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Promises and Challenges of the Type Three Secretion System-Injectisome as an Anti-Virulence Target

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CHAPTER SUMMARY

Antibiotic resistance is a major public health threat that has stimulated the scientific community to search for non-traditional therapeutic targets. Because virulence, but not the growth, of many Gram-negative bacterial pathogens depends on the multi-component type three secretion system injectisome (T3SSi), the T3SSi has been an attractive target for identifying small molecules, peptides, and monoclonal antibodies that inhibit its function to render the pathogen avirulent. While many small molecule lead compounds have been identified in whole cell-based high throughput screens (HTSs), only a few protein targets of these compounds are known, an important step to developing more potent and specific inhibitors. Evaluation of the efficacy of compounds in animal studies is ongoing. Some efforts involving the development of antibodies and vaccines that target the T3SSi are further along and include an antibody that is currently in phase II clinical trials. Continued research into these anti-virulence therapies, used alone or in combination with traditional antibiotics, requires combined efforts from both pharmaceutical companies and academic labs.

INTRODUCTION

Antibiotic resistance is a great and growing threat to public health motivating scientists to find innovative strategies to cure infections (1–3). An alternative approach to classical antibiotics is to target virulence factors (4) – bacterial factors required for infection or damage but not for growth outside the host (2, 5, 6). An anti-virulence factor should render the bacteria non-pathogenic by neutralizing a critical virulence element thereby allowing clearance of the pathogen by the host immune system (5–8).

The type 3 secretion system/injectisome (T3SSi) is expressed in a broad spectrum of Gram-negative bacteria and is usually crucial for virulence (4, 9). This needle and syringe-like

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apparatus functions as a conduit for the delivery of effector proteins from the bacterial cytoplasm into host cells (Fig 1A). These T3SSi systems share homology with 8 essential core components of flagellar T3SS and contain an additional 20–30 proteins involved in expression, secretion and translocation of effector proteins (9–11). Therapeutic strategies against the T3SSi have been pursued that include interfering with transcriptional regulation, chaperone-effector interaction, assembly of various structures (outer ring, needle, tip complex), or effector translocation or function (4, 5, 12–18).

Targeting the T3SSi as an effective means of curtailing infection has been rationalized in several ways. Since the injectisome is absent in many resident microbiota, one proposed advantage is that more of the microbiome would be preserved during treatment. Furthermore, the likelihood of developing resistance in resident microbiota that can be transferred by horizontal gene transfer to pathogenic bacteria is minimal. However, due to the homology between some components of the T3SSi and flagella, some inhibitors also affect flagella (13, 19, 20), an observation that may mitigate this advantage. Another potential benefit is that since these anti-virulence agents should minimally affect bacterial growth, they may exert low selective pressure in the environment and therefore drug resistance may develop infrequently. To our knowledge this has not been experimentally tested in an animal model of infection. On the other hand, disadvantages to be considered include that anti-T3SSi agents may not impede bacterial growth in infected immunocompromised individuals and that some infections require bactericidal agents. Nonetheless, discovering and studying reagents that inhibit the T3SSi remains attractive both for the potential therapeutic benefits and their use as important tools to elucidate the structure-functional relationships of this complex machinery.

This review focuses on advances in T3SSi-targeted therapies in the past 4 years (Tables 1–2) including small molecules, antibodies, and vaccines, whose molecular targets are known (Fig. 1B). Excellent in-depth reviews covering progress of the field until 2014–2015 and structure of molecules include (2, 21, 22). Some previously well-studied compounds are also summarized in Table 1.

SMALL MOLECULES

Many studies use HTSs to identify small molecule inhibitors of T3SSi via phenotypic readouts of T3SSi functions including inhibition of T3SSi expression in bacteria (13, 15, 23–25), secretion of effectors into the extracellular supernatant (14, 17, 25–27), or translocation of effector proteins into host cells (14, 18). A benefit of such approaches is that identified molecules are effective in the context of the bacterium. However, complications include that the inhibitors may target more than one protein, may target a host protein, or may alter T3SSi function by generally affecting bacterial cell physiology rather than a specific component of the machinery. Consequently, identification of the specific targets of many small molecule inhibitors has lagged and structure activity relationship (SAR) studies are complicated if the molecule targets several proteins.

Recently, several exciting advances have been made in both target identification and in identifying lead compounds with sufficiently low IC_{50} for *in vivo* studies. More classical

pharmacological approaches that identify compounds that bind to a protein or inhibit its biochemical activity have been fruitfully employed (16, 28–30). Increasingly, the structures of T3SS components are being exploited to elucidate the design of potential inhibitors to these proteins (31–34).

Salicylidene Acylhydrazides

Salicylidene acylhydrazides (SAHs) are the first identified and most widely studied class of synthetic small molecules that target the T3SSi across many bacterial species (13, 14). Several studies suggest that some of these molecules have multiple targets or act indirectly on the T3SSi by impacting bacterial physiology (19, 25, 35–37). Of the derivatives generated, many show promising results. Modifications to improve stability and selectivity of SAH ME0055 resulted in two new synthesized compounds, RCZ12 and RCZ20, that inhibit secretion of EHEC T3SS translocon protein, EspD, as effectively as ME0055 (Fig. 1B). Unlike the parent compound, RCZ12 and RCZ20 have no effect on bacterial growth suggesting they are more specific (38). Affinity-chromatography experiments revealed the coiled-coil domain 1 of EspD as the inhibitors' key domain-binding site (38). These compounds show dual functionality by also downregulating transcription of the locus of enterocyte effacement (LEE) that encodes the T3SS (38). Recent mechanistic analysis of another SAH, INP0341, shows that it prevents T3SS expression in *P. aeruginosa* clinical isolates without affecting growth (39).

A very recent study employed a multiple-assay approach to elucidate the mechanism of action of a group of previously identified T3SS inhibitors (40). Compound SAH INP0007 disrupts YscD puncta formation suggesting interference with needle assembly and significantly decreases flagellar motility. Whether inhibition occurs by directly binding to a common core component between the T3SSi and flagella, or by interfering with other processes that render bacteria less able to build both systems, is still unknown (40). Compound 4 (C4), a haloid-containing sulfonamidobenzamide (SAB), which was originally identified along with SAHs as inhibitors of the T3SS (13), is now postulated to have an indirect effect on T3SS transcription by inhibiting the secretion process (40).

Compounds Targeting the T3SS ATPase

Using the known structure of the EPEC EscN ATPase, a computational HTS identified compounds predicted to block the protein's active site (29). One lead compound (WEN05–03) competitively inhibits hydrolysis of ATP by EscN and reduces toxicity to infected HeLa cells (29). Another study using molecular docking and virtual screening identified a series of N-arylbenzylamines predicted to target the SctN T3SS ATPase of *C. trachomatis* (30). Two of these compounds block translocation of the T3SS effector, IncA, into cultured cells and reduce chlamydial survival in these cells (30). Hydroxyquinoline (HQ) derivatives were first described as inhibitors of T3SSi gene expression in *Y. pseudotuberculosis* and *C. trachomatis* (41). HQ INP1855 inhibits YscN ATPase activity *in vitro* as well as impairs flagellar motility providing evidence that it might target conserved ATPases found in T3SS and flagella (28). In addition, HQ INP1855 reduces *P. aeruginosa* T3SS-mediated cytotoxicity in cultured cells, blocks secretion of ExoS effector protein, as well as enhances survival and reduces bacterial burden and lung pathology of mice infected intranasally with

P. aeruginosa (28). HQ INP1750 acts similarly to HQ INP1855 and inhibits both ExoS secretion as well as flagellar motility (39). However, a direct interaction between these HQ derivatives and T3SS ATPases remains to be shown.

Compounds Targeting Needles or Needle Assembly

Phenoxyacetamide (PXA) was first discovered as an inhibitor of the T3SSi in *P. aeruginosa* and SAR analysis demonstrated strict stereoselectivity suggesting an interaction with a specific target or site (42). Isolation of several mutants in PscF resistant to PXA inhibitors provides genetic evidence that PXAs target the needle protein (34, 43). Modeling of PXA inhibitors supports the idea that these molecules intercalate within the needle and interact simultaneously with several assembled PscF subunits; however, biochemical and structural studies are needed to demonstrate a direct interaction. Importantly, injection of PXA (MBX2359) into abscesses formed by P. aeruginosa significantly reduces abscess size providing evidence that these inhibitors are efficacious in infection models in mammals (44).

Piericidins, a class of compounds derived from *Actinomycetales*, inhibits translocation of YopM into cultured cells (45). A follow-up study showed that *Yersinia* treated with Piericidin A1 has fewer needles, suggesting that it inhibits a step prior to or during needle assembly (46). The related Psc T3SS of *P. aeruginosa* and the Ysa T3SS of *Y. enterocolitica* are not inhibited, indicating its specificity but potentially limiting its usefulness without additional SAR analysis (46).

Compounds Targeting Translocon and/or Effector Secretion and Activity

Using click chemistry, the flavonoids baicalein and quercetin were found to covalently modify *S.* Typhimurium translocases and effectors, resulting in changes to stability or activity (47). The N-terminal chaperone-binding domain is proposed to be the modified site (47). These flavonoids inhibit invasion of *S.* Typhimurium into cultured cells but have no effect on effector secretion or needle assembly (47). Screening libraries for compounds that bind to *Salmonella* SipD (48) or *Shigella* IpaD tip proteins (49) identified a new class of small molecules based on the indole scaffold as potential inhibitors of the T3SSi. Malic diamide (42), a compound structurally related to PXA, significantly inhibits the secretion of YopB and YopD proteins required for translocation, without disrupting needle YscF puncta formation indicating that it targets the translocon (40).

In the past few years, several natural compounds have been identified, typically in screens for secretion (50–53), translocation into target cells (54) or by inhibiting the effects on T3SSi-mediated functions on targeted host cells (55). Potentially promising compounds are listed in Table 1, but to our knowledge, the specificity against T3SSi or protein targets have not been investigated in depth.

Anti-T3SS Compounds Tested Against Plant Pathogens

Plants are also susceptible to infection by bacteria harboring T3SSs, and there have been several recent exciting findings. Natural and synthetic compounds were screened for the ability to reduce expression of the *R. solanacearum* T3SS pilus gene *hrpY* (56). The most potent inhibitors were SAHs, which inhibit secretion of T3SS effector AvrA and limit

bacterial growth on tomato plants (56). SAHs also reduce the expression of T3SS genes of *Erwinia amylovora* and reduce disease symptoms on inoculated crab apple pistils (57). Phenolic compounds repress the expression of T3SS transcriptional regulators *hrpG* and *hrpX* of *Xanthomonas oryzae* and reduce disease symptoms on rice leaves (58). Thiazolidine-2-cyanamide compounds also reduce relative expression of *X. oryzae hrpG* and *hrpX* and disease symptoms on rice (59).

ANTIBODIES, VACCINES, AND PEPTIDES

Recent advances in targeting T3SSi using antibodies, vaccines, and polypeptides are summarized below and in Table 2.

Antibodies

A monoclonal antibody, KB001, that binds to the *P. aeruginosa* T3SS tip protein, PcrV, initially showed promise in the treatment of patients with airway-associated *P. aeruginosa* infection or colonization, but failed in phase II clinical trials for not meeting efficacy endpoints (60–62). By contrast, a bispecific antibody, MEDI3902, against *P. aeruginosa* PcrV and the Psl exopolysaccharide, is effective against a wide range of clinical isolates and is currently in phase II clinical trials for prevention of ventilator nosocomial pneumonia (63, 64).

Single-domain antibodies that consist of the N-terminal variable region of an immunoglobulin heavy chain (VHH) but not the light chain can be isolated from camelid species (65). A panel of VHH single-domain antibodies was raised against the *Shigella flexneri* IpaD tip protein (66). Four such antibodies that bound IpaD significantly inhibit hemolysis of sheep red blood cells, a measure of T3SS translocon functionality (66). Structural binding analysis revealed that these inhibitory VHHs mostly bound to the distal domain of IpaD, suggesting the importance of this region in T3SS function (66).

Vaccines

Work towards a plague vaccine has led to testing a recombinant vaccine consisting of the *Yersinia pestis* F1 protein and the T3SS tip protein LcrV, reviewed in (67). The FDA has granted Orphan Drug status for the development of this rF1V vaccine, as a prophylactic for high risk individuals (68, 69). Efforts to lessen Shiga toxin-producing *Escherichia coli* (STEC) disease burden in cattle to reduce transmission to humans are ongoing. Cohorts of cattle immunized against serotype O157 have reduced shedding of O157 but not of other STEC serotypes due to serotype specificity (70). To develop vaccines against a different prevalent serotype, anti-sera to five T3SS proteins, EspA, EspB, EspF, NleA and Tir, of STEC serotype 0103 were studied. These anti-sera block STEC adherence to HEp-2 cells (71). In efficacy studies, mice developed strong serum IgG titers against four of these five proteins, but still shed 0103 after oral administration indicating that the bacteria could still be transmitted (71).

Recent attempts to develop T3SS-targeted vaccines against *Salmonella enterica* show some success in mouse studies. A peptide vaccine that elicits a CD4 T cell response against T3SS effector protein SseI, protects mice against acute infection, a tantalizing result given that

only a single peptide elicits protection (72). Mice were immunized by different routes with *Salmonella* T3SS proteins SipD and PrgI in combination or alone; oral immunization with SipD provides the highest level of protection against lethal challenge (73). Increased protection is observed when flagellin is added to a vaccine against *Salmonella* T3SS protein SseB (74). A subunit vaccine against *Salmonella* consisting of two components, S1 (a genetic fusion of SPI-1 translocon proteins SipB and SipD) and S2 (a genetic fusion of SPI-2 proteins SseB and SseC) elicits strong IgG titers to all four proteins in mice (75). These mice are significantly protected against challenge with *S. typhimurium* and *S. enteritidis* and experience reduced cecal inflammation (75). These results warrant studies on long-term protection.

Peptides

Anti-T3SS peptides (Table 2) have been identified against *Salmonella* (76), EPEC (77), and EHEC (78) and more recently, in *Yersinia* (79). Derivatives of the natural compound phepropeptin D that contained various peptoid substitutions on the cyclic peptide backbone, significantly inhibits NF-kB signaling, secretion of the effector protein YopE, and translocation of YopM into HeLa cells by *Yersinia* (79). The peptomers do not affect *Yersinia* growth or flagellar motility indicating their potential specificity to the T3SSi. Several derivatives also inhibit secretion of the *P. aeruginosa* effector protein ExoU suggesting that they might target a conserved component of these two injectisome systems (79).

CONCLUSION AND PERSPECTIVE

Discovery of and research into inhibitors of the T3SSi is a highly active area with many candidates from different classes that are effective in blocking the function of T3SS. Although antibodies and vaccines are further along in the pipeline, many small molecule inhibitors show promise. Some molecules have a narrower spectrum of activity, while others have broader spectrums including those that target components conserved between the T3SSi and flagella. Both have benefits and disadvantages. For instance, an effective, but narrow spectrum molecule against the T3SSi of the multi-drug resistant *P. aeruginosa* could save many lives each year. By contrast, a narrow spectrum molecule effective towards *Y. pestis* would not save many lives annually unless a major outbreak occurred. Yet importantly, study of such a molecule could help elucidate structure-function relations of the T3SSi and be used as a platform to develop molecules highly effective against homologous components in other T3SSi. Resistance mutants, biochemical assays, structural modeling, and rational designs are helping to identify targets and generate more potent inhibitors. Validating their efficacy in animal systems is ongoing. Both basic science and clinical translational research from academic and pharmaceutical groups is crucial to the advancement of these molecules to combat the rising threat of antibiotic resistance.

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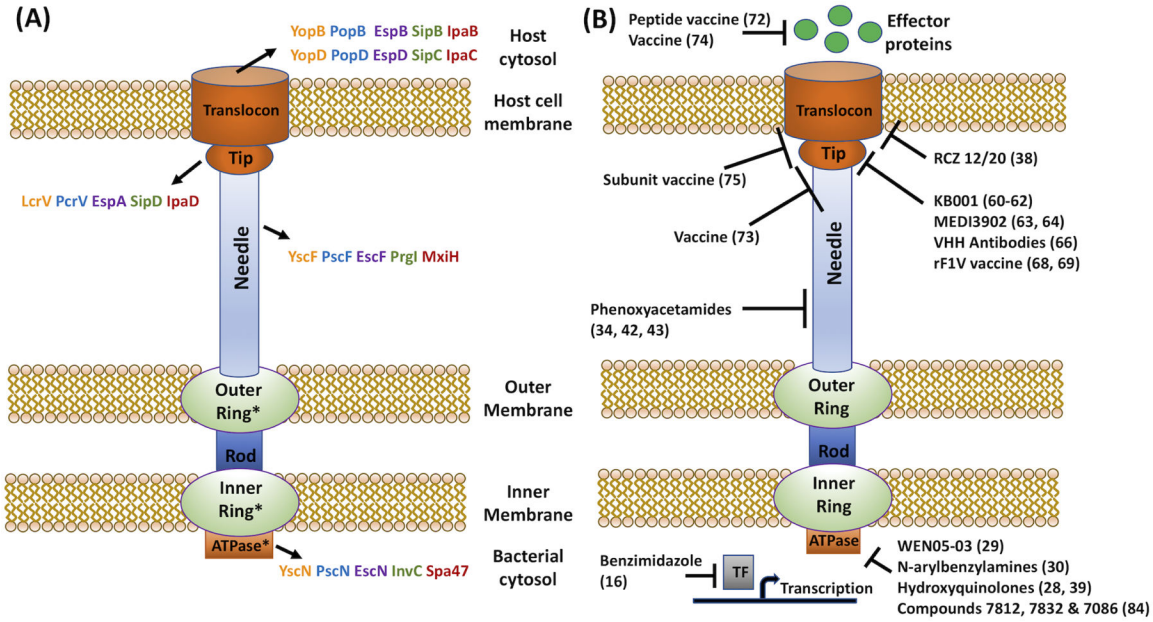

Figure 1.

(A) Structure of T3SSi. * indicate regions with conserved components between T3SSi and flagella. *Yersinia* = orange; *Pseudomonas* = blue; EPEC/EHEC = purple; *Salmonella* = green; *Shigella* = red. (B) Potential targets of compounds based on inhibition of T3SSi function, biochemical or binding studies, genetic resistance, or animal studies.

Table 1:

Possible Targets and Function of Small Molecule Inhibitors of the T3SS

Compound	Organism	Target	Inhibits bacterial growth?	Toxic to cells?	In vivo studies?	Phenotype/Readout	Refs.
SAH (C1, C2) SABC4	*Yersinia pseudotuberculosis*		No	NT	No	Inhibits T3SS transcription and Yop secretion; C2 and C4 inhibit flagellar motility	(13)
SAH (C1–C23) C1-INP0007	*Y. pseudotuberculosis*		No	No	No	Inhibits secretion and translocation	(14)
SAH C1-INP0007 SAH C11-INP0403	*Salmonella enterica*		No	No	Yes	Inhibits secretion and blocks invasion; First study to validate SAH *in vivo* using bovine intestinal ligated loops	(27)
SAH C11-INP0403 (ME0053)	*S. enterica*	Suggested indirect effect-iron chelation	No	No	No	Inhibits T3SS transcription and secretion; Upregulation of iron acquisition	(25)
SAH INP0341 SAH INP0400	*Chlamydia trachomatis*	Suggested indirect effect-iron chelation	No	No	Yes	Inhibits T3SS transcription; Upregulation of iron acquisition; Protects mice against vaginal infection when administered topically	(35, 80, 81)
SAH INP0341	*C. trachomatis*		No	No	No	Mutations isolated in HemG suggesting indirect effect on T3SS	(37)
SAH INP0400 SAHINP0402 (C15)	*Shigella flexneri*	Suggested to inhibit T3SS basal needle assembly	No	No	No	Inhibits secretion and blocks invasion; Fewer and shorter needle assembly	(17)
SAHME0052(C8, INP0010) SAHMEOO53(C11, INP0403) SAHME0054(C10, INP0401) SAH ME0055(C17; INPOO31)	EHEC	Suggested to inhibit T3SS regulators	No	No	No	Inhibits secretion	(15)
SAH ME0052(C8, INPOO10) SAH ME0055(C17, INPOO31)	*Y. pseudotuberculosis* *Escherichia coli*		No	No	No	Inhibits secretion; Pull down assays identified WrbA, FoIX and Tpx bind to SAH suggesting indirect effect on T3SS	(36)
SAH INP0404 SAH INP0405	*S. enterica*		No	No	NA	Mutations isolated in FlhAgene suggest targeting of T3SS basal body	(19)
SAH INP0341	*Pseudomonas aeruginosa*		No	No	No	Inhibits T3SS transcription and ExoS secretion	(39)

Compound	Organism	Target	Inhibits bacterial growth?	Toxic to cells?	In vivo studies?	Phenotype/Readout	Refs.
SAH RCZ12 and RCZ20	EHEC	EspD – needle pore protein	No	No	No	Inhibits EspD secretion; Fewer/shorter needle assembly	(38)
SAB Compound 4	*Y. pseudotuberculosis*		No	No	No	Inhibits secretion	(40)
SAH INP0007	*Y. pseudotuberculosis*		No	No	No	Affects YscD puncta formation	(40)
SAH INP0010	*Y. pseudotuberculosis*		No	Yes	No	Affects YscD puncta formation	(40)
Salicylideneanilide C3	*Y. pseudotuberculosis*		No	NT	No	Inhibits secretion and transcription	(13)
Salicylideneanilide	EPEC		No	No	No	Inhibits T3SS transcription and EspB secretion	(26)
Benzimidazole	*Y. pseudotuberculosis*	LcrF – T3SS master regulator	No	No	Yes	Reduces cytotoxicity in infected cells; Protective in a murine model	(16)
C15, C19, C22, C24 and C38	*Y. pseudotuberculosis* *P. aeruginosa*		No	No	No	Inhibits effector translocation	(18)
C20	*Y. pseudotuberculosis* *P. aeruginosa*	Suggested to interfere with adherence	No	No	No	Inhibits effector translocation	(18)
Compound D	*Y. pseudotuberculosis* *Yersinia pestis* *P. aeruginosa*	Suggested to target YopD – translocon	NT	Yes	No	Inhibits effector secretion	(82)
Thiazolidinones	*S. enterica* *P. aeruginosa* *Yersinia Entercolitica* *Psuedomonas syringae*	Inhibits T2SS suggesting common target with T3SS such as secretin	No	No	Yes tobacco plants	Inhibits transcription and secretion; Reduces needle complex formation; Reduces hypersensitivity response in plant leaves	(83)
Phenoxyacetamides	*P. aeruginosa*	Suggested to target PscF – needle protein	No	No	No	Isolation of *PscF* mutants resistant to phenoxyacetamide inhibitors	(34, 42, 43)
Phenoxyacetamides	*P. aeruginosa*		NT	NT	Yes	Reduces abscess size in mouse model of *P. aeruginosa* abscess formation	(44)
Piericidins	*Y. pseudotuberculosis*		No	No	No	Inhibits T3SS-dependent NF-kB activation	(45)
Piericidin A1	*Y. pseudotuberculosis*	Suggested to target YscF-needle protein	NT	NT	No	Reduces number of needles present	(46)

Compound	Organism	Target	Inhibits bacterial growth?	Toxic to cells?	In vivo studies?	Phenotype/Readout	Refs.
Library of compounds	*Salmonella spp.*	SipD – tip protein SipB – translocon protein	NT	NT	No	Surface plasmon resonance screen to find compounds that bind to SipD and SipB	(48)
Library of compounds	*Shigella spp.*	IpaD – tip protein	NT	NT	No	Surface plasmon resonance screen to find compounds that bind to IpaD	(49)
Malic diamide	*Y. pseudotuberculosis*		No	No	No	Inhibits secretion of YopB and YopD	(40)
Flavonoids	*S. enterica*	Covalent labeling ofSPI-1 substrates	No	NT	No	Inhibits bacterial invasion of host cells	(47)
Compounds 7812, 7832, 7086	*Y. pestis*	T3SS ATPase YscN	No; 7086 -Yes	No	No	Inhibits secretion	(84)
WEN05–03	EPEC	T3SS ATPase EscN	No	No	No	Inhibits ATP hydrolysis; Reduces toxicity to infected HeLa cells	(29)
N-arylbenzylamines	*C. trachomatis*	Suggested to target T3SS ATPase SctN	No	No	No	Reduces secretion and chlamydial inclusions in host cells	(30)
Hydroxyquinolines INP1750INP1767 INP1855	*C. trachomatis* *Y. pseudotuberculosis*		No	No	No	Inhibits cytotoxicity	(41)
Hydroxyquinoline INP1855	*P. aeruginosa*	Suggested to target T3SS ATPase	No	No	Yes	Reduces cytotoxicity on host cells; Reduces bacterial burden and lung pathology in infected mice; Reduces activity of homologous T3SS ATPase YscN	(28)
Hydroxyquinoline INP1750	*P. aeruginosa* *Y. pseudotuberculosis*	Suggested to target T3SS ATPase	No	No	No	Inhibits secretion and flagellar motility; Reduces activity of *Yersinia* T3SS ATPase YscN	(39)
Licoflavonol	*S. enterica*		No	NT	No	Reduces expression of chaperone *sicA* and *invF*-transcriptional regulator for SPI-1 effector proteins	(50)
Epigallocatechin gallate	EPEC/EHEC *S. enterica* *Y. pseudotuberculosis*		No	NT	No	Reduces adherence of EHEC/EPEC; Reduces *Salmonella* invasion into host cells; Reduces *Yersinia* induced cell death	(52)
Epigallocatechin gallate	*S. enterica*		No	NT	No	Reduces *Salmonella* invasion into host cells	(51)

Compound	Organism	Target	Inhibits bacterial growth?	Toxic to cells?	In vivo studies?	Phenotype/Readout	Refs.
Psidium guajava leaf extract	EPEC/EHEC *S. enterica* *Y. pseudotuberculosis*		No	NT	No	Reduces adherence of EHEC/EPEC; Reduces *Salmonella* invasion into host cells; Reduces *Yersinia* induced cell death	(53)
Sanguinarine chloride	*S. enterica*		No	Yes at higher conc.	No	Inhibits bacterial invasion of host cells	(54)
Thymol	*S. enterica*		Slightly at higher conc.	Slightly at higher conc.	Yes	Inhibits bacterial invasion of host cells; Protects mice against infection	(85)
Obovatol	*S. enterica*		No	NT	No	Reduces hemolysis of sheep red blood cells	(55)
7-hydroxycoumarin – Umbelliferone	*Ralstonia solanacearum*		Yes(86)	NT	Yes tobacco plants	Reduces expression of T3SS effector genes; Reduces disease progression on tobacco plants	(87)
SAHs	*R. solanacearum*		Minimal	NT	Yes tomato plants	Inhibits translocation; reduces bacterial growth on tomato plants	(56)
SAHs	*Erwinia amylovora*		No	NT	Yes apple plants	Reduces expression of T3SS genes; reduces disease symptoms on apple plants	(57)
Phenols	*Xanthomonas oryzae*		No	NT	Yes rice plants	Reduces expression of *hrpG* and *hrpX*-regulators of *hrp* genes which regulate T3SS effector expression; Reduces disease symptoms on rice plants	(58)
Thiazolidin-2-cyanamide derivatives	*X. oryzae*		No	NT	Yes rice plants	Reduces expression of *hrpG* and *hrpX*-regulators of *hrp* genes which regulate T3SS effector expression; Reduces disease symptoms on rice plants	(59)

NT = Not Tested; EHEC = Enterohemorrhagic *Escherichia coli*; EPEC = Enteropathogenic *Escherichia coli*; T3SS = Type III secretion system

Table 2:

Antibodies, vaccines, and peptomers against T3SS components

Class	Organism	Target	Phenotype/Readout	Therapeutic Potential	Refs.
Antibody – KB001	*Pseudomonas aeruginosa*	PcrV – tip	Protects host cells against T3SS mediated toxicity and protects mice against acute pulmonary infection (reviewed in (88))	Did not meet efficacy endpoints in phase II clinical trials	(60–62)
Bispecific Antibody – MEDI3902	*P. aeruginosa*	PcrV – tip Psl – exopolysaccharide	*In vitro* cytotoxicity protection and *in vivo* protection of acute pneumonia model in mice	Currently in phase II clinical trials	(63, 64)
Single-VH Domain Antibodies	*Shigella flexneri*	IpaD – tip	Reduces hemolysis of sheep red blood cells		(66)
rF1V Vaccine	*Yersinia pestis*	LcrV – tip F1 protein	Enhances survival of cynomolgus macaques infected with lethal aerosol challenge of *Y. pestis*	Orphan Drug designation by FDA	(69)
Rabbit polyclonal anti-sera	STEC	$STEC_{O103}$ T3SS proteins	Blocks adherence of STEC to host cells; Immunized mice not protected against fecal shedding		(71)
Peptide vaccine	*Salmonella enterica*	Ssel – effector	Protects mice against acute infection		(72)
Vaccine	*S. enterica*	PrgI – needle SipD - tip	Protects mice against infection		(73)
Vaccine	*S. enterica*	SseB – effector Flagellin	Protects mice against infection		(74)
Subunit vaccine	*S. enterica*	S1: Fusion of SipD and SipB-tip and translocon S2: Fusion of SseB and SseC-tip and translocon	Protects mice against lethal challenge		(75)
Polypeptide	*S. enterica* *S. flexneri*	SipB – translocon IpaB – translocon	Inhibits bacterial invasion into host cells	Polypeptide too large for therapeutic potential	(76)
Peptides	EPEC	EspA-tip	Inhibits EspA polymerization thereby preventing A/E lesions		(77)
Peptides	EHEC *Citrobacter rodentium*	EspA-tip	Protects mice against colon damage after *C. rodentium* challenge		(78)
Peptomers – phepropeptin D derivatives	*Yersinia pseudotuberculosis* *P. aeruginosa*		Inhibits secretion of T3SS proteins; Inhibits *Yersinia* YopM effector translocation and reduces cell rounding		(79)

STEC = Shiga-toxin producing *Escherichia coli;* EPEC = Enteropathogenic *Escherichia col*