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Design and Synthesis of C-2 Substituted Thiazolo and Dihydrothiazolo Ring-Fused 2-Pyridones: Pilicides with Increased Antivirulence Activity†

Erik Chorell,‡ Jerome S. Pinkner,§ Gilles Phan,∥ Sofie Edvinsson,‡ Floris Buelens,∥ Han Remaut,∥,⊥ Gabriel Waksman,∥ Scott J. Hultgren,*,§ and Fredrik Almqvist*,‡

‡*Department of Chemistry, Umeå University, SE-90187 Umeå, Sweden,* §*Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110,* ∥*Institute of Structural and Molecular Biology, University College London/Birkbeck, Malet Street, London WC1E 7HX, U.K., and* ⊥*Department of Cellular and Molecular Interactions, Laboratory for Structural and Molecular Microbiology, Structural Biology Brussels, Vrije Universiteit Brussel/VIB, 1050 Brussels, Belgium*

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Pilicides block pili formation by binding to pilus chaperones and blocking their function in the chaperone/usher pathway in *E. coli*. Various C-2 substituents were introduced on the pilicide scaffold by design and synthetic method developments. Experimental evaluation showed that proper substitution of this position affected the biological activity of the compound. Aryl substituents resulted in pilicides with significantly increased potencies as measured in pili-dependent biofilm and hemagglutination assays. The structural basis of the PapD chaperone−pilicide interactions was determined by X-ray crystallography.

Introduction

The increasing bacterial resistance to many antibiotics has resulted in a growing interest in new antibacterial agents with new modes of action directed toward Gram-negative bacteria.[1] Contrary to broad-range bactericidal or bacteriostatic antibiotics, drugs that specifically target bacterial virulence factors would exert less selective pressure on bacteria and minimize the risk for horizontal spread of drug-resistant genes.[2−4] It is estimated that approximately 11 million cases of urinary tract infection (UTI[a]) occur in the U.S. each year, primarily in women. Treatment of UTI, like other microbial infections, is exacerbated by increasing antimicrobial resistance. This has been described as an impending "public health crisis". In addition, over 900 000 women and men in the U.S. experience three or more UTI episodes per year. Thus, antibiotics do not stop recurrence in this population, independent of antibiotic resistance, highlighting the need for alternative therapeutics.

Many Gram-negative bacteria, such as uropathogenic *E. coli* (UPEC), produce long filamentous structures called pili/fimbriae assembled by the chaperone/usher pathway (CUP pili)[5,6] that mediate colonization and invasion of host tissues.[4,7,8] Compounds that target CUP pili have the potential to block virulence in a broad range of bacterial strains. The chaperones that are required for pilus assembly bind to pilus subunits and facilitate their folding by a mechanism termed donor strand complementation.[9−11] Chaperone−subunit complexes are targeted to the outer membrane usher, which catalyzes pilus assembly by a process known as donor strand exchange.[5,12,13] The chaperone comprises two immunoglobulin-like domains arranged in a boomerang shape. The usher has a 24-stranded β barrel that forms a channel across the

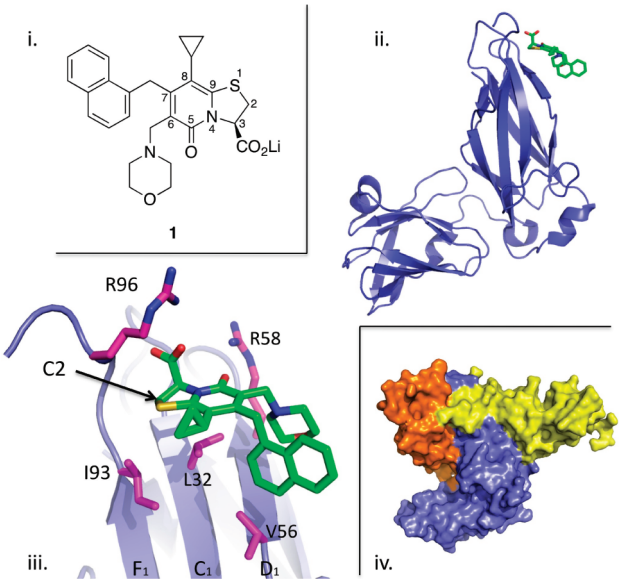

Figure 1. (i) Structure of pilicide **1**. (ii) The pilicide **1** (green) binds to the N-terminal domain of the chaperone PapD (blue). (iii) The pilicide (green) occupies a hydrophobic patch formed by I93, L32, and V56 (purple). (iv) Through this interaction the pilicide occupies the usher N-terminal binding site on the chaperone−subunit complex and thus pauses pilus assembly (chaperone in blue, subunit in orange, usher N-terminal domain in yellow).

†Protein coordinates for the PapD−PapH−pilicide **1** and the PapD−PapH−pilicide **5d** crystal complexes have been deposited with the Protein Data Bank with PDB codes 2xg4 and 2xg5, respectively.

*To whom correspondence should be addressed. For S.J.H.: phone, 314-362-6772; fax, (314) 362-1998; e-mail, hultgren@borcim.wustl.edu. For F.A.: phone, +46 90 7866925; fax, +46 90 7867655; e-mail, fredrik.almqvist@chem.umu.se.

[a] Abbreviations: UTI, urinary tract infection; UPEC, uropathogenic *E. coli*; CUP, chaperone usher pathway; LDA, lithium diisopropylamide; MWI, microwave irradiation; HA, hemagglutination.

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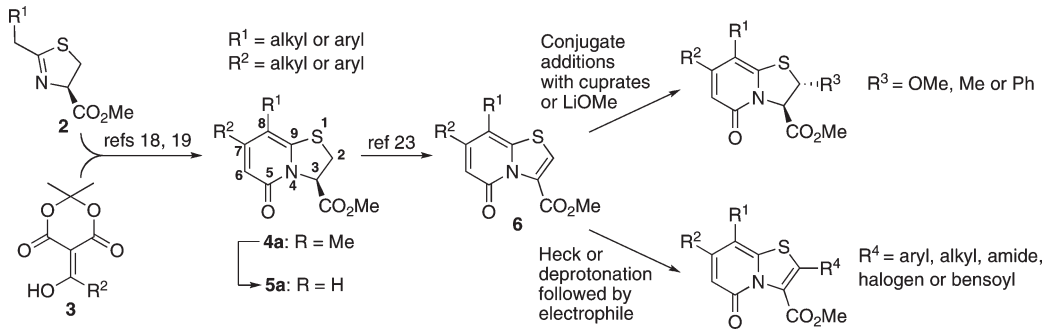


Figure 2. Synthesis of the dihydrothiazolo ring-fused 2-pyridone scaffold (**4**) from Δ^2-thiazolines (**2**) and Meldrum's acid derivatives (**3**). Prior to biological evaluation all compounds are hydrolyzed (e.g., **4a** to **5a**). From the oxidized scaffold (**6**), methods to introduce substituents in position C-2 on the scaffold have been developed.

outer membrane. The channel is gated by a plug domain. Ushers also have two periplasmic domains. The N terminal periplasmic domain interacts in part with a hydrophobic patch on the N terminal domain of the chaperone exposed on incoming chaperone/subunit complexes. One class of compounds termed pilicides, consisting of a dihydrothiazolo ring-fused 2-pyridone scaffold, has been shown to prevent the formation of pili by blocking the interaction of chaperone/subunit complexes with the N terminal domain of the usher, thus preventing the donor strand exchange reaction and the formation of chaperone-usher assembled pili, exemplified in UPEC.[14,15] The X-ray crystal structure of pilicide **1** bound to the P pilus chaperone PapD revealed that **1** binds to the hydrophobic patch on the N terminal domain of the chaperone (formed by residues I93, L32, and V56) which forms the well-conserved usher binding site (Figure 1).[14,16] Competition binding studies confirmed that the pilicide **1** prevents binding of chaperone−subunit complexes to the usher N-terminal domain. Pilicide **1** therefore functions by blocking delivery of subunits to the usher, thus preventing donor strand exchange and inhibiting pilus assembly (Figure 1). Besides being useful as potential antibacterial agents, pilicides have been used as chemical tools to study details of pilus assembly and their role in disease processes.[14,17]

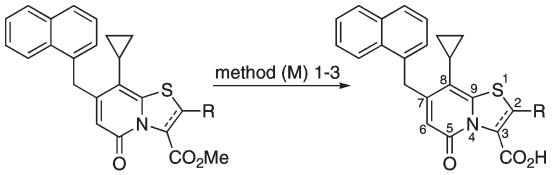
In the crystal structure showing pilicide **1** bound to the chaperone−subunit complex there is unoccupied space near the thiazolo part of the pilicide scaffold (Figure 1iii). We hypothesized that introducing substituents in this part of the scaffold could thus lead to favorable interactions between the chaperone and the pilicide and thereby result in increased biological activity. We decided to decorate the C-2 position on the scaffold to create interactions with the unoccupied space in the crystal structure to improve pilicide activity. The dihydrothiazolo ring-fused 2-pyridone scaffold (**4**), which constitutes the platform for design of new pilicides, can be synthesized via an acyl ketene imine cyclocondensation.[18,19] This method introduces substituents directly in the C-7 and C-8 positions on the scaffold, leaving positions C-6 and C-2 open for further improvements of the pilicides (Figure 2). Previous studies have also described a number of methods for the introduction of various substituents in position C-6 on the core structure.[20−22] Although this did not lead to any substantially increased biological activity, substitutions at C-6 allow for the introduction of solubility enhancing substituents. The C-2 position on the scaffold had not been varied until recently, when we reported on the decoration of this position starting from the α,β-unsaturated methyl ester (**6**) (Figure 2).[23] From this α,β-unsaturated methyl ester substituents were introduced by conjugate additions resulting in an addition with complete trans selectivity. In addition, substituents were introduced with a retained double bond by the use of Heck couplings or deprotonation using Lithium diisopropylamide (LDA) and subsequently react the lithiated scaffold with different electrophilic reagents. Together, these different methods opened up the possibility of introducing a wide range of substituents with varying size, electronic properties, and spatial arrangement (Figure 2).

Here, we present biological and crystallographic data on second generation compounds that were synthesized via C-2 substitution of the thiazolo ring-fused 2-pyridone **6**, based on the pilicide **1**−PapD complex crystal structure. The C-6 morpholinomethyl substituent in pilicide **1**, earlier introduced for solubility reasons, was not included in this study, as it is not vital for pilicide activity. The activities of the compounds were evaluated in pilus dependent biofilm and hemagglutination assays. Compared to the parent pilicide **5a**, C-2 substituted compounds have significantly improved IC_{50} in inhibiting pilus formation. The molecular basis of the observed improved biological activity of the compounds was determined by elucidating the X-ray crystal structures of pilicides bound to the PapD−PapH chaperone−subunit complex (PapH is the P pilus termination subunit[24]). The pilicides bound to the previously identified binding site on the chaperone N-terminal domain near strands F_1, C_1, and D_1. The introduced substituents were accommodated in a shallow hydrophobic pocket on the chaperone surface, formed by residues P30, L32, I93, and P95.

Results and Discussion

The developed methods for C-2 substitution were implemented to introduce a diverse set of substituents on a known pilicide (**4**)[23] to increase the potency of the compounds in inhibiting pilus assembly. The generated set of compounds (**4a**−**d** and **6a**−**f**) were then subjected to hydrolysis by three different methods depending on the nature of the substituents to give the corresponding carboxylic acids (**5a**−**d** and **7a**−**f**) in 80−95% yield (Table 1). The synthesized C-2 substituted thiazolo and dihydrothiazolo ring-fused 2-pyridones were biologically evaluated for their ability to block pilus formation as measured in a pili-dependent biofilm assay on polyvinylchloride plastic.[25] In this assay, deletion of the type 1 pilus gene cluster in *E. coli* strain UTI89 completely destroys the ability of the bacteria to form biofilm. Thus, the amount of biofilm that is formed in UTI89 grown in the presence of pilicide is related to the potency of the compound in blocking piliation. This whole bacteria assay is suitable for screening of

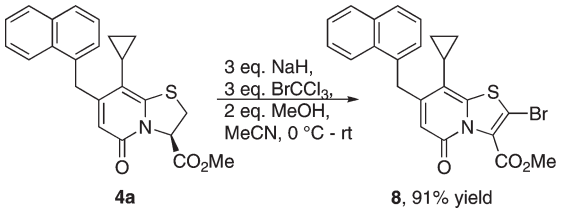
Table 1. Synthesized Compounds Were Hydrolyzed and Evaluated for Their Ability To Prevent Pili Dependent Biofilm Formation



bond C-2/C-3	R	M[a]	compd	yield[b]	IC_{50}[c] (μM)
single	H-	1	**5a**	quant	> 800[d]
single	Me-	1	**5b**	92	54
single	MeO-	*e*	**5c**	75[e]	167
single	Ph-	1	**5d**	95	22
double	Me-	2	**7a**	85	90
double	$HO(CH_3)_2C$-	1	**7b**	93	304
double	Ph-	2	**7c**	85	11
double	4-MeO-Ph-	2	**7d**	90	NA[f]
double	PhCO-	3	**7e**	80	NA[f]
double	PhNHCO-	2	**7f**	88	159

[a] Method 1: (i) LiOH in THF/MeOH, room temp. (ii) H^+ Method 2: (i) KOH in THF/MeOH, MWI, 90 °C 25 min. (ii) H^+ Method 3: (i) Et_3N, LiBr in $MeCN/H_2O$, room temp; (ii) H^+. [b] Isolated yield. [c] Calculated from 16−32 data points on every concentration (Figure S1 in Supporting Information). [d] Figure S1 in Supporting Information. [e] Hydrolyzed directly in the methoxy addition.[23] [f] Not active.

Scheme 1

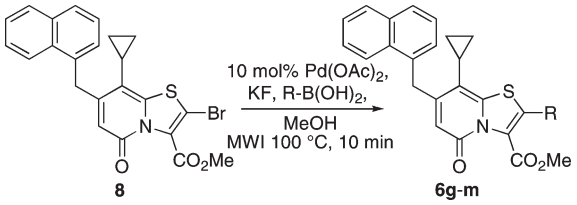


the synthesized compounds and provides more relevant biological information than elementary in vitro binding assays. The results are summarized in Table 1.

These experiments revealed that the addition of substituents in the C-2 position of the pilicide scaffold significantly enhanced the potency of the pilicides, resulting in increased inhibition of pili dependent biofilm formation. The phenyl substituted **5d** and **7c** proved to be the best pilicides with significantly improved potency compared to their parent lead **5a**. Also, the methyl substituents proved to increase the potency. However, the influence of the spatial arrangement on activity is different between the phenyl and methyl substituents. In the phenyl case, the unsaturated **7c** is more potent than the saturated counterpart **5d**, whereas in the methyl case the situation is the opposite; saturated **5b** is more potent than the unsaturated **7a**. The remaining substituents resulted in 15−30 times lower potency compared to the most active **7c** (**5c**, **7f**, and **7b**) or were completely inactive (**7d** and **7e**).

On the basis of the results with phenyl substituents and the unsaturated analogue in particular, an additional investigation with C-2 aryl and heteroaryl substituents was performed. We decided to use the Suzuki−Miyaura cross-coupling to introduce these substituents.[26] To implement these cross-coupling reactions, a bromo-substituted unsaturated ring-fused 2-pyridone **8** was desired. The synthesis of **8** had previously been accomplished by a two-step process starting with oxidation of **4a** followed by lithiation and bromination.[23] To avoid unnecessary purification and isolation of the oxidized ring-fused 2-pyridone **6**, a one-pot procedure directly from **4a** to **8** by modifying the developed oxidation method was envisioned. Consequently, by adjustment of the reaction conditions and treatment of **4a** with 3 equiv of NaH followed by 3 equiv of $BrCCl_3$ and finally 2 equiv of MeOH, the desired bromo derivative **8** was synthesized in 91% yield (Scheme 1).

Table 2. Isolated Yields of C-2 Substituted Unsaturated Thiazolo Ring-Fused 2-Pyridones



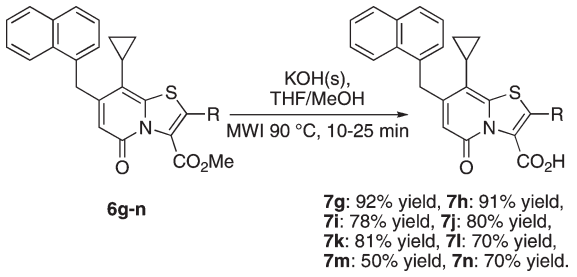
compd	R	yield (%)[a]
6g		96
6h		96
6i		72
6j		94
6k		90
6l		46
6m		34

[a] Isolated yield.

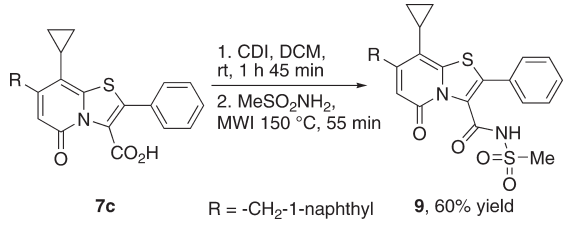
After we successfully established a robust one-pot method for the key compound **8**, our focus was directed to the subsequent Suzuki−Miyaura cross-coupling. For this reaction we adapted a method previously used within our lab for Suzuki−Miyaura cross-couplings.[27] Interestingly, we discovered that this reaction could be performed with no use of ligands and still give the same or increased yields without influencing reaction time and temperature. Hence, seven different aryl- or heteroarylboronic acids could be coupled to give C-2 substituted thiazolo ring-fused 2-pyridones **6g**−**m** by running the reaction at 100 °C for 10 min by microwave irradiation (MWI) with 10 mol % $Pd(OAc)_2$, 2 equiv of boronic acid, and 1.9 equiv of KF in dry MeOH (Table 2). The reaction resulted in good to excellent yields (72−97%, Table 2) with the furane boronic acids as the only exceptions resulting in thiazolo ring-fused 2-pyridones **6l** and **6m** in 46% and 34% isolated yield, respectively (Table 2).

To expand this set of compounds, benzyl substituted analogue **6n** was synthesized in 75% yield via lithiation followed by addition of benzyl bromide using the previously described procedure.[23] Before being evaluated, the compounds were hydrolyzed (KOH in THF/MeOH and MWI for 10−20 min at 90 °C, method 2). This rendered the corresponding carboxylic acids (**7g**−**n**) in 50−92% yield (Scheme 2).

Scheme 2



Scheme 3



In addition to this, it has been shown that the carboxylic acid is important for retaining biological activity[28] and that the use of carboxylic acid isosteres (e.g., tetrazoles or acylsulfonamides) could lead to increased pilicide activity.[29] Hence, **7c** was also transformed into its corresponding methylacylsulfonamide **9**, which had proven to be one of the most promising isosteres, in 60% yield (Scheme 3).

Finally, the Suzuki coupled aryl and heteroaryl compounds, the benzyl derivative, and the methylacylsulfonamide acid isostere were all biologically evaluated for their ability to prevent pili dependent biofilm formation (Table 3).

We found that many of the aryl- and heteroaryl-substituted compounds efficiently inhibited formation of biofilm. The benzyl substituted compound **7n** had an even lower IC_{50} than the most active compound from the first biofilm evaluation (**7c**) (Table 3). The tolyl substituted compounds **7g** and **7h** were both highly active; 3-tolyl (**7g**) resulted in a similar activity as the phenyl substituted analogue **7c** (Table 3). Of the smaller heteroaryls, the thiophene substituted **7k** proved to be more potent than the furan substituted **7l** and **7m** (Table 3). Sterically more demanding substituents as in **7i** and **7j** were not tolerated, and both of them turned out to be inactive (Table 3). Interestingly, the acid isosteric acylsulfonamide in **9** did not give any additive effect but instead gave a 3-fold decreased potency compared to the carboxylic acid **7c** (Table 3). Next, the best compounds from the biofilm evaluations were further tested using a hemagglutination (HA) assay (Table 3).[14] In this assay, the degree of piliation of the culture is related to the HA titer. Thus, after growth in the presence of pilicide, the HA titers of the cultures were determined to evaluate the relative potencies of the compounds in blocking pilus formation. Growth of UTI89 in the presence of all five tested compounds resulted in significantly lower HA titers. The benzyl substituted **7n** and the 3-tolyl substituted **7g** resulted in 16 times increased activity compared to the hydrogen substituted compound **5a** (Table 3). Interestingly, **7n**, **7g**, and the thiophene substituted compound **7k** were all more active than the phenyl substituted **5d** and **7c** (Table 3). Importantly, the compounds did not affect the bacterial growth (Figure S2 in Supporting Information).

Table 3. Biofilm IC_{50} and HA Titer Data for the C-2 Aryl Substituted **7g−n** and **9**

compd	bond C-2/C-3	C-2 substituent	IC_{50}[a] (μM)	HA titer[b]
5a	single	H-	> 800[c]	7
5d	single	Ph-	22	4
7c	double	Ph-	11	4.5
7g	double	3-tolyl-	11	3
7h	double	4-tolyl-	13	
7i	double	5-indole-	NA[d]	
7j	double	6-(1,4-bensodioxane)-	NA[d]	
7k	double	3-thiophene-	25	3.5
7l	double	3-furane-	78	
7m	double	2-furane-	61	
7n	double	Bn-	7	3
9	double	Ph-	39	
DMSO[e]				8
f				8

[a] Calculated from 16−32 data points on every concentration (Figure S1 in Supporting Information). [b] Number of wells with agglutination (2^n). *E. coli* was grown in the presence of pilicide (250 μM), and HA titers were determined, average of dublicate runs. [c] Figure S1 in Supporting Information. [d] Not active. [e] All compounds were dissolved in DMSO. [f] Solely UTI89.

The X-ray crystal structure of pilicide **1** bound to the P pilus chaperone PapD previously revealed that the pilicide binds a conserved hydrophobic patch on the chaperone N-terminal domain formed by residues L32, V56, and I93, which coincides with the usher binding site. Subsequently, pilicide **1** was shown to block the binding of chaperone−subunit complexes to the usher in an in vitro binding assay. Thus, we hypothesized that pilicide **1** should be capable of binding to chaperone−subunit complexes to exert its mechanism in blocking binding of the complex to the usher. This hypothesis was tested by determining the X-ray crystal structure of pilicide **1** bound to the PapD−PapH chaperone−subunit complex. Pilicide **1** was found to bind to the same global binding site as seen in the PapD−pilicide **1** structure; however, substantial reorientation was observed, possibly as a result of the conformational changes in the loop between strands F_1 and G_1 in the subunit-bound and free chaperone state (Figure 3i). In the pilicide **1**−PapD structure, the pilicide carboxylic acid and carbonyl group make an electrostatic interaction with R96 and R58, respectively (Figure 1iii). In the PapD−PapH bound structure, these interactions are broken. We note that in the PapD−pilicide **1** complex, the morpholine ring of **1** is in contact with a neighboring molecule such that the effect of crystal contacts in the observed reorientation in the PapD- and PapD−PapH-bound compound cannot be excluded. Finally, to investigate the structural basis for the observed improved IC_{50} after introduction of C-2 substituents, PapD−PapH crystals were soaked with the best compounds. Soaks with **5d** generated a crystal, showing the pilicide bound to the chaperone−subunit (PapD−PapH) complex near the same position as the C-2 unsubstituted **1** (Figure 3ii). Compared to the pilicide **1**-bound form, L32 undergoes a conformational change when bound to **5d**, which creates a shallow pocket that accommodates the phenyl substituent at the C2 position (Figure 3, ii and iii). The crystal structure of the N terminal domain of the FimD usher bound to the FimC−$FimH_{158-279}$[16] chaperone−subunit complex is known. Modeling, based on this information, clearly shows the clash between pilicide and the usher binding site (Figure 3iv).

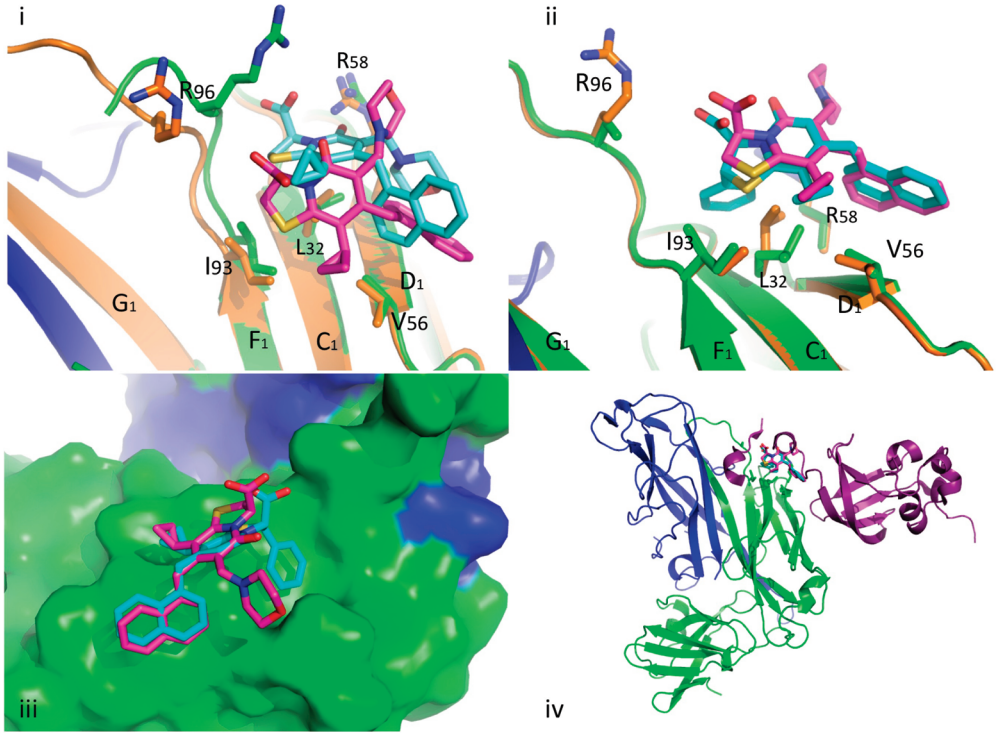


Figure 3. (i) Pilicide **1** bound to the PapD−PapH chaperone−subunit complex (pink) undergoes a reorientation compared to pilicide **1** bound to the PapD chaperone (cyan). (ii) Pilicide **5d** (cyan) binds near the same position as C-2 unsubstitued pilicide **1** (pink) in the PapD−PapH chaperone−subunit complex. (iii) L32 reorients and thus creates a shallow pocket for the C-2 substituent in **5d** (cyan). (iv) Clash between the usher FimD N-terminal (yellow) and the pilicides (cyan and pink) binding site (chaperone FimC in green and subunit FimH in blue).

Conclusion

A new route for fast and convenient synthesis of C-2 aryl substituted pilicides was developed and applied. Biofilm and HA assays revealed that proper decoration of this position can significantly improve the antivirulence activity of the compounds. The most rewarding substituents proved to be phenyls in general and the benzyl and tolyl substituents in particular. Smaller heteroaryls (thiophene and furanes) also improved activity, whereas sterically more demanding groups as indoles and 1,4-benzodioxanes abolished all activity. Combining a C-2 phenyl substituent with an acid isoster did not give any additive effect; instead a drop in activity was observed.

X-ray crystallography of the pilicides bound to the PapD-PapH chaperone−subunit complex revealed that introduction of a C-2 phenyl substituent induced a reorientation of one of the chaperone (PapD) residues (L32), generating a shallow pocket that accommodates this substituent.

Experimental Section

General. Flash column chromatography (eluents given in brackets) was performed on silica gel (Matrex, 60 Å, 35−70 μm, Grace Amicon). Parallel flash chromatography was performed on a Gradmaster parallel Jones chromatograph. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 in $CDCl_3$ [residual $CHCl_3$ (δH 7.26 ppm) or $CDCl_3$ (δC 77.0 ppm) as internal standard] at 298 K. IR spectra were recorded on an ATI Mattson Genesis series FTIR spectrometer. Microwave reactions were carried out using a monomode reactor (Smith Creator, Biotage AB) in Teflon septa capped 0.5−2 or 2−5 mL Smith process vials with stirring. Reaction times refer to irradiation time at target temperature, as measured by IR sensor. Purities of key compounds were >95% as determined by ^{1}H NMR and HPLC.

General Procedure for the Preparation of 6g−m. The procedure for 8-cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-2-*m*-tolyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid methyl ester (**6g**) is representative. Dry MeOH (0.9 mL) was added to pyridone **8** (20 mg, 0.043 mmol), $Pd(OAc)_2$ (1 mg, 0.004 mmol), KF (5 mg, 0.81 mmol), and boronic acid (12 mg, 0.085 mmol). The mixture was heated in a sealed tube by MWI at 100 °C for 10 min. The resulting mixture was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were concentrated under reduced pressure and purification by parallel flash chromatography [heptane/EtOAc 100:0 to 0:100] to give pyridone **6g** (20 mg, 96% yield) as a yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77−0.84 (m, 2H), 1.02−1.10 (m, 2H), 1.76−1.86 (m, 1H), 2.41 (s, 3H), 3.88 (s, 3H), 4.55 (s, 2H), 5.94 (s, 1H), 7.21−7.26 (m, 2H), 7.30−7.36 (m, 1H), 7.37−7.44 (m, 3H), 7.45−7.52 (m, 2H), 7.75−7.81 (m, 1H), 7.81−7.91 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.9 (2C), 10.9, 21.4, 36.2, 53.3, 111.7, 111.9, 123.7, 125.4, 125.5 (2C), 125.7, 126.2, 127.3, 127.6, 128.6, 128.8, 128.9, 129.0, 129.1, 130.8, 131.9, 133.9, 134.2, 139.0, 146.3, 153.4, 158.9, 161.8.

General Procedure for the Preparation of 7a,c,d,f−n. The procedure for 8-cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-2-*m*-tolyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (**7g**) is representative. KOH (s) (20 equiv) was added to **6g** (17 mg, 0.0355 mmol) dissolved in THF (1 mL) and MeOH (0.5 mL). The suspension was heated by microwave irradiation for 25 min at 90 °C before being cooled to room temperature. The mixture was diluted in CH_2Cl_2 and washed twice with 1 M HCl. The combined organic layers were concentrated and purified by column chromatography [CH_2Cl_2/MeOH 97:3 → CH_2Cl_2/MeOH 95:5 and 1% AcOH] to give **7g** (15.2 mg, 92% yield) which was lyophilized from MeCN/H_2O to give a light-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.68−0.75 (m, 2H), 0.94−1.03 (m, 2H), 1.66−1.76 (m, 1H), 2.32 (s, 3H), 4.45 (s, 2H), 6.12 (s, 1H), 7.02−7.09 (m, 1H), 7.14−7.18 (m, 1H), 7.20−7.25 (m, 1H), 7.27−7.38 (m, 1H), 7.37−7.41 (m, 1H),

7.41−7.50 (m, 3H), 7.70−7.80 (m, 2H), 7.82−7.88 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.3 (2C), 11.4, 21.5, 36.7, 111.3, 113.7, 124.2, 126.0, 126.1, 126.2, 126.7, 127.9, 128.1, 128.3, 128.7, 129.3, 129.38, 129.43, 129.6, 131.1, 132.4, 134.5, 134.8, 139.4, 148.0, 154.4, 160.0, 164.5.

Structure Determination of Pilicides 1 and 5d Bound to PapD−PapH. The PapD−PapH complex (where for PapH, residues 1−22 of the mature protein are removed for stability) was purified and crystallized as previously described.[30] Complexes with pilicide **1** and **5d** were formed by a 24 h incubation of PapD−PapH crystals in the crystallization solution (10 mM cobalt chloride, 100 mM MES, pH 6.5, and 1.8 M ammonium sulfate) containing 2 mM compound.

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Supporting Information Available: Synthetic procedures and characterization data of all compounds, growth curves for **5d**, **7c**, **7f**, and **9**, details of data quality and structure refinement for structure determination of **1** and **5d** bound to PapD−PapH. This material is available free of charge via the Internet at http://pubs.acs.org.

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