

Ups and Downs of Structurally *Un-enabled* Fragment Optimization

Fragment-Based Lead Discovery Conference

Philadelphia, October, 12 2010

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Sandler Center for Drug Discovery
Advancing treatments for neglected tropical diseases



Journal of
**Medicinal
Chemistry**
Letter

Divergent Modes of Enzyme Inhibition in a Homologous Structure–Activity Series

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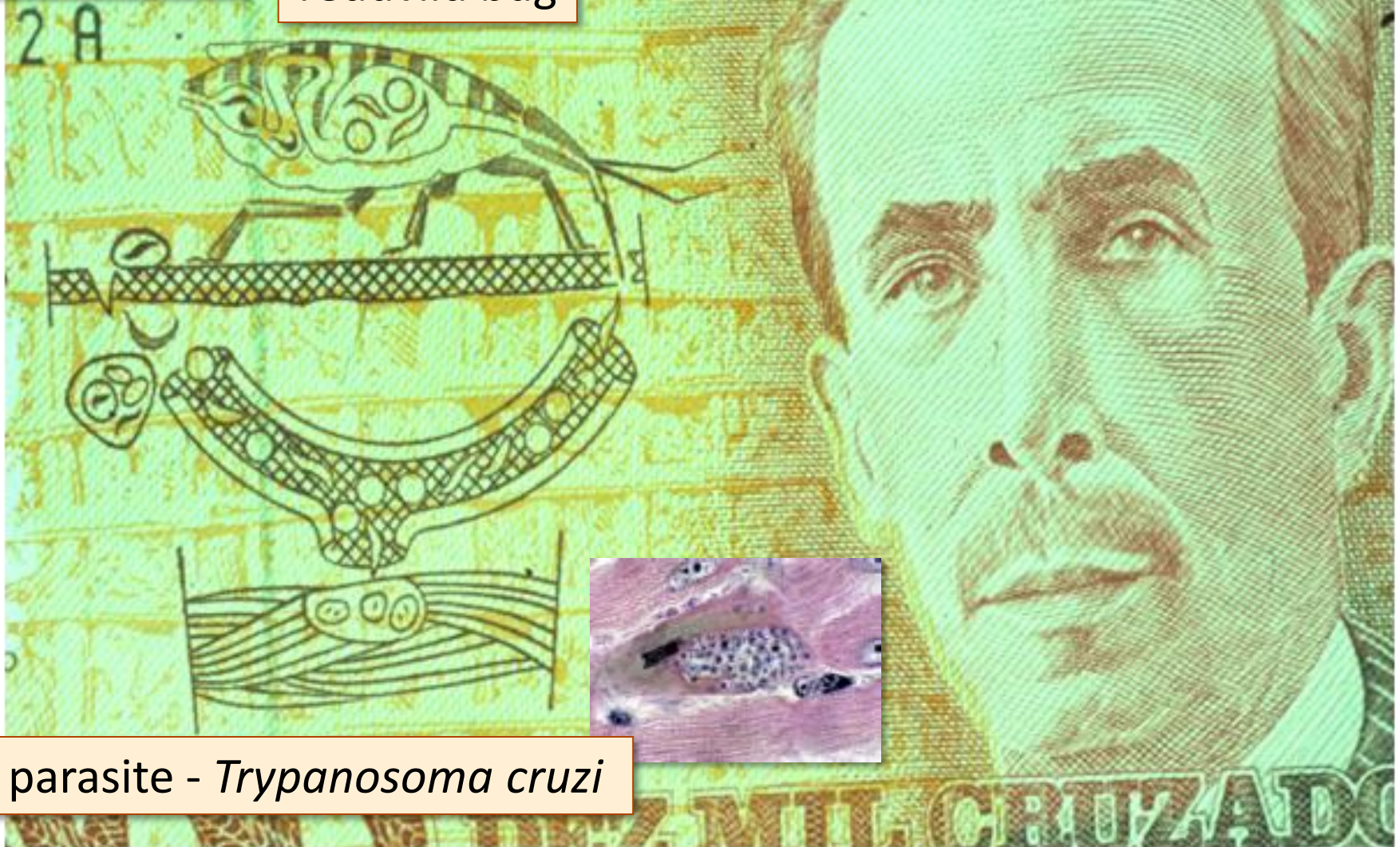
Received June 22, 2009

J. Med. Chem. **2009**, 52, 5005

Dr. Carlos Chagas

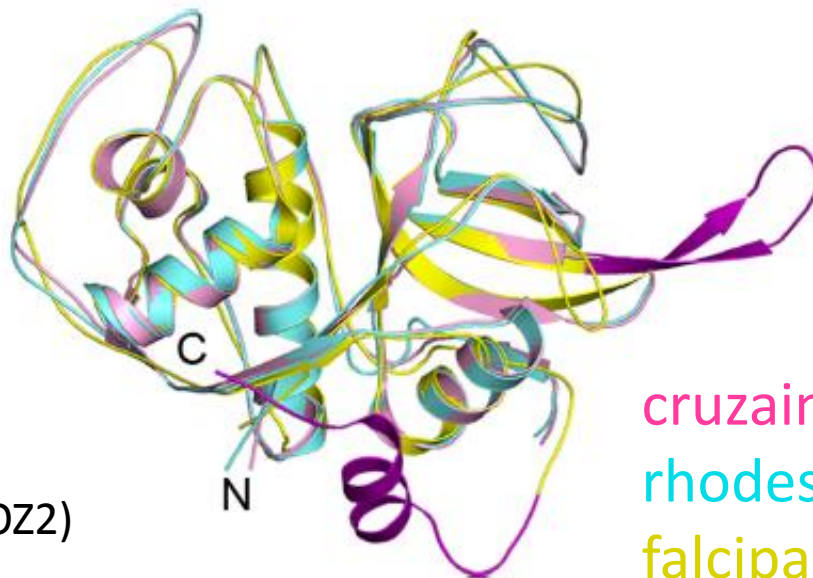


reduviid bug



parasite - *Trypanosoma cruzi*

Cruzain as a target in Chagas' disease

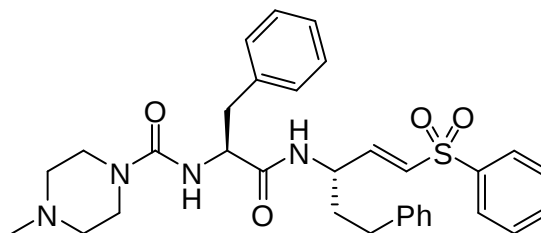
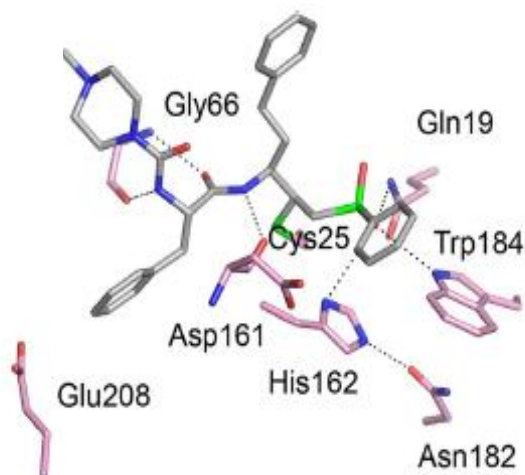


cruzain (*T. cruzi*)

rhodesain (*T. brucei*)

falcipain-3 (*P. falciparum*)

cruzain-K777 (PDB ID 2OZ2)

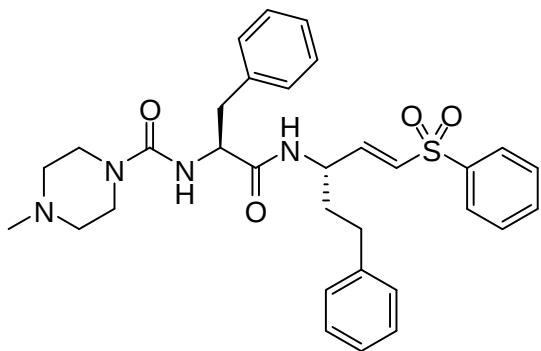


K-777 (Kepri/Celera)

Biochemistry, **2000**, 39, 12543

K777 - A vinylsulfone-based irreversible inhibitor of cruzain

JBC, **2009**, 284, 25697



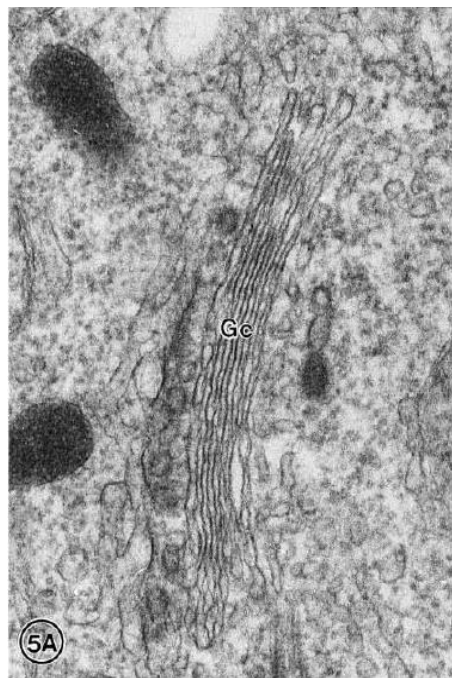
K-777 (Kepri/Celera)

cruzain inhibition: $k_{\text{inact}}/K_i = 510,000 \text{ s}^{-1} \text{ M}^{-1}$

effective against *T. cruzi* in vitro at $\sim 10 \text{ } \mu\text{M}$

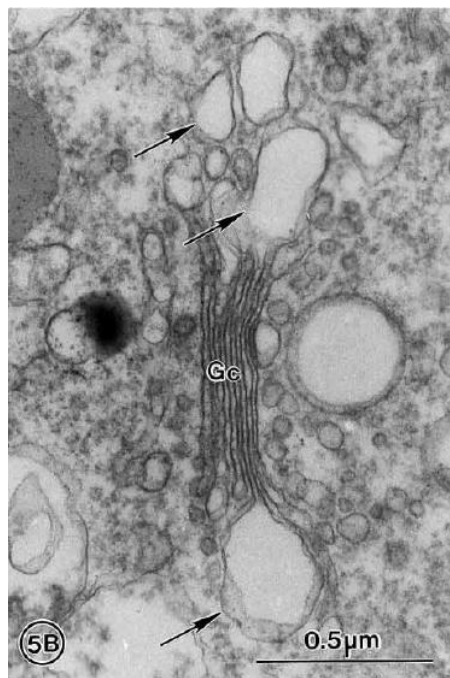
curative in Chagas' mouse model ($\text{ED}_{100} = 50 \text{ mg/kg}$)

J. Exp. Med., **1998**, 725



untreated

J. Cell. Sci., **1998**, 111, 597



48h (20 μM CPI)

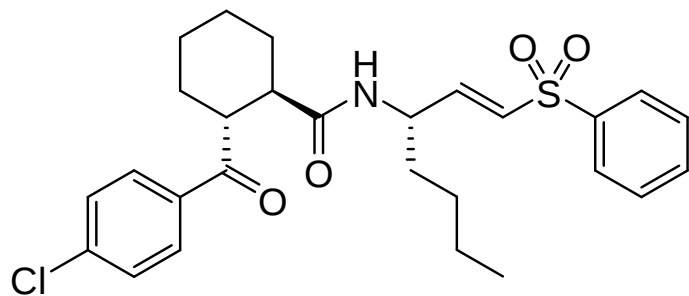


CPI-resistant *T. cruzi*

J. Cell. Sci., **2000**, 113, 2000

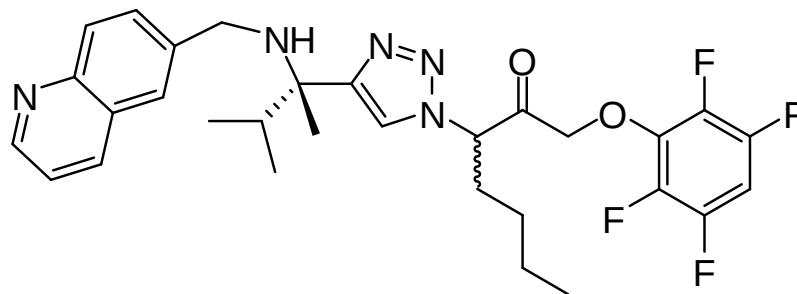
Two recent non-peptidic inhibitors of cruzain

fragments $\rightarrow$ inhibitors $\rightarrow$ screen

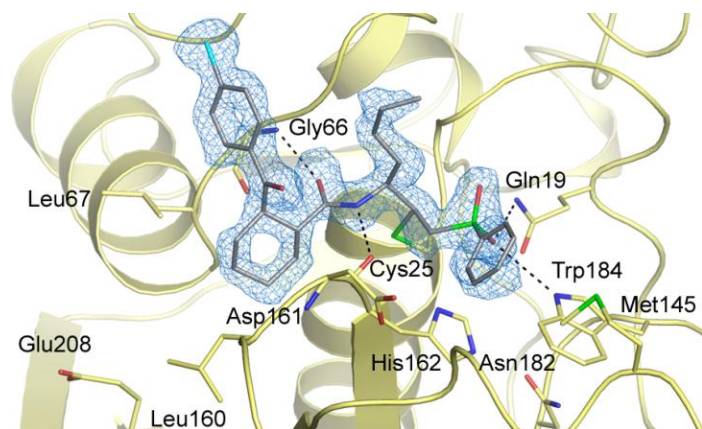


BMCL, 2009, 19, 6218

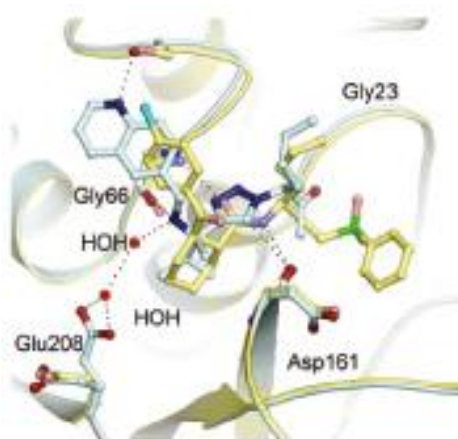
fragments $\rightarrow$ substrates $\rightarrow$ screen $\rightarrow$ inhibitors



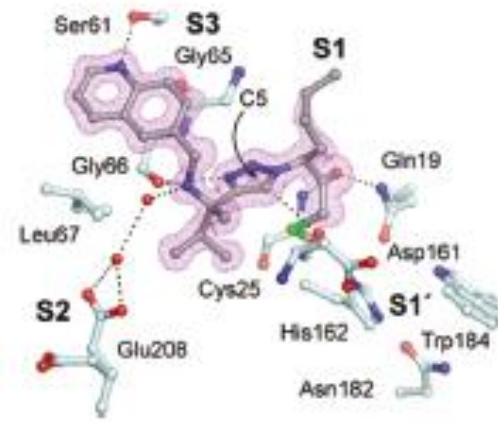
JMC, 2010, 53, 1763



PDB ID 3HD3



3HD3/3IUT



PDB ID 3IUT

In silico screen of Lead/Fragment Library in ZINC

Lead-like database from ZINC: 416,857 compounds

Library properties:

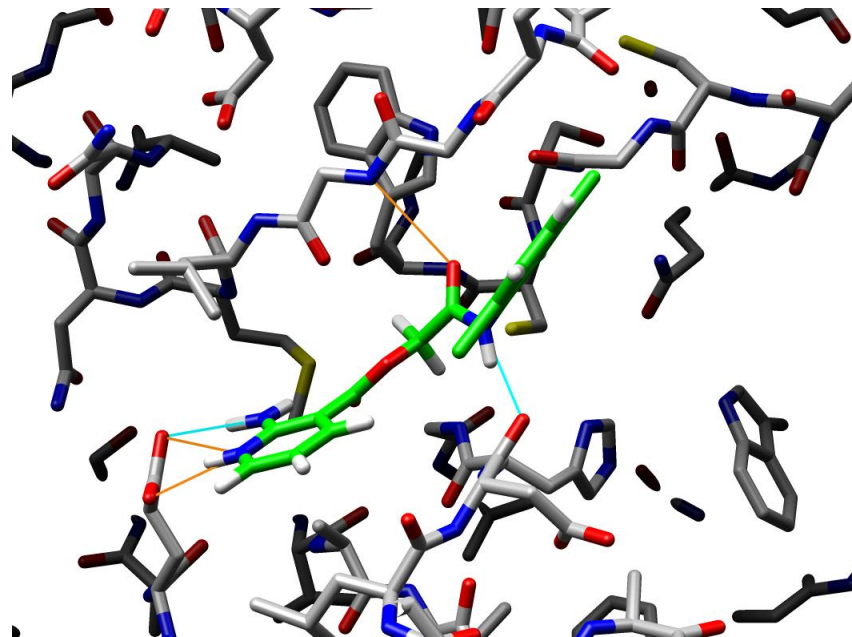
$$-2 < \log P < 4$$

$$150 < \text{molecular weight} < 350$$

$$\text{Hydrogen bond donors} \leq 3$$

$$\text{Hydrogen bond acceptors} \leq 6$$

$$\text{Rotatable bonds} < 7$$



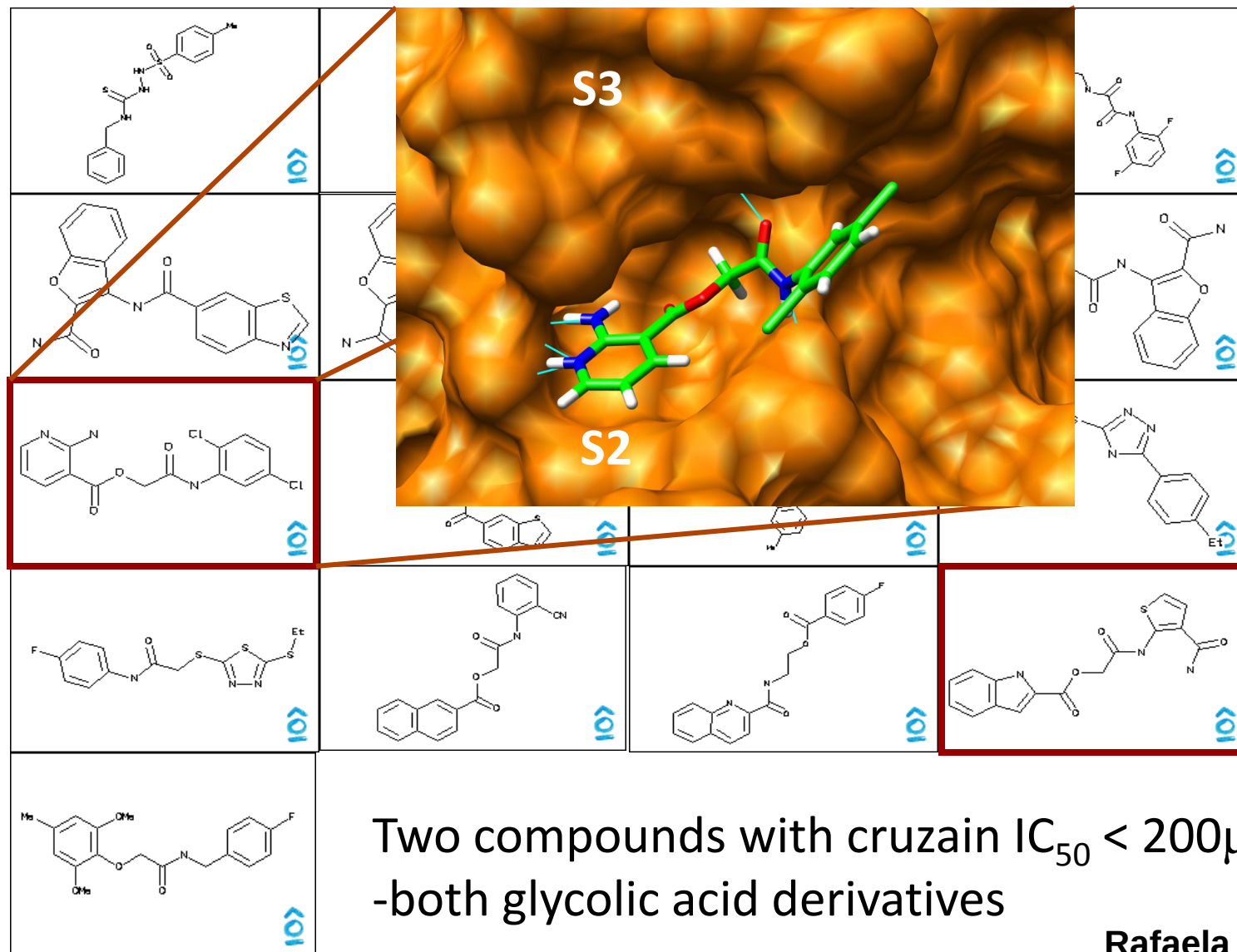
DOCK 3.5.54, CHEMGRID, Delphi

Model built after inspecting 13 cruzain structure and 10 bound inhibitors

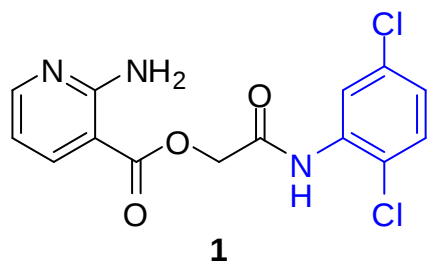
Two conformations screened (Glu 208 'in' and Glu 208 'out')

Partial charges altered on important H-bonding atoms

Triage – 500 docking poses examined



A series of reversible cruzain inhibitors

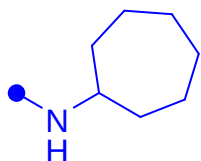


1

cruzain $IC_{50} = 77 \mu M$

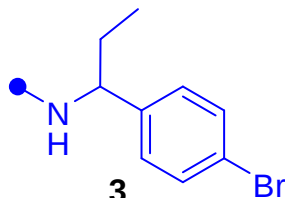
cruzain $K_i = 32 \mu M$

L.E. ~ 0.26



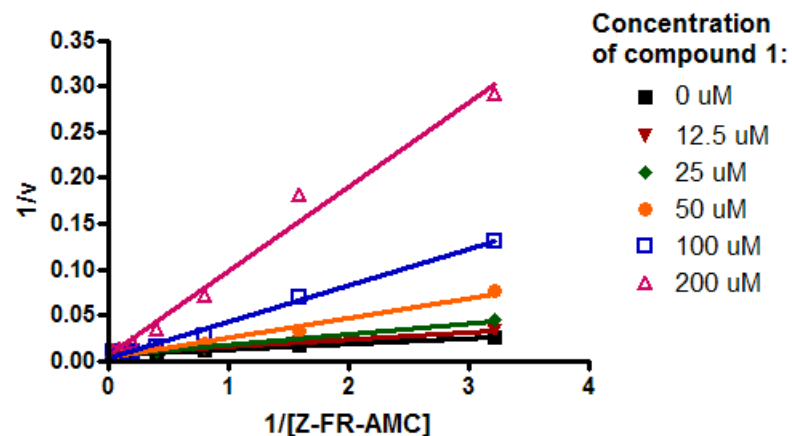
2

87 μM

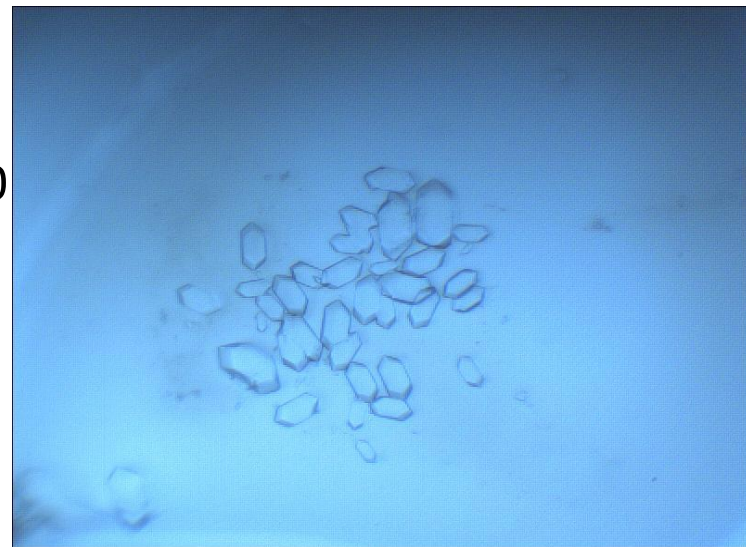


3

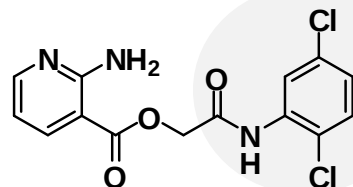
84 μM



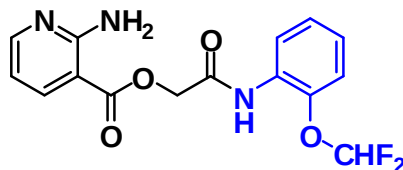
- **Competitive** inhibition kinetics for **1**, **2**, and **3**
- No pre-incubation time dependence
- IC_{50} and K_i determinations with 0.01% Triton X-100
- Early crystallography effort suggested possible ligand density on prime side of active site



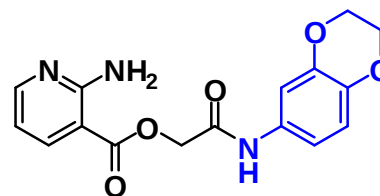
Initial pharmacophore profiling



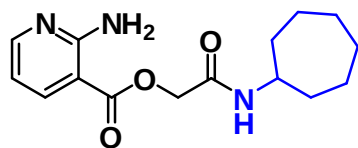
cruzain $IC_{50} = 77 \mu M$



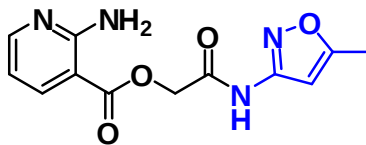
$IC_{50} = 126 \mu M$



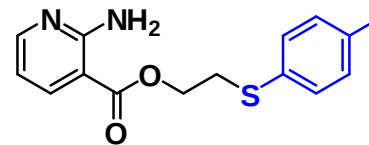
$IC_{50} = 182 \mu M$



$IC_{50} = 87 \mu M$



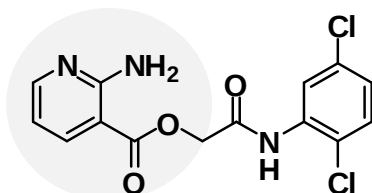
$IC_{50} = 350 \mu M^*$



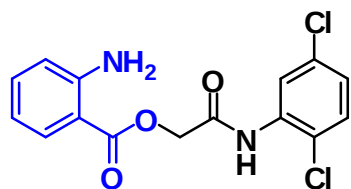
$IC_{50} = 485 \mu M^*$

glycolyl amide function possibly important

Initial pharmacophore profiling

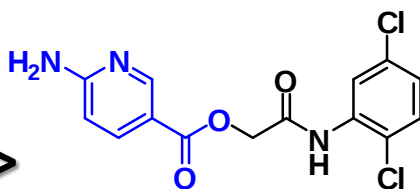


cruzain $IC_{50} = 77 \mu M$

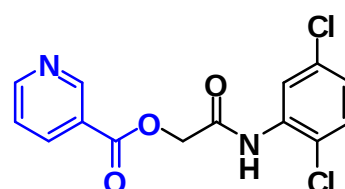


$IC_{50} = 58 \mu M$

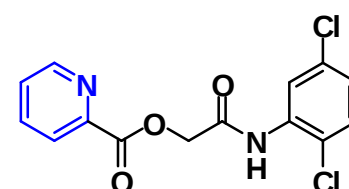
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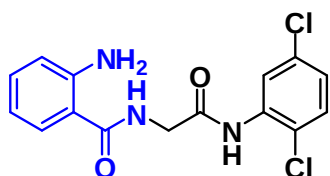
$IC_{50} = 470 \mu M^*$



$IC_{50} = 770 \mu M^*$

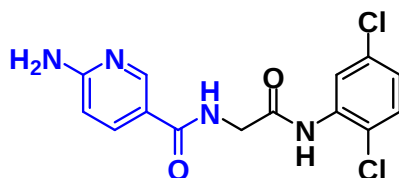


$IC_{50} = 4300 \mu M^*$

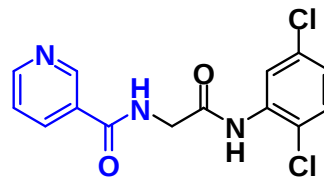


$IC_{50} = 128 \mu M$

>



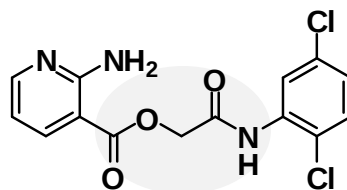
$IC_{50} = 480 \mu M^*$



$IC_{50} = 940 \mu M^*$

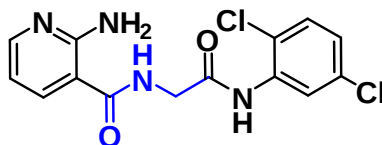
ortho amine is important; ester preferred to amide

Linker SAR

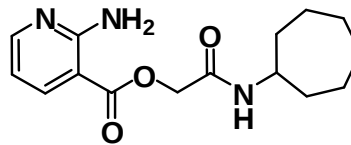


cruzain $IC_{50} = 77 \mu M$
LE = 0.26

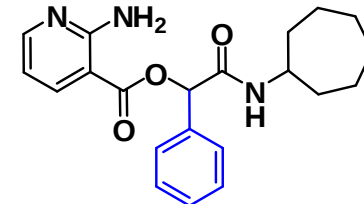
Acyclic linkers



cruzain $IC_{50} (\mu M) = 350$

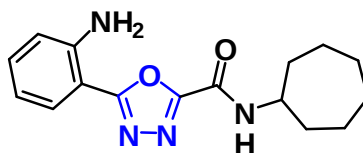


87



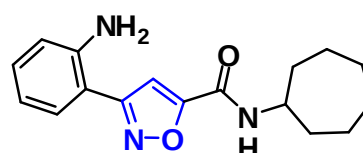
> 100

Heterocyclic linkers



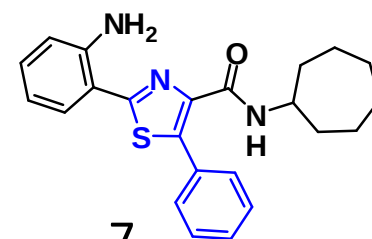
cruzain $IC_{50} (\mu M) = 14$

LE = **0.30**



17

0.30

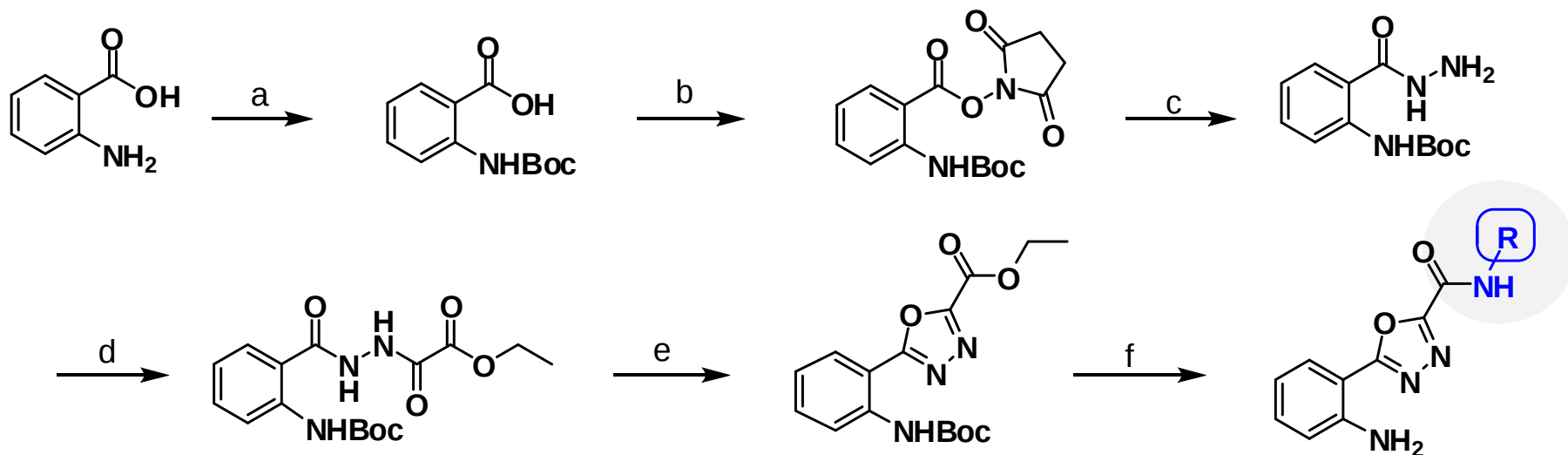


7

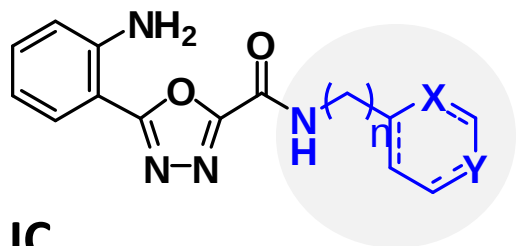
0.25

Oxadiazole linker selected for further SAR studies

Oxadiazole synthesis with late diversification



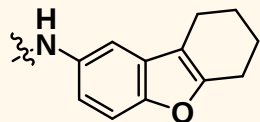
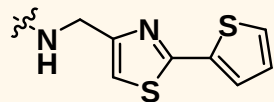
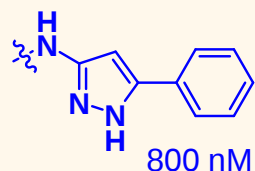
Scheme 1. Synthesis of oxadiazole analogs. Conditions: a) Boc anhydride, NaOH, acetonitrile-water-dioxane, 81%; b) NHS-OH, EDC, dichloromethane, 70%; c) 10 equiv. hydrazine hydrate, MeOH, 91%; d) ethyl oxalyl chloride, DIPEA, THF, 96%; e) i) thionyl chloride DIPEA, ether, ii) toluene reflux 45%; f) i) 4N HCl-dioxane, ii) R-NH₂, DIPEA, EtOH 88°C, sealed vial.



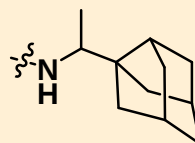
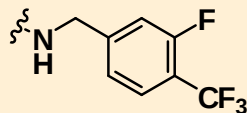
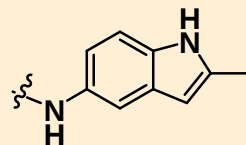
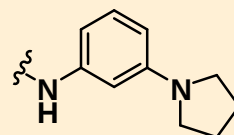
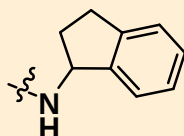
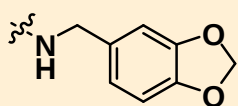
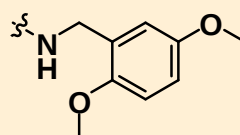
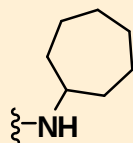
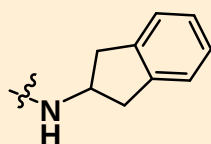
SAR of the eastern ring system

cruzain IC₅₀

< 2 μ M

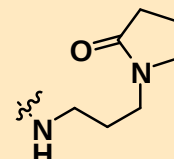
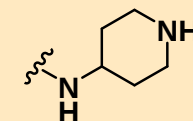
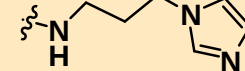
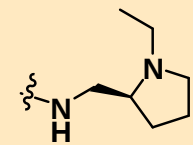
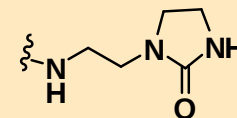
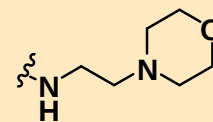


5-20 μ M



Tbb 17 μ M

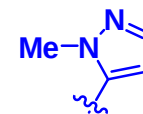
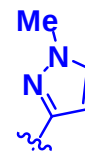
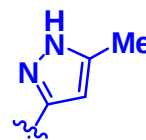
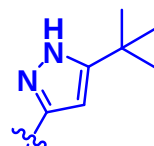
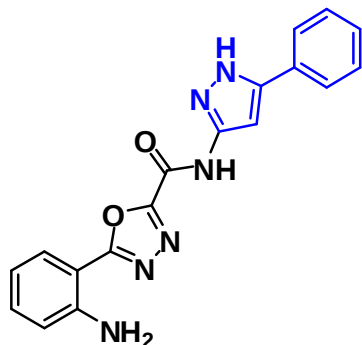
>100 μ M



- Basic aliphatic C-rings are poorly tolerated
- Non-basic aliphatic, benzylic, and aromatic C-rings are well tolerated
- Pyrazole was the best C-ring identified and was selected for further study

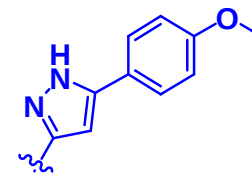
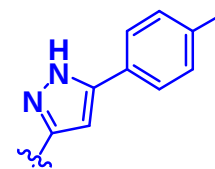
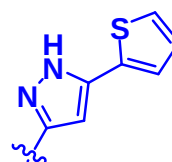
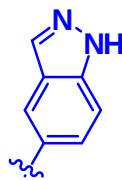
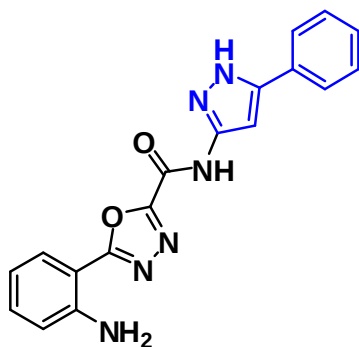
Pyrazole breakthrough!

aliphatics

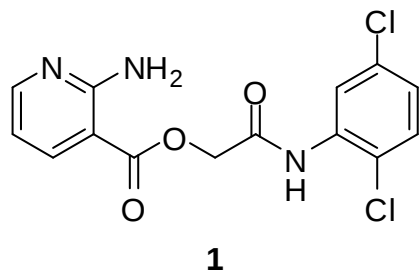


cruzain IC ₅₀ (μM) =	0.80	2.0	11	> 100	> 100
LE =	0.32	0.32	0.32		

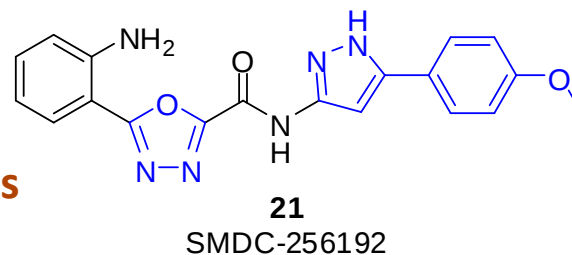
aromatics



cruzain IC ₅₀ (μM) =	0.80	41	1.0	0.50	0.20
LE =	0.32	0.25	0.33	0.32	0.33

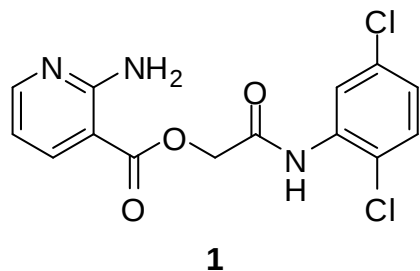


< 50 analogs

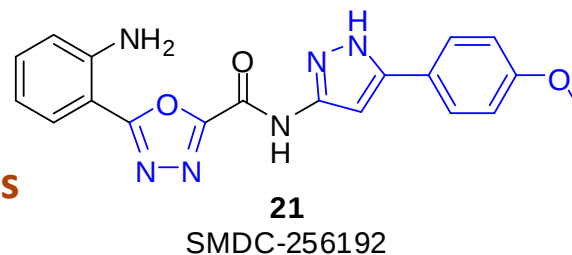


- 400x more potent
- LE 0.26 → 0.33
- no ester liability
- sharp SAR

SMDC#	Cruz IC ₅₀ (μM)	Cat L IC ₅₀ (μM)	<i>T. cruzi</i> (days)	Rhod IC ₅₀ (μM)	TbCat B IC ₅₀ (μM)	<i>Tbb</i> GI ₅₀ (μM)	Jurkat CC ₅₀ (μM)
256192	0.2	0.1	5	0.3	> 100	> 100	---
281573	4	0.2	---	3	> 100	---	---
281568	24	0.3	---	10	> 100	> 100	---
256194	2	0.4	---	12	> 100	> 100	---
281564	2	1	5	6	> 100	> 100	---
281567	7	1.5	5	20	> 100	37	> 100
256171	18	2	19	30	> 100	17	> 100
256199	12	3.3	5	14	> 100	19	> 100
256200	7	4.9	---	8	> 100	> 100	---
256188	6	7.9	---	18	> 100	> 100	---
281563	11	9	---	45	> 100	> 100	---
256185	7	10.7	---	12	> 100	> 100	---
281562	> 100	> 200	---	> 100	> 100	> 100	---

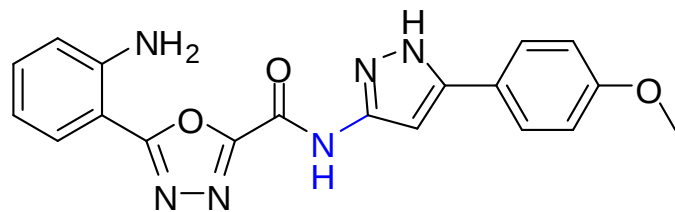


< 50 analogs



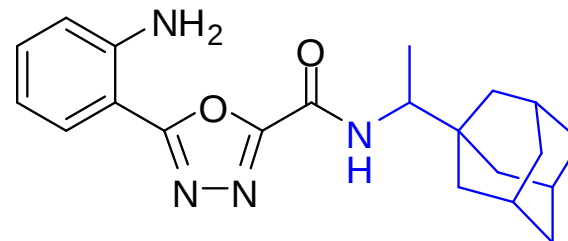
- 400x more potent
- LE 0.25 → 0.37
- no ester liability
- sharp SAR

SMDC#	Cruz IC ₅₀ (μM)	Cat L IC ₅₀ (μM)	<i>T. cruzi</i> (days)	Rhod IC ₅₀ (μM)	TbCat B IC ₅₀ (μM)	<i>Tbb</i> GI ₅₀ (μM)	Jurkat CC ₅₀ (μM)
256192	0.2	0.1	5	0.3	> 100	> 100	---
281573	4	0.2	---	3	> 100	---	---
281568	24	0.3	---	10	> 100	> 100	---
256194	2	0.4	---	12	> 100	> 100	---
281564	2	1	5	6	> 100	> 100	---
281567	7	1.5	5	20	> 100	37	> 100
256171	18	2	19	30	> 100	17	> 100
256199	12	3.3	5	14	> 100	19	> 100
256200	7	4.9	---	8	> 100	> 100	---
256188	6	7.9	---	18	> 100	> 100	---
281563	11	9	---	45	> 100	> 100	---
256185	7	10.7	---	12	> 100	> 100	---
281562	> 100	> 200	---	> 100	> 100	> 100	---



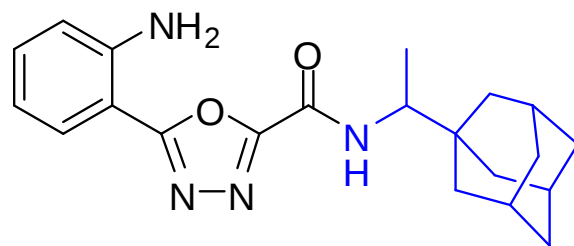
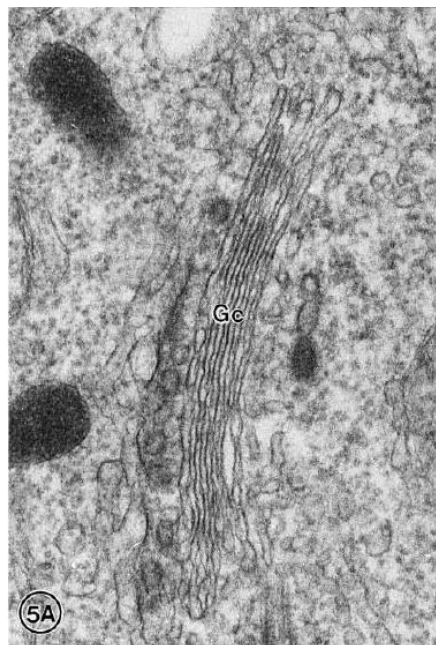
21

SMDC-256192

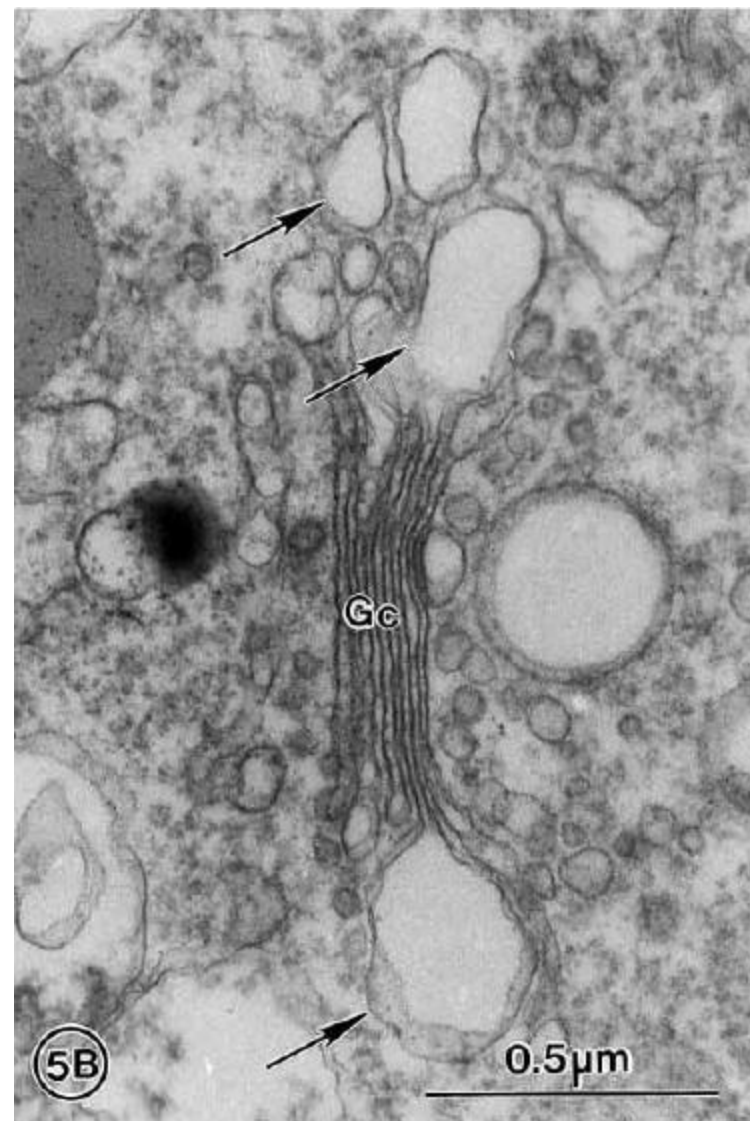
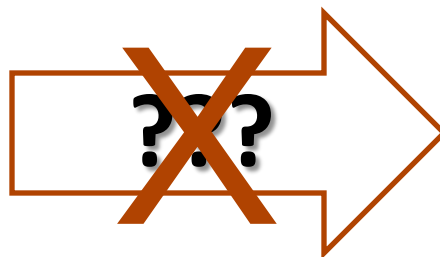


SMDC-256171

SMDC#	Cruz IC ₅₀ (μ M)	Cat L IC ₅₀ (μ M)	<i>T. cruzi</i> (days)	Rhod IC ₅₀ (μ M)	TbCat B IC ₅₀ (μ M)	<i>Tbb</i> GI ₅₀ (μ M)	Jurkat CC ₅₀ (μ M)
256192	0.2	0.1	5	0.3	> 100	> 100	---
281573	4	0.2	---	3	> 100	---	---
281568	24	0.3	---	10	> 100	> 100	---
256194	2	0.4	---	12	> 100	> 100	---
281564	2	1	5	6	> 100	> 100	---
281567	7	1.5	5	20	> 100	37	> 100
256171	18	2	19	30	> 100	17	> 100
256199	12	3.3	5	14	> 100	19	> 100
256200	7	4.9	---	8	> 100	> 100	---
256188	6	7.9	---	18	> 100	> 100	---
281563	11	9	---	45	> 100	> 100	---
256185	7	10.7	---	12	> 100	> 100	---
281562	> 100	> 200	---	> 100	> 100	> 100	---

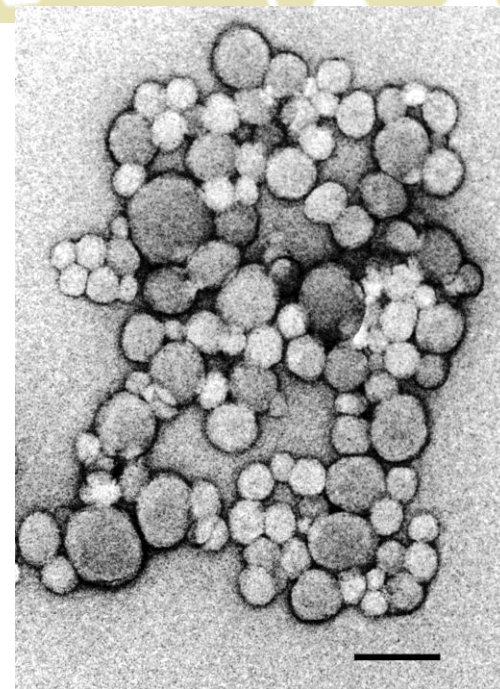


SMDC-256171

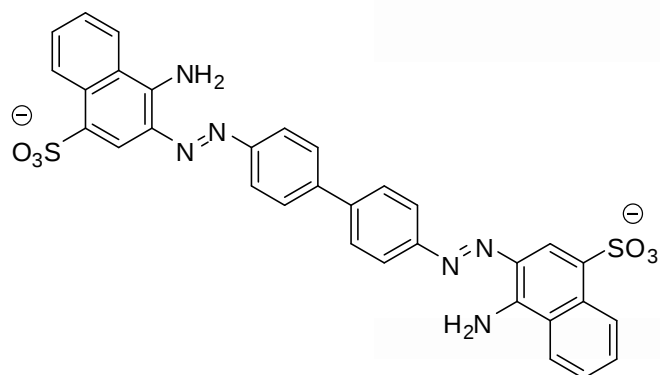


Aggregation???

1. Non-specific
2. Time-dependent
3. Reversible by dilution
4. Steep IC_{50} curves
5. Decreased by increasing $[E]$



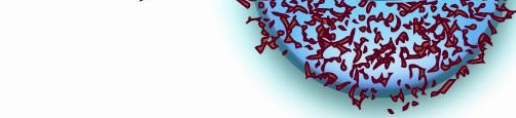
McGovern, *JMC* 2002



$\times 10^8$

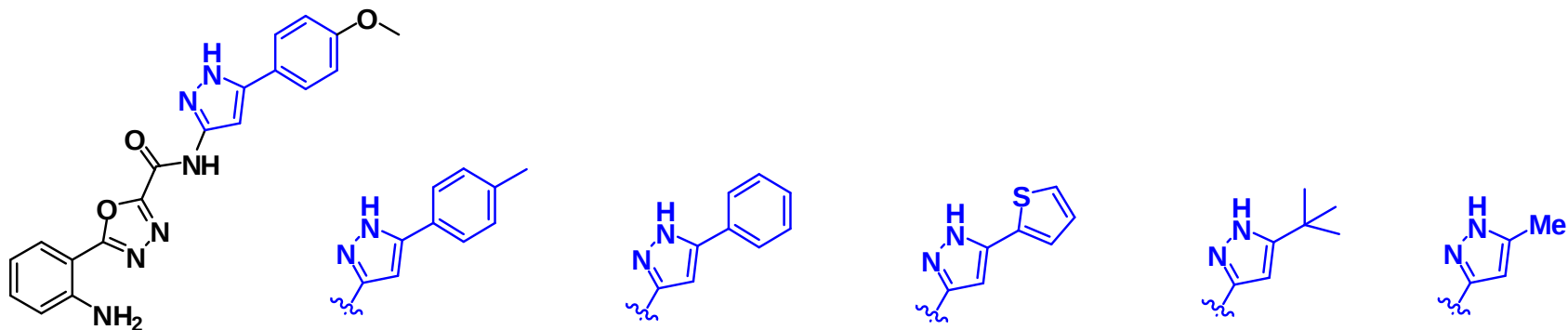


+ 10^4 enzyme molecules



Coan, *JACS* 2008

Hill slopes



cruzain IC₅₀ (μM) 0.001% Triton X-100

0.20

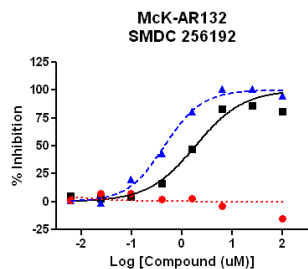
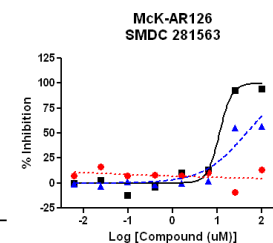
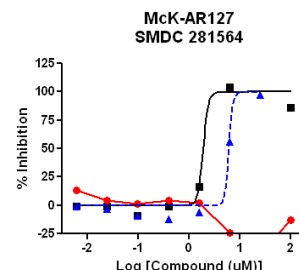
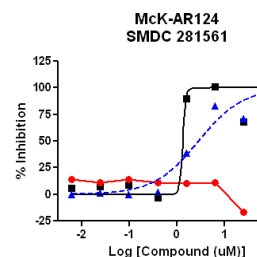
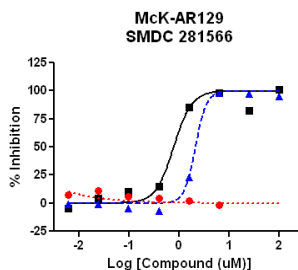
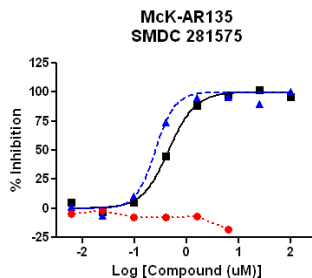
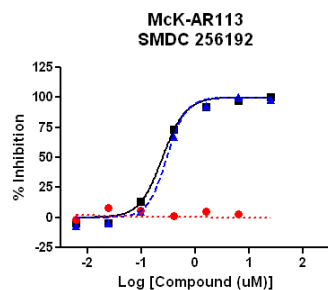
0.50

0.80

1.0

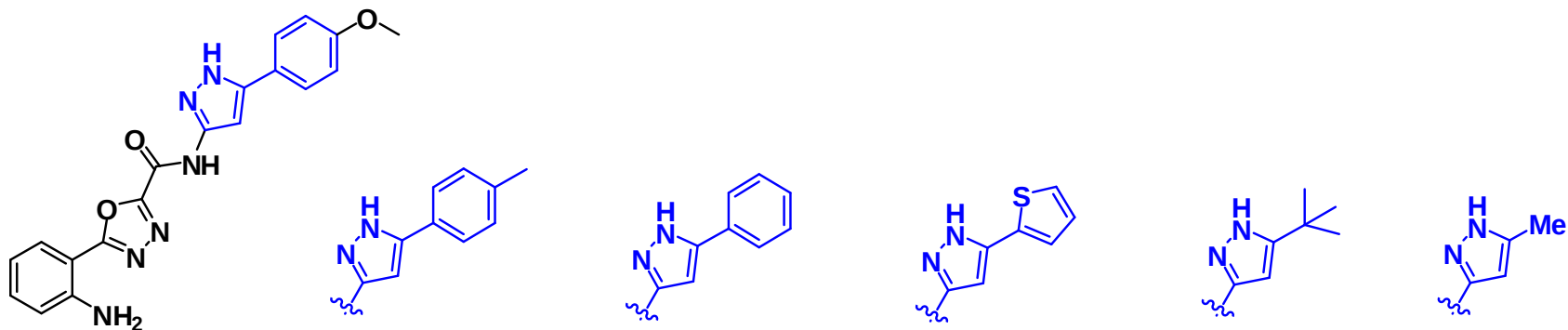
2.0

11



Lot 2
repeat

Detergent effects



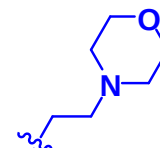
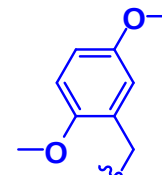
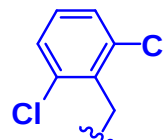
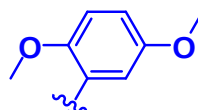
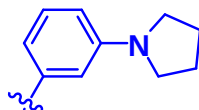
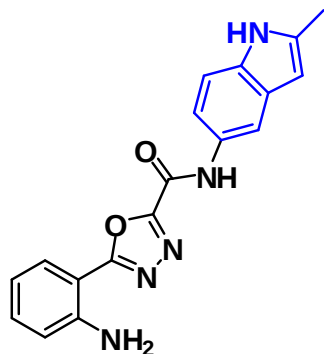
0.001% Triton X-100

$IC_{50} = 0.20$	0.50	0.80	1.0	2.0	11
------------------	------	------	-----	-----	----

0.01% Triton X-100

$IC_{50} = >100$	>100	>100	83	>100	>100
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Hill slopes



cruzain IC₅₀ (μM) 0.001% Triton X-100

6.0

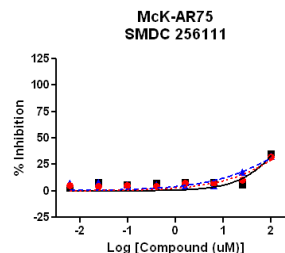
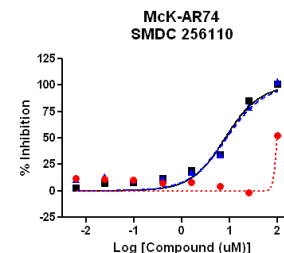
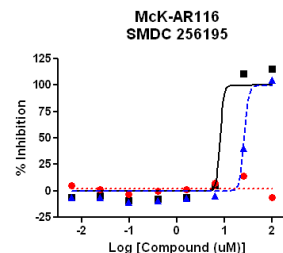
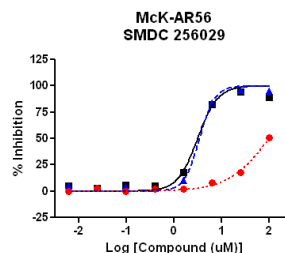
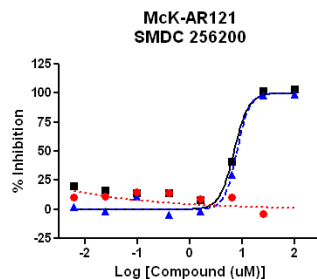
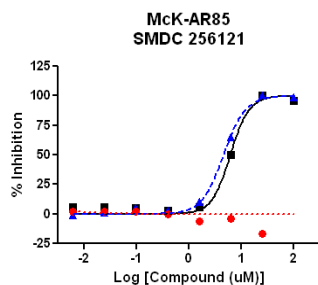
7.0

3.0

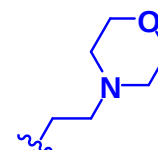
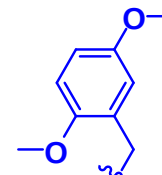
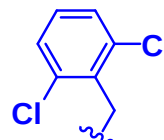
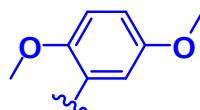
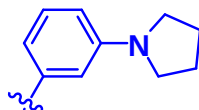
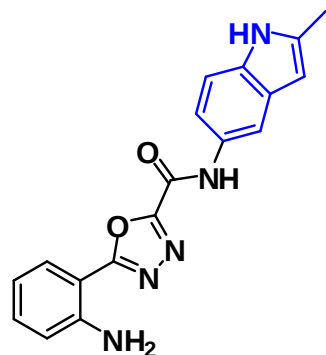
10

8.0

>100



Hill slopes



0.001% Triton X-100

$IC_{50} = 6.0$

7.0

3.0

10

8.0

>100

0.01% Triton X-100

$IC_{50} = >100$

>100

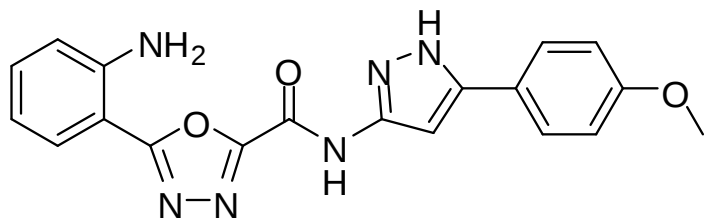
>100

>100

44

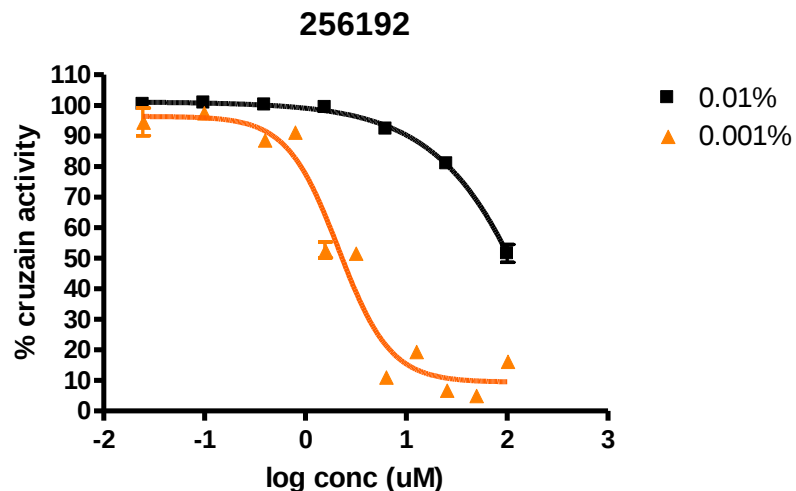
>100

SMDC-256192



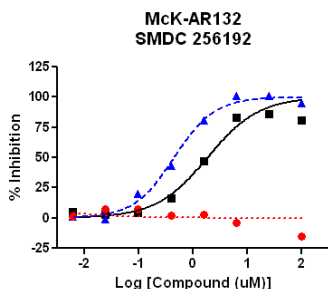
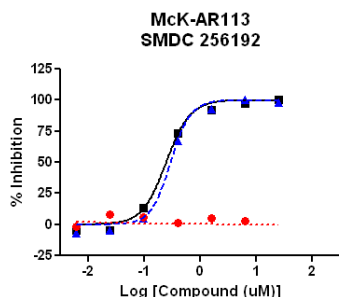
SMDC-256192

cruzain $IC_{50} = 0.20 \mu M$
 rhodesain $IC_{50} = 0.30 \mu M$
 Cat L $IC_{50} = 0.10 \mu M$
 Tb Cat B $IC_{50} = >100 \mu M$



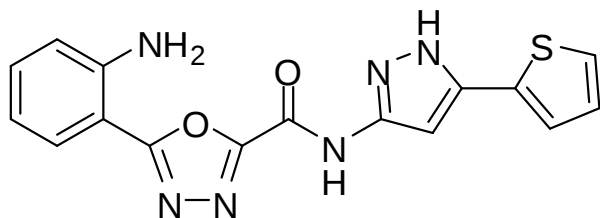
0.01% triton
0.001% triton

cruzain IC_{50}
107 μM
2 μM



- Strong detergent effect
- No inhibition if solution is spun down
- Detergent reversible ampC inhibition (50% inhibit. @ 13 μM)
- **Non-competitive** @ 5 μM (0.001% triton)
- Particles at 3 μM (DLS, 0.001% triton)

SMDC-281561

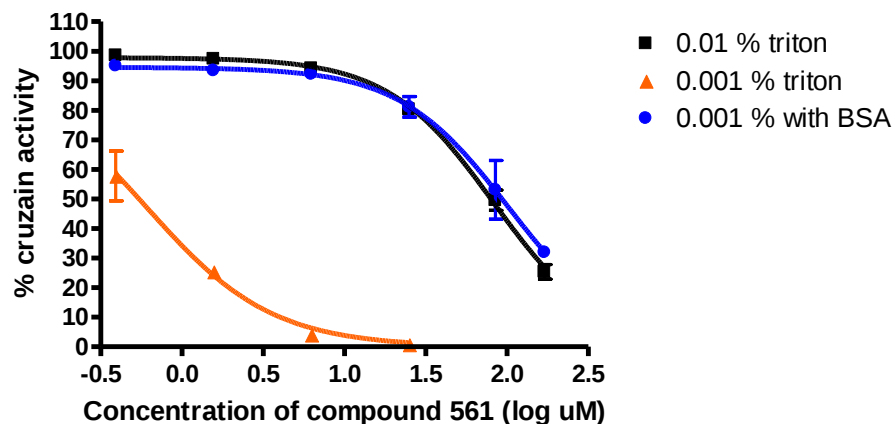


SMDC-281561

cruzain $IC_{50} = 1.0 \mu M$

rhodesain $IC_{50} = 3.0 \mu M$

Tb Cat B $IC_{50} = >100 \mu M$



0.01% triton

0.001% triton

0.001% + BSA

cruzain IC_{50}

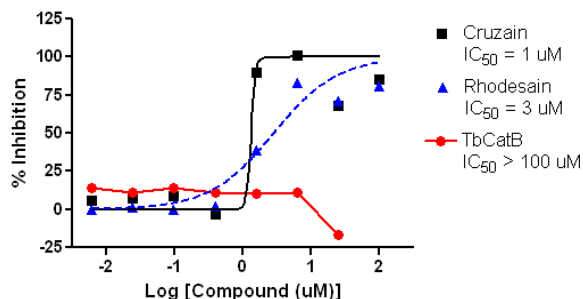
82 μM

< 1 μM

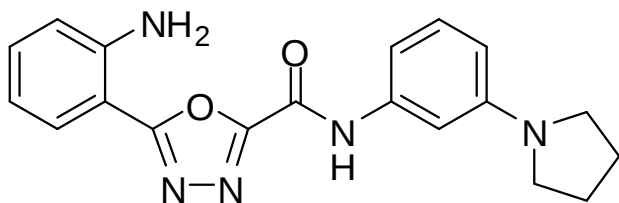
102 μM

- Strong detergent and BSA effect
- Very modest inhibition of ampC (18% inhibit. @ 5 μM)
- **Non-competitive** at 5 μM (0.001% triton)
- **Competitive** at 50 μM (0.01% triton)

McK-AR124
SMDC 281561

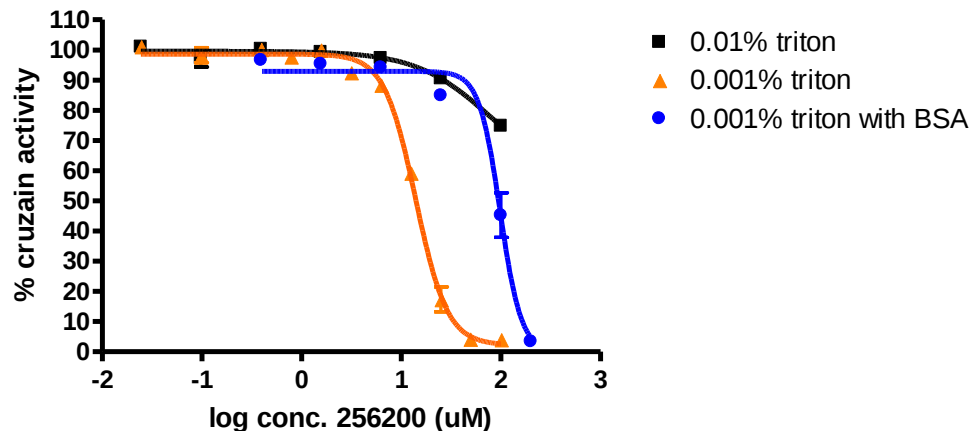
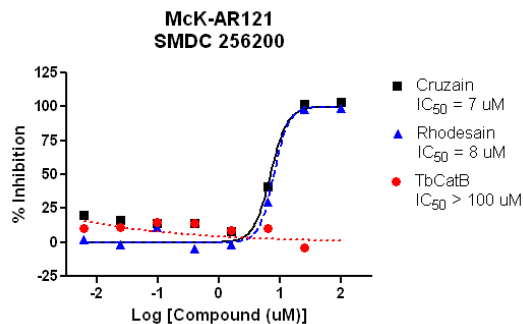


SMDC-256200



SMDC-256200

cruzain $IC_{50} = 7.0 \mu M$
 rhodesain $IC_{50} = 8.0 \mu M$
 Cat L $IC_{50} = 4.9 \mu M$
 Tb Cat B $IC_{50} = >100 \mu M$



0.01% triton

0.001% triton

0.001% + BSA

cruzain IC_{50}

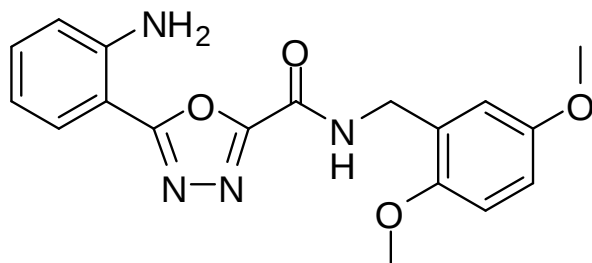
> 100 μM

14 μM

100 μM

- Strong detergent and BSA effect
- Detergent reversible ampC inhibition (63% inhibit. @ 50 μM)
- Particles at 50 μM (DLS, 0.001% triton)

SMDC-256110

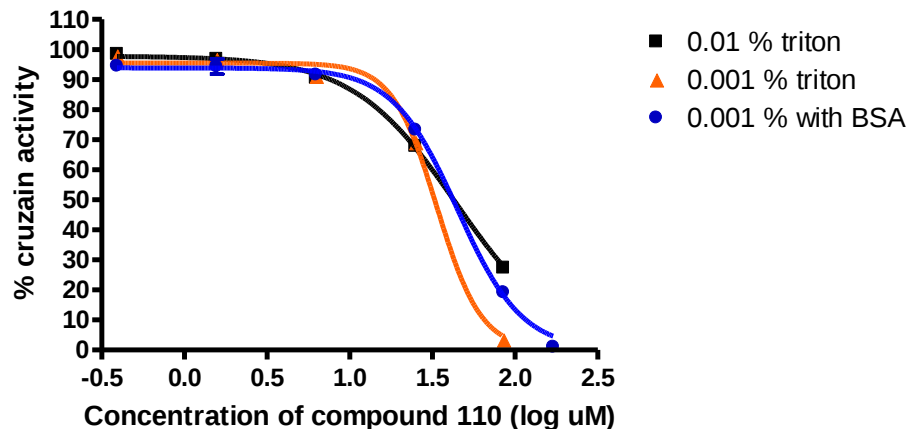
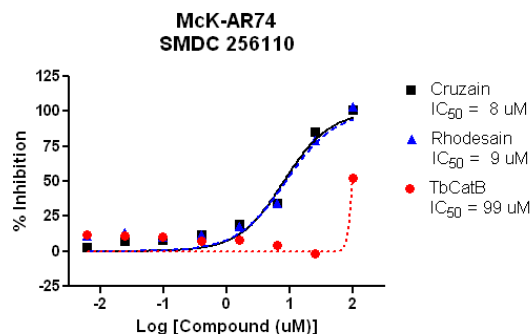


SMDC-256110

cruzain $IC_{50} = 8.0 \mu M$

rhodesain $IC_{50} = 9.0 \mu M$

Tb Cat B $IC_{50} = 100 \mu M$



0.01% triton

0.001% triton

0.001% + BSA

cruzain IC_{50}

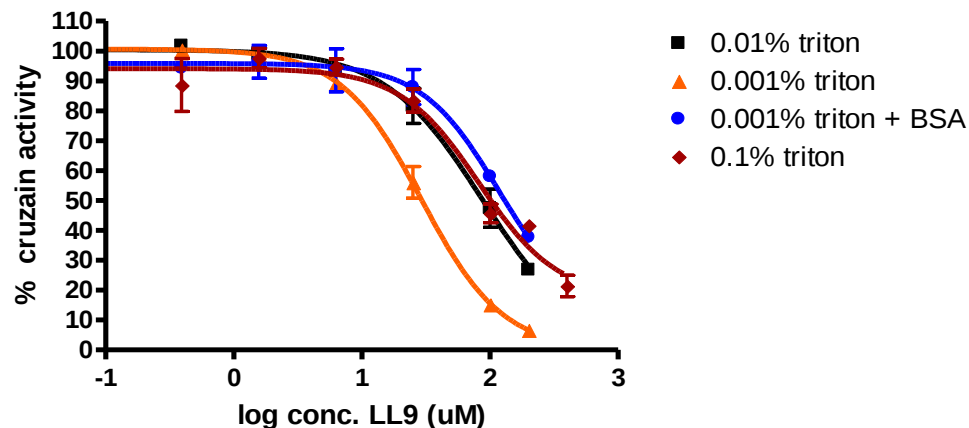
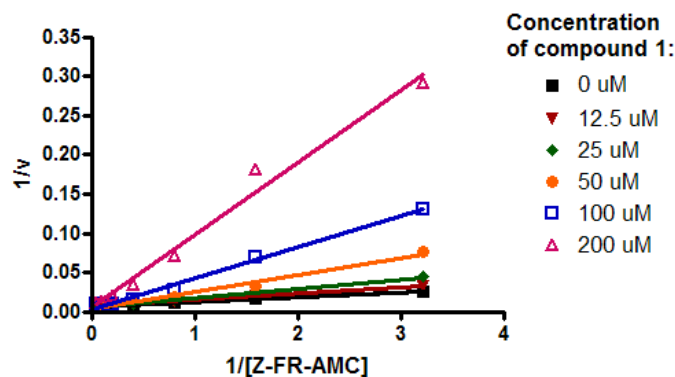
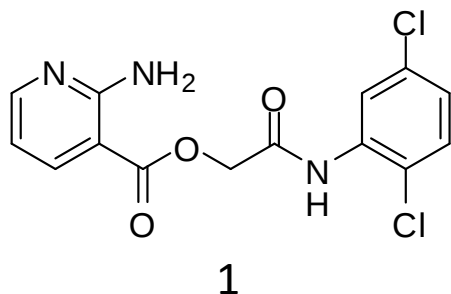
44 μM

34 μM

45 μM

- No detergent or BSA effect
- Detergent reversible ampC inhibition (88 % @ 100 μM)
- **Competitive** at 50 μM (0.01% triton)

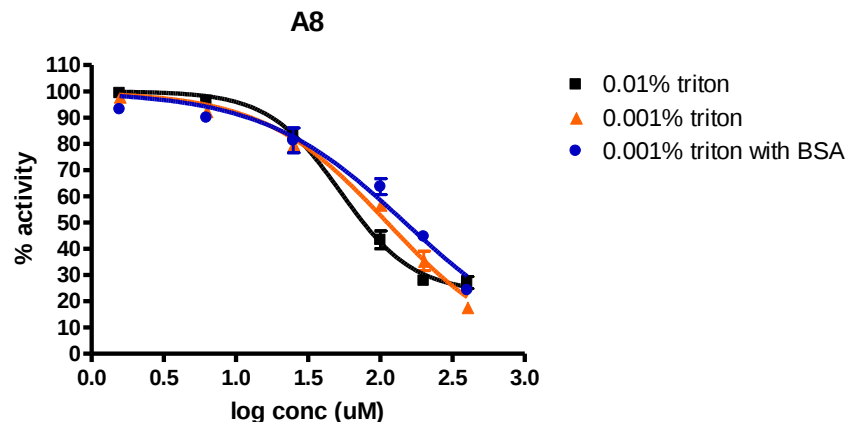
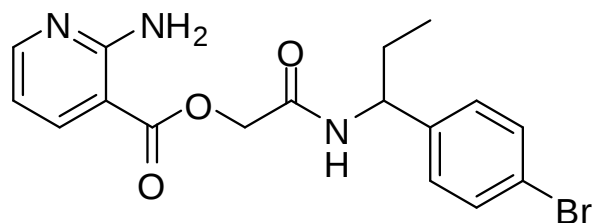
Revisiting the original leads



	IC ₅₀ (uM)
0.01% triton	88 μM
0.001% triton	29 μM
0.001% + BSA	117 μM
0.1% triton	82 μM

- More potent at low triton condition
- Particles at 100 μM (0.001% triton)
- Detergent reversible ampC inhibition (99 % @ 100 μM)
- **Competitive** at 100 μM (0.01% triton)
- **Non-competitive** at 25 μM (0.001% triton)

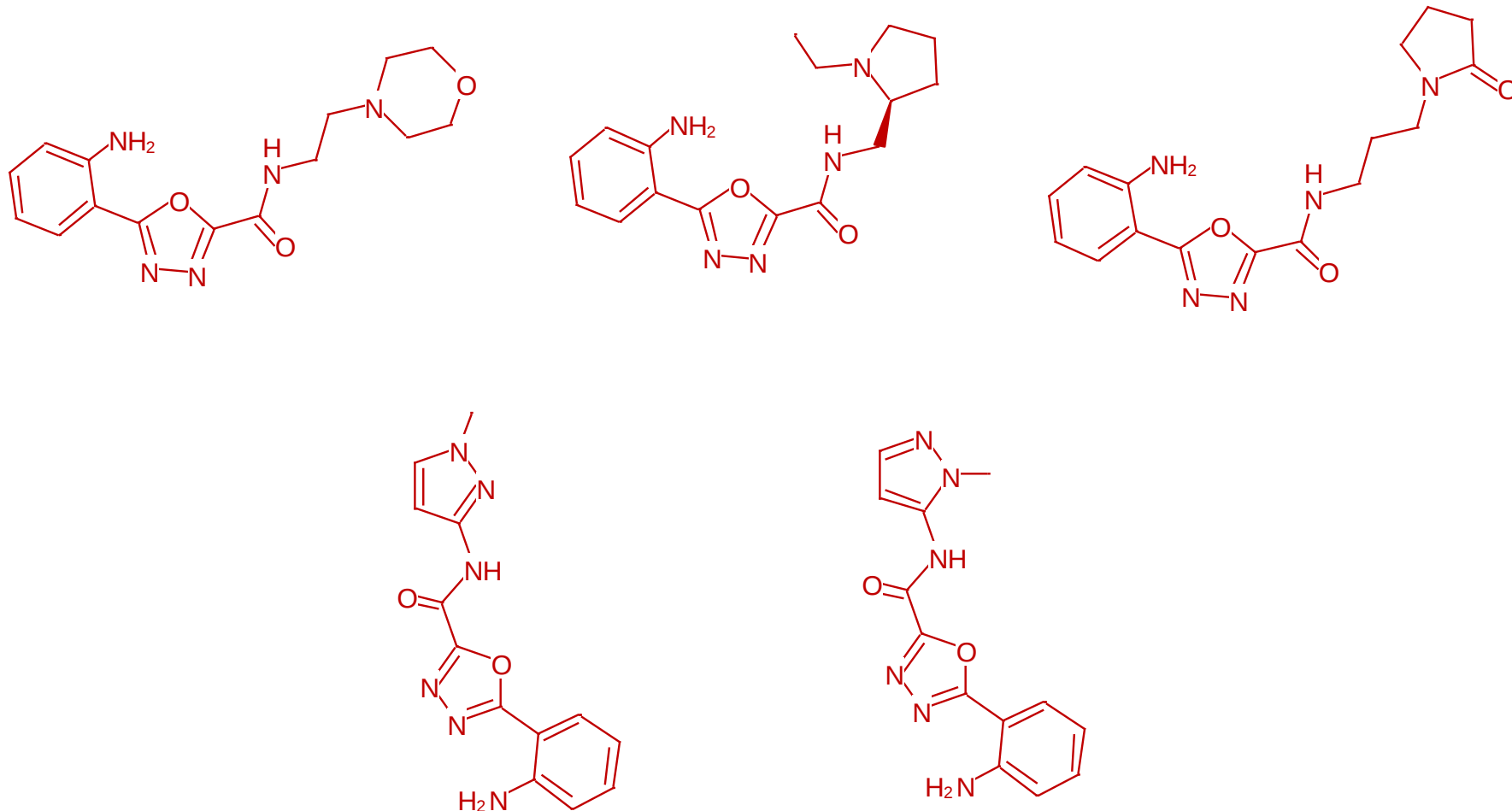
Revisiting the original leads



	cruazin IC ₅₀
0.01% triton	94 μ M
0.001% triton	123 μ M
0.001% + BSA	186 μ M

- No detergent effect (slightly *more* potent in high triton X-100)
- Weak ampC inhibition (23 % @ 200 μ M, no triton)
- **Competitive** at 100 μ M (0.01% triton)

Inactive analogs show no particles by DLS

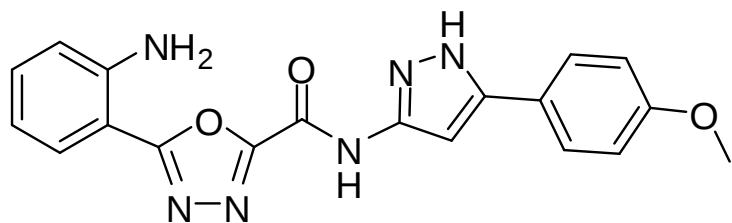




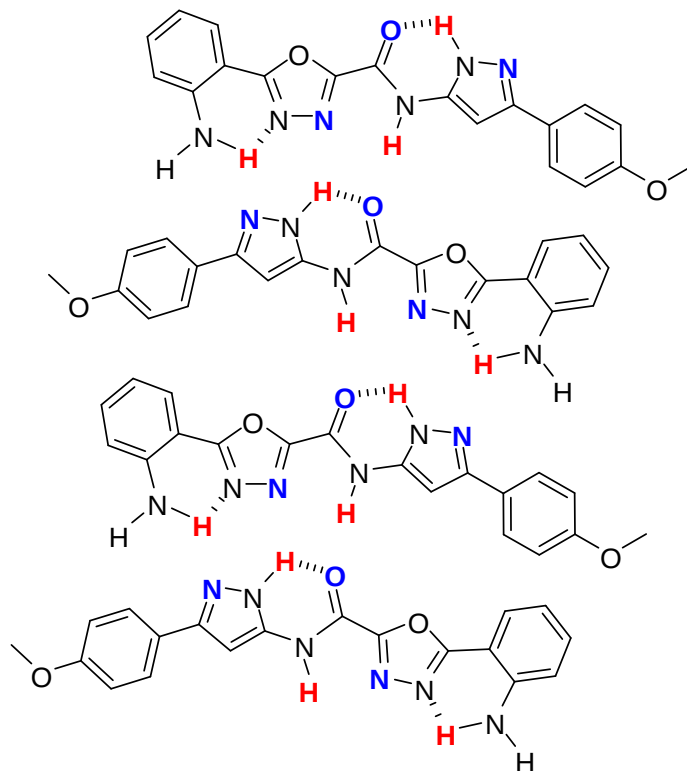
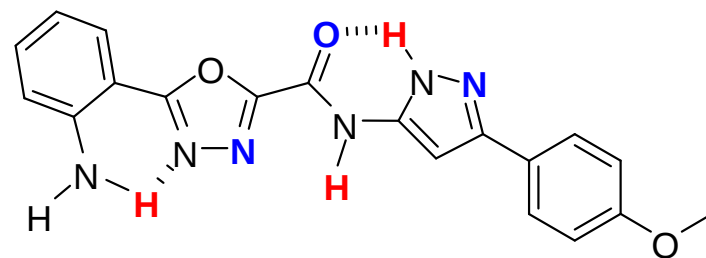
What we learned...

- Assay conditions are crucial – *you find what you look for*
- If possible, employ an orthogonal binding assay (SPR, etc)
- Interpretable SAR does not necessarily imply specific binding
- Nanomolar potencies can be conferred by non-specific mechanisms
- Hill-slope alone is insufficient to rule out an artifactual mechanism
- Mechanism of inhibition should be assessed throughout the course of an optimization effort

Optimization for aggregation??



SMDC-256192





A final thought...

Small molecules are much stranger than we've been led to believe



Acknowledgements

Medicinal Chemistry

Clifford Bryant

Computational Studies

Rafaela Ferreira

John Irwin

Brian Shoichet

Screening & Parasite Biology

Kenny Ang

Rafaela Ferreira

Patricia Doyle-Engel

Juan Engel

Jim McKerrow

SPR Screen

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