2016

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Microbiology Spectrum
American Society for Microbiology Press

Innovative Solutions to Sticky Situations: Antiadhesive Strategies for Treating Bacterial Infections

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ABSTRACT Bacterial adherence to host tissue is an essential process in pathogenesis, necessary for invasion and colonization and often required for the efficient delivery of toxins and other bacterial effectors. As existing treatment options for common bacterial infections dwindle, we find ourselves rapidly approaching a tipping point in our confrontation with antibiotic-resistant strains and in desperate need of new treatment options. Bacterial strains defective in adherence are typically avirulent and unable to cause infection in animal models. The importance of this initial binding event in the pathogenic cascade highlights its potential as a novel therapeutic target. This article seeks to highlight a variety of strategies being employed to treat and prevent infection by targeting the mechanisms of bacterial adhesion. Advancements in this area include the development of novel antivirulence therapies using small molecules, vaccines, and peptides to target a variety of bacterial infections. These therapies target bacterial adhesion through a number of mechanisms, including inhibition of pathogen receptor biogenesis, competition-based strategies with receptor and adhesin analogs, and the inhibition of binding through neutralizing antibodies. While this article is not an exhaustive description of every advancement in the field, we hope it will highlight several promising examples of the therapeutic potential of antiadhesive strategies.

INTRODUCTION

The discovery of penicillin in 1928 and its subsequent introduction as a therapeutic in the 1940s sparked the antibiotic era, ushering in effective treatment options for many common bacterial infections (1). Following the end of World War II, several pharmaceutical companies including Bayer, Merck, and Pfizer became household names through the discovery and clinical success of a number of additional antibiotics, which were identified by screening soil samples for antimicrobial activity (1). Compounds identified during this screening became the founding members of many now-ubiquitous groups of antibiotics, including the tetracycline, rifamycin, quinolone, and aminoglycoside families. In the early 1970s, declining rates of novel antibiotic discovery from microbial sources shifted the onus of antimicrobial development to synthetic chemists, who were tasked with designing and screening new compounds based on known principles of antibiotic design. These synthetic chemists were faced with many practical challenges,

Received: 10 July 2015, **Accepted:** 16 September 2015, **Published:** 18 March 2016

Editors: Indira T. Kudva, National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, IA; and Bryan H. Bellaire, Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA

Citation: Cusumano ZT, Klein RD, Hultgren SJ. 2016. Innovative solutions to sticky situations: antiadhesive strategies for treating bacterial infections. *Microbiol Spectrum* 4(2):VMBF-0023-2015. doi:10.1128/microbiolspec.VMBF-0023-2015.

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including poor penetration into bacterial cells, bacterial enzymes, and/or efflux pumps that degrade or expel the compounds, respectively, innate resistance mechanisms, and the requirement of high concentrations of some compounds that result in toxic side effects (2, 3).

As the difficulty of novel antimicrobial discovery increased and the incidence of vaccine-preventable disease continued to fall, the apparent ease and speed with which most infections were cured decreased the incentives driving antimicrobial development. As a result, for-profit drug companies shifted their foci away from the development of antimicrobials and toward drugs designed to treat chronic, noncommunicable diseases. Indeed, the increase in patient life expectancy, brought on in part by the decrease in deaths from infectious diseases, coupled with the rising prevalence of metabolic diseases, dramatically increased the population of patients requiring treatment for cancer, diabetes, and hyperlipidemia. In contrast to the 5- to 7-day treatment course for most antibiotic infections, chronic diseases require constant medication, providing pharmaceutical companies with a much higher return on investment. Investigation into these more lucrative therapeutic areas largely halted the research and development of new antimicrobials by for-profit companies (2, 3). Concurrently, resistance to existing antimicrobials has continued to rise as a result of their sustained misuse in both agriculture and clinical settings (4, 5), propelling us into a postantibiotic era defined by dwindling treatment options for many common infections. Recently, the CDC has recognized several pathogens as "urgent" or "serious" threats, including *Clostridium difficile*, carbapenem-resistant *Enterobacteriaceae*, multidrug-resistant *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus*, and others (6). The prevalence of these pathogens and their associated morbidity and mortality has highlighted the need for the identification of new canonical antibiotics and innovative therapeutic strategies to fight what were once considered easily curable bacterial infections.

Currently, common antibiotics function by inhibiting or disrupting important bacterial cellular processes, including cell wall synthesis, RNA transcription, DNA replication, and protein synthesis needed for cell viability. While this has resulted in the development of effective broad-spectrum antibiotics, it has also generated a strong selective pressure that fosters development of bacterial resistance. To circumvent this problem, researchers have begun targeting specific virulence mechanisms critical to the ability of specific bacteria to cause disease (7–9). These "antivirulence" therapeutics are designed to neutralize pathogenesis and promote efficient clearance by the host immune system without affecting overall bacterial viability. It is believed that targeting these nonessential processes will weaken the selective pressure currently driving the development of resistance, increasing the effective therapeutic lifetime of these drugs (10). Additionally, the specific targeting of pathogenic bacteria eliminates the nonspecific killing of the beneficial human microbiota, which occurs during broad-spectrum antibiotic therapy (11–14). Recent research into the role of the human microbiota in human health and disease has led to our understanding of the microbiota as a bacterial organ within the host that trains the immune system and provides essential metabolic functions for the host. Perturbation of this system has been linked to significant decreases in overall health and a plethora of numerous disease complications (15). Thus, regular insults to the human microbiota through antibiotic treatment can result in a detrimental state of dysbiosis (16). To overcome resistance and protect the commensal microbiota, researchers are actively pursuing antibiotic-sparing therapeutic strategies to target and disrupt pathways related to virulence but not to general bacterial viability (9, 10, 17).

Antivirulence Therapies

Bacterial virulence factors are defined by their role in pathogen replication and formation of the disease state within the host environment. These bacterial determinants can provide a fitness advantage by mediating a variety of processes, including evasion of the host immune system, extraction of required nutrients from the host, or colonization of a particular niche. Toxins, cytolysins, bacterial secretion systems, and proteases are a few common examples of such factors that have been the subject of intensive investigation. To date, many successful examples of antivirulence therapeutic studies have focused on abrogating the effectiveness of toxins, be it through direct inhibition of activity, delivery, or attachment to the host cell (18–20). One such study utilized a glycomimetic approach to inhibit Shiga toxin's recognition of the host receptor, globotriaosylceramide, which has proven to be effective *in vitro* and in animal models of enterohemorrhagic *Escherichia coli* infections (21, 22). Similarly, the monoclonal antibody raxibacumab targets the protective antigen component of anthrax toxin (23) and was approved by the FDA in 2012 for protection against and treatment of inhaled anthrax (23–25). Despite this great promise, however, only a handful of antivirulence therapeutics have made it to human clinical trials to date.

In addition to secreted factors, investigators have also targeted virulence factors on the cellular surface. Two such targets, the polysaccharide capsule and flagellar appendages of *E. coli*, have been studied extensively for their role in phagocytosis of bacteria by host immune cells and bacterial chemotaxis and motility, respectively (26, 27). Structures mediating bacterial adhesion have also proven to be promising antivirulence targets, because nearly all bacterial pathogens utilize specific adhesion modalities to colonize biotic and abiotic surfaces (28–32). This adhesion is required to resist the natural clearance mechanisms of the host, including high liquid flow rates on mucosal surfaces in the gastrointestinal, upper-respiratory, and genito-urinary tracts. Thus, the importance of adhesion in establishing an infection makes it one of the most attractive targets for new therapeutics.

BACTERIAL ADHESIVE STRATEGIES

Pili

Pili (or fimbriae) are long proteinaceous filaments that are utilized by both Gram-negative and Gram-positive bacteria to adhere to host surfaces while maintaining a separation between the cell membranes, preventing the electrostatic repulsion that occurs as a result of the net negative charge found on the surface of both the bacteria and host. Pili are generally composed of hundreds or thousands of repeating protein subunits interacting covalently (in the case of Gram-positive bacteria) or noncovalently (in the case of Gram-negative bacteria) to form the shaft of the pilus. For both Gram-negative and Gram-positive bacteria to interact with the host, additional protein subunits are often incorporated into the pilus, including tip adhesins that mediate stereochemically specific interactions with a host receptor (33). Like lectins, adhesin domains frequently recognize oligosaccharides found on glycoproteins or glycolipids (34). The specificity of these interactions often dictates a pathogen's tropism for a particular host tissue. In addition to cell surface carbohydrate receptors, some pili also interact with proteinaceous components of the basement membrane or extracellular matrix and may bind the collagen, fibronectin, and fibrinogen found extensively throughout the host (30, 35). Despite a common overall function of Gram-negative and Gram-positive pili of mediating attachment, the structure and biosynthetic machinery required to generate these adhesive structures vary drastically (28) (Fig. 1).

Chaperone usher pathway (CUP) pili

Members of the CUP family of pili have been extensively characterized in Gram-negative bacteria. CUP pili are a diverse set of homologous appendages distributed throughout the *Enterobacter* genus. A recent analysis identified 458 CUP pili operons, which represent 38 distinct CUP pilus types based on usher phylogeny in *Escherichia* alone (36, 37). CUP pili tipped with specific adhesins enable *E. coli* to bind to distinct ligands on host cells with stereochemical specificity. CUP pilus biogenesis is defined by the utilization of the eponymous chaperone and usher, which function to coordinate and catalyze pilus assembly. CUP chaperones are localized to the bacterial periplasm and consist of two immunoglobulin (Ig) domains that are required for the folding and stability of the secreted pilin structural subunits. Each structural subunit is composed of an incomplete immunoglobulin fold lacking the C-terminal beta strand, which results in the presence of a hydrophobic grove with, in the case of the well-studied Pap (P) pilus system, five defined hydrophobic pockets, termed P1 to P5. Chaperone-assisted folding of pilin domains occurs by a reaction termed donor strand complementation. During this process, a series of conserved exposed hydrophobic residues on the cognate chaperone's G1 strand are buried in the hydrophobic pockets comprising the groove of the pilus subunit, thus forming a complex in which the subunit's Ig fold is completed (38, 39). Incorporation of the chaperone's G1 β-strand occurs in a noncanonical parallel fashion, generating a stable yet high-energy intermediate.

Chaperone-subunit complexes are next targeted to the outer membrane, where they interact with the membrane-localized usher, which both catalyzes pilus assembly and acts as a gated pore (Fig. 1). Ushers contain five functional domains: a 24-stranded transmembrane β-barrel translocation domain, a β-sandwich plug domain (PD) that resides in the pore of the TD in the apo-usher, an N-terminal periplasmic domain (NTD), and two C-terminal periplasmic domains (CTD1 and 2) (40–42). These domains function as components of a molecular machine that catalyzes pilus biogenesis and secretes pili across the outer membrane. The crystal structures of both an usher-chaperone-adhesin ternary complex in the well-studied type 1 pilus system (FimCDH) and a fimbrial tip (FimFGH) in complex with the chaperone and usher have been solved (41, 43). Binding of the chaperone-adhesin complex to the usher results in translocation of the PD into the periplasmic space (40, 41) and a conformational change in the translocation domain from the apo, kidney-shaped

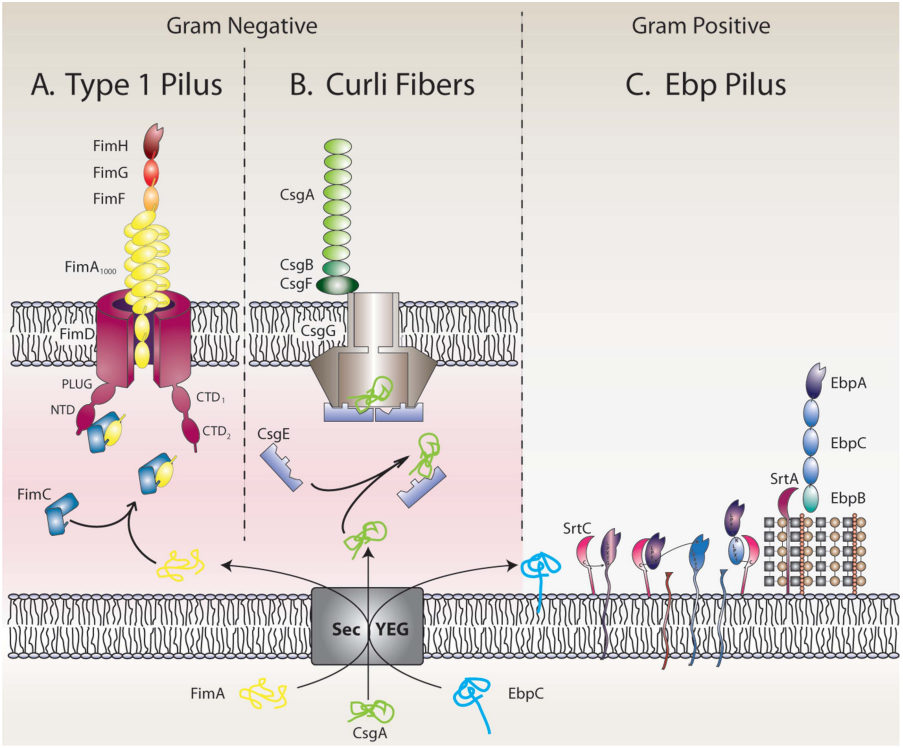

FIGURE 1 Comparison of structure and assembly mechanism of common extracellular adhesive organelles. **(A)** Following translocation through the SecYEG apparatus, the FimA structural subunits are bound by the FimC chaperone via the donor strand complementation reaction before delivery to the FimD usher, which catalyzes a donor strand exchange reaction that links subunits of the growing pilus. **(B)** In the periplasmic space, soluble CsgA binds the CsgE chaperone, which delivers it to the CsgG pore for secretion to the outer membrane. From there, its folding and polymerization is nucleated by CsgB, which is anchored to the outer membrane by the CsgF assembly factor. **(C)** Ebp pilus subunits integrate themselves into the membrane, where the dedicated pilus assembly sortase, SrtC, cleaves the sorting sequence and facilitates the nucleophilic attack by a new incoming subunit. The fully assembled pilus is then integrated into the membrane by the housekeeping sortase, SrtA.

conformation (52 x 28 Å) to a circular form (44 x 36 Å). This conformational change likely facilitates the extrusion of folded pilins (~20 to 25 Å in diameter) across the outer membrane. After translocation into the periplasmic space, the PD mediates a high-affinity interaction with the NTD of the usher (41, 44). Thus, the PD gates the translocation domain such that, in the absence of pili, the PD prevents large molecules from flowing freely across the outer membrane (45). The PD, NTD, CTD1, and CTD2 work together in the assembly function of this molecular machine. Mutations in either NTD or CTDs or deletions of the PD completely inhibit pilus assembly, implicating their direct role in catalysis of pilus assembly (44, 46).

Polymerization of pilus subunits occurs via a process known as donor strand exchange (DSE) and is dependent upon a hydrophobic N-terminal extension encoded by all pilus subunits, excluding the adhesin. (45, 47–49). Pilus DSE occurs at the usher when the chaperone is displaced, and an incoming subunit's N-terminal extension zips into the previously chaperone-bound groove of a nascently incorporated subunit at the growing terminus of the pilus (Fig. 2B and C). It is believed that interaction of the donated N-terminal extension with the vacant pocket of the acceptor pilus subunit results in initiation of a "zip-in-zip-out mechanism," displacing the chaperone's G1 β-strand and facilitating the final folding of the pilus subunit. This process is repeated for each round of subunit incorporation into the fiber such that every subunit in the pilus completes the Ig fold of its neighbor. In contrast to the chaperone's donated β-strand, which interacts in a parallel fashion, the incoming N-terminal extension binds in the canonical antiparallel fashion (Fig. 2B and C). This results in a

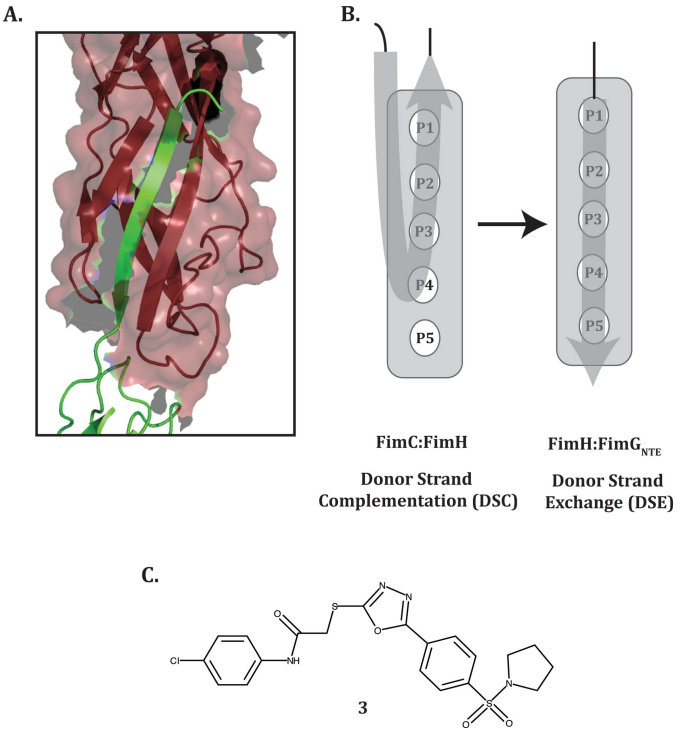

FIGURE 2 Inhibitors of the donor strand exchange reaction between pilus subunits are able to abrogate pilus biogenesis. **(A)** Crystal structure of the FimG adaptor's donor strand exchange reaction with the pilin domain of the FimH adhesin (PDB ID code 3JWN). FimG donates its hydrophobic N-terminal beta strand to FimH, which is shown residing in the P5 pocket. **(B)** Schematic of the donor strand complementation and donor strand exchange pathways. The donor strand complementation reaction between the chaperone G1 strand and the bound pilin results in a noncanonical parallel fashion (left panel), while the zip-in, zip-out process underlying the DSE reactions results in the formation of an antiparallel, low-energy interaction (adapted from reference 240). **(C)** Chemical structure of compound 3, first identified from an *in silico* docking assay before further refinement in *in vitro* DSE assays.

folded pilin domain in a much lower energy state than its chaperone-bound form. It is believed that the transition from the chaperone-bound high-energy state to the NTE-bound low-energy state that occurs during DSE provides the energy necessary to drive pilus formation in the periplasmic space, which lacks ATP and is not coupled to the proton motive force (50).

Type 1 and pap pili: roles in urinary tract infections (UTIs)

CUP pili have been identified as key virulence determinants in murine UTIs, making them exciting targets for novel therapeutics. UTIs affect more than 150 million people annually and are a significant cause of morbidity in women throughout their lifespan, (51, 52). UTI is generally divided into two major diseases, demarcated by their location within the urinary tract. Infection and colonization of the bladder in healthy women is commonly referred to as uncomplicated cystitis. Upon introduction of bacteria into the bladder, bacteria can ascend the ureters and colonize the kidneys, causing pyelonephritis. The clinical sequelae of pyelonephritis are particularly concerning, because an uncontrolled bacterial infection in the renal pelvis and calyces can spread to the bloodstream, leading to sepsis and death.

Uropathogenic *E. coli* (UPEC) is the most common causative agent of UTI, responsible for 80 to 90% of all infections (53–55). UPEC tropism for the murine bladder is largely mediated via type 1 (fim) pili. The type 1 pilus adhesin, FimH, binds mannosylated uroplakins on the bladder surface and β1-3 integrin receptors throughout the bladder tissue. The rod of the type 1 pilus is composed of ~1,000 FimA protein subunits, which are wound in a helical manner to create a force-sensitive cylindrical shaft (56). At the distal tip of the rod is a flexible fibrillum composed of two adaptor proteins, FimF and FimG, and the two-domain tip adhesin FimH (57). It is the lectin domain ($FimH_L$) of this adhesin that mediates interaction with host cell receptors and facilitates invasion of the bacteria into the uroepithelial cells, also called superficial facet cells (58). The pilin domain ($FimH_P$) interacts with the FimG adaptor. Once internalized, a single bacterium can rapidly replicate in the host cytoplasm to form a biofilm-like intracellular bacterial community (IBC) (58–60). Once these communities reach maturation, bacteria within the IBC disperse and flux out, becoming filamentous. These filamentous bacteria can then go on to adhere to and invade neighboring superficial facet cells, reinitiating IBC formation and the pathogenic cycle.

Following this acute pathogenic cycle, the outcome of UPEC bladder infection in naïve mice often resolves, leading to sterilization of the urine within days of inoculation. However, fairly frequently, this acute infection results in chronic cystitis, which is characterized by persistent high titer bacteriuria accompanied by chronic bladder inflammation. Clinical evidence of chronic inflammation in women suffering from recurrent UTIs (61, 62), as well as the observation of IBCs and bacterial filaments in women diagnosed with acute UTIs (63), supports the validity of the IBC pathogenic cycle and the ability of the mouse model to recapitulate human disease.

In contrast to colonization of the bladder, adherence to and infection of the kidneys is believed to occur

primarily via interactions of P pili with Galα-4Gal-containing glycolipid receptors, which are expressed throughout the kidneys and ureters of mammals (64). Like the type 1 pilus, P pili are comprised of a rod generated from repeating major subunits (PapA) and a distal fibrillum tip containing minor pilins (PapK, PapE, and PapF) and the adhesin PapG (65, 66). To date, three alleles of PapG have been discovered, each mediating attachment to a slightly different host receptor and consequently determining host tropism (67–69). Human kidneys, for example, abundantly express the ligands for PapG-II, globoside, and as a result, human pyelonephritis usually involves colonization of UPEC that expresses PapG-II alleles. Conversely, PapG-III binds strongly to Forssman glycolipid, which is present in dog but not in human kidneys, and most cases of pyelonephritis in dogs involve UPEC encoding the PapG-III allele (67–69). Unfortunately, the lack of these receptors in small mammals, specifically mice, has limited the ability to dissect the molecular details of pathogenesis with regard to pyelonephritis. Nevertheless, the unique role of these pili in mediating tissue-specific tropism makes them excellent targets for preventing infection throughout the urinary tract.

Gram-positive pili

While pili expressed by Gram-negative bacteria have been extensively studied over the last several decades, the identification and characterization of pili from Gram-positive bacteria has occurred relatively recently. Although pili were observed in *Corynebacterium renale* as early as 1968 (70), the mechanism of biogenesis remained unknown until a decade ago, when work with *Corynebacterium diphtheriae* revealed the unique function of a pilus-specific sortase on the highly conserved pilus domain structure (71).

In Gram-positive bacteria, each pilus subunit domain contains a highly conserved sortase recognition motif (LPXTG) followed by a hydrophobic transmembrane domain and a positively charged C-terminal tail (72, 73). Pilus assembly is initiated by the Sec-dependent secretion of pilin subunits, which become anchored to the cytoplasmic membrane via their C-terminal hydrophobic membrane-spanning region (74). Subsequently, following insertion in the membrane, the pilus-dedicated sortase recognizes and cleaves between the threonine and glycine residues of the LPXTG motif to produce an acyl-enzyme intermediate (75, 76). This intermediate is resolved by nucleophilic attack from an amino group on a specific lysine side chain from an incoming pilin subunit, resulting in the covalent attachment of the two pilin subunits (71, 77). The lysine responsible for the nucleophilic attack is located within a pilin motif whose sequence varies between pilus subtypes (72, 78). Repetition of this process results in growth of the pilus fiber from the base as sortase cleavage of each subsequent pilus subunit is resolved by nucleophilic attack by a newly incorporating subunit. Pilus assembly is terminated by a housekeeping sortase enzyme encoded outside the pilus operon, which covalently attaches the pilus to the cell wall through a final transpeptidation reaction (71) (Fig. 1). Similar to Gram-negative bacteria, minor subunits can be commonly found at the distal end of Gram-positive pili, including adhesins that mediate interactions with host receptors (79). Since their identification nearly 50 years ago, pili have been identified and implicated in diseases for several Gram-positive pathogens including *Streptococcus agalactiae* (80), *Streptococcus pneumonia* (81), and *Enterococcus faecalis* (82, 83).

Role of Ebp pilus in *Enterococcus* catheter-associated UTI (CAUTI)

The endocarditis- and biofilm-associated (Ebp) pilus is encoded by several Gram-positive bacteria including *E. faecalis* (84). *E. faecalis* is a leading cause of CAUTI, because its ability to adhere to both host and abiotic surfaces as well as its resistance to multiple antibiotics makes it difficult to prevent and treat (85, 86). A common feature of *E. faecalis* infections is their dependence on an abiotic surface, such as a catheter, to cause an infection (87). This reliance can be recapitulated in a mouse model of CAUTI using a small piece of silicone tubing to mimic catheterization in humans (88). Consistent with clinical findings, *E. faecalis* is rapidly cleared from the mouse bladder in the absence of a catheter.

Establishment of the mouse CAUTI model has allowed investigation of the molecular mechanisms of *E. faecalis* pathogenesis, identifying the unique interplay between the host and pathogen. Catheterization in mice stimulates a robust inflammatory response, increasing the levels of inflammatory cytokines while causing edema and the release of fibrinogen, a glycoprotein shown to adhere to implanted catheters (82, 89). Within 24 hours, the surface of the indwelling catheter is completely coated with fibrinogen (82). Many of these immunological findings have been demonstrated in humans and verified by a number of clinical studies, giving further credence to the robustness of the murine CAUTI model (90–92). Accumulation of fibrinogen on the urinary catheter provides a surface for the attachment of *E. faecalis*, which is mediated by the Ebp pilus and involves direct recognition

of fibrinogen (82). Deletion of the pilus operon eliminates the ability of the bacteria to adhere to the catheter *in vivo* and abolishes the infection, demonstrating the essential role for the pilus in mediating attachment to the catheter and establishing disease (82, 83).

The Ebp pilus is composed of three proteins, EbpA, EbpB, and EbpC, and is encoded in the enterococcal genome as a single operon along with the pilus-dependent sortase, SrtC (Fig. 1) (93). The shaft of the pilus is comprised of a polymer of EbpC subunits, with EbpA localized at the distal tip of the pilus and EbpB at the base (93) (Fig. 1). Deletion of *ebpA* disrupted bacterial binding to fibrinogen *in vitro* and completely attenuated virulence *in vivo* (82, 83). The N-terminal region of EbpA, which contains a Von Willebrand factor A domain with a conserved metal-ion-dependent adhesion site (MIDAS) motif, is required for recognition of fibrinogen (82, 83). MIDAS motifs are commonly found in proteins responsible for mediating interactions with extracellular matrix proteins (94). Mutation of the MIDAS motif in *ebpA* eliminates binding to fibrinogen *in vitro* and phenocopies an *ebpA* mutant *in vivo* (82, 83). Together, this work has elucidated the molecular recognition of fibrinogen by EbpA, identifying it as a putative target for prevention and treatment of CAUTI.

Biofilm Formation

Biofilms are loosely defined as surface-associated microbial communities and have been shown to play a central role in bacterial persistence in both commensal environmental and pathogenic colonization of the host niches (95, 96). Biofilm formation is generally triggered by an environmental cue that initiates a change in the physiological state of the bacteria, drastically altering the biological properties of the bacteria compared to a planktonic state (97, 98). The expression of pili or nonpilus adhesins is considered central to this transition, because they allow bacteria to interact with cellular or abiotic surfaces and other bacteria during formation of the extracellular matrix, which often consists of both proteinaceous and polysaccharide components (78, 99, 100). Disruption of this adherence through genetic deletion of specific pili or adhesins completely abolishes biofilm formation in many bacterial systems (101–104).

Upon establishment, bacteria embedded within a biofilm are able to survive a number of environmental stresses, contributing to bacterial pathogenesis and disease in a variety of chronic infections (105). Encapsulation within a biofilm decreases bacterial susceptibility to changes in environmental pH and osmolarity while conferring resistance to phagocytosis, desiccation, and UV light (106). In addition, bacteria within biofilms are commonly recalcitrant to antibiotic treatment due to a number of mechanisms, including a decrease in antibiotic penetration, expression of antibiotic-modifying enzymes, and the formation of persister cells whose metabolic dormancy promotes the resistance of colonization (107, 108). Given the high association of biofilms with indwelling medical devices, the increased use of these devices has resulted in a concurrent increase in the incidence of chronic, antibiotic-resistant infections.

Curli

Curli were first described in *Salmonella* in 1989 and have been extensively studied in both *Salmonella* and *E. coli* (109). Curli fibers mediate the formation of bacterial biofilms and have also been to shown to interact with extracellular DNA as part of the biofilm. Although this interaction is not essential for biofilm formation (110), it has been shown to increase the rate of biofilm formation (111).

The biochemical and biophysical properties of curli fibers have long been known to mirror those of pathologic amyloids (112). Indeed, structural characterization has revealed that, like known amyloids, curli fibers are 4 to 12 nm wide, highly resistant to denaturation, possess a cross β-sheet structure, and bind to amyloid-specific dyes such as Congo red and thioflavin T (113). A variety of pathogenic, eukaryotic amyloids have been implicated in several neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases, making prevention of amyloid formation therapeutically relevant. Identification of curli fibers as functional amyloids has opened up an interesting avenue of research focused on understanding the molecular mechanisms of curli assembly and the processes by which bacteria regulate the spatio-temporal formation of curli fibers (114–116). Ultimately, this approach may aid in the identification of novel therapeutics to target bacterial production of curli-associated biofilms while elucidating new treatment options for common neurodegenerative diseases. Further, Rapsinski et al. reported that extracellular DNA is bound tightly by bacterial amyloid fibrils during biofilm formation and that amyloid/DNA composites are powerful immune stimulators when injected into mice, leading to autoimmunity (111).

Curli assembly in bacteria is directed by a unique, highly regulated process (114–116) (Fig. 1). The extracellular curli fibers consist primarily of a major component, CsgA, and a minor component, CsgB (112).

Formation of curli fibers requires the periplasmic assembly factor CsgE and outer membrane assembly factor CsgF, which both associate with the outer membrane channel protein CsgG (117, 118). Nine CsgG subunits form a 36-stranded β-barrel that traverses the lipid bilayer, forming a 0.9-nM channel through which CsgA, CsgB, and CsgF are secreted as disordered monomers (119, 120). Once secreted, CsgF associates with CsgG on the outside of the cell and anchors CsgB to the pore and/or outer membrane (117). CsgB in turn anchors curli fibers to the cell surface and nucleates CsgA polymerization (121–123). Deletion of CsgB or CsgF results in attenuation of curli formation and the release of CsgA monomers into the surrounding milieu (117, 123). CsgE is believed to function as a pore gating factor and curli-specific chaperone, sequestering unfolded CsgA subunits in the periplasm and facilitating their interactions with CsgG subunits within the pore (118, 120). Deletion of *csgE* attenuates curli formation and results in the promiscuous transport of proteins and small molecules through the CsgG pore (118).

Role of curli in biofilm formation and UPEC pathogenesis

Curli have been implicated in *E. coli* and *Salmonella* colonization of the gastrointestinal tract (124, 125) and, in the case of UPEC, promote infection. A *csgA* mutant or *csgB*/*csgG* double mutant are both attenuated during acute infection in a murine model of cystitis, suggesting that curli fibers contribute to UTI pathogenesis (126). This defect in virulence could be partially explained by the binding of curli fibers with the human antimicrobial peptide LL-37 and the murine ortholog, cathelicidin-related antimicrobial peptide (CRAMP) (127). This interaction is believed to sequester these peptides and attenuate their antimicrobial activity. Curli have also been implicated in the binding of several additional host proteins including the extracellular matrix protein fibronectin (128, 129).

Nonpili adhesins

In addition to pili and curli, bacteria have evolved a number of additional surface-associated proteins to interact with host cell receptors and aid in adhesion and invasion. The majority of these adhesins are anchored to the membrane through a transmembrane region or, in the case of Gram-positive bacteria, through attachment to the cell wall via the activity of a housekeeping sortase. Some of these adhesins recognize the cell adhesion molecules, which mediate specific interactions with other cells and with the extracellular matrix (130–132). One of the best-characterized nonpilus bacterial adhesins is the invasin protein from the Gram-negative pathogen *Yersinia enterocolitica*, which mediates high-affinity binding to a subset of β1-integrins, resulting in bacterial invasion (133, 134). Internalin, an adhesin from the Gram-positive pathogen *Listeria monocytogenes*, functions in a similar manner through the binding of E-cadherin (135).

In addition to recognition of cell adhesion molecules, many nonpilus adhesins contain lectin domains and function in a manner similar to the adhesins incorporated into pili: through the recognition of specific sugar moieties. Two well-characterized soluble adhesins from *P. aeruginosa* are LecA (PA-IL) and LecB (PA-IIL), which have been determined to make significant contributions to both biofilm formation and *Pseudomonas* pathogenesis (102, 103, 136).

Role of LecA and LecB adhesins in *P. aeruginosa* pathogenesis

P. aeruginosa is an opportunistic pathogen often associated with hospital-acquired infections and is the most common bacteria found in the sputum of patients with cystic fibrosis (CF) (137, 138). The presence of *P. aeruginosa* in the lower respiratory tract of CF patients is associated with poor lung function and a decreased quality of life and is the leading cause of mortality among CF patients (139). Following colonization of the airway, *P. aeruginosa* is believed to encapsulate itself in a biofilm, thus promoting its persistence by increasing resistance to antibiotic treatment and aiding in the ability to adapt to the harsh host environment (140, 141). Biofilm formation and the pathogenesis of *P. aeruginosa* have been shown to involve several virulence factors, including the type III secretion system and several adhesins (142). Investigation into the adhesive properties of *P. aeruginosa* revealed a significant role of the two soluble lectin domains, LecA and LecB, which were both found to contribute to the attachment of *P. aeruginosa* to the human lung epithelial cell line A549 (136, 143). LecA and LecB are tetrameric adhesins with four identical binding sites (144, 145). Although originally isolated from the cytoplasm of *P. aeruginosa*, these lectins have since been shown to accumulate on the outer membrane in high quantities (103, 146). Subsequent studies have determined that LecA has specificity for D-galactose and binds α-galactosyl residues found in the glycosphingolipids of the lung epithelial membranes (147, 148). Conversely, LecB has been demonstrated to recognize L-fucose and its derivatives and has high affinity for Lewis-a oligosaccharides (149). In addition

to their role in bacterial adhesion to lung epithelial cells, both LecA and LecB have been shown to contribute to *in vitro* biofilm formation, likely by mediating contact with biotic and abiotic surfaces as well as initiating interactions with other bacterial cells (102, 103, 136).

In vivo analysis of *lecA* and *lecB* mutants has demonstrated a significantly decreased bacterial lung burden 16 hours following inoculation compared to wild-type bacteria. LecA and LecB were also found to mediate alveolar capillary barrier injury, facilitating the dissemination of *P. aeruginosa* into the bloodstream (136). This phenotype may be due in part to the cytotoxic effect seen by purified LecA in primary epithelial cells in culture (143). These varied and significant contributions of LecA and LecB to *P. aeruginosa* pathogenesis make them exciting therapeutic targets. Indeed, inhibition of LecA and LecB could have a 2-fold effect on *P. aeruginosa* pathogenesis, preventing adherence to epithelial cells to decrease invasion while disrupting preformed biofilms to render the pathogens more susceptible to antimicrobial therapy.

SMALL-MOLECULE ANTIVIRULENCE THERAPEUTICS

Inhibition of Pathogen Receptor Biogenesis

The assembly and anchoring of pili to the cell surface of pathogens requires the coordinated expression and interaction of several proteins. An understanding of the complexity of this assembly process has uncovered a plethora of targets for the disruption of adhesive strategies utilized by both Gram-negative and Gram-positive pathogens summarized in this section and Table 1.

TABLE 1 Antiadhesive small molecules

Compound number	Synonyms	References
1	MP048, 2c	150
2	ec240	153
3	AL1	155
4	FN075	126
5	6e	170
6	Heptyl mannoside	180
7	5a	241
8	FIM-2238, 2ZFH238, 8e	181
9	FIM-4269, 4ZFH269, 8	188
10	7a	193
11	7b	193
12	19	194
13	4d	196

Small-molecule inhibitors of CUP pili biogenesis

Pilicides were the first small molecules to be utilized for the inhibition of CUP assembly (150). They belong to a class of molecules known collectively as pyrisides. These compounds are based upon a bicyclic 2-pyridone scaffold that maintains a rigid, peptide-like conformation that closely mimics a β-strand (Fig. 3A). Measurement of pilicide activity in culture revealed remarkable success in inhibiting the assembly of both P and type 1 pili as monitored via electron microscopy (150) (Fig. 3B) and as determined in a variety of *in vitro* assays, including hemagglutination, biofilm formation, and adherence to a bladder tissue cell line. Pilicides were originally designed to disrupt the formation of chaperone-subunit complexes by targeting the chaperone-subunit interface. However, X-ray crystallographic studies with the P pilus chaperone, PapD, in complex with pilicide 1 (see Table 1) determined that the pilicide was instead binding a conserved hydrophobic region on the chaperone known to interact with the N-terminal domain of the usher pore, suggesting a disruption of a key interaction between the chaperone-subunit complexes and the usher (150) (Fig. 3C). *In vitro* binding studies with the type 1 FimCH chaperone-subunit complex and the N-terminal domain of the FimD usher confirmed this hypothesis, because increasing concentrations of pilicide were shown to inhibit binding between these two species (150).

Continued development of pilicides has led to a detailed understanding of the structure-activity relationship of these compounds and has resulted in the synthesis of molecules with vastly improved inhibition of type 1 pilus biogenesis (151, 152). The most efficacious compounds inhibit type 1 pilus-mediated *E. coli* biofilm formation in the low μM and high nM range. One such pilicide, compound 2, was found to disrupt several virulence-associated pili, including type 1 pili, P pili, and S pili (153) (Fig. 3A), as well as flagellar motility (153). Growth of the cystitis isolate UTI89 in compound 2 results in a dramatic downregulation of the type 1 pilus genes. Type 1 pilus expression is controlled by inversion of the *fimS* promoter element, which can oscillate between phase ON and phase OFF orientations. Growth in compound 2 results in *fimS* orientating into the OFF phase. In addition, it results in increased levels of the transcriptional regulators SfaB and PapB, which also promote the phase OFF orientation of the *fimS* promoter (153). Thus, the potency of pilicide 2 is in part due to the unexpected mechanism of inducing a phase OFF orientation of the type 1 pilus promoter. Additionally, pilicide

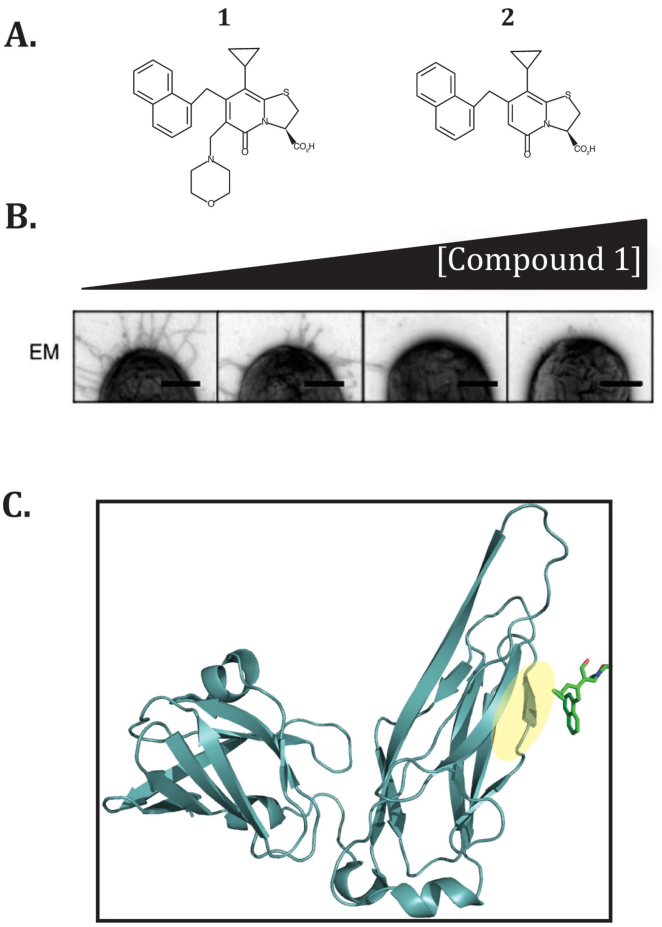

FIGURE 3 Small molecules known as "pilicides" disrupt pilus biogenesis. **(A)** Structures of two potent curlicides. Curlicide 1 disrupts type 1, P, and S pili. Curlicide 2 binds to the P pilus chaperone PapD, inhibiting its interaction with the PapC usher. **(B)** Electron micrographs demonstrating a loss of P pili on uropathogenic *Escherichia coli* cells exposed to increasing concentrations of curlicide 2 (adapted from reference 150, with permission; copyright [2006] National Academy of Sciences, USA). **(C)** Crystal structure of the two-domain adhesin FimH complexed with pilicide (PDB ID code 2J7L).

activity against Dr pili, another type of CUP pili known to play a role in pyelonephritis in mice and humans, has also been confirmed, further expanding the therapeutic potential of these compounds in targeting UTI (154). The exciting ability of these compounds to target multiple CUP pili suggests that these compounds could demonstrate broad therapeutic coverage in the clinic. However, further testing of these compounds in animal models of infection along with pharmacological development will be required to solidify their role as a therapeutic.

In addition to the bicyclic 2-pyridone pilicides, another class of small-molecule compounds has been identified to target the P5 pocket of the FimH pilus subunit (155). This rationally designed molecule was developed to prevent the donor strand exchange reaction between FimH and the FimG rod adaptor (Fig. 2A and B). Because assembly of the pilus rod initiated by FimH binding to the usher complex followed by FimG incorporation is necessary for pilus biogenesis, abrogation of the FimH-FimG interaction completely abolishes pilus formation (156, 157). To identify such a molecule, a virtual screen was performed that measured *in silico* docking of 2,000 compounds in the P5 pocket of FimH of the usher-bound FimCH protein structure (155). The top compounds from this screen were further examined in an *in vitro* DSE assay, resulting in the identification of the most potent inhibitor, compound 3 (Fig. 2C) (155). This compound was found to completely inhibit type 1 pili expression when the bacteria were grown in the presence of a 200-μM compound. Interestingly, addition of compound 3 to growing bacterial cells rapidly resulted in the decrease of surface-localized pili, suggesting that this compound was capable of facilitating the disassembling and/or shedding of preformed pili (155). Based on the existing crystal structures and known mechanism of action, it is hypothesized that compound 3 may disrupt the anchoring of type 1 pili in the outer membrane by disrupting the terminal chaperone-subunit complex. Further verification of this mechanism of action, along with identification of the compound 3 binding site, will be necessary to continue the development of this compound as a therapeutic.

Small-molecule inhibitors of curli biogenesis

Another class of pyrisides has been shown to inhibit curli assembly. The 2-pyridone scaffold's modular nature allows for the manipulation of chemical activity through the substitution of various R groups onto the ring. When substituted compounds were screened for their ability to inhibit curli-dependent biofilms, it was found that replacement of a cyclopropyl group with a CF_3-phenyl substituent (Fig. 4A) inhibited curli fiber formation at a concentration of 250 μM (Fig. 4B) (126). To determine if this inhibition occurred during the ordered assembly of CsgA into amyloid fibers, various concentrations of compound 4 were added *in vitro* to purified CsgA (126). Compound 4 was able to completely prevent amyloid formation of purified CsgA when present in 5-fold excess. This inhibition is believed to occur via a direct interaction with soluble CsgA, thus preventing its transition into an amyloid-competent state prior to polymerization.

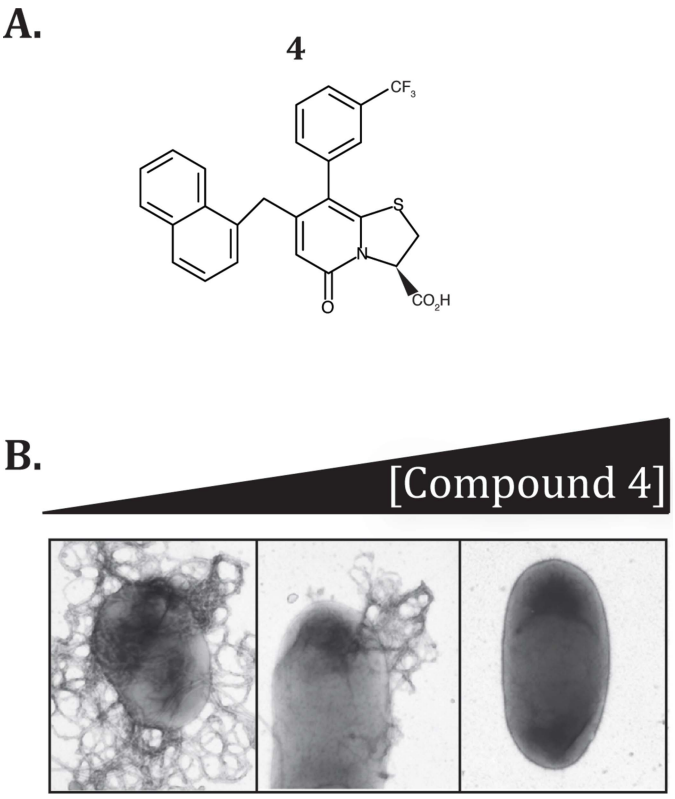

FIGURE 4 Curlicides inhibit biofilm formation. **(A)** Structure of the curlicide 4 compound. Curlicide compounds are based on 2-pyridine scaffold functionalized with a variety of substituents. **(B)** Inhibition of extracellular curli formation in the presence of increasing concentrations of curlicide 4 (adapted from reference 126).

Interestingly, compound 4 has also been demonstrated to inhibit type 1 pilus biogenesis, suggesting its ability to inhibit multiple adhesive strategies utilized by UPEC to colonize the bladder and underscoring its promise as a therapeutic for UTI. Indeed, studies in a murine model of cystitis demonstrated that *E. coli* pretreated with compound 4 is significantly attenuated during infection when compared to untreated bacteria (126). Iterative rounds of synthetic chemistry and structural studies continue to provide further insight into the relationship between curlicide structure and potency (158). Additionally, structural similarities between curli fibers and other amyloid proteins have prompted investigators to test the inhibitory effects of curlicide compounds in other disease states. Indeed, specific curlicides have shown excellent efficacy in the *in vitro* inhibition of Aβ and α-synuclein polymerization, two amyloids associated with Alzheimer's and Parkinson's disease, respectively (159, 160). This ability to target amyloids in a nonspecific manner has provided generalizable insights at the molecular level into the process of amyloid formation. Further knowledge of aggregative mechanisms and their role in disease pathology will help inform the development of therapeutics that target specific regions vital to amyloid pathology.

Small-molecule inhibitors of sortase

Sortase enzymes play a unique role in Gram-positive bacterial physiology and are essential for the virulence of many pathogens. Gram-positive organisms encode up to four distinct classes of sortases that can be classified based on the substrates they act upon and the nucleophile they employ to resolve the acyl enzyme intermediate (76). The class A and B sortases are responsible for the covalent attachment of surface-anchored proteins to the cell wall. Thus, the pentaglycine cross-bridge of the peptidoglycan precursor lipid II acts as the nucleophile in the transpeptidation reaction (161). Although they are structurally homologous to one another, the class A sortase (commonly referred to as the housekeeping sortase) acts on a majority of surface-associated proteins, while the class B sortase functions to specifically anchor heme transporters to the cell wall (162). Class C sortases are responsible for the covalent attachment of pilin subunits in the assembly of pili and are encoded in a pilus-specific manner within the pilus operons (71). Thus, while pilus biogenesis requires sortase C, anchoring of the pilus to the cell wall requires the activity of sortase A. Finally, the class D sortase mediates the attachment of envelope proteins to the cell wall and is believed to play a specific role in sporulation (163).

The conservation of the class A sortase and the integral nature of the process it facilitates have prompted investigators to aggressively pursue therapeutic inhibitors of sortase function. Indeed, virulence of Gram-positive pathogens, including *E. faecalis* and *Staphylococcus aureus*, is severely attenuated in animal models when the class A sortase is deleted (164, 165). These findings have resulted in the pursuit of many strategies to discover inhibitors of sortase A function, including screening of natural products, high-throughput screening of chemical libraries, and structure-based *in silico* screening of compounds (Table 2). While all of these inhibitors were originally screened against sortase A, further analysis demonstrated inhibition of both sortase B and C for many compounds, demonstrating the conservation of sortase protein structure and its mechanism of action.

The first major attempt to identify natural products with sortase inhibitory activity focused its efforts on the

TABLE 2 Antiadhesive strategies targeting the Gram-positive sortase[a,b]

Inhibitor	Origin of inhibitor	IC_{50} measured from *in vitro assay*	Surface protein measured during inhibition of sortase in culture	References
Methanethiosulfonate	Synthetic	N.D.	Seb anchoring	242
p-hydroxymercuribenzoic acid	Synthetic	N.D.	Seb anchoring	242
β-Sitosterol-3-*O*-glucopyranoside	*Fritillaria verticillata* (plant)	18 μg/ml	Binding to fibronectin	168
Berberine chloride	*Callosobruchus chinensis* (plant)	SrtA: 8.7 μg/ml SrtB: 6.3 μg/ml	Binding to fibronectin	167, 243
Psammaplin A1	*Aplysinella rhax* (sponge)	SrtA: 39 μg/ml SrtB: 23 μg/ml	Binding to fibronectin	243
Bromodeoxytopsentin	*Topsentia genitrix* (sponge)	19.4 μg/ml	Binding to fibronectin	244
Curcumin	*Curcuma longa* (plant)	13 μg/ml	Binding to fibronectin	245
Flavonoid phenols	*Rhus verniciflua* (bark) and natural products	SrtA: 37-52 μM SrtB: 8-36 μM	Clumping	246
Diazo/chloromethyl ketone	Synthetic, substrate mimetic	N.D.	N.D.	247
3,3,3-trifluoro-1-(phenylsulfonyl)-1-propene	Synthetic	190 μM	Binding to fibronectin	248
Phosphinic-peptidomimetic	Synthetic, transition state mimic	10 Mm	N.D.	249
Diarylacrylonitrile	Small-molecule library	SrtA: 2.7 μg/ml SrtB: 10 μg/ml	Binding to fibronectin	243, 250
Aryl β-amino(ethyl)ketones	Small-molecule library	SrtA: 4.8 μM SrtB: 14 μM SrtC: 15 μM	N.D.	169
3-(4-pyridinyl)-6-(2-sodiumsulfonatephenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole] (5), see Table 1	Small-molecule library	*Staphylococcus aureus* SrtA: 9.3 μM *Streptococcus pyogenes* SrtA: 0.82 μM	Spa anchoring Binding to fibrinogen	170

[a]N.D., not determined
[b]Adapted from reference 251

screening of extracts from 80 Korean medicinal plants (166). This work led to the identification of several compounds (summarized in Table 2) that demonstrate varying degrees of inhibition *in vitro* and *in vivo*. Most notable were the isoquinoline alkaloids from the rhizomes of *Coptis chinensis* (167) and β-sitosterol-3-*O*-glucopyranoside from *Fritillaria verticillata*, which were determined to have an IC_{50} of 18.3 μg/mL and 15 μg/ml, respectively (168). These compounds demonstrated inhibition of sortase enzymatic activity *in vitro*, but their utilization in bacterial culture resulted in a growth defect (167, 168). Given that a *sortase A* mutant behaves similarly to wild type when grown in culture, these findings suggest that some compounds may target more than one cellular process, resulting in the measured pleiotropic effects. Future work will look to expand on these studies in an attempt to understand the mechanism of inhibition of sortase activity, such that specific inhibitors of sortase function can be identified and optimized for therapeutic use.

In addition to the screening of natural products, high-throughput screens of chemical libraries have led to the identification of several compounds that demonstrate both reversible and nonreversible inhibition of sortase activity. One class of inhibitors which demonstrated the most promise were the aryl β-amino(ethyl) ketones (AEEK) (169) (Table 1). These compounds irreversibly inhibit sortase A with an IC_{50} in the low micromolar range and have a simple, drug-like structure. Preliminary investigations into the structure-activity relationship have identified the value of anionic substituents in the para position on the aryl ring. Structural studies have also helped elucidate a model of inhibition, which involves the generation of an electrophilic intermediate that reacts with the catalytic cysteine, resulting in irreversible inactivation of the thiol active site (169). While these inhibitors hold promise, *in vivo* analysis of these compounds will be required for further verification.

Currently, the most successful class of sortase inhibitors has come from a rational design approach using the crystal structure of sortase A:substrate complex (PDB ID code 2KID) to virtually screen 300,000 compounds for putative binding to the active site (170) (Fig. 5A). From this screen, 105 compounds were selected for further *in vitro* characterization. Identification

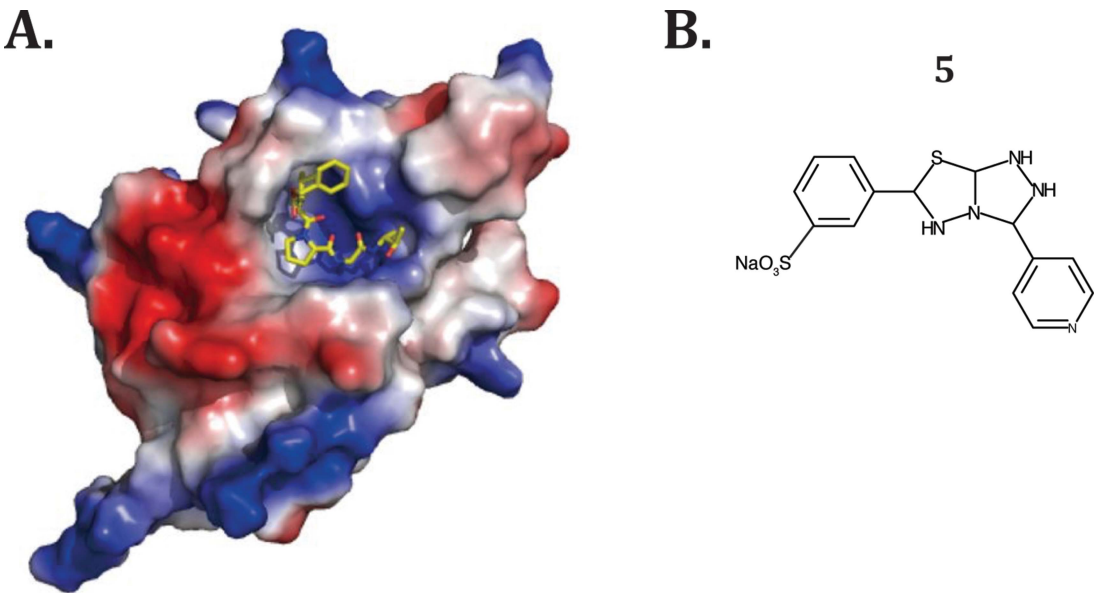

FIGURE 5 Potent inhibitors of sortase A function. **(A)** X-ray crystal structure of sortase A from *Staphylococcus aureus* (PDB ID code 2KID) with *in silico* docking of compound 5, which binds directly to the active site of the enzyme (adapted from reference 170, with permission). **(B)** Structure of the sortase inhibitor compound 5, which inhibits sortase A from *S. aureus* with an IC_{50} of 9.3 μM and *Streptococcus pyogenes* with an IC_{50} of 0.82 μM.

of a potent inhibitor of recombinant *S. aureus* sortase A activity followed by synthetic optimization produced compound 5 (Fig. 5B), which demonstrated an IC_{50} of 9.3 μM (170). This compound was found to inhibit sortase A in a reversible fashion and was demonstrated to directly bind to sortase A with a K_d = 8.8 μM. *In vivo* analysis of the influence of compound 5 on *S. aureus* in culture demonstrated no influence on growth but revealed a decrease in incorporation of cell wall–anchored proteins (170). One such protein showing decreased incorporation is protein A (SpA), which is known to aid bacterial subversion of phagocytosis by host immune cells by the binding the Fcγ and Fab domains of host immunoglobins (171). To determine compounds 5's potential as a therapeutic, the ability of intraperitoneal doses of the compound to protect mice from a lethal challenge of *S. aureus* was tested. These studies found that intraperitoneal dosing of compound 5 in mice resulted in a significant increase in murine survival, demonstrating the anti-infective capabilities of sortase inhibitors (170). Interestingly, compound 5 was also found to inhibit the sortase from *Streptococcus pyogenes* with an IC_{50} of 0.82 μM, suggesting that it could have broad therapeutic use in the clinic (170). Further optimization of compound 5 and validation in additional animal models could make the targeting of sortase activity a legitimate therapeutic option.

Inhibition of Pathogen Binding by Receptor Analogs

Recognition of surface-exposed glycans by a pathogen is often characterized by a relatively weak association between the pathogen carbohydrate-binding domain (CBD) and the host glycoconjugate (34). To compensate for this relatively weak interaction, bacteria typically express multiple copies of the CBD to increase the avidity for the target and strengthen the interaction between the pathogen and the host. One strategy that is commonly employed to target this interaction involves the introduction of small "glycomimetics" to the system, which saturates the CBDs by imitating their natural ligand. This saturation weakens the pathogen's interaction with host tissue, increasing susceptibility to natural mechanical expulsion. The efficacy of this treatment approach is thus based on the generation of high-affinity glycomimetics that can outcompete the natural ligand for the CBD at physiologically plausible concentrations. To accomplish this task, both high-affinity monovalent as well as multivalent inhibitors have proven to hold great promise (Table 3).

FimH antagonists: mannosides

The type 1 pilus adhesin, FimH, mediates adherence of UPEC to the bladder epithelium and is essential for infection in a murine model of cystitis (172). The lectin

TABLE 3 Antiadhesive strategies utilizing receptor and adhesin analogs[a]

Bacterium	Method of validation	Inhibitor	References
Receptor analogs			
Campylobacter jejuni	Murine gastrointestinal model	Fucosyloligosaccharides of human milk	252
Helicobacter pylori	Rhesus model	3-sialyllactose	253
Listeria monocytogenes	Human epithelial colorectal cell line	Xylo-oligosaccharides	254
Streptococcus pneumoniae	Rabbit and rat nasopharynx	6′-sialylneolactotetraose	255
Yersinia pestis	Human epithelial respiratory cell line	GalNAcβ1-3Gal and GalNAcβ1-4Gal	256
Streptococcus sobrinus	Rat oral cavity	Oxidized α1,6glucan	257
Streptococcus suis	Murine model of peritonitis	Tetravalent galabiose	258–262
Escherichia coli (type 1 pili)	Murine model of cystitis	Mannosides	181, 182, 188–190, 263–267
E. coli (P pili)	*In vitro* binding assay	Multivalent galabiose	268–270
E. coli (F1C pili)	*In vitro* binding assay	Multivalent GalNAcβ1-4Gal	271
Pseudomonas aeruginosa (LecA)	Murine model of lung infection	Galactosides	193, 272–276
P. aeruginosa (LecB)	Murine model of lung infection	Fucosides/mannosides	193, 195, 196, 277–279
Adhesin analogs			
Streptococcus mutans	Human studies monitoring recolonization of *S. mutans*	Full length streptococcal antigen (SA) I/II and 22 residue peptide	280, 281
Streptococcus gordonii	*In vitro* binding and biofilm assay	Peptides of the adhesin *Streptococcus gordonii* surface protein SspB	282
Enterotoxigenic *Escherichia coli*	Horse red blood cells and calf ileal enterocytes	Truncated versions of K99 pili	283
Gram-negative bacteria and *Staphylococcus aureus*	Multiple human tissue culture cell lines	MAM7	197, 200

Adapted from references 284, 285.

domain of FimH has been demonstrated to mediate binding to several glycoproteins, including uroplakin Ia (UPIa), Tamm-Horsfall protein, and β1 and α3 integrins (173–175). Recognition of this diverse set of host ligands occurs through a stereochemically specific interaction with mannose. Crystallographic studies of FimH complexed with a number of mannose derivatives have revealed the structural basis of mannose recognition on a molecular level. The FimH lectin domain ($FimH_L$) is composed of an 11-stranded elongated β-barrel with a jelly roll–like topology with a mannose-binding pocket located at the tip of the two-domain protein (176–178). This binding pocket is comprised of several residues, which make extensive hydrogen bonding and hydrophobic interactions with D-mannose. Outside of this pocket is a hydrophobic ridge, which includes two tyrosine residues that form the so-called tyrosine gate (Fig. 6A). Genetic analyses of hundreds of *fimH* sequences have found these distinct regions to be invariant, further arguing for their importance in the pathogenic cascade (177, 179). Indeed, interactions with the tyrosine gate and other hydrophobic residues found within the ridge are believed to mediate the increase in affinity seen for many mannose-containing oligosaccharides, including Manα1,3Manβ1,4GlcNAcβ1,4GlcNAc (oligomannose-3). Crystallization of $FimH_L$ with oligomannose-3 confirmed this hypothesis and has directed the development of mannosides that initiate interactions with this hydrophobic ridge (180). Interestingly, one of the first mannosides, butyl α-D-mannoside, was initially identified when it was serendipitously copurified and crystallized with $FimH_L$ (178). This mannoside displayed a 15-fold increase in affinity compared to D-mannose, largely resulting from the hydrophobic interaction between the alkyl chain and the tyrosine gate (178).

Subsequent rounds of rational compound design and testing continued to examine the influence of alkyl chain length on affinity. In this manner, heptyl α-D-mannoside (compound 6) was identified as a lead compound, because its binding was shown to be 30-fold tighter than butyl α-D-mannoside and 600 times tighter than the natural ligand, D-mannose (Fig. 6B) (178). The therapeutic efficacy of this compound was tested in a murine model of cystitis, which demonstrated that incubation of UPEC with compound 6 prior to infection in mice resulted in a significant decrease in bacterial burden in the bladder 6 hours postinoculation (180). This was the first study demonstrating the utility of mannosides *in vivo*, highlighting the therapeutic potential of targeting FimH.

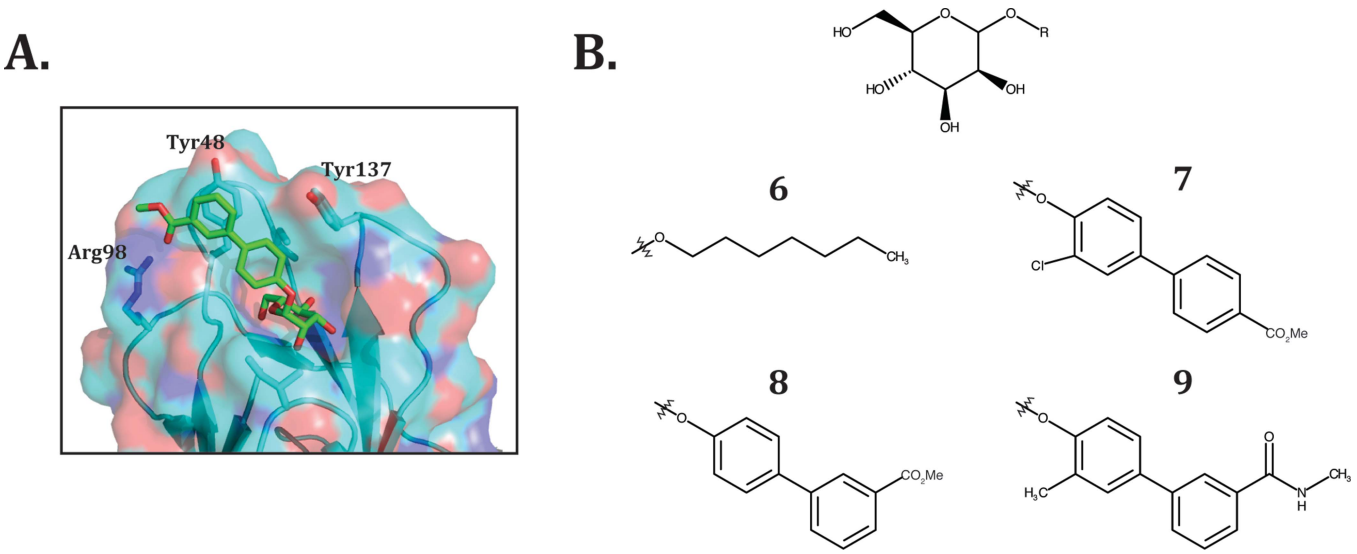

FIGURE 6 Mannosides are potent inhibitors of FimH binding. **(A)** Crystal structure of FimH complexed with mannoside 8, which binds to FimH with an affinity over 1 million times higher than its natural substrate, D-mannose (PDB ID code 3MCY). **(B)** Structure of a variety of mannosides, each rationally designed to increase affinity for FimH by interacting with the hydrophobic ridge outside of the mannose-binding pocket. Although heptyl α-D-mannoside 6 successfully bound FimH with a 600 times increased affinity when compared to D-mannoside, the biphenyl substituents ultimately proved to be the most effective compounds (7, 8, and 9).

Since these initial findings, further development of mannosides has continued to focus on structure-based optimization of affinity. To exploit π-π stacking interactions with the tyrosine gate and interactions beyond the binding pocket, the alkyl chain was substituted with a variety of aromatic substituents. Ultimately, it was found that the affinity of biphenyl α-D-mannosides (compounds 7, 8, and 9) for FimH was much higher than aryl- or heptyl-mannose (compound 6), resulting in a new line of potent therapeutic candidates (Fig. 6B). Further substitutions to these rings have focused on the addition of electron withdrawing groups to decrease the electron density of the aryl ring and increase π-π stacking (Fig. 6B). These charged residues have also been found to facilitate hydrogen bond formation with Arg98, which resides right outside the binding pocket (Fig. 6A). Additionally, it is believed that increased binding affinity of compounds can also be related to a decrease in conformational flexibility and thus a decrease in the entropic cost of binding (181–183).

Iterative refinement of these compounds has necessitated the use of multiple modalities to assess mannoside potency. Inhibition of epitope binding can be measured directly by examining the inhibition of the type 1–dependent hemagglutination of guinea pig red blood cells, which present mannosylated epitopes on the surface of the cell (181). Additional assays include inhibition of bacterial adherence to plastic plates that have been coated in mannosylated bovine serum albumin or inhibition of type 1–dependent adherence to human epithelial carcinoma bladder 5637 cells expressing mannosylated uroplakins (184, 185). Measurement of mannoside binding to $FimH_L$ can also be measured by isothermal titration calorimetry, differential scanning calorimetry, or biolayer interferometry. Comparative analyses of mannosides using a multitude of these distinct methods have consistently produced similar results, demonstrating that each assay measures aspects of mannoside affinity. Combined with structural studies, this research has led to the development of one of the most potent mannosides, compound 9 (Fig. 6B), which can inhibit FimH function in the nanomolar concentration range as measured by hemagglutination. Indeed, compound 9 is over 1 million times more potent than D-mannose as measured by hemagglutination.

Continued improvement of this potent compound and others has focused on improving their pharmacokinetic behavior through a number of approaches, including the use of bioisosteres, as well as the development of prodrugs (186, 187). The culmination of this work has led to the development of small, orally bioavailable compounds capable of preventing acute UTIs and treating chronic UTIs (188). Furthermore, prophylactic use of mannosides was found to significantly reduce bacterial burden in a murine

model of CAUTI (189). Ongoing research continues to improve the pharmacokinetic behavior of these drugs to optimize their therapeutic potential in human disease.

While these efforts have largely been based around monovalent inhibition of FimH, other investigations have been directed at the development of multivalent compounds designed to interact with more than one FimH lectin domain. Attachment of compound 6 to a cyclodextrin core has resulted in compounds capable of binding multiple adhesins, thus increasing compound affinity relative to their monovalent counterparts (190). This increase in affinity has resulted in a significant improvement in the *in vivo* efficacy during acute UTI compared to the monovalent heptyl mannoside. However, delivery of these compounds was performed by transurethral administration (190), making their potential as a prophylactic tool or treatment in patients susceptible or suffering with a UTI uncertain.

Multivalent inhibitors of *P. aeruginosa* LecA and LecB

Interaction of lectin domains with specific glycosidic ligands is often relatively weak and usually relies upon multiple interactions to increase the overall avidity. The affinity of LecA and LecB for monomeric galactose and fucose has been determined to be 87.5 μM and 3 μM, respectively (191, 192). However, several multivalent inhibitors have been found to bind in the nanomolar range, supporting the utility of a multivalent approach to lectin inhibition (Table 3). This "clustering" effect has been exploited in the design of multivalent glycoconjugate inhibitors of the LecA and LecB soluble adhesins from *P. aeruginosa*. These synthetic glycoclusters have utilized a variety of scaffolds including peptides (192), modified oligonucleotides (192), fullerenes (192), and trithiotriazine (192) with either galactose or fucose attached to target LecA and LecB, respectively.

Two of the most thoroughly examined inhibitors of the LecA and LecB adhesins are the multivalent galactosylated and fucosylated calixarenes (193). These tetravalent compounds contain triethylene glycol linkers that are attached to the calixarene core and functionalized with either galactose (compound 11) or fucose (compound 10) at the distal tip (Fig. 7A). Measurements of the affinity of these compounds by isothermal titration calorimetry were able to demonstrate nanomolar K_ds of 48 and 176 nM for compounds 10 and 11, respectively (193). These experiments also demonstrated that these compounds functioned in a multivalent manner, as determined by analysis of the isothermal titration calorimetry measurements (193). Given the spacing between subunits within the tetrameric proteins and the length of the ethylene glycol linkers, structural analysis suggested that these inhibitors interacted with separate epitopes on individual tetrameric proteins. Binding to glycomimetic compounds was also found to correlate with *in vitro* bacterial phenotypes. Micromolar concentrations of either drug were found to significantly inhibit *P. aeruginosa* adherence to A549 epithelial cells, reducing binding to between 70 and 90% of wild type levels (193). Additionally, both compounds 10 and 11 were found to inhibit biofilm formation, albeit at the fairly high concentration of 5 mM (193). The efficacy of these compounds *in vivo* was also investigated by preincubating *P. aeruginosa* with compound prior to intranasal instillation in a mouse model of pneumonia. Incubation with either 1 or 5 mM of either compound resulted in a significant reduction in bacterial burden in the lung and was associated with decreased alveolar capillary permeability, which is directly correlated with *P. aeruginosa*–induced lung injury (193). Despite the high concentrations needed to obtain *in vivo* phenotypes, these studies validate this anti-LecA and -LecB approach, providing an impetus to continue biochemical and pharmacokinetic optimization of these compounds as therapies for *P. aeruginosa* infections.

In addition to a multivalent approach, development of both divalent and monovalent inhibitors of LecA and LecB has also been attempted (Table 3). These efforts have involved modification of galactose and fucose with aglycon structures to generate additional contacts with protein residues outside of the binding pocket, thus increasing the affinity and specificity of the compound for their cognate lectin. In the case of LecA, aromatic aglycon structures have proved to be successful, especially those containing a naphthalene ring (Fig. 7B compound 12) (194). For LecB, optimization of the disaccharide substructure L-Fucβ1-GlcNAc from Lewis-a identified a relatively high-affinity molecule with a K_d = 290 nM (195). However, cross-reactivity of this compound with the lectin DC-SIGN, which is found on the surface of macrophages and dendritic cells, was identified as a potentially harmful side effect (195). To circumvent this problem, additional compounds utilizing mannose in place of fucose have been generated. While LecB has a relatively low affinity for mannose compared to fucose, modification of C6 carbon on mannose has resulted in high-affinity compounds, with IC_{50} approaching the low μM range (Fig. 7C compound 13) (196). Continued development in this field will hopefully lead to new therapeutics for the treatment of *P. aeruginosa* infections.

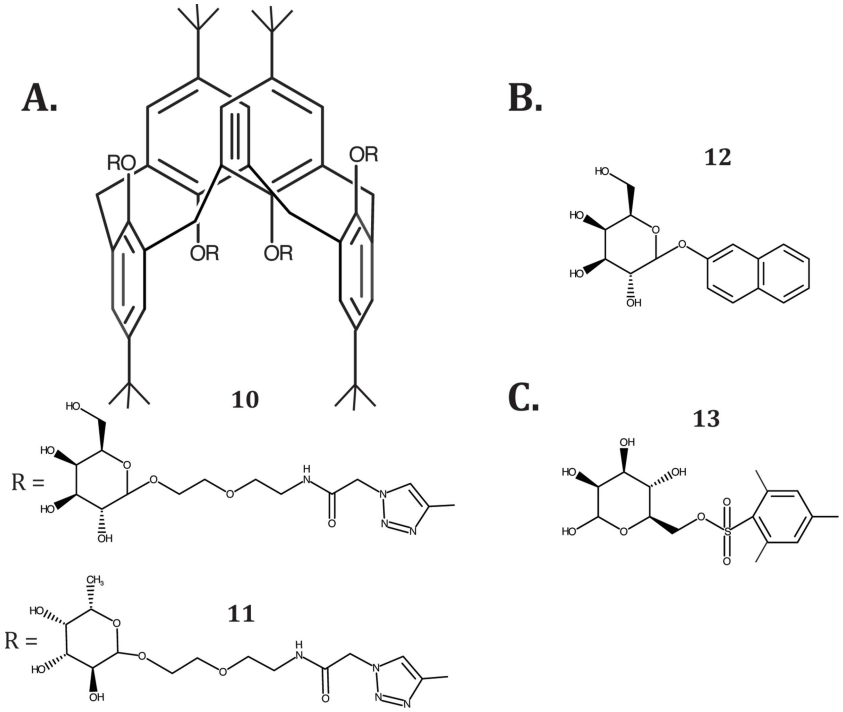

FIGURE 7 Inhibitors of soluble lectins LecA and LecB. **(A)** Structural depiction of the tetravalent calixarene scaffold, which can be functionalized with galactose and fucose moieties using triethylene glycol linkers to form compounds 11 and 10, respectively. **(B)** Monovalent inhibitor of LecA 12 binds with a K_d of 4.2 μM. **(C)** Monovalent inhibitor of LecB 13 activity binds with a K_d of 3.3 μM.

Inhibition of Pathogen Binding by Adhesin Analogs

While the approaches described above have attempted to competitively inhibit adhesin binding by mimicking the adhesin's natural ligand, inhibition of adherence can also be achieved through delivery of adhesin analogs that compete with the bacteria for their natural receptor (Table 3). This approach often utilizes peptide-like inhibitors as opposed to the glycomimetics described above, but it requires the same stable, high-affinity interactions to be successful. Additionally, adhesin analogs must avoid disruption of the host cell function, which can occur with host cell receptor recognition.

MAM7, a peptide-like inhibitor of Gram-negative and Gram-positive infections

The Gram-negative outer membrane protein multivalent adhesion molecule 7 (MAM7) was first identified in the pathogen *Vibrio parahaemolyticus* and found to consist of a transmembrane motif followed by seven mammalian cell entry (mce) domains, all of which are required for attachment to cultured HeLa epithelial cells (197). Utilizing a bioinformatics approach, it was determined that MAM7, or its 6-mce domain counterpart MAM6, was highly conserved among Gram-negative pathogens but absent in Gram-positive bacteria (197). MAM7 is believed to mediate attachment to host cells via interactions with host fibronectin and the host membrane lipid phosphatidic acid and was shown to augment cell death mediated by the type III secretion system (197, 198). Interestingly, prior addition of nonpathogenic bacteria expressing MAM7 was able to ameliorate infection of cultured HeLa epithelial cells infected with a variety of Gram-negative pathogens, including additional *Vibrio* species, *Yersinia pseudotuberculosis*, enteropathogenic *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii-calcoaceticus*, and *P. aeruginosa* (197, 199).

It has also been demonstrated that nonpathogenic bacteria expressing MAM7 or purified recombinant MAM7 immobilized on latex beads are able to prophylactically inhibit cytotoxicity in a tissue culture model of infection of either HeLa epithelial cells or 3T3 fibroblasts (197, 199). Delivery of MAM7 involved a 30-minute preincubation of the tissue culture cells with either the MAM7-expressing bacteria at a multiplicity of infection of 100 or the addition of MAM7-coated latex beads at a concentration of 7.5 mg protein/10^6

beads/well (199). This decrease in cytotoxicity results from a decrease in MAM-7-mediated adhesion, which is believed to occur primarily through competition with the pathogen's MAM7 homolog.

MAM7 can also be effective at outcompeting non-MAM7 adhesins for the same receptor. For example, *S. aureus* is known to interact with the extracellular glycoprotein fibronectin. Preincubation of HaCaT, human dermal fibroblast, or HeLa tissue culture cells with 500 nM of bead-coupled MAM7 significantly reduced attachment of *S. aureus* (200). This reduction is similar to that observed when beads coated with the staphylococcal fibronectin-binding protein (FnBPA) or the *S. pyogenes* fibronectin-binding protein F1 are preincubated with tissue culture cells prior to the introduction of *S. aureus* (200). It should be noted that treatment with F1- or FnBPA-coated beads disrupted host cell function, resulting in a delay in wound healing due to impaired matrix formation and cellular adhesion, even in the absence of a pathogen (200). Thus, MAM7 is uniquely able to prevent adherence of *S. aureus* without contaminant perturbation of the host cell environment. The broad coverage of MAM7 as an inhibitor of both Gram-positive and Gram-negative infections makes it an exciting candidate as a broad-spectrum antivirulence therapeutic. However, the size of MAM7 (~840 amino acids) and the lack of *in vivo* data still represent major obstacles to overcome in the course of development of this concept into a stable, high-affinity therapeutic.

INHIBITION OF PATHOGEN BINDING BY ANTIADHESION ANTIBODIES AND VACCINES

Adhesin-based vaccines have proven to be highly successful in the prevention of bacterial infections in a number of animal models (Table 4). This strategy relies upon vaccination with an essential adhesin required for colonization and disease. Generation of antibodies against the adhesin can result in disruption of adhesin-receptor interactions by occlusion of the binding pocket in an orthosteric manner. Alternatively, antibodies may disrupt binding via allosteric interactions by blocking a

TABLE 4 Antiadhesive strategies utilizing antiadhesin vaccines or antibodies

Bacterium	Adhesin targeted	Details	References
Salmonella enterica serovar Typhi	T2544	Active and passive immunization provides limited protection	286
S. enterica serovar Typhi	SadA	Active vaccination provides limited protection	287
Enterotoxigenic *Escherichia coli* (ETEC)	K88ab, K88ac, FedA, and FedF	Vaccination of proteins expressed in live attenuated *Salmonella* Typhimurium strain	209, 210
Enterotoxigenic *E. coli*	FaeG major subunit of K88ac fimbriae	Active vaccination with heat-labile (LT) toxin and the A subunit of shiga toxin (STa) is protective	288
Enterohemorrhagic *E. coli*	Intimin	Active vaccination with fusion protein of intimin and two shiga toxin antigens provides protection	289
Uropathogenic *E. coli*	FimCH	Active and passive vaccination provides protection	202, 203
Pseudomonas aeruginosa	Type IV pilin adhesin	Generation of an effective antibody response relied on coupling of the N- and C-terminal to a carrier, provides protection against multiple strains	290
Proteus mirabilis	MrpH, MR/P pilus adhesin	Active vaccination as fusion with cholera toxin provides significant protection	205
Enterococcus faecalis	EbpA	Active vaccination of full length EbpA or its N-terminal provides protection	82
Bordetella pertussis	Hemagglutinin adhesin and pertactin	Both adhesins are included in the vaccine against whooping cough and function partly by inhibition of adhesion	291
Streptococcus mutans	Streptococcal antigen (SA) I/II	Application of a monoclonal antibody prevents tooth colonization	292, 293
Staphylococcus aureus	Clumping factor A, fibronectin-binding protein A, and fibronectin-binding protein B	Both active and passive vaccination provided protection against prosthetic-device infection	294
S. aureus	Collagen-binding protein (CNA)	DNA vaccine generated antibodies against CNA, but does not provide protection against intra-peritoneal infection	211
S. aureus	Clumping factor A (ClfA), fibronectin-binding protein (FnBPa)	DNA vaccine including the sortase enzyme provides strain-dependent protection	212
Streptococcus pneumoniae	Surface adhesin A and surface protein A	Intranasal vaccination reduced colonization	295

conformational change within the adhesin required for ligand recognition (201). Either of these mechanisms, when fully realized, result in the host's elimination of the pathogen by natural mechanisms. This protection has been seen following both active and passive vaccination, suggesting that the development of a monoclonal antibody may also be highly effective at treating active infections.

Vaccines for urinary tract infections

Elucidation of the role of type 1 pili and the FimH adhesin led to one of the first adhesin-based vaccines. Vaccination of mice with a truncated version of FimH corresponding to the lectin domain or with the FimC-FimH chaperone-adhesin complex produced high serum titers of anti-FimH IgG (202). These antibodies were also detectable in the urine and were shown to reduce colonization of the bladder mucosa by 99% following transurethral challenge in mice with a model UPEC strain (202). Independent *ex vivo* studies further verified that these antibodies were able to recognize FimH and prevent the binding of UPEC to human bladder epithelial cells (202). Protection of neutropenic mice from UTI by vaccination with FimH further demonstrated that elimination of bacteria was occurring independently of neutrophilic involvement, supporting the notion that protection occurs via inhibition of bacterial adhesion and subsequent mechanical elimination of UPEC strains (202).

Nonhuman primate trials subsequently demonstrated that immunization of monkeys with FimCH was found to be effective, generating a strong IgG response and preventing infection in three out of four animals, compared to a 100% infection rate in the control group (203). Further, vaccination was found to have no impact on the *E. coli* niche in the gut microbiota, demonstrating the specific targeting of pathogenic *E. coli* in the urinary tract (203). Continued development of this vaccine has primarily been focused on inducing a greater immune stimulation in an attempt to increase the concentration of protective antibodies near the mucosal surface. To this end, investigators have attempted to fuse FimH to the flagellin FliC to stimulate a stronger acute inflammatory response (204). Similarly, coadministration of the FimC-FimH vaccine with a synthetic analogue of monophosphoryl lipid A has resulted in a phase 1 clinical trial that began in January 2014.

Adhesin-based vaccines for other uropathogens, including *Proteus mirabilis* and *E. faecalis*, have been shown to be successful in animal models. Vaccination with the adhesin capping the MR/P pilus from *P. mirabilis* was found to significantly reduce bladder bacterial burdens compared to unvaccinated controls (205). Additionally, vaccination with the pilus adhesin EbpA from *E. faecalis* reduced the bladder bacterial burden 1,000 times, representing a significant amount of protection against infection (82). Similar to the FimH vaccine, this protection was found to be specific to the adhesin, EbpA, and was demonstrated to disrupt EbpA's interaction with fibrinogen. Interestingly, vaccination with an EbpA construct lacking the metal-binding MIDAS motif necessary for fibrinogen recognition did not result in the production of protective antibodies (82).

Additional antiadhesive vaccine strategies

In addition to the straightforward adhesin-based vaccine strategies described above wherein soluble protein is introduced directly into the animal, investigators are also pursuing a number of alternative approaches designed to ensure that adhesins are properly oriented and displayed in a physiologically relevant conformation for antibody generation. For example, development of a vaccine against the Gram-negative pathogen *Neisseria meningitidis* required the delivery of adhesin antigens encapsulated in outer membrane vesicles to ensure proper presentation. Previous attempts at vaccination had focused simply on the delivery of adhesins: NadA, factor H binding protein (fHBP) and *Neisseria* heparin binding antigen (NHBA) (206–208). Similarly, a vaccine against enterotoxigenic *E. coli* (ETEC) successfully developed for postweaning pigs utilizes a *Salmonella enterica* serovar Typhimurium strain that expresses and displays the *E. coli* fimbrial components (K88ab, K88ac, FedA, and FedF) (209, 210). Although this vaccine is effective, additional care must be taken when vaccinating with live strains to ensure that no live, genetically modified bacteria are introduced into the environment.

Finally, some studies have been directed toward the development of DNA vaccines, which involve the direct delivery of DNA encoding the pathogen-derived antigen. Expression, processing, and presentation of a DNA antigen are believed to occur in a more efficient manner, leading to both a humoral and cellular response. DNA vaccines against adhesins from enterotoxigenic *E. coli*, *S. pneumoniae*, and *S. aureus* have been attempted with variable success (211–214). As a whole, the targeting of adhesin-receptor interactions through vaccination has shown great promise, leading to the successful development of several novel therapies for both Gram-negative and Gram-positive pathogens (Table 4).

DIETARY SUPPLEMENTS AND PROBIOTICS AS INHIBITORS OF BACTERIAL ADHESION

There are several cited examples of fruits, plants, and milk that possess the ability to inhibit bacterial adherence to a variety of tissues (Table 5) (215). Extraction and characterization of the active constituents from these products suggest that they often function as receptor analogs or adhesin inhibitors. A number of extracted plant phenols have been shown to prevent attachment by a number of bacteria, including *Streptococcus mutans* and *Helicobacter pylori*, which are known to cause dental caries and gastric ulcers, respectively (216–218). However, the exact mechanism or active component of the majority of these inhibitors is unknown. Some of the most thoroughly studied extracts come from cranberries (*Vaccinium macrocarpon*), which have long been recognized for their possible preventative and therapeutic utility toward UTIs (219). Although some studies of elderly and young women suggested that regular intake of cranberry juice results in a significant reduction in bacteriuria, additional studies have not shown a significant difference (220–222). However, high-molecular-weight polyphenols extracted and purified from cranberry extracts have demonstrated the ability to inhibit bacterial binding of *E. coli* (223), *N. meningitidis* (224), and *S. mutans* (225) to host tissue *in vitro*. While the mechanism of inhibition in many of these cases has not yet been fully elucidated, in the case of UTIs it is possible that the high level of fructose present in most cranberry juices may bind to the

TABLE 5 Antiadhesive strategies utilizing dietary supplements[a]

Plant	Active ingredient	Bacterium	References
Plant derivatives			
Camilla sinensis (green tea)	Green tea extract, (-) epicatechin gallate, (-) gallocatechin gallate	*Helicobacter pylori, Staphylococcus aureus, Porphyromonas gingivalis*	218
Vaccinium spp.	Cranberry polyphenols	*Escherichia coli, Neisseria meningitidis, Streptococcus mutans, H. pylori*	223–225
Curcuma longa (turmeric)	Essential oil components	*S. mutans*	296
Nidus vespae (honeycomb extract from *Polistes* spp.)	Chloroform/methanol fraction	*S. mutans*	297
Paullinia cupana (guarana)	Tannins	*Streptococcus mutans*	216
Psidium guajava	Guaijaverin	*S. mutans*	298
Vitis (red grape marc)	Polyphenols	*S. mutans*	217
Azadirachta indica (neem stick)	N.D.	*Streptococcus sanguis*	299
Gilanthus nivalis (snowdrop)	Mannose-sensitive lectin	*E. coli*	300
Gloiopeltis furcata and *Gigatina teldi* (seaweeds)	Sulfated polysaccharides	*Streptococcus sobrinus*	301
Melaphis chinensis	Gallotannin	*S. sanguis*	299
Persea americana (avocado)	Tannins	*S. mutans*	302
Legume storage proteins	Glycoprotein	*E. coli*	303
Milk constituents			
Human milk	Fucosyloligosaccharides	*E. coli*	304
Mammalian milk	Free oligosaccharides	*Neisseria meningitidis*	305
Human milk	Polymeric glycan	*Pseudomonas aeruginosa* and *Chromobacterium violaceum*	306
Human milk	Lactoferrins	*Shigella* spp.	307
Human milk	Caseins	*S. mutans*	308
Human milk	Caseinoglycopeptides	*H. pylori*	309
Human milk	Glycoprotein	*Staphylococcus aureus*	310
Human milk	Neutral oligosaccharides	*Streptococcus pneumonia* and *Haemophilus influenzae*	311
Human milk	Sialylated glycoproteins	*Mycoplasma pneumoniae*	312
Porcine milk	Glycosylated proteins	*H. pylori*	227
Human milk	Sialylated poly(*N*-acetyl lactosamine)	*Mycoplasma pneumoniae*	313
Human milk	Sialylated poly(*N*-acetyl lactosaminoglycans)	*Streptococcus suis*	314
Human milk	Sialyl-3′-Lac and sialylated glycoproteins	*E. coli* (S pili)	315
Human milk	Sialylgalactosides	*E. coli* (S pili)	316

[a]Adapted from references 284, 285.

FimH adhesin in type 1 pili and compete with the natural mannosylated receptors. However, this mechanism of action cannot explain the ability of cranberry extract to inhibit P pili–mediated adhesion *in vitro*, suggesting that multiple inhibitors may be present (226).

Milk from humans and other mammals has also been determined to contain a number of antibodies, glycoproteins, and oligosaccharides that inhibit or reduce bacterial binding (Table 5). A murine model of *H. pylori* infection revealed that the oligosaccharides Lewis-b and sialyl Lewis-x present in porcine milk have the ability to reduce colonization of the gastrointestinal tract through the inhibition of bacterial adherence to host receptors (227). Human milk oligosaccharides have also been demonstrated to inhibit binding of the enteric pathogens *E. coli*, *Vibrio cholerae*, and *Salmonella fyris* to epithelial cell lines (228). This observation likely explains the correlation between protection against diarrhea and the quantity of oligosaccharides detected in breast milk (229). Taken together, the inhibition of bacterial adhesion by naturally occurring products in milk and plant tissue may represent an evolved approach to targeting bacterial adhesion as a host defense mechanism. Indeed, many attempts at inhibiting bacterial adhesion have been informed by investigation into the activity of naturally occurring products. Further screening of these natural products for inhibitors of bacterial binding will likely serve as an important source of novel therapeutics for targeting pathogen adherence.

Additional strategies to prevent adhesion and colonization by human pathogens have included the use of commensal or probiotic strains to reduce binding of detrimental microorganisms by saturating host surface receptors and eliminating pathogen-binding sites. This form of protection is known as colonization resistance (230). Interestingly, administration of antibiotics has been shown to perturb the beneficial commensal bacteria that generate this resistance, resulting in increased colonization of opportunistic pathogens (231). Probiotic bacteria can also compete with pathogens for vital nutrients required for growth, as well as influence production of host mucins that improve barrier function (232, 233). Ultimately, utilization of probiotics can function to prevent pathogen colonization through a diverse array of mechanisms.

SMALL-MOLECULE INHIBITORS OF ADHESIN EXPRESSION

In addition to interruption of protein complex assembly and inhibition of adhesin-receptor interactions, disruption of adhesin transcription is also a viable mechanism of preventing and treating bacterial infections. In many pathogens, regulation of adhesin transcription is integrated into large regulons that impact a number of additional virulence factors, including toxins and secretion systems. Expression of these regulatory networks is often controlled by a variety of cellular and environmental signals, including bacterial density. Detection of bacterial populations can occur by a number of mechanisms but often involves quorum sensing, a mode of bacterial communication utilized by several pathogens to regulate expression of growth and virulence factors as a function of population density. Quorum sensing typically involves the secretion of a small signaling molecule that accumulates in the extracellular space until a critical threshold is reached, resulting in the transcriptional upregulation of a number of virulence genes, including adhesins (234). There are a number of examples in which targeting and disruption of these regulatory pathways has prevented the expression of adhesins and other known virulence factors (235–238).

CONCLUDING REMARKS: THE ADVANTAGES OF TARGETING PATHOGEN ADHESIN

As the effectiveness of broad-spectrum antibiotics continues to decline at an alarming rate, the need for the development of novel antimicrobial agents has never been more immediate. The current paradigm of antibiotic discovery is largely based on continued expansion of existing classes of antibiotics to circumvent evolved bacterial resistance mechanisms. While this approach may provide short-term solutions, the strong selective pressure conferred by drugs that target vital cell processes will ultimately limit the effective lifetime of these derivatives. To mitigate this selective pressure, investigators are instead pursuing novel mechanisms of antimicrobial action that target virulence factors central to the bacterial pathogenic cascade. These factors play a role in a variety of processes, beginning with the colonization of host niches and continuing with engagement of secretion systems, formation of biofilms, and others.

A necessary first step in the progression of many bacterial infections is adherence to host tissues in the niche targeted by the pathogen. There are many structurally distinct but functionally overlapping mechanisms by which bacteria mediate this adhesion. These mechanisms depend on the bacteria's location, identity, and local environment. As we continue to build our understanding of these systems at the molecular level, we will

elucidate several new strategies for the targeting of these vital bacterial processes.

Further refinement of these antivirulence compounds, peptides, and vaccines will lead to the development of therapeutics that target pathogens in the niche in which they cause disease. By directing these strategies toward a diverse array of disease processes, investigators hope to provide clinicians with a formidable arsenal of tools for use against a wide variety of infections. The ability of these therapeutics to target pathogens within their specific host niches eliminates the concomitant disruption of the host commensal microbiota that commonly accompanies treatment with current, broad-spectrum antibiotics. This, in turn, eliminates the blooming of other pathogenic bacteria that can occur in states of host dysbiosis.

An additional advantage conferred by the utilization of therapeutics that target bacterial virulence rather than essential cellular metabolic processes is a possible reduction in the rate of antimicrobial resistance. It is conceivable that any mutations made to escape the therapeutic mechanism will result in a concomitant decrease in the ability of the adhesin to interact with its natural receptor. While this hypothesis has not yet been vigorously tested, this may represent a unique scenario wherein development of resistance to antiadhesive therapeutics may occur, but doing so will result in a significant attenuation of virulence.

Recent advances in the targeting of bacterial adhesion have come from *in silico* docking with solved protein structures. The ability to virtually screen thousands of compounds reduces costs and decreases the time necessary to identify promising targets. Thus, it is necessary to continue to pursue a structural understanding of host-pathogen interactions on a molecular level through NMR and X-ray crystallography to inform computational identification and rational design of potent and effective compounds. Additionally, consideration of the pharmacokinetic behavior of compounds early in their development will also be crucial in identifying successful antiadhesive approaches that will be conducive to a clinical setting.

Finally, while the development of small molecules and vaccines can provide exciting therapeutic options, their development is also fundamental to obtaining a clear and complete understanding of bacterial pathogenesis. As with genetic manipulation, these compounds provide investigators with molecular scalpels that can dissect host-pathogen interactions, allowing one to understand the contribution of these interactions to disease in animal models, as well as to identify putative host targets. Indeed, there are a number of examples in which chemical biology through the generation of molecular probes has aided in the study of bacterial pathogenesis (239). Ultimately, continued development of antiadhesive strategies will further our understanding of bacterial virulence as it relates to human disease and provide unique approaches to the treatment of infectious diseases.

ACKNOWLEDGMENTS

Scott Hultgren has an ownership interest in Fimbrion and may financially benefit if the company is successful in marketing the mannosides that are related to this research.

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