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Identifying small molecule and peptide inhibitors against ricin and Shiga toxin

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INTRODUCTION & AIM

Ricin is a ribosome inactivating protein, present in the seeds of the castor bean (Ricinus communis)

Ricinus communis

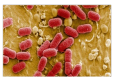
Castor Bean

Castor Oil

- Processed worldwide for biodiesel and lubricant production
- More than 5% of total protein in seed
- Category B biothreat agent, anticancer agent
- There are no antidotes or vaccines
- Mucorales fungi produce a ricin-like toxin

Shiga toxins

- Shiga toxin 1 and 2 (Stx1 and Stx2) are produced by *E. coli* (STEC), which causes food poisoning and is a public health concern.
- STEC causes hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS).
- Shiga toxin is also produced by *Shigella dysenteriae*.
- Stx2 producing *E.coli* was the cause of a major foodborne outbreak in Germany in July 2011, which was the deadliest on record.
- At least 35 people were confirmed sick in an *E. coli* (STEC) outbreak linked to the San Diego restaurant around November 2023.
- There are no FDA-approved vaccines or therapeutics against STEC.

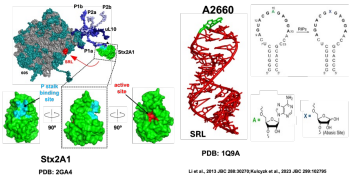


***E. coli* outbreak linked to McDonald's Quarter Pounders**

Cases: 90 (15 new)
Hospitalizations: 27 (5 new)
Deaths: 1 (0 new)
States: 13 (3 new)

https://www.cdc.gov/ecoli/outbreaks/

The A1 subunit Shiga toxins bind to the ribosomal P stalk to depurinate the SRL



Li et al., 2013 JBC 288 36270;Kulczyk et al., 2023 JBC 299:102795

RESULTS & DISCUSSION

Comparative study of binding affinities using FA and SPR

Peptide	Peptide Sequence	K_i (μM) using FA assay[a]		K_D (μM) using SPR assay		IC_{50} (μM) using qRT-PCR[d]	
		RTA	Stx2A1	RTA[b]	Stx2A1[c]	RTA	Stx2A1
P3	LFD	85.6 ± 8	>1000	> 10 mM	> 1 M	U.D [e]	U.D.
P4	GLFD	41.6 ± 7	>1000	451 ± 17	> 2 mM	102 ± 45	U.D.
P5	FGLFD	46.5 ± 7	14.9 ± 2	497 ± 30	125 ± 27	121 ± 44	U.D.
P6	GFGLFD	28.0 ± 7	9.6 ± 1	399 ± 20	71 ± 13	63 ± 13	U.D.
P7	MGFGLFD	20.6 ± 2	6.0 ± 0.4	294 ± 47	66 ± 3	34 ± 10	60 ± 6
P8	DMGFGLFD	6.0 ± 0.9	2.4 ± 0.4	299 ± 5	36 ± 8	23 ± 4	23 ± 4
P9	DDMGFGLFD	4.3 ± 0.3	2.1 ± 0.1	309 ± 7	29 ± 5	15 ± 2	26 ± 4
P10	DDDMGFGLFD	1.5 ± 0.1	1.8 ± 0.1	272 ± 6	20 ± 4	8 ± 2	28 ± 4
P11	SDDDMGFGLFD	0.5 ±0.04	1.2 ±0.04	196 ± 17	22 ± 3	5 ± 1	30 ± 4

P11 binding at the P-stalk site of ricin & Shiga toxin inhibits depurination of the SRL validating that the P-stalk site as a target for inhibition of both toxins.
Differences between the way the peptides interacts with the P-stalk site of each toxin
The relative importance of certain amino acids differ b/w ricin and Shiga toxin.

Comparative study of peptide binding affinities between ricin & Shiga toxin

Peptide	Peptide Sequence	K_i (μM) using FA assay		IC_{50} (μM) using qRT-PCR	
		RTA	Stx2A1	RTA	Stx2A1
P3	LFD	86 ± 8	> 1000	U.D.	U.D.
P4	GLFD	42 ± 7	> 1000	102 ± 45	U.D.
P5	FGLFD	47 ± 7	15 ± 2	121 ± 44	U.D.
P6	GFGLFD	28 ± 7	10 ± 1	63 ± 13	U.D.
P7	MGFGLFD	21 ± 2	6 ± 0.4	34 ± 10	60 ± 6
P8	DMGFGLFD	6 ± 0.9	2.4 ± 0.4	23 ± 4	23 ± 4
P9	DDMGFGLFD	4.3 ± 0.3	2.1 ± 0.1	15 ± 2	26 ± 4
P10	DDDMGFGLFD	1.5 ± 0.1	1.8 ± 0.1	8 ± 2	28 ± 4
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- P11 binding at the P-stalk site of ricin & Shiga toxin inhibits depurination of the SRL validating that the P-stalk site is a target for inhibition of both toxins.
- Differences between the way the peptides interact with the P-stalk site of each toxin show that the relative importance of certain amino acids differ between ricin and Shiga toxin

Chemical structure, Affinity (K_i), and inhibitory activity (IC_{50}) of small molecule inhibitors targeting toxin-ribosome interaction

RU-NT	Structure	MW (dalton)	K_i FA[a] (μM)	IC_{50}[b] FA (μM)	IC_{50} Rib.[c] (μM)	EC_{50} Vero[d] (μM)
CC10501		204	32	119	427 n=1.7	UD
93		232	3	12	40 n=1	UD
202		250	4	15	31.5 n=1	169 n=1
135		311	3	12	49 n=1.2	135
102		232	2	9	38 n=1.3	120 n=1
165		246	2	9	45 n=1.5	98 n=1.5
124		288	5	21	23 n=1.4	52 n=1.2
192		260	1	6	18.5 n=1.2	36
206		258	0.6	4	18 n=1.6	29.5 n=1

[1]The K_i values were measured by fluorescence anisotropy (FA) using a Synergy H1 plate reader.
[2]The IC_{50} value determined by FA is the inhibitor concentration required to replace 50% of the fluorescent P11 probe from RTA. The IC_{50} value determined by qRT-PCR is the inhibitor concentration required to inhibit *in vitro* depurination of rat liver ribosomes by RTA by 50%.
[3]The EC_{50} value is the half-maximal concentration required to inhibit depurination by ricin holotoxin in Vero cells as determined by qRT-PCR. U.D., unable to determine.

Chemical structure, Affinity (K_i), and inhibitory activity (IC_{50}) of small molecule inhibitors targeting toxin-ribosome interaction

RU-NT	Structure	MW (Dalton)	K_i (μM) using FA	IC_{50} (μM) qRT-PCR	EC_{50} (μM) Vero cells
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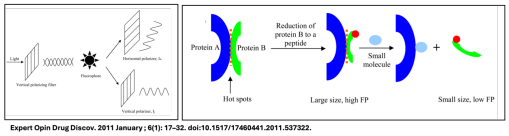
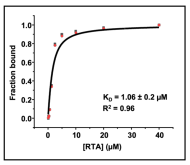
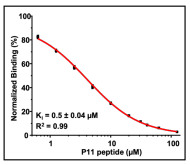
METHOD

Fluorescence Anisotropy (FA)

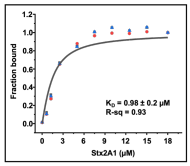


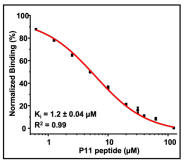
Expert Opin Drug Discov. 2011 January ; 6(1): 17–32. doi:10.1517/17460441.2011.537322.





Direct binding and unbinding of BODIPY-TMR labeled P11 peptide (5'-SDDDMGFGLFD-3') with purified RTA





Direct binding and unbinding of BODIPY-TMR labeled P11 peptide (5'-SDDDMGFGLFD-3') with purified Stx2A1

CONCLUSION

- Peptide data validated the P-stalk pocket as a novel target for inhibitors against toxin-ribosome interactions.
- Using FBLD with SPR we screened the Maybridge Ro3 Core library and identified CC10501, which binds at the P-stalk site of RTA and inhibits activity.
- We improved CC10501 using a structure-based design and a new fluorescence anisotropy (FA) assay and identified RU-NT-206 and RU-NT-192, which showed over 50- and 30-fold improved affinity, respectively.
- Ki data measured using FA assay showed a positive correlation with the Vero cell protection assay.

FUTURE WORK / REFERENCES

Acknowledgements:

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References:

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B. McLaughlin JE, Rudolph MJ, Dutta A, Li XP, Tsymbal AM, Chen Y, Bhattacharya S, Algava B, Goger M, Roberge JY, Tumer NE (2025) *Journal of Biological Chemistry* 301(3):108310.