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C-Terminal properties are important for ring-fused 2-pyridones that interfere with the chaperone function in uropathogenic *E. coli*†

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Virulence-associated organelles, termed pili or fimbriae, are assembled *via* the highly conserved chaperone–usher pathway in a vast number of pathogenic bacteria. Substituted bicyclic 2-pyridones, *pilicides*, inhibit pilus formation, possibly by interfering with the active site residues Arg8 and Lys112 of chaperones in uropathogenic *E. coli*. In this article we describe the synthesis and evaluation of nine analogues of a biologically active pilicide. Derivatization was performed with respect to its C-terminal features and the affinities for the chaperone PapD were studied with ^{1}H relaxation-edited NMR spectroscopy. It could be concluded that the carboxylic acid functionality and also its spatial location was important for binding. In all cases, binding was significantly reduced or even abolished when the carboxylic acid was replaced by other substituents. In addition, *in vivo* results from a hemagglutination assay are presented where the derivatives have been evaluated for their ability to inhibit pilus formation in uropathogenic *E. coli*.

Introduction

Targeting bacterial pathogenicity in general and, more specifically, pilus biogenesis is considered an attractive approach to overcoming existing and increasing antibiotic resistance.[1,2] The virulence of uropathogenic *E. coli* (UPEC) depends on adhesive organelles that are expressed on the cell surface of the bacteria. These organelles, termed pili or fimbriae, are assembled *via* the conserved chaperone–usher pathway present in a large number of pathogenic microbes.[3] UPEC is the most common cause of urinary tract infections (UTIs) and require pili for the recognition of, and attachment to, host cells.

Pili are macromolecular fibers consisting 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). Altogether, the repeating subunits form a right-handed helical structure of the pilus fiber where the major component is PapA or FimA in P pili and type 1 pili, respectively. The carbohydrate-recognizing subunit called the adhesin (PapG or FimH) is located at the tip of each pilus fiber.[4,5] P pili mediate kidney infections (pyelonephritis) while type 1 pili are related to bladder infections (cystitis).[6–8] Recently, pili have also been shown to be present in the later stages of the infection cascade, possibly facilitating escape of host defences and thereby causing severe problems with recurrent UTIs.[9]

In brief, pilus assembly *via* the chaperone–usher pathway proceeds in a three-step process. First, pilus subunits are translocated into the periplasm where they fold and form stable chaperone–subunit complexes together with the chaperone PapD or FimC. Then the chaperones transport the subunits through the periplasm and target an outer membrane assembly site in the form of an usher (PapC or FimD). Finally, the subunit is incorporated into the growing pilus rod.[3]

The subunits have an immunoglobulin (Ig)-like fold but lack their seventh β-strand.[10] Owing to this they are unstable as monomers and easily misfold and/or aggregate, leading to proteolytical degradation.[11,12] Stabilization of the subunits is possible if their Ig-fold is completed by an external source. Either the lacking β-strand is provided by (i) the N-terminal of its neighboring subunit as in the mature pilus fiber, or by (ii) the G1 β-strand of the chaperone as in the chaperone–subunit complex.[13–15] Thus, when the complex is dissociated at the usher location, the chaperone G1 β-strand is replaced by the N-terminal of the most recently incorporated subunit in a 'donor-strand exchange' mechanism. Subunit stabilization by the chaperone is termed 'donor-strand complementation'.[3,16]

The features of the subunits necessitate chaperones for subunit folding, stabilization and transport, and chaperone-deficient strains are unable to produce pili.[17,18]

The crucial role of the chaperones in pilus biogenesis has resulted in thorough studies of their structures and function with biochemical methods, genetics, and X-ray crystallography.[10,14,15,19,20] Specific chaperone–subunit interactions anchor the subunit C-terminus into the cleft between two Ig-like domains of the boomerang-shaped chaperone. Importantly, the binding site on the chaperone is highly conserved throughout the chaperone family.[12,21] The high sequence homology is reflected by the fact that PapD is capable of assembling type 1 pili.[18] The C-terminal carboxylate of the subunit forms ionic- and hydrogen-bonds to the invariant chaperone residues Arg8 and Lys112. These residues are conserved in numerous chaperones and it has been shown that single site mutations of Arg8 or Lys112 in PapD abolish subunit binding and, thus, pilus assembly.[19] Recent reports also conclude that the folding of the subunits is chaperone-catalyzed and that Arg8 is essential in this event.[22,23]

Studies of subunits and subunit peptides provide additional information of the important subunit–chaperone interactions. Length series of subunit peptides have previously been evaluated for the inhibitory activity of PapD and FimC using inhibition ELISA (enzyme-linked immunosorbent assay). The C-terminal 8-mer peptide of PapG was reported to function as an inhibitor of PapD and to have a significantly higher potency than the corresponding 7- and 6-mer peptide.[24] However, the peptide C-terminal carboxylate has been argued to be of only minor

† Electronic supplementary information (ESI) available: ^{13}C NMR spectra of compounds **3**–**10**, and chiral HPLC chromatograms and conditions for compounds (+)-**1**, (−)-**3** and (−)-**5**. See http://dx.doi.org/10.1039/b509376g

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importance for the anchoring to PapD based on studies of a reduced 8-mer (PapG307-314*red*), where the carboxylate was replaced with a methyl group, and a proline-deficient derivative PapG306-313. Both peptides retained all or most of their inhibitory power.[24] Still, the subunit C-terminal is essential for subunit–chaperone complex formation. For example, it has been shown that deletion of the 13 C-terminal residues of the PapG adhesin prevented complex formation with PapD.[11]

In view of the detailed knowledge of chaperone–subunit interactions we believed that a small synthetic compound that specifically targets the active site residues of the chaperone could inhibit the formation of chaperone–subunit complexes and thereby block pilus assembly.

We have reported earlier the use of rational design to produce small, non-peptidic molecules, *pilicides*, which interfere with the critical subunit–chaperone interactions.[25] Three classes of compounds have been synthesized to mimic the C-terminus of PapG and one of these classes, substituted 2-pyridone carboxylic acids, is illustrated in Fig. 1.[26–32] Several compounds displayed affinity for the chaperones PapD and FimC and the most potent binders were found to be the bicyclic 2-pyridones. The R^2 substituent mimics the hydrophobic properties of Phe313 and Leu311 in PapG, and further substitutions of positions R^1 and R^3 provide improved interaction possibilities. This class of compounds also proved to dissociate chaperone–subunit (FimC–FimH) complexes and exhibited *in vivo* activity, if it had a suitable substitution pattern.[25,33] Pili formation of several different UPEC strains producing both P pili and type 1 pili was blocked by the compounds in a dose-dependent manner.[33] The disruption of pilus biogenesis, when incubating with pilicide, was assayed by hemagglutination (HA), biofilm formation, and colonization ability. The reduced levels of pili were also visually confirmed with electron microscopy and general bacterial toxicity was ruled out with growth studies. Included as a control was an inactive 2-pyridone with an intact 2-pyridone scaffold but where the important hydrophobic naphthyl moiety in R^2 had been exchanged for a small methyl substituent. This negative control did not affect pilus biogenesis as determined in a HA-assay.[33]

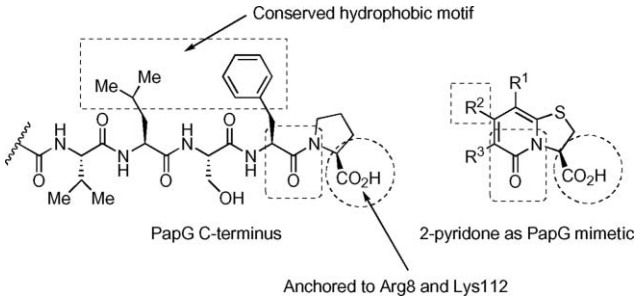


Fig. 1 Substituted 2-pyridones are designed to inhibit chaperones as C-terminal mimetics of the pilus adhesin PapG.

NMR spectroscopy has been used for more detailed studies of the pilicide–chaperone interactions.[34] It could be concluded that the di-substituted 2-pyridones (R^1 and R^2) displayed low mM affinity for PapD. Furthermore, ^{15}N-labeled FimC was used in an HSQC (heteronuclear single quantum coherence) experiment to locate their binding site as judged by induced chemical shift changes of the residues affected upon binding of the pilicide. This study revealed two possible binding sites for the biologically active 2-pyridones: one located in the cleft, involving Arg8 and Lys112, and one located at the flexible, so-called F1–G1 loop of the chaperone (Fig. 2).[34]

Although the F1–G1 loop was not the primary target binding site it is still plausible that interacting with that region could prevent pilus assembly since it participates in the donor-strand complementation process.[3] In addition to the described *in vivo*

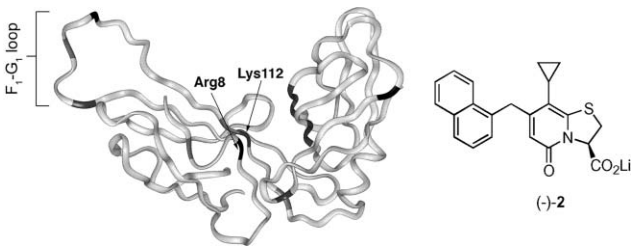


Fig. 2 Affected residues of ^{15}N-labeled chaperone FimC (marked in dark grey) upon the binding of pilicide (−)-**2**.[34]

studies, the theorized chaperone effect is supported also by other studies. First, pilicides have been shown to dissociate FimC–FimH complexes in an FPLC (fast protein liquid chromatography) assay[25] and, secondly, pilicides compete with the C-terminal peptide of PapG for the chaperone binding site as shown in NMR spectroscopy studies.[34] The chaperone affinity of the same peptide has been evaluated with surface plasmon resonance and displayed binding in the same range as the pilicides.[25]

Here we describe the syntheses and evaluation of nine different analogues of the di-substituted 2-pyridone (−)-**2** that exhibits pilicide activity *in vivo* and has been suggested to interact with the invariant residues Arg8 and Lys112 involved in the anchoring of pilus subunits. Derivatization has been performed with respect to its carboxylic acid functionality in order to elucidate the importance of the C-terminal character of the 2-pyridones for chaperone affinity. The objective was to establish structure–activity relationships (SARs) for pilicide–chaperone interactions and to use these SARs to outline a direction of future synthetic work. If it could be shown that the carboxylic acid was important it would support the preceding design and encourage research on affinity enhancement with substituents R^1–R^3. The carboxylic acid could either be preserved or replaced by an isoster. On the other hand, high acceptance for changes would enable utilization of this position to address future issues such as pharmacokinetic properties. Relative binding data for the analogues to chaperone PapD are presented as determined in a ^{1}H relaxation-edited NMR spectroscopic assay. The analogues have also been evaluated in a hemagglutination assay for their ability to inhibit pili formation *in vivo*.

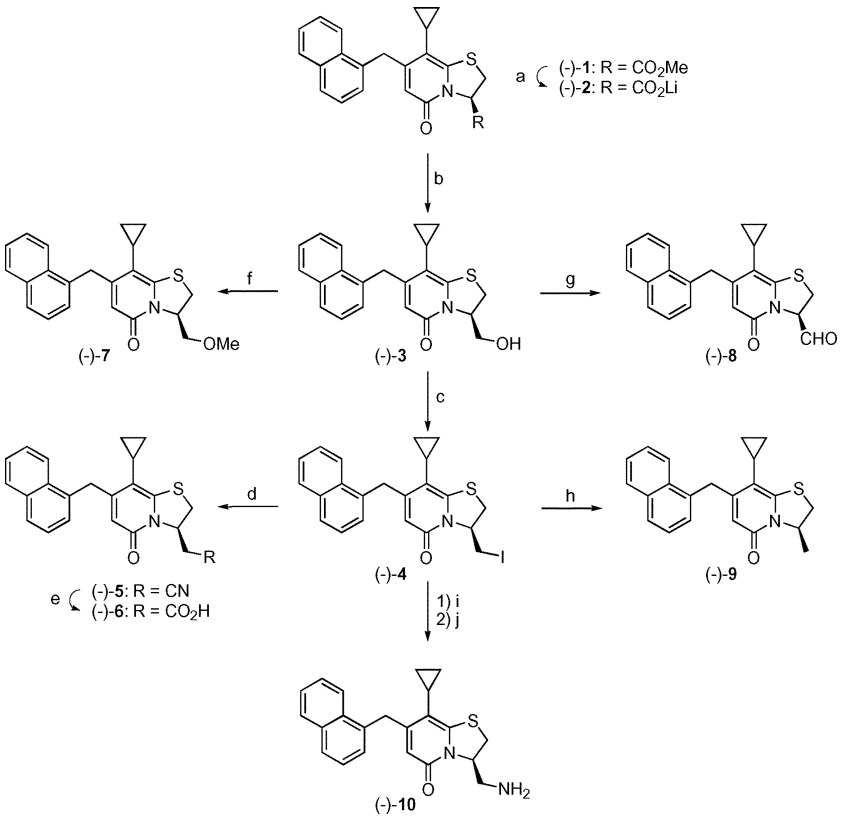
Results

Synthesis of analogues

A set of nine analogues of the biologically active pilicide (−)-**2** was synthesized with the intention to obtain derivatives with varying charge, polarity and hydrogen bonding properties (Scheme 1, compounds **1**, **3**, and **7**–**10**). The influence of the spatial location of the carboxylic acid was studied by including the enantiomer (+)-**2**, as well as a carboxylic acid derivative, which was extended with one carbon atom, (−)-**6**.

The methyl ester (−)-**1** was produced in five steps according to previously published procedures[27,29] and the corresponding lithium carboxylate (−)-**2** was obtained by saponification (Scheme 1). A straightforward borane reduction of (−)-**2** afforded the alcohol (−)-**3** in excellent yield and without racemization (see Experimental section and ESI† for details regarding enantiomeric purity). DMP (Dess–Martin periodinane) oxidation of the alcohol (−)-**3** afforded the aldehyde (−)-**8**, and the methyl ether (−)-**7** was obtained from (−)-**3** in a Williamson's ether synthesis.

Alcohol (−)-**3** also served as precursor for the iodinated derivative (−)-**4**, which was obtained in 76% yield. The moderate yield was due to formation of an elimination byproduct. $LiEt_3BH$ reduction of (−)-**4** yielded the methyl analogue (−)-**9**. (−)-**4** also served as a key intermediate for the synthesis of the one-carbon extended carboxylic acid (−)-**6**. The iodine was cyano-substituted to (−)-**5** and subsequent hydrolysis was



Scheme 1 *Reagents and conditions*: (a) 0.1 M aq. LiOH, MeOH–THF (4 : 1), rt, quant.; (b) $BH_3 \cdot Me_2S$, THF, rt, 98%; (c) PPh_3, I_2, imidazole, toluene, rt, 82%; (d) KCN, DMF, 70 °C, 70%; (e) 2 M aq. NaOH, EtOH, MW 130 °C, 300 s, 71%; (f) NaH, MeI, THF, rt, 98%; (g) Dess–Martin periodinane, CH_2Cl_2, rt, 85%; (h) $LiEt_3BH$, THF, rt, 82%; (i) NaN_3, DMF, rt, 6 h, then (j) Pd/C (10 wt%), atm. H_2(g) 12 h, 87%.

performed using microwave heating. The alkaline hydrolysis of (−)-**5** proceeded smoothly and full conversion of starting material to the desired product (−)-**6** was observed within 5 min at 130 °C. The cyanation was also accompanied by the elimination byproduct observed in the iodination step, which explains the moderate yield of 70%. Finally, the amino-functionalized analogue (−)-**10** was synthesized from the iodo-derivate (−)-**4** in a one-pot procedure *via* the corresponding azide intermediate. The azide was not isolated but could be reduced directly by adding Pd/C to the crude reaction mixture, followed by hydrogenation at atmospheric pressure.

In addition to these eight analogues the (*S*)-enantiomer (+)-**2** was synthesized according to the same procedure as for (−)-**2**, but starting from D-cysteine. In total, a collection of nine compounds with varying polarity, charge, hydrogen bonding properties, and spatial arrangement of the carboxylate was produced and evaluated.

Ranking of chaperone affinity and evaluation of *in vivo* activity as pilicides

Relaxation-edited one-dimensional ^{1}H NMR spectroscopy was used to estimate the affinities of the nine pilicide analogues for the chaperone PapD.[35] The technique relies on the fact that when a small ligand binds to a large protein it adopts the slow tumbling rate and fast T_2 relaxation of the protein. Hence, by applying an appropriate spin-lock filter it is possible to remove the signals from both the protein and the bound pilicide and only signals originating from the free pilicide are detected. This discrimination results in a reduced signal intensity from the pilicide if it binds to PapD (Fig. 3). In this study, 1-naphthylacetic acid was included as a non-binding control to allow determination of the reduced signal intensities of the pilicides in the presence of PapD (pilicide–PapD, 1 : 4). The relative affinities of the pilicides are given as percentages compared to the parent compound (−)-**2** (100%, Table 1, entry 1).

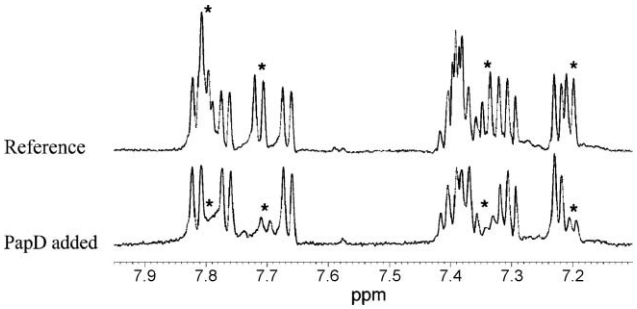
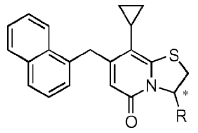


Fig. 3 Pilicide affinity for PapD was determined with relaxation-edited NMR spectroscopy. Shown here is the reduction in signal intensity upon binding of pilicide (−)-**2** (marked with*) compared with a non-binding control, 1-naphthylacetic acid (unmarked).

The analogues were also evaluated for their *in vivo* effect in a hemagglutination (HA) assay with the P pili-producing *E. coli* strain HB101/pPAP5. Bacteria were incubated in the presence of pilicide (3.5 mM in agar) and the expression of pili was compared to an untreated control. The results are presented in Table 1, where a low HA-titer denotes reduced levels of pili, while the number four represents an unaffected expression of pili, comparable to the fully piliated control. It is worth noting that several of the analogues precipitated in the agar and, consequently, reliable data on their biological activities could not be determined.

Transformation of the carboxylic acid into other substituents led to substantially reduced binding (0–62%). The analogues (−)-**7**, (−)-**1**, and (−)-**9** did not bind to the target chaperone PapD (Table 1, entry 7–9, Relative affinity) whereas the five derivatives (+)-**2**, (−)-**3**, (−)-**6**, (−)-**8**, and (−)-**10** did bind, although with a reduced affinity (11–62%, Table 1, entry 2–6) compared with (−)-**2**. The carboxylic acid-functionalized enantiomers (+)-**2** and (−)-**2** exhibited comparable *in vivo* activities and both reduced the expression of P pili in *E. coli*

Table 1 Relative binding of analogues to chaperone PapD, and pilicide activity on P pili-producing *E. coli* HB101/pPAP5



Entry	Compound	*R	Relative affinity (%)[a]	HA-titer (no of wells)[g]
1	(−)-**2**	$-CO_2Li$	100[b]	0
2	(+)-**2**	$-CO_2Li$	58[c]	0
3	(−)-**6**	$-CH_2CO_2Li$	62[b]	0/1
4	(−)-**10**	$-CH_2NH_2$	60[b]	[e,f]
5	(−)-**3**	$-CH_2OH$	25[b]	4[f]
6	(−)-**8**	–CHO	11[b]	4[f]
7	(−)-**7**	$-CH_2OMe$	0[d]	4[f]
8	(−)-**1**	$-CO_2Me$	0[d]	4[f]
9	(−)-**9**	–Me	0[d]	4[f]

[a] 6% RSD from triplicate runs. [b] 84% ee. [c] 78% ee. [d] 30% ee. [e] Inhibition of bacterial growth. [f] Precipitation of compound in agar. [g] Number of wells with agglutination, see Experimental section for more details.

(Table 1, entry 1–2, HA-titer). Also the one-carbon extended carboxylic acid (−)-**6** was biologically active but slightly less potent than the other carboxylic acids (Table 1, entry 3). The amine (−)-**10** inhibited bacterial growth at a 3.5 mM concentration and could not be evaluated for its effect on pilus assembly.

Discussion

The carboxylic acid-functionalized 2-pyridone (−)-**2** was found to be the most potent binder among the nine investigated analogues. Except for the three carboxylates, derivatives unable to interact as hydrogen bond donors displayed a significantly reduced (Table 1, entries 7–9) or completely abolished affinity (Entry e–9) for PapD. The acceptance for transformations into hydrogen bond donating substituents, *i.e.* alcohol (−)-**3** and amine (−)-**10**, was higher, although a drop in affinity was seen also for these derivatives. Interestingly, a decrease in binding was also observed for both the (*S*)-enantiomer (+)-**2**, and for the one-carbon extended carboxylic acid (−)-**6** (Table 1, entry 2–3). Thus, the spatial arrangement of the carboxylic acid appears to be of importance for binding to PapD. It can be speculated that the beneficial (*R*)-configuration of the carboxylate, which is also present in the C-terminal Pro314 of PapG, indicates a specific, and possibly matching, binding site involving Arg8 and Lys112. In contrast to the results obtained in the previous study of the 8-mer peptide of PapG,[24] the C-terminal properties are important for the mimicking 2-pyridones. However, it is not unlikely that the carboxylic acid functionality has a more pronounced role in the smaller, mimicking 2-pyridones, which lack the additional interaction contributions from the rest of the peptide sequence.

Computer modeling using the crystal structure of PapD showed that the targeted binding site is wide enough to allow alternative binding poses with a maintained carboxylate anchoring to Arg8 and Lys112 (not shown). This supports the fact that all three carboxylate-bearing compounds (−)-**2**, (+)-**2** and (−)-**6** were good binders, still with (−)-**2** as the top ranked.

The (*S*)-enantiomer (+)-**2**, the one-carbon extended carboxylic acid (−)-**6**, and the amine (−)-**10** were found to exhibit similar affinities for PapD. The affinity displayed by the positively charged amine cannot be explained at this point. However, regarding the pilicide design as C-terminal mimetics and the proposed anchoring to Arg8 and Lys112, a different binding site is considered possible for this analogue. It should be kept in mind that ^{1}H relaxation-edited NMR spectroscopy does not provide information about the location of the binding.

All carboxylic acid-functionalized compounds displayed pilicide activity *in vivo* (Table 1, entry 1–3, HA-titer) whereas the other analogues did not affect pilus formation. This may result from their poor solubility in the agar. Inhibition of bacterial growth was observed with the amine (−)-**10** and a different mechanism of action for this compound has to be considered. As expected, it is difficult to correlate chaperone affinity *in vitro* (Table 1, Relative affinity) to the complex biological activity *in vivo* (Table 1, HA-titer). However, the biological activity of the carboxylic acids in this study may suggest that this functionality is necessary for solubility reasons and plausibly also for bioavailability and uptake of the pilicides.

Conclusions

A set of nine analogues of a biologically active 2-pyridone has been synthesized. Straightforward transformations rendered derivatives with varying C-terminal features affecting polarity, charge, and hydrogen bond-donating and -accepting properties. Affinity ranking of the analogues was performed using relaxation-edited NMR spectroscopy, and all analogues displayed a significantly reduced or even abolished affinity for PapD compared with the parent carboxylic acid-substituted pilicide (−)-**2**. It can thus be concluded that a carboxylic acid is favorable, given that it has the conserved spatial location of a subunit carboxylate. In contradiction to earlier studied peptides, the chaperone affinity for bicyclic 2-pyridones is clearly dependent on the substituent in the investigated position, and the low tolerance for changes encourage future work to investigate carboxylic acid isosteres. The presented results open up possibilities to enhance chaperone binding with preserved or fine-tuned carboxylic acid functionality in combination with variations of the additional substituents (R^1–R^3) on the 2-pyridone scaffold.

Experimental

General synthesis

All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. CH_2Cl_2 and 1,2-dichloroethane were distilled from calcium hydride. THF was distilled from potassium, and toluene was distilled from sodium. DMF was distilled and dried over 3 Å molecular sieves. EtOH was dried over 3 Å molecular sieves. HCl (g) was passed through concentrated H_2SO_4 prior to use. All microwave reactions were carried out in a monomode reactor (Smith Synthesizer, Biotage AB) using Smith Process Vials™ sealed with Teflon septa and an aluminium crimp top. TLC was performed on Silica Gel 60 F_{254} (Merck) using UV light detection. Flash column chromatography (eluents given in brackets) employed normal phase silica gel (Matrex, 60 Å, 35–70 μm, Grace Amicon). The ^{1}H and ^{13}C NMR spectra were recorded at 298 K with a Bruker DRX-400 spectrometer in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.26 ppm) or $CDCl_3$ (δ_C 77.0 ppm) as internal standard], or MeOH-d_4 [residual CD_2HOD (δ_H 3.30 ppm) or CD_3OD (δ_C 49.0 ppm) as internal standard]. IR spectra were recorded on an ATI Mattson Genesis Series FTIR™ spectrometer. Optical rotations were measured with a Perkin-Elmer 343 polarimeter at 20 °C. Enantiomeric excess was determined with HPLC using an (S, S)-Whelk-O1 column from Chrom Tech AB at a flow rate of 1.0 ml min^{-1} and UV-detection at 254 nm. See ESI† for eluents and retention times. It should be noted that the ee of evaluated batches of (−)-**1**, (−)-**7** and (−)-**9** for PapD affinity was 30% due to the use of a different batch of starting material of (−)-**1**. Importantly, these compounds displayed no affinity for PapD, which rules out the possibility of ambiguous data.

HRMS data were recorded with fast atom bombardment (FAB^+) ionization on a JEOL JMS-SX 102 spectrometer.

Affinity measurements using ^{1}H relaxation-edited NMR spectroscopy

Each sample contained PapD (95 μM), pilicide (25 μM) and 1-naphthylacetic acid (included as a non-binding reference, 25 μM) in phosphate buffer with 5% DMSO-d_6. The samples were prepared from a solution of PapD in phosphate buffer (50 mM, pH 5.9) and stock solutions of pilicide and 1-naphthylacetic acid (2.5 mM in DMSO-d_6). Additional DMSO-d_6 was added to obtain a final 5% content. A 200 ms cpmg spin-lock filter was used to efficiently remove the signals from PapD and bound pilicide. Suppression of the water signal was accomplished with a WATERGATE sequence.[36] Spectra were recorded at 25 °C on a Bruker DRX 600 MHz spectrometer.

In vivo activity evaluated with hemagglutination

E. coli HB101/pPAP5 was grown in the absence or presence of 3.5 mM compound in TSA (trypticase soy agar) plates for 24 h at 37 °C. The plates were prepared from compound stock solutions in pure DMSO and the final DMSO content in the plate was 5%. Positive controls were used from plates containing no compound but still 5% DMSO. 50 μg ml^{-1} carbenicillin was included in all plates. Bacteria were harvested and normalized for optical density in phosphate buffered saline (PBS) pH 7.4 at 540 nm (OD_{540}) before being evaluated for their level of piliation with hemagglutination using rabbit red blood cells (RBC). The blood was washed with PBS and the OD_{640} was adjusted to 1.4 using PBS. Bacteria were serially diluted in a V-bottomed 96-well microtiter plate prior to addition of RBC and the plates were kept at 4 °C. The last well with agglutination was visually determined and reported (HA-titer, no of wells). The number of wells reflects the concentration of bacteria needed for agglutination to occur, *i.e.* a high number demonstrates low presence of pili. An unaffected expression of pili, comparable to the fully piliated positive control, gave rise to agglutination for 4 wells. The non-pili-producing strain HB101/pBR322 was included as a negative control.

Syntheses

Compounds (−)-**1**, (+)-**1**, (−)-**2**, and (+)-**2** were prepared according to published procedures.[25,27,29] (−)-**1** and (+)-**1** were determined to have 84, and 78% ee, respectively, as determined by chiral HPLC (see ESI†). Data are in agreement with published data.[25,28,29]

Lithium (3*S*)-8-Cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate [(+)-2]. Prepared according to published procedures. Data are in agreement with published data for (−)-**2**. $[\alpha]_D$ 189 (*c* 0.7, MeOH).

(3*R*)-8-Cyclopropyl-3-hydroxymethyl-7-naphthalen-1-ylmethyl-2,3-dihydro-thiazolo[3,2-*a*]pyridin-5-one [(−)-3]. 2 M $BH_3{\cdot}Me_2S$ in THF (1.6 ml, 3.2 mmol) was slowly added to a stirred solution of (−)-**2** (300 mg, 0.78 mmol) in THF (12 ml) at rt. After 13 h MeOH (8 ml) was added dropwise and the solution was stirred for 30 min before being concentrated. Purification by silica gel chromatography (heptane–EtOAc–MeOH, 1 : 9 : 1) gave (−)-**3** as a white foam (280 mg, 98%): $[\alpha]_D$ −87 (*c* 0.5, $CHCl_3$); 84% ee as determined with chiral HPLC (see ESI†); IR ν/cm^{-1} 3360, 2921, 2853, 1639, 1488; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91–7.71 (m, 3H), 7.56–7.33 (m, 3H), 7.25 (d, J = 7.14 Hz, 1H), 5.70 (s, 1H), 5.21 (m, 1H), 4.54–4.29 (m, 2H), 3.93 (dd, J = 11.25, 6.22 Hz, 1H), 3.72 (m, 1H), 3.48 (dd, J = 11.53, 8.05 Hz, 1H), 3.26 (dd, J = 11.53, 1.83 Hz, 1H), 1.66 (m, 1H), 1.07–0.83 (m, 2H), 0.81–0.59 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9, 156.6, 148.2, 133.9 (2C), 131.8, 128.8, 127.6, 127.5, 126.1, 125.6, 125.5, 123.7, 114.8, 114.2, 64.4, 60.8, 36.0, 30.5, 11.1, 7.8, 7.4; HRMS (FAB) calc. for $[M + H]^+$ $C_{22}H_{22}NO_2S$ 364.1371, found 364.1380.

(3*R*)-8-Cyclopropyl-3-iodomethyl-7-naphthalen-1-ylmethyl-2,3-dihydro-thiazolo[3,2-*a*]pyridin-5-one [(−)-4]. I_2 (330 mg, 1.4 mmol), PPh_3 (430 mg, 1.6 mmol) and imidazole (127 mg, 1.9 mmol) were added to a stirred solution of (−)-**3** (250 mg, 0.7 mmol) in toluene (10 ml) at rt. After stirring overnight the reaction mixture was diluted with EtOAc and washed with 5% aqueous $Na_2S_2O_5$. The aqueous layer was extracted with EtOAc and the combined organic layers were dried and concentrated. Purification by silica gel chromatography (heptane–EtOAc, 1 : 9) gave (−)-**4** as a white foam (248 mg, 76%): $[\alpha]_D$ −170 (*c* 0.5, $CHCl_3$); 84% ee as determined with chiral HPLC on the starting material (−)-**3**; IR ν/cm^{-1} 3066, 3002, 1648, 1574, 1487; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (m, 1H), 7.80–7.74 (m, 2H), 7.50–7.39 (m, 3H), 7.25 (d, J = 7.14 Hz, 1H), 5.69 (s, 1H), 5.28 (m, 1H), 4.48–4.30 (m, 2H), 3.59–3.42 (m, 4H), 1.63 (m, 1H), 1.02–0.88 (m, 2H), 0.77–0.68 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.8, 156.5, 146.4, 133.9, 133.8, 131.9, 128.8, 127.6, 127.5, 126.1, 125.7, 125.5, 123.7, 115.3, 113.5, 63.0, 36.1, 32.9, 11.0, 7.8, 7.4, 2.4; HRMS (ES) calc. for $[M + H]^+$ $C_{22}H_{21}INOS$ 474.0389, found 474.0376.

(3*R*)-(8-Cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridin-3-yl)-acetonitrile [(−)-5]. KCN (22 mg, 0.34 mmol) was added to a stirred solution of (−)-**4** (78 mg, 0.16 mmol) in DMF (1 ml) at rt. After heating at 65 °C for 2.5 h the solution was allowed to reach room temperature and was then diluted with EtOAc and washed with brine and H_2O. The organic layer was dried (Na_2SO_4) and concentrated. Purification by silica gel chromatography (heptane–EtOAc, 1 : 9) gave (−)-**5** as a pale yellow foam (43 mg, 70%): $[\alpha]_D$ −124 (*c* 0.5, $CHCl_3$); 84% ee as determined with chiral HPLC (see ESI†); IR ν/cm^{-1} 3001, 2922, 2250, 1643, 1571, 1486; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (m, 1H), 7.83–7.71 (m, 2H), 7.54–7.36 (m, 3H), 7.25 (d, J = 7.14 Hz, 1H), 5.67 (s, 1H), 5.30 (m, 1H), 4.51–4.29 (m, 2H), 3.66 (dd, J = 12.08, 7.78 Hz, 1H), 3.31 (d, J = 12.08 Hz, 1H), 3.07 (m, 2H), 1.66 (m, 1H), 1.08–0.87 (m, 2H), 0.81–0.68 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.7, 157.0, 146.0, 134.0, 133.7, 131.9, 128.9, 127.8, 127.6, 126.2, 125.7, 125.5, 123.7, 116.3, 115.4, 113.9, 58.0, 36.2, 32.3, 18.9, 11.10, 7.8, 7.5; HRMS (ES) calc. for $[M + H]^+$ $C_{23}H_{21}N_2OS$ 373.1375, found 373.1353.

(3*R*)-(8-Cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridin-3-yl)-acetic acid [(−)-6]. 2 M aqueous NaOH (450 μl, 0.9 mmol) was added to a stirred solution of (−)-**5** (27 mg, 0.07 mmol) in EtOH (1.5 ml) at rt. The reaction vessel was capped and heated using microwave irradiation at 130 °C for 5 min. After cooling, the solution was acidified with HCl (2 M aq.) and concentrated. The residue was dissolved in CH_2Cl_2 and washed with H_2O and the combined aqueous layers were extracted with CH_2Cl_2. The combined organic layers were concentrated and lyophilized from MeCN–H_2O (2 : 3) giving (−)-**6** as a white powder (20 mg, 71%): $[\alpha]_D$ −13 (*c* 0.24, DMSO); 84% ee as determined by chiral HPLC on the starting material (−)-**5**; IR ν/cm^{-1} 3196, 2868, 1721, 1629, 1547, 1487; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.62 (bs, 1H), 7.95 (m, 1H), 7.89–7.82 (m, 2H), 7.55–7.45 (m, 3H), 7.35 (d, J = 7.14 Hz, 1H), 5.23 (s, 1H), 5.13 (m, 1H), 4.50–4.30 (m, 2H), 3.68 (dd, J = 11.80, 7.60 Hz, 1H), 3.24 (d, J = 11.80 Hz, 1H), 2.69–2.52 (m, 2H), 1.69 (m, 1H), 0.97–0.82 (m, 2H), 0.77–0.61 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 159.9, 156.4, 148.0, 135.0, 133.9, 132.0, 129.1, 128.0, 127.8, 126.8, 126.3, 126.2, 124.5, 114.0, 112.8, 58.8, 35.7, 34.5, 33.2, 11.3, 7.9, 7.6; HRMS (ES) calc. for $[M + H]^+$ $C_{23}H_{22}NO_3S$ 392.1320, found 392.1299.

(3*R*)-8-Cyclopropyl-3-methoxymethyl-7-naphthalen-1-ylmethyl-2,3-dihydro-thiazolo[3,2-*a*]pyridin-5-one [(−)-7]. (−)-**3** (50 mg, 0.14 mmol) was added to a stirred suspension of NaH (60% in mineral oil, 13 mg, 0.32 mmol) in THF at rt and after stirring for 5 min MeI (20 μl, 0.31 mmol) was added. After one hour

additional amounts of NaH and MeI (10 mg and 20 μl, respectively) were added followed by stirring for another hour and then concentration. The residue was dissolved in THF and MeOH was added slowly prior to concentration. Purification by silica gel chromatography (heptane–EtOAc, 1 : 9) gave (−)-**7** as a pale yellow foam (51 mg, 98%): $[a]_D$ −58 (*c* 0.3, $CHCl_3$); 30% ee as determined by chiral HPLC on the starting material (−)-**3**; IR ν/cm^{-1} 2981, 2926, 2854, 1643, 1572, 1486; ^1H NMR (400 MHz, $CDCl_3$) δ 7.86 (m, 1H), 7.80–7.74 (m, 2H), 7.51–7.36 (m, 3H), 7.25 (d, $J = 7.14$ Hz, 1H), 5.68 (s, 1H), 5.26 (m, 1H), 4.49–4.27 (m, 2H), 3.65–3.53 (m, 2H), 3.52–3.33 (m, 2H), 3.38 (s, 3H), 1.63 (m, 1H), 1.03–0.83 (m, 2H), 0.78–0.66 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 156.0, 147.7, 134.1, 133.9, 132.0, 128.8, 127.6, 127.5, 126.1, 125.7, 125.5, 123.8, 115.2, 113.3, 68.0, 60.9, 59.0, 36.1, 30.5, 11.10, 7.8, 7.4; HRMS (ES) calc. for $[M + H]^+$ $C_{23}H_{24}NO_2S$ 378.1528, found 378.1504.

(3*R*)-8-Cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carbaldehyde [(−)-8]. Dess–Martin periodinane (0.47 M in CH_2Cl_2, 1 ml, 0.09 mmol) was added to a stirred solution of (−)-**3** (20 mg, 0.06 mmol) in CH_2Cl_2 (1 ml) at rt. After 1.5 h the solution was diluted with EtOAc and washed first with an ice-cold 1 : 1 solution of $NaHCO_3$ (half sat. aqueous) : $Na_2S_2O_5$ (5% aqueous) and then H_2O. The organic layer was dried, (Na_2SO_4) concentrated and purified by silica gel chromatography (heptane–EtOAc–MeOH, 1 : 9 : 1) giving (−)-**8** as a white foam (17 mg, 85%): $[a]_D$ −153 (*c* 0.5, $CHCl_3$); 84% ee as determined with chiral HPLC on the starting material (−)-**3**; IR ν/cm^{-1} 2957, 2923, 2852, 1638, 1561, 1485; ^1H NMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 7.94–7.69 (m, 3H), 7.55–7.36 (m, 3H), 7.28 (d, $J = 7.04$ Hz, 1H), 5.77 (s, 1H), 5.45 (dd, $J = 8.51$, 3.02, 1H), 4.54–4.32 (m, 2H), 3.62 (dd, $J = 11.53$, 3.11 Hz, 1H), 3.50 (dd, $J = 8.51$, 11.53 Hz, 1H), 1.66 (m, 1H), 1.08–0.85 (m, 2H), 0.80–0.61 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.4, 161.34, 157.3, 146.9, 134.0, 133.8, 131.9, 128.9, 127.8, 127.6, 126.3, 125.8, 125.5, 123.7, 115.2, 114.0, 68.4, 36.3, 28.3, 11.1, 7.7, 7.5; HRMS (FAB) calc. for $[M + H]^+$ $C_{22}H_{20}NO_2S$ 362.1215, found 362.1209.

(3*R*)-8-Cyclopropyl-3-methyl-7-naphthalen-1-ylmethyl-2,3-dihydro-thiazolo[3,2-*a*]pyridin-5-one [(−)-9]. $LiEt_3BH$ (1 M in THF, 170 μl, 0.17 mmol) was slowly added to a stirred solution of (−)-**4** (50 mg, 0.11 mmol) in THF at rt. After 30 min the reaction was quenched by dropwise addition of MeOH and then concentrated. Purification by silica gel chromatography (heptane–EtOAc, 1 : 9) gave (−)-**9** as a pale yellow solid (30 mg, 82%): $[a]_D$ −46 (*c* 0.25, $CHCl_3$); 30% ee as determined with chiral HPLC on the precursor (−)-**3**; IR ν/cm^{-1} 2968, 2925, 2852, 1641, 1568, 1486; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90–7.73 (m, 3H), 7.50–7.37 (m, 3H), 7.25 (d, $J = 7.14$ Hz, 1H), 5.69 (s, 1H), 5.24 (m, 1H), 4.50–4.29 (m, 2H), 3.57 (dd, $J = 11.16$, 7.59 Hz, 1H), 2.90 (d, $J = 11.16$ Hz, 1H), 1.64 (m, 1H), 1.45 (d, $J = 6.40$ Hz, 3H), 1.01–0.87 (m, 2H), 0.79–0.67 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 155.7, 146.8, 134.2, 133.9, 132.0, 128.8, 127.6, 127.5, 126.1, 125.6, 125.5, 123.8, 115.3, 113.2, 58.1, 36.1, 35.1, 16.9, 11.1, 7.8, 7.4; HRMS (ES) calc. for $[M + H]^+$ $C_{22}H_{22}NOS$ 363.1422, found 363.1414.

(3*R*)-3-Aminomethyl-8-cyclopropyl-7-naphthalen-1-ylmethyl-2,3-dihydro-thiazolo[3,2-*a*]pyridin-5-one [(−)-10]. NaN_3 (18 mg, 0.28 mmol) was added to a stirred solution of (−)-**4** (80 mg, 0.17 mmol) in DMF (2 ml) at rt. After 6 h Pd/C (10 wt%, 54 mg) was added and hydrogenation was carried out at atmospheric pressure overnight. The catalyst was removed by filtration through Celite and the solid phase was rinsed with MeOH. The filtrate was concentrated and the residue was diluted with EtOAc and washed with H_2O. The organic layer was concentrated and after lyophilization from MeCN–H_2O (2 : 3) (−)-**10** was obtained as a white powder (53 mg, 87%): $[a]_D$ −98 (*c* 0.5, $CHCl_3$); 84% ee as determined with chiral HPLC on the precursor (−)-**3**; IR ν/cm^{-1} 3062, 3001, 2961, 2864, 1641, 1564, 1486; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90–7.74 (m, 3H), 7.50–7.36 (m, 3H), 7.25 (d, $J = 7.14$ Hz, 1H), 5.68 (s, 1H), 5.02 (m, 1H), 4.49–4.28 (m, 2H), 3.52 (dd, $J = 11.43$, 7.50 Hz, 1H), 3.38 (d, $J = 11.43$ Hz, 1H), 3.12 (dd, $J = 12.35$, 3.20 Hz, 1H), 2.92 (dd, $J = 12.35$, 3.20 Hz, 1H), 1.64 (m, 1H), 1.04–0.83 (m, 2H), 0.78–0.67 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.3, 156.0, 147.6, 134.1, 134.0, 132.0, 128.8, 127.64, 127.58, 126.2, 125.7, 125.5, 123.8, 115.3, 113.5, 64.2, 41.1, 36.1, 31.2, 11.1, 7.9, 7.4; HRMS (ES) calc. for $[M + H]^+$ $C_{22}H_{24}N_2OS$ 363.1531, found 363.1518.

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