Pilicides—small molecules targeting bacterial virulence

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In a time of emerging bacterial resistance there is a vital need for new targets and strategies in antibacterial therapy. Using uropathogenic *Escherichia coli* as a model pathogen we have developed a class of compounds, *pilicides*, which inhibit the formation of virulence-associated organelles termed pili. The pilicides interfere with a highly conserved bacterial assembly and secretion system called the chaperone–usher pathway, which is abundant in a vast number of Gram-negative pathogens and serves to assemble multi-protein surface fibers (pili/fimbriae). This class of compounds provides a platform to gain insight into important biological processes such as the molecular mechanisms of the chaperone–usher pathway and the sophisticated function of pili. Pili are primarily involved in bacterial adhesion, invasion and persistence to host defenses. On this basis, pilicides can aid the development of new antibacterial agents.

Introduction

To meet the important need of innovation in antimicrobial therapy, bacterial virulence factors have gained attention as new targets.[1–4] By inhibiting virulence-associated functions, the rationale is to deprive the pathogen of its infectious ability and thus make it susceptible to host immune responses. There is a reason to believe that this strategy, as compared to traditional antibiotics that either kill the pathogen or prevent its growth, would induce bacterial resistance at a much slower rate, since non-essential genes and/or functions that are under less selective pressure to mutate are inhibited. Another speculative advantage of impairing virulence is that the host will be subjected to intact but harmless bacteria, allowing the host to develop an adequate immune response against the virulent pathogen. This would increase the host's chances to efficiently respond to and eradicate the intruder in case of recurrent exposure.

Bacterial virulence factors vary between pathogens and range from adhesive surface fibers such as pili/fimbriae to invasins, toxins and different secretion systems (Fig. 1). Bacteria also benefit from the ability to communicate *via* 'quorum sensing' and establish intracellular bacterial communities (IBCs) and biofilms. Several of these virulence factors, *e.g.* quorum sensing,[5–7] type III secretion,[8] biofilm formation[3,9,10] and the assembly of adhesive organelles,[11] have been recognized as interesting targets.

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Fredrik Almqvist received a BS in chemistry at Umeå University, Sweden, 1991. He received a PhD in organic chemistry in 1996 from Lund University where he was a member of Professor Torbjörn Frejd's research group. From 1997–8, Almqvist was a Knut and Alice Wallenberg post-doctoral fellow in the laboratory of Professor Garland Marshall at Washington University in St. Louis. In 1998 Almqvist joined the Umeå University in Sweden as an assistant professor and in July 1999 he was tenured and appointed Associate Professor in Organic Chemistry. Currently he is research director for biological chemistry at the Department of Chemistry, Umeå University. His research interests focus on design and synthesis of molecules that interact with macromolecules, and in particular inhibition of protein–protein interactions. Examples of the latter involve development of inhibitors of bacterial macromolecular assembly and amyloid formation.

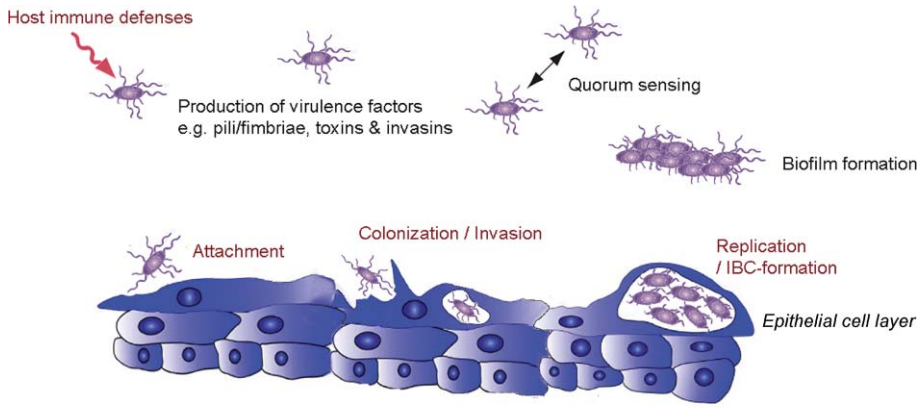


Fig. 1 Pathogenic bacteria can express a range of virulence factors, which initiate and set the course of a disease process. The virulence factors are often important for host attachment and invasion but can also play a critical role for the persistence and spread of the pathogen *via*, *e.g.*, quorum sensing and matrix (biofilms/intracellular bacterial communities) formation.

Virulence factors are often involved in several events and at different stages in the infectious process. They can mediate attachment and invasion and can also be essential in subsequent events in the pathogenic cascade and thereby often set the course of an infection. With the help of virulence factors the pathogen can withstand innate defenses and in some cases antibiotics, thus facilitating its persistence and spread in the host. Hence, inhibition of virulence factors may not only prevent the initial (and crucial) pathogen–host interaction, but could also change the course of an infection by hampering the following, highly dynamic interplay either between pathogen–host or pathogen–pathogen (quorum sensing). The latter inter-bacterial communication is essential for a sustained infection and allows the formation of a critical bacterial density (quorum) and bacterial networks, *e.g.* biofilms and IBCs, which facilitate the escape from innate defenses.[12,13]

To date, several reports of small molecules with inhibitory activity on virulence factors have been presented. In addition to the novelty of the targets and the anti-pathogenic approach, the use of small molecules is in itself intriguing since chemical attenuation of virulence would be a more clear-cut approach than genetics.[14,15] The apparent drawbacks of genetic manipulation of gene clusters are first, that other genes may unfavorably be affected and second, limitations in terms of fine-tuning. Chemomodulation, on the other hand, offers the opportunity of a selective (and at the same time dose-dependent) regulation of virulence factors. In addition, it provides means to interfere in a reversible manner with dynamic biological processes in real-time. Thus, small molecules that inhibit virulence-associated mechanisms open up possibilities to gain, alongside their encouragement of future applications as therapeutics, fundamental insights into disease processes at a molecular level. Consequently, chemical up- or down-regulation can serve to study the roles of different virulence factors in important disease processes such as adherence, invasion and evasion from host defenses. For example, small synthetic molecules have been applied to block virulence by inhibiting type III secretion in different strains of *Yersinia*, *Pseudomonas*, *Escherichia coli* and *Chlamydiae*.[16–20] In the latter case, the chemical approach is the only alternative, owing to the impossibility of the use of genetics on *Chlamydiae*. Small molecules have also been utilized to disrupt the expression of ToxT,[21] which is a virulence factor in *Vibrio cholerae*, and as inhibitors of quorum sensing and biofilm formation of *Pseudomonas aeruginosa*.[9,22]

Pilicides regulate the chaperone–usher pathway in *E. coli*

We have designed and synthesized small synthetic compounds, *pilicides*, which inhibit the assembly of bacterial pili. Pili/fimbriae are multi-protein fibers, which commonly serve as sophisticated virulence factors for a vast number of pathogens. Pili/fimbriae are often assembled *via* a highly conserved mechanism called the chaperone–usher pathway utilized by Gram-negatives such as *E. coli*, *P. aeruginosa*, *Yersinia enterocolitica*, *Haemophilus influenzae* and *Bordetella pertussis*.[23] Thus, pilicides directed against conserved targets (see below) of pilus biogenesis have the potential for broad-spectrum activity as virulence inhibitors. The pili assembly machinery has been well studied in uropathogenic *E. coli* (UPEC) in view of the function and structural details of implicated proteins.[23–25] Consequently, UPEC has served as a prototype pathogen in our studies of both type 1 and P pili.

Pili—function and assembly

Pili consist of a number of repeating protein subunits (PapA, E, F, G, H and K in the case of P pili and FimA, F, G and H in type 1 pili) that are arranged in a helical structure (Fig. 2a). The subunits PapA and FimA are the major subunits in P and type 1 pili, respectively.

The assembly of pili relies on periplasmic chaperones (PapD or FimC) and on outer membrane protein complexes, ushers (PapC or FimD). The chaperones are essential for the folding, stabilization and transport of the subunits and chaperone-deficient or dysfunctional strains are unable to assemble pili.[27–30] The usher is required for the incorporation of the subunits into the growing pilus fiber. Both the usher C- and N-terminal have been reported to be crucial for the assembly.[31–35] The N-terminal of the usher essentially recognizes and associates with incoming chaperone–subunit complexes, while the C-terminal is critical for the subsequent processing.

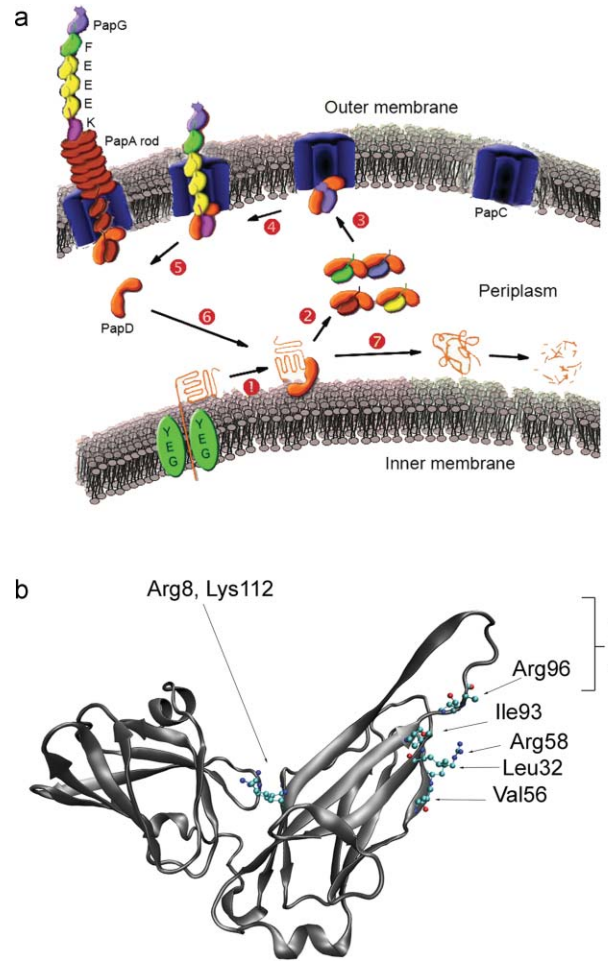


Fig. 2 (a) Model of P pilus assembly *via* the chaperone–usher pathway. Newly translocated subunits are folded (step 1) and stabilized (step 2) by the chaperone PapD. Two invariant chaperone cleft residues, Arg8 and Lys112 (shown in Fig. 2b), are critical for the chaperone–subunit complex formation. The chaperone–subunit complex targets the outer membrane assembly site, *i.e.* the usher PapC (step 3). The pilus subunit is incorporated into the pilus rod in a top to bottom fashion (step 4), starting with the PapG adhesin. PapD is then released (step 5) and recycled (step 6). In cases of chaperone deficiency or dysfunction, the premature subunits are proteolytically degraded (step 7). The assembled subunits form a surface pilus fiber consisting of PapG at the distal end, followed by PapF, E, K, A and finally PapF as the anchoring subunit. PapA is the major subunit in the complete P pilus rod. (b) Structure of the chaperone PapD.[26] The two immunoglobulin (Ig)-like domains form the characteristic boomerang shape with the two invariant cleft residues Arg8 and Lys112, which anchors the subunit's C-terminal carboxylate. A second conserved binding site is located near the F1-G1 loop of PapD and binds to the N-terminal of the usher (PapC) in the process where the subunit is released from the chaperone.

At the distal end of each pilus fiber there is an adhesin (PapG or FimH),[36,37] which recognizes and binds to specific carbohydrate receptors (galabiose[38,39] on the kidney, or mannose[40,41] on bladder cells) on host cells. Type 1 pili also mediate the invasion[42] of bladder epithelium cells and, furthermore, are involved in the formation of biofilms[43] and IBCs[44–46] where UPEC mature into a biofilm-like state. Both biofilms and IBCs facilitate the escape of innate defenses and allow bacteria to persist and spread in the urinary tract.[13,47] Since pilicides block the formation of type 1 pili, one could speculate that a pilicide has the potential to interfere not only with the establishment of an infection, but also with biofilm and IBC formation.

In addition to the adhesive function of pili, which aids the initial attachment and invasion, recent studies have revealed more sophisticated functions of the pilus fibers and, for example, the structure of the pilus appears to affect the binding specificity of the adhesin.[48] In addition, pili have a quaternary, helical structure that, when subjected to external forces such as urine flow, has the ability to elongate and contract.[49–52] These mechanical properties have proved to be ideal for maintaining attachment to the host, as they govern flow-enhanced cell adhesion[51,53] (catch bonds) and sustained host contact *via* the elongation possibilities of the pilus rod.

Development and utility of pilicides

Creating a chemical platform and establishing SARs. Structure-based design was initially applied to outline the synthesis of a first generation of potential pilicides that was directed against chaperone function.[54] The strategy was to target the chaperone family, which has as high level of structural preservation in a variety of Gram-negative pathogens utilizing the chaperone–usher pathway.[26,55] In addition, the prototype UPEC chaperones PapD and FimC had been extensively studied and characterized by NMR spectroscopy and X-ray crystallography.[26,29,56–60] Based on the known molecular details of the subunits' interactions with the chaperones, we envisioned that substituted bicyclic 2-pyridones would serve as dipeptidomimetics and competitively inhibit the highly conserved cleft site on the chaperone (Fig. 3a-b).

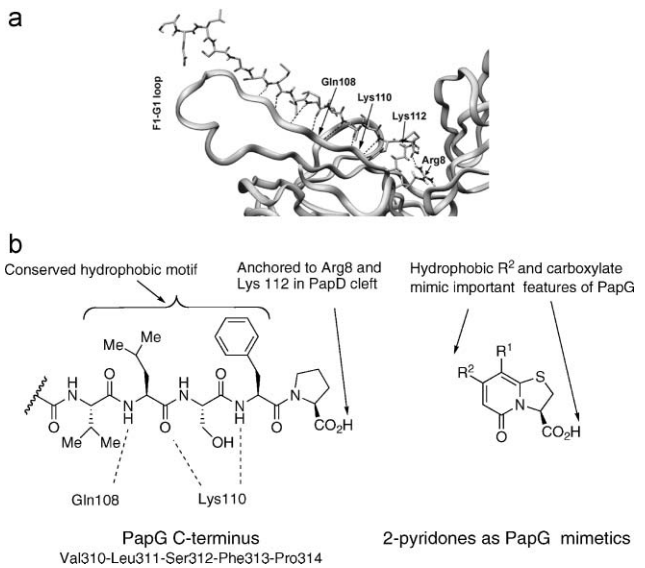


Fig. 3 (a) Crystal structure of the chaperone PapD and the 19-mer C-terminal peptide of PapG.[29] The C-terminal carboxylate of PapG is anchored to Arg8 and Lys112 in the chaperone cleft-site. (b) Substituted, bicyclic 2-pyridones were initially designed[54] to inhibit chaperones as C-terminal mimetics of the subunit PapG. Hydrogen bonds between PapG and PapD are illustrated with dashed lines.

The cleft site has two essential and invariant residues, Arg8 and Lys112, to which the subunit C-terminus anchors as a key step in

the assembly process (Fig. 3a). Competitive pilicide binding to the chaperone cleft would thus prevent the formation of chaperone–subunit complexes and consequently inhibit the formation of pili.

Computer-based modelling[54] supported the hypothesized binding, and a synthetic route[61] to bicyclic 2-pyridones that offered valuable variability in the substitution pattern was developed within our group. The synthesis of the di-substituted scaffold **C** (R^1 and R^2 = aryl/alkyl, Fig. 4) is straightforward *via* a two-component building block synthesis suitable for combinatorial chemistry. The building blocks are thiazolines **A** and acyl Meldrum's acid derivatives **B**, which are derived from nitriles and carboxylic acids, respectively (Fig. 4). Conveniently, this gives access to substituents R^1 and R^2 and, if desired, position R^1 can be left unsubstituted. The developed synthetic routes have been shown to be efficient both in solution, using either conventional[61] (i) or microwave heating[62] (ii), as well as on the solid-phase[63] (iii) (Fig. 4). In addition, the cyclocondensation reaction has later been developed and applied to imines other than thiazolines, rendering multi ring-fused 2-pyridones.[64] Moreover, an efficient and reagent-free microwave-assisted decarboxylation procedure has been developed that provides the 2-pyridone scaffold **C** with a hydrogen in position 3 (Fig. 4).[65]

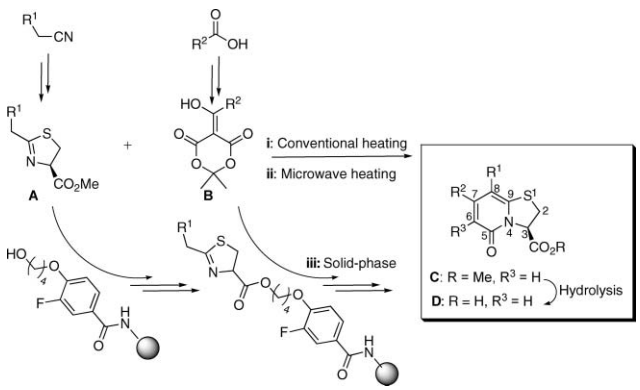


Fig. 4 We have reported three synthetic procedures to 2-pyridones **C** employing conventional heating[61] (i: 64 °C, 14 h, HCl (g) in 1,2-dichloroethane), a microwave-assisted technique[62] (ii: 140 °C, 120 s) and solid-phase supported chemistry[63] (iii). The building blocks, thiazolines **A** and Meldrum's acid derivatives **B**, can be derived from simple starting materials (nitriles, L-cysteine, carboxylic acids and Meldrum's acid). Subsequent alkaline hydrolysis (aq. LiOH in MeOH–THF) of **C** yields carboxylic acids **D** as C-terminal dipeptide mimetics. Note that route (iii) gives **D** directly after cleavage.

Encouragingly, several of the di-substitued 2-pyridones in class **D** bound to the chaperones PapD and FimC, as determined by relaxation-edited ^{1}H NMR-spectroscopy and surface plasmon resonance (SPR).[54,63] Out of 22 compounds, four pilicides (**1**–**4**) were further identified as hits, which with millimolar activity were able to prevent pilus assembly in UPEC without causing growth defects (Fig. 5).[66,67]

The following chemistry on the 2-pyridone scaffold was outlined based on three main objectives. First, to introduce hydrophilic functionalities in the open R^3-position (Fig. 4) in order to increase the water solubility, second, to improve the peptide mimicking properties of the identified hits and third, to establish structure–activity relationships (SARs) regarding pilicide activity. The R^3-

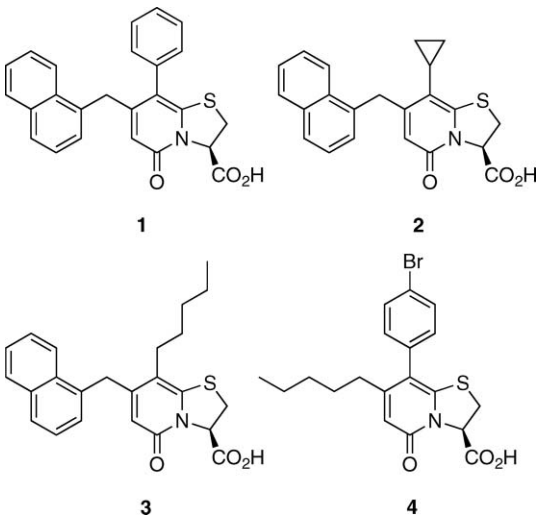


Fig. 5 Di-substituted 2-pyridones **1**–**4** display pilicide activity. **1** and **2** were the most potent inhibitors of pilus biogenesis and were thus considered lead compounds.

position has mainly been investigated using structures **1** and **2** (Fig. 5), which were the most potent pilicides among the four hits.

A number of useful key intermediates could be synthesized *via* facile electrophilic aromatic substitution of position R^3 (framed with dashed lines, Fig. 6). Halogenations[68] (**E**), formylations[69] (**I**) and nitrations[70] (**N**) could be performed and further transformations of these functionalities yielded additional interesting precursors such as nitriles[68] **F**, alcohols[69] **J** and carboxylic acids[69] **K**.

Next, to address the solubility issue, the scaffold was substituted with primary, secondary and tertiary methylamines. The primary methylamines **G** were synthesized either from the nitriles **F** *via* borane reduction or directly from **C** *via* Vilsmeyer formylation (**I**), oxime formation (**L**) and subsequent reduction.[68,69] The oxime-route could be performed advantageously without loss in enantiomeric purity. Symmetrical, tertiary amines **H** could be obtained *via* a microwave-assisted Mannich-type reaction,[68] whereas both secondary and tertiary methylamines **M** were synthesized by reductive amination[69] of aldehydes **I**.

The solubility increased for most of the amino methyl derivatives, which significantly facilitated biological evaluation. Several of the secondary and tertiary methylamines in classes **H** and **M** also displayed a retained or improved ability to inhibit pilus biogenesis.[67,69] On the other hand, the primary methylamines **G** were shown to lose potency and were less active than the parent di-substituted lead compounds in class **D**.[71] Collectively, the results demonstrated that the R^3 position could be substituted to increase solubility, while activity was maintained or improved.

To enhance the resemblance of the mimicked peptide sequence, the R^3 position was utilized to design and synthesize extended peptidomimetics (classes **P**, **Q** and **R**) *via* the key-intermediate amines **O** (Fig. 6).[70] It should be noted that bicyclic scaffolds similar to **O** have previously been recognized as dipeptide mimetics in the literature and that application areas other than as pilicides have been presented.[72–75] Nitration of **C** to **N** followed by reduction gave **O** and next, traditional amino acid couplings rendered the extended peptidomimetics **P**.[70] SARs of the extended peptidomimetics were further investigated by including structures **Q** and **R** obtained *via*

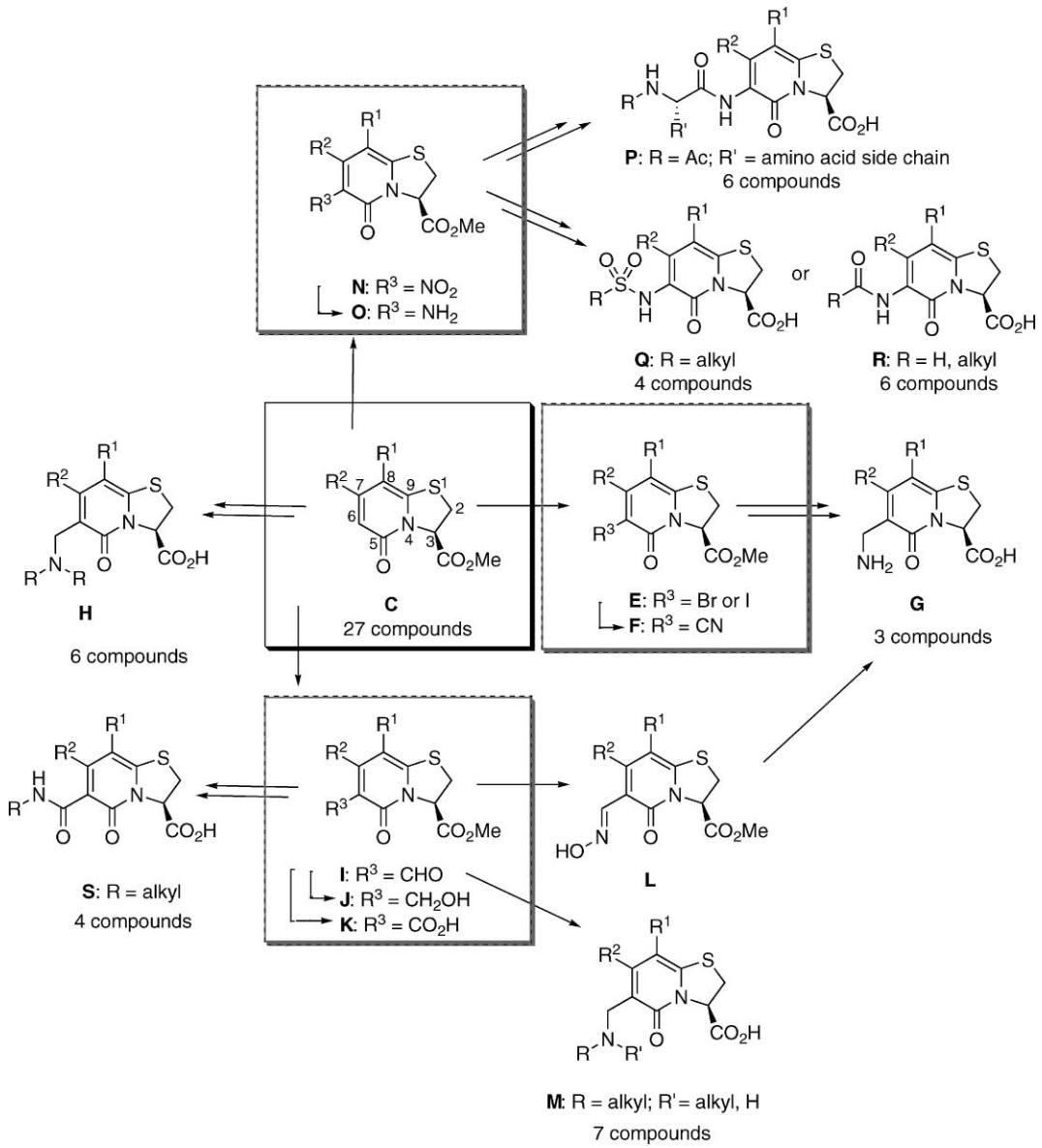


Fig. 6 A chemical synthesis platform has been created from the bicyclic 2-pyridone scaffold **C**. A number of useful key intermediates (framed with dashed lines) can be synthesized and utilized for further transformations.

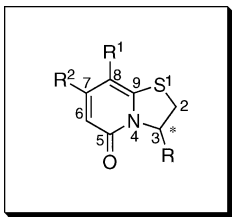
sulfonylation and acylation of **O**, respectively. In addition, the formylated derivatives **I** gave access to peptidomimetics **S**, with a reversed order of the amide bond compared to **R**.

From the biological evaluation of the compounds in classes **N**, **O**, **P**, **Q**, **R** and **S**, it could be concluded that, except for class **P**, the affinities for the chaperone PapD were retained or even increased as compared to the lead of class **D**. In spite of this, the ability to inhibit pilus formation in *E. coli* was substantially reduced for all derivatives within all classes **N**–**S**. We speculate that the poor potencies are due to permeability problems of these substances.

Finally, the importance of the carboxylic acid functionality of **D** has been investigated by synthesizing and evaluating a series of analogues with selected physico-chemical properties (classes **T**–**AC**, Table 1).[76] The analogues, in which hydrogen-bonding properties, size, lipophilicity and spatial location of the carboxylate were varied, exhibited chaperone affinities for PapD that were clearly dependent on the introduced R-substituents (Table 1). The ability to prevent pilus biogenesis in *E. coli* was strictly restricted to the carboxylate-bearing derivatives (**D**, **T** and **U**). Since the carboxylic acid functionality also proved to be critical for solubility reasons, we are at present investigating this position using isosteric replacement. The amine **V** inhibited bacterial growth, and bactericidal and/or bacteriostatic properties have occasionally been observed in the biological evaluations. Compounds causing these effects are discarded as pilicides but could still be interesting antibacterial agents.

Altogether, our synthetic routes provide a solid chemistry platform to synthesize, substitute and functionalize rigid 2-pyridone scaffolds, which from a more general point of view can serve as building blocks in peptidomimetic related research. At present, synthetic routes to further derivatize the scaffold are under development in our laboratories.

Table 1 Carboxylic acid derivatives (R^1 = phenyl or cyclopropyl, R^2 = $–CH_2$-1-naphthyl, in total 17 compounds)[71,76]



Class	R^1	R[a]	Inhibits pili assembly[b]
D	Ph/Cyclopropyl	$–CO_2H$	Yes
T	Ph/Cyclopropyl	(*S*)–CO_2H	Yes
U	Cyclopropyl	$–CH_2CO_2Li$	Yes
V	Cyclopropyl	$–CH_2NH_2$	No[c]
X	Ph/Cyclopropyl	$–CH_2OH$	No[c]
Y	Ph/Cyclopropyl	–CHO	No[c]
Z	Cyclopropyl	$–CH_2OMe$	No[c]
AA	Ph/Cyclopropyl	$–CO_2Me$	No[c]
AB	Ph/Cyclopropyl	$–CH_3$	No[c]
AC	Ph/Cyclopropyl	–H	No[c,d]

[a] (*R*)-Configuration unless otherwise stated. [b] Published data of HA-assay.[70,76] [c] Precipitated. [d] Synthesized from **D** according to a published decarboxylation procedure.[65]

Mode of action and biological effects. The pilicides had been shown to bind to chaperones, which are key proteins in the chaperone–usher pathway. However, more detailed studies to elucidate the pilicide mode of action at a molecular level led to reconsideration of the initial hypothesis of pilicides as competitive inhibitors of chaperone–subunit association. First, the binding location was investigated using NMR spectroscopy and chemical shift mapping with ^{15}N-labelled chaperone FimC.[77] As anticipated, binding of the pilicides induced chemical shift changes in the cleft region of FimC through which the pilus subunits are anchored to Arg8 and Lys112, but unexpectedly the pilicides also affected a second location near the flexible F1-G1 loop of the chaperone (Fig. 2b). This site was known to be part of a preserved hydrophobic surface that is involved in the interactions with the N-terminal of the usher and could thus be targeted to block the subunit delivery process. Subsequent X-ray crystallography of a pilicide–PapD complex[67] verified this latter binding site and the competitive binding to the N-terminal of the usher was further confirmed by a binding assay using surface plasmon resonance (SPR), showing that binding between chaperone–subunit complex FimC–FimH and the N-terminal of the usher FimD was prohibited in the presence of a pilicide.[67] This interaction is critical in the pilus assembly process and accordingly, the ability of pilicides to interfere with this event could be monitored as reduced pili abundance. The relevance of the proposed mechanism of action was further supported by a single-site mutation of Arg58 in the pilicide binding site in PapD. The Arg58 deficient PapD mutant was indeed able to bind and stabilize subunits, while it was incapable of pilus assembly *in vivo*.

An overview of the biological evaluation is presented in Fig. 7. Hemagglutination[67,69,70,76] and biofilm assays[67] have shown that the formation of both type 1 and P pili could be inhibited. Consistently, the impaired pili biogenesis was also reflected by the loss in UPEC's ability to colonize bladder cells.[67]

The pilicides exhibit a dose-dependent regulatory effect on pilus assembly, as shown by their titratable effects on biofilm formation and HA and also using Western immunoblot analysis and electron- and atomic force microscopy.[67,78] Thus, pilicides display a potential utility as chemical tools to study the role of

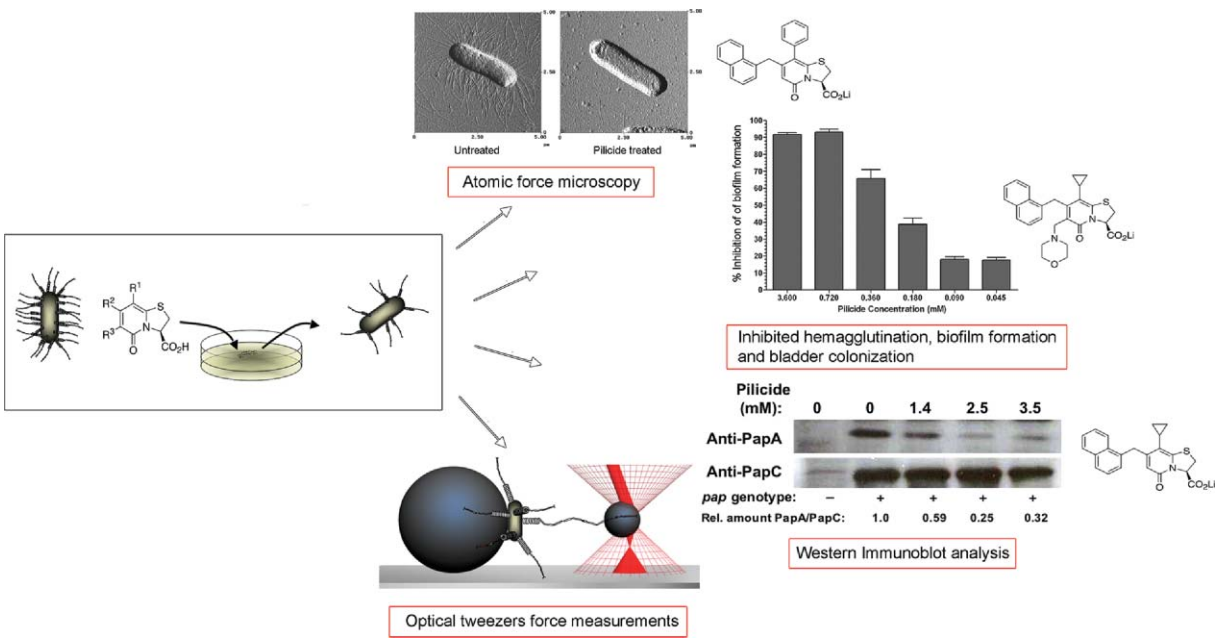


Fig. 7 Schematic illustration of how pilicides have been demonstrated to regulate pilus biogenesis using electron microscopy, atomic force microscopy,[67,78] Western immunoblot analysis[78] and assays of hemagglutination,[67,69,71,76,78] biofilm formation and adherence.[67] In addition, optical tweezers force measurements have verified that sub-inhibitory concentrations of pilicides suppress pili abundance, but do not affect the biofunctional properties of assembled pili.[78] (See text and references for details.) Electron micrographs courtesy of Jana Jass.

pili at stages important for the establishment and persistence of an infection. A prerequisite for this application was that the pilicides did not affect the properties of the pilus rod, which had previously been demonstrated by other research groups to influence adhesion and adaptive ability to shear forces. We addressed this issue by comparing the biophysical properties of individual pilus fibers expressed either on pilicide-treated or normal, untreated, *E. coli*.[78] The expression of P pili in *E. coli* was first suppressed by pilicide treatment and, next, the biophysical properties of the pilus rod were investigated using force measuring optical tweezers. The biodynamic properties of pili fibers (formed in suppressed amounts under pilicide treatment) were found to be intact and the presented results establish a potential use of pilicides as chemical tools to study important biological processes such as pilus biogenesis and the role of pili in critical events during an infection cascade.

Future

Our research on pilicides represents one example of how small synthetic molecules can serve as tools to elucidate fundamental knowledge of bacterial virulence. A robust chemical platform for synthesis of highly substituted, bicyclic 2-pyridones has been created to give access to compounds that have already shown value as chemical tools to gain insights into molecular details of the chaperone–usher pathway, an essential virulence mechanism in a number of pathogenic bacteria. In a future perspective, these studies could aid the identification of new antibacterial targets and thus, the development of new therapeutics. Given the pilicides' mode of action, a prospective broad-spectrum activity can be anticipated and remains to be investigated.

From a more general viewpoint, finding antibiotics with novel functions, such as inhibitors of virulence, must be considered crucial. The industrial downscaling of antibacterial research in past years has slowed this process at the same time as the level of scientific challenge becomes increasingly obvious. The hurdles and limitations of target based screening approaches were recently described in a report from GlaxoSmithKline, presenting largely unfruitful results over a period of seven years.[79] Here, they also emphasize the benefits of performing whole-cell screening assays, since their experience suggests that it is easier to identify the cellular target of an antibacterial compound than it is to engineer permeability into an enzyme inhibitor. Interestingly, they also clearly stress the need for more chemists in the antibacterial therapeutic area, an area that has been dominated by genomics during the past decade.

With very few exceptions, marketed antibiotics originate from a prevailing "me-too" concept where derivatives of already existing drugs are directed against the same bacterial targets. This is accompanied with a great risk, since derivatives within one class of antibacterials can be undermined by a single resistance mechanism. In contrast, chemical attenuation of virulence would not only offer new targets but is also believed to induce resistance at a much slower rate. The utility and efficiency of virulence inhibitors remain unknown until their impact has been investigated in relevant model systems or, ideally, *in vivo* where adequate host defenses are present. Presumably, the potency of anti-virulence inhibitors will be acknowledged first when acting synergistically with innate defenses. Another prospective is co-administration together with traditional antibiotics. Finally, although the envisioned therapeutic value of small-spectrum virulence inhibitors depends on the development of new and efficient tools to identify the disease causing pathogen in clinics, the potential applications are intriguing and remain to be further explored.

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References

1 R. M. Goldschmidt, M. J. Macielag, D. J. Hlasta and J. F. Barrett, *Curr. Pharm. Des.*, 1997, **3**, 125.
2 M. N. Alekshun and S. B. Levy, *Drug Discovery Today: Ther. Strategies*, 2004, **70**, 451.
3 A. Marra, *Drugs R&D*, 2006, **7**, 1.
4 K. A. Melström, J. W. Smith, R. L. Gamelli and R. Shankar, *J. Burn Care Res.*, 2006, **27**, 251.
5 M. Hentzer, H. Wu, J. B. Andersen, K. Riedel, T. B. Rasmussen, N. Bagge, N. Kumar, M. A. Schembri, Z. J. Song, P. Kristoffersen, M. Manefield, J. W. Costerton, S. Molin, L. Eberl, P. Steinberg, S. Kjelleberg, N. Hoiby and M. Givskov, *EMBO J.*, 2003, **22**, 3803.
6 R. B. Raffa, J. R. Iannuzzo, D. R. Levine, K. K. Saeid, R. C. Schwartz, N. T. Sucic, O. D. Terleckyj and J. M. Young, *J. Pharmacol. Exp. Ther.*, 2005, **312**, 417.
7 T. B. Rasmussen and M. Givskov, *Int. J. Med. Microbiol.*, 2006, **296**, 149.
8 S. Müller, M. F. Feldman and G. R. Cornelis, *Expert Opin. Ther. Targets*, 2001, **5**, 327.
9 D. J. Musk and P. J. Hergenrother, *Curr. Med. Chem.*, 2006, **13**, 2163.
10 J. W. Costerton, P. S. Stewart and E. P. Greenberg, *Science*, 1999, **284**, 1318.
11 Y. M. Lee, F. Almqvist and S. J. Hultgren, *Curr. Opin. Pharmacol.*, 2003, **3**, 513.
12 D. Davies, *Nat. Rev. Drug Discovery*, 2003, **2**, 114.
13 S. M. Soto, A. Smithson, J. P. Horcajada, J. A. Martinez, J. P. Mensa and J. Vila, *Clin. Microbiol. Infect.*, 2006, **12**, 1034.
14 G. E. Ward, K. L. Carey and N. J. Westwood, *Cell. Microbiol.*, 2002, **4**, 471.
15 D. P. Walsh and Y. T. Chang, *Chem. Rev.*, 2006, **106**, 2476.
16 A. M. Kauppi, R. Nordfelth, H. Uvell, H. Wolf-Watz and M. Elofsson, *Chem. Biol.*, 2003, **10**, 241.
17 R. Nordfelth, A. M. Kauppi, H. A. Norberg, H. Wolf-Watz and M. Elofsson, *Infect. Immun.*, 2005, **73**, 3104.
18 A. Gauthier, M. L. Robertson, M. Lowden, J. A. Ibarra, J. L. Puente and B. B. Finlay, *Antimicrob. Agents Chemother.*, 2005, **49**, 4101.
19 S. Muschiol, L. Bailey, A. Gylfe, C. Sundin, K. Hultenby, S. Bergstrom, M. Elofsson, H. Wolf-Watz, S. Normark and B. Henriques-Normark, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 14566.
20 K. Wolf, H. J. Betts, B. Chellas-Gery, S. Hower, C. N. Linton and K. A. Fields, *Mol. Microbiol.*, 2006, **61**, 1543.
21 D. T. Hung, E. A. Shakhnovich, E. Pierson and J. J. Mekalanos, *Science*, 2005, **310**, 670.

22 G. D. Geske, R. J. Wezeman, A. P. Siegel and H. E. Blackwell, *J. Am. Chem. Soc.*, 2005, **127**, 12762.
23 F. G. Sauer, H. Remaut, S. J. Hultgren and G. Waksman, *Biochim. Biophys. Acta*, 2004, **1694**, 259.
24 D. Hung and S. J. Hultgren, *J. Struct. Biol.*, 1998, **124**, 201.
25 D. G. Thanassi, E. T. Saulino and S. J. Hultgren, *Curr. Opin. Microbiol.*, 1998, **1**, 223.
26 F. G. Sauer, K. Futterer, J. S. Pinkner, K. W. Dodson, S. J. Hultgren and G. Waksman, *Science*, 1999, **285**, 1058.
27 F. Lindberg, J. M. Tennent, S. J. Hultgren, B. Lund and S. Normark, *J. Bacteriol.*, 1989, **171**, 6052.
28 P. Klemm, *Res. Microbiol.*, 1992, **143**, 831.
29 M. J. Kuehn, D. J. Ogg, J. Kihlberg, L. N. Slonim, K. Flemmer, T. Bergfors and S. J. Hultgren, *Science*, 1993, **262**, 1234.
30 D. L. Hung, S. D. Knight and S. J. Hultgren, *Mol. Microbiol.*, 1999, **31**, 773.
31 D. G. Thanassi, C. Stathopoulos, K. Dodson, D. Geiger and S. J. Hultgren, *J. Bacteriol.*, 2002, **184**, 6260.
32 M. Nishiyama, M. Vetsch, C. Puorger, I. Jelesarov and R. Glockshuber, *J. Mol. Biol.*, 2003, **330**, 513.
33 T. W. Ng, L. Akman, M. Osisami and D. G. Thanassi, *J. Bacteriol.*, 2004, **186**, 5321.
34 M. Nishiyama, R. Horst, O. Eidam, T. Herrmann, O. Ignatov, M. Vetsch, P. Bettendorff, I. Jelesarov, M. G. Grutter, K. Wuthrich, R. Glockshuber and G. Capitani, *EMBO J.*, 2005, **24**, 2075.
35 S. S. K. So and D. G. Thanassi, *Mol. Microbiol.*, 2006, **60**, 364.
36 M. J. Kuehn, J. Heuser, S. Normark and S. J. Hultgren, *Nature*, 1992, **356**, 252.
37 K. W. Dodson, J. S. Pinkner, T. Rose, G. Magnusson, S. J. Hultgren and G. Waksman, *Cell*, 2001, **105**, 733.
38 G. Källenius, R. Mollby, S. B. Svenson, J. Winberg, A. Lundblad, S. Svensson and B. Cedergren, *FEMS Microbiol. Lett.*, 1980, **7**, 297.
39 H. Leffler and C. Svanborg-Edén, *Infect. Immun.*, 1981, **34**, 920.
40 S. N. Abraham, D. X. Sun, J. B. Dale and E. H. Beachey, *Nature*, 1988, **336**, 682.
41 K. A. Krogfelt, H. Bergmans and P. Klemm, *Infect. Immun.*, 1990, **58**, 1995.
42 J. J. Martinez, M. A. Mulvey, J. D. Schilling, J. S. Pinkner and S. J. Hultgren, *EMBO J.*, 2000, **19**, 2803.
43 G. A. O'Toole and R. Kolter, *Mol. Microbiol.*, 1998, **28**, 449.
44 G. G. Anderson, J. J. Palermo, J. D. Schilling, R. Roth, J. Heuser and S. J. Hultgren, *Science*, 2003, **301**, 105.
45 S. S. Justice, C. Hung, J. A. Theriot, D. A. Fletcher, G. G. Anderson, M. J. Footer and S. J. Hultgren, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 1333.
46 S. S. Justice, D. A. Hunstad, P. C. Seed and S. J. Hultgren, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 19884.
47 M. A. Mulvey, Y. S. Lopez-Boado, C. L. Wilson, R. Roth, W. C. Parks, J. Heuser and S. J. Hultgren, *Science*, 1998, **282**, 1494.
48 M. J. Duncan, E. L. Mann, M. S. Cohen, I. Ofek, N. Sharon and S. N. Abraham, *J. Biol. Chem.*, 2005, **280**, 37707.
49 J. Jass, S. Schedin, E. Fällman, J. Ohlsson, U. J. Nilsson, B. E. Uhlin and O. Axner, *Biophys. J.*, 2004, **87**, 4271.
50 E. Fällman, S. Schedin, J. Jass, B. E. Uhlin and O. Axner, *EMBO Rep.*, 2005, **6**, 52.
51 M. Forero, O. Yakovenko, E. V. Sokurenko, W. E. Thomas and V. Vogel, *PLoS Biol.*, 2006, **4**, 1509.
52 E. Miller, T. Garcia, S. Hultgren and A. F. Oberhauser, *Biophys. J.*, 2006, **91**, 3848.
53 W. E. Thomas, E. Trintchina, M. Forero, V. Vogel and E. V. Sokurenko, *Cell*, 2002, **109**, 913.
54 A. Svensson, A. Larsson, H. Emtenäs, M. Hedenström, T. Fex, S. J. Hultgren, J. S. Pinkner, F. Almqvist and J. Kihlberg, *ChemBioChem*, 2001, **2**, 915.
55 D. L. Hung, S. D. Knight, R. M. Woods, J. S. Pinkner and S. J. Hultgren, *EMBO J.*, 1996, **15**, 3792.
56 A. Holmgren and C. I. Branden, *Nature*, 1989, **342**, 248.
57 C. H. Jones, J. S. Pinkner, A. V. Nicholes, L. N. Slonim, S. N. Abraham and S. J. Hultgren, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 8397.
58 M. Pellecchia, P. Guntert, R. Glockshuber and K. Wuthrich, *Nat. Struct. Biol.*, 1998, **5**, 885.
59 G. E. Soto, K. W. Dodson, D. Ogg, C. Liu, J. Heuser, S. Knight, J. Kihlberg, C. H. Jones and S. J. Hultgren, *EMBO J.*, 1998, **17**, 6155.
60 D. Choudhury, A. Thompson, V. Stojanoff, S. Langermann, J. Pinkner, S. J. Hultgren and S. D. Knight, *Science*, 1999, **285**, 1061.
61 H. Emtenäs, L. Alderin and F. Almqvist, *J. Org. Chem.*, 2001, **66**, 6756.
62 H. Emtenäs, C. Taflin and F. Almqvist, *Mol. Diversity*, 2003, **7**, 165.
63 H. Emtenäs, K. Åhlin, J. S. Pinkner, S. J. Hultgren and F. Almqvist, *J. Comb. Chem.*, 2002, **4**, 630.
64 N. Pemberton, L. Jakobsson and F. Almqvist, *Org. Lett.*, 2006, **8**, 935.
65 V. Åberg, F. Norman, E. Chorell, A. Westermark, A. Olofsson, A. E. Sauer-Eriksson and F. Almqvist, *Org. Biomol. Chem.*, 2005, **3**, 2817.
66 H. Emtenäs, *Synthesis and Biological Evaluation of Bicyclic* β*-lactams and 2-pyridinones – Pilicides targeting Pilus Biogenesis in Pathogenic Bacteria*, Umeå University, Umeå, 2003.
67 J. S. Pinkner, H. Remaut, F. Buelens, E. Miller, V. Åberg, N. Pemberton, M. Hedenström, A. Larsson, P. Seed, G. Waksman, S. J. Hultgren and F. Almqvist, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 17897.
68 N. Pemberton, V. Åberg, H. Almstedt, A. Westermark and F. Almqvist, *J. Org. Chem.*, 2004, **69**, 7830.
69 N. Pemberton, J. S. Pinkner, L. Jakobsson, S. J. Hultgren and F. Almqvist, *Tetrahedron Lett.*, under revision.
70 V. Åberg, M. Sellstedt, M. Hedenström, J. S. Pinkner, S. J. Hultgren and F. Almqvist, *Bioorg. Med. Chem.*, 2006, **14**, 7563.
71 V. Åberg, *Peptidomimetics based on ring-fused 2-pyridones – Probing pilicide function in uropathogenic* E. coli *and identification of A*β*-peptide aggregation inhibitors*, Umeå University, Umeå, 2006.
72 J. Cluzeau and W. D. Lubell, *Biopolymers*, 2005, **80**, 98.
73 P. S. Dragovich, T. J. Prins, R. Zhou, T. O. Johnson, Y. Hua, H. T. Luu, S. K. Sakata, E. L. Brown, F. C. Maldonado, T. Tuntland, C. A. Lee, S. A. Fuhrman, L. S. Zalman, A. K. Patick, D. A. Matthews, E. Y. Wu, M. Guo, B. C. Borer, N. K. Nayyar, T. Moran, L. J. Chen, P. A. Rejto, P. W. Rose, M. C. Guzman, E. Z. Dovalsantos, S. Lee, K. McGee, M. Mohajeri, A. Liese, J. H. Tao, M. B. Kosa, B. Liu, M. R. Batugo, J. P. R. Gleeson, Z. P. Wu, J. Liu, J. W. Meador and R. A. Ferre, *J. Med. Chem.*, 2003, **46**, 4572.
74 P. S. Dragovich, T. J. Prins, R. Zhou, T. O. Johnson, E. L. Brown, F. C. Maldonado, S. A. Fuhrman, L. S. Zalman, A. K. Patick, D. A. Matthews, X. J. Hou, J. W. Meador, R. A. Ferre and S. T. Worland, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 733.
75 X. J. Zhang, A. C. Schmitt and C. P. Decicco, *Tetrahedron Lett.*, 2002, **43**, 9663.
76 V. Åberg, M. Hedenström, J. S. Pinkner, S. J. Hultgren and F. Almqvist, *Org. Biomol. Chem.*, 2005, **3**, 3886.
77 M. Hedenström, H. Emtenäs, N. Pemberton, V. Åberg, S. J. Hultgren, J. S. Pinkner, V. Tegman, F. Almqvist, I. Sethson and J. Kihlberg, *Org. Biomol. Chem.*, 2005, **3**, 4193.
78 V. Åberg, E. Fällman, O. Axner, B. E. Uhlin, S. Hultgren and F. Almqvist, *Mol. BioSyst.*, 2007, **3**, 214.
79 D. J. Payne, M. N. Gwynn, D. J. Holmes and D. L. Pompliano, *Nat. Rev.Drug Discovery*, 2006, **6**, 29.

