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Supplemental Data

An Inhibitor of Gram-Negative

Bacterial Virulence Protein Secretion

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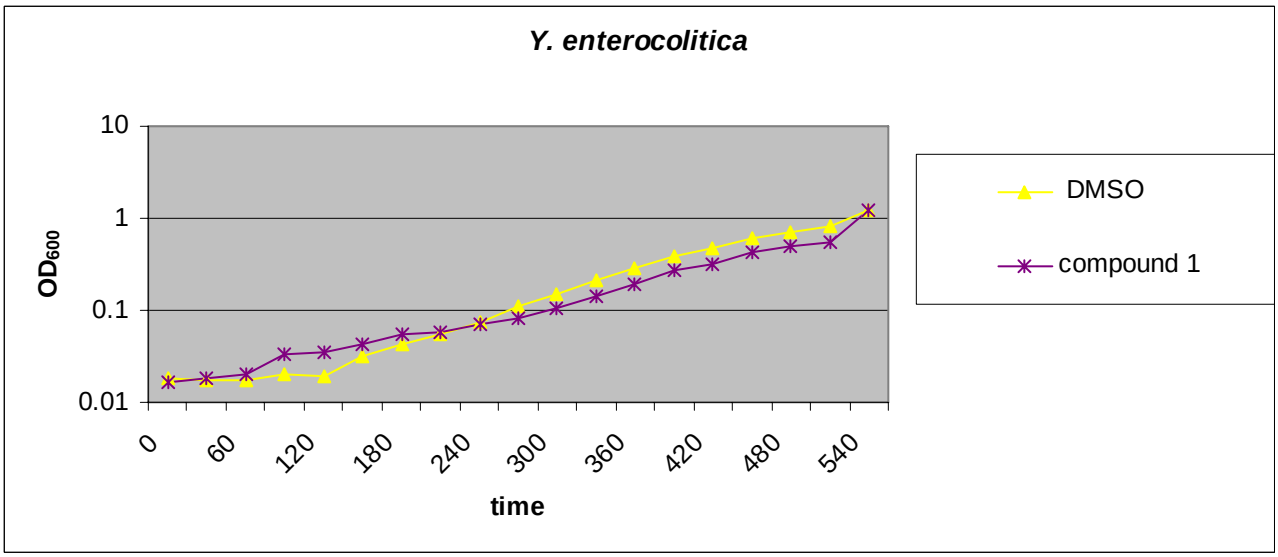
Screening For our HTS a novel strain of the Gram-negative bacterium *Salmonella enterica* serovar Typhimurium was engineered, which secretes a phospholipase A2 reporter construct in a T3 dependent manner. Specifically, this strain contains a protein fusion between the *S. typhimurium* T3S substrate SipA and the *Yersinia enterocolitica* T3 secreted substrate YplA. The first 59 amino acids have been removed from YplA, because they have been shown to be the signal sequence for its secretion by the *Y. enterocolitica* Ysa T3S system. Phospholipase activity is frequently used as a reporter in HT assays because of the availability of phospholipase substrates with a cleavage product that is fluorescent. Our assay was based on cleavage, by our phospholipase A2 reporter construct, of the substrate PED6: N-((6-(2,4-dinitro-phenyl)amino)-hexanoyl)-2-(4,4-difluro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Invitrogen, Carlsbad, CA). Such cleavage results in an increase in absorbance readily measured using a fluorometer. When PED6 is added directly to a culture of the engineered *Salmonella* strain, fluorescence is proportionally to the amount of phospholipase reporter secreted by the T3S system.

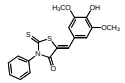
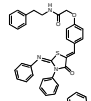
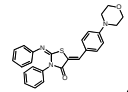
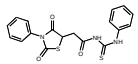
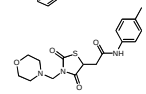
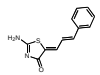
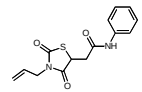
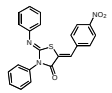
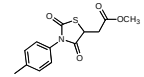
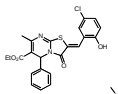
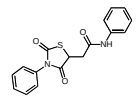
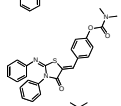
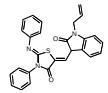
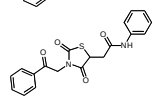
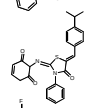
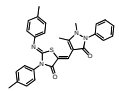
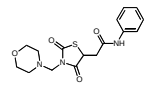
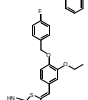
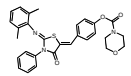
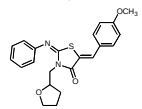
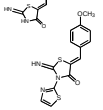
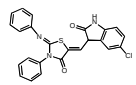
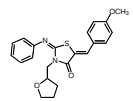
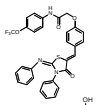
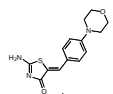
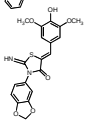
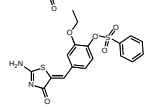
To perform the screening 30 μl of LB with bacteria (diluted 1:100 from an overnight culture) were aliquoted into 384-well black bottom plates (Corning), approximately 1μl of compounds were arrayed into wells (approximate concentrations of 5mg/ml in DMS0) and plates were incubated overnight at 37 degrees C. The next day, 30 μl of PLA buffer (10mM Tris HCl pH8.0, 100mM NaCl, 10mM $CaCl_2$) with PED6 (Invitrogen, Carlsbad, CA) was aliquoted to each well and plates were incubated at RT for 4 h. Fluorescence was read at 515nm (Envision, St. Louis, MO). Screening was performed at the National Screening Laboratory for the Regional Centers of Excellence in Biodefense and Emerging Infectious Disease (Boston, MA).

Figure S1. Growth curve for *Yersinia enterocolitica* in LB medium with compound **1** or an equal volume of the solvent (DMSO).



Supplemental Figure 1

SUPPLEMENTAL TABLE 1. Effect of thiazolidinone anologs on type III secretion in *S. typhimurium*

Compound	Structure	% secretion	Compound	Structure	% secretion	Compound	Structure	% secretion
10		81 ± 15	21		74 ± 5	32		48 ± 12
11		ND	22		68 ± 11	33		32 ± 5
12		96 ± 5	23		64 ± 24	34		3 ± 4
13		103 ± 1	24		67 ± 21	35		60 ± 18
14		91 ± 5	25		52 ± 9	36		2 ± 2
15		83 ± 5	26		66 ± 11	37		73 ± 7
16		89 ± 13	27		40 ± 31	38		24 ± 4
17		+	28		0 ± 0	39		70 ± 9
18		+	29		61 ± 10	40		90 ± 2
19		107 ± 21	30		35 ± 5			
20		+	31		39 ± 32			

+ = Secreted SipA protein levels were similar to WT. Protein levels were not quantitated for these samples.