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Functionalization of bicyclic 2-pyridones targeting pilus biogenesis in uropathogenic *Escherichia coli*

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Abstract—Substituted bicyclic 2-pyridones, termed pilicides, prevent pilus assembly in uropathogenic *Escherichia coli*. Based on the bioactive methyl ester protected 2-pyridone **4**, further functionalization at C-6 has yielded a set of new compounds, which have been evaluated for their ability to inhibit pilus formation in uropathogenic *E. coli*. The key intermediate in the synthesis was formylated 2-pyridone **5**, which could be obtained via a Vilsmeier reaction. This versatile intermediate was converted into secondary and tertiary amines via reductive amination and could also be converted to other interesting functionalities using simple chemical transformations.

Several Gram negative pathogens use adhesive organelles called pili/fimbriae to initiate infection of host cells. The adhesive pili fibers are assembled via a highly conserved mechanism called the chaperone/usher pathway which is used by a multitude of bacterial pathogens. We have recently shown that the bicyclic 2-pyridone **1** and its aminomethylated derivative **2a** (Fig. 1) block the formation of both type 1 and P pili in uropathogenic *Escherichia coli*,[1] thus paving the way for these compounds to serve as possible leads towards broad-spectrum antibacterial agents.

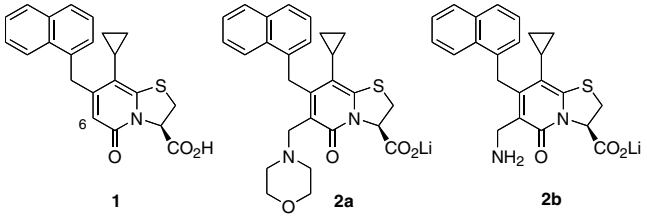

Figure 1. 2-Pyridone **1** prevents pili formation in uropathogenic *E. coli*. Further functionalization at C-6 has yielded the more water soluble aminomethylated compounds **2a** and **2b**.

An efficient synthesis of ring-fused di-substituted 2-pyridones has previously been developed and the key step was a cyclocondensation between a Δ^2-thiazoline and an acyl-ketene generated from acyl Meldrum's acids.[2–4] However, the large hydrophobic substituents present in the active di-substituted 2-pyridones results in poor water solubility that limited their utility. This issue was addressed by introducing more hydrophilic substituents at C-6 of the 2-pyridone scaffold (see Fig. 1). By using a modified Mannich reaction, symmetrical tertiary aminomethylated 2-pyridones **2a** could be prepared and primary amine **2b** was synthesized via a three-step procedure based on a cyano-group as the key intermediate.[5] The cyano functionality was introduced via a microwave-assisted Rosenmund von Braun reaction, and could then be reduced to yield the primary aminomethylated 2-pyridone **2b** in good yield. Unfortunately, the somewhat harsh reaction conditions did result in low optical purity of the final product (8% ee). In vivo evaluations of these compounds showed that several of the tertiary aminomethylated 2-pyridones **2** (e.g. **2a**) had maintained or in some cases even improved their ability to attenuate pilus assembly in *E. coli*. In addition, these more water soluble compounds were much easier to evaluate in biological assays.[1]

Keywords: 2-Pyridone; Formylation; Antibacterial agent; Pilicide; Biofilm inhibitor.
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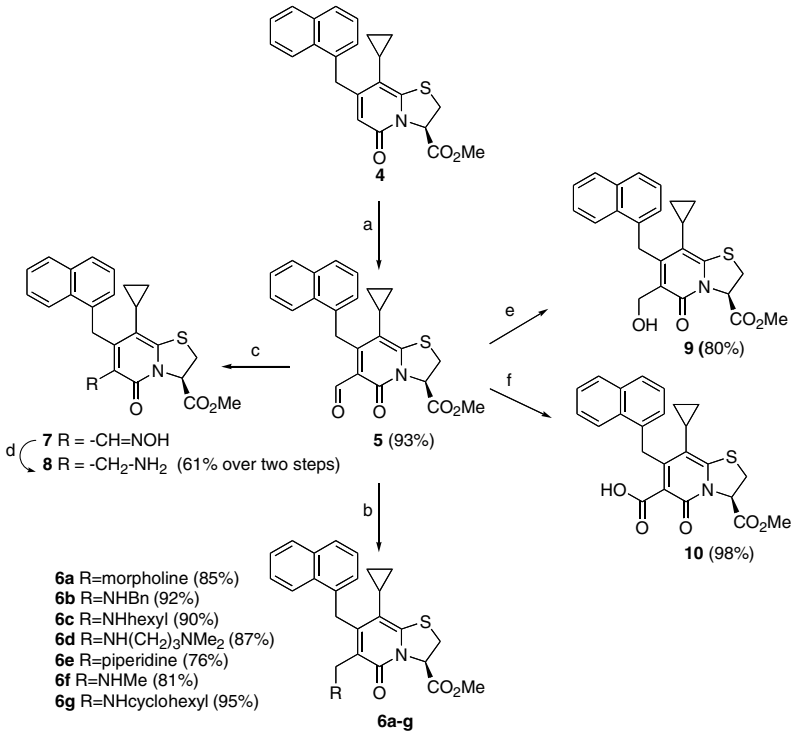
Encouraged by these positive results we were interested in investigating the possibility to further fine-tune the aminomethyl-substituent to obtain compounds with enhanced potency. The limitations of the earlier developed methodologies did not make them suitable for this purpose, as the previously developed Mannich reaction was performed by first preparing the methylene iminum salts in two steps, and the route via the cyano-group was performed in a time consuming manner over several steps and yielded the final product with poor ee. Thus a new method for the preparation of a small series of aminomethylated 2-pyridones was needed. In this Letter, we present synthetic routes to primary, secondary and tertiary amines. All the synthetic routes developed were based on a formyl substituted 2-pyridone as the key intermediate. The formyl group could also be transformed to an alcohol and a carboxylic acid by using robust and efficient transformations.

To introduce the desired formyl functionality the Vilsmeier reaction was employed.[6] Starting with classical conditions using DMF and $POCl_3$, the desired formylated 2-pyridone was obtained, however, the conversion was slow and after 15 h at room temperature a large amount of unreacted starting material still remained. Therefore, a preformed chloromethylene iminium salt was used instead, providing a higher concentration of the reactive electrophilic species. Commercially available $Cl^-Me_2N^+{=}CHCl$ (Arnold's reagent) and 2-pyridone **4** were reacted in refluxing acetonitrile. This resulted in full conversion after 3 h and compound **5** was isolated in excellent yield (93%) after simple filtration through a silica plug.[7]

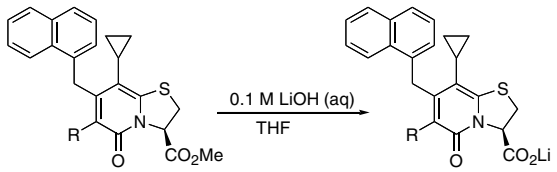
The formylated 2-pyridone was then reductively aminated to secondary and tertiary aminomethylated 2-pyridones **6a**–**g** in good to excellent yields (76–95%) (Scheme 1).[8] The reactions were performed in CH_2Cl_2 and MeOH, allowing the amine and the aldehyde to react for 30 min in the presence of 3 Å molecular sieves prior to the addition of $NaBH(OAc)_3$. This was done to prevent the formation of alcohol **9**, which was obtained as a by-product if all the reagents were added together at the start. Primary aminomethylated 2-pyridone **8** was prepared by first converting aldehyde **5** to the corresponding oxime **7**, which was transformed to the desired primary amine in moderate yield using zinc in acetic acid.[9] This synthetic pathway had to be developed as initial efforts to perform reductive amination with different sources of ammonia (NH_4OAc, NH_3 saturated MeOH) resulted only in dimerization. Importantly, this pathway also yielded the primary aminomethylated 2-pyridone without any loss of optical purity, which had been the major drawback of the previously developed method via Rosenmund von Braun cyanation followed by reduction.

The formylated 2-pyridone **5** could also easily be reduced to alcohol **9** using $BH_3{\cdot}SMe_2$ in THF (Scheme 1).[10] Oxidation of **5** to the carboxylic acid **10** also proved straightforward. Compound **10** was prepared in excellent yield using a chemoselective sodium chlorite oxidation.[11,12] DMSO was used as the solvent which prevents oxidation of the sulfide by functioning as a hypochlorous acid scavenger and being oxidized to dimethyl sulfone. All 12 compounds were then hydrolyzed to their corresponding lithium carboxylates **11a**–**k** (0.1 M aq



Scheme 1. Reagents and conditions: (a) $Cl^-Me_2N^+{=}CHCl$, MeCN, reflux; (b) amine, CH_2Cl_2–MeOH 7:3, 3 Å mol sieves, $NaBH(OAc)_3$; (c) $NH_2OH{\cdot}HCl$, pyridine, EtOH, reflux; (d) Zn powder, AcOH, rt; (e) 2 M $BH_3{\cdot}SMe_2$, THF, rt; (f) $NaClO_2$, NaH_2PO_4, H_2O, DMSO, rt.

Table 1. The ability to prevent pili formation was evaluated in a hemagglutination assay



5, 6a-g, 7-10 **2a, 11a-k**

Compound	R	HA-titer[a] 3.6 mM pilicide	HA-titer[a] 1.8 mM pilicide	HA-titer[a] 0.72 mM pilicide
2a	–CH_2-morpholine	16[b]	16	16
11a	–CHO	128	—	—
11b	–CH_2NHBn	16[c]	—	16[c]
11c	–CH_2NHhexyl	32[c]	—	32[c]
11d	–$CH_2NH(CH_2)_3NMe_2$	—[d]	—	—[d]
11e	–CH_2-piperidine	8[b]	16	32
11f	–CH_2NHMe	—[d]	—	—[d]
11g	–CH_2NHcyclohexyl	32[c]	—	32[c]
11h	–CH=NOH	128	—	—
11i	–CH_2NH_2	—[c]	—	—
11j	–CH_2OH	8[b]	16	16
11k	–CO_2Li	128	—	—
No pilicide	—	128[b]	128	128

E.coli was grown in the presence of pilicide at different concentrations and HA-titers were determined.
[a] Highest dilution that still provides hemagglutination (see text for details).
[b] Duplicate runs.
[c] Precipitation of compound in broth.
[d] Inhibition of bacterial growth.

LiOH in THF) and evaluated for their ability to prevent pili formation in uropathogenic *E. coli*. (Table 1). Bacteria were grown in the presence of these substrates at different concentrations (3.6, 1.8, 0.7 mM) and the level of piliation was subsequently determined in a hemagglutination assay (HA).[13] A low HA-titer indicates that less pili are formed, and is thus a verification of an efficient pilicide. Compound **2a** bearing a morpholine moiety has previously been evaluated in several different assays and has been recognized as the most promising pilicide so far.[1] Several of the new compounds: **11b**, **11c**, **11e**, **11g** and **11j** exhibited improved or comparable in vivo activity compared to **2a** (Table 1). Unfortunately, the low water solubility of secondary amines **11b**, **11c** and **11g** resulted in some precipitation in the bacterial broth even at the lowest concentrations (Table 1, column 5). However, it should be noted that even though the limited water solubility complicates the evaluation of these compounds they still seem to have a clear effect.

Tertiary amine **11e** and alcohol **11j** also maintained their effect at lower concentrations (Table 1) and further evaluation of **2a**, **11e** and **11j** was performed in a biofilm assay.[1] (data not shown) confirming their ability to inhibit pili formation. In agreement with the data from the HA-titer, **11j** was confirmed as the most efficient pilicide with 50% inhibition of biofilm growth at 90 μM.

In conclusion, by developing a method to introduce a formyl functionality at C-6 of the bioactive 2-pyridone **4,** we have been able to prepare a set of new derivatives which have been evaluated as inhibitors for pilus assembly in *E. coli*. Several of the new compounds show maintained or improved in vivo activity compared to the previously most effective compound **2a**, suggesting that further substitution in this readily accessible position is a viable approach towards more potent pilicides.

Acknowledgement

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7. *Experimental procedure for formylation:* 2-Pyridone **4** was added to a stirred solution of Arnold's reagent ($Cl^-Me_2N^+$=CHCl) (4 equiv) in acetonitrile (0.12 mmol/ml) at rt. The solution was refluxed for 3 h, then concentrated and dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$ (aq), extracted with CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4(s), filtered and concentrated. Further purification by filtration through a short silica plug (EtOAc) yielded compound **5**

(93%): $[\alpha]_D$ −297 (*c* 1.0, $CHCl_3$); IR λ 1748, 1633, 1470, 1406, 1211, 1154, 1012, 792; ^1H NMR (400 MHz, $CDCl_3$) δ 10.39 (s, 1H), 8.18 (d, *J* = 8.8, 1H), 7.87 (d, *J* = 8.4, 1H), 7.70 (d, *J* = 8.3, 1H), 7.50–7.61 (m, 2H), 7.24–7.29 (m, 1H), 6.76 (d, *J* = 6.6, 1H), 5.76 (dd, *J* = 8.9, 2.5, 1H), 5.23 (d, *J* = 14.6, 1H), 5.09 (d, *J* = 14.6, 1H), 3.89 (s, 3H), 3.77 (dd, *J* = 11.9, 9.0, 1H), 3.59 (dd, *J* = 11.9, 2.5, 1H), 1.30–1.39 (m, 1H), 0.63–0.76 (m, 2H), 0.51–0.58 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.6, 167.9, 162.0, 160.6, 156.9, 134.4, 133.4, 131.8, 128.5, 126.5, 125.9, 125.5, 125.2, 123.0, 122.9, 118.6, 115.3, 63.2, 53.2, 31.4, 31.1, 11.0, 7.6, 7.1; HRMS (FAB) calcd for $[M+H]^+$ $C_{24}H_{22}NO_4S$ 420.1270, obsd 420.1267.

8. *General procedure for reductive amination:* 1.0 equiv of amine was added to a stirred solution of aldehyde **5** in CH_2Cl_2–MeOH 7:3 and 3 Å mol sieves at 0 °C, and stirring was continued for 30 min. Sodium triacetoxyborohydride (1.8 equiv) was then added and the mixture was allowed to attain rt and stirred for 3 h. The reaction was washed with saturated $NaHCO_3$ (aq), extracted with CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4(s). Purification by column chromatography yielded the aminomethylated 2-pyridones **6a**–**g**. Data for compound **6b**: $[\alpha]_D$ −128 (*c* 1.2, $CHCl_3$); IR λ 3009, 2953, 1748, 1631, 1557, 1500, 1209, 791, 726; ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 8.3 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.71 (d, *J* = 8.3 Hz, 1H), 7.51–7.62 (m, 2H) 7.10–7.36 (m, 6H) 6.80 (d, *J* = 7.0 Hz, 1H), 5.68 (dd, *J* = 8.6, 2.5 Hz, 1H), 4.66 (d, *J* = 16.3 Hz, 1H), 4.57 (d, *J* = 16.3 Hz, 1H) 3.85 (s, 3H) 3.49–3.74 (m, 6H) 1.35–1.43 (m, 1H) 0.45–0.69 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 161.4, 153.0, 145.8, 139.4, 134.3, 133.4, 131.6, 128.6, 128.0 (split), 126.8, 126.6, 126.0, 125.6, 125.3, 124.2, 123.8, 122.9, 114.4, 63.2, 53.1 (split), 45.2, 31.8, 31.3, 11.6, 7.3, 7.0; HRMS (FAB) calcd for $[M+H]^+$ $C_{31}H_{31}N_2O_3S$ 511.2055, obsd 511.2042. The enantiomeric excess was 76% as determined by chiral HPLC (compared to 76% ee for the starting material **4**).

   Data for compound **6c**: IR λ 2948, 2927, 2858, 2362, 2337, 1749, 1631, 1498, 1452, 1211, 1170, 790, 771; HRMS (FAB) calcd for $[M+H]^+$ $C_{30}H_{37}N_2O_3S$ 505.2525, obsd 505.2508.

   Data for compound **6d**: IR λ 2942, 2816, 2765, 1747, 1629, 1501, 1211, 792, 732; HRMS (FAB) calcd for $[M+H]^+$ $C_{29}H_{36}N_3O_3S$ 506.2474, obsd 506.2477.

   Data for compound **6e**: IR λ 2930, 2849, 1751, 1633, 1497, 1207, 793, 771; HRMS (FAB) calcd for $[M+H]^+$ $C_{29}H_{33}N_2O_3S$ 489.2212, obsd 489.2205.

   Data for compound **6f**: IR λ 1747, 1629, 1449, 1209, 791, 772; HRMS (FAB) calcd for $[M+H]^+$ $C_{25}H_{27}N_2O_3S$ 435.1742, obsd 435.1743.

   Data for compound **6g**: IR λ 2923, 2850, 1750, 1632, 1501, 1209, 791, 771; HRMS (FAB) calcd for $[M+H]^+$ $C_{30}H_{35}N_2O_3S$ 503.2368, obsd 503.2361.

9. *Experimental procedure for the synthesis of primary amine* **8**: Aldehyde **5** was dissolved in ethanol (0.05 mmol/ml) and hydroxylamine hydrochloride (6 equiv) and pyridine (0.5 mmol/mL) were added. The mixture was refluxed for 3 h, allowed to cool to rt and concentrated. The residue was dissolved in CH_2Cl_2 and washed with water. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases dried over Na_2SO_4(s), filtered and concentrated to yield the oxime as a yellow solid in quantitative yield. The oxime and Zn dust (6 equiv) were dissolved in acetic acid (0.020 mmol/mL) and stirred at room temperature for 20 h. The zinc dust was removed by filtration and the organic phase carefully neutralized with saturated $NaHCO_3$ (aq). The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases dried over Na_2SO_4(s), filtered and concentrated. Purification by column chromatography yielded primary amine **8** (61%): Data was in agreement with published data.[5] The enantiomeric excess was 86% as determined by chiral HPLC (compared to 86% ee for the starting material **4**).

10. *Experimental procedure for reduction*: Formylated 2-pyridone **5** was dissolved in THF (0.07 mmol/ml) at 0 °C, then 2 M $BH_3 \cdot SMe_2$ in THF (1.1 equiv) was added dropwise over 15 min. The mixture was stirred at rt for 1 h, quenched with methanol and concentrated. The residue was co-concentrated from methanol and then purified by column chromatography to yield **9** (80%): $[\alpha]_D$ −148 (*c* 2.0, $CHCl_3$); IR λ 3419, 3001, 2950, 2872, 1739, 1627, 1501, 1210, 1008, 792, 773; ^1H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 8.1 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.50–7.62 (m, 2H) 7.28–7.33 (m, 1H) 6.83 (d, *J* = 6.9 Hz,1H), 5.68 (dd, *J* = 8.7, 2.4 Hz, 1H), 4.71 (d, *J* = 16.3 Hz, 1H), 4.62 (d, *J* = 16.3 Hz, 1H), 4.52 (d, *J* = 12.8 Hz, 1H), 4.43 (d, *J* = 12.8 Hz, 1H) 3.84 (s, 3H) 3.68 (dd, *J* = 11.8, 8.7 Hz, 1H) 3.51 (dd, *J* = 11.8, 2.4 Hz, 1H) 1.34–1.44 (m, 1H) 0.60–0.69 (m, 2H) 0.43–0.56 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 161.7, 151.9, 146.4, 133.9, 133.6, 131.6, 128.7, 127.0, 126.2, 125.7, 125.5, 125.4, 124.1, 122.8, 114.7, 63.1, 58.6, 53.2, 31.6, 31.4, 11.6, 7.3, 7.0; HRMS (FAB) calcd for $[M+H]^+$ $C_{24}H_{24}NO_4S$ 422.1426, obsd 422.1423. The enantiomeric excess was 76% as determined by chiral HPLC (compared to 76% ee for the starting material **4**).

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12. *Experimental procedure for oxidation*: To a solution of formylated 2-pyridone **5** in DMSO (0.1 mmol/mL), NaH_2PO_4 (2 equiv), dissolved in water (0.5 mmol/mL), was added dropwise at room temperature; the mixture was then kept on ice and $NaClO_2$ (4 equiv), dissolved in water (2 mmol/mL), was added dropwise over 30 min. After stirring the reaction for 1 h at room temperature, the reaction mixture was poured into a separation funnel containing ice-cooled 1 M HCl. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases concentrated. The residue was dissolved in H_2O: CH_3CN, 8:2 and lyophilized to yield carboxylic acid **10** (98%): $[\alpha]_D$ −155 (*c* 1.1, $CHCl_3$); IR λ 1712, 1592, 1450, 1216, 1141, 791, 773; ^1H NMR (400 MHz, DMSO-d_6) δ 13.86 (s, 1H), 8.26 (d, *J* = 8.3 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 8.3 Hz, 1H) 7.54–7.66 (m, 2H) 7.33–7.40 (m, 1H), 6.79 (d, *J* = 7.2 Hz, 1H), 5.79 (dd, *J* = 9.3, 1.8 Hz, 1H) 4.93 (m, 2H) 3.95 (dd, *J* = 12.0, 9.5 Hz, 1H) 3.78 (s, 3H) 3.70 (dd, *J* = 12.0, 1.8 Hz, 1H), 1.22–1.32 (m, 1H) 0.41–0.69 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 165.1, 165.0, 163.6, 154.2, 134.8, 133.7, 132.0, 128.7, 126.6, 126.0, 125.6, 125.4, 123.3, 123.1, 118.5, 112.6, 64.0, 53.7, 33.2, 31.4, 12.0, 8.2, 7.5; HRMS (FAB) calcd for $[M+H]^+$ $C_{24}H_{22}NO_5S$ 436.1219, obsd 436.1199. The enantiomeric excess was 76% as determined by chiral HPLC after methylation (TMSCl, MeOH) (compared to 76% ee for the starting material **4**).

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