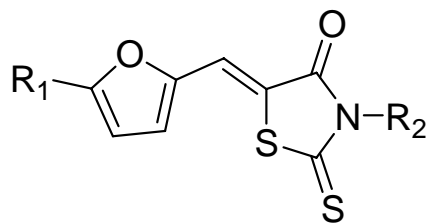
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Pellechia's Scaffold

- Fragment-based design
- Hit with an IC_{50} of 140 μM



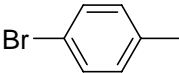
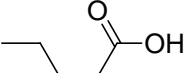
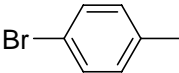
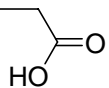
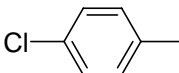
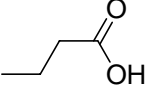
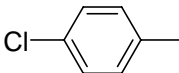
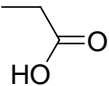
MP-1

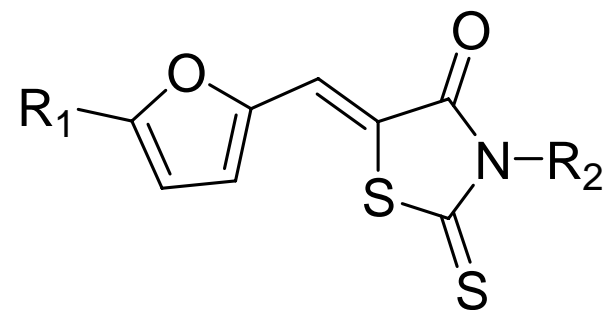


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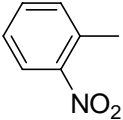
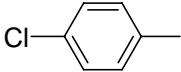
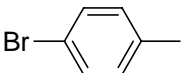
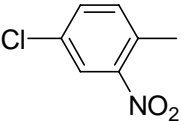
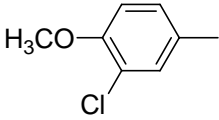
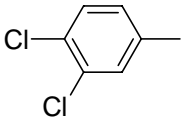
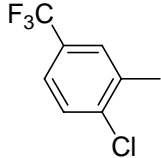
ID	R ₁	R ₂	IC ₅₀ (μM)	ID	R ₁	R ₂	IC ₅₀ (μM)
MP-2			300	MP-7			20
MP-3			150	MP-8			12.8
MP-4			37.7	MP-9			9.1
MP-5			36.3	MP-10			5.5
MP-6			31.9	MP-11			1.7

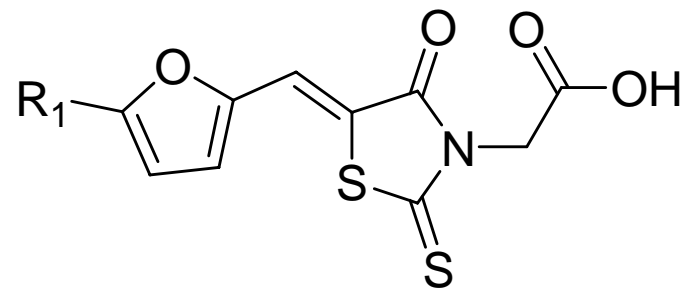
Varying the Acid Side Chain

ID	R ₁	R ₂	IC ₅₀ (μM)
MP-12			2.3
MP-13			0.85
MP-11			1.7
MP-14			0.90

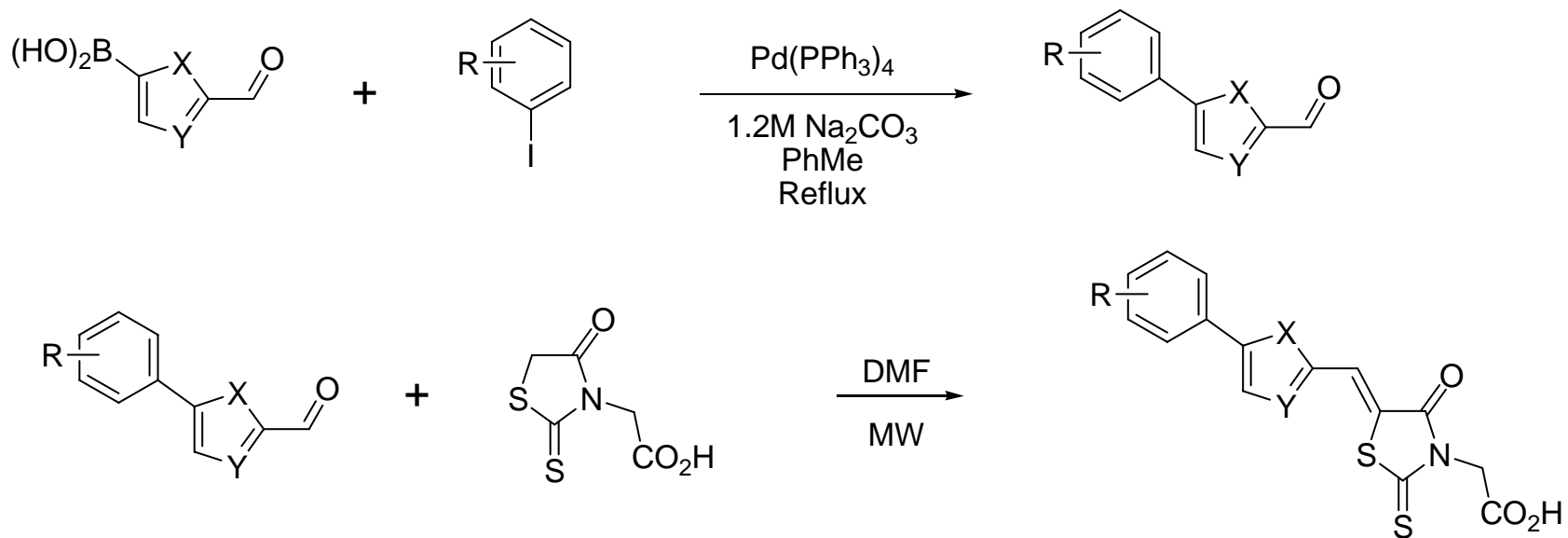


Varying the R₁ Ring

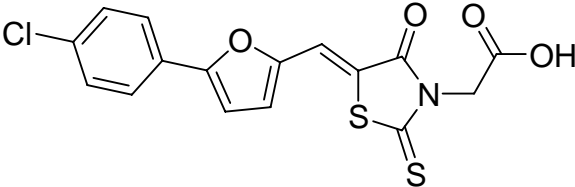
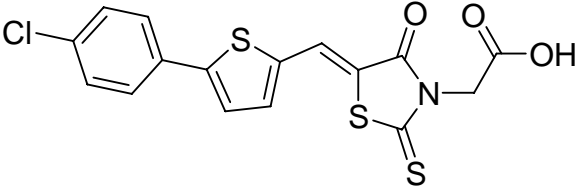
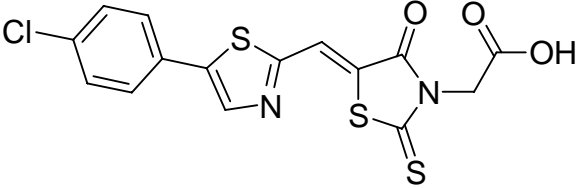
ID	R ₁	IC ₅₀ (μM)
MP-16		3.10
MP-14		0.90
MP-13		0.85
MP-17		0.50
MP-18		0.30
MP-19		0.27
MP-20		0.19



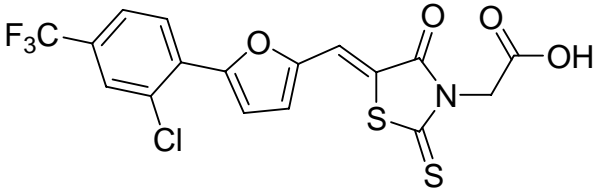
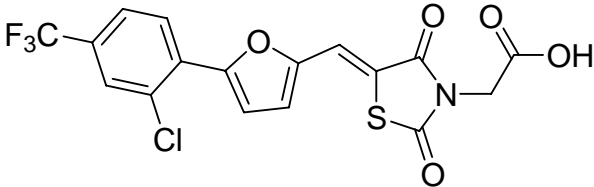
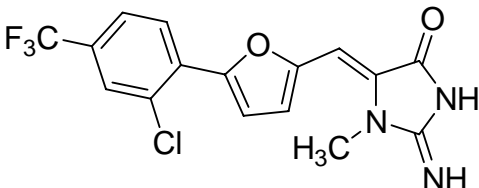
Synthesis of MP-1 Derivatives



Varying the Furan Ring

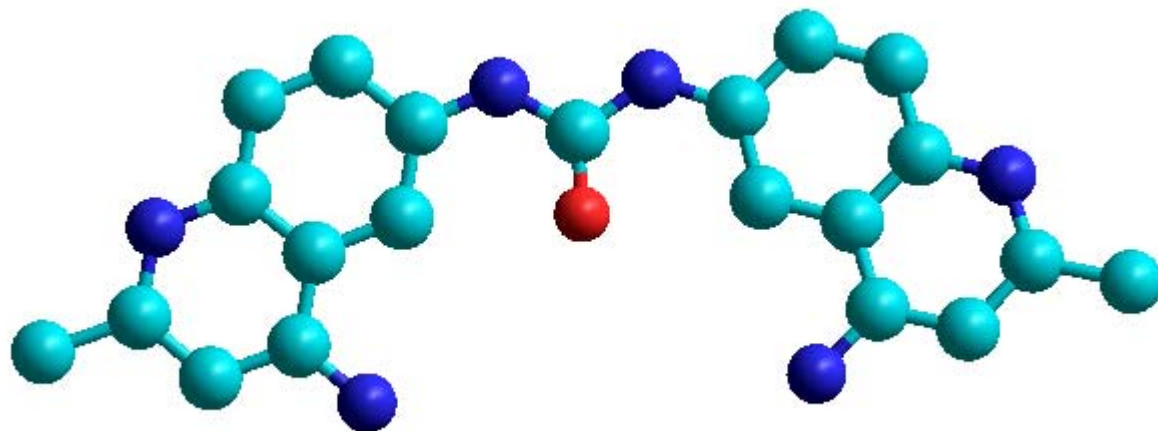
ID		IC ₅₀ (μM)
MP-14		0.9
MP-21		3.2
MP-22		10.0

Varying the Thiazolidinone Ring

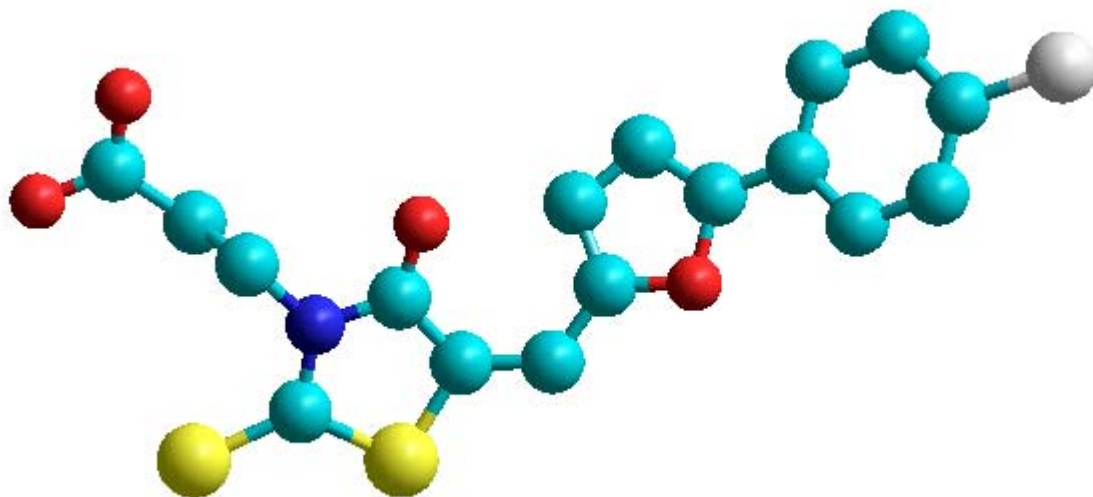
ID		IC ₅₀ (μM)
MP-20		0.19
MP-23		5.9
MP-24		100

Applying the Pharmacophore

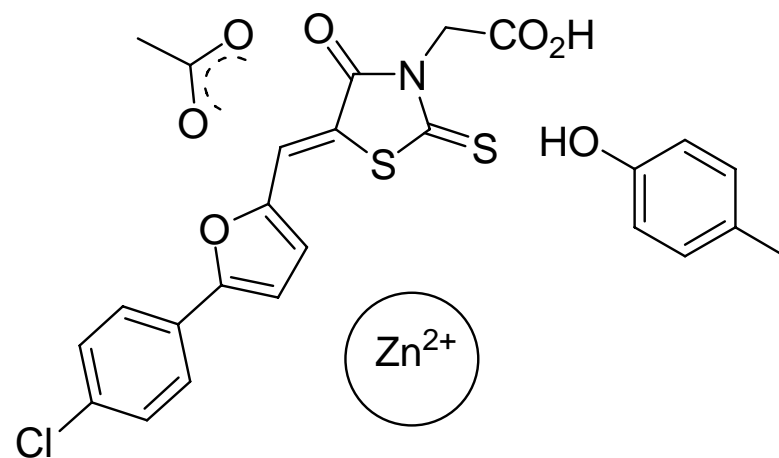
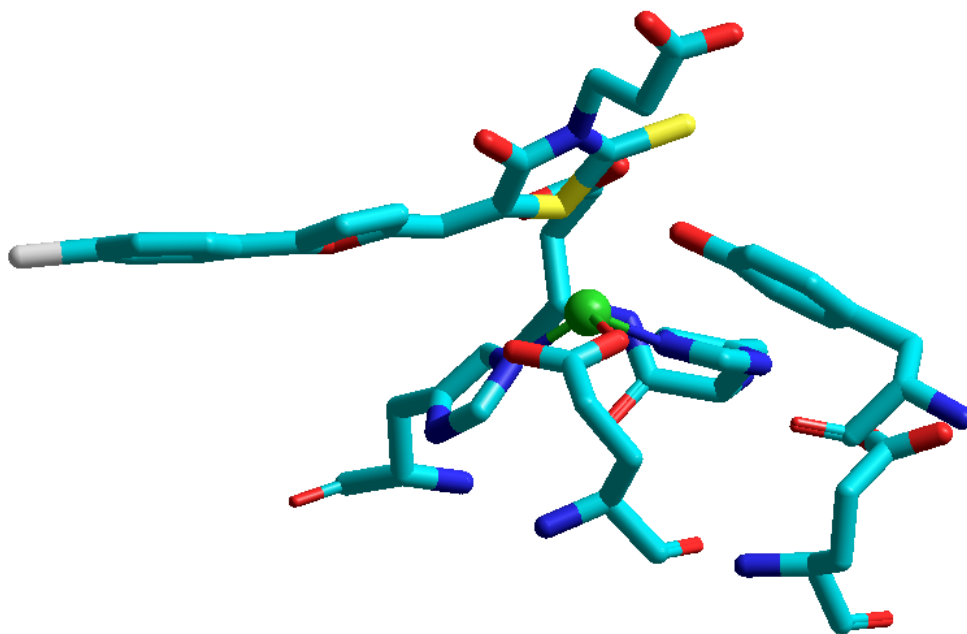
PB-1



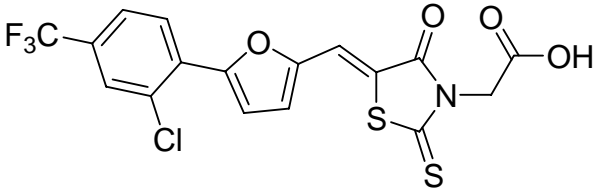
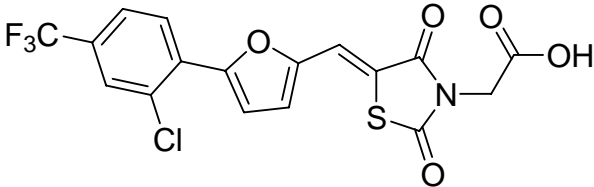
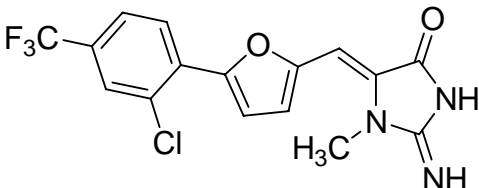
MP-11



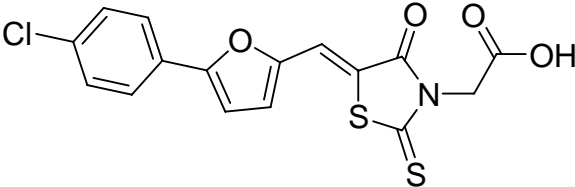
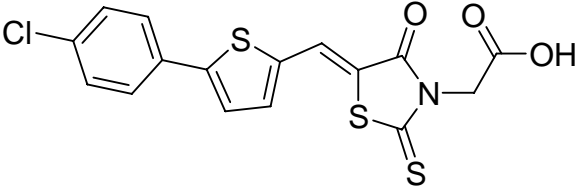
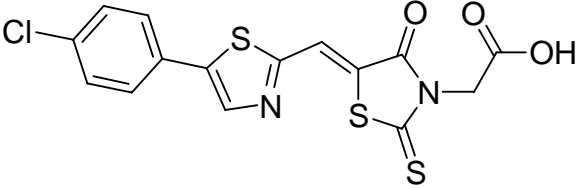
LF Complexed With MP-11



Varying the Thiazolidinone Ring

ID		IC ₅₀ (μM)
MP-20		0.19
MP-23		5.9
MP-24		100

Varying the Furan Ring

ID		IC ₅₀ (μM)
MP-14		0.9
MP-21		3.2
MP-22		10.0

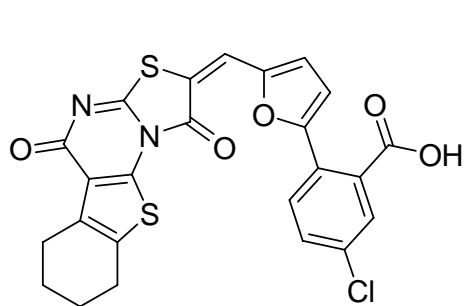
Pellechia Summary

- 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

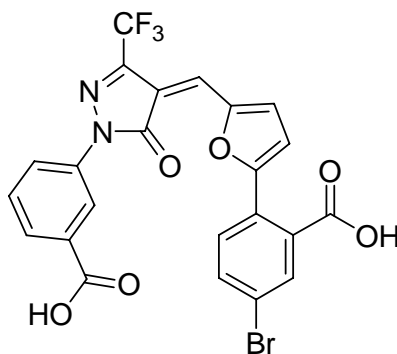
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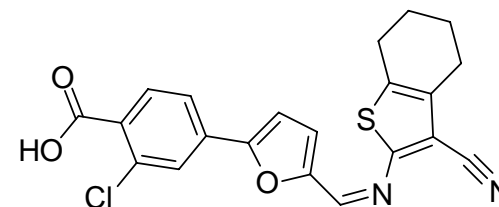
Quinn's Lead Compounds



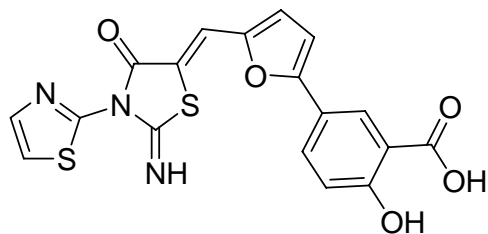
MQ-1
 $IC_{50} = 1.1 \mu M$



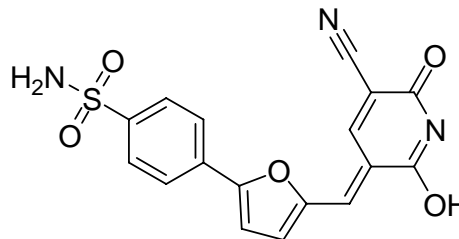
MQ-2
 $IC_{50} = 1.8 \mu M$



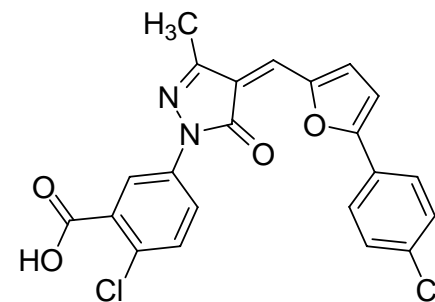
MQ-3
 $IC_{50} = 5.2 \mu M$



MQ-4
 $IC_{50} = 4.8 \mu M$



MQ-5
 $IC_{50} = 8.3 \mu M$

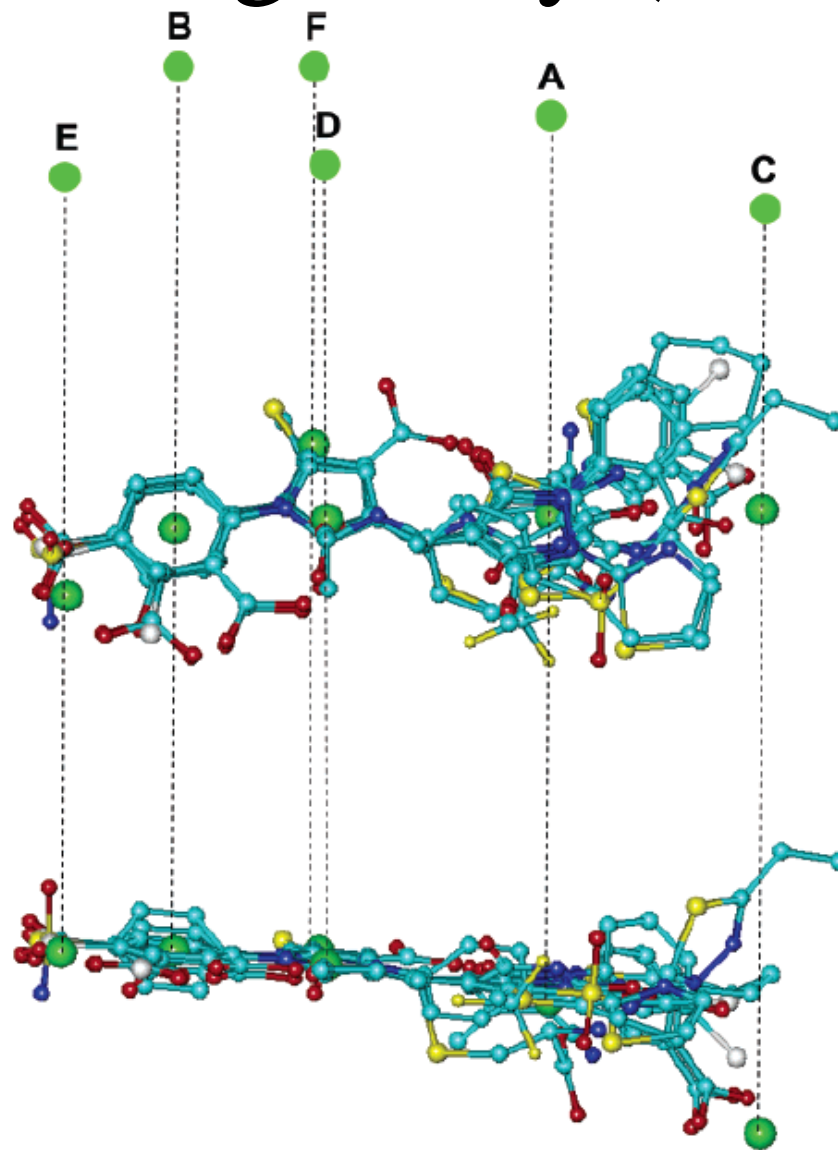


MQ-6
 $IC_{50} = 10.0 \mu M$

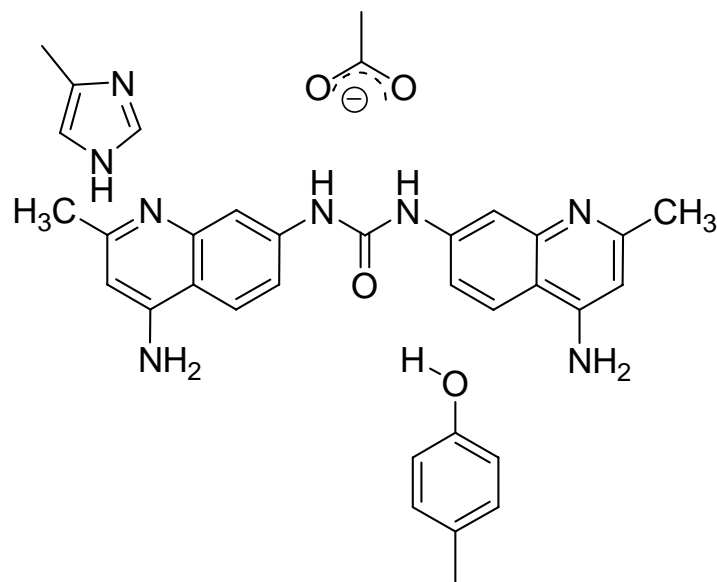
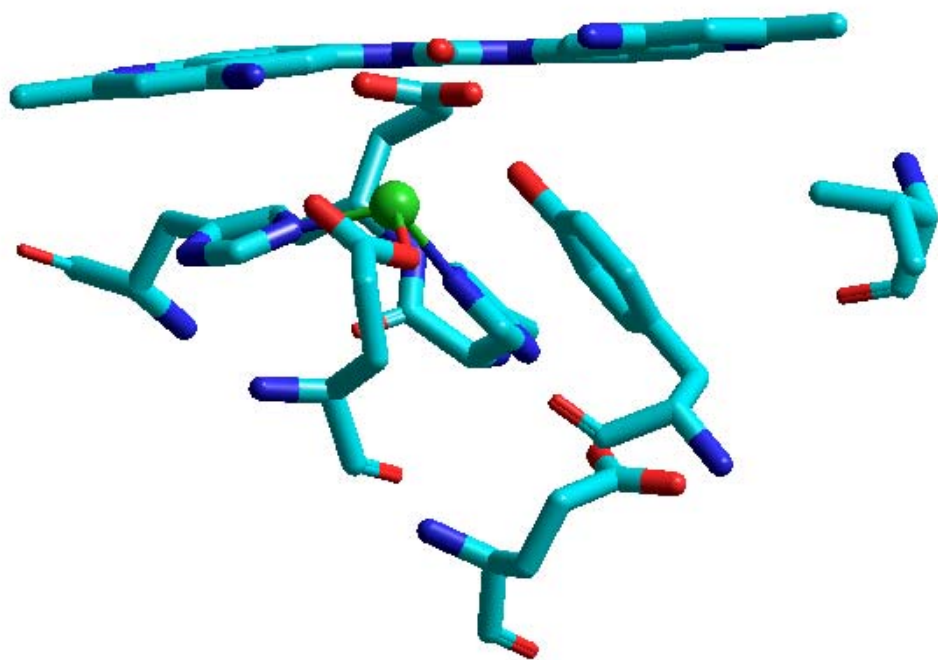
Quinn's Modeling Study

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

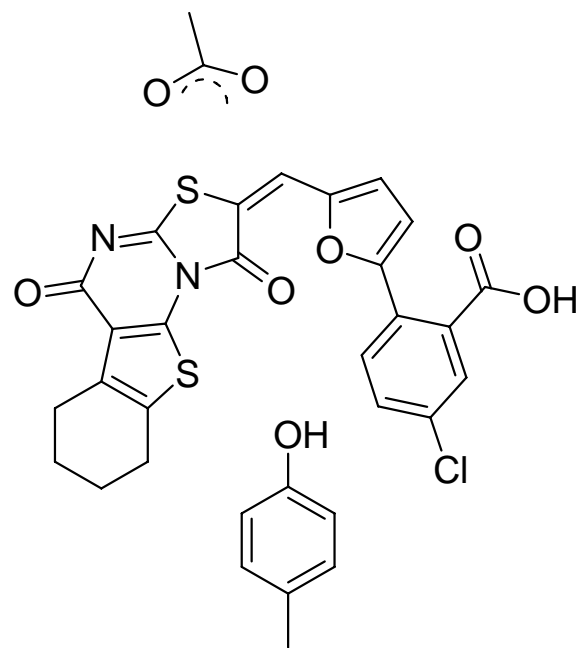
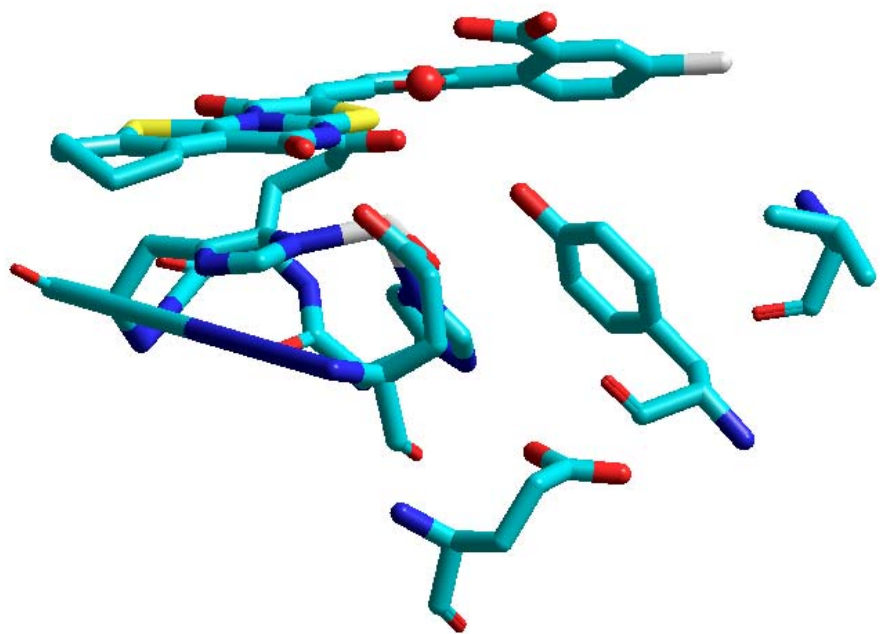
Quinn's Modeling Study (cont.)



PB-1 Complexed with LF



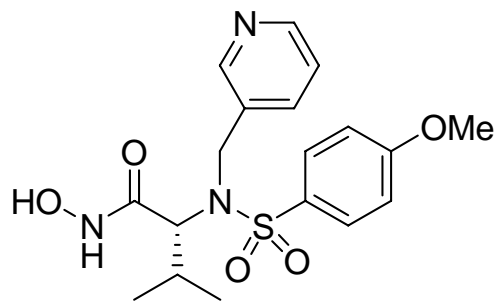
MQ-1 Manually Moved Into the Active Site



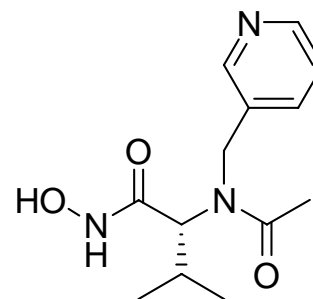
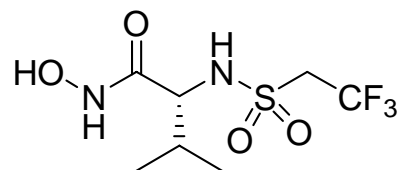
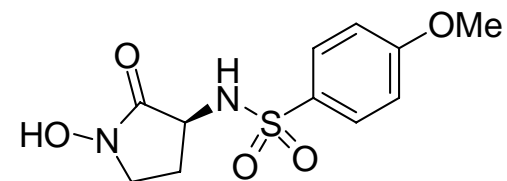
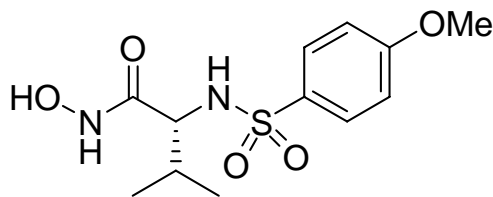
Outline

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

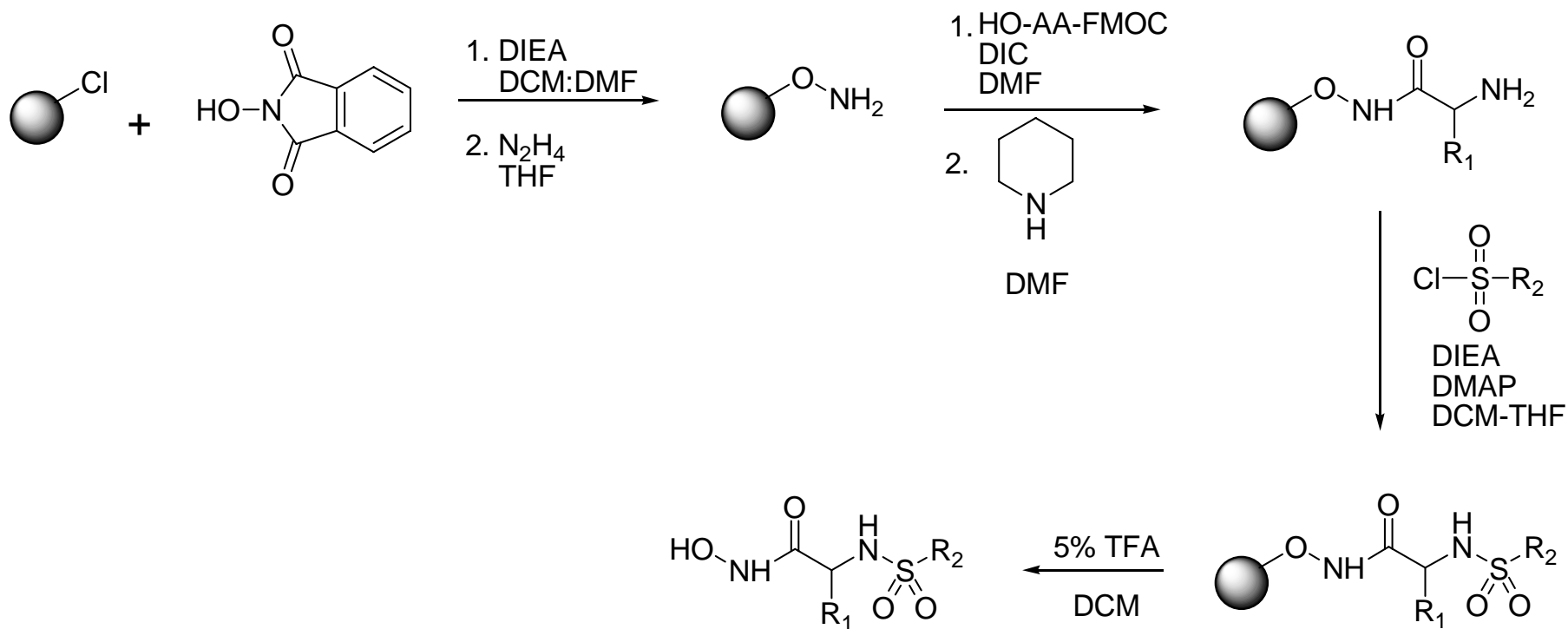
Merck Compounds



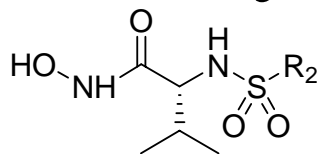
YX-1
 $IC_{50} = 1.2\mu M$



Synthesis of Derivatives

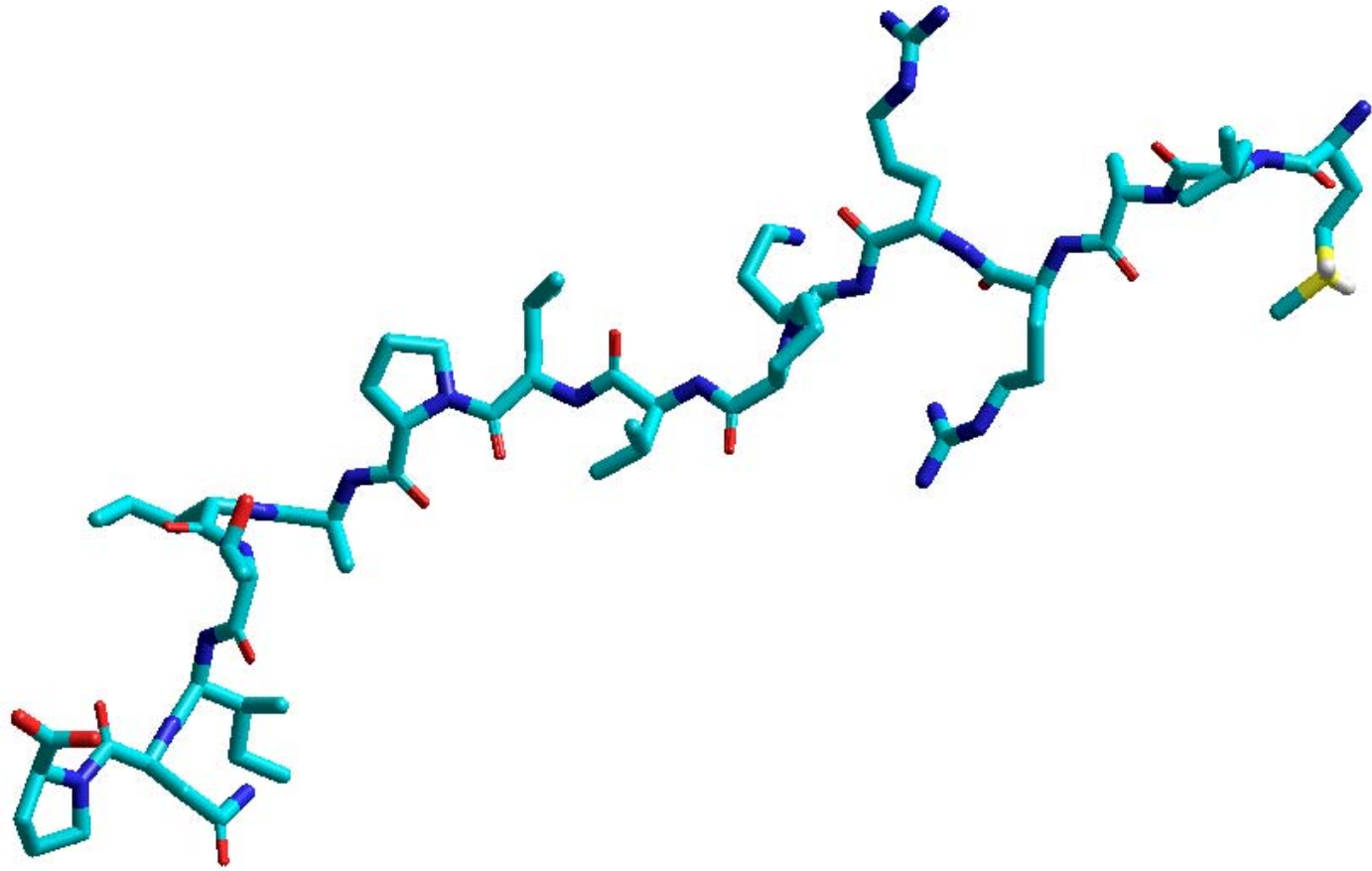


Structure Activity Relationship Study

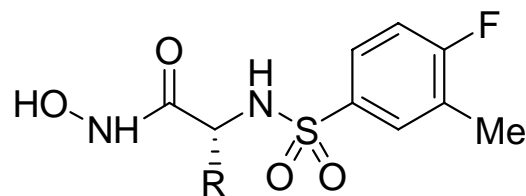


ID	R	IC ₅₀ (μM)	ID	R	IC ₅₀ (μM)
YX-2		1.10	YX-6		3.00
YX-3		6.60	YX-7		0.40
YX-4		2.80	YX-8		0.13
YX-5		0.29	YX-9		5.18

MAPK Kinase in the Active Site



SAR Study (cont.)



Fluorescence

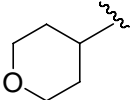
Cell Culture

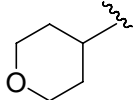
ID **R** **IC₅₀ (μM)**

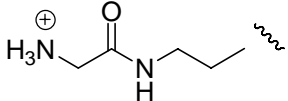
ID **R** **IC₅₀ (μM)**

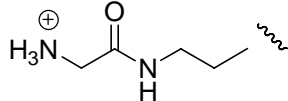
YX-10 CH₃ 0.130

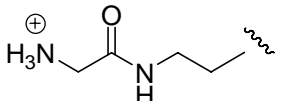
YX-10 CH₃ 2.10

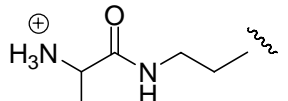
YX-11  0.054

YX-11  0.19

YX-12  0.037

YX-12  7.40

YX-13  0.059

YX-13  7.47

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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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

Acknowledgements

- Dr. Tepe
- Dr. Walker
- Monica
- Aman
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