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# Mapping pilicide anti-virulence effect in *Escherichia coli*, a comprehensive structure–activity study

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## Abstract

Pilicides prevent pili formation and thereby the development of bacterial biofilms in *Escherichia coli*. We have performed a comprehensive structure activity relationship (SAR) study of the dihydrothiazolo ring-fused 2-pyridone pilicide central fragment by varying all open positions. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) was used to distinguish active from inactive compounds in which polarity proved to be the most important factor for discrimination. A quantitative SAR (QSAR) partial least squares (PLS) model was calculated on the active compounds for prediction of biofilm inhibition activity. In this model, compounds with high inhibitory activity were generally larger, more lipophilic, more flexible and had a lower HOMO. Overall, this resulted in both highly valuable SAR information and potent inhibitors of type 1 pili dependent biofilm formation. The most potent biofilm inhibitor had an $EC_{50}$ of 400 nM.

### Keywords

Pilicide; Antivirulence; 2-Pyridone; Peptidomimetic; Structure–activity; Biofilm inhibitor

## 1. Introduction

The discovery of compounds with antimicrobial activity in the 1940s resulted in tremendous advances for humanity. However, the reduced efforts put into antibacterial research in combination with the increasing development of bacterial resistance have resulted in the re-emergence of bacterial diseases as a global health problem. Therefore, the development of new antibacterial targets and strategies, especially those directed towards Gram-negative bacteria, is of great importance.[1]

Bacterial pathogens have an arsenal of virulence factors that are critical in promoting host–pathogen interactions. One such factor is type 1 pili, assembled by the chaperone/usher pathway (CUP), which constitute a critical virulence factor in uropathogenic *Escherichia*



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*coli* (UPEC).[2] Type 1 pili facilitate the attachment to and invasion of the bladder epithelium and is important for IBC formation.[3–6] In addition, recent studies concluded that type 1 pili play an important role in human cystitis,[7] and it has been reported that type 1 pili fulfill 'Molecular Koch's postulates' of microbial pathogenesis.[8]

Biofilm formation is a severe complicating factor in bacterial infections due to the increased resistance of bacteria within biofilms to immune- or drug-mediated clearance, leading to persistent, drug-tolerant infections.[9–11] Thus, biofilms exacerbate bacterial infections, such as urinary tract infection (UTI), causing significant human morbidity and mortality and billions of dollars in health care expenditures.[12]

Type 1 pili and other CUP pili are promising targets of anti-virulence therapeutics as they are critical factors for binding and invading the bladder tissue and forming biofilms.[13,14]

Pilicides are a class of small molecular weight compounds that prevent formation of pili in UPEC.[15–17] The assembly proteins of the CUP pilus systems have a high degree of structural conservation and compounds that target pili formation can thus potentially have broad-spectrum activity.[18] The pilicide core structure (**5**) is based on a dihydrothiazolo ring-fused 2-pyridone that can be synthesized via an acyl-ketene imine cyclocondensation of acyl-Meldrum's acid derivatives (**3**) and thiazolines (**4**).[19,20] The acyl-Meldrum's acid derivatives (**3**) and thiazolines (**4**) are generally obtained from commercially available carboxylic acids (**1**) and nitriles (**2**), respectively. This synthetic procedure introduces substituent diversity in position C-7 and C-8 already in the scaffold formation by the choice of carboxylic acids (**1**) and nitriles (**2**). Furthermore, various synthetic methodologies for the introduction of substituents at the C-2 and C-6 positions of the scaffold have been developed (Fig. 1).[16,21–24]

Rational alteration of the pilicide scaffolds substituents using multivariate design could generate valuable new insights into the intricate relationship between pilicide structure and biological activity and ultimately result in comprehensive structure–activity relationship (SAR) information. Multivariate design can be used to decipher the complex correlation patterns between chemical structures and specific biological responses and properties. In multivariate design, a representative subset of compounds is selected from a more complete virtual set. This considerably reduces the number of compounds to be synthesized and evaluated while retaining much of the chemical information associated with the full compound set. To enable this subset selection, all compounds are described by numerical molecular descriptors. To visualize the data set of different compounds with their corresponding descriptors, the use of principal component analysis (PCA)[25] is often applied. From this, a representative selection of a subset can be performed by applying statistical experimental designs, that is, statistical molecular design (SMD).[26,27] This results in a balanced subset of compounds that encompass as much as possible of the significant variation from the entire data set.[28]

Here we present a multivariate design strategy on di-substituted derivatives of the pilicide scaffold from which the most promising compounds could be further substituted to give highly active tri- and tetra-substituted derivatives. Furthermore, the set of di-substituted derivatives could also be used to construct a reliable SAR model that could correctly rank the external validation set. Overall the strategy was rewarding and significantly improved pilicides and valuable SAR information was obtained. The results described herein will guide future studies of these compounds as research tools to study the biological processes they interfere with, which ultimately may lead to novel anti-virulence therapeutics.

## 2. Results and discussion

### 2.1. Synthesis and evaluation of di-substituted derivatives

Starting from suitable commercially available carboxylic acids and nitriles, two sets of potential C-7 and C-8 substituents were identified and their chemical diversity was characterized by molecular descriptors. Eight substituents at each position were manually selected from the chemical space generated by PCA. The choice of substituents was based on two known pilicides that both had a $CH_2$-1-naphthyl substituent in C-7 and a phenyl or cyclopropyl in C-8 (Fig. 1).[15] Hence, substituents were selected from the chemical space in the vicinity of these substituents with varying sizes, polarities and flexibility (Fig. 2).

All combinations of the eight C-7 and eight C-8 substituents, resulted in a virtual set of 64 different pilicides. An SMD using D-optimal design was used to select a balanced subset of 24 compounds from this set, in which all of the substituents were represented three times (Table 1).

The 24 derivatives of the pilicide scaffold were next synthesized in a five step synthetic sequence to the target compounds.[20] In general, the acyl-ketene imine cyclocondensation produced the desired compounds in good yields (66–93%). However, the polar C-7 substituent in building block **1g** proved problematic and resulted in lower yields (15–50%). The C-8 indole building block **2f** resulted in both atropisomers, probably due to increased sterical clash with adjacent substituents, and low yields in the acyl-ketene imine cyclocondensation, primarily due to acylation of the indole by the acyl-ketene.

Before biological evaluation could be undertaken, the compounds had to be hydrolyzed to the corresponding lithium carboxylates. This was straightforward and all 24 target compounds were isolated in yields above 90%. The 24 compounds were evaluated using a pili dependent biofilm assay.[29] In this assay, blocking the formation of type 1 pili formation in the clinical isolate *E. coli* strain, UTI89, completely prevents the bacteria's ability 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 the formation of pili. The results are summarized in Table 1.

We discovered that many of the di-substituted compounds efficiently prevented pilus-dependent biofilm formation. In general, the potency of the compounds turned out to be highly dependent upon the nature of both substituents. The best compound in the set was the C-8 indole and C-7 naphthoxy substituted **24** with an $EC_{50}$ of 12 μM. Compounds incorporating a thiophene or 3,4-methylenedioxyphenyl group at C-8 (**18–22**) were also effective, except when combined with a C-7 coumarin substituent as in **17**. The C-8 phenyl substituent was also favorable, but pyridine and the smaller methoxy, cyclopropyl, and isopropyl substituents resulted in reduced potency. The most useful C-7 substituents were the 1-naphthylmethyl, naphthoxymethyl, 3-tolylethyl, and 2,3-dimethylphenoxymethyl groups. Exchange of the 1-naphthylmethyl group and extension of the C-7 linker were well tolerated, but heteroaryls were not.

### 2.2. Multivariate design

A structure–activity investigation was conducted based on the balanced set of di-substituted compounds and biological responses using a two-step modeling strategy. First, a model to distinguish active from inactive compounds was constructed followed by a QSAR model based on the active compounds for prediction of biofilm inhibition activity. Consequently, all di-substituted compounds (**6–31**) were characterized with descriptors (see Supplementary data for complete list) and a OPLS-DA model was calculated, in which fourteen of the 26 di-substituted compounds (**6–9, 14, 16, 18–25**) were classified as active and the remaining

in-active. The resulting two-component OPLS-DA model[30] ( $R^2_X$=0.70, $R^2_Y$=0.79, $Q^2$= 0.71) discriminated between the two classes of compounds (Fig. 3a) with acceptable accuracy considering the challenge of QSAR modeling when complex whole cell responses are used. The polarity of the compounds appears to be the most important factor for discrimination, where active compounds are more lipophilic than the in-active ones. In the second step, the di-substituted compounds were correlated to their biofilm inhibition activity using PLS.[31,32] This resulted in a one-component model ( $R^2_X$=0.50, $R^2_Y$=0.62, $Q^2$ = 0.50) that explained the relationships between the chemical structure of the designed molecules and their ability to prevent biofilm formation (Fig. 3b). The compounds with high inhibitory effects were generally larger, more lipophilic, more flexible and had lower HOMO energies compared to the analogues with lower activity. To follow up on the results of the balanced compound set, and to validate and challenge the generated QSAR model, 14 additional di-substituted derivatives of the pilicide scaffold (**32–45**; Table 2) were synthesized (for predictions see Table 2 and Fig. 3). Four derivatives of the most potent biofilm inhibitor (**24**) from the tested compounds were prepared. In the first analogue (**32**), the C-7 naphthoxy was replaced with the C-7 naphthyl substituent that also had proved promising. The second analogue (**33**) retained the C-7 naphthoxy group but contained an N-methylated indole at C-8 rather than the free N–H indole found in **24**. In the third analogue (**34**), the indole was similarly methylated and in addition, the carboxylic acid was replaced with a methyl acylsulfonamide. The use of such groups as bioisosteres of carboxylic acids has been reported to enhance pilicide activity.[33,34] In the fourth analogue (**35**), the sulfur atom in the pilicide scaffold was exchanged with oxygen using a one-pot procedure starting from acylated L-serine derivatives.[19] Two additional oxygen analogues (**36** and **37**) of promising compounds from the tested derivatives were prepared using the same procedure. Furthermore, six compounds with presumed high activities that really challenge the model (**38–43**), one compound predicted to have intermediate activity (**44**), and one compound that was predicted to be inactive (**45**) were synthesized. All compounds (**32–45**) were biologically evaluated using the pili-dependent biofilm inhibition assay (Table 2).

As predicted, several of the compounds based on the SAR data from the first set of di-substituted compounds proved to be highly potent inhibitors of pili-dependent biofilm formation. Considering the differences between the first training set of compounds and the validation set of compounds, the predicted activity correlates well to the experimentally determined activity (Fig. 3b). The biofilm evaluations suggest that the introduction of sterically demanding substituents in position C-7 can greatly enhance biofilm inhibition capacity. Moreover, N-methylation of the C-8 indole substituent in **24** to give **33** does not have a negative effect on the biological response, which greatly facilitates the synthesis of such compounds (Table 2). The use of a naphthoxy group as the C-7 substituent seems to be superior to the C-7 naphthyl substituent (Table 2, **24** and **32**). The introduction of the methyl acylsulfonamide carboxylic acid isostere as in **34** was well tolerated, with an $EC_{50}$ value of 9 μM. The oxygen analogues retain much of the biological activity of the parent sulfur derivatives, which is encouraging in case metabolic sulfur oxidation would emerge as a problem. The oxygen analogue **36** retains all biological activity whereas **35**, and **37** result in a two and threefold decrease in activity, respectively as compared to compound **33** and **18** (Table 2, and **35**–**37** vs **18, 22,** and **33**).

### 2.3. Synthesis and evaluation of tri- and tetra-substituted derivatives

To generate more comprehensive SAR of the pilicide central fragment, the effect of substitution at the C-2 and C-6 positions, respectively, were evaluated by a new SMD (Table 3). The C-7 and C-8 di-substituted compounds **33**, **22**, and **18** were selected as starting points for exploring C-2 and C-6 variations based on their low molecular weight and

moderate to high potency ($EC_{50}$ values of 10, 21, 39 μM, respectively). The choice of substituents in position C-2 or C-6 on the pilicide scaffold was based on both previous studies of these positions as well as the size of the substituent.[16,17,24] Position C-2 was thus substituted with either a phenyl or methoxy substituent and C-6 with a morpholinomethyl or a hydroxymethyl substituent. The C-2 phenyl and methoxy substituents were introduced using a two-step process starting with C-2/C-3 oxidation of **33, 22,** and **18** followed by conjugate additions to the corresponding α,β-unsaturated derivatives to give **46–51** as the pure trans stereoisomers.[22]

Addition of a morpholinomethyl or a hydroxymethyl substituent in position C-6 was performed by following previously published protocols.[24] Formylation of **33, 22,** and **18** gave the corresponding aldehydes from which reduction or reductive amination gave the desired morpholinomethyl or hydroxymethyl substituted compounds **52–57**. After hydrolysis the 12 tri-substituted derivatives **46–57** were evaluated using the pili-dependent biofilm formation assay and the results are summarized in Table 3.

The C-2 phenyl substituent was the most effective in terms of increasing the pilicides potency. The most potent of the tri-substituted compounds was the C-2 phenyl substituted **48** with an $EC_{50}$ of 400 nM, which is almost a 100-fold more potent than the C-2 unsubstituted **18** (Table 3). The incorporation of C-2 phenyl substituents in **46** and **47** resulted in almost 10- and 20-fold increased activities, respectively, relative to **33** and **22**. The C-2 methoxy substitution increased the potency of **49** from **33** although in both **50** and **51** the same substitution reduced potency compared to their parent compounds. Both C-6 substituents (morpholinomethyl and hydroxymethyl) reduced the potencies of the compounds. However, **52–57** retained the majority of their potency with C-6 substituents, which could be valuable to improve the compounds solubility properties.

The synthesis and biological evaluation of the di-substituted compounds showed that the incorporation of indole or 3,4-methylenedioxyphenyl substituents at the C-8 position and a naphthoxymethyl substituent at the C-7 position of the pilicide scaffold results in compounds with enhanced potency. Moreover, the introduction of an appropriate C-2 substituent can further increase the compounds potency, while substituents can be incorporated at the C-6 position to improve the pilicides solubility. In using this SAR data to synthesize tetra-substituted pilicide derivatives, **33** and **22** were used as substrates for the introduction of substituents at both C-2 and C-6. Based on a recent study,[16] we incorporated a 3-tolyl substituent at the C-2 position of the oxidized scaffold. Our previously developed one-pot oxidation–bromination procedure followed by Suzuki–Miyaura couplings was performed on substrates **33** and **22** to obtain the C-2 substituted intermediates.[16] To increase the compounds solubility properties the morpholinomethyl substituent was introduced in position C-6 as described above. After subsequent hydrolysis the tetra-substituted derivatives **58** and **59** and the tri-substituted intermediates **60** and **61** were biologically evaluated for their ability to prevent pili-dependent biofilm formation. The results are shown in Table 4.

We have previously described that C2/C3 oxidized pilicides bearing a 3-tolyl C-2 substituent were more active than saturated pilicides bearing a phenyl group at the C-2 position.[16] However, the incorporation of a 3-tolyl substituent at the C-2 positions of **60** and **61** resulted in decreased activities compared to the analogous unsubstituted (**33** and **22**) and C-2 phenyl substituted (**46** and **47**) compounds (Tables 3 and 4). Interestingly, the introduction of a C-6 morpholinomethyl substituent, which previously was found to decrease activity (Table 3), resulted in 17- and 4-fold increases in the respective activities of the tetrasubstituted derivatives **58** and **59** relative to their C-6 unsubstituted counterparts **60** and **61** and also increased their water solubility.

## 3. Conclusion

Various synthetic methods for the introduction of substituents onto the basic pilicide scaffold were used to prepare a series of 51 new and highly-substituted analogues to generate more comprehensive pilicide SAR information. Biological testing of the 24 di-substituted compounds produced in the initial step of the design process revealed several compounds with improved potency and important QSAR information. Three efficient di-substituted inhibitors of biofilm formation were used to study the effects of further substituting the C-2 and C-6 positions. A set of 14 trisubstituted and two tetra-substituted compounds was synthesized. This showed that substitution of the scaffold's C-2 position can increase pilicide potency and both the C-2 and C-6 positions can be used to optimize the compounds solubility properties with retained activity. However, the effect of C-2 and C-6 substitution is dependent on the other substituents on the scaffold and is not simply an additive effect. Interestingly, the most potent tri-substituted compound **48** ($EC_{50}$ 400 nM) was obtained from the least potent of the three di-substituted compounds from the initial set that were chosen for further substitution. Compounds in which the sulfur atom in the pilicide scaffold was exchanged for oxygen retained much of the potency of the parent compounds and will thus be useful complements for use in oxidative environments. The use of a methyl acyl sulfonamide as a carboxylic acid isostere was tolerated but did not substantially increase activity.

In addition to generating potent pilicides and valuable SAR, the generated data from the set of di-substituted compounds could be used to construct a multivariate model. The multivariate model could correctly rank the external validation set consisting of 16 compounds with different substituents and substitution pattern. The herein generated comprehensive SAR information can be used to optimize biological activity and properties of these antivirulence compounds, which will be highly useful both from a future therapeutic perspective but also as research tools to study the complex biological processes involved in pili and biofilm formation.

## 4. Experimental section

### 4.1. Multivariate design

ChemFinderACX2002Prod[35] was used to search for carboxylic acids and nitriles with molecular weights below 250. These building blocks were imported to MOE[36] and molecular descriptors were calculated. The nitrile data set was manually filtered to remove undesired substituents mainly based on chemical compatibility giving 531 nitriles. The carboxylic acid data set was imported to SIMCA-P[37] and a PCA model was calculated with six components having an eigenvalue over two. The principal components were normalized to unit variance and centered. Building blocks with a Euclidean distance over 1.5 from $CH_2$-1-naphthyl carboxylic acid were discarded in all principal components resulting in a set of 822 carboxylic acids. The carboxylic acid and the cyanomethyl fragments were removed from the respective building blocks, as they are not part of the target compounds. The data sets were washed and molecular descriptors were calculated MOE.[36] The filtered datasets were imported to SIMCA-P[37] and PCA models were created. The carboxylic acids gave a model with five components with an eigenvalue over two ($R^2$: 0.82) and the nitriles similarly gave a model with five components with an eigenvalue over two ($R^2$: 0.84). From these models, eight C-7 and eight C-8 substituents were manually selected with respect to the properties of **1a** and **2a,b** from the lead compounds **6** and **7**. Combining these substituents in position C-7 and C-8, respectively on the pilicide scaffold resulted in a set of 64 derivatives for which molecular descriptors were calculated (see Supplementary data). The principal component data from the PCA model of the 64 derivatives was imported to Modde[38] and a D-optimal design was calculated to select 24 compounds (all substituents

represented three times). To discriminate active from inactive compounds, an orthogonal partial least square discriminant analysis (OPLS-DA) model was calculated. Fourteen of the 26 di-substituted compounds (**6–9, 14, 16, 18–25**) were classified as active and the rest as inactive, resulting in a two-component OPLS-DA model ( $R^2_X$=0.70, $R^2_Y$=0.79, $Q^2$ = 0.71). A PLS model was created with the active di-substituted compounds using the $pEC_{50}$ values as response giving a one-component model ( $R^2_X$=0.50, $R^2_Y$=0.62, $Q^2$ = 0.50). Compounds from the validation set (**32–45**) with a DModX below three were used as a prediction set to validate the models.

## 4.2. Synthesis

**4.2.1. General remarks—**All reactions were carried out under inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. THF was freshly distilled from potassium, $CH_2Cl_2$ was distilled from calcium hydride, MeCN and MeOH was dried over activated 3 Å molecular sieves. TLC was performed on Silica Gel 60 F254 (Merck) with detection by UV light (254 nm). 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 Chromatography using silica gel (Matrex, 60 Å, 35–70 μm, Grace Amicon). $^1$H and $^{13}$C NMR spectra were recorded on a Bruker DRX-400 or Bruker DRX-360 in $CDCl_3$ [residual $CHCl_3$ ($\delta_H$ 7.26 ppm) or $CDCl_3$ ($\delta_C$ 77.0 ppm) as internal standard], $CD_3OD$ [residual $CD_3OD$ ($\delta_H$ 3.31 ppm) or $CD_3OD$ ($\delta_C$ 49.0 ppm) as internal standard], DMSO-$d_6$ [residual DMSO ($\delta_H$ 2.50 ppm) or DMSO-$d_6$ ($\delta_C$ 40.0 ppm) as internal standard] or $CDCl_3$/$CD_3OD$ mixtures using $CD_3OD$ (see above) as internal standard at 298 K. Microwave reactions were carried out using a monomode reactor (Smith Creator, Biotage AB) in Teflon septa capped 0.5–2 ml or 2–5 ml Smith TM process vials with stirring. Purities of key compounds were >95% as determined by $^1$H NMR and HPLC.

The synthesis was performed by following previously reported procedures.[14,16,19,22,24] The acyl-ketene imine cyclocondensations were typically performed in 0.5 mmol scale. Hydrolysis was generally performed in 0.15 mmol scale.

**4.2.2. (3*R*)-7-(3-Methylphenethyl)-5-oxo-8-phenyl-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (8)—**Isolated in 76% overall yield as a light grey foam. $[\alpha]_D$ −16 (*c* 0.5, $CH_2Cl_2$/MeOH 9:1); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.20 (s, 3H), 2.44–2.62 (m, 4H), 3.43–3.51 (m, 1H), 3.76–3.85 (m, 1H), 5.49 (dd $J_1$ = 1.38 Hz, $J_2$ = 8.95 Hz, 1H), 6.16 (s, 1H), 6.67–6.76 (m, 2H), 6.91–6.97 (m, 1H), 7.04–7.11 (m, 1H), 7.21–7.56 (m, 4H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 20.9, 31.3, 34.7, 34.8, 63.4, 113.1, 114.3, 125.0, 126.5, 128.06, 128.10, 128.7, 128.8 (2C), 129.98, 130.04,136.4, 137.2, 140.8, 147.6,154.0, 160.2, 169.6. HRMS (electrospray ionization) calcd for [M–H] $C_{23}H_{20}NO_3S$ 390.1164, obsd 390.1161.

**4.2.3. (3*R*)-7-((2,3-Dimethylphenoxy)methyl)-5-oxo-8-phenyl-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (9)—**Isolated in 77% overall yield as a light grey foam. $[\alpha]_D$ −48 (*c* 0.5, DMSO); $^1$H NMR (400 MHz, MeOD) $\delta$ 2.15 (s, 3H), 2.22 (s, 3H), 3.56 (dd $J_1$ = 1.73 Hz, $J_2$= 11.83 Hz, 1H), 3.76 (dd $J_1$ = 8.79 Hz, $J_2$= 11.84 Hz, 1H), 4.56–4.68 (m, 2H), 5.66 (dd $J_1$ = 1.68 Hz, $J_2$ = 8.73 Hz, 1H), 6.40 (d, $J$ = 8.15 Hz, 1H), 6.64 (s, 1H), 6.72 (d, $J$ = 7.55 Hz, 1H), 6.90 (t, $J$ = 7.88 Hz, 1H), 7.24–7.33 (m, 2H), 7.36–7.46 (m, 3H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 12.0, 20.2, 32.3, 64.6, 67.5, 109.7, 112.3, 115.5, 123.6, 125.8, 126.4, 129.4, 129.8 (2C), 130.0, 130.4, 135.6, 138.7, 149.0, 152.4, 156.4, 163.1, 170.2. HRMS (electrospray ionization) calcd for [M–H] $C_{23}H_{20}NO_4S$ 406.1113, obsd 406.1110.

**4.2.4. (3*R*)-7-((7-Methoxy-2-oxo-2*H*-chromen-4-yl)methyl)-5-oxo-8-phenyl-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (10)—**Isolated in 70% overall yield as a light yellow non-crystalline solid. $[\alpha]_D$ −17 (*c* 0.25, $CHCl_3$); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.49 (dd, $J_1$ = 1.79 Hz, $J_2$ = 11.82 Hz, 1H), 3.72–3.88 (m, 6H), 5.48 (dd, $J_1$ = 1.63 Hz, $J_2$ = 9.01 Hz, 1H), 5.93 (s, 1H), 6.01 (s, 1H), 6.88 (dd, $J_1$ = 2.50 Hz, $J_2$ = 8.83 Hz, 1H), 6.96 (d, $J$ = 2.49, 1H), 7.20–7.28 (m, 1H), 7.29–7.36 (m, 2H), 7.36–7.44 (m, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 32.4, 35.7, 56.8, 64.6, 101.9, 112.8, 113.1, 115.0, 115.3 (2C), 127.0, 129.2, 129.8 (2C), 130.8 (broad, 2C), 136.8, 149.6, 150.8, 154.0, 155.8, 160.79, 160.83, 163.3, 170.4. HRMS (electrospray ionization) calcd for [M+H] $C_{25}H_{20}NO_6S$ 462.1011, obsd 462.1018.

**4.2.5. (3*R*)-8-Cyclopropyl-7-((7-methoxy-2-oxo-2*H*-chromen-4-yl)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (11)—**Isolated in 65% overall yield as a light yellow non-crystalline solid. $[\alpha]_D$ −58 (*c* 0.20, $CHCl_3$); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.51–0.71 (m, 2H) 0.76–0.88 (m, 2H), 1.56–1.66 (m, 1H), 3.52 (dd, $J_1$ = 1.76 Hz, $J_2$= 11.86 Hz, 1H), 3.82 (dd, $J_1$ = 9.08 Hz, $J_2$= 11.87 Hz, 1H), 3.86 (s, 3H), 4.10–4.27 (m, 2H), 5.40 (dd, $J_1$ = 1.75 Hz, $J_2$ = 9.08 Hz, 1H), 5.79 (s, 1H), 6.03 (s, 1H), 6.97 (dd, $J_1$ = 2.55 Hz, $J_2$ = 8.87 Hz, 1H), 7.03 (d, $J$ = 2.51 Hz, 1H), 7.66 (d, $J$ = 8.88 Hz, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 7.6, 7.8, 11.2, 32.2, 34.9, 56.5, 63.8, 101.6, 111.8, 112.4, 112.7, 112.9, 114.5, 127.0, 149.6, 152.9, 154.3, 155.6, 160.4, 160.6, 163.0, 170.1. HRMS (electrospray ionization) calcd for [M+H] $C_{22}H_{20}NO_6S$ 426.1011, obsd 426.1016.

**4.2.6. (3*R*)-7-(Benzo[*b*]thiophen-3-ylmethyl)-8-cyclopropyl-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (12)—**Isolated in 57% overall yield as a light yellow non-crystalline solid. $[\alpha]_D$ −99 (*c* 0.5, DMSO); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.53–0.73 (m, 2H), 0.81–0.97 (m, 2H), 1.56–1.67 (m, 1H), 3.50 (dd $J_1$ = 1.81 Hz, $J_2$ = 11.93 Hz, 1H), 3.78 (dd $J_1$ = 9.12 Hz, $J_2$ = 11.91 Hz, 1H), 4.15–4.30 (m, 2H), 5.37 (dd $J_1$ = 1.78 Hz, $J_2$ = 9.10 Hz, 1H), 5.61 (s, 1H), 7.35–7.43 (m, 2H), 7.47 (s, 1H), 7.71–7.77 (m, 1H), 7.97–8.04 (m, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 7.9, 8.4, 12.0, 33.0, 34.0, 67.2, 114.8, 115.8, 122.9, 123.8, 125.1, 125.1, 125.4, 134.5, 140.0, 141.9, 151.2, 157.2, 163.7, 174.0. HRMS (electrospray ionization) calcd for [M–Li] $C_{20}H_{16}NO_3S_2$ 382.0572, obsd 382.0578.

**4.2.7. (3*R*)-7-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-8-cyclopropyl-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (13)—**Isolated in 46% overall yield as a light pink non-crystalline solid. $[\alpha]_D$ −33 (*c* 0.5, $CH_2Cl_2$/MeOH 9:1); $^1$H NMR (400 MHz, MeOD) $\delta$ 0.77–0.89 (m, 2H), 0.91–1.10 (m, 2H), 1.59–1.70 (m, 1H), 3.59 (dd $J_1$ = 1.31 Hz, $J_2$= 11.34 Hz, 1H), 3.73 (dd $J_1$ = 8.64 Hz, $J_2$ = 11.30 Hz, 1H), 5.34 (s, 1H), 5.37 (dd $J_1$ = 1.36 Hz, $J_2$ = 8.61 Hz, 1H), 5.98–6.16 (m, 2H), 7.43–7.51 (m, 1H), 7.54–7.62 (m, 1H), 7.68–7.76 (m, 1H), 8.01–8.07 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 8.0, 8.5, 11.3, 34.1, 50.0, 67.3, 111.6, 112.2, 113.9, 120.1, 125.9, 129.2, 134.7, 146.8, 152.46, 152.50, 163.3, 173.8. HRMS (electrospray ionization) calcd for [M–Li] $C_{18}H_{15}N_4O_3S$ 367.0865, obsd 367.0868.

**4.2.8. (3*R*)-8-Isopropyl-7-(3-methylphenethyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (14)—**Isolated in 84% overall yield as a light grey foam. $[\alpha]_D$ −21 (*c* 0.09, $CH_2Cl_2$/MeOH 9:1); $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 1.31 (m, 6H), 2.31 (s, 3H), 2.78–2.83 (m, 4H), 3.07–3.22 (m, 1H), 3.62 (dd $J_1$ = 1.54 Hz, $J_2$ = 11.27 Hz, 1H), 3.72 (dd $J_1$ = 8.45 Hz, $J_2$ = 11.27 Hz, 1H), 5.41 (dd $J_1$ = 1.55 Hz, $J_2$ = 8.42 Hz, 1H), 6.12 (s, 1H), 6.97–7.06 (m, 3H), 7.13–7.19 (m, 1H). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 20.0, 21.2, 21.5, 29.7, 34.2, 36.8, 37.4, 66.6, 115.1, 121.6, 126.4, 128.0, 129.5,

130.1, 139.2, 142.2, 147.5, 156.9, 163.5, 173.9. HRMS (electrospray ionization) calcd for [M–H] $C_{20}H_{22}NO_3S$ 356.1320, obsd 356.1315.

**4.2.9. (3*R*)-7-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-8-isopropyl-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (15)—**Isolated in 13% overall yield as a light pink non-crystalline solid. $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 1.94–1.24 (m, 3H), 1.25–1.31 (m, 3H), 3.22–3.30 (m, 1H), 3.61–3.67 (m, 1H), 3.79 (dd $J_1$ = 8.66 Hz, $J_2$ = 11.52 Hz, 1H), 5.48 (d, $J$ = 8.77 Hz, 1H), 5.63 (br s, 1H), 5.90–5.98 (m, 2H), 7.44–7.50 (m, 1H), 7.54–7.61 (m, 1H), 7.67–7.73 (m, 1H), 8.01–8.07 (m, 1H). $^{13}$C NMR (90 MHz, $CD_3OD$) $\delta$ 19.6, 20.9, 29.6, 34.3, 50.8, 66.7, 111.7, 114.1, 120.0, 120.2, 125.9, 129.3, 134.7, 146.8, 149.1, 149.7, 162.9, 173.5. HRMS (electrospray ionization) calcd for [M–Li] $C_{18}H_{17}N_4O_3S$ 369.1021, obsd 369.1019.

**4.2.10. (3*R*)-8-Isopropyl-7-(naphthalen-1-ylmethyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (16)—**Isolated in 56% overall yield as a light grey non-crystalline solid. $[\alpha]_D$ −9 (*c* 0.5, $CH_2Cl_2$/MeOH 9:1); $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 1.20–1.32 (m, 6H), 3.01–3.17 (m, 1H), 3.63 (dd $J_1$ = 1.22 Hz, $J_2$ = 11.29 Hz, 1H), 3.74 (dd $J_1$ = 8.51 Hz, $J_2$ = 11.28 Hz, 1H), 4.34 (s, 2H), 5.39 (dd $J_1$ = 1.13 Hz, $J_2$ = 8.33 Hz, 1H), 5.81 (s, 1H), 7.22–7.27 (m, 1H), 7.38–7.44 (m, 1H), 7.46–7.54 (m, 2H), 7.76–7.83 (m, 1H), 7.85–7.97 (m, 2H). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 19.7, 20.9, 29.9, 34.3, 37.5, 66.6, 116.3, 121.8, 124.6, 126.6, 126.8, 127.3, 128.2, 128.6, 129.8, 133.1, 135.4, 135.7, 147.5, 155.8, 163.3, 173.9. HRMS (electrospray ionization) calcd for [M–Li] $C_{22}H_{20}NO_3S$ 378.1164, obsd 378.1166.

**4.2.11. (3*R*)-7-((7-Methoxy-2-oxo-2*H*-chromen-4-yl)methyl)-5-oxo-8-(thiophen-2-yl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (17)—**Isolated in 72% overall yield as a light yellow non-crystalline solid. $[\alpha]_D$ −17 (*c* 0.69, $CHCl_3$); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.51 (dd, $J_1$ = 1.77 Hz, $J_2$ = 11.61 Hz, 1H), 3.78–3.83 (m, 1H), 3.85 (s, 2H), 5.43 (dd, $J_1$ = 1.52 Hz, $J_2$ = 9.16 Hz, 1H), 5.91 (s, 1H), 6.04 (s, 1H), 6.91 (dd, $J_1$ = 2.56 Hz, $J_2$ = 8.88 Hz, 1H), 6.98 (d, $J$ = 2.54 Hz, 1H), 7.03–7.07 (m, 2H), 7.48 (d, $J$ = 8.88 Hz, 1H), 7.56–7.60 (m, 1H) $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 32.6, 35.8, 56.9, 65.1, 101.9, 107.1, 112.78, 112.81, 113.1, 115.5, 126.9, 128.4, 128.9, 130.4, 136.8, 151.5, 152.4, 154.2, 155.8, 160.7, 160.9, 163.3, 170.4. HRMS (electrospray ionization) calcd for [M+H] $C_{23}H_{18}NO_6S_2$ 468.0576, obsd 468.0587.

**4.2.12. (3*R*)-7-(Naphthalen-1-ylmethyl)-5-oxo-8-(thiophen-2-yl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (18)—**Isolated in 81% overall yield as a light yellow non-crystalline solid. $[\alpha]_D$33 (*c*0.05, $CH_2Cl_2$/MeOH 9:1); $^1$H NMR (400 MHz, MeOD) $\delta$ 3.50–3.56 (m, 1H), 3.63–3.72 (m, 1H), 4.01–4.16 (m, 2H), 5.36–5.42 (m, 1H), 5.70 (s, 1H), 7.04–7.09 (m, 2H), 7.23–7.28 (m, 1H), 7.34–7.45 (m, 3H), 7.46–7.50 (m, 1H), 7.66–7.77 (m, 2H), 7.79–7.85 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 34.0, 37.6, 68.2, 110.2, 114.7, 124.9, 126.5, 126.7, 127.2, 128.38, 128.41, 128.6, 128.8, 129.7, 130.5, 133.1, 135.35, 135.43, 137.9, 153.1, 156.9, 163.7, 173.8. HRMS (electrospray ionization) calcd for [M–Li] $C_{23}H_{16}NO_3S_2$ 418.0572, obsd 418.0575.

**4.2.13. (3*R*)-7-((Naphthalen-1-yloxy)methyl)-5-oxo-8-(thiophen-2-yl)-3,5-dihydro-2*H*–thiazolo[3,2-*a*]pyridine-3-carboxylic acid (19)—**Isolated in 87% overall yield as a light yellow foam. $[\alpha]_D$ −23 (*c* 0.36, $CH_2Cl_2$/MeOH 9:1); $^1$H NMR (400 MHz, MeOD) $\delta$ 3.55 (dd $J_1$ = 1.55 Hz, $J_2$ = 11.90 Hz, 1H), 3.87 (dd $J_1$ = 9.14 Hz, $J_2$ = 11.89 Hz, 1H), 4.94–5.03 (m, 2H), 5.55 (dd $J_1$ = 1.51 Hz, $J_2$ = 9.05 Hz, 1H), 6.47 (s, 1H), 6.79 (d, $J$ = 7.70 Hz, 1H), 7.11–7.15 (m, 1H), 7.18–7.20 (m, 1H), 7.34–7.40 (m, 1H), 7.47–7.57 (m, 3H), 7.64–7.67 (m, 1H), 7.84–7.90 (m, 1H), 8.13–8.18 (m, 1H). $^{13}$C NMR (100 MHz,

MeOD) δ 31.4, 63.7, 66.4, 104.8, 105.4, 112.2, 120.6, 121.4, 124.7, 125.6, 126.0, 126.6, 127.5, 127.6, 127.9, 129.2, 134.0, 135.0, 149.8, 151.0, 152.9, 159.9, 169.4. HRMS (electrospray ionization) calcd for [M–H] $C_{23}H_{16}NO_4S_2$ 434.0521, obsd 434. 0530.

**4.2.14. (3*R*)-8-(Benzo[*d*][1,3]dioxol-5-yl)-7-((2,3-dimethylphenoxy)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (20)—**Isolated in 84% overall yield as a light grey foam. $[\alpha]_D$ −9 (*c* 0.36, DMSO); $^1$H NMR (400 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.26 (s, 3H), 3.62–3.72 (m, 1H), 3.76–3.83 (m, 1H), 4.57–4.71 (m, 2H), 5.77–5.82 (m, 1H), 6.03 (s, 2H), 6.43–6.48 (s, 1H), 6.68–6.90 (m, 5H), 6.93–6.99 (m, 1H), 8.95 (s, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 11.9, 20.1, 30.8, 64.8, 66.7, 101.5, 109.0, 109.2, 110.0, 112.0, 115.5, 122.8, 123.1, 123.5, 125.5, 125.7, 127.9, 138.3, 148.1, 148.6, 152.6, 155.7, 162.9, 168.5. HRMS (electrospray ionization) calcd for [M–H] $C_{24}H_{20}NO_6S$ 450.1011, obsd 450.1006.

**4.2.15. (3*R*)-7-(Benzo[*b*]thiophen-3-ylmethyl)-8-(benzo[*d*][1,3]dioxol-5-yl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (21)—**Isolated in 72% overall yield as a light yellow non-crystalline solid. $[\alpha]_D$ −5 (*c*0.1, DMSO); $^1$H NMR (400 MHz, MeOD) δ 3.49–3.57 (m, 1H), 3.68–3.78 (m, 1H), 3.80–3.92 (m, 2H), 5.42–5.49 (m, 1H), 5.84–5.97 (m, 2H), 6.07 (s, 1H), 6.64–6.81 (m, 3H), 7.14–7.19 (m, 1H), 7.24–7.34 (m, 2H), 7.45–7.53 (m, 1H), 7.78–7.86 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) δ 32.2, 34.0, 68.1 (split, 68.12, 68.07), 102.6, 109.4 (split, 109.34, 109.48), 111.4 (split, 111.08, 111.73), 114.7 (split, 114.65, 114.70), 117.8, 122.7, 123.6, 124.8 (split, 124.55, 124.09), 125.0, 125.3 (2C), 131.2 (split, 131.23, 131.25), 134.1, 139.7, 141.6, 148.9 (split, 148.86, 148.95), 149.2, 151.0, 155.1 (split, 155.05, 155.09), 163.8, 174.0 (split, 173.97, 174.04). HRMS (electrospray ionization) calcd for [M–Li] $C_{24}H_{16}NO_5S_2$ 462.0470, obsd 462.0480.

**4.2.16. (3*R*)-8-(Benzo[*d*][1,3]dioxol-5-yl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (22)—**Isolated in 78% overall yield as a light grey non-crystalline solid. $[\alpha]_D$ −2 (*c* 0.65, DMSO); $^1$H NMR (400 MHz, MeOD) δ 3.55–3.60 (m, 1H), 3.77 (dd $J_1$ = 8.70 Hz, $J_2$ = 11.31 Hz, 1H), 4.88–4.99 (m, 2H), 5.49–5.53 (m, 1H), 5.97 (d, *J* = 13.38 Hz, 1H), 6.64 (s, 1H), 6.68 (d, *J* = 7.64 Hz, 1H), 6.83–6.94 (m, 3H), 7.27–7.33 (m, 1H), 7.39–7.43 (m, 1H), 7.44–7.51 (m, 2H), 7.76–7.81 (m, 1H), 8.21–8.27 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) δ 34.1, 68.0, 68.1, 102.7, 106.3, 109.6 (split), 111.3 (split, 110.9, 111.6), 112.5, 115.7, 121.8, 122.8, 124.7 (split, 124.4, 125.0), 126.3, 126.82, 126.84, 127.5, 128.5, 130.3, 136.1, 149.2 (split), 149.5, 151.3, 151.9, 154.9, 163.9, 173.9 (split). HRMS (electrospray ionization) calcd for [M–Li] $C_{26}H_{18}NO_6S$ 472.0855, obsd 472.0845.

**4.2.17. (3*R*)-8-(1*H*-Indol-3-yl)-7-(3-methylphenethyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (23)—**Isolated in 59% overall yield as a light pink non-crystalline solid. As a 1:1.8 mixture of atropisomers. $[\alpha]_D$ –29 (*c* 0.8, DMSO); $^1$H NMR (400 MHz, MeOD) δ 2.11–2.16 (m, 3H), 2.47–2.65 (m, 4H), 3.45–3.52 (m, 1H), 3.63–3.72 (m, 1H), 5.46–5.52 (m, 1H), 6.21–6.26 (m, 1H), 6.42–6.58 (m, 2H), 6.80–6.87 (m, 1H), 6.90–7.07 (m, 2H), 7.12–7.29 (m, 3H), 7.40–7.48 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) δ 21.31 (maj), 21.33 (min), 33.68 (maj), 33.74 (min), 36.9 (maj), 37.0 (min), 37.3 (min), 37.4 (maj), 68.4 (maj), 68.5 (min), 111.18 (maj), 111.24 (min), 111.6 (min), 112.1 (maj), 112.5 (min), 112.7 (maj), 113.66 (maj), 113.69 (min), 120.1 (maj), 120.4 (min), 120.5 (maj), 120.6 (min), 122.8 (min), 122.9 (maj), 125.5 (min), 126.16 (maj), 126.19 (min), 126.6 (maj), 127.4 (maj), 127.5 (min), 127.7 (maj), 128.4 (min), 129.0, 129.88 (maj), 129.92 (min), 137.8 (min), 137.9 (maj), 138.8, 142.5, 152.2 (maj), 152.3 (min), 158.8 (min),

158.9 (maj), 164.2, 174.1 (min), 174.2 (maj). HRMS (electrospray ionization) calcd for [M–Li] $C_{25}H_{21}N_2O_3S$ 429.1273, obsd 429.1282.

**4.2.18. (3*R*)-8-(1*H*-Indol-3-yl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (24)—**Isolated in 41% overall yield as a light pink non-crystalline solid. As a 1:1.1 mixture of atropisomers. $[\alpha]_D$ −52 (*c* 1, DMSO); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.45–3.53 (m, 1H), 3.77–3.90 (m, 1H), 4.80–5.12 (m, 2H), 5.54–5.62 (m, 1H), 6.47 (s, 1H, maj), 6.48 (s, 1H, min), 6.61 (d, *J*=7.67 Hz, 1H, maj), 6.70 (d, *J*=7.68 Hz, 1H, min), 7.00–7.08 (m, 1H), 7.11–7.18 (m, 1H), 7.24–7.33 (m, 1H), 7.39–7.56 (m, 6H), 7.82–7.88 (m, 1H), 8.11–8.19 (m, 1H), 11.39 (s, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 31.0 (maj), 31.2 (min), 63.6 (min), 63.7 (maj), 66.5 (maj), 66.7 (min), 105.0 (min), 105.3 (maj) 105.3 (maj), 105.4 (min), 108.0 (min), 108.5 (maj), 111.1 (maj), 111.3 (min), 112.0 (maj/min), 118.8 (min), 118.9 (maj), 119.1 (min), 119.3 (maj), 120.5, 121.30 (min), 121.33 (maj), 121.5 (min), 121.6 (maj), 124.7 (maj), 125.1 (min), 125.5–125.6 (2C, maj/min), 125.8 (min), 125.9 (maj), 126.0 (maj), 126.1 (min), 126.53 (maj), 126.55 (min), 127.5, 134.0, 135.9 (min), 136.0 (maj), 149.7 (maj), 149.9 (min), 150.8 (maj), 151.1 (min), 153.0, 160.2, 169.61 (maj), 169.64 (min). HRMS (electrospray ionization) calcd for [M–Li] $C_{27}H_{19}N_2O_4S$ 467.1066, obsd 467.1056.

**4.2.19. (3*R*)-7-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-8-(1*H*-indol-3-yl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (25)—**Isolated in 27% overall yield as a light pink non-crystalline solid. As a 1:1.3 mixture of atropisomers. $[\alpha]_D$ −1 (*c* 0.5, $CH_2Cl_2$/MeOH 9:1); $^1$H NMR (400 MHz, MeOD) $\delta$ 3.44–3.62 (m, 3H), 3.64–3.75 (m, 1H), 5.58 (br s, 1H), 5.79–5.85 (m, 2H), 6.07–6.13 (m, 1H), 6.27–6.35 (m, 2H), 6.52–6.57 (m, 1H), 6.97–7.19 (m, 3H), 7.24–7.31 (m, 1H), 7.38–7.45 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 35.1 (maj), 35.2 (min), 42.79 (maj), 42.84 (min), 68.6, 104.7, 111.5, 112.89 (min), 112.94 (maj), 113.7 (maj), 114.0 (min), 114.1 (min), 114.3 (maj), 115.2 (min), 115.4 (maj), 117.2, 122.5 (maj), 122.6 (min), 123.1 (min), 123.3 (maj), 125.5 (min), 125.6 (maj), 125.7 (min), 12.8 (maj), 128.3 (min), 129.1 (maj), 130.2 (maj), 130.7 (min), 136.0 (maj), 136.2 (min), 140.3 (min), 140.4 (maj), 150.06 (min), 150.10 (maj), 151.6, 154.3 (maj), 154.5 (min), 162.1 (min), 162.2 (maj), 166.6 (maj), 167.5 (min), 174.2 (broad). HRMS (electrospray ionization) calcd for [M–Li] $C_{24}H_{17}N_2O_5S$ 445.0858, obsd 445.0863.

**4.2.20. (3*R*)-7-((2,3-Dimethylphenoxy)methyl)-5-oxo-8-(pyridin-3-yl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (26)—**Isolated in 53% overall yield as a light pink non-crystalline solid. $[\alpha]_D$ −11 (*c* 1.1, DMSO); $^1$H NMR (400 MHz, MeOD) $\delta$ 2.09 (s, 3H), 2.22 (s, 3H), 3.60 (dd $J_1$ = 1.17 Hz, $J_2$ = 11.34 Hz, 1H), 3.80 (dd $J_1$ = 8.74 Hz, $J_2$ = 11.25 Hz, 1H), 4.68 (s, 2H), 5.51 (dd $J_1$ = 1.07 Hz, $J_2$ = 8.52 Hz, 1H), 6.50 (d, *J* = 8.18 Hz, 1H), 6.56 (s, 1H), 6.72 (d, *J* = 7.53 Hz, 1H), 6.88–6.94 (m, 1H), 7.46–7.53 (m, 1H), 7.87–7.93 (m, 1H), 7.51–7.60 (m, 2H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 11.9, 20.1, 34.3, 68.1, 68.2, 110.2, 112.0, 113.5, 123.9, 125.4, 125.9, 126.9, 134.0, 139.0, 140.1, 149.9, 151.2, 151.68, 151.75, 157.1, 163.9, 173.7. HRMS (electrospray ionization) calcd for [M–Li] $C_{22}H_{19}N_2O_4S$ 407.1066, obsd 407.1057.

**4.2.21. (3*R*)-7-((1*H*-Benzo[*d*][1,2,3]triazol-1-yl)methyl)-5-oxo-8-(pyridin-3-yl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (27)—**Isolated in 33% overall yield as a light pink non-crystalline solid. $[\alpha]_D$0 (*c* 0.36, $CH_2Cl_2$/MeOH 9:1); $^1$H NMR (400 MHz, MeOD) $\delta$ 3.58 (dd $J_1$ = 1.12 Hz, $J_2$ = 11.42 Hz, 1H), 3.74–3.84 (m, 1H), 5.48 (dd $J_1$ = 1.21 Hz, $J_2$ = 8.56 Hz, 1H), 5.58–5.75 (m, 2H), 5.98 (s, 1H), 7.30–7.44 (m, 2H), 7.46–7.54 (m, 2H), 7.58–7.74 (m, 1H), 7.91–7.96 (m, 1H), 8.28–8.46 (m, 2H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 34.4, 49.9, 68.2, 111.4, 111.9, 114.7, 120.0, 125.4, 125.7, 129.1, 133.4, 134.4, 139.9 (split, 139.7, 140.2), 146.5, 149.0, 149.9 (split,

149.8,150.0), 151.0 (split, 150.9,151.1), 153.1, 163.3, 173.4. HRMS (electrospray ionization) calcd for [M–Li] $C_{20}H_{14}N_5O_3S$ 404.0817, obsd 404.0814.

**4.2.22. (3*R*)-7-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-5-oxo-8-(pyridin-3-yl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (28)—**Isolated in 40% overall yield as a light pink non-crystalline solid. $[\alpha]_D$ −2 (*c* 0.9, DMSO); $^1$H NMR (400 MHz, MeOD) $\delta$ 3.52–3.60 (m, 3H), 3.71–3.82 (m 1H), 5.45–5.50 (m, 1H), 5.86 (s, 2H), 6.18 (s, 1H), 6.29 (d *J* = 7.80 Hz, 1H), 6.37 (s, 1H), 6.59 (d *J* = 7.92 Hz, 1H), 7.40–7.47 (m, 1H), 7.62–7.70 (m, 1H), 8.21–8.31 (m, 1H), 8.46–8.51 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 34.2, 40.1, 67.9, 102.1, 108.9, 110.0, 113.8, 115.6, 122.9, 125.1, 132.5, 134.6, 140.2 (split, 140.0, 140.4), 147.5, 149.0, 149.3 (split, 149.2, 149.4), 151.2, 151.3, 155.5, 163.7, 173.6. HRMS (electrospray ionization) calcd for [M–Li] $C_{21}H_{15}N_2O_5S$ 407.0702, obsd 407.0709.

**4.2.23. (3*R*)-7-(Benzo[*b*]thiophen-3-ylmethyl)-8-methoxy-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (29)—**Isolated in 63% overall yield as a light yellow non-crystalline solid. $[\alpha]_D$ −8 (*c* 0.5, DMSO); $^1$H NMR (400 MHz, MeOD) $\delta$ 3.67 (dd $J_1$ = 1.48 Hz, $J_2$ = 11.38 Hz, 1H), 3.69 (s, 3H), 3.82 (dd $J_1$ = 8.52 Hz, $J_2$= 11.35 Hz, 1H), 4.06–4.17 (m, 2H), 5.37 (dd $J_1$ = 1.45 Hz, $J_2$ = 8.47 Hz, 1H), 5.89 (s, 1H), 7.31–7.40 (m, 3H), 7.72–7.77 (m, 1H), 7.85–7.90 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 29.4, 34.7, 61.0, 67.8, 114.5, 122.9, 123.8, 125.2, 125.4, 125.5, 133.8, 138.8, 139.9,141.8,142.8,151.7,162.6,173.6. HRMS (electrospray ionization) calcd for [M–Li] $C_{18}H_{14}NO_4S_2$ 372.0364, obsd 372.0374.

**4.2.24. (3*R*)-8-Methoxy-7-(naphthalen-1-ylmethyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (30)—**Isolated in 80% overall yield as a light grey non-crystalline solid. $[\alpha]_D$ −1 (*c* 0.36, $CH_2Cl_2$/MeOH 9:1); $^1$H NMR(400 MHz, DMSO-$d_6$) $\delta$ 3.56–3.63 (m, 1H), 3.70 (s, 3H), 3.89 (dd $J_1$ = 8.93 Hz,$J_2$ = 11.71 Hz, 1H), 4.24–4.36 (m, 2H), 5.31–5.39 (m, 2H), 7.41–7.58 (m, 4H), 7.85–7.99 (m, 3H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 33.4, 34.7, 61.0, 67.8, 114.6, 125.1, 126.6, 126.8, 127.3, 128.7, 128.9, 129.7, 133.3, 135.3, 135.5, 138.7, 142.7, 153.0, 162.6, 173.6. HRMS (electrospray ionization) calcd for [M–Li] $C_{20}H_{16}NO_4S$ 366.0800, obsd 366.0803.

**4.2.25. (3*R*)-7-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-8-methoxy-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (31)—**Isolated in 76% overall yield as a light yellow foam. $[\alpha]_D$ −1 (*c* 0.5, $CH_2Cl_2$/MeOH 9:1); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.56–3.61 (m, 4H), 3.71 (s, 2H), 3.88 (dd $J_1$ = 8.99 Hz, $J_2$ = 11.70 Hz, 1H), 5.37 (dd $J_1$ = 124 Hz, $J_2$ = 8.79 Hz, 1H), 5.79 (s, 1H), 5.98 (s, 2H), 6.70–6.75 (m, 1H), 6.82–6.87 (m, 2H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 32.0, 34.8, 60.1, 62.9, 100.8, 108.2, 109.4, 113.5, 122.1, 131.8, 135.3, 139.7, 145.8, 147.3, 151.4, 159.1, 169.4. HRMS (electrospray ionization) calcd for [M–H] $C_{17}H_{14}NO_6S$ 360.0542, obsd 366.0543.

**4.2.26. (3*R*)-8-(1*H*-Indol-3-yl)-7-(naphthalen-1-ylmethyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (32)—**Isolated in 50% overall yield as a light pink foam. As a 1:1.3 mixture of atropisomers. $[\alpha]_D$ −33 (*c* 0.6, DMSO);$^1$H NMR (400 MHz, MeOD) $\delta$ 3.43–3.49 (m, 1H), 3.67–3.77 (m, 1H), 3.95–4.09 (m, 2H), 5.57–5.63 (m, 1H), 5.73 (s, 1H, maj), 5.76 (m, 1H, min), 7.06–7.40 (m, 7H), 7.41–7.49 (m, 2H), 7.54–7.60 (m, 1H), 7.66–7.71 (m, 1H), 7.74–7.79 (m, 1H), 10.77 (s, 1H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 32.4 (maj), 32.5 (min), 37.7, 65.7 (maj), 65.8 (min), 111.0 (min), 111.4 (maj), 111.48 (min), 111.51 (maj), 112.8 (min), 112.9 (maj), 114.3, 120.0 (maj), 120.1 (min), 120.7 (min), 120.8 (maj), 123.0 (min), 123.1 (maj), 124.99 (min), 125.01 (maj), 125.7, 126.4 (min), 126.5 (maj), 126.56 (min), 126.62 (maj), 127.00 (maj), 127.02 (min),

127.6 (maj), 128.1 (min), 128.52 (min), 128.55 (maj), 128.8 (min), 128.9 (maj), 129.6, 133.0 (min), 133.1 (maj), 135.3, 135.7 (maj), 135.9 (min), 137.8 (min), 138.0 (maj), 151.6 (maj), 151.7 (min), 159.2 (min), 159.3 (maj), 163.81 (maj), 163.83 (min), 171.2. HRMS (electrospray ionization) calcd for [M–Li] $C_{27}H_{19}N_2O_3S$ 451.1117, obsd 451.1126.

**4.2.27 (3*R*)-8-(1-Methyl-1*H*-indol-3-yl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-3,5-dihydro-2*H*–thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (33)—** Isolated in 76% overall yield as a light pink foam. As a 1:1.3 mixture of atropisomers. $[\alpha]_D$ −18 (*c* 0.45, DMSO); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.45–3.52 (m, 1H), 3.78 (s, 3H, min), 3.79 (s, 3H, maj), 3.81–3.89 (m, 1H), 4.81–5.13 (m, 2H), 5.55–5.63 (m, 1H), 6.47–6.50 (m, 1H), 6.60 (d,*J* = 7.73 Hz, 1H, maj), 6.70 (d,*J* = 7.67 Hz, 1H, min), 7.03–7.10 (m, 1H), 7.17–7.55 (m, 8H), 7.81–7.86 (m, 1H), 8.11–8.18 (m, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 31.0 (maj), 31.2 (min), 32.58 (maj), 32.62 (min), 63.62 (min), 63.64 (maj), 66.6 (maj), 66.7 (min), 104.7 (maj), 104.9 (min), 105.5 (maj), 105.6 (min), 107.2 (min), 107.7 (maj), 110.26 (maj), 110.31 (min), 111.08 (maj), 111.13 (min), 119.0 (min), 119.2 (maj), 119.4 (min), 119.5 (maj), 120.5, 121.35 (min), 121.37 (maj), 121.6 (min), 121.7 (maj), 124.8, 125.6, 125.94 (maj), 125.97 (min), 125.99 (maj), 126.4 (min), 126.6, 127.5, 129.3 (min), 130.0 (maj), 134.1, 136.5 (min), 136.6 (maj), 149.8 (min), 150.0 (maj), 151.0 (min), 151.2 (maj), 153.0, 160.3, 169.6 (maj), 169.7 (min). HRMS (electrospray ionization) calcd for [M–Li] $C_{28}H_{21}N_2O_4S$ 481.1222, obsd 481.1217.

**4.2.28. (*R*)-8-(1-Methyl-1*H*–indol-3-yl)-*N*-(methylsulfonyl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxamide (34)—**Isolated in 51% yield as a light grey foam starting from 0.08 mmol of **33**. As a 1:1.9 mixture of atropisomers. $[\alpha]_D$ 2 (*c* 0.5, DMSO); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.32 (s, 3H), 3.52–3.68 (m, 1H), 3.75 (s, 3H, min), 3.83 (s, 3H, maj), 3.85–3.93 (m, 1H), 4.81–5.06 (m, 2H), 5.77–5.84 (m, 1H), 6.46 (d, *J* = 7.60 Hz, 1H, maj), 6.50 (d, *J* = 7.64 Hz, 1H, min), 6.97 (s, 1H, min), 6.99 (s, 1H, maj), 7.06 (s, 1H, min), 7.14 (s, 1H, maj), 7.15–7.54 (m, 9H), 7.74–7.80 (m, 1H), 8.30–8.38 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 28.6 (maj), 29.2 (min), 33.0 (min), 33.1 (maj), 41.36 (min), 41.4 (maj), 65.6 (maj), 65.7 (min), 66.5 (maj), 66.7 (min), 105.06 (maj), 105.11 (min), 107.5 (min), 108.1 (maj), 108.6 (maj), 108.8 (min), 109.7 (min), 110.0 (maj), 111.9 (maj), 112.0 (min), 119.1 (maj), 119.6 (min), 120.26 (min), 120.33 (maj), 121.02 (maj), 121.04 (min), 122.0, 122.5 (min), 122.6 (maj), 125.39 (min, 2C), 125.43 (maj, 2C), 125.57 (maj), 125.60 (min), 126.1 (min), 126.59 (maj), 126.61 (maj), 126.7 (min), 127.4, 128.0 (min), 129.1 (maj), 134.4, 136.8 (min), 137.0 (maj), 149.9, 153.4, 153.6 (min), 153.9 (maj), 162.7 (min), 162.9 (maj), 166.1 (maj), 166.2 (min). HRMS (electrospray ionization) calcd for [M–H] $C_{29}H_{24}N_3O_5S_2$ 558.1158, obsd 558.1173.

**4.2.29. (3*S*)-7-((Naphtalen-1-yloxy)methyl)-5-oxo-8-(1-metyhyl-1*H*-indol-3-yl)-2,3-dihydro-5*H*-oxazolo[3,2-*a*]pyridine-3-carboxylic acid (35)—**Isolated in 29% overall yield as a light pink non-crystalline solid. $[\alpha]_D$ −23 (*c* 0.2, $CHCl_3$/$CH_2Cl_2$/MeOH 1:1:1); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.77 (s, 3H), 4.69 (m, 1H), 4.86 (m, 1H), 5.07 (m, 3H), 6.28 (s, 1H), 6.71 (d, *J* =7.52 Hz, 1H), 7.04 (m, 1H), 7.18 (m, 1H), 7.31 (m, 1H), 7.37 (m, 1H), 7.46 (m, 3H), 7.25 (m, 2H), 7.86 (m, 1H), 8.16 (m, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 33.0, 58.2, 67.4, 72.7, 89.0, 104.3, 106.0, 107.2, 110.4, 119.6, 119.8, 120.9, 121.8, 121.8, 125.3, 126.0, 126.5, 127.0, 127.8, 128.0, 130.2, 134.5, 136.9, 153.2, 153.5, 155.7, 158.8, 169.9. HRMS (electrospray ionization) calcd for [M–H] $C_{28}H_{21}N_2O_5$ 465.1451, obsd 465.1451.

**4.2.30. (3*S*)-7-((Naphtalen-1-yloxy)methyl)-5-oxo-8-(benzo[*d*][1,3]dioxol-5-yl)-2,3-dihydro-5*H*-oxazolo[3,2-*a*]pyridine-3-carboxylic acid (36)—**Isolated in 39% overall yield as a light yellow non-crystalline solid. $[\alpha]_D$ −37 (*c* 0.2, $CHCl_3$/MeOH 1:1); $^1$H

NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.74 (dd, $J_1$ = 4.01 Hz, $J_2$ = 9.05 Hz, 1H), 4.90 (t, $J$ = 9.31 Hz, 1H), 5.00 (d, $J$ = 14.00 Hz, 1H), 5.10 (d,$J$ = 14.00 Hz, 1H), 5.16 (dd, $J_1$ = 4.01 Hz, $J_2$ = 9.44 Hz, 1H), 6.01 (s, 2H) 6.26 (s, 1H), 6.78 (d, $J$ = 7.65 Hz, 1H), 6.86 (m, 1H), 6.91 (m, 1H), 6.97 (m, 1H), 7.36 (m, 1H), 7.47 (m, 1H), 7.52 (m, 2H), 7.86 (m,1H), 8.11 (m, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 58.1, 67.2, 72.9, 96.7, 101.5, 106.0, 107.8, 108.7, 111.3, 120.9, 121.8, 124.4, 125.3, 125.8, 126.0, 126.5, 127.0, 128.0, 134.5, 147.0, 147.7, 151.8, 153.5, 155.3, 158.6, 167.8. HRMS (electrospray ionization) calcd for [M–H] $C_{25}H_{19}NO_7$ 456.1084, obsd 456.1079.

**4.2.31. (3*S*)-7-((Naphthalen-1-yloxy)methyl)-5-oxo-8-(thiophen-2-yl)-2,3-dihydro-5*H*-oxazolo[3,2-*a*]pyridine-3-carboxylic acid (37)—**Isolated in 34% overall yield as a light grey non-crystalline solid. $[\alpha]_D$ −67 ($c$ 0.75, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.16 (d, $J$ = 17.26 Hz, 1H), 4.23 (d, $J$ = 17.26 Hz, 1H), 4.80 (m, 1H), 5.20 (m, 1H), 5.35 (m, 1H), 5.80 (s, 1H), 7.05 (m, 1H), 7.10 (m, 1H), 7.24 (m, 1H), 7.38–7.48 (m, 4H), 7.62 (m, 1H), 7.78 (m, 1H), 7.85 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 37.1, 59.2, 70.8, 96.0, 109.9, 123.6, 125.5, 125.8, 126.3, 127.4, 127.4, 127.9, 128.0, 128.8, 129.5, 131.5, 131.6, 133.4, 133.9, 155.0, 160.5, 161.0, 167.5. HRMS (electrospray ionization) calcd for [M–H] $C_{23}H_{16}NO_4S$ 402.0800, obsd 402.0794.

**4.2.32. (3*R*)-8-(1-Methyl-1*H*-indol-3-yl)-7-(3-((naphthalen-1-yloxy)methyl)benzyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (38)—**Isolated in 40% yield as a light grey foam in a 1:1.3 mixture of atropisomers. $[\alpha]_D$ −30 ($c$ 0.82, DMSO); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.47–3.68 (m, 4H), 3.71 (s, 3H, maj), 3.73 (s, 3H, min) 5.05–5.15 (m, 2H), 5.24–5.32 (m, 1H), 5.85–5.90 (m, 1H), 6.88–7.02 (m, 4H), 7.10–7.26 (m, 4H), 7.28–7.33 (m, 1H), 7.36–7.44 (m, 2H), 7.45–7.55 (m, 3H), 7.83–7.89 (m, 1H), 8.11–8.17 (m, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 32.88 (maj), 32.93 (min), 33.1 (broad), 39.05 (maj), 39.09 (min), 66.8, 69.82 (min), 69.84 (maj), 106.2, 106.6 (broad and split), 109.7 (min), 110.2 (maj), 110.5, 114.1 (broad and split), 119.5, 119.6 (maj), 119.7 (min), 120.6, 121.8 (min), 121.9 (maj), 122.0,125.5, 125.7 (broad), 125.9, 126.6 (min), 126.7 (maj), 126.9, 127.0 (maj), 127.5 (min), 127.9, 128.4 (min), 128.5 (maj), 128.8, 128.9, 129.6 (min), 130.2 (maj), 134.5, 136.8 (min), 136.9 (maj), 137.5 (split), 139.5 (maj), 139.8 (min), 150.49 (min), 150.52 (maj), 154.17 (min), 154.19 (maj), 154.7 (min), 154.8 (maj), 161.2 (broad and split), 170.1 (broad and split). HRMS (electrospray ionization) calcd for [M–$CO_2$H] $C_{34}H_{27}N_2O_2S$ 527.1793, obsd 527.1786.

**4.2.33. (3*R*)-7-[3-(Naphthalen-1-yloxymethyl)-benzyl]-5-oxo-8-(3-trifluoromethyl-phenyl)-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (39)—**Isolated in 35% overall yield as a colorless foam. $[\alpha]_D$ 5 ($c$ 0.25, CHCl3); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.46–3.53 (m, 1H), 3.58–3.71 (m, 2H), 3.76–3.84 (m, 1H), 5.17 (s, 2H), 5.46–5.52 (m, 1H), 6.11 (s, 1H), 6.77–6.84 (m, 1H), 6.97–7.04 (m, 2H), 7.18–7.25 (m, 1H), 7.30–7.60 (m, 8H), 7.63–7.69 (m, 1H), 7.84–7.89 (m, 1H), 8.14–8.19 (m, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 31.7, 38.6, 63.6, 69.2, 105.7, 112.8, 114.7, 120.1, 121.5, 123.9 (q, $J$ = 271 Hz, 1C), 124.7 (broad), 125.0, 125.4 (2C), 126.1, 126.4, 126.8 (broad splitted), 127.4, 127.6 (broad), 128.1, 128.4, 129.3 (q, $J$ = 31 Hz, 1C), 129.7, 134.0, 134.4, 137.1, 137.3 (broad), 138.2, 148.5, 153.0 (broad), 153.7, 160.1, 169.5. HRMS (electrospray ionization) calcd for [M–H] $C_{33}H_{23}F_3NO_4S$ 586.1300, obsd 586.1294.

**4.2.34. (3*R*)-8-Cyclopropyl-7-[3-(naphthalen-1-yloxymethyl)-benzyl]-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (40)—**Isolated in 30% overall yield as a colorless foam. $[\alpha]_D$ −17 ($c$ 0.25, $CHCl_3$/MeOH 9:1); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.44–0.62 (m, 2H), 0.69–0.86 (m, 2H), 1.26–1.37 (m, 1H), 3.44–3.53 (m, 1H), 3.67–3.77 (m, 1H), 3.91–4.06 (m, 2H), 5.38 (m, 1H), 5.86 (s, 1H), 7.00–7.06 (m, 1H), 7.19–

7.24 (m, 1H), 7.34–7.55 (m, 7H), 7.84–7.89 (m, 1H), 8.17–8.23 (m, 1H);$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 7.4, 7.7, 10.9, 31.3, 38.2, 62.8, 69.3, 105.8, 111.8, 114.2, 120.1, 121.5, 125.0, 125.4, 125.5, 126.1, 126.5, 127.5, 128.1, 128.5, 128.7, 134.1, 137.4, 138.9, 148.4, 153.6, 156.2, 160.1, 169.7. HRMS (electrospray ionization) calcd for [M–H] $C_{29}H_{24}NO_4S$ 482.1426, obsd 482.1416.

**4.2.35. (3*R*)-7-(Anthracen-9-ylmethyl)-5-oxo-8-(3-(trifluoromethyl)phenyl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (41)—**Isolated in 57% overall yield as a white non-crystalline solid by following a previously published procedure and the use of naphthalene-1-boronic acid. $[\alpha]_D$ −4 (*c* 0.5, DMSO); $^1$H NMR (400 MHz, MeOD/$CDCl_3$ (1:1)) $\delta$ 3.48–3.56 (m, 1H), 3.63–3.75 (m, 1H), 4.20–4.38 (m, 2H), 5.36 (s, 1H), 5.41–5.50 (m, 1H), 7.32–7.43 (m, 4H), 7.65–7.94 (m, 8H), 8.27 (s, 1H); $^{13}$C NMR (100 MHz, MeOD/$CDCl_3$ (3:2)) $\delta$ 32.4, 32.6, 65.1 (broad), 114.6, 116.0, 124.4 (2C), 124.7 (q, *J* = 272.8 Hz), 125.5 (2C), 126.1 (broad and split), 126.9 (2C), 127.6 (split *J* = 36.5 Hz), 127.84, 129.3, 129.8 (2C), 130.7, 130.9 (2C), 132.1 (2C), 132.3 (q, *J* = 33.5 Hz), 134.5 (split *J* = 47.5Hz), 137.9 (split *J* = 4.3 Hz), 149.3, 155.4, 162.8, 170.6 (broad). HRMS (electrospray ionization) calcd for [M–H] $C_{30}H_{19}F_3NO_3S$ 530.1038, obsd 530.1038.

**4.2.36. (3*R*)-8-(1-Methyl-1*H*-indol-3-yl)-5-oxo-7-((3-phenoxyphenoxy)methyl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (42)—**Isolated in 50% overall yield as a light pink foam in a 1:1.2 mixture of atropisomers. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.41–3.51 (m, 1H), 3.68–3.81(m, 4H), 4.57–4.85 (m, 2H), 5.44–5.51 (m, 1H), 6.23 (s, 1H, maj), 6.25 (s, 1H, min), 6.37 (t, *J* = 2.25 Hz, 1H, maj), 6.45 (t, *J* =2.24 Hz, 1H, min), 6.49–6.54 (m, 1H), 6.55–6.62 (m, 1H), 6.93–7.04 (m, 3H), 7.12–7.25 (m, 4H), 7.35–7.41 (m, 2H), 7.43–7.49 (m, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 32.0 (maj), 32.2 (min), 33.0 (maj), 33.1 (min), 65.0, 67.0 (maj), 67.1 (min), 104.8 (maj), 105.0 (min), 105.4 (maj), 105.6 (min), 107.7 (min), 108.2 (maj), 110.2 (min), 110.3 (maj), 110.6, 111.3 (min), 111.4 (maj), 111.7 (maj), 111.9 (min), 119.25, 119.31, 119.5, 119.7 (min), 119.9 (maj), 121.95 (min), 122.05 (maj), 124.11 (min), 124.13 (maj), 126.5 (maj), 126.9 (min), 129.7 (min), 130.3 (maj), 130.5 (2C), 131.00 (min), 131.03 (maj), 136.8 (min), 136.9 (maj), 150.4 (min), 150.60 (maj), 150.65 (min), 150.8 (maj), 156.62 (min), 156.63 (maj), 158.2 (maj), 158.3 (min), 159.4 (maj), 159.5 (min), 160.8, 170.3 (maj), 170.4 (min). HRMS (electrospray ionization) calcd for [M–H] $C_{30}H_{23}N_2O_5S$ 523.1328, obsd 523.1334.

**4.2.37. 5-Oxo-7-((3-phenoxy-phenoxy)methyl)-8-(3-trifluoromethyl-phenyl)-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (43)—**Isolated in 54% overall yield as a white non-crystalline solid. $[\alpha]_D$ −32 (*c* 0.5, DMSO); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.49–3.58 (m, 1H), 379–3.91 (m, 1H), 4.73 (s, 2H), 5.51–5.60 (m, 1H), 6.31–6.41 (m, 2H), 6.48–6.59 (m, 2H), 6.91–7.01 (m, 2H), 7.10–7.25 (m, 2H), 7.33–7.44 (m, 2H), 7.50–7.76 (m, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 32.0, 63.9, 67.0, 105.4, 110.1, 111.5, 111.7, 113.5, 119.3, 124.1, 124.4 (q,*J* = 277.36 Hz), 125.4 (d, *J* = 3.43 Hz), 126.8 (d, *J* = 3.3 Hz), 130.0 (q,*J* = 31.93 Hz), 130.4, 130.5, 131.0, 134.4, 136.9, 149.1, 149.4, 156.6, 158.2, 159.2, 160.4, 169.9. HRMS (electrospray ionization) calcd for [M–H] $C_{28}H_{19}F_3NO_5S$ 538.0936, obsd 538.0926.

**4.2.38. (3*R*)-8-Cyclopropyl-7-((3-phenoxy-phenoxy)methyl)-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (44)—**Isolated in 61% overall yield as a grey non-crystalline solid. $[\alpha]_D$ −44 (*c* 0.5, DMSO); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.49–0,63 (m, 2H), 0.78–0.84 (m, 2H), 1.58–1.64 (m, 1H), 3.50–3.55 (m, 1H), 3.75–3.82 (m, 1H), 5.14 (s, 2H), 5.38–5.43 (m, 1H), 6.14 (s, 1H), 6.55–6.59 (m, 1H), 6.65 (s, 1H), 6.77–6.81 (m, 1H), 7.00–7.05 (m, 2H), 7.13–7.18 (m, 1H), 7.27–7.33 (m, 1H) 7.36–7.42 (m,

2H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 6.7, 6.8, 9.8, 31.4, 62.7, 66.1, 105.1, 109.7, 109.9, 110.8, 111.3, 119.0, 123.7, 130.0, 130.6, 148.5, 151.7, 156.1, 158.0, 159.2, 160.0, 169.6. HRMS (electrospray ionization) calcd for [M–H] $C_{24}H_{20}NO_5S$ 434.1062, obsd 434.1051.

**4.2.39. 8-Cyclopropyl-7-indol-1-ylmethyl-5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (45)—**Isolated in 21% overall yield as a yellow non-crystalline solid. $[\alpha]_D$ −22 (*c* 0.33, $CHCl_3$/MeOH 9:1); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.83–0.78 (m, 2H), 1.07–0.91 (m, 2H), 1.75–1.69 (m, 1H), 3,58 (d, J = 11.36 Hz, 1H), 3.75–3.68 (m, 1H), 5.22 (s, 1H), 5.35 (d, J = 8.59 Hz, 1H), 5.49 (s, 2H), 6.53 (d, J = 3.10 Hz, 1H), 7.07–7.01 (m, 1H), 7.16–7.09, (m, 1H), 7.28–7.23 (m, 2H), 7.57 (d, J = 7.88 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 7.9, 8.4, 11.25, 34.1 (2C), 67.1, 102.8, 110.7, 111.7, 113.9, 120.6, 121.8, 122.8, 129.7, 130.2, 137.8, 151.3, 155.5, 163.7, 173.9. HRMS (electrospray ionization) calcd for [M–Li] $C_{20}H_{17}N_2O_3S$ 365.0960, obsd 365.0970.

**4.2.40. 8-(1-Methyl-1*H*-indol-3-yl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-2-phenyl-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (46)—**Isolated in 54% yield over three steps starting from 0.4 mmol of **33**. As a light pink foam in a 1:1.5 mixture of atropisomers. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.78 (s, 3H, min), 3.80 (s, 3H, maj), 4.85–5.07 (m, 2H), 5.29–5.33 (m, 1H), 5.84–5.90 (m, 1H), 6.49 (d, *J* = 7.66 Hz, 1H, maj), 6.54 (d, *J* =7.62 Hz, 1H, min), 7.06–7.54 (m, 15H), 7.75–7.81 (m, 1H), 8.33–8.39 (m, 1H), 8.76 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 33.0, 48.9 (maj), 49.3 (min), 66.7 (maj), 66.9 (min), 72.5 (min), 72.7 (maj), 105.1 (maj), 105.2 (min), 107.5 (min), 108.0 (min), 108.3 (maj), 108.5 (min), 109.7 (min), 109.9 (maj), 112.0 (maj), 112.3 (min), 119.0 (maj), 119.6 (min), 120.30 (min), 120.31 (maj), 121.0 (maj), 121.1 (min), 121.98 (min), 122.02 (maj), 122.5, 125.4 (min), 125.46 (maj), 125.49 (min), 125.5 (maj, 2C), 126.2 (maj), 126.4, 126.5 (2C), 126.7 (min), 127.41 (maj), 127.45 (min), 128.1, 128.69 (maj), 128.74 (min), 129.1, 129.2 (maj), 129.3 (min), 134.46 (maj), 134.48 (min), 136.8 (min), 137.0 (maj), 139.6 (maj), 139.7 (min), 149.8 (min), 149.9 (maj), 153.47 (maj), 153.50 (min), 153.8 (min), 154.2 (maj), 163.1 (min), 163.3 (maj), 167.99 (min), 168.04 (maj). HRMS (electrospray ionization) calcd for [M–H] $C_{34}H_{25}N_2O_4S$ 557.1535, obsd 557.1543.

**4.2.41. 8-(Benzo[*d*][1,3]dioxol-5-yl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-2-phenyl-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (47)—**Isolated in 62% yield as a grey foam starting from 0.4 mmol of methylester protected **22**. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.76–4.93 (m, 2H), 5.23 (s, 1H), 5.84 (s, 1H), 5.98 (s, 2H), 6.60 (d, *J* = 7.29 Hz, 1H), 6.67–6.87 (m, 3H), 7.04 (s, 1H), 7.24–7.35 (m, 6H), 7.40–7.53 (m, 3H), 7.75–7.81 (m, 1H), 8.31–8.37 (m, 1H), 11.87 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 50.0 (split, 50.01, 50.09), 65.6, 71.9 (split, 71.88, 71.93), 101.4, 105.1, 109.0, 109.7 (split, 109.4, 110.1), 112.5, 114.9, 121.1, 122.0, 123.2 (split, 122.9, 123.6), 125.4, 125.5, 125.6, 126.4, 126.5, 126.6, 127.4, 127.8, 128.7, 129.2 (2C), 134.5, 139.3 (split, 139.3, 139.4), 148.0, 148.1 (split, 148.1, 148.2), 148.4, 151.9, 153.5, 162.7, 168.6. HRMS (electrospray ionization) calcd for [M–H] $C_{32}H_{22}NO_6S$ 548.1168, obsd 548.1170.

**4.2.42. 7-((Naphthalen-1-yloxy)methyl)-5-oxo-2-phenyl-8-(thiophen-2-yl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (48)—**Isolated in 65% yield as a light yellow foam starting from 0.4 mmol of methylester protected **18**. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.10–4.24 (m, 2H), 5.27 (d, *J* = 2.07 Hz, 1H), 5.66 (d, *J* = 2.19 Hz, 1H), 5.96 (s, 1H), 7.06–7.10 (m, 2H), 7.23–7.36 (m, 6H), 7.39–7.50 (m, 4H), 7.67–7.72 (m, 1H), 7.76–7.80 (m, 1H), 7.83–7.88 (m, 1H), 9.04 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 36.9, 49.4, 72.5, 110.4, 114.8, 123.7, 125.5, 125.8, 126.4, 126.6 (2C), 127.6, 127.7, 128.0 (2C), 128.8 (2C), 129.29 (2C), 129.33, 131.7, 133.2, 133.9, 135.6, 139.1, 150.2, 157.4, 162.7,

167.8. HRMS (electrospray ionization) calcd for [M–H] $C_{29}H_{20}NO_3S_2$ 494.0885, obsd 494.0892.

**4.2.43. 2-Methoxy-8-(1-methyl-1*H*-indol-3-yl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (49)—**Isolated in 65% yield starting from 0.4 mmol of methylester protected **33**. As a light pink foam in a 1:1.5 mixture of atropisomers. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.34 (s, 3H, maj), 3.38 (s, 3H, min), 3.81 (s, 3H, min), 3.83 (s, 3H, maj), 4.84–5.06 (m, 2H), 5.70 (s, 1H, maj), 5.71 (s, 1H, min), 5.89 (s, 1H, maj), 5.92 (s, 1H, min), 6.46 (d, $J$ = 7.64 Hz, 1H, maj), 6.52 (d, $J$ = 7.73 Hz, 1H, maj), 7.03–7.24 (m, 4H), 7.28–7.53 (m, 6H), 7.75–7.81 (m, 1H), 8.30–8.36 (m, 1H), 9.11 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$/MeOD (70:30)) $\delta$ 33.07 (maj), 33.09 (min), 56.4 (maj), 56.6 (min), 67.1 (maj), 67.3 (min), 71.8, 87.9 (maj), 88.3 (min), 105.48 (maj), 105.51 (min), 107.89 (min), 107.93 (maj), 108.1 (min), 108.5 (maj), 110.06 (min), 110.14 (maj), 112.2 (maj), 112.6 (min), 119.7, 120.2 (min), 120.3 (maj), 121.09 (maj), 121.13 (min), 122.1, 122.56 (min), 122.63 (maj), 125.62 (maj), 125.64 (min), 125.7, 125.8, 126.66 (maj), 126.73 (maj), 126.74 (min), 127.2 (min), 127.7, 128.7 (min), 129.5 (maj), 134.8, 137.1 (min), 137.3 (maj), 148.0 (maj), 148.1 (min), 153.0 (min), 153.3 (maj), 153.77 (min), 153.79 (maj), 162.7, 167.5. HRMS (electrospray ionization) calcd for [M–H] $C_{29}H_{23}N_2O_5S$ 511.1328, obsd 511.1335.

**4.2.44. 8-(Benzo[*d*][1,3]dioxol-5-yl)-2-methoxy-7-((naphthalen-1-yloxy)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (50)—**Isolated in 65% yield as a light grey foam starting from 0.4 mmol of methylester protected **22**. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.36 (s, 3H), 4.73–4.91 (m, 2H), 5.63 (s, 1H), 5.89 (s, 1H), 5.98–6.04 (m, 2H), 6.54–6.60 (m, 1H), 6.64–6.89 (m, 2H), 6.99 (s, 1H), 7.24–7.30 (m, 1H), 7.39–7.51 (m, 3H), 7.74–7.80 (m, 1H), 8.26–8.33 (m, 1H), 11.81 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 56.6, 66.6, 71.9, 87.9, 101.4, 105.1, 109.0 (split, 108.9, 109.0), 109.8 (split, 109.4, 110.1), 112.6, 115.8, 121.1, 122.0, 123.3 (split, 123.0, 123.6), 125.4, 125.5, 125.6, 126.6, 127.4, 127.9 (split, 127.8, 127.9), 134.5, 146.9, 148.0 (split), 148.2 (split), 151.8, 153.5, 162.8, 166.7. HRMS (electrospray ionization) calcd for [M–H] $C_{27}H_{20}NO_7S$ 502.0961, obsd 502.0948.

**4.2.45. 2-Methoxy-7-((naphthalen-1-yloxy)methyl)-5-oxo-8-(thiophen-2-yl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (51)—**Isolated in 65% yield as a light grey foam starting from 0.4 mmol of methylester protected **18**. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.36 (s, 3H), 4.01–4.22 (m, 2H), 5.60 (s, 1H), 5.74 (s, 1H), 5.93 (s, 1H), 7.01–7.10 (m, 2H), 7.19–7.23 (m, 1H), 7.35–7.49 (m, 4H), 7.62–7.68 (m, 1H), 7.73–7.78 (m, 1H), 7.81–7.87 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 37.7, 56.8, 72.8, 89.6, 111.2, 115.3, 124.8, 126.5, 126.8, 127.3, 128.6, 128.7, 128.79, 128.83, 129.7, 130.7, 133.0, 135.1, 135.4, 137.2, 150.3, 157.9, 163.4, 168.2. HRMS (electrospray ionization) calcd for [M–H] $C_{24}H_{18}NO_4S_2$ 448.0677, obsd 448.0691.

**4.2.46. (3*R*)-8-(1-Methyl-1*H*-indol-3-yl)-6-(morpholinomethyl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (52)—**Isolated in 65% yield starting from 0.4 mmol of methylester protected **33**. As a pink non-crystalline solid as a 1:1.4 mixture of atropisomers. $[\alpha]_D$ −58 ($c$ 0.27, DMSO); $^1$H NMR (400 MHz, MeOD) $\delta$ 2.36–2.54 (m, 4H), 3.37–3.56 (m, 8H), 3.59–3.74 (m, 3H), 4.80–5.32 (m, 2H), 5.56–5.62 (m, 1H), 6.51 (d, $J$ = 7.62 Hz, 1H maj), 6.61 (d, $J$ = 7.65 Hz, 1H, min), 6.83–6.51 (m, 9H), 7.71–7.78 (m, 1H), 8.04–8.09 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 32.6 (min), 32.7 (maj), 33.4 (maj), 33.7 (min), 53.9 (maj), 54.1 (min), 54.67 (2C, min), 54.70 (2C, maj), 65.9 (min), 66.0 (maj), 67.95 (2C, min), 67.99 (2C, maj), 69.1 (min), 69.2 (maj), 105.7 (maj), 105.9 (min), 110.3 (min), 110.4 (maj), 110.5 (min),

110.8 (maj), 111.1 (min), 111.3 (maj), 120.3 (min), 120.4 (maj), 120.5 (maj), 121.2, 121.3 (min), 122.8, 122.85 (maj), 122.87 (min), 122.92 (maj), 122.94 (min), 126.0 (maj), 126.2 (min), 126.71 (maj), 126.75 (min), 126.81 (maj), 126.9 (min), 127.27 (maj), 127.35 (min), 128.38 (min), 128.42 (maj), 128.5 (maj), 128.6 (min), 130.1 (min), 131.0 (maj), 135.88 (maj), 135.92 (min), 138.0 (min), 138.1 (maj), 150.4 (min), 150.7 (maj), 151.5 (min), 151.7 (maj), 155.6 (min), 155.7 (maj), 163.99 (maj), 164.03 (min), 173.9 (min), 174.0 (maj). HRMS (electrospray ionization) calcd for [M–Li] $C_{33}H_{30}N_3O_5S$ 580.1906, obsd 580.1929.

**4.2.47. (3*R*)-8-(Benzo[*d*][1,3]dioxol-5-yl)-6-(morpholinomethyl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (53)—**Isolated in 56% yield as a light pink non-crystalline solid starting from 0.4 mmol of methylester protected **22**. $[\alpha]_D$ −29 (*c* 0.22, DMSO); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.23 (br s, 4H), 3.35 (br s, 4H), 3.46–3.60 (m, 3H), 3.78–3.89 (m, 1H), 4.97–5.17 (m, 2H), 5.53–5.60 (m, 1H), 5.82–5.99 (m, 2H), 6.70–6.92 (m, 4H), 7.31–7.37 (m, 1H), 7.40–7.53 (m, 3H), 7.80–7.86 (m, 1H), 7.94–8.00 (m, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 31.0, 52.1, 52.9 (2C), 64.0, 64.5, 65.8 (2C), 100.9, 105.0, 108.0 (split, 108.0, 108.1), 109.9 (split, 109.8, 110.0), 140.1, 120.0, 121.3, 121.7, 123.3, 124.7, 125.0, 125.8, 126.2, 127.1, 129.9, 133.8, 146.4, 146.6, 147.0, 147.5, 153.7, 160.7, 169.2. HRMS (electrospray ionization) calcd for [M–Li] $C_{31}H_{27}N_2O_7S$ 571.1539, obsd 571.1535.

**4.2.48. (3*R*)-6-(Morpholinomethyl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-8-(thiophen-2-yl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (54)—**Isolated in 47% yield as a light pink non-crystalline solid starting from 0.4 mmol of methylester protected **18**. $[\alpha]_D$ −7 (*c* 0.5, DMSO); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.24 (br s, 4H), 3.19–3.33 (m, 6H), 3.52 (dd $J_1$ = 1.71 Hz, $J_2$ = 11.78 Hz, 1H), 3.86 (dd $J_1$ = 9.17Hz, $J_2$= 11.76 Hz, 1H), 4.21–4.49 (m, 2H), 5.57 (dd $J_1$ = 1.65 Hz, $J_2$ = 9.17Hz, 1H), 6.79–6.87 (m, 2H), 6.96 (d, $J$ = 6.99 Hz, 1H), 7.37–7.43 (m, 2H), 7.46–7.53 (m, 2H), 7.73–7.78 (m, 1H), 7.87–7.98 (m, 2H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 31.1, 31.7, 52.6, 52.9 (2C), 64.7, 65.7 (2C), 107.4, 120.5, 122.7, 123.9, 125.2, 125.5, 125.9, 126.3, 126.7, 127.2, 128.3, 128.7, 131.1, 133.0, 135.0, 136.8, 149.6, 152.2, 160.8, 169.3. HRMS (electrospray ionization) calcd for [M–Li] $C_{28}H_{25}N_2O_4S_2$ 517.1256, obsd 517.1270.

**4.2.49. (3*R*)-6-(Hydroxymethyl)-8-(1-methyl-1*H*-indol-3-yl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (55)—**Isolated in 62% yield starting from 0.4 mmol of methylester protected **33**. As a pink non-crystalline solid in a 1:1.2 mixture of atropisomers. $[\alpha]_D$ −21 (*c* 0.5, DMSO); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.44–3.50 (m, 1H), 3.56 (s, 3H, min), 3.62 (s, 3H, maj), 3.74–3.80 (m, 1H), 4.54–4.68 (m, 2H), 4.88–5.18 (m, 2H), 5.51–5.58 (m, 1H), 6.63 (d,$J$ = 7.69 Hz, maj), 6.72 (d, $J$ = 7.65 Hz, min), 6.87–7.00 (m, 1H), 7.07–7.15 (m, 1H), 7.18–7.27 (m, 2H), 7.31–7.51 (m, 5H), 7.77–7.82 (m, 1H), 7.93–8.02 (m, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 31.0 (maj), 31.2 (min), 32.3, 54.91 (min), 54.93 (maj), 64.5, 64.57 (min), 64.62 (maj), 105.3 (maj), 105.5 (min), 106.9 (maj), 107.0 (min), 108.6 (min), 109.2 (maj), 109.9 (maj), 110.1 (min), 119.1 (2C), 120.18 (maj), 120.2 (min), 121.5 (2C), 124.8, 125.27 (min), 125.29 (maj), 125.8 (min), 125.89 (maj), 125.93, 126.37, 126.41 (maj), 126.7 (min), 127.3, 129.0 (min), 129.9 (maj), 133.9, 136.15 (min), 136.24 (maj), 146.5 (min), 146.6 (maj), 148.6 (min), 148.7 (maj), 153.65 (min), 153.70 (maj), 160.50 (min), 160.53 (maj), 169.6. HRMS (electro-spray ionization) calcd for [M–Li] $C_{29}H_{23}N_2O_5S$ 511.1328, obsd 511.1335.

**4.2.50. (3*R*)-8-(Benzo[*d*][1,3]dioxol-5-yl)-6-(hydroxymethyl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (56)—**Isolated in 50% yield as a yellow non-crystalline solid starting from 0.4

mmol of methylester protected **22**. $[\alpha]_D$ −10 (*c* 0.45, DMSO); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.50 (dd $J_1$ = 2.08 Hz, $J_2$ = 11.80 Hz, 1H), 3.84 (dd $J_1$ = 9.11 Hz, $J_2$ = 11.78 Hz, 1H), 4.51–4.59 (m, 2H), 4.96–5.06 (m, 2H), 5.57 (dd $J_1$ = 2.02 Hz, $J_2$ = 9.04 Hz, 1H), 5.80–6.00 (m, 2H), 6.70–6.90 (m, 4H), 7.30–7.37 (m, 1H), 7.42–7.53 (m, 3H), 7.80–7.86 (m, 1H), 7.96–8.02 (m, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 31.3, 54.6, 64.1, 64.3 (split, 64.28, 64.35), 101.1, 105.3, 108.3 (split, 123.4), 124.7, 125.3, 126.0 (2C), 126.4, 127.3, 129.9 (split, 129.8, 129.9), 133.9, 145.3 (split, 145.23, 145.80), 146.8 (split, 146.79, 146.86), 147.2, 147.5, 153.6, 160.3, 169.5. HRMS (electrospray ionization) calcd for [M–Li] $C_{27}H_{20}NO_7S$ 502.0961, obsd 502.0964.

**4.2.51. (3*R*)-6-(Hydroxymethyl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-8-(thiophen-2-yl)-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (57)—**Isolated in 53% yield as a yellow non-crystalline solid starting from 0.4 mmol of methylester protected **18**. $[\alpha]_D$ −3 (*c* 1.0, DMSO); $^1$H NMR (400 MHz, MeOD) $\delta$ 3.59 (dd $J_1$ = 1.45 Hz, $J_2$ = 11.36 Hz, 1H), 3.77 (dd $J_1$ = 8.83 Hz, $J_2$ = 11.40 Hz, 1H), 4.28–4.48 (m, 4H), 5.59 (dd $J_1$ = 1.41 Hz, $J_2$ = 8.70 Hz, 1H), 6.68–6.77 (m, 2H), 7.05–7.09 (m, 1H), 7.18–7.21 (m, 1H), 7.32–7.37 (m, 1H), 7.41–7.47 (m, 2H), 7.67–7.73 (m, 1H), 7.80–7.89 (m, 2H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 33.5, 33.9, 57.8, 68.9, 110.9, 123.8, 125.1, 126.0, 126.5, 126.7, 127.1, 127.7, 127.9, 128.0, 129.6, 130.2, 132,9, 135.1, 136.1, 138.3, 152.8, 153.4, 163.5, 174.0. HRMS (electrospray ionization) calcd for [M–Li] $C_{24}H_{18}NO_4S_2$ 448.0677, obsd 448.0673.

**4.2.52. 8-(1-Methyl-1*H*-indol-3-yl)-6-(morpholinomethyl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-2-m-tolyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (58)—**Isolated in 34% yield as a yellow non-crystalline solid starting from 0.5 mmol of methylester protected **33**. $^1$H NMR (400 MHz, MeOD) $\delta$ 2.30 (s, 3H), 2.48 (br s, 4H), 3.48–3.54 (m, 7H), 3.79 (s, 2H), 5.07–5.40 (m, 2H), 6.61 (d, $J$ = 7.71 Hz, 1H), 6.93–6.98 (m, 1H), 7.11–7.24 (m, 5H), 7.28–7.49 (m, 6H), 7.53 (s, 1H), 7.72–7.76 (m, 1H), 7.99–8.04 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 21.3, 32.8, 54.3, 54.7 (2C), 66.2, 68.0 (2C), 106.1, 109.7, 110.3, 110.8, 118.6, 120.3, 120.7, 121.4, 122.8, 123.2, 125.2, 126.1, 126.5, 126.8, 126.9, 127.3, 128.0, 128.5, 129.79, 129.84, 130.6, 130.8, 131.4, 135.7, 135.9, 138.4, 139.9, 147.0, 149.9, 155.7, 161.4, 167.9. HRMS (electrospray ionization) calcd for [M–$CO_2$Li] $C_{39}H_{34}N_3O_3S$ 624.2321, obsd 624.2307.

**4.2.53. 8-(Benzo[*d*][1,3]dioxol-5-yl)-6-(morpholinomethyl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-2-*m*-tolyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylate lithium salt (59)—**Isolated in 42% yield as a yellow non-crystalline solid starting from 0.5 mmol of methylester protected **22**. $^1$H NMR (400 MHz, MeOD) $\delta$ 2.34 (s, 3H), 2.45 (s, 4H), 3.48 (s, 4H), 3.72 (s, 2H), 5.19–5.29 (m, 2H), 5.85 (d, $J$ = 36.04 Hz, 2H), 6.72–6.76 (m, 1H), 6.78–6.82 (m, 1H), 6.85–6.92 (m, 2H), 7.17–7.21 (m, 1H), 7.24–7.32 (m, 2H), 7.37–7.48 (m, 3H), 7.50–7.55 (m, 1H), 7.58 (s, 1H), 7.75–7.80 (m, 1H), 8.00–8.06 (m, 1H). $^{13}$C NMR (100 MHz, MeOD) $\delta$ 21.4, 54.2, 54.6 (2C), 65.8, 68.0 (2C), 102.7, 106.0, 109.8, 111.4, 116.8, 118.8, 121.6, 122.8, 124.9, 125.0, 126.2, 126.6, 126.8, 126.9, 127.4, 128.5, 129.87, 129.94, 130.96, 130.98, 131.3, 135.7, 136.0, 140.0, 145.6, 148.9, 149.3, 149.6, 155.7, 161.2, 167.7. HRMS (electrospray ionization) calcd for [M–$CO_2$Li] $C_{37}H_{31}N_2O_5S$ 615.1954, obsd 615.1969.

**4.2.54. 8-(1-Methyl-1*H*-indol-3-yl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-2-*m*-tolyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (60)—**Isolated in 66% yield as a yellow foam starting from 0.5 mmol of methylester protected **33**. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.26 (s, 3H), 3.83 (s, 3H), 4.96–5.18 (m, 2H), 6.55 (s, 1H), 6.66 (d, $J$ = 7.61 Hz, 1H), 7.02–7.09 (m, 1H), 7.14–7.18 (m, 1H), 7.19–7.30 (m, 3H), 7.36–7.56 (m, 7H), 7.62

(s, 1H), 7.82–7.87 (m, 1H), 8.12–8.18 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$/MeOD (9:20)) $\delta$ 21.4, 33.2, 67.3, 105.8, 107.4, 107.9, 108.3, 110.6, 119.6, 120.7, 121.4, 122.1, 123.0, 125.95, 125.99, 126.1, 126.2, 126.4, 126.7, 127.2, 128.0, 129.35, 129.42, 129.44 (2C), 130.0, 130.8, 135.2, 137.8, 139.4,149.0,150.1,154.1,160.7,167.5. HRMS (electrospray ionization) calcd for [M–$CO_2$H] $C_{34}H_{25}N_2O_2S$ 525.1637, obsd 525.1630.

**4.2.55. 8-(Benzo[*d*][1,3]dioxol-5-yl)-7-((naphthalen-1-yloxy)methyl)-5-oxo-2-*m*-tolyl-5*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (61)—**Isolated in 67% yield as a yellow foam starting from 0.5 mmol of methylester protected **22**. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.31 (s, 3H), 5.11 (s, 2H), 6.08 (s, 2H), 6.54 (s, 1H), 6.81 (d, $J$ = 7.59 Hz, 1H), 6.99–7.06 (m, 2H), 7.13 (s, 1H), 7.22–7.28 (m, 1H), 7.30–7.39 (m, 2H), 7.40–7.57 (m, 5H), 7.84–7.90 (m, 1H), 8.12–8.18 (m, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$/MeOD (9:20)) $\delta$ 21.4, 40.4, 67.3, 102.3, 105.9, 108.7, 110.0, 110.3, 114.2, 121.6, 122.3, 123.9, 125.9, 126.1, 126.2, 126.2, 127.1, 128.0, 128.4, 129.0, 129.6, 129.7, 129.8, 131.5, 135.3, 139.7, 148.0, 148.4, 149.1, 149.4, 154.1, 160.3, 163.5. HRMS (electrospray ionization) calcd for [M–$CO_2$H] $C_{32}H_{22}NO_4S$ 516.1270, obsd 516.1263.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **SAR** | structure activity relationship |
| **QSAR** | quantitative structure activity relationship |
| **PLS** | partial least squares |
| **OPLS-DA** | orthogonal projections to latent structures discriminant analysis |
| **UTI** | urinary tract infection |
| **SMD** | statistical molecular design |
| **PCA** | principal component analysis |
| **MRSA** | methicillin-resistant *Staphylococcus aureus* |
| **UPEC** | uropathogenic *Escherichia coli* |

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37. SIMCA-P+ 12.0. Umetrics AB; Box 7960, Umeå, Sweden: 2008.
38. MODDE 7.0. Umetrics AB; Box 7960, Umeå, Sweden:

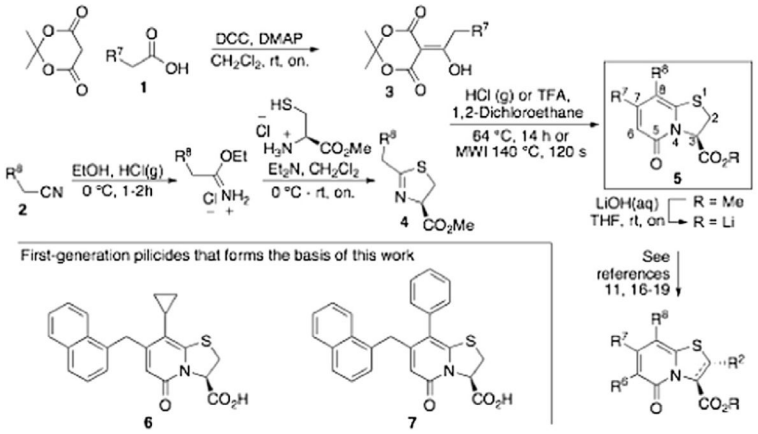



**Figure 1.**

Synthesis of the dihydrothiazolo ring-fused 2-pyridone pilicide scaffold (**5**) from commercially available carboxylic acids (**1**) and nitriles (**2**) via acyl-Meldrum's acid derivatives (**3**) and thiazolines (**4**), respectively. Firs-generation pilicides with a $CH_2$-1-naphthyl substituent in C-7 and a cyclopropyl (**6**) or phenyl (**7**) in C-8 that forms the basis of this work.

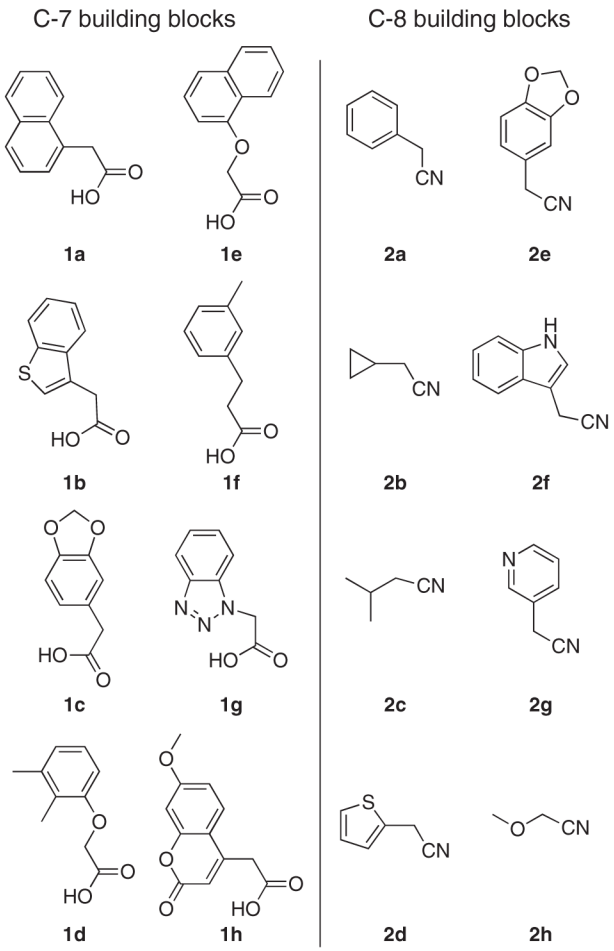



**Figure 2.**
The building blocks used when constructing the library of C-7 and C-8-substituted pilicides.

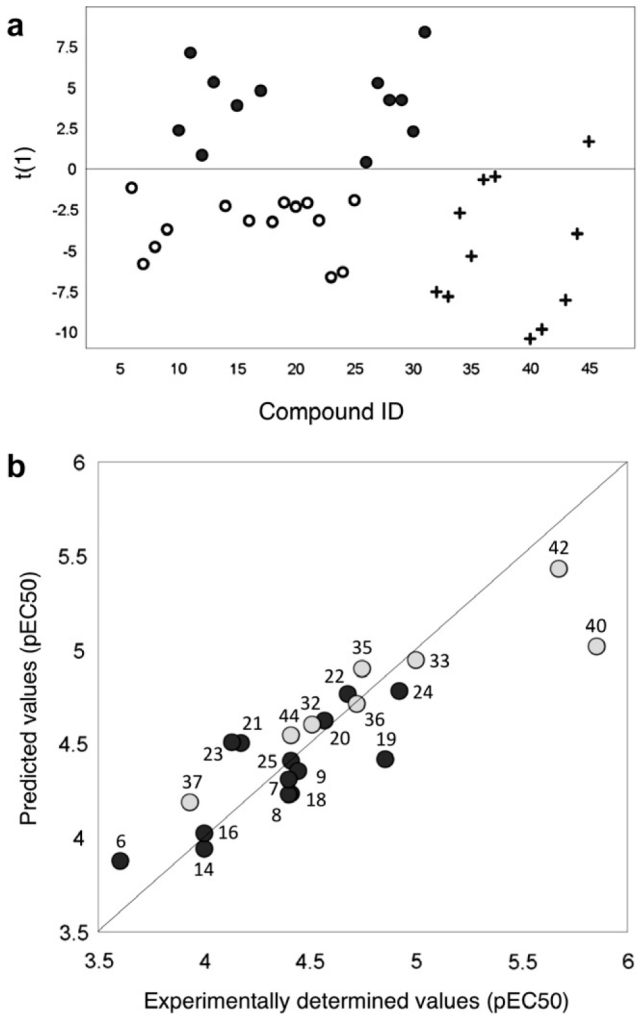
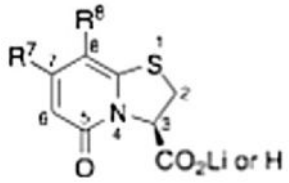
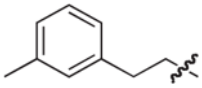
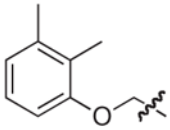
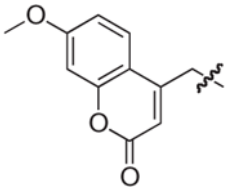
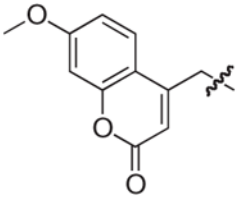
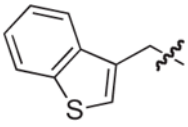
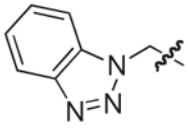
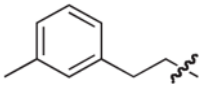


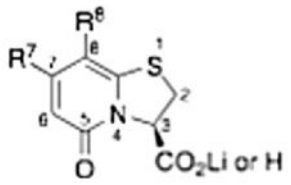

**Figure 3.**
(a) A OPLS-DA score plot showing active compounds (circles), inactive compounds (filled circles), and the validation set (plus signs). (b) Observed versus predicted plot of the PLS model showing calculated and predicted values of the model set (black) and the validation set (grey) versus experimentally determined values. The chemical structures can be seen in Tables 1 and 2.

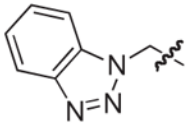
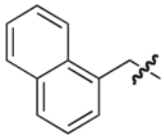
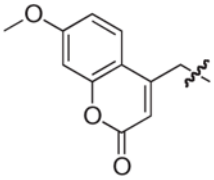
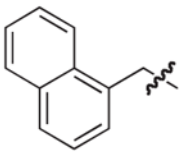
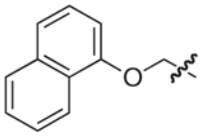
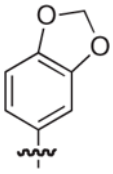
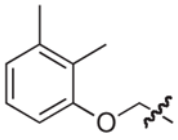
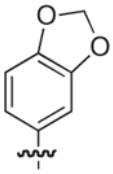
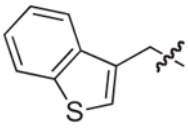
## Table 1

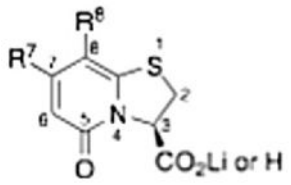
Chemical structures and biological evaluation of the balanced set of di-substituted compounds

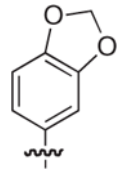
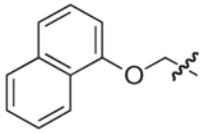
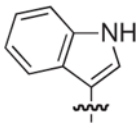
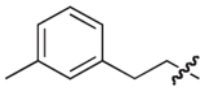
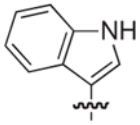
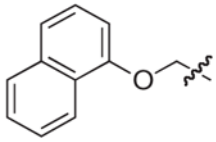
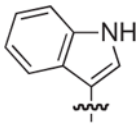
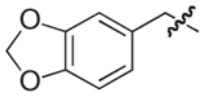
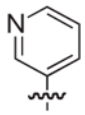
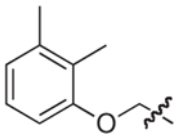
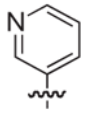
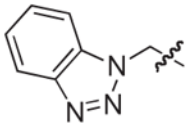
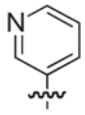
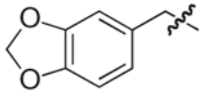


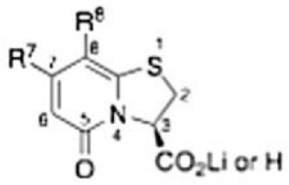

| ID | R[8] (C-8) | R[7] (C-7) | EC[50] (μM)[a] |
|---|---|---|---|
| **8**[b,c] |  |  | 40 |
| **9**[b,c] |  |  | 36 |
| **10**[b] |  |  | NA[d] |
| **11**[b] |  |  | NA[d] |
| **12**[b] |  |  | NA[d] |
| **13**[b] |  |  | NA[d] |
| **14**[b,c] |  |  | 100 |

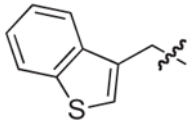
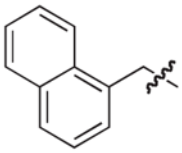
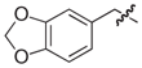
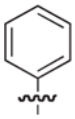
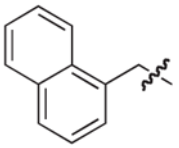
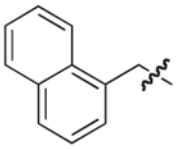



| ID | R[8] (C-8) | R[7] (C-7) | EC$_{50}$ (μM)[a] |
|---|---|---|---|
| **15**[b] |  |  | NA[d] |
| **16**[b] |  |  | 100 |
| **17**[b] |  |  | NA[d] |
| **18**[b] |  |  | 39 |
| **19**[b,c] |  |  | 14 |
| **20**[b,c] |  |  | 27 |
| **21**[b] |  |  | 67 |




| ID | R[8] (C-8) | R[7] (C-7) | $EC_{50}$ (μM)[a] |
|---|---|---|---|
| 22[b] |  |  | 21 |
| 23[b] |  |  | 74 |
| 24[b] |  |  | 12 |
| 25[b] |  |  | 39 |
| 26[b] |  |  | NA[d] |
| 27[b] |  |  | NA[d] |
| 28[b] |  |  | NA[d] |




| ID | R[8] (C-8) | R[7] (C-7) | $EC_{50}$ (μM)[a] |
|---|---|---|---|
| **29**[b] |  |  | NA[d] |
| **30**[b] |  |  | NA[d] |
| **31**[b,c] |  |  | NA[d] |
| **7**[e] |  |  | 40 |
| **6**[e] |  |  | 250 |

[a] Calculated from 16–72 data points per concentration.

[b] Compound is part of the design.

[c] For solubility reasons these compounds were tested as carboxylic acids and not as lithium carboxylates.

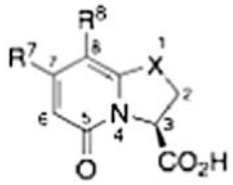
[d] Not active.

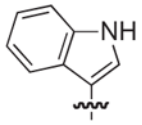
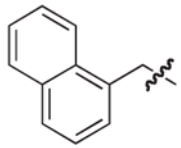
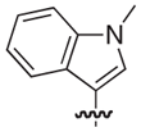
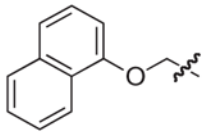
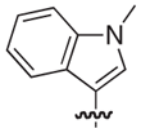
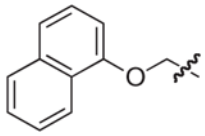
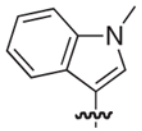
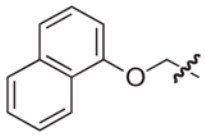
[e] First generation pilicides that the design was based on.

*Bioorg Med Chem*. Author manuscript; available in PMC 2013 August 26.

**Table 2**

Chemical structures and biofilm inhibition activities (predicted and experimentally determined) for the compounds synthesized to validate the QSAR model that was generated based on the balanced set of 24 di-substituted compounds shown in Table 1




| ID | $R^8$ (C-8) | $R^7$ (C-7) | Pred. | | |
|---|---|---|---|---|---|
| | | | X | $EC_{50}$ (μM) | $EC_{50}$ (μM)[a] |
| **32** |  |  | S | 25 | 31 |
| **33** |  |  | S | 11 | 10 |
| **34**[b] |  |  | S | _[c] | 9 |
| **35** |  |  | O | 13 | 18 |

| ID | $R^8$ (C-8) | $R^7$ (C-7) | Pred. | | |
|---|---|---|---|---|---|
| | | | X | $EC_{50}$ (μM) | $EC_{50}$ (μM)[a] |
| 36 |  |  | O | 19 | 19 |
| 37 |  |  | O | 65 | 117 |
| 38 |  |  | S | _c | 1.1 |
| 39 |  |  | S | _c | 1.9 |

| ID | $R^8$ (C-8) | $R^7$ (C-7) | X | Pred. $EC_{50}$ (μM) | $EC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| 40 |  |  | S | 9.6 | 1.4 |
| 41 |  |  | S | _c | 3 |
| 42 |  |  | S | 3.7 | 2.1 |
| 43 |  |  | S | _c | 4.3 |

| ID | $R^8$ (C-8) | $R^7$ (C-7) | Pred. | | |
|---|---|---|---|---|---|
| | | | X | $EC_{50}$ (μM) | $EC_{50}$ (μM)[a] |
| **44** |  |  | S | 28 | 39 |
| **45** |  |  | S | NA[d] | NA[d] |
| **24** |  |  | S | 17 | 12 |
| **22** |  |  | S | 17 | 21 |
| **18** |  |  | S | 58 | 39 |
| – | – | – | – | – | – |

[a] Estimated from 16 to 72 data points per concentration.

[b] In this derivative, the C-3 carboxylic acid was replaced with a methyl acylsulfonamide carboxylic acid isostere.

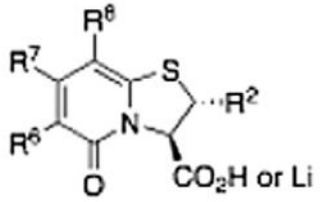
[c] A reliable predicted $EC_{50}$ value could not be determined because the compounds were too chemically different from the set of di-substituted compounds the model is based upon (see Section 4 for details).

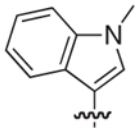
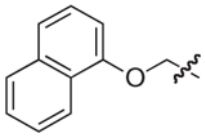
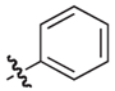
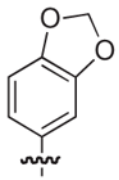
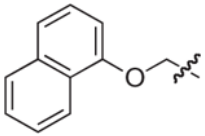
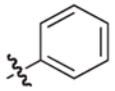
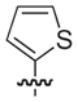
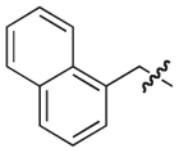
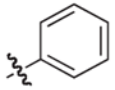
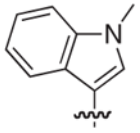
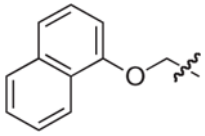
[d] Not active.

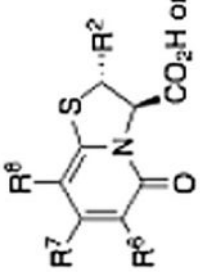
*Bioorg Med Chem*. Author manuscript; available in PMC 2013 August 26.

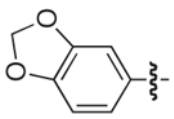
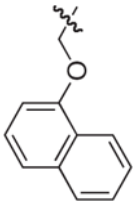
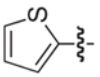
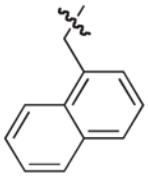
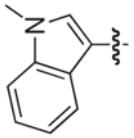
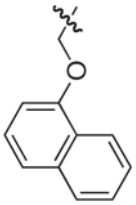
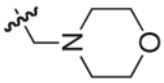
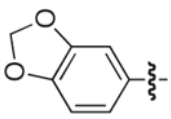
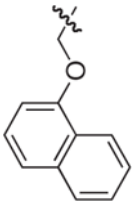
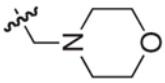
**Table 3**

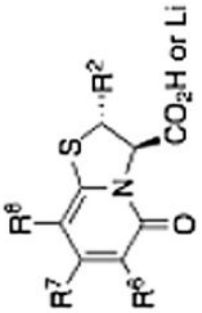
The chemical structures and biological evaluation of tri-substituted derivatives included in the designed set to explore the C-2 and C-6 positions

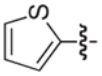
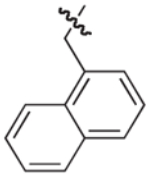
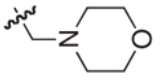
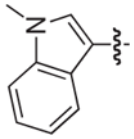
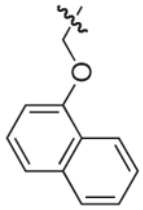
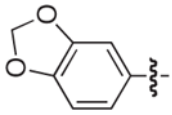
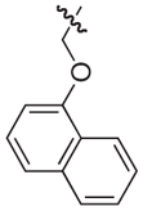
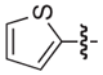
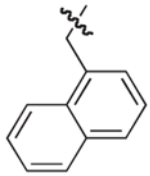



| ID | $R^8$ (C-8) | $R^7$ (C-7) | $R^6$ (C-6) | $R^2$ (C-2) | $EC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| **46** |  |  | –H |  | 1.0 |
| **47** |  |  | –H |  | 1.2 |
| **48** |  |  | –H |  | 0.4 |
| **49** |  |  | –H |  | 2.5 |



| ID | $R^8$ (C-8) | $R^7$ (C-7) | $R^6$ (C-6) | $R^2$ (C-2) | $EC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| 50 |  |  | –H |  | 30 |
| 51 |  |  | –H |  | 92 |
| 52 |  |  |  | –H | 18 |
| 53 |  |  |  | –H | 32 |



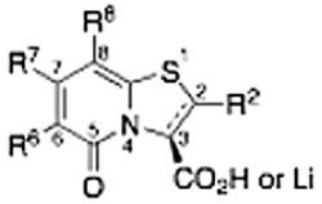

| ID | $R^8$ (C-8) | $R^7$ (C-7) | $R^6$ (C-6) | $R^2$ (C-2) | $EC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| 54 |  |  |  | –H | 88 |
| 55 |  |  |  | –H | 16 |
| 56 |  |  |  | –H | 30 |
| 57 |  |  |  | –H | 250 |

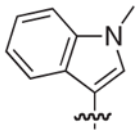
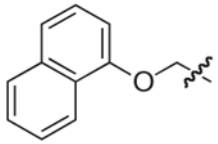
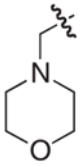
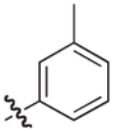
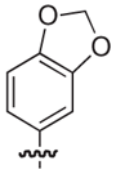
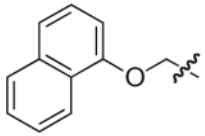
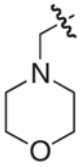
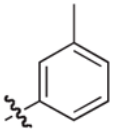
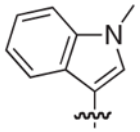
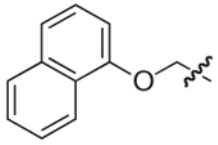
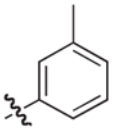
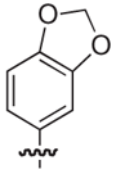
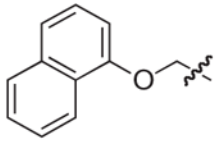
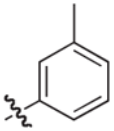
| ID | $R^8$ (C-8) | $R^7$ (C-7) | $R^6$ (C-6) | $R^2$ (C-2) | $EC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| **33** |  |  | –H | –H | 10 |
| **22** |  |  | –H | –H | 21 |
| **18** |  |  | –H | –H | 39 |
| – | – | – | – | – | – |

[a]Estimated from 16 to 72 data points per concentration.

**Table 4**

The chemical structure and biological evaluation of tri- and tetra-substituted derivatives of the pilicide scaffold




| ID | $R^8$ (C-8) | $R^7$ (C-7) | $R^6$ (C-6) | $R^2$ (C-2) | $EC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| **58** |  |  |  |  | 5 |
| **59** |  |  |  |  | 5 |
| **60** |  |  | –H |  | 86 |
| **61** |  |  | –H |  | 20 |

| ID | $R^8$ (C-8) | $R^7$ (C-7) | $R^6$ (C-6) | $R^2$ (C-2) | $EC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| **33** |  |  | –H | –H | 10 |
| **22** |  |  | –H | –H | 21 |

[a]Estimated from 16 to 72 data points per concentration.