Supporting Information

Small molecule inhibitors target *E. coli* amyloid biogenesis and biofilm formation

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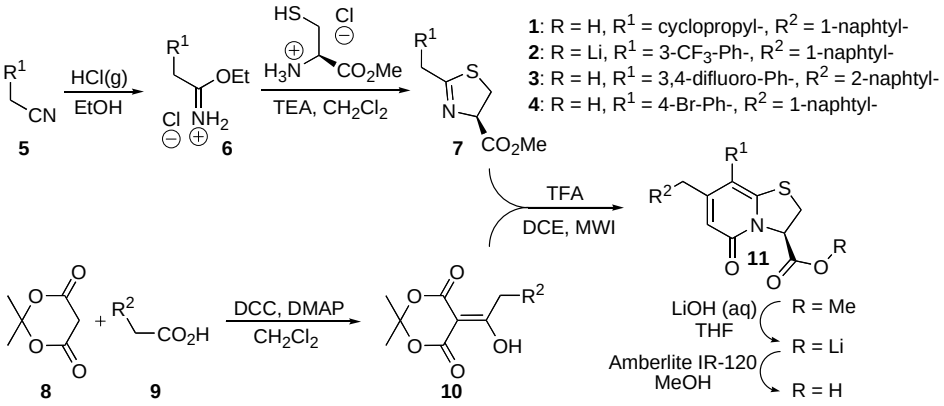
Supplementary Methods

Synthesis of ring-fused 2 pyridones



General procedure for the preparation of **7**:
5 was dissolved in dry EtOH (0.1mL/mmol) (in some cases small amounts of CH_2Cl_2 was added because of solubility problems). Dry HCl(g) was bubbled through the solution for approximately 2h before the reaction mixture was concentrated. The resulting imino ether (**6**) (1 mol equiv.) and cystein methyl ester hydrochloride (1 mol equiv.) was dissolved in dry CH_2Cl_2 (1.6 mL/mmol) at 0 °C. After 15 min of stirring triethylamine (TEA) (1 mol equiv.) was added dropwise at 0 °C and the suspension was left stirring over night. The reaction mixture was washed with water/brine (1/1) and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases was dried with Na_2SO_4, filtrated and concentrated. Purification by column chromatography in heptane/ethylacetate gave **7**.

General procedure for the preparation of **10**:
9 (1 mol equiv.) and DCC (1.15 mol equiv.) dissolved in CH_2Cl_2 (8 mL/mmol) was stirred for 30 min at 0 °C before **8** (1.1 mol equiv.) and DMAP (1.6 mol equiv.) were added. The suspension was allowed to stir over night before being quenched by 6% aq. $KHSO_4$. The resulting urea precipitation was filtered off and the filtrate was washed 2 times with 6% aq. $KHSO_4$. The organic phase was dried with Na_2SO_4, filtrated and concentrated to give **10** that normally was used without further purification.

General procedure for the preparation of **11:**
To **7** (1 mol equiv.) and **10** (3 mol equiv.) dissolved in 1,2-dichloroethane (DCE)(5 mL/mmol) was trifluoroacetic acid (TFA)(1 mol equiv.) added dropwise. The solution was stirred for 5 min before heated by microwave irradiation (MWI) for 2 min and 20 s at 120 °C. The resulting solution was washed with $NaHCO_3$ (1/2 sat.) and brine, dried with Na_2SO_4, filtrated and concentrated. Purified by column chromatography in heptane/ethylacetate gave **11**.
General procedure for the hydrolysis of **11**:
11 (1 mol equiv.) was dissolved in tetrahydrofuran (THF) (40 mL/mmol) and 0.1M aq. LiOH (1 mol equiv.) was added dropwise. The solution was left stirring over night before being concentrated to give the corresponding lithium carboxylate. If desired, the lithium carboxylate

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could be protonated to its corresponding carboxylic acid by treatment with Amberlite IR-120 (H^+) in MeOH, followed by filtration and concentration.

1

(3R)-8-cyclopropyl-7-(naphthalen-1-ylmethyl)-5-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylic acid.
Characterization of **1** and intermediates agreed with previously reported data[1].

2

lithium (3R)-7-(naphthalen-1-ylmethyl)-5-oxo-8-(3-(trifluoromethyl)phenyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylate.
Characterization of **2** and intermediates agreed with previously reported data[2].

3

(3R)-8-(3,4-difluorophenyl)-7-(naphthalen-2-ylmethyl)-5-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylic acid.
Characterization of **3** and intermediates agreed with previously reported data[1].

4

(3R)-8-(4-bromophenyl)-7-(naphthalen-1-ylmethyl)-5-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylic acid. 1H NMR (400 MHz, DMSO-d6) 7.88-7.96 (m, 1H), 7.80-7.86 (m, 1H), 7.67-7.74 (m, 1H), 7.58-7.66 (m 2H), 7.24-7.53 (m, 6H), 5.43-5.49 (m, 2H), 3.99 (s, 2H), 3.83 (dd, J = 11.94, 9.07 Hz, 1H), 3.50 (dd, J = 11.97, 1.50 Hz, 1H); 13C NMR (100 MHz, DMSO-d6) 169.4, 159.9, 153.4, 148.0, 135.4, 134.0, 133.3, 132.3 (broad, 2C), 131.9 (2C), 131.3, 128.6, 127.6, 127.4, 126.3, 125.8, 125.5, 123.8, 121.6, 113.7, 113.2, 63.3, 35.8, 31.4. MS (ES+) calcd for $[M+H]^+$ $C_{25}H_{18}BrNO_3S$ 492/494, obsd 492/494.
Characterization of intermediates agreed with previously reported data[1, 3].

UTI89Δ*csgA*, UTI89*ΔcsgA/pLR5, and* UTI89*ΔcsgA/pLR5 construction,* UTI89*ΔBG*

UTI89Δ*csgA* was constructed according to the method of Datsenko and Wanner [4]. A linear knockout product was produced by PCR using the template pKD4 and the following primers with homologous ends specific to *csgA*: 5'-ATGAAACTTTTAAAAGTA GCAGCAATTGCAGCAATCG TATTCTCTGGTAGCATATGAATATCCTCCTTAG and 5'-TTAGTACTGATGAGCGGTCGCGTTGTTACCAAA GCCAACCTGAGTGACG TGTGTAGGCTGGAGCTGCTTC. Gene deletion was confirmed with the *csgA*-flanking primers 5'-TGGCTATTCGCGTGACACAA and 5'-GGCTTGCGCCCTGTTTCTT T. The kanamycin cassette was excised by introduction of the Flp recombinase-expressing vector pCP20[4]. Subsequent passage at 42°C eliminated the temperature sensitive replicon of pCP20.

The *csgA* expressing plasmid construct pLR5 was previously described[5]. pLR1 was constructed by inserting only the *csgBA* promoter in plasmid pACYC177[6].

Each plasmid was introduced in the UTI89Δ*csgA* via electroporation and selected on antibiotic.

UTI89 Δ*csgBG* was created through P1 phage transduction of a *csgB-csgG* deletion construct according to standard protocol. Briefly, P1 phage lysate was generated from MHR420[7]. Overnight culture of UTI89 was then infected with MHR420 P1 phage lysate and selected on LB agar plates containing 20 μg/ml chloramphenical.

Curli promoter-gfp transcriptional fusion construction

PCR primers csg #1 (TTATAGGATCCGTTTTTCCTGCTCAAAGTATCC) and csg #2 (ATTATGGATCCTGCGCAACAACCGCCAAAAG) were used to amplify a 1207 bp product from the genome of the prototypical cystitis strain UTI89, including the intergenic region between the *csgD* and *csgB* genes. This region contains all of the described regulatory regions involved in *csgBA* transcription. The BamHI-cut product was ligated into the like-cut integration vector pPSSH10 containing a promoterless *gfp*. A clone in which the *csgBA* promoter fragment was oriented to direct *gfp* transcription was selected, confirmed by sequencing, and integrated into the lambda site of *E. coli* MG1655 as previously described[8]. The integrated reporter was subsequently transferred into UPEC UTI89 by P1 phage generalized transduction.

References

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Supplementary Figures

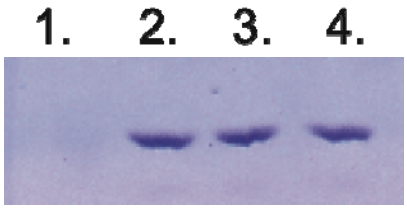

Figure S1. Inhibition of Aβ protein polymerization by BibC6 and VA028 demonstrated in a polyacrylamide gel-shift assay. Aβ peptide 1-40 was dissolved in 50 mM phosphate, 100mM NaCl, pH 6.8, and was incubated for 24 hours at 37 ºC with agitation. All substances were dissolved in DMSO at a final concentration corresponding to 5% (V/V) and incubated at a 1:10 ratio (peptide:substance). Aggregated material was preciptated through centrifugation to discriminate between aggregated and non-aggregated protein. The SDS PAGE gel indicates the presence of peptide in the superanatant after centrifugation. **1**. Control (verifying 100% aggregation); **2** VA028; **3**. BibC6; **4**. FN075; (positive control).

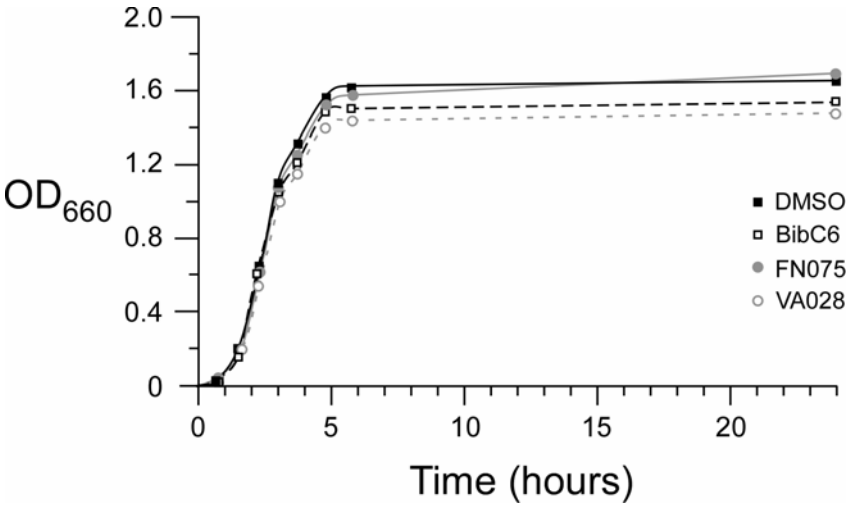

Figure S2. UTI89 growth curves in the presence of DMSO carrier and curlicides. Curlicides had no effect on growth rates of UTI89 when media was amended with 400 μM of each curlicide, relative to cells grown in an equivalent volume of DMSO. Bacteria were grown in LB broth at 37 °C with a shaking speed of 250 rpm for 24 hours. Optical densities as a function of time were measured spectrophotometrically at 660 nm.

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