Design, Synthesis & Evaluation of dihydro oxazolo ring-fused 2-pyridones; Potential Novel Antibacterial Agents

Master thesis 30hp
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Abstract
Substituted thiazolo ring-fused 2-pyridones, called pilicides, possess novel antibacterial properties and inhibit pilus assembly in Uropathogenic *Escherichia coli*. Recently, a small number of oxygen analogues of active pilicides have shown maintained ability to disrupt pilus assembly. Here an improved methodology for the synthesis of oxazolo ring-fused 2-pyridones is presented, allowing dihydro oxazolo ring-fused 2-pyridones to be synthesized in good yields and with excellent enantiomeric excess. Evaluation of three novel oxazolo 2-pyridones in a biofilm assay proved these analogues to be potent pilicides and able to reduce biofilm formation in UPEC at low µM-concentrations. Additionally, applying Suzuki-Miyaura cross coupling, a set of C-2 substituted unsaturated thiazolo ring-fused 2-pyridones have been synthesized using ligand-free conditions in modest to excellent yields. These C-2 substituted analogues are to be hydrolyzed and evaluated as pilicides and will result in valuable structure-activity information for the pilicide project and hopefully the discovery of new potent pilicides.
# List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcOH</td>
<td>acetic acid</td>
</tr>
<tr>
<td>Arg</td>
<td>arginine</td>
</tr>
<tr>
<td>aq.</td>
<td>aqueous</td>
</tr>
<tr>
<td>±BINAP</td>
<td>$(\pm)$-$(1,1'$'$-binaphthalene-2,2'$'$-diyl)bis(diphenylphosphine)</td>
</tr>
<tr>
<td>b.p.</td>
<td>boiling point</td>
</tr>
<tr>
<td>calcd</td>
<td>calculated</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DDQ</td>
<td>2,3-dichloro-5,6-dicyano-1,4.benzoquinone</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N'$-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>E. coli</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>ee</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
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<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>eq.</td>
<td>equivalents</td>
</tr>
<tr>
<td>HMTA</td>
<td>hexamethylenetetramine</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>50% inhibitory concentration</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography mass spectrometry</td>
</tr>
<tr>
<td>LDA</td>
<td>lithium diisopropylamide</td>
</tr>
<tr>
<td>Lys</td>
<td>lysine</td>
</tr>
<tr>
<td>MeCN</td>
<td>acetonitrile</td>
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<tr>
<td>MeOH</td>
<td>methanol</td>
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<tr>
<td>mol.</td>
<td>molecular</td>
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<tr>
<td>m.p.</td>
<td>melting point</td>
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<tr>
<td>MP-TsOH</td>
<td>macroporous polystyrenesulfonic acid</td>
</tr>
<tr>
<td>MWI</td>
<td>microwave irradiation</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>OAc</td>
<td>acetate</td>
</tr>
<tr>
<td>obsd</td>
<td>observed</td>
</tr>
<tr>
<td>Pap</td>
<td>pyelonephritis associated pili</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>PLS</td>
<td>partial least square projection to latent structures</td>
</tr>
<tr>
<td>PPTS</td>
<td>$p$-pyridinium tolenesulfonate</td>
</tr>
<tr>
<td>pTsOH</td>
<td>$p$-tolenesulfonic acid</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
<tr>
<td>sat.</td>
<td>saturated</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>UPEC</td>
<td>Uropathogenic <em>Escherichia coli</em></td>
</tr>
<tr>
<td>UTI</td>
<td>urinary tract infection</td>
</tr>
<tr>
<td>UV</td>
<td>ultra violet</td>
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</table>
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1. Introduction

It has become an indisputable fact that bacterial resistance to traditional antibiotic treatments poses a threat to modern society.\(^1\)\(^-\)\(^2\) Infectious diseases are the second major cause of death in the world\(^3\) and treatments of resistant bacterial strains are becoming ever more costly. Today's antibiotics that target vital cell processes in bacteria and thereby cause death or inhibition of growth, have been readily used and saved many lives. Unfortunately, they have also resulted in a worldwide emergence of multi-drug resistant pathogens.\(^4\)

Through selective pressure, pathogens have adapted and evolved to evade the actions of antibacterial agents in use today. After all, bacteria have been on earth for more than three billion years and have learned to survive most extreme and harsh conditions.\(^5\) Needless to say, there is an immense need for novel targets and innovative strategies in antimicrobial therapies. One novel approach is to target virulence, a pathogen's ability to establish infection and cause disease.\(^6\) In recent years virulence factors, products that aid in the infectious process, have been recognized as attractive novel targets for new antibacterial agents.\(^7\)\(^-\)\(^8\) The objective is to render the pathogen non-infectious without affecting its viability. Targeting virulence in this fashion could theoretically impose weaker selective pressure on the bacteria and resistance would therefore develop more slowly.\(^6\)

Pilicides are small, rationally designed molecules aimed at inhibiting pilus biogenesis in Uropathogenic \textit{E. coli}.\(^9\)\(^-\)\(^10\) Pili are virulence factors essential for host adherence in many Gram-negative pathogens and in absence of pili the bacteria become incapable of causing infection. Pilicides could therefore act as leads towards new types of antibacterial agents capable of depriving the bacteria of its ability to cause infection without killing it.
2. Background

2.1 Pili and the chaperone-usher pathway

2.1.1 Pili, adhesive fibers on the bacterial surface

When a pathogen first enters the host, adhesion to host tissue is a crucial step in the infectious cascade. To facilitate the adherence process many pathogenic bacteria express pili, hair-like fibers, on their surface.\textsuperscript{11, 12} Pili assist the pathogen in many steps of the infectious process such as adhesion, invasion and in biofilm formation.\textsuperscript{13}

Uropathogenic \textit{E. coli}, UPEC, causes urinary tract infection, UTI, and is a Gram-negative strain of bacteria that produces pili. Mainly two types of pili, P pili and type 1 pili, are involved in the development of UTI by UPEC.\textsuperscript{12} Pili consist of repeating protein subunits, named Pap in P pili and Fim in type 1 pili. Situated at the tip of each P and type 1 pilus rod is a carbohydrate-recognizing adhesin, PapG and FimH respectively.\textsuperscript{13} PapG attaches to receptors in the kidney and are involved in the establishment of pyelonephritis.\textsuperscript{14} FimH, on the other hand, attaches to receptors in the bladder and is required for the development of bladder infections, cystitis.\textsuperscript{15} The biogenesis of P and type 1 pili in UPEC facilitated by a secretion system known as the chaperone-usher pathway. This pathway is highly conserved and required in a wide range of Gram-negative bacteria for the assembly of adhesive organelles.\textsuperscript{12}

2.1.2 The chaperone-usher pathway

In UPEC, pili biogenesis is dependent upon two types of assembly proteins, periplasmic chaperones and outer membrane bound ushers. In P pili biogenesis chaperone, PapD, is required for folding and stabilization of the pili subunits as well as for their transportation across the periplasm to the usher.\textsuperscript{12, 13, 16} The usher, PapC, is the outer membrane assembly site. When the chaperone-subunit complex reaches the usher, the subunit is incorporated into the growing pilus rod. Subsequent release and recycling of the chaperone completes the pathway (Figure 1).\textsuperscript{17} Type 1 pili are assembled top to bottom, by chaperone FimC and usher FimD.
2.2 Design of ring-fused 2-pyridones as pilicides

2.2.1 Chaperones, periplasmic transport proteins

The periplasmic chaperones are considered attractive targets for novel antibacterial agents since they are essential for pilus biogenesis, have a well-conserved primary structure and function. Their structures, as well as the structures of several PapD-subunit complexes, have been elucidated by X-ray crystallography. Chaperones consist of two domains arranged in a boomerang-shape, with the pilu subunit binding site located in the cleft of the chaperone (Figure 2).
Two preserved residues, Arg 8 and Lys 112, are responsible for anchoring the subunit to the chaperone. Further, the chaperone-subunit complex is stabilized by hydrophobic side-chains near the C-terminal of the protein subunit, which interact with hydrophobic pockets in the chaperone.

2.2.2 Structure based design

Based on a PapD-PapG complex, dihydro thiazolo ring-fused 2-pyridones have been designed as dipeptidomimetics of the PapG C-terminus (Figure 3). The aim was to inhibit the cleft region of the chaperone and thus interfere with crucial chaperone-subunit interactions. The designed 2-pyridone scaffold succeeded in the correct placement of important pharmacophores and allowed variation of the R₁- and R₂-substituents.
Figure 3. Structure based design resulted in ring-fused 2-pyridones as peptidomimetics of the PapG subunit C-terminus. Important features such as the C-terminal carboxylic acid and the hydrophobic side-chains are retained in the rigid ring-fused 2-pyridone framework.

2.3 Pilicides and their mode of action

2.3.1 Potent pilicides

Biologically active pilicides, with the general structure shown in Figure 4, commonly have hydrophobic substituents in the C-7 and C-8 position of the 2-pyridone containing scaffold. Another important feature is the carboxylic acid functionality in the C-3 position, which has proven crucial for pilicide activity (Figure 4). A recent study also showed that the ring-fused system is required for pilicide activity.

Figure 4. General structure of the rigid ring-fused 2-pyridone containing scaffold.
Some of the most potent pilicides are shown in Figure 5. Biological evaluation of these compounds showed that pilicides A-D were capable of inhibiting both P and type 1 pil formation in UPEC without effecting its bacterial growth.\textsuperscript{24, 25}

![Chemical structures of compounds A-D](image)

**Figure 5.** A and B were the most promising compounds from the first screening of pilicides. Introduction of hydrophilic substituents in C-6 resulted in compounds C and D, which exhibited improved solubility and maintained or increased potency compared to A and B.\textsuperscript{24, 25}

### 2.3.2 The pilicide mode of action

The first step towards understanding the mechanism by which pilicides disrupts pilus assembly was to determine the pilicide binding site. NMR spectroscopy and chemical shift mapping using \textsuperscript{15}N-labelled FimC showed changes in the cleft region of the chaperone, but also near the F1-G1 loop, and thus gave no clear answer to the question of binding site.\textsuperscript{26} A cocrystal of a chaperone-pilicide complex was able to illustrate the pilicide mode of action. X-ray crystallography showed pilicide D bound near the F1-G1 loop but not in the cleft.\textsuperscript{24} The F1-G1 loop is located in the part of the chaperone that interacts with the usher, indicating that pilicides do in fact inhibit critical chaperone-usher interactions (Figure 6). This hypothesis was further supported by point mutation of a crucial residue found in the pilicide binding site of the chaperone. The mutation resulted in decreased pilus assembly, demonstrating that this region is of great importance in pilus biogenesis.\textsuperscript{13}
Figure 6. Left: Atomic force spectroscopy images of P pili expressing, untreated E. coli HB101/pPAP5 (a) and E. coli treated with pilicide (b). The scale bar indicates 2.5 μm. Right: X-ray structure of a chaperone-pilicide cocrystal showing the bound pilicide near the F1-G1 loop. \(^\text{24}\)

### 2.4 Functionalization of the thiazolo ring-fused 2-pyridone scaffold

Ring-fused 2-pyridones have been synthesized in solution using both conventional and microwave heating. \(^\text{9, 21, 27, 28}\) Starting from commercially available nitriles and carboxylic acids, ring-fused 2-pyridones, III, have been made from two building blocks, \(\Delta^2\)-thiazolines, I, and Meldrum’s acid derivatives, II, resulting in a wide range of possible \(R^1\)- and \(R^2\)-substituents (a) (Figure 7).

Figure 7. Thiazolo ring-fused 2-pyridones III are synthesized (a) from two building blocks, thiazolines I and Meldrum’s acid derivatives II. \(^\text{9, 21, 27, 28}\) Methods to further functionalize the 2-pyridone scaffold have been developed (b-d). \(^\text{23, 25, 29-31}\)
Methods have been developed to allow great variation in the substitution pattern of the ring-fused 2-pyridones. Substituents such as nitriles, aminomethylene, halogens and aldehydes have been incorporated at the C-6 position (b).\textsuperscript{25, 29, 30} The use of Raney-Ni reduction has resulted in monocyclic 2-pyridones without the thiazolo ring (c).\textsuperscript{23} Further functionalizations have been performed at the C-2 position where a diversity of substituents have been incorporated by either conjugate addition, microwave assisted Heck couplings or lithiations (d).\textsuperscript{31} To enable these substitutions, the thiazolo ring needed to be oxidized to its unsaturated analogue according to Scheme 1.

\textbf{Scheme 1}

\begin{center}
\begin{tikzpicture}
\node[anchor=east] (R1) at (0,0) {$\text{R}^1$};
\node[anchor=east] (R2) at (0,-0.5) {$\text{R}^2$};
\node[below=0.5cm of R1] (R3) {$\text{CO}_2\text{Me}$};
\node[below=0.5cm of R2] (R4) {$\text{CO}_2\text{Me}$};
\node[below=0.5cm of R1] (R5) {$\text{N}$};
\node[below=0.5cm of R2] (R6) {$\text{N}$};
\node[below=0.5cm of R1] (R7) {$\text{S}$};
\node[below=0.5cm of R2] (R8) {$\text{S}$};
\node[below=0.5cm of R1] (R9) {$\text{O}$};
\node[below=0.5cm of R2] (R10) {$\text{O}$};
\node at (0,0.5) {$\text{1}$};
\draw[thick, rounded corners] (0,0) -- (0.5,0) -- (0.5,-0.5) -- (0,-0.5) -- cycle;
\draw[thick, rounded corners] (0,0) -- (0.5,0) -- (0.5,-0.5) -- (0,-0.5) -- cycle;
\end{tikzpicture}
\end{center}

\begin{center}
$\text{NaH, BrCCl}_3$
MeOH (1.5 eq.), MeCN, rt
\end{center}

$\text{R}^1 = \text{cyclopropyl}$

$\text{R}^2 = \text{CH}_2-1$-naphtyl

**2.5 Dihydro oxazolo ring-fused 2-pyridones**

Cytochrom P450 is a collective name for a number of proteins involved in the metabolism of drugs and foreign compounds in the human body.\textsuperscript{32} P450 reactions catalyze the incorporation of oxygen into substrates such as the oxidation of sulfur to S-oxides.\textsuperscript{33-35} Metabolism of a compound can lead to destabilization or deactivation of the drug or induce toxicity.\textsuperscript{36} It is reasonable to consider the sulfur atom, present in the thiazole moiety of the active pilicides, to be susceptible to metabolic oxidation with a potential loss of activity as a result. This has been indicated in an initial study of a small number of sulfone analogues, which had lost their pilicide activity (unpublished data). Since the ring-fused system is required for pilicide activity,\textsuperscript{23} exchanging sulfur for oxygen could circumvent this issue. In an initial study, potentially more metabolically stable oxygen analogues of three active pilicides have been synthesized (Scheme 2) and biological evaluation showed maintained ability to inhibit pilus formation.\textsuperscript{37}
A convenient one-pot method has been developed by the Almqvist group for the synthesis of oxazolo fused 2-pyridones (Scheme 2). In this synthetic route, the formation of oxazolo ring-fused 2-pyridones 5 from 3 proceeds via the corresponding oxazolines. It has recently been shown that molybdenum oxides possess excellent catalytic activities for the cyclization of acylated serine, threonine and cysteine derivatives and the dehydrative cyclization of 3 is facilitated by a tetra ammonium molybdenum oxide catalyst and continuous removal of water by azeotropic reflux. Under acidic conditions, the formed oxazoline reacts with the acyl ketene generated from acylated Meldrum’s acid derivative 4 in an acylketene-imine cyclocondensation reaction to yield the desired ring-fused 2-pyridone.

Employing the one-pot method, oxazolo ring-fused 2-pyridones, 5a-c, have been prepared in modest to good yields. However, this method resulted in almost complete racemization of the stereogenic center and efforts to improve the enantiomeric excess by decreasing the amount of TFA from 5 eq. to 0.1 eq. resulted in a significant drop in yields. A plausible explanation for this, taking into account the relatively low boiling point of TFA and that the reaction is run in refluxing toluene, would be that most of the acid quickly evaporates up to the Soxhlet apparatus and remain there until the Soxhlet is emptied. Hence, when decreasing the number of equivalents of acid, the amount of acid remaining in the reaction vessel would not be enough for the reaction to proceed.
3. Results and discussion

3.1 Method improvement by experimental design

3.1.1 Acid selection and experimental design

Motivated by the results from the first screening of the oxygen analogues we wanted to improve the one-pot synthesis of oxazolo ring-fused 2-pyridones. In order to determine the optimal reaction conditions experimental design was employed. The goal of the design was to improve the enantiomeric purity of the reaction while maintaining a good yield. First and foremost, we wanted to explore the possibility of exchanging the acid used in the reaction and a screening of acids was therefore performed. The acids were chosen with considerations to boiling point and acidity (\(pK_a\)). Since the reaction is run in refluxing toluene high-boiling acids are advantageous. The acids should also be of varying acidity and thus one stronger and one weaker acid, as compared to TFA, were chosen. The acids included in the screening, their \(pK_a\) values and melting or boiling points are presented in Table 1. The reaction time and number of equivalents of acid were also regarded influential factors for the outcome of the reaction. Hence, by varying acid, reaction time and equivalents of acid, while measuring two responses, yield and enantiomeric excess, a D-optimal design\(^{39}\) with eight experimental runs was created using the chemometric software MODDE 8.0 (Table 2).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acid</th>
<th>(pK_a)</th>
<th>m.p. (°C)</th>
<th>b.p.°</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>MP-TsOH,</strong></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macroporous polystyrenesulfonic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><strong>PPTS,</strong></td>
<td>~6</td>
<td>117-120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyridinium (p)-toluenesulfonate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><strong>pTsOH,</strong></td>
<td>-2.8</td>
<td>103-106</td>
<td></td>
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<td></td>
<td>(p)-Toluenesulfonic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><strong>TFA,</strong></td>
<td>-0.25</td>
<td>72°</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trifluoroacetic acid</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
3.1.2 Model computation and evaluation

5a was chosen as a model compound for the screening and was synthesized according to the design matrix following the previously developed method (Scheme 3).

Scheme 3

Ten experiments, eight experiments and two replicates, were run and the results are shown in Table 2. The use of polymer bound sulfonic acid, MP-TsOH, yielded no product, probably due to lack of swelling of the polystyrene in toluene. 2-pyridone 5a synthesized using PPTS exhibited excellent ee 92-99% according to chiral HPLC and was isolated in 41-57% yield. Using 0.1 equivalents of ρTsOH, 5a could be obtained in 36-57% with 88-92% ee, but no 2-pyridone was formed when 5 equivalents were used (Table 2).

Table 2. Results from the experimental design. Eight experiments and two replicates were made. ee was determined by chiral HPLC using a WHELK-O 1 column.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Exp No</th>
<th>Acid</th>
<th>Time (h)</th>
<th>Eq</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
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<tr>
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<td>1</td>
<td>MP-TsOH</td>
<td>6</td>
<td>0.1</td>
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<td>2</td>
<td>MP-TsOH</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>PPTS</td>
<td>6</td>
<td>0.1</td>
<td>41</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>PPTS</td>
<td>6</td>
<td>0.1</td>
<td>57</td>
<td>93</td>
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<td>PPTS</td>
<td>5</td>
<td>5</td>
<td>56</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>ρTsOH</td>
<td>5</td>
<td>0.1</td>
<td>36</td>
<td>92</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>ρTsOH</td>
<td>5</td>
<td>0.1</td>
<td>57</td>
<td>88</td>
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<tr>
<td>8</td>
<td>6</td>
<td>ρTsOH</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>TFA⁺</td>
<td>5</td>
<td>0.1</td>
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<tr>
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<td>8</td>
<td>TFA⁺</td>
<td>6</td>
<td>5</td>
<td>78</td>
<td>2</td>
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</table>

*Previously collected data.*
A PLS model relating the X matrix (experimental factors Acid, Time, Eq.) to the responses Yield and ee (Y matrix) was calculated. Using cross-validation, three significant components were extracted, describing 77.7% of the variation in the response Y and predicting 40.9% of the variation in Y. Interpretation of the model coefficients showed a strong positive correlation between PPTS and both responses (Figure 8). According to the model, going from low to high settings of both reaction time and equivalents of acid negatively affects the ee. The optimal reaction conditions predicted by the model were catalytic amounts of mild acid and short reaction times. These predictions could be verified by the raw data (Table 2).

Figure 8. Coefficient plots for Yield (left) and ee (right). The coefficient plot displays the effect of a factor on the response, when going from the low setting to the high setting of the factor. The error bars show the confidence intervals at 0.95 confidence level.

There are several examples in the literature showing PPTS to be a mild, effective and versatile catalyst. PPTS can be readily prepared from pyridine and p-toluenesulfonic acid monohydrate and is easy to handle. This made additional screenings to further optimize the reaction conditions excessive and catalytic amounts of PPTS was considered the conditions of choice for the synthesis of the oxygen analogues.
3.2 Synthesis of three novel dihydro oxazolo ring-fused 2-pyridones

### 3.2.1 Acylation of L-serine

Encouraged by the results from the screening, the improved one-pot method, using catalytic amounts of PPTS, was employed for the synthesis of three novel oxazolo fused 2-pyridones. The substitution patterns of these analogues were based on promising results from a parallel study containing thiazolo fused 2-pyridones made by the Almqvist group (unpublished data).

In the synthesis of dihydro oxazolo ring-fused 2-pyridones the chiral moiety of the compounds is derived from L-serine methyl ester 6. The acylated serine derivatives, 8a-c, required in the one-pot synthesis were synthesized in good to excellent yields following a previously published procedure (Scheme 4).\(^{38}\) 7b was prepared from the corresponding carboxylic acid according to a previously published procedure\(^ {44}\) and 7c was synthesized from indole-3-acetic acid by N-methylation\(^ {45}\) followed by conversion of the acid to the acid chloride.\(^ {46}\)

Scheme 4

\[
\begin{align*}
6 & \quad 7a: R^1 = \text{thiophen-2-yl} \\
 & \quad 7b: R^1 = 3,4-(\text{methylenedioxy})\text{phenyl} \\
 & \quad 7c: R^1 = 1\text{-methyl-1H-indol-3-yl} \\
8a: R^1 = \text{thiophen-2-yl} (81\%) \\
8b: R^1 = 3,4-(\text{methylenedioxy})\text{phenyl} (93\%) \\
8c: R^1 = 1\text{-methyl-1H-indol-3-yl} (74\%)
\end{align*}
\]

3.2.2. Synthesis of novel oxazolo ring-fused 2-pyridones with catalytic amounts of PPTS

As described earlier (see section 2.5) the key intermediate for the synthesis of oxazolo 2-pyridones, the oxazoline, is formed by cyclization of the acylated serine derivative. The oxazoline is then reacted with the acylketene generated from an acylated-Meldrum’s acid derivate to form the ring-fused 2-pyridone. The second building block needed for the one-pot synthesis, Meldrum’s acid derivatives 4a-b, were synthesized according to published procedures\(^ {47}\) and used without further purification.
In order to obtain the oxazolo fused 2-pyridones 8a-c was allowed to reflux in the presence of (NH₄)₂MoO₄ with removal of water using a Soxhlet apparatus containing activated 3Å molecular sieves. Reflux was continued until TLC showed complete conversion to oxazoline 9 (4-5 h). The ring-fused 2-pyridones were then formed by adding 2-3 eq. of Meldrum’s acid derivative, 4a or 4b, and 0.2 eq. PPTS (Scheme 5). In order to obtain good yields of 10a and 10b, subsequent additions of Meldrum’s acid derivative (0.5-2 eq.) were required. The optimized reaction conditions allowed isolation of oxygen analogues, 10a-c, in 61-71% yield (Table 3).

Scheme 5

![Scheme 5](image)

**Table 3.** Isolated yields and ee of the synthesized oxazolo ring-fused 2-pyridone methyl esters 10a-c.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>Yield %</th>
<th>ee %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10a</td>
<td>S</td>
<td></td>
<td>71</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>10b</td>
<td>O</td>
<td></td>
<td>67</td>
<td>97</td>
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<tr>
<td>3</td>
<td>10c</td>
<td>N</td>
<td></td>
<td>61</td>
<td>96</td>
</tr>
</tbody>
</table>
With the purpose of verifying the enantiomeric excess of the reaction, ee was determined for 2-pyridone methyl esters 10a-c by chiral HPLC. Excellent separation of the two enantiomers was achieved using a (S,S) – WHELK-O1 column (Figure 9) and comparisons with racemic 10a-c confirmed that the ee was as high as 95, 97 and 96% for 10a-c respectively (Table 3) (see experimental section). Further, it cannot be excluded that the epimerization originates from serine derivative 8.

Figure 9. Determination of enantiomeric excess by chiral HPLC using a (S,S) – WHELK-O1 column and a 4:4:1 CH₂Cl₂:heptane:EtOH eluent system. Comparisons with racemic 10b confirmed that ee for compound 10b was 97%.

3.2.3 Hydrolysis of methyl esters

Hydrolysis of the obtained methyl esters, 10a-c, was accomplished using LiBr and TEA in aqueous MeCN, a mild method developed by Mattson et al. which had been successfully employed for the previously synthesized oxygen analogues. Unfortunately, purification of the obtained acids by silica gel chromatography was not as straightforward as expected. Still, this could be solved by slightly changing the work-up procedure (see experimental section for details) and 11a-c was isolated in 59-64% yield.

Scheme 6
3.3 C-2 substituted unsaturated ring-fused 2-pyridones

Having improved the methodology for the synthesis of oxazolo ring-fused 2-pyridones, the attention was turned towards the possibility to further decorate the 2-pyridone containing scaffold. Results from a previous study, where a phenyl substituent at the C-2 position of the scaffold had resulted in increased potency (unpublished data), inspired us to explore the possibility of introducing various aryl substituents at this position. Hence, with the aim to synthesize a number of C-2 substituted unsaturated sulfur and oxygen analogues, our interest fell upon the Suzuki-Miyaura cross coupling. The creation of a small library of C-2 substituted analogues would not only result in possibly active pilicides and valuable structure-activity information for the pilicide project, but the use of Suzuki-Miyaura couplings would also further exemplify the versatility of the 2-pyridone scaffold.

There are examples in the literature where Suzuki couplings have successfully been applied to 2-pyridones, thiazoles and oxazoles. This cross-coupling method therefore seemed like an attractive approach to introduce substituents at the C-2 position in the ring-fused 2-pyridone system. Fundamental to the cross coupling reaction was a bromo-substituted unsaturated ring-fused 2-pyridone as shown in Figure 10.

![Figure 10](image-url)

**Figure 10.** Retrosynthetic analysis of C-2 substituted unsaturated ring-fused 2-pyridones. Key intermediate in the synthetic route was a bromo substituted unsaturated 2-pyridone.
3.3.1 Oxidation-halogenation of thiazolo ring-fused 2-pyridones

Starting with the thiazolo 2-pyridone, we set out to find a straightforward and time efficient synthetic route to key intermediate 12. The synthesis of 12 had previously been accomplished by oxidation of 1, followed by lithiation and bromination.\(^\text{31}\) In order to avoid unnecessary purification and isolation of the oxidized ring-fused 2-pyridone 2, a one-pot procedure going directly from 1 to 12 by modifying the developed oxidation method was envisioned. Consequently, 1 was synthesized according to published procedures\(^\text{27, 28}\) and then treated with 3 eq. NaH followed by 3 eq. BrCCl\(_3\) (Scheme 7). As previously described, the oxidation of 1 does not proceed without the addition of MeOH and also here the addition of MeOH proved to be crucial. A total of 4.2 eq. of MeOH was added dropwise in portions over 2 h and the reaction was monitored by TLC. Upon the last addition of MeOH, the reaction mixture turned dark brown and was quenched. After purification by silica gel chromatography this one-pot procedure gave 12 in 72\% yield.

![Scheme 7](image)

3.3.2 Microwave assisted Suzuki-Miyaura cross coupling

With key intermediate 12 in hand, the next mission was the cross coupling reaction. Previously, microwave assisted Heck couplings had resulted in high yields of unsaturated products.\(^\text{31}\) Hence, it was believed that microwave heating would be superior compared to using conventional heating for the Suzuki-Miyaura cross couplings. Microwave accelerated organic chemistry has considerably reduced reaction times, improved yields and reduced byproduct formation in a number of different chemical transformations including transition-metal-catalyzed carbon-carbon bond formations.\(^\text{59-61}\) Moreover, the Almqvist group has successfully applied microwave heating to a number of reactions involving the ring-fused 2-pyridone scaffold.\(^\text{28, 29, 31}\)
Initial attempts to couple m-tolyl boronic acid to 12 were made by heating a mixture of 12, 10 mol% Pd(OAc)$_2$, ± BINAP, boronic acid and KF in dry MeOH to 140°C using microwave irradiation. These conditions had previously been used by other members of the Almqvist group$^{62}$ and appeared to be a good starting point. After purification these conditions allowed isolation of coupled product 13a in 68% yield, but the reaction conditions seemed unnecessarily harsh. Attempts showed that the reaction temperature could be lowered to 100°C and to our delight we saw that the reaction worked equally well without the use of ± BINAP as a ligand. Ligand-free conditions are advantageous since ligands can be difficult to synthesize or be very expensive and can make purification procedures troublesome.$^{52,63-67}$

Scheme 8

Running the reaction at 100°C for 10 min with 10 mol% Pd(OAc)$_2$, 2 eq. of m-tolyl boronic acid and 1.9 eq. KF in dry MeOH allowed 13a to be obtained in excellent yield (97%) and we now moved on to couple five other aryl or heteroaryl boronic acids (Scheme 8). The coupling products 13a-c of aryl boronic acids were readily synthesized in 94-97% yield (Table 4). Unfortunately, the coupling was more difficult when heteroaryl boronic acids were used and dehalogenated starting material was seen as a major byproduct. Still 13d and 13e were isolated in 72 and 34% yield respectively, while only traces of 13f could be seen by LC-MS analysis and no product could be isolated. Attempts to improve the low yield of 13e, by adding 10 mol% of ±BINAP and changing the solvent to DMF, were made but these efforts did not increase the yield.
Table 4. Isolated yields of C-2 substituted unsaturated thiazolo ring-fused 2-pyridones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13a</td>
<td></td>
<td>97</td>
</tr>
<tr>
<td>2</td>
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<td>97</td>
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<tr>
<td>3</td>
<td>13c</td>
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<td></td>
<td>72</td>
</tr>
<tr>
<td>5</td>
<td>13e</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>13f</td>
<td></td>
<td>Trace</td>
</tr>
</tbody>
</table>

3.3.3 Oxidation of oxazolo ring-fused 2-pyridones

The one-pot oxidation-halogenation method developed for the thiazolo ring-fused 2-pyridones proceeds via the unsaturated counterpart 2. Hence, the first objective when working with the oxygen analogues was to find a method for this oxidation (Figure 11).

![Oxidation of oxazolo ring-fused 2-pyridones](image)

Figure 11. Oxidation of oxazolo ring-fused 2-pyridones.
To the best of our knowledge, there are no methods reported in the literature for the transformation of dihydro oxazolo ring-fused 2-pyridone systems to their unsaturated analogues. However, the method used to oxidize the thiazolo 2-pyridones, NaH and BrCCl$_3$, and a typical method for the oxidation of oxazolines to oxazoles, DBU and BrCCl$_3$, were considered good starting points. Unfortunately, no oxidized product 14 could be detected following these procedures, instead the formation of a major byproduct 15 was seen (Scheme 9). Still, the method using NaH and BrCCl$_3$ had worked well for the corresponding thiazolo analogues and some further efforts to apply this reaction to the oxazolo ring-fused 2-pyridones were made. These efforts included running the reaction at different temperatures (-78°C, -15°C, 0°C and rt), reducing the number of equivalents of NaH, altering the solvent (MeCN, THF or MeCN:THF 1:1) and changing the base from NaH to a more bulky base, LDA. Another method using CuBr$_2$, DBU and HMTA was also attempted. Generally, these conditions either resulted in no conversion of the starting material or in the formation of the previously detected byproduct. The byproduct was identified as 2,6 pyridinedione 15, which is formed by a ring-opening of the oxazolo ring of the ring-fused system (Scheme 9). $^1$H- and $^{13}$C-NMR data, collected from one-dimensional spectra, cosy, nosey, hetcor and dept experiments, all agreed with the proposed structure 15, which could be isolated in 68% yield.

Scheme 9
Having realized the instability of the oxygen analogues under basic conditions, milder and base-free oxidation methods were attempted. MnO$_2$ has previously been used to oxidize oxazolines to oxazoles$^{70}$ but no conversion of the starting material occurred when this method was applied to the oxazolo 2-pyridones. Trials with other typical dehydrogenation reagents, such as $S_8$ in refluxing xylene, microwave assisted DDQ in either CHCl$_3$ $^{69}$ or dioxane$^{71, 72}$ and Pd/C in AcOH$^{73}$ using microwave heating, were made. To our disappointment, no product formation could be detected using any of the methods.

### 3.4 Biological evaluation

#### 3.4.1 Inhibition of biofilm formation

Biofilms, communities of bacteria attached to a surface, allows the bacteria to evade host defenses and spread in the host.$^{15, 74}$ In UPEC, biofilm formation is dependent on type 1 pili, hence bacteria lacking pili lose the ability to form biofilms.$^{75}$ Oxazolo 2-pyridones, 11a-c, were evaluated in an in vitro biofilm assay (Figure 12). The biofilm assay is a good initial screen for identifying potent pilicides, but it is not conclusive since biofilm formation can be inhibited by other factors than the absence of pili. Further tests, e.g a hemagglutination assay, is necessary to confirm that the tested compounds inhibit pili assembly.$^{24}$

![Figure 12](image-url) Oxazolo 2-pyridones 11a-c were evaluated in a biofilm assay.
In the biofilm assay Uropathogenic *E. coli*, UTI89, were grown in Luria broth in presence of the compound to be tested. The assay was performed in microtiter plates and the initial concentration of compound was 400 μM. The ability to form biofilm was quantified after 48 h after the wells had been rinsed and subsequently stained with crystal violet. This procedure was repeated at different concentrations of pilicide and the results of the biological evaluation are shown in Figure 13. No solubility problems were experienced during the evaluation.

![Biofilm assay](image)

**Figure 13.** Compounds 11a-c were screened in a type 1 dependent biofilm assay. All compounds were tested from a concentration of 400 μM down to 50 μM. The most potent pilicide 11c was tested down to 1.5 μM. All of the compounds behaved in a dose-dependent manner. Inhibition is shown as percent relative an untreated control in each assay and, where shown, error bars represent the standard deviation of the mean.

When tested at 400 μM pilicides 11a-c were all able to reduce biofilm formation *in vitro* by over 90% as compared to an untreated control. Tests at lower concentrations showed that the compounds behaved in a dose-dependent manner and identified 11c as the most potent of the oxygen analogues. The concentration of 11c needed to obtain 50% inhibition of biofilm formation was estimated to 12-25 μM, compared to compound D which has an estimated IC<sub>50</sub> value of ~190 μM. In conclusion, these results clearly show that it is possible to exchange sulfur for oxygen in the ring-fused scaffold and still obtain potent pilicides. Further, should S-oxidation of active pilicides become an issue in the future, the oxygen analogues could very likely be the way forward.
4. Conclusions and future perspectives

An improved methodology for the synthesis of the dihydro oxazolo ring-fused 2-pyridones has been developed. Using a molybdenum oxide catalyst and azeotropic removal of water followed by the addition of 0.2 eq. of PPTS and Meldrum’s acid derivative, oxazolo fused 2-pyridones have been synthesized with excellent enantiomeric excess. Biological evaluation showed that three novel highly substituted oxygen analogues were able to inhibit biofilm formation in UPEC, identifying them as potential pilicides. The most potent oxygen analogue was able to reduce biofilm formation at low μM-concentrations and had an estimated IC$_{50}$ value of 12-25 μM, clearly demonstrating that it is possible to exchanging sulfur for oxygen in the 2-pyridone scaffold and still obtain active pilicides.

A straightforward synthetic route to bromo-substituted unsaturated thiazolo 2-pyridones have been developed, avoiding time consuming purification and isolation of the oxidized intermediate. Suzuki-Miyaura cross couplings have successfully been applied to the thiazolo 2-pyridone structure. The coupling reaction could be performed under ligand-free conditions with excellent yields of C-2 aryl substituted unsaturated thiazolo ring-fused 2-pyridones. Hydrolysis and subsequent biological evaluation of the synthesized C-2 substituted analogues will result in important structure-activity information for the pilicide project and hopefully new potent pilicides.

Efforts to oxidize the oxazolo 2-pyridones resulted in the discovery of a new interesting compound, a 2, 6 pyridinedione. It would be of great interest to explore the utility and biological applications of these compounds. Further investigations remain to find mild and base-free conditions for the oxidation of the oxazolo ring-fused 2-pyridones.

5. Acknowledgements

First and foremost I would like to thank my supervisor Fredrik Almqvist for believing in me and giving me the opportunity to do this project. I have had a lot fun and learned even more! A special thank you to Erik Chorell, your help, encouragement and support has been invaluable. Thanks also to the other members of the group, Hans Andersson, Magnus Sellstedt and Ida Andersson, whom have all contributed to this work. Finally I would like to mention Henrik Antti, for helping with the experimental design, and Jerome Pinkner, Washington University in St. Louis, for performing the biological testing.
6. Experimental section

6.1 Screening of acids using D-optimal design

Macroporous polystyrenesulfonic acid, pyridinium \( p \)-toluenesulfonate, \( p \)-toluenesulfonic acid and TFA were chosen for the screening. For TFA, the previously obtained results were used in the model.\(^{37}\) A D-optimal design with 8 experimental runs (Table 5), varying three factors, acid (Acid), reaction time (Time) and equivalents of acid (Eq.) was created using MODDE 8.0 (Umetrics AB, Umeå, Sweden). The two responses yield (Yield) and enantiomeric excess (ee) were measured in percent. The factors were varied according to the design matrix \( X \) with 5 and 6 h and 0.1 and 5 equivalents as low and high settings for reaction time and equivalents of acid respectively.

Table 5. Design matrix \( X \).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Exp No</th>
<th>Acid</th>
<th>Time (h)</th>
<th>Eq</th>
</tr>
</thead>
<tbody>
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<td>1</td>
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<td>MP-TsOH</td>
<td>6</td>
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<td>( p )TsOH</td>
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<td>( p )TsOH</td>
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<td>7</td>
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<td>8</td>
<td>8</td>
<td>TFA</td>
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<td>5</td>
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</tbody>
</table>

Ring-fused 2-pyridone \( 5a \) was chosen as a model compound and was synthesized according to the general method in agreement with the design matrix. Replicates were synthesized of experiment number 3 and 5. The data was centered and scaled to unit variance prior to model calculation. A PLS model was fitted to the obtained data (Table 6) correlating the three factors to the two responses. Three model components were extracted using cross-validation and the calculated model explained 77.7\% of the variation in the responses \( Y \) (\( R^2 = 0.777 \)) and predicted 40.9\% of the variation in \( Y \) (\( Q^2 = 0.409 \)).
6.2 Synthesis

**General method.** All reactions were carried out under inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. CH$_2$Cl$_2$ 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) and staining with a solution of Ce(SO$_4$)$_2$ 4 H$_2$O (2g), PMA (5g) and concentrated sulphuric acid (12 ml) in H$_2$O (188 ml) or KMnO$_4$ (1.5 g), K$_2$CO$_3$ (10 g) and 10% NaOH (1.25 ml) in H$_2$O (200 ml). Flash column chromatography (eluents given in brackets) was performed on silica gel (Matrex, 60 Å, 35-70 µm, Grace Amicon). Centrifugal preparative thin layer chromatography (eluents in brackets) was performed on a Chromatotron® (Harrison Research, model 7924T) using Merk type 60 PF$_{254}$ silica gel (1 mm thick plates). Parallel flash chromatography was performed on a Gradmaster parallel, Jones Chromatography using silica gel (Matrex, 60 Å, 35-70 µm, Grace Amicon) and gradient [heptane:EtOAc 100:0→0:100] eluents. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker DRX-400 in CDCl$_3$ [residual CHCl$_3$ ($\delta_H$ 7.26 ppm) or CDCl$_3$ ($\delta_C$ 77.0 ppm) as internal standard] or DMSO-d$_6$ [residual DMSO ($\delta_H$ 2.50 ppm) or DMSO-d$_6$ ($\delta_C$ 40.0 ppm) as internal standard] at 298 K. IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer. Optical rotation was measured with a Perkin-Elmer 343 polarimeter at 298 K and 589 nm. Chiral HPLC was performed on a PRIKLE COVALENT, (S,S) WHELK-O 1 10/100 Krom FEC, 25cm*4.6mm column. UV-detection and a 4:4:1 CH$_2$Cl$_2$:heptane:EtOH eluent was used. Microwave reactions were carried out using a monomode reactor (Smith Creator, Biotage AB) in Teflon septa capped 0.5-2ml or 2-5 ml Smith TM process vials with stirring. Reaction times refer to irradiation time at target temperature, as measured by IR sensor. LC-MS data was recorded by detecting positive (ES+) molecular ions with an electrospray Water Micromass ZG instrument using an XTerra MS C$_{18}$ 5 μm 4.6 _ 50 mm column and a H$_2$O:acetonitrile:formic acid eluent system.

**General procedure for the preparation of 5a in the experimental design.** (NH$_4$)$_2$MoO$_4$ (4 mg, 0.021 mmol) was added to a stirred solution of serine derivative 3a (50 mg, 0.211 mmol) dissolved in 21 ml of toluene. The reaction mixture was heated to reflux with azeotropic removal of water using a Soxhlet apparatus containing activated 3Å molecular sieves. After 4 h at reflux the reaction mixture was allowed to cool at room temperature for 10 min when Meldrum’s acid derivative 4a (132 mg, 0.422 mmol), followed by 0.1 or 5 eq. of acid (Table 6) were added. After 1-2 h (Table 6) of reflux the reaction mixture was allowed to attain room temperature, filtered through celite and concentrated under reduced pressure. The residue was dissolved in CH$_2$Cl$_2$, washed with brine and the aqueous layer was extracted with EtOAc. The combined organic layers were concentrated under reduced pressure and purified by parallel flash chromatography [heptane:EtOAc 100:0→0:100] to give 5a.
(3S)-7-(Naphthalen-1-ylmethyl)-5-oxo-8-phenyl-2,3-dihydro-5H-oxazolo[3,2-a]pyridine-3-carboxylic Acid Methyl Ester (5a). 3a (50 mg, 0.211 mmol) gave 5a as light brown foam, yields for the different experimental runs are shown in Table 6. Data in agreement with published data.\(^{37}\)

Table 6. Yields and ee of compound 5a synthesized in the experimental design.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Exp No</th>
<th>Acid</th>
<th>Time</th>
<th>Eq</th>
<th>Yield (%)</th>
<th>ee (%)</th>
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</tr>
</tbody>
</table>

General procedure for the preparation of 8a-c. TEA (2 eq.) followed by 7a\(^7\) (0.95 eq.) or 7b\(^b\)-c\(^b\) (1 eq. dissolved in 1 ml dry CH\(_2\)Cl\(_2\)) were added dropwise at -8°C to serine methyl ester hydrochloride 6 suspended in CH\(_2\)Cl\(_2\) (2.7 ml/mmol) while stirring. The reaction mixture was stirred at -8°C for approximately 1h before being allowed to attain rt. After another 2 h the resulting mixture was diluted with CH\(_2\)Cl\(_2\), washed with sat. NaHCO\(_3\)(aq.) and brine. The aqueous layers were extracted once with CH\(_2\)Cl\(_2\) and twice with EtOAc and the combined organic layers were dried over anhydrous Na\(_2\)SO\(_4\), filtered and concentrated under reduced pressure. Purification by column chromatography [heptane:EtOAc 1:4→0:1 for 8a-b and 1:9→0:1 for 8c] gave 8a-c.\(^\text{a}\) Commercially available.\(^\text{b}\) Synthesized according to previously published procedures.\(^{44-46}\)

N-(2-Thiophen-2-ylacetetyl)-L-serine Methyl Ester (8a). 6 (1.00 g, 6.43 mmol) gave 8a as a light brown solid (1.26 g, 81% yield): [\(\alpha\)]\(_D\) = 35 (c = 1.0, CHCl\(_3\)); IR (neat) 3332 (br), 2952, 1739, 1648, 1531, 1436 and 1241 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 2.37 (br s, 1H), 3.76 (s, 3H), 3.84 (s, 2H), 3.89 (dd, \(J_1 = 3.39\) Hz, \(J_2 = 11.24\) Hz, 1H), 3.95 (dd, \(J_1 = 3.87\) Hz, \(J_2 = 11.24\) Hz, 1H), 4.65 (m, 1H), 6.60 (d, \(J = 5.67\) Hz, 1H), 7.00 (m, 2H), 7.26 (m, 1H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 37.3, 52.8, 54.9, 63.3, 77.0, 125.7, 127.4, 127.4, 135.4, 170.3, 170.6.
N-(2-(3,4-Methylenedioxy)phenylacetyl)-L-serine Methyl Ester (8b). 6 (933 mg, 6.0 mmol) gave 8b as a white solid (1.57 g, 93% yield): [α]D = 33 (c = 1.0, CHCl3); IR (neat) 3348 (br), 2956, 1741, 1650, 1490, 1245 and 1037 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl3) δ 3.54 (s, 2H), 3.76 (s, 3H), 3.89 (dd, \(J_1 = 3.43\) Hz, \(J_2 = 11.20\) Hz, 1H), 3.94 (dd, \(J_1 = 4.02\) Hz, \(J_2 = 11.20\) Hz, 1H), 4.64 (m, 1H), 5.96 (s, 2H), 6.42 (d, \(J = 5.68\) Hz, 1H), 6.73 (m, 1H), 6.78 (m, 2H), \(^13\)C NMR (100 MHz, CDCl3) δ 42.2, 52.3, 54.5, 62.1, 100.8, 108.1, 109.3, 122.1, 127.8, 146.4, 147.5, 170.7, 171.9.

N-(2-(1-Methyl-1H-indol-3-ylacetyl))-L-serine Methyl Ester (8c). 6 (778 mg, 5.0 mmol) gave 8c as a light brown oil (1.08 g, 74% yield): [α]D = -22 (c = 1.2, CHCl3); IR (neat) 3344 (br), 2956, 1743 and 1650 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl3) δ 3.09 (br s, 1H), 3.66 (s, 3H), 3.73 (s, 5H), 3.77 (dd, \(J_1 = 3.57\) Hz, \(J_2 = 11.25\) Hz, 1H), 3.84 (dd, \(J_1 = 4.07\) Hz, \(J_2 = 11.25\) Hz, 1H), 4.60 (m, 1H), 6.75 (d, \(J = 7.47\) Hz, 1H), 7.00 (s, 1H), 7.12 (m, 1H), 7.22-7.31 (m, 2H), 7.56 (m, 1H); \(^13\)C NMR (100 MHz, CDCl3) δ 32.1, 32.5, 52.0, 54.4, 62.0, 106.5, 109.0, 118.4, 118.9, 121.5, 127.1, 127.9, 136.7, 170.5, 172.2.

5-(1-Hydroxy-2-(naphthalen-1-ylxyloxy)ethylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (4b). Starting from 1-naphthoxyacetic acid (5g, 24.7 mmol) Meldrum’s acid derivative 4b was synthesized in quantitative yields according to published procedures and was used without further purification: IR (neat) 3316, 2927, 2850, 1731, 1664, 1571, 1394, 1353, 1270, 1201, 1155, 1018 and 769 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl3) δ 1.78 (s, 6H), 5.66 (s, 2H), 6.82 (d, \(J = 7.52\) Hz, 1H), 7.37 (m, 1H), 7.52 (m, 3H), 7.82 (m, 1H), 8.37 (m, 1H).

(3S)-7-(Naphthalen-1-ylmethyl)-5-oxo-8-(thiophen-2-yl)-2,3-dihydro-5H-oxazolo[3,2-a]pyridine-3-carboxylic Acid Methyl Ester (10a). (NH₄)₂MoO₄ (22 mg, 0.11 mmol) was added to a stirred solution of serine derivative 8a (268 mg, 1.1 mmol) dissolved in 110 ml of toluene. The reaction mixture was heated to reflux with azeotropically removal of water using a Soxhlet apparatus containing activated 3Å molecular sieves. After 4 h of reflux the reaction mixture was allowed to cool at rt for 10 min when Meldrum’s acid derivative 4a (687 mg, 2.2 mmol), followed by PPTS (55 mg, 0.22 mmol) were added. The mixture was refluxed for 45 min when a second addition of Meldrum’s acid 4a (172 mg, 0.55 mmol) was made. After an additional 30 min of reflux the reaction mixture was allowed to attain rt and was washed with 1:1 sat. NaHCO₃(aq.):brine and brine. The aqueous layers were extracted four times with EtOAc. The combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Purification by column chromatography [heptane:EtOAc 3:2→1:4 ] and radial chromatography [CH₃Cl₂:MeOH 100:1] gave 10a as a light brown foam (328 mg, 71% yield): [α]D = -87 (c = 1.0, CHCl₃) (corresponding to 95% ee); IR (neat) 1754, 1671, 1608 and 1511 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl₃) δ 3.84 (s, 3H), 4.11 (d, \(J = 17.19\) Hz, 1H), 4.21 (d, \(J = 17.19\) Hz, 1H), 4.70 (dd, \(J_1 = 4.72\) Hz, \(J_2 = 9.38\) Hz, 1H), 4.85 (t, \(J = 9.38\) Hz, 1H), 5.22 (dd, \(J_1 = 4.72\) Hz, \(J_2 = 9.38\) Hz, 1H), 5.68 (s, 1H), 7.03 (dd, \(J_1 = 1.14\) Hz, \(J_2 = 3.49\) Hz, 1H), 7.07 (m, 1H), 7.25 (m, 1H), 7.38 (m, 2H), 7.44 (m, 2H), 7.69 (m, 1H), 7.76 (m, 1H), 7.84 (m, 1H); \(^13\)C NMR (100 MHz, CDCl₃) δ 36.9, 53.3, 57.1, 71.5, 92.2, 111.2, 123.8,
(3S)-7-((Naphtalen-1-ylxy)methyl)-5-oxo-8-(benzo[d][1,3]dioxol-5-yl)-2,3-dihydro-5H-oxazolo[3,2-a]pyridine-3-carboxylic Acid Methyl Ester (10b). (NH₄)₂MoO₄ (13 mg, 0.06 mmol) was added to a stirred solution of serine derivative 8b (180 mg, 0.64 mmol) dissolved in 64 ml of toluene. The reaction mixture was heated to reflux with azeotropic removal of water using a Soxhlet apparatus containing activated 3Å molecular sieves. After 4.5 h of reflux the reaction mixture was allowed to cool at room temperature for 20 min and Meldrum’s acid derivative 4b (420 g, 1.28 mmol), followed by PPTS (32 mg, 0.13 mmol) were added. Reflux was continued and two additions of Meldrum’s acid derivative 4b (210 mg, 0.64 mmol) were made after 1h and 2 h of reflux. The reaction mixture was allowed to reflux for 40 min after the last addition of Meldrum’s acid derivative and was then cooled at rt. The resulting solution was washed with 1:1 sat. NaHCO₃, filtered and concentrated under reduced pressure. Purification by column chromatography [heptane:EtOAc 1:4→0:1+] and radial chromatography [CH₂Cl₂:MeOH 100:1] gave 10b as a light brown foam (201 mg, 67% yield): [α]D = -68 (c = 1.0, CHCl₃) (corresponding to 97% ee); IR (neat) 1751, 1671, 1596, 1486, 1220 and 1033 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.87 (s, 3H), 4.69 (dd, J₁ = 4.65 Hz, J₂ = 9.30 Hz, 1H), 4.83 (t, J = 9.28 Hz, 1H), 4.92 (s, 2H), 5.30 (dd, J₁ = 4.65 Hz, J₂ = 9.30 Hz, 1H), 6.00 (s, 2H) 6.62 (d, J = 7.58 Hz, 1H), 6.68 (s, 1H), 6.79 (m, 1H), 6.86 (m, 1H), 7.28 (m, 1H), 7.43 (m, 1H), 7.50 (m, 2H), 7.79 (m, 1H), 8.31 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 53.1, 56.9, 66.6, 71.3, 96.9, 101.1, 104.8, 108.2, 108.3, 110.4, 120.7, 121.8, 123.6, 124.4, 125.2, 125.2, 125.3, 126.3, 127.2, 134.2, 147.2, 147.7, 152.6, 153.3, 153.7, 158.9, 167.9; LC-MS calcd for [M+H]⁺ C₂₉H₂₂NO₇ 472.14, obsd 472.04.

(3S)-7-((Naphtalen-1-ylxy)methyl)-5-oxo-8-(1-methyl-1H-indol-3-yl)-2,3-dihydro-5H-oxazolo[3,2-a]pyridine-3-carboxylic Acid Methyl Ester (10c). (NH₄)₂MoO₄ (29 mg, 0.15 mmol) was added to a stirred solution of serine derivative 8c (430 mg, 1.48 mmol) dissolved in 150 ml of toluene. The reaction mixture was heated to reflux with azeotropic removal of water using a Soxhlet apparatus containing activated 3Å molecular sieves. After 5 h of reflux the reaction mixture was allowed to cool at rt for 10 min when Meldrum’s acid derivative 4b (1.46 g, 4.44 mmol), followed by PPTS (75 mg, 0.30 mmol) were added. The reaction mixture was refluxed for 1 h and then allowed to attain rt, concentrated under reduced pressure to approximately 60 ml, diluted with EtOAc, washed with 1:1 sat. NaHCO₃, filtered and concentrated under reduced pressure. The aqueous layers were extracted three times with EtOAc and the combined organic layers were dissolved over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Purification by column chromatography [EtOAc:EtOH 100:1→20:1] gave 10c as a red-brown foam (433 mg, 61% yield): [α]D = -75 (c = 1.4, CHCl₃) (corresponding to 96% ee); IR (neat) 3071, 2953, 1717, 1642, 1586, 1490, 1249 and 1220 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.78 (s, 3H), 3.89 (s, 3H), 4.66 (dd, J₁ = 4.55 Hz, J₂ = 9.32 Hz, 1H), 4.79 (t, J = 9.32 Hz, 1H), 4.95 (d, J
= 14.60 Hz, 1H), 5.00 (d, J = 14.60 Hz, 1H), 5.33 (dd, J₁ = 4.55 Hz, J₂ = 9.32 Hz, 1H), 6.53 (d, J = 7.58 Hz, 1H), 6.74 (s, 1H), 7.08 (s, 1H), 7.14-7.31 (m, 3H), 7.39 (m, 2H), 7.50 (m, 2H), 7.78 (m, 1H), 8.35 (m, 1H); 13C NMR (100 MHz, CDCl₃) δ 32.9, 53.4, 57.3, 67.2, 71.4, 89.7, 104.1, 105.1, 108.6, 109.7, 113.7, 119.4, 120.8, 122.1, 122.2, 125.4, 125.5, 125.6, 127.4, 127.6, 128.8, 134.4, 136.8, 153.6, 154.4, 154.6, 159.4, 168.2; LC-MS calc'd for [M+H]+ C₂₉H₂₅N₂O₅ 481.18, obsd 481.11.

**General procedure for preparation of 11a-c.** LiBr (10 eq.) was added to 10a-c (30 mg) suspended in 1 ml of MeCN (2 v/v % water) cooled on ice while stirring. TEA (3 eq.) was added dropwise and the reaction mixture was stirred on ice for 30 min before stirring was continued at rt for 4-5 h. The reaction mixture was then diluted with EtOAc, washed twice with 2% KHSO₄(aq.) and the aqueous layer was extracted with EtOAc. The combined organic layers were concentrated under reduced pressure and purified by column chromatography [CH₂Cl₂:MeOH 98:2→ CH₂Cl₂:MeOH 97:3 and 1% AcOH for 11a, CH₂Cl₂:MeOH 97:3→ CH₂Cl₂:MeOH 97:3 and 1% AcOH for 11b and CH₂Cl₂:MeOH 97:3→ CH₂Cl₂:MeOH 95:5 and 1% AcOH for 11c] to give 11a-c, which were lyophilized from MeCN and water.

(3S)-7-(Naphthalen-1-ylmethyl)-5-oxo-8-(thiophen-2-yl)-2,3-dihydro-5H-oxazolo[3,2-a]pyridine-3-carboxylic Acid (11a). 10a (30 mg, 0.072 mmol) gave 11a as a light grey solid (17 mg, 59%); [α]D = -67 (c = 0.75, CHCl₃); IR (neat) 2949, 2863, 1617, 1474, 1243, 1040, 757 and 716 cm⁻¹; 1H NMR (400 MHz, CDCl₃) δ 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); 13C NMR (100 MHz, CDCl₃) δ 37.1, 59.2, 70.8, 96.0, 109.9, 123.6, 125.5, 125.8, 126.3, 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; LC-MS calc'd for [M+H]+ C₂₉H₁₈N₂O₅ 404.10, obsd 404.07.

(3S)-7-((Naphthalen-1-yl)oxy)methyl)-5-oxo-8-(benzo[d][1,3]dioxol-5-yl)-2,3-dihydro-5H-oxazolo[3,2-a]pyridine-3-carboxylic Acid (11b). 10b (30 mg, 0.06 mmol) gave 11b as a light yellow solid (17 mg, 62% yield); [α]D = -37 (c = 0.2, CHCl₃:MeOH 1:1); IR (neat) 2903, 1634, 1511, 1495, 1256 and 1025 cm⁻¹; 1H NMR (400 MHz, DMSO-d₆) δ 4.74 (dd, J₁ = 4.01 Hz, J₂ = 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, J₁ = 4.01 Hz, J₂ = 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); 13C NMR (100 MHz, DMSO-d₆) δ 58.1, 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; LC-MS calc'd for [M+H]+ C₂₆H₂₀NO₇ 458.12, obsd 458.03.
(3S)-7-((Naphthalen-1-yloxy)methyl)-5-oxo-8-(1-methyl-1H-indol-3-yl) 2,3-dihydro-5H-oxazolo[3,2-a]pyridine-3-carboxylic Acid (11c). 10c (30 mg, 0.06 mmol) gave 11c as a light pink solid (18 mg, 64% yield): [α]D = -23 (c = 0.2, CHCl3:CH2Cl2:MeOH 1:1:1); IR (neat) 2921, 2852, 1668 and 1517 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 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); ¹³C NMR (100 MHz, DMSO-d₆) δ 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; LC-MS: calcd for [M+H]⁺ C₂₈H₂₃N₂O₅ 467.16, obsd 467.17.

2-Bromo-8-cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-5H-thiazolo[3,2-a]pyridine-3-carboxylic Acid Methyl Ester (12). NaH (74 mg, 3.07 mmol, washed with n-pentane) was added to 1 (400 mg, 1.02 mmol) dissolved in dry MeCN (8 ml) cooled on ice while stirring. After 10 min BrCCl₃ (302 μl, 3.07 mmol) was added dropwise and stirring was continued for 35 min before the reaction mixture was allowed to attain rt. After 15 min at rt 30 μl dry MeOH (0.7 eq.) was added dropwise. After the first addition, an additional 145 μl dry MeOH (3.5 eq.) was added dropwise in portions over 2 h. Upon the last addition of dry MeOH the reaction mixture turned dark brown and was quenched by dropwise addition of 2% KHSO₄(aq.). The mixture was acidified with 2M HCl and extracted three times with EtOAc. The combined organic layers were washed with brine, the aqueous layer was extracted with EtOAc, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Purification by column chromatography [heptane:EtOAc 3:1] gave 12 as a yellow foam (345 mg, 72% yield). Data in agreement with published data.¹³

General procedure for preparation of 13a-e. Dry MeOH (21 ml/mmol) was added to pyridine 12, Pd(OAc)$_₂$ (10 mol%), KF (1.9 eq.) and the boronic acid to be coupled (2 eq.). The reaction mixture was heated in a sealed tube by MWI at 100 °C for 10 min. The resulting mixture was diluted with CH₂Cl₂, washed with sat. NaHCO₃(aq.) and the aqueous layer was extracted with CH₂Cl₂. The combined organic layers were concentrated under reduced pressure and purified by parallel flash chromatography [heptane:EtOAc 100:0→0:100 ] to give 13a-e.

2-m-Toly-8-cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-5H-thiazolo[3,2-a]pyridine-3-carboxylic Acid Methyl Ester (13a). 12 (20mg, 0.043mmol) gave 13a as a yellow foam (20 mg, 97%): IR (neat) 3056, 2934, 1771, 1688, 1588, 1468, 1249, 1200, 786, 763 and 711 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.80 (m, 2H), 1.05 (m, 2H), 1.81 (m, 1H), 2.41 (s, 3H), 3.88 (s, 3H), 4.55 (s, 2H), 5.94 (s, 1H), 7.24 (m, 1H), 7.33 (m, 1H), 7.41 (m, 3H), 7.48 (m, 2H), 7.78 (m, 1H), 7.86 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 7.9 (2C), 10.9, 36.2, 53.3, 111.7, 111.9, 123.7, 125.4, 125.5(2C), 125.7, 126.2, 127.3, 127.6, 128.6, 128.8, 128.9, 129.0, 129.1, 130.8, 131.9, 133.9, 134.2, 139.0, 146.3, 153.4, 158.9, 161.8; LC-MS calcd for [M+H]⁺ C₃₀H₂₆NO₅S 480.16, obsd 480.21.
2-p-Toly-8-cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-5H-thiazolo[3,2-a]pyridine-3-carboxylic Acid Methyl Ester (13b). 12 (20 mg, 0.043 mmol) gave 13b as a yellow foam (20 mg, 96% yield): IR (neat) 2992, 2934, 1771, 1690, 1613, 1440, 1247, 1177, 1023, 784 and 711 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.80 (m, 2H), 1.05 (m, 2H), 1.81 (m, 1H), 2.40 (s, 3H), 3.88 (s, 3H), 4.55 (s, 2H), 5.94 (s, 1H), 7.24 (m, 2H), 7.41 (m, 1H), 7.47 (m, 4H), 7.78 (m, 1H), 7.86 (m, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 7.9 (2C), 10.9, 21.3, 36.2, 53.3, 111.7, 111.9, 123.6, 125.1, 125.5, 125.7, 125.7, 126.2, 127.3, 127.6, 128.3 (2C), 128.8, 129.0, 129.9 (2C), 131.9, 133.9, 134.2, 140.2, 140.2, 153.3, 153.3, 158.9, 161.9; LC-MS calcd for [M+H]\(^+\) \(C_{30}H_{26}NO_3S\) 480.16, obsd 480.21.

2-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-8-cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-5H-thiazolo[3,2-a]pyridine-3-carboxylic Acid Methyl Ester (13c). 12 (40 mg, 0.085 mmol) gave 13c as a yellow foam (42 mg, 94% yield): IR (neat) 2979, 1735, 1648, 1471, 1288, 1247, 1068 and 752 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.78 (m, 2H), 1.04 (m, 2H), 1.79 (m, 1H), 3.90 (s, 3H), 4.28 (s, 4H), 4.54 (s, 2H), 5.93 (s, 1H), 6.92 (d, \(J = 7.95\) Hz, 1H), 7.09 (m, 2H), 7.23 (m, 1H), 7.40 (m, 1H), 7.47 (m, 2H), 7.77 (m, 1H), 7.85 (m, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 7.8 (2C), 10.9, 36.1, 53.3, 64.2, 64.4, 111.6, 111.8, 117.4, 118.0, 121.6, 121.7, 123.6, 124.8, 125.5, 125.7, 126.2, 127.2, 128.5, 131.9, 133.9, 134.2, 143.9, 145.2, 146.1, 153.2, 158.8, 161.8; LC-MS calcd for [M+H]\(^+\) \(C_{31}H_{26}NO_5S\) 524.15, obsd 524.21.

2-(1H-Indol-5-yl)-8-cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-5H-thiazolo[3,2-a]pyridine-3-carboxylic Acid Methyl Ester (13d). 12 (20 mg, 0.043 mmol) gave 13d as a yellow solid (16 mg, 72% yield): IR(neat) 1688, 1602, 1517, 1467, 784 and 747 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO, \(d_6\)) \(\delta\) 0.84 (m, 2H), 1.04 (m, 2H), 1929 (m, 1H), 3.72 (s, 3H), 4.60 (s, 2H), 5.56(s, 1H), 6.56 (s, 1H), 7.29 (m, 1H), 7.38 (m, 1H), 7.48 (m, 1H), 7.53 (m, 4H), 7.81 (s, 1H), 7.89 (m, 1H), 7.92 (m, 1H), 7.98 (m, 1H), 11.44 (s, 1H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 8.0 (2C), 11.1, 35.9, 53.3, 102.4, 110.2, 112.0, 112.9, 118.9, 120.9, 121.3, 123.7, 124.5, 126.2, 126.3, 126.9, 127.8 (2C), 128.0, 128.5, 129.1, 130.9, 132.0, 134.0, 135.1, 137.0, 146.3, 154.0, 158.0, 161.8; LC-MS calcd for [M+H]\(^+\) \(C_{31}H_{25}N_2O_3S\) 505.16, obsd 505.17.

2-(2,3-Furan-2-yl)-8-cyclopropyl-7-naphthalen-1-ylmethyl-5-oxo-5H-thiazolo[3,2-a]pyridine-3-carboxylic Acid Methyl Ester (13e). 12 (40 mg, 0.085 mmol) gave 13e as a red oil (13 mg, 34% yield): IR (neat) 3117, 2932, 1702, 1603, 1588, 1440, 1251, 1220, 765 and 713 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.80 (m, 2H), 1.05 (m, 2H), 1.81 (m, 1H), 2.40 (s, 3H), 3.88 (s, 3H), 4.55 (s, 2H), 5.94 (s, 1H), 7.24 (m, 2H), 7.41 (m, 1H), 7.47 (m, 4H), 7.78 (m, 1H), 7.86 (m, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 7.9 (2C), 10.9, 21.3, 36.2, 53.3, 111.7, 111.9, 123.6, 125.1, 125.5, 125.7, 125.7, 126.2, 127.3, 127.6, 128.3 (2C), 128.8, 129.0, 129.9 (2C), 131.9, 133.9, 134.2, 140.2, 146.3, 153.3, 158.9, 161.9; LC-MS calcd for [M+H]\(^+\) \(C_{27}H_{22}NO_4S\) 456.13, obsd 456.15.
Methyl 2-(4-(naphthalen-1-ylmethyl)-2,6-dioxo-3-(thiophen-2-yl)-5,6-dihydropyridin-1(2H)-yl)acrylate (15). NaH (6 mg, 0.24 mmol, washed with n-pentane) was added to 10a (50 mg, 0.12 mmol) suspended in 1ml of dry MeCN and 0.4 ml of dry THF at 0°C while stirring. After 10 min BrCCl$_3$ (12 μl, 0.12 mmol) was added dropwise and the reaction mixture turned dark brown. The reaction mixture was stirred at 0°C for an additional 45 min before being allowed to attain rt. After 15 min at rt, 2 μl dry MeOH was added and stirring was continued for 3 h when the reaction mixture was quenched by dropwise addition of 2% KHSO$_4$(aq.). The mixture was acidified with 2M HCl and extracted three times with EtOAc. The combined organic layers were concentrated under reduced pressure and purified by column chromatography [heptane:EtOAc 1:4→0:1] to give 15 as dark purple oil (34 mg, 68% yield): IR (neat) 3050, 2948, 2910, 1720, 1671, 1392, 1265, 1203, 1153, 781, 732 and 701 cm$^{-1}$; $^{1}$H NMR (400 MHz, CDCl$_3$) δ 3.38 (s, 2H), 3.77 (s, 3H), 4.22 (s, 2H), 5.85 (s, 1H), 6.68 (s, 1H), 7.13 (m, 1H), 7.18 (m, 1H), 7.33 (m, 1H), 7.45 (m, 1H), 7.51 (m, 3H), 7.70 (m, 1H), 7.82 (m, 1H), 7.88 (m, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 38.0, 38.2, 52.7, 123.1, 124.2, 125.5, 126.7, 126.7, 127.4, 127.7, 128.3, 128.7, 129.0, 129.5, 131.7, 131.8, 132.3, 133.1, 133.9, 151.6, 162.6, 163.9, 168.4; LC-MS calcd for [M+H]$^+$ C$_{24}$H$_{20}$NO$_4$S 418.11, obsd 418.01.
6.3 ee measurements

Figure 14. Chromatogram from ee measurements of 10a by chiral HPLC using a (S,S) – WHELK-O1 column and a 4:4:1 CH₂Cl₂:heptane:EtOH eluent system. ee for compound 10a was 95%.

Figure 15. Chromatogram from ee measurements of epimerized 10a by chiral HPLC using a (S,S) – WHELK-O1 column and a 4:4:1 CH₂Cl₂:heptane:EtOH eluent system.
Figure 16. Chromatogram from ee measurements of 10b by chiral HPLC using a (S,S) – WHELK-O1 column and a 4:4:1 CH₂Cl₂:heptane:EtOH eluent system. ee for compound 10b was 97%.

Figure 17. Chromatogram from ee measurements of epimerized 10b by chiral HPLC using a (S,S) – WHELK-O1 column and a 4:4:1 CH₂Cl₂:heptane:EtOH eluent system.
Figure 18. Chromatogram from ee measurements of 10c by chiral HPLC using a (S,S) – WHELK-O1 column and a 4:4:1 CH$_2$Cl$_2$:heptane:EtOH eluent system. ee for compound 10c was 96%.

Figure 19. Chromatogram from ee measurements of epimerized 10c by chiral HPLC using a (S,S) – WHELK-O1 column and a 4:4:1 CH$_2$Cl$_2$:heptane:EtOH eluent system.
References


