

**Small Molecule Inhibitors of the Artemis Endonuclease and  
Thiadiazines as HSP70 Agonists**

by

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## **Small Molecule Inhibitors of the Artemis Endonuclease and Thiadiazines as HSP70 Agonists**

Leila Terrab, PhD

University of Pittsburgh, 2021

The first chapter of this dissertation covers the analog synthesis of three screening hit compounds previously identified as Artemis inhibitors. Artemis is a metalloenzyme nuclease involved in the nonhomologous end-joining pathway, a DNA damage repair pathway. The screening hits included the hydroxamic-acid containing Droxinostat, a 2-amino-3-carboxythiophene, and a 2-aminothiazole. Previous work by our group identified chromane analogs of Droxinostat to show Artemis inhibition, and this work mostly focuses on exploring new substitution patterns of the chromane scaffold for our structure-activity relationship (SAR) studies. While a few of the chromane analogs inhibited Artemis, they have also been shown to target histone deacetylases (HDACs), another family of metalloenzymes. None of the 2-amino-3-carboxythiophene nor 2-aminothiazole analogs have shown Artemis inhibition. The Artemis crystal structure has only become available in February 2020, and our group used an Artemis homology model and SAR studies to guide our compound design.

The second chapter covers the selective functionalization of the 1,2,6-thiadiazine 1,1-dioxide scaffold for the synthesis of medicinally relevant compounds. We employed chemoselective transformations of the thiadiazine's sulfamide nitrogens and vinylogous carbamate. We were interested in using the sulfamide group as a bioisosteric replacement to the urea substructure of MAL1-271, an HSP70 agonist. HSP70 proteins are ATP-dependent

molecular chaperones that regulate protein folding, and HSP70 modulation has shown a therapeutic potential in neurodegenerative diseases, such as Huntington's Disease. Our collaborators evaluated our compounds in a Huntington Disease model, where their ability to reduce polyglutamine (polyQ) aggregates was assayed. A few analogs have shown higher efficacy than MAL1-271. We also decided to utilize our method for the synthesis of thiadiazine-containing HDAC6 inhibitors; however, our assays did not show a promising HDAC inhibition profile.

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## List of Abbreviations

$\mu\text{M}$	Micromolar
$\mu\text{W}$	Microwave
ALL	Acute lymphoblastic leukemia
AMC	Aminomethyl coumarin
aq	Aqueous
Bn	Benzyl
calcd	Calculated
CD1	Catalytic domain 1
CD2	Catalytic domain 2
COSY	Homonuclear correlation spectroscopy
DAPI	4',6-Diamidino-2-phenylindole
dba	Dibenzylideneacetone
DBAD	Di- <i>tert</i> -butyl azodiformate
DCC	<i>N,N'</i> -Dicyclohexylcarbodiimide
DEMS	Diethoxymethylsilane
DIPEA	<i>N,N</i> -Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMAc	Dimethylacetamide
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethylsulfoxide
DNA-PK <sub>cs</sub>	DNA-dependent protein kinase catalytic subunit complex

DSB.....Double-strand break  
 DPPA.....Diphenylphosphoryl azide  
 EDCI.....1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide  
 ELSD.....Evaporating light scattering detector  
 Equiv.....Equivalents  
 ESI.....Electrospray ionization  
 Et.....Ethyl  
 EtOAc.....Ethyl acetate  
 FDA.....United States Food and Drug Administration  
 FT-IR.....Fourier-transform infrared spectroscopy  
 GC-MS.....Gas chromatography-mass spectrometry  
 GSH.....Glutathione  
 HATU.....2-(7-Aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate  
 h.....Hour(s)  
 HD.....Huntington's Disease  
 HEK293.....Human embryonic kidney 293 cells  
 HDAC.....Histone deacetylases  
 HFIP.....Hexafluoroisopropanol  
 HIF.....Hypoxia inducible factor  
 HMBC.....Heteronuclear multiple bond correlation  
 HOMO.....Highest occupied molecular orbital  
 HPLC.....High performance liquid chromatography

HRMS.....	High-resolution mass spectrometry
HSP70.....	70 Kilodalton heat shock proteins
HSQC.....	Heteronuclear single quantum coherence spectroscopy
HTS.....	High-throughput screen
HTT.....	Huntingtin
IC <sub>50</sub> .....	Concentration of inhibitor required for achieving 50% inhibition
Ig.....	Immunoglobulin
IR.....	Infrared spectroscopy
JohnPhos.....	(2-Biphenyl)di-tert-butylphosphine
LC/MS.....	Liquid chromatography/mass spectrometry
LUMO.....	Lowest unoccupied molecular orbital
M.....	Molar
Me.....	Methyl
MBL.....	Metallo-β-lactamase
min.....	Minute
MNase.....	Micrococcal nuclease
Mp.....	Melting point
NHEJ.....	Non-homologous end joining
nM.....	Nanomolar
NMR.....	Nuclear magnetic resonance
Ph.....	Phenyl
<i>p</i> -TSA.....	<i>p</i> -Toluenesulfonic acid
PDB.....	Protein Data Bank



PPTS.....Pyridinium *p*-toluenesulfonate  
 PyBOP.....Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate  
 rac.....Racemic  
 RBF.....Round-bottom flask  
 RM..... Reactive metabolites  
 RAG.....Recombination-activating gene  
 RSS.....Recombination-signal sequences  
 rt .....Room temperature  
 (*S*)-DM-Segphos.....(*S*)-(-)-5,5'-bis(diphenylphosphino)-4,4'-bi-1,3-benzodioxole  
 SAR.....Structure-activity relationship  
 sat .....Saturated  
 SCID.....Severe combined immunodeficiency  
 SFC.....Supercritical fluid chromatography  
 sm.....Starting material  
 SNM1A.....Sensitive to nitrogen mustard 1A  
 SNM1B.....Sensitive to nitrogen mustard 1B  
 T<sub>3</sub>P.....Propylphosphonic anhydride  
 TBAF.....Tetrabutylammonium fluoride  
 TBDPS.....*Tert*-Butyl diphenyl silyl  
 TBTU.....2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethylammonium tetrafluoroborate  
 TCR.....T-Cell receptor  
 TEA.....Triethylamine  
 TFA.....Trifluoroacetic acid

THF.....Tetrahydrofuran  
THP.....Tetrahydro-2*H*-pyran  
TLC.....Thin layer chromatography  
TMS .....Trimethylsilyl  
Ts.....Toluenesulfonyl  
UV.....Ultraviolet

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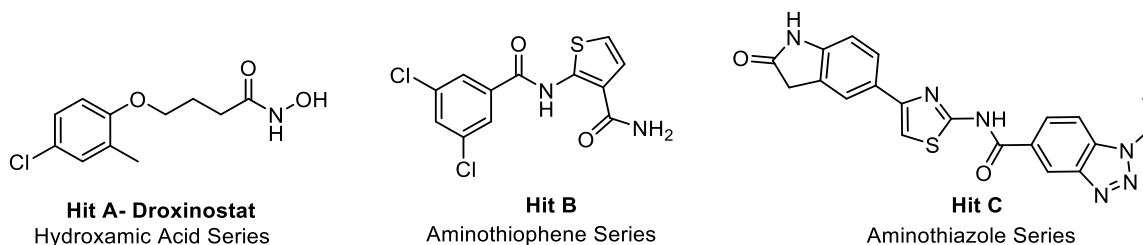
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## 1.0 Small Molecule Inhibitors of the Artemis Endonuclease

### 1.1 Introduction

Artemis (SNM1C) is a 692 amino acid<sup>1</sup> vertebrate nuclease that belongs to the  $\beta$ -CASP family of enzymes, a subgroup of the metallo- $\beta$ -lactamase (MBL) superfamily of nucleases.<sup>2</sup> Artemis is the nuclease that repairs DNA double-strand breaks (DSBs).<sup>2a,3</sup> DSBs are a form of DNA damage and may be physiological or pathological, such as those formed during programmed V(D)J recombination or from ionizing radiation, respectively.<sup>4</sup> Since acute lymphoblastic leukemia (ALL) cells undergo V(D)J recombination, Artemis inhibition may potentially be used against ALL.<sup>5</sup> Additionally, Artemis inhibition may be used as part of a combination treatment to stop the cancer cell's repair of the DSBs caused by radiation therapy.<sup>6</sup> A high-throughput screen (HTS) of 433,360 compounds was conducted at Sanford Burnham Prebys Medical Discovery Institute and our group selected three hit compounds (**Figure 1**) based on their: in-vitro activity (see **Section 1.2.2** for assay) (**Table 1**), specificity (Out of class nuclease – Micrococcal Nuclease Assay), and selectivity (In-class nucleases: SNM1A and SNM1B). This chapter focuses on the synthesis of analogs of these three hit compounds in an attempt to develop Artemis inhibitors.



**Figure 1.** Hits Selected from a High-Throughput Screen.

**Table 1.** Assay Data for Selected Hits.<sup>a</sup>

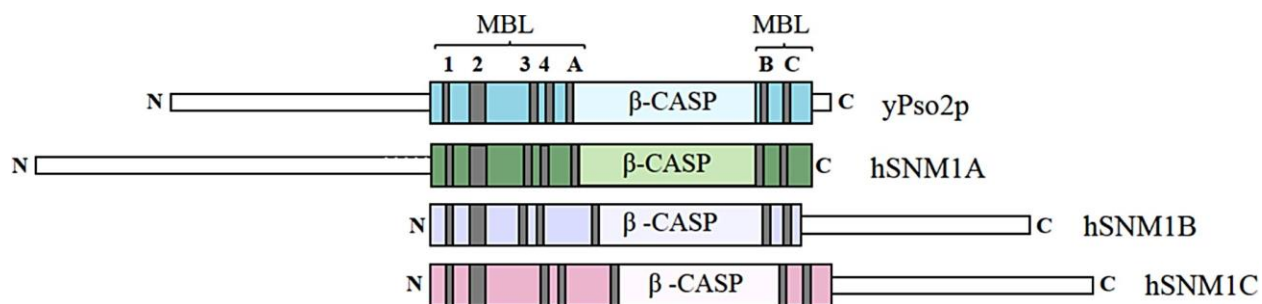
Compound ID	Hit A	Hit B	Hit C
Artemis IC <sub>50</sub>	0.56 μM	7.0 μM	8.8 μM
Specificity (MNase)	> 40 μM	> 40 μM	> 40 μM
Selectivity (SNM1A)	> 80 μM	> 80 μM	2.5 μM
Selectivity (SNM1B)	> 80 μM	> 80 μM	> 80 μM

<sup>a</sup>Obtained from Sanford Burnham Prebys Medical Discovery Institute.

### 1.1.1 Artemis Structure and Biological Function

SNM1A, Apollo (SNM1B), and Artemis (SNM1C) are members of the mammalian SNM1/PSO2 gene family.<sup>2c</sup> SNM1A, B, and C are involved in DNA damage repair through their nuclease activity: SNM1A in interstrand cross-link repair, Apollo in maintaining overhangs in telomeres, and Artemis in V(D)J Recombination.<sup>7</sup> The characteristic regions of the SNM1/PSO2 family consist of the highly conserved motifs (1-4) of the canonical MBL domain and the conserved motifs (A-C) of the β-CASP domain (**Figure 2**).<sup>7-8</sup> Motif 1 is an acidic residue; motif 2, the sequence HxHxDH; motif 3, a histidine residue; motif 4, an acidic or Cys residue; motif A, an acidic residue (D or E) after a stretch of hydrophobic residues found in a β-strand structure; motif B, a histidine ending an amphiphilic β-strand structure and preceding an α-helical structure; motif C, a valine at the end of a β-strand. In Artemis, the motifs include: motif 1, Asp17; motif 2, His33, His35, Asp37, and His38; motif 3, His 115; motif 4, Asp 136; motif A, Asp165; motif B,

His319; motif C, Val341.<sup>6,8-9</sup> The second MBL domain motif, HxHxDH is believed to bind to the metal ions for enzymatic activity.<sup>10</sup>

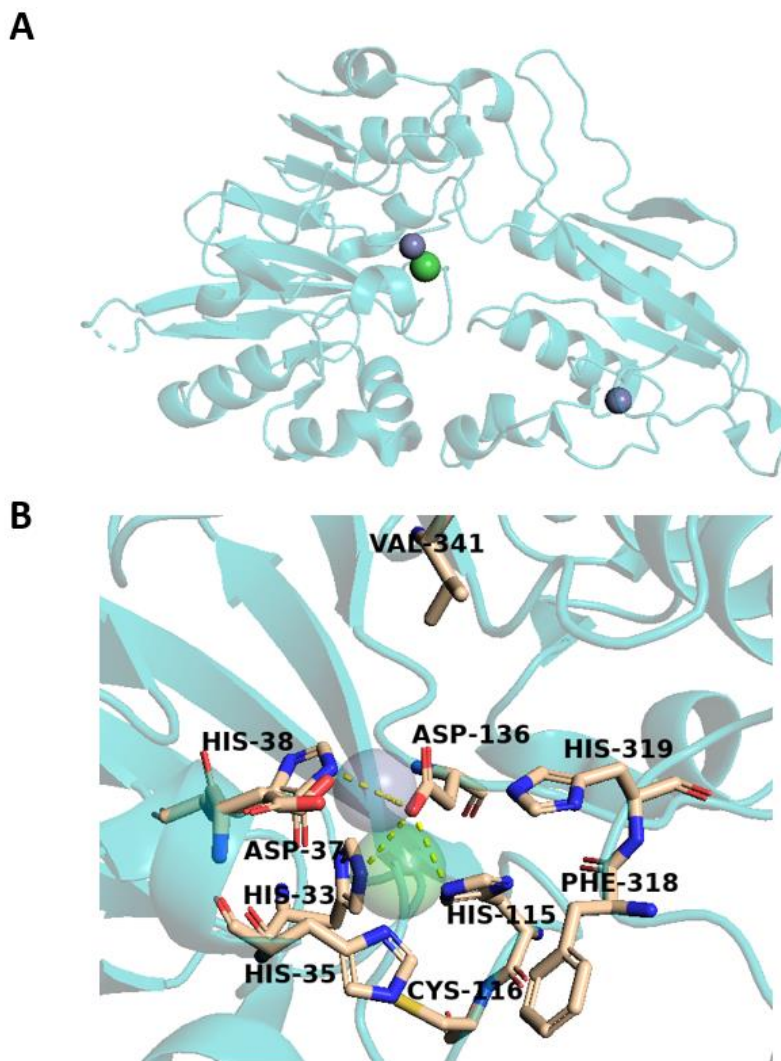


**Figure 2.** The  $\beta$ -CASP Family of Metallo- $\beta$ -Lactamases.<sup>7</sup>

Figure reproduced from *DNA Repair*, <https://doi.org/10.1016/j.dnarep.2020.102941> (Permission not required).

The first crystal structure of the truncated Artemis metalloenzyme has only been available as of February 2020 (pdb: 6TT5) (**Figure 3A**),<sup>11</sup> while those of SNM1A and SNM1B have been published previously and have therefore been more extensively studied.<sup>12</sup> We previously used a homology model to guide our synthetic efforts as the compounds presented in this dissertation were completed prior to February 2020. The Artemis enzyme crystal structure shows two catalytic sites, with the first catalytic site, CD1, (**Figure 3B**), consisting of one  $Zn^{2+}$  and one  $Ni^{2+}$  atom, and the second catalytic site, CD2, consisting of one  $Zn^{2+}$  atom. More recent Artemis crystal structures<sup>6</sup> (June 2020; pdb: 6W00 and pdb: 6WNL) include two  $Zn^{2+}$  atoms in the Artemis CD1 instead. Artemis acquires 5' and 3' endonuclease activity when in complex with autophosphorylated DNA-PKcs (DNA-dependent protein kinase catalytic subunit).<sup>2a</sup> Interaction with DNA-PKcs occurs at the Artemis C-terminal segment (residues 448-462). Artemis has nuclease activity at CD1 of its N-terminal catalytic domain (~370 residues).<sup>6</sup> Mutations at motifs 1-4, A, and B in the N-catalytic

domain have been studied and it was found that all their residues, except for His38, are critical for endonucleolytic activity of Artemis.<sup>6</sup>

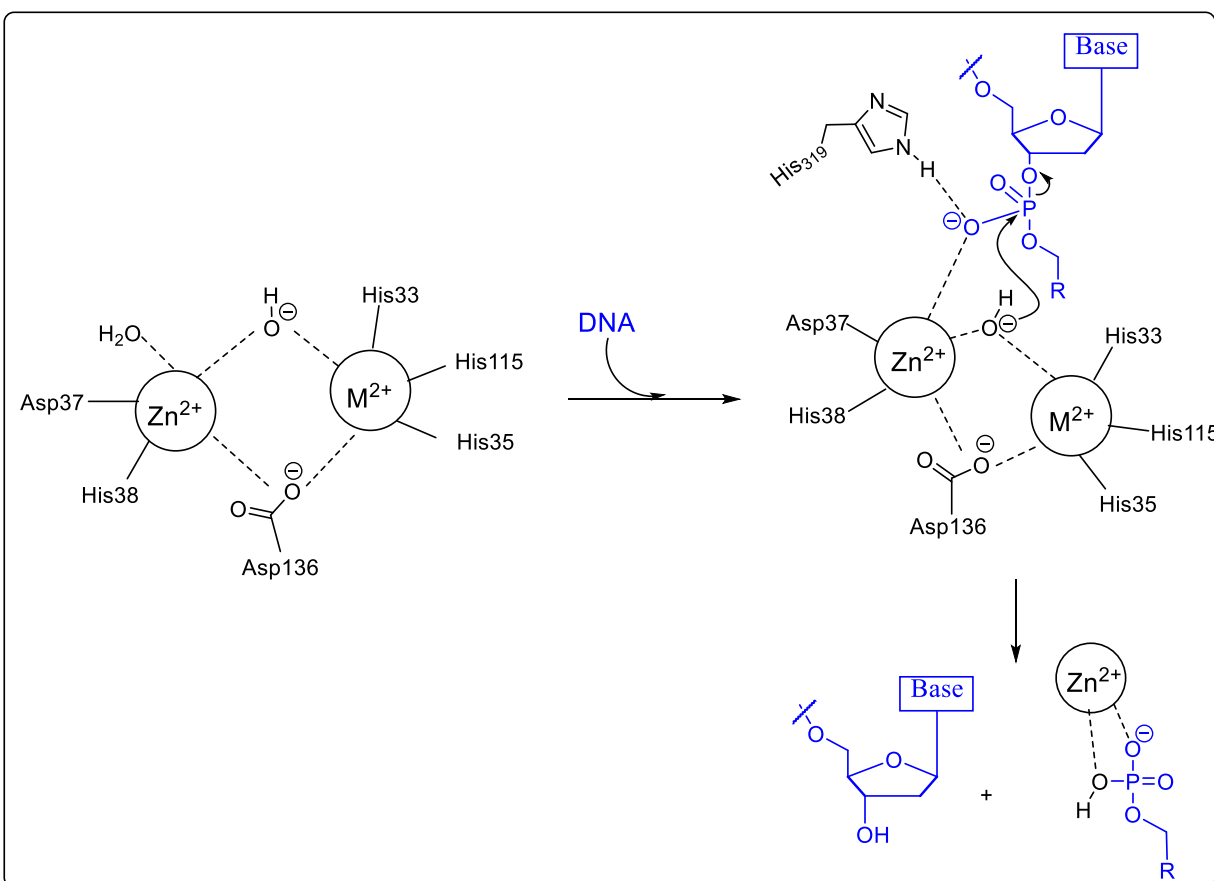


**Figure 3.** Artemis Crystal Structure, February 2020.

Artemis protein X-ray structure (pdb: 6TT5) was visualized using PyMol. **A** and **B**: Artemis protein is indicated as the cyan cartoon; gray and green spheres indicate the active site zinc and nickel atoms, respectively. **A**: 362 AA of Artemis are shown with its two active sites; **B**: the residues within 6Å of the CD1 active site metals are depicted as wheat-colored licorice; yellow dashes indicate polar contacts: hydrogen bonding or anion chelation to the active site metals.



I propose the nucleolytic cleavage of a DNA phosphodiester bond by Artemis (**Figure 4**) based on a similar mechanism shown for the exonuclease SNM1B.<sup>13</sup> Water completes the coordination sphere of  $Zn^{2+}$ , and deprotonation of the coordinated water by the Lewis acidic  $Zn^{2+}$  generates the nucleophilic hydroxide ion. The phosphorous atom serves as the electrophilic partner as the phosphate group forms a hydrogen bond with His319. The hydroxide ion attacks the phosphorous atom, cleaving the phosphodiester bond in an  $S_N2$  fashion.<sup>14</sup> In addition to designing compounds that may chelate to the active site metals, the polar side chains of histidine and aspartate in the active site may serve as hydrogen bonding partners to the inhibitors.



**Figure 4.** Proposed Hydrolysis of a Phosphodiester Bond by Artemis.

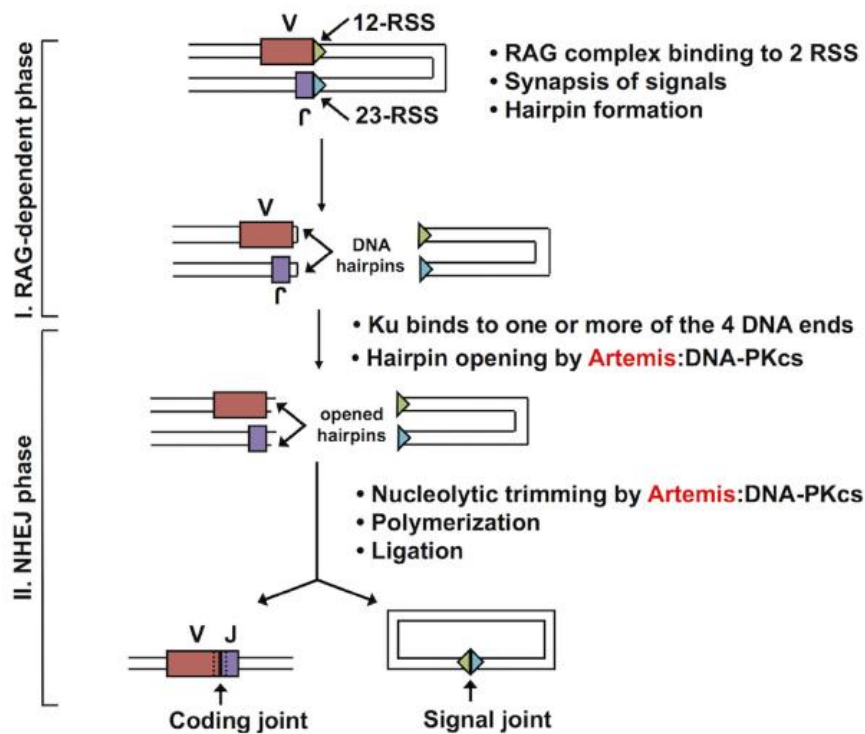
### 1.1.1.1 V(D)J Recombination and Acute Lymphoblastic Leukemia

Our immune system relies on a variety of T and B lymphocyte antigen receptors to help us fight off the diverse set of pathogens we encounter in our daily lives. The antigen receptors expressed by T and B lymphocytes are known as T cell receptors (TCRs) and immunoglobulins (Igs), respectively. To diversify the TCR and Ig receptors, developing T and B cells need to assemble a diverse set of TCR and Ig genes, respectively, in a process known as V(D)J recombination.<sup>15</sup>

V(D)J recombination (**Figure 5**)<sup>3</sup> is initiated by the recombination activating gene (RAG) protein complexes, RAG-1 and RAG-2, which are only expressed in specific developmental stages of lymphoid cells.<sup>16</sup> RAG-1 and RAG-2 selectively target the recombination signal sequences (RSS), 12-RSS and 23-RSS (triangles). The RAG proteins bind to and cleave the RSS, which consist of conserved heptamer and nonamer sequence elements that are either separated by 12 non-conserved base pairs (12-RSS) or 23 non-conserved base pairs (23-RSS). The RSS are bound to each of the V, D, and J segments (rectangles), and for every recombination event, RAG can only bind to and cleave one 12-RSS and one 23-RSS (known as the 12/23 rule), resulting in a hairpin at the coding ends (black loop) and blunt signal ends. The rest of the DNA segments that do not consist of the (V) of (J) genes nor the RSS are shown as the black “double-strand” lines in **Figure 5**.

The nonhomologous end-joining (NHEJ) pathway is used to repair DNA DSBs, and in the case of V(D)J recombination, it is used to open the hairpin intermediate (**Figure 5**).<sup>3</sup> The NHEJ phase starts when the Ku protein binds to the DNA ends, and recruits the Artemis-DNA PK<sub>cs</sub> (DNA-dependent protein kinase catalytic subunit) complex, which opens the hairpinned V, D, or

J ends. Artemis is the only vertebrate nuclease to open the DNA hairpin. The opened ends can be further processed by Artemis-DNA PKcs and a DNA polymerase. A NHEJ ligase complex ligates the DNA coding ends, forming the coding joints. A wide repertoire of V, D, and J genes can be recombined through this process to generate the necessary B cell and T cell receptor diversity. The blunt ends are also ligated, forming a circularized DNA fragment.



**Figure 5.** V(D)J recombination Mechanism.<sup>3</sup>

Reproduced with permission from *Nucleic Acids Res*, <https://doi.org/10.1093/nar/gkw456>.

Defects in V(D)J recombination lead to oncogenic translocations, which can affect developing lymphocytes, resulting in acute lymphoblastic leukemia (ALL).<sup>16a</sup> ALL is a type of blood cancer that is most common in children under the age of five.<sup>17</sup> The risk of developing ALL declines slowly until the mid-twenties, and then slowly rises after the age of fifty. About 60% of

ALL patients are children, and 80% of deaths occur in adults. For 2020, the American Cancer Society estimates 6,150 new ALL cases and 1,520 ALL deaths. The most common treatment for ALL is chemotherapy, and it is typically better tolerated in children than in adults.<sup>17</sup>

Most ALL cells express the RAG-1 and RAG-2 proteins that are required in V(D)J recombination.<sup>5</sup> However, Artemis-deficient cells are not tumor prone.<sup>18</sup> Humans with Artemis inactivation do not have T- or B-lymphocytes, are diagnosed with severe combined immunodeficiency (SCID), and are sensitive to ionizing radiation (IR).<sup>2a,3,16a</sup> With Artemis being the only enzyme that efficiently opens DNA hairpins encountered during V(D)J recombination in developing B- and T-cell precursors, it is hypothesized that blocking hairpin opening by Artemis would result in preferential apoptosis in ALL cells.<sup>19</sup> Mature lymphocytes and other cell types do not express RAG and should therefore be less affected. Artemis inhibition would block a key step unique to RAG-expressing cells, such as ALL, pre-B, and pre-T cells, with minimal and reversible effects on the immune system. Developing lymphocytes can repopulate after the removal of the Artemis inhibitor. However, there are no published studies that measure the extent in which Artemis inhibition affects the population of pre-B and pre-T cells versus ALL cells. Such study would be valuable when evaluating the superiority of this therapy compared to the current ALL therapies.

Artemis as a target is appealing as it has limited roles in DNA recombination, whereas the other proteins in NHEJ are involved in multiple cellular processes.<sup>6</sup> There are potent and selective DNA-PKcs inhibitors, such as Nedisertib, AZD7648, and VX-984 that have entered clinical trials.<sup>20</sup> Nedisertib has been actively developed as a combination treatment with chemotherapy, irradiation, and immune checkpoint inhibition.<sup>20</sup> Inhibition of DNA-PKcs by Nedisertib is not sufficient to inhibit Artemis, and it is therefore better to directly inhibit Artemis.<sup>21</sup> Esguerra *et al.*

propose that it may be possible that Nedisertib does not affect the portion of DNA-PKcs that interacts with Artemis or that the residual level of kinase activity post-Nedisertib treatment may be sufficient for Artemis to function.<sup>21</sup> There are no known Artemis inhibitors in development.<sup>6</sup>

#### **1.1.1.2 Double-strand Breaks from Ionizing Radiation**

Radiation therapy is used for the treatment of a variety of cancers.<sup>22</sup> Exposure to ionizing radiation results in a variety of DNA lesions, including DSBs, which are the main mechanism driving therapeutic efficacy.<sup>22</sup> However, many cancers contain cells with high DSBs repair activity, and therefore show resistance to radiation therapy. Since NHEJ repairs the DSBs caused by ionizing radiation, targeting proteins in the DNA DSB repair pathways may sensitize cancer cells when used simultaneously with radio/chemotherapy.<sup>22-23</sup> An Artemis inhibitor may potentially sensitize cancer cells to radiation and type II topoisomerase inhibitor cancer treatments.<sup>19</sup>

#### **1.1.2 Metalloenzymes**

Metalloproteins depend on the active site metal ion to achieve a functional or structural purpose. Functional purposes include substrate recognition/binding, electron transfer, and catalysis to achieve a biological function.<sup>24</sup> The metalloprotein with a catalysis function is known as a metalloenzyme.<sup>24</sup> The metal cations are bound in the protein and are coordinated by the oxygen, nitrogen, or sulfur atoms of amino acid residues, mostly cysteine, histidine, aspartic acid, or glutamic acid.<sup>25</sup> Zinc finger proteins are the most abundant class of metalloproteins, and are an example of metalloproteins that use the metal ion for structural purposes.<sup>24,25-26</sup> The zinc ion ( $Zn^{2+}$ )

is abundant in biological systems and it displays multiple characteristics that make it a suitable metal for metalloproteins. Amongst these properties are: (a) a great stability towards redox reactions with its one oxidation state of  $Zn^{2+}$  (b) a  $d^{10}$  electronic configuration where it can form four-, five-, and six coordinated complexes without relevant energetic penalty; (c) an intermediate polarizability or borderline hardness allowing coordination of N, S, and O donor atoms; and (d) a Lewis acid character useful to activate coordinated substrates, while maintaining ligand nucleophilicity.<sup>25-26</sup>

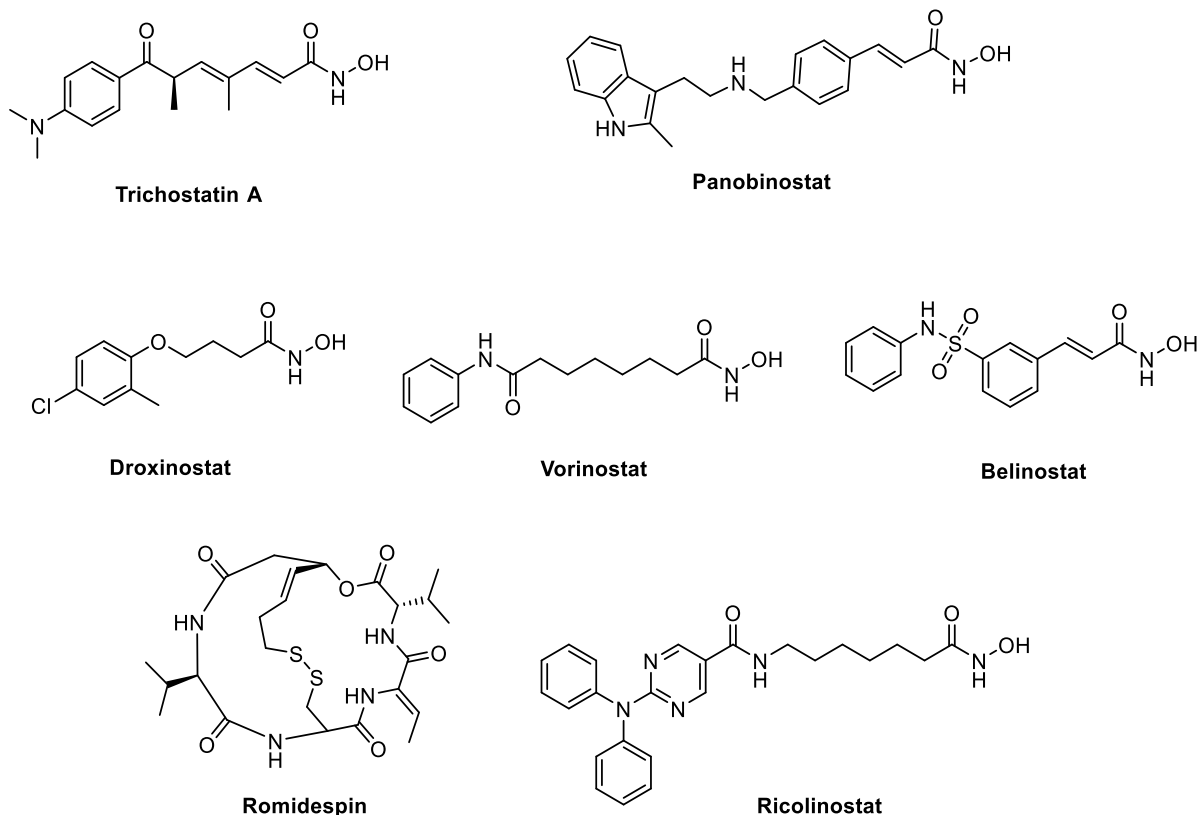
There are numerous drugs approved that work as inhibitors of the metalloproteins such as carbonic anhydrase, histone deacetylase, angiotensin converting enzyme, HIV-1 integrase, and lipoxygenase.<sup>25</sup> In recent years, research groups have been working on protein design by engineering new metal-binding sites into existing native proteins to improve new activities.<sup>27</sup>

The common zinc binding groups (ZBGs) of inhibitors of metalloenzymes include hydroxamic acids, hydrazides, *N*-hydroxyurea, carboxylic acids, sulfonyl hydrazide, pyrimidine-2,4,6-trione, and picolinic acid, to name a few.<sup>28</sup> One of the unique features of hydroxamic acids is their low acidity, with pKa values around 8.5.<sup>29</sup> They are neutral species at physiological pH, and their deprotonation occurs after coordination to the zinc cation.<sup>29</sup> Since the hydroxamic acid series contains a hydroxamic acid, a ZBG common amongst histone deacetylases (HDAC) inhibitors, we tested our compounds on HDAC assays and found many of them inhibited HDAC. Molecular Docking Studies in the HDAC6 protein will be discussed in **Section 1.2.2**.

### 1.1.2.1 Histone Deacetylases

Histone deacetylases (HDACs) are a class of metalloenzymes that catalyze the hydrolysis of acetyl groups from the  $\epsilon$ -amino substituent of specific Lys residues of histone and non-histone proteins.<sup>25,30</sup> This allows the histones to wrap the DNA more tightly, blocking DNA and repressing transcription.<sup>25,31</sup> With this role, HDACs are involved in various biological functions: cell differentiation, embryogenesis, cancer, neurodegenerative diseases, immunological responses, and metabolic homeostasis.<sup>25</sup> There are 18 HDAC isoforms grouped into four classes, with Classes I, II, and IV depending on a metal cofactor.

In 1990, Trichostatin A (**Figure 6**) was identified by Yoshida as an HDAC inhibitor, decreasing the proliferation of the FM3A tumor cell lines.<sup>32</sup> Over the past thirty years, numerous HDAC inhibitors were discovered and have been shown to induce cell differentiation, cell cycle arrest, and/or apoptosis.<sup>25</sup> For example, vorinostat, romidespin, and belinostat were approved by the FDA as treatments for T-cell lymphoma; panobinostat for the treatment of multiple myeloma; ricolinostat is currently going through phase 2 trials for diabetic neuropathic pain as an HDAC6 selective inhibitor.<sup>33</sup> Droxinostat has not been approved by the FDA, but has been shown to inhibit HDAC.<sup>34</sup>



**Figure 6.** Structures of HDAC Inhibitors.

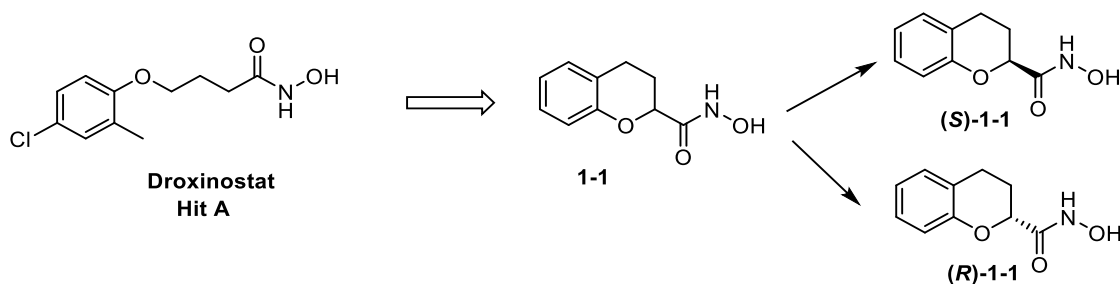
HDAC inhibitors have proven to exhibit anti-tumor effects; however, their side effects have limited their clinical potential.<sup>25</sup> Current research is involved with identifying selective isoform or class HDAC inhibitors. Class I (HDACs 1-3,8) and IIb (HDACs 6,10) isoforms are overexpressed in most solid and hematological tumors and not in resting endothelial cells and normal organs.<sup>25</sup> HDAC 6 (Class IIb) is unique amongst the HDAC family as its substrates are not limited to histones, but also include  $\alpha$ -tubulin, HSP90, cortactin, and peroxiredoxin.<sup>35</sup> Additionally, HDAC6 bears two catalytic sites and a zinc finger ubiquitin-binding domain.<sup>36</sup> Ricolinostat and citarinostat are the first HDAC6-selective inhibitors in clinical trials.<sup>30</sup> The active sites for HDAC classes I,



II, and IV are highly conserved.<sup>26</sup> A key characteristic of HDAC active sites is the presence of a narrow hydrophobic channel leading to the Zn<sup>2+</sup> chelation site.

### 1.1.3 Series A: Chromane Hydroxamic Acid Derivatives

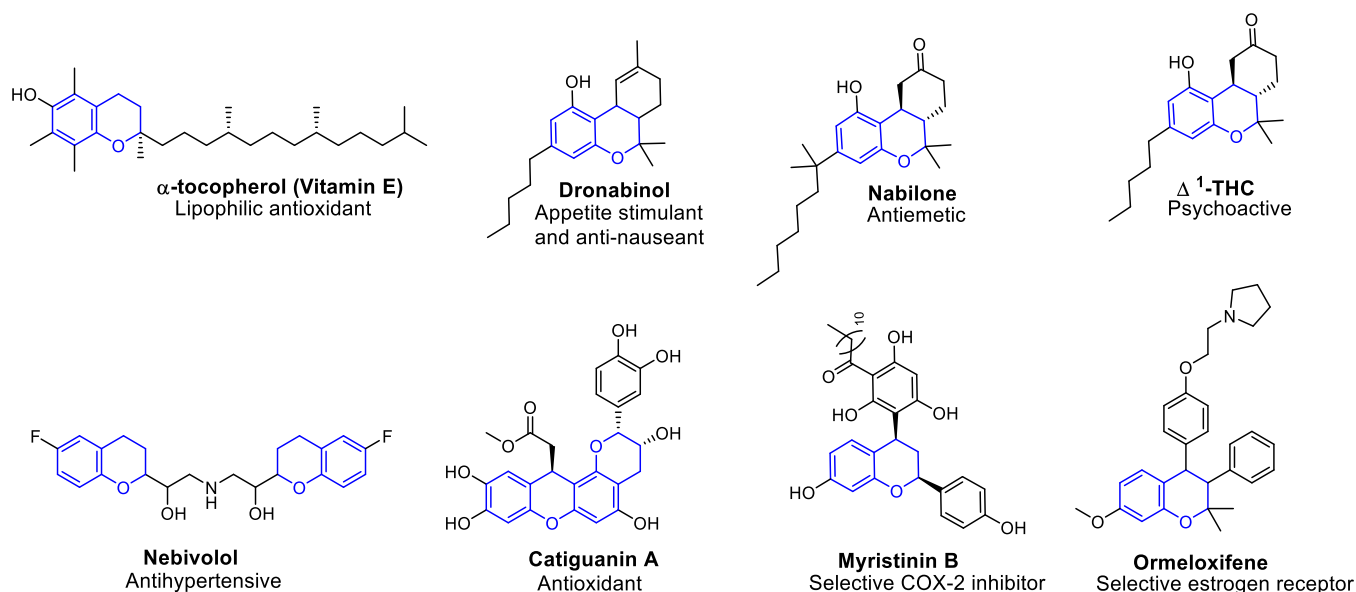
Members of our group synthesized about fifty analogs of **Hit A-Droxinostat** (**Figure 7**) before concluding that the chromane analogs were the most potent. Chromane (**S**)-**1-1** was three-fold more active than its enantiomer (**R**)-**1-1**, and our work focused on making chiral analogs of the former.



**Figure 7.** Chromane Derivative of Hit A.

#### 1.1.3.1 Chromanes in Medicinal Chemistry

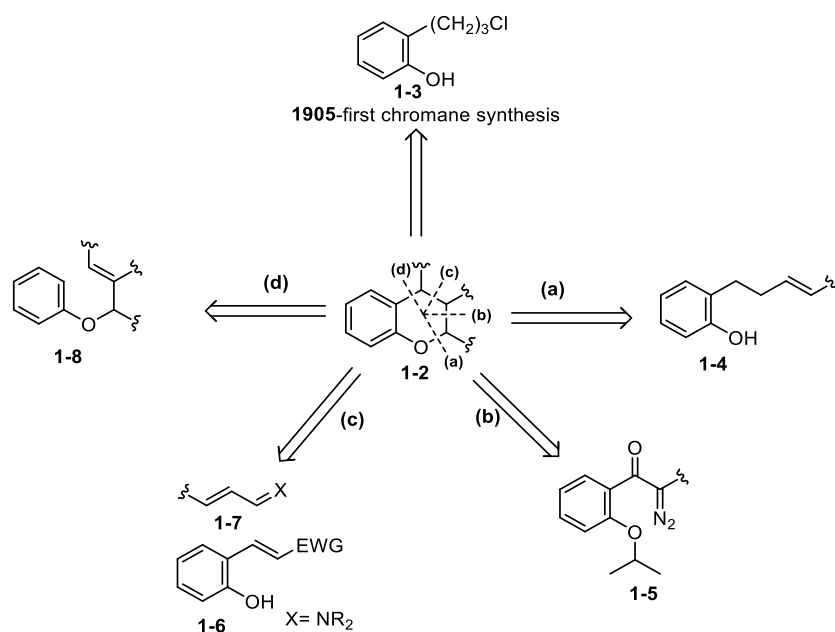
The chromane heterocycle is a privileged scaffold in natural products and pharmaceuticals.<sup>37</sup> Examples of the chromane motif include Vitamin E (tocopherol class), Dronabinol, Nabilone, THC, Nebivolol,<sup>38</sup> catiguanins A,<sup>39</sup> myristinin B,<sup>40</sup> and Ormeloxifene (**Figure 8**).<sup>41</sup>



**Figure 8.** Chromane-Containing Pharmaceutical Compounds and Natural Products.

### 1.1.3.2 Syntheses of Chromanes

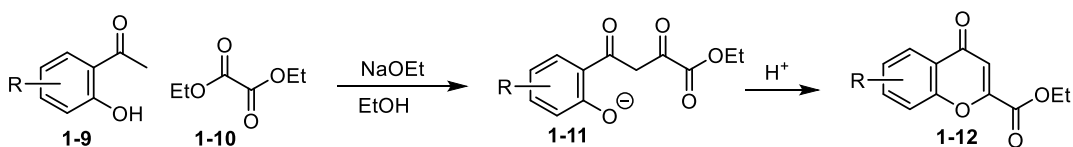
The first chromane compound was synthesized in 1905 from heating a sodium hydroxide solution of 2-(3-chloropropyl)phenol (**1-3**) (**Figure 9**).<sup>42</sup> Since then, numerous sophisticated methods have been employed to install the chromane scaffold. Those include: (a) cyclization of a 2-allylphenol (**1-4**), (b) C-H functionalization of an *O*-alkyl phenol (**1-5**), (c) tandem Michael additions involving the phenol (**1-6**) with an unsaturated carbonyl derivative (**1-7**) (d) intramolecular Friedel-Crafts-type cyclization of an *O*-allylphenol (**1-8**).<sup>43</sup>



**Figure 9.** Syntheses of Chromane.

(a)  $\text{Pd}_2(\text{dba})_3 \cdot \text{CHCl}_3$  (2 mol%), Trost ligand, AcOH,  $\text{CH}_2\text{Cl}_2$ <sup>44</sup> (b)  $\text{Rh}_2(\text{OAc})_4$ ,  $\text{C}_6\text{H}_6$  or  $\text{CH}_2\text{Cl}_2$ , reflux<sup>45</sup> (c) benzoic acid additive (20 mol%),  $\text{PhCl}$ <sup>46</sup> (d)  $\text{PdCl}_2(\text{CH}_3\text{CN})_2$  (10 mol%),  $\text{CuCl}_2$ , CO balloon, MeOH, DCE<sup>37a</sup>

A straightforward method to access chromanes is through the reduction of a chromone derivative. One of the earliest reported syntheses of chromones is through the Kostanecki reaction where the condensation product of *ortho*-hydroxyacetophenone with diethyl oxalate in sodium ethoxide and ethanol is subjected to an acid-mediated cyclization (**Scheme 1**).<sup>47</sup> All chromane derivatives presented in this document have been obtained via the reduction of commercially available chromone derivatives.

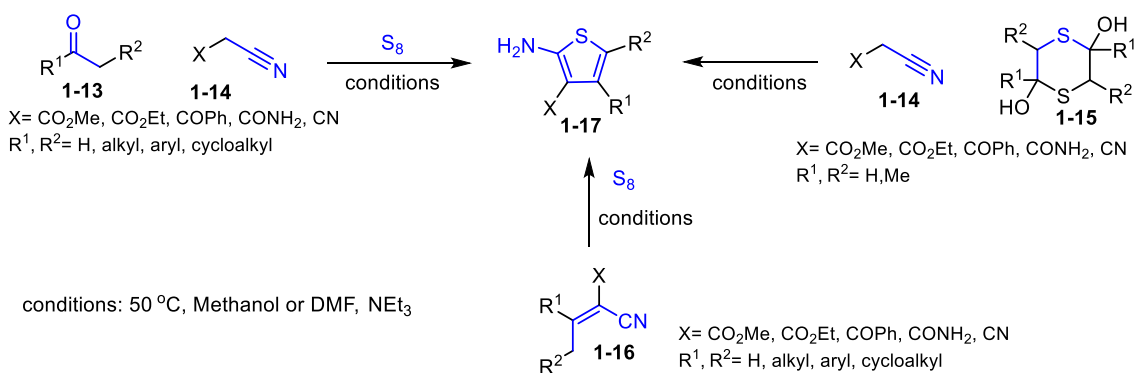


**Scheme 1.** Kostanecki Reaction.

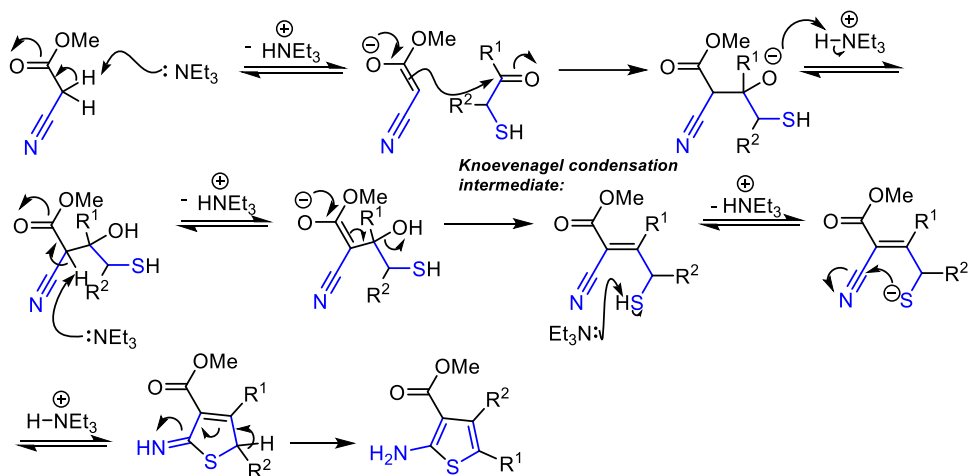
### 1.1.4 Series B: 2-Aminothiophenes Derivatives

2-Aminothiophenes are another class of privileged scaffolds that have proven to hold great therapeutic value.<sup>48</sup> Drugs containing the 2-aminothiophene core include Olanzapine (marketed as Zyprexa® by Eli-Lilly), an antipsychotic drug used for treating schizophrenia and bipolar disorder; <sup>48b</sup> PD81723, a selective allosteric enhancer for the Adenosine A1 receptor;<sup>48b</sup> and Raltitrexed, an antimetabolite used in chemotherapy for colorectal cancer.<sup>49</sup> A subclass, the 2-amino-3-carboxythiophene moiety exhibits intramolecular hydrogen-bonding between the 2-amino and 3-carbonyl groups, forming a favorable 6-membered ring that reduces the mobility of the 2- and 3-substituents.<sup>48b</sup>

2-Aminothiophene derivatives are typically synthesized via the three-component Gewald reaction (**Figure 10**).<sup>50</sup> The three components include a cyanoacetic acid, sulfur, and an oxo-component. The Gewald reaction provides a fast, convergent synthesis for diverse thiophene-based compound libraries. This multicomponent reaction has not only been used to install the 2-amino and 3-carboxy (or 3-cyano) substituents, but also for substitutions at the 4- and/or 5- position.



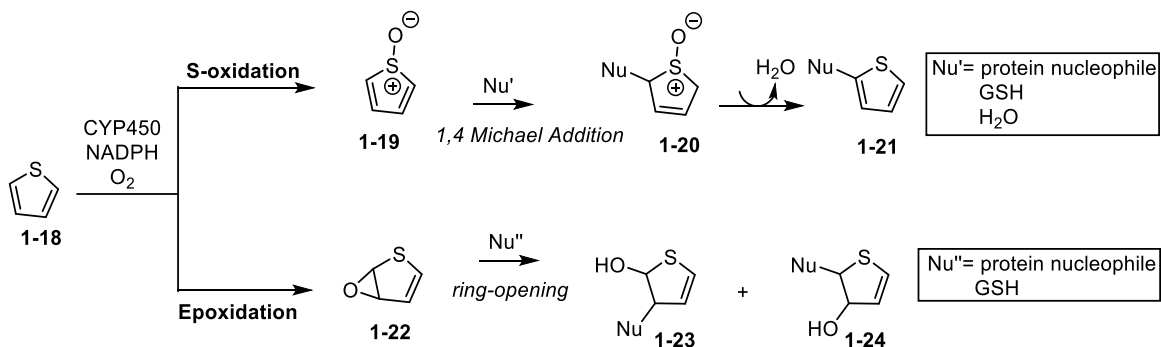
**Proposed Mechanism:**



**Figure 10.** Substituted Thiophenes from the Gewald Reaction.

Due to the electron-rich nature of the thiophene ring, it is prone to undergo oxidative metabolism, leading to reactive metabolites (RMs).<sup>51</sup> Adverse effects of drugs containing the thiophene ring are caused by cytochrome P450 (CYP450) mediated biotransformation, forming thiophene S-oxides and thiophene epoxides (**Figure 11**).<sup>51a</sup> These highly reactive metabolites can rapidly react with small molecule nucleophiles, such as water, glutathione, and protein nucleophilic residues.<sup>51b</sup> The 2-position of the thiophene S-oxide is proposed to undergo a Michael addition in the presence of a nucleophile.<sup>51b,52</sup> Thiophene epoxides can undergo ring-opening in the presence of glutathione and protein nucleophiles.<sup>51b</sup> Although glutathione is able to detoxify

small amounts of RMs, if the intermediates are highly reactive, they are capable of reacting with numerous targets before reacting with GSH.<sup>51b</sup>

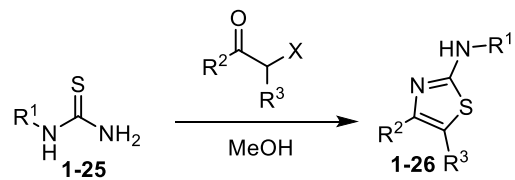


**Figure 11.** Metabolism of the Thiophene Ring.

### 1.1.5 Series C: 2-Aminothiazoles Derivatives

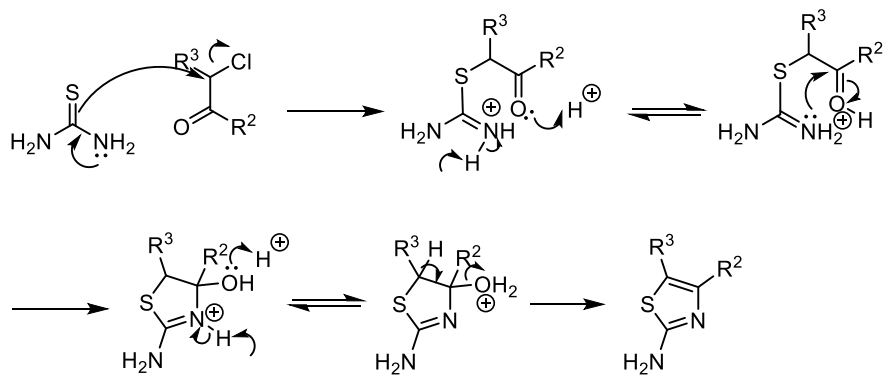
The 2-aminothiazoles group is another privileged scaffold known to display anticancer and antitumor activities.<sup>53</sup> Relative to the thiophene ring, the thiazole is less  $\pi$ -electron rich as the presence of the nitrogen lowers the energy levels of the  $\pi$  orbitals.<sup>54</sup> The additional nitrogen atom also adds a protonation site, altering the basicity of the original thiophene.

2-Aminothiazoles are synthesized via the Hantzsch-Traumann reaction (**Figure 12**).<sup>55</sup> The biotransformations that aminothiazoles undergo include oxidative P450-catalyzed ring opening (**Figure 13**).<sup>54</sup> For example, metabolic studies on sudoxicam showed thiohydantoic acid (**1-28**) and a thiourea derivative (**1-29**) as metabolites. This suggests that the thiazole ring opening occurs via the hydrolysis of the thiazolone intermediate. Compared to sudoxicam, meloxicam has a methyl group at the 5-position, and metabolic studies showed that the methyl group is oxidized to the alcohol (**1-30**), which undergoes further oxidation to the carboxylic acid (**1-31**).

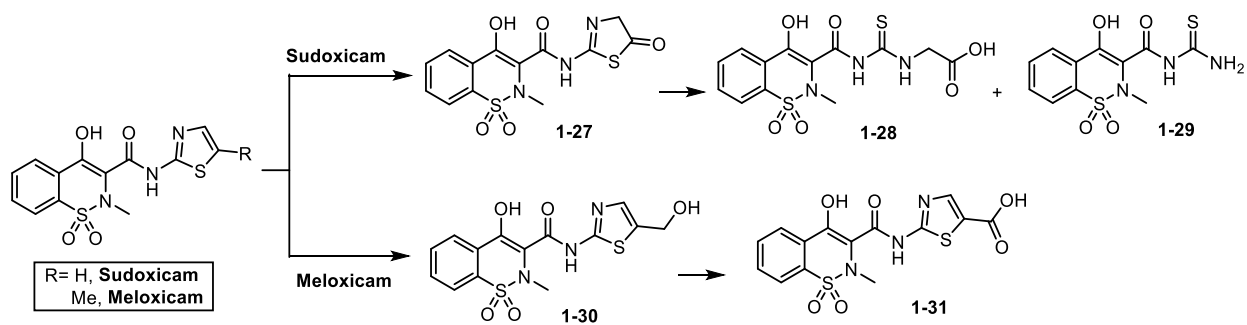


X = Cl, Br, I, OTs, OMs

**Proposed Mechanism:**



**Figure 12.** Hantzsch-Traummann Reaction.

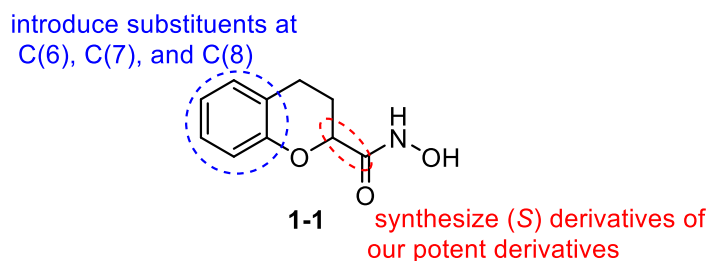


**Figure 13.** Metabolism of the Aminothiazoles Sudoxicam and Meloxicam.

## 1.2 Results and Discussion

### 1.2.1 Chromane Hydroxamic Acid Series

With members of our group finding that **1-1** displays more effective Artemis inhibition than **Hit A** (Droxinostat) from their SAR studies, we wanted to continue working with the chromane scaffold by introducing substituents around the aryl group (**Figure 14**). Additionally, since they found that (*S*)-**1-1** was more active than (*rac*)-**1-1** and (*R*)-**1-1**, we sought to synthesize (*S*) enantiomers of our promising candidates.



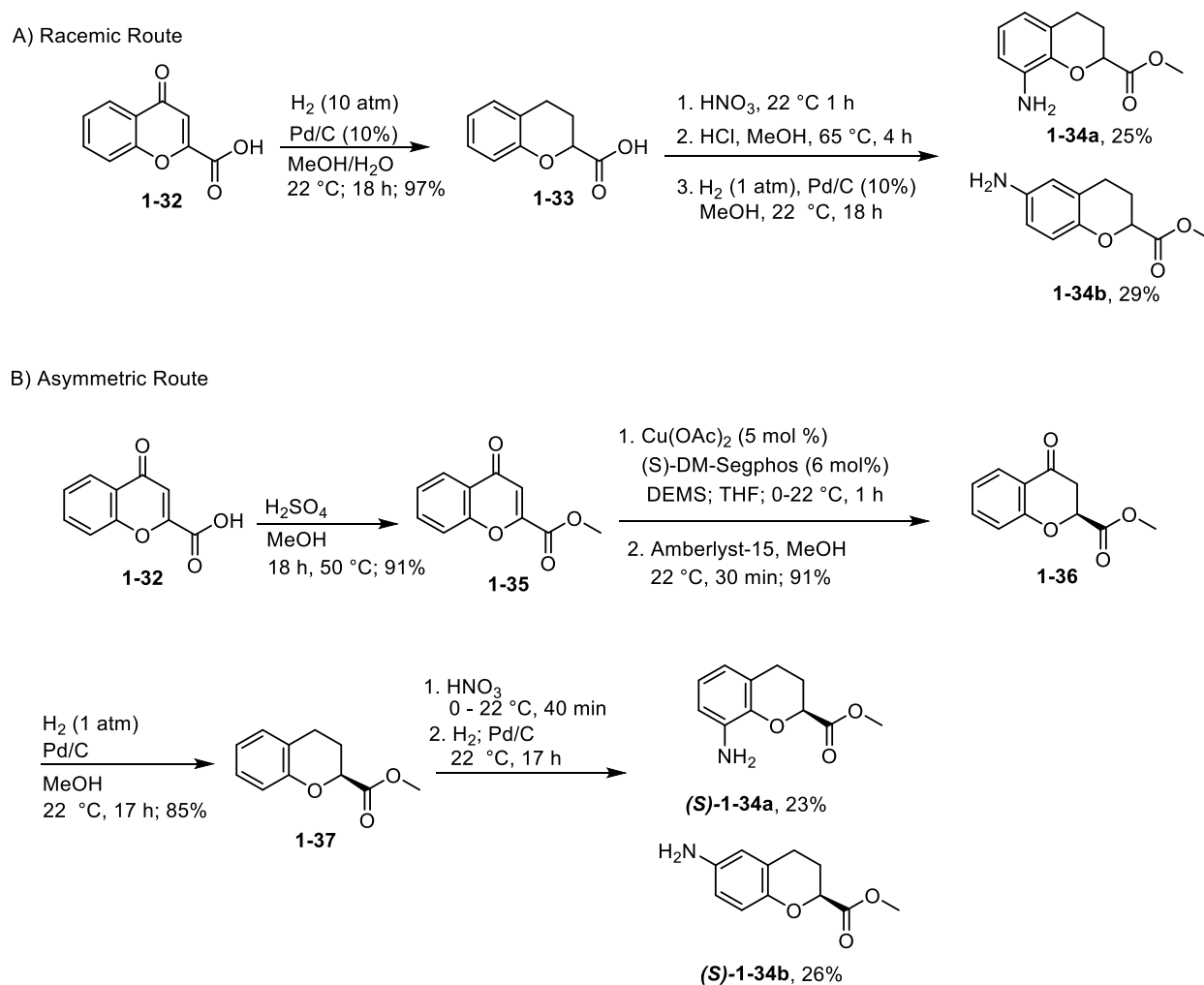
**Figure 14.** Zone Break-down of **1-1**.

#### 1.2.1.1 Synthesis of 6-Amino Derivatives

The racemic chromane derivatives were synthesized from the commercially available chromone **1-32** (**Scheme 2A**). A Pd/C hydrogenation to the chromane **1-33**, followed by nitration, esterification, and Pd/C hydrogenation provided the aniline regioisomers **1-34a**<sup>56</sup> and **1-34b**. A literature protocol<sup>57</sup> showed that the chromone **1-35** (**Scheme 2B**) is reduced to the chromanone **1-36** enantioselectively through a ligated CuH species generated from the Takasago ligand, (*S*)-DM-Segphos (11 mol%), Cu(OAc)<sub>2</sub> (10 mol%), and Et<sub>3</sub>SiH. CuH as a 1,4-reductant has first been



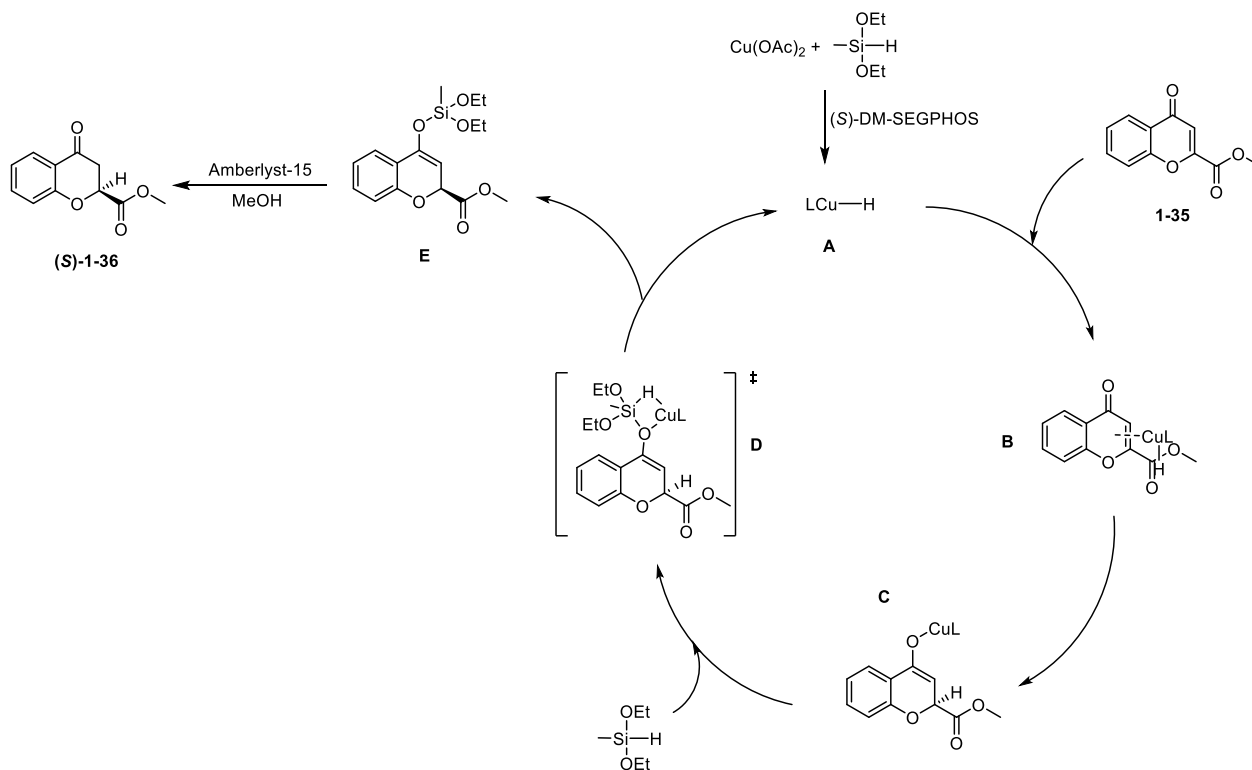
introduced by Stryker.<sup>58</sup> As an alternative to using Stryker's reagent, *in situ* generation of CuH through hydrosilanes and copper salts have been developed by Hiyama<sup>59</sup> and Lipshutz.<sup>60</sup> Asymmetric 1,4-reductions have been completed using BINAP by Buchwald<sup>61</sup> and Takasago Ligands by Lipshutz.<sup>62</sup>



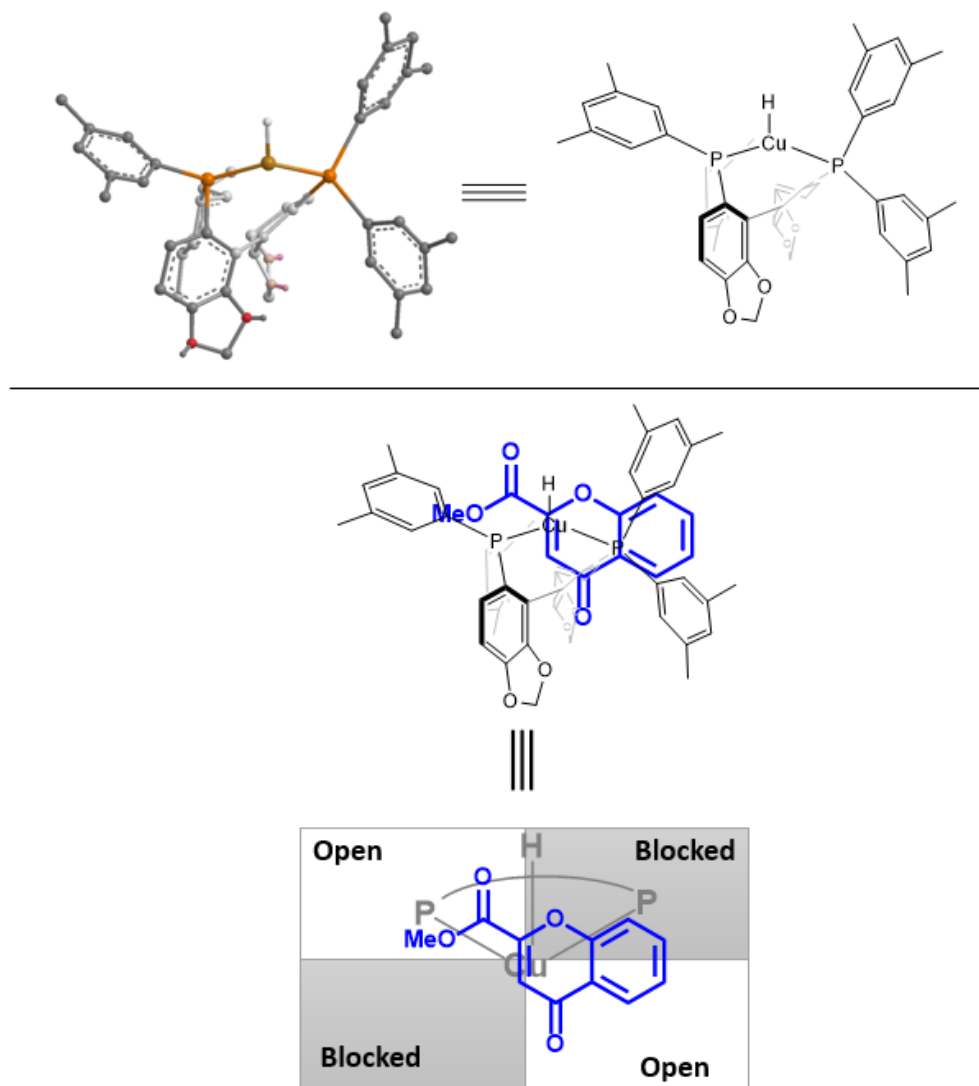
**Scheme 2.** Synthesis and Scaleup of Racemic and Chiral Aminochromanes.

We propose the mechanism in **Figure 15** for this reduction, following the Buchwald and Lipshutz precedence on ligated CuH species. The ligated carbophilic CuH species **A** reacts with

the Michael acceptor, enone **1-35**, forming  $\pi$ -complex **B**. Conjugate reduction occurs, with the hydride delivery occurring preferentially via *si* face attack. The copper enolate intermediate **C** is generated and subsequently undergoes  $\sigma$ -bond metathesis (**D**) with the stoichiometric hydride source, DEMS, to form the silyl enol ether **E**, regenerating the catalytic CuH species **A**. The enantiotopic facial selectivity<sup>63</sup> may be rationalized by the structure of the transition states in the quadrant diagram (**Figure 16**). The methyl ester group of **1-35** is located in the open region, where the equatorial aryl groups of the ligand are located. With the ligand's axial aryl groups being in close proximity to the methyl ester group of **1-35**, the favored transition state leads towards (*S*)-**1-36**.



**Figure 15.** Proposed CuH-mediated Asymmetric Reduction.

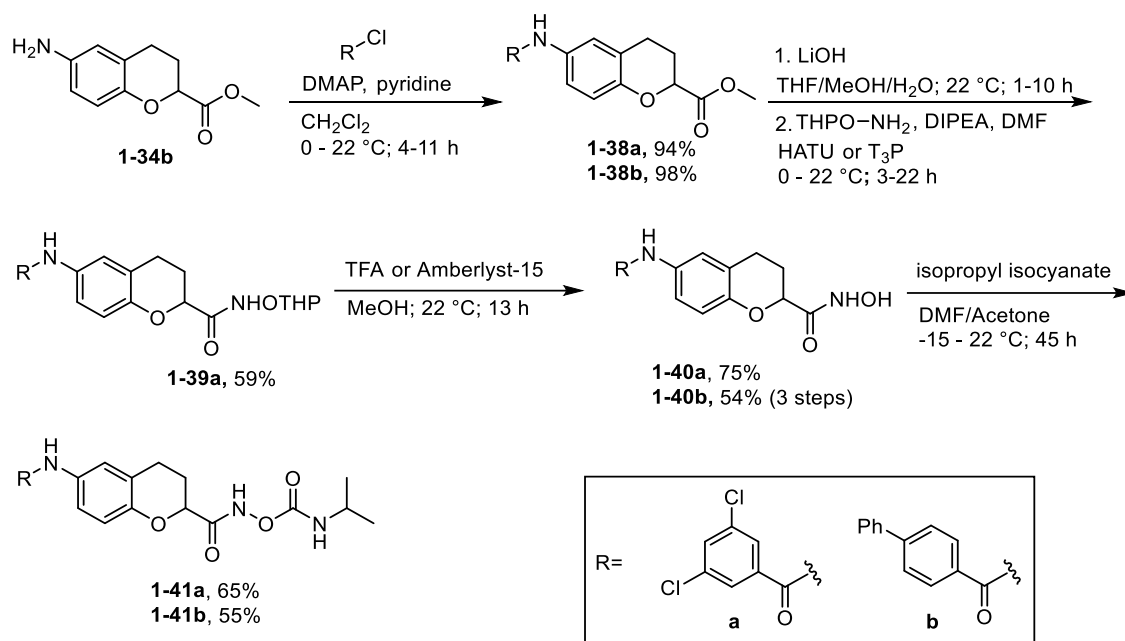


**Figure 16.** Catalyst Model and Quadrant Diagram of the Cu-H Asymmetric Reduction.

In reproducing this reduction, we determined that the catalyst and ligand loading may be reduced in half. The reaction resulted in the desired ketone **1-36** along with its silyl enol ether derivative, and subsequent solvolysis of the product mixture yielded **1-36** in >96% ee, as determined by HPLC analysis on a chiral stationary phase. This asymmetric reduction was scaled up to 15 g, where the desired 1,4-reduction product was obtained along with the overreduction alcohol byproduct (~15:1, silyl enol ether : alcohol). Overreduction has been observed in the CuH

reduction of  $\alpha,\beta$ -unsaturated aldehydes in the presence of water.<sup>58b</sup> Since no water was used in our reactions, we suspect a possible interference of diethoxymethylsilanol that may have been present in the DEMS (95% purity) bottle. Protic solvents quench the copper enolate species,<sup>62a</sup> generating the ketone that is prone to reduction. In fact, one of our smaller scale batches contaminated with trace methanol has shown overreduction with the silyl enol ether : alcohol ratio reaching 7:1. An overall 25 g of **1-36** and about 2.8 g each of (*S*)-**1-34a** and (*S*)-**1-34b** was collected.

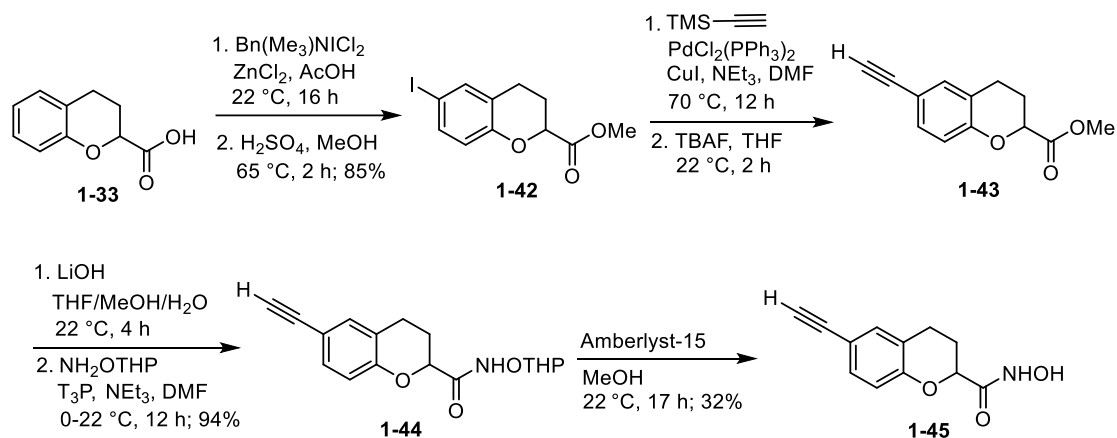
With the 6-aminoanilines in hand, we introduced benzamide derivatives from the acylation of **1-34b** with benzoyl chlorides (**Scheme 3**). **Scheme 3** only highlights two examples; other members of our group incorporated a variety of substitution patterns around the benzamide's phenyl ring. They also introduced ureas, carbamates, acetamides, and sulfonamides, with an overall 64 analogs synthesized from the anilines **1-34a** and **1-34b**. The hydroxamic acids **1-40a,b** were obtained following the saponification of the methyl esters **1-38a,b**, coupling with the THP-protected hydroxylamine, and deprotection. Treatment of the hydroxamic acids with isopropyl isocyanate produced the isopropyl carbamate derivatives **1-41a** and **1-41b** that were of interest as potential prodrugs.<sup>64</sup>



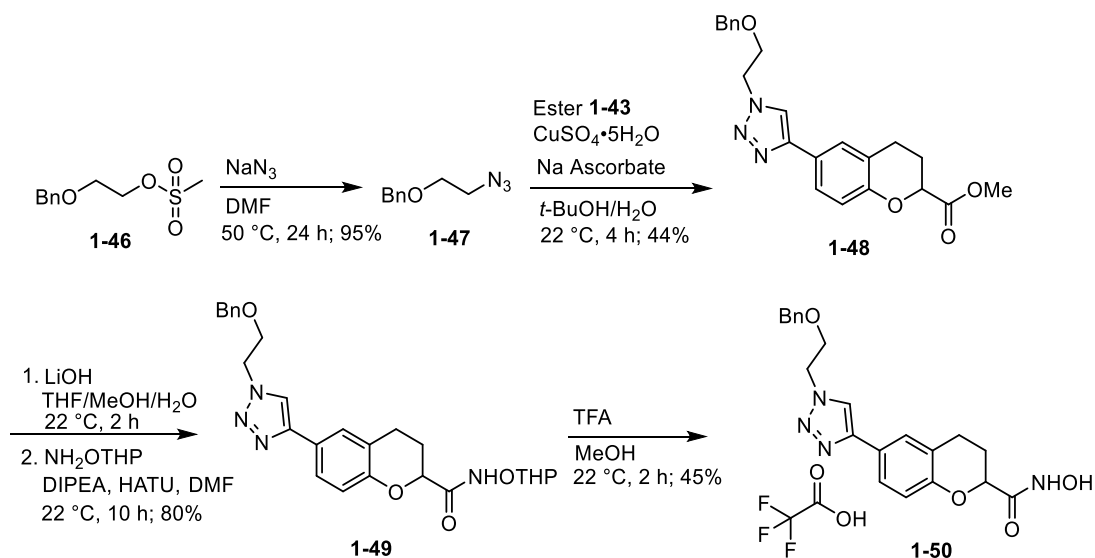
**Scheme 3.** Synthesis of 6-Aminochromane Derivatives.

### 1.2.1.2 Synthesis of 6-Carbo Derivatives

Iodination and esterification of **1-33** to the 6-iodochromane methyl ester **1-42** (**Scheme 4**), followed by Sonogashira coupling with trimethylsilylalkyne and TBAF deprotection, yielded the alkyne **1-43**. The alkyne intermediate was used for the synthesis of the chromane hydroxamic acid **1-45** and the synthesis of the triazole hydroxamic acid **1-50** (**Scheme 5**). Regioselective [3+2] cycloaddition conditions, using  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and sodium ascorbate, of **1-43** with the azide **1-47** formed the triazole **1-48**. The methyl ester was subjected to saponification, followed by peptide coupling with THP-protected hydroxylamine, and deprotection with TFA, to yield the hydroxamic acid **1-50**.



**Scheme 4.** Synthesis of 6-Ethynyl Chromane Derivative **1-45**.

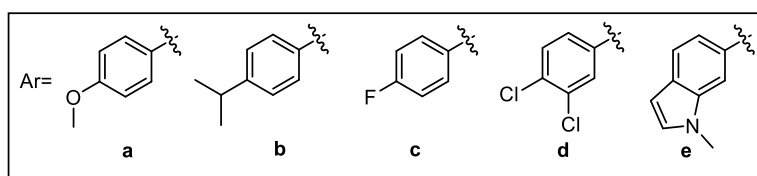
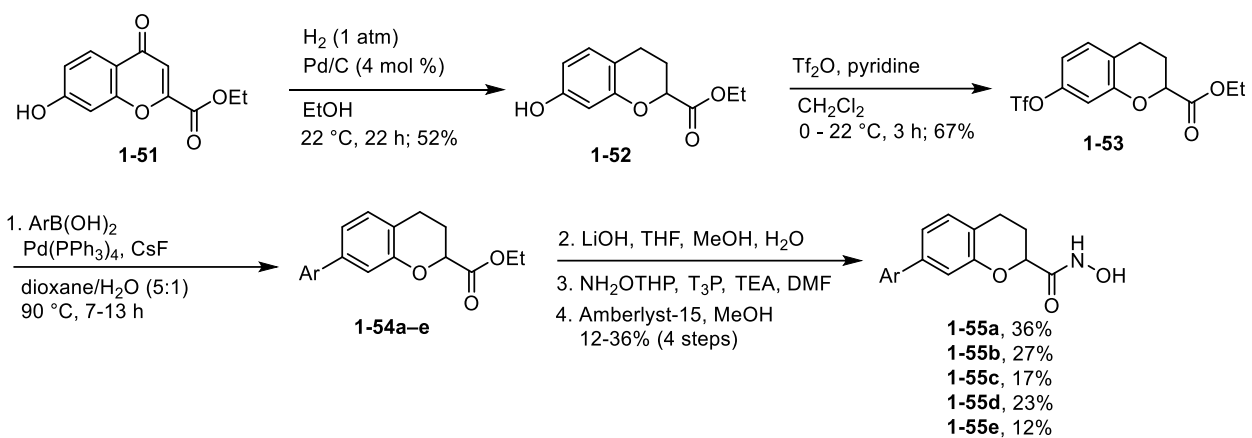


**Scheme 5.** Synthesis of 6-Triazole Chromane Derivative **1-50**.

### 1.2.1.3 Synthesis of *N*-Hydroxy-7-arylchromane-2-carboxamide Analogs

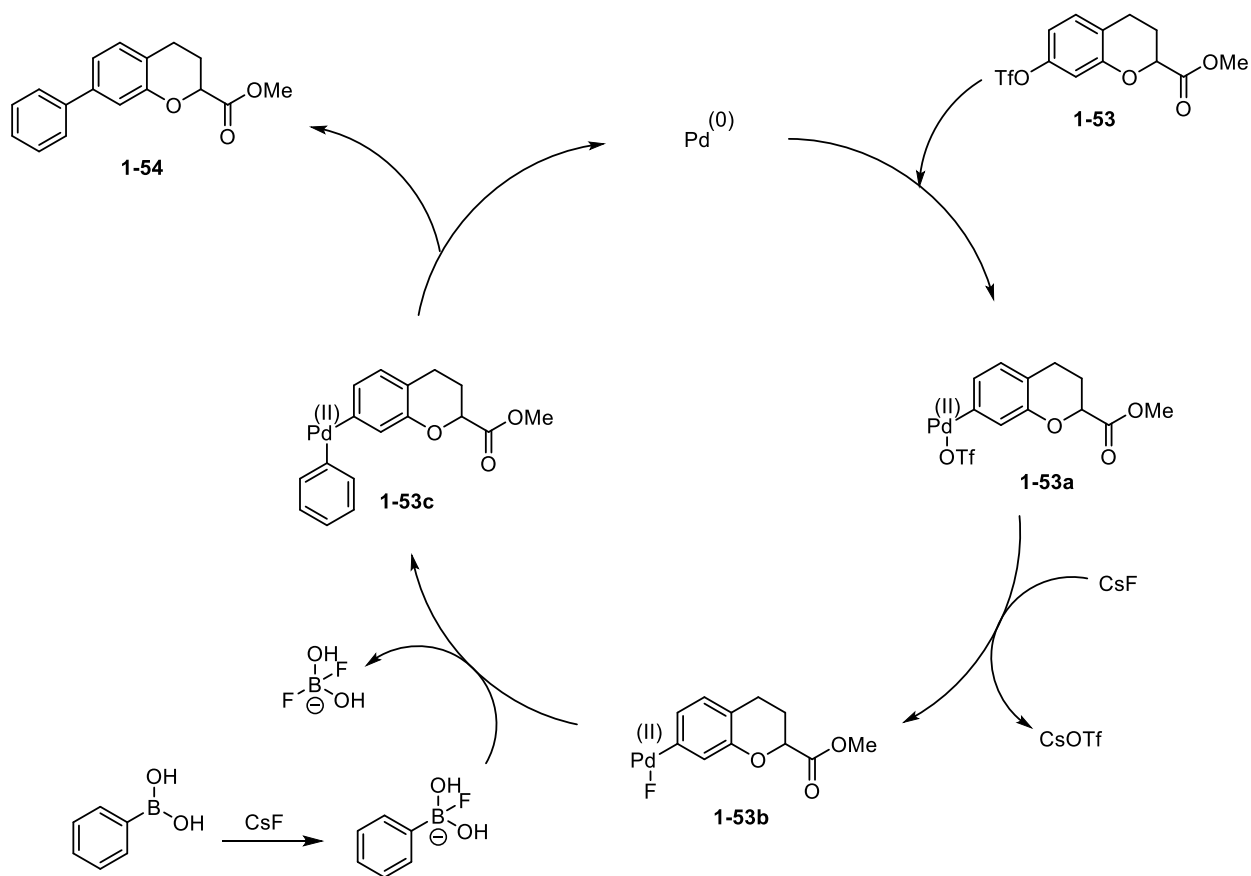
The C(6)-aryl analogs (synthesized by other members of our group) proved to be more potent than the C(6)-amino derivatives, and we were interested in exploring aryl derivatives on

other positions of the chromane. Initial assays of C(7)-aryl derivatives revealed a significant increase in potency, and we decided to pursue enantioenriched derivatives. For the racemic analogs, the commercially available 7-hydroxy chromone **1-51** was reduced to **1-52** (Scheme 6), followed by sulfonylation of the alcohol to the triflate **1-53**. Suzuki couplings with boronic acids, followed by the three-step ester to hydroxamic acid transformation gave the hydroxamic acids **1-55a-e**. The Suzuki cross-coupling mechanism (Figure 17)<sup>65</sup> starts with oxidative addition of Pd<sup>(0)</sup> to the triflate, forming the Pd<sup>(II)</sup>-species **1-53a**. Next, metathesis with the base, CsF, gives intermediate **1-53b**. The base also reacts with the arylboronic acid to make the boron-ate complex, which undergoes transmetallation with **1-53b** to give the organopalladium species **1-53c**. Recent studies<sup>66</sup> indicate the formation of Pd-O-B linkages prior to the transmetallation step. Reductive elimination provides the coupled product **1-54** and regenerates Pd<sup>0</sup>. For our starting point, we selected the five aromatic substituents that provide varying electron density. The electron density of an aromatic ring system influences the orientation and nature of electrostatic interactions (cation- $\pi$ , for example). Additionally, the methoxy and fluoro substituents may serve as hydrogen bond acceptors, and the isopropyl group may participate in hydrophobic interactions.



**Scheme 6.** Synthesis of Racemic C(7)-Aryl Chromane Derivatives.

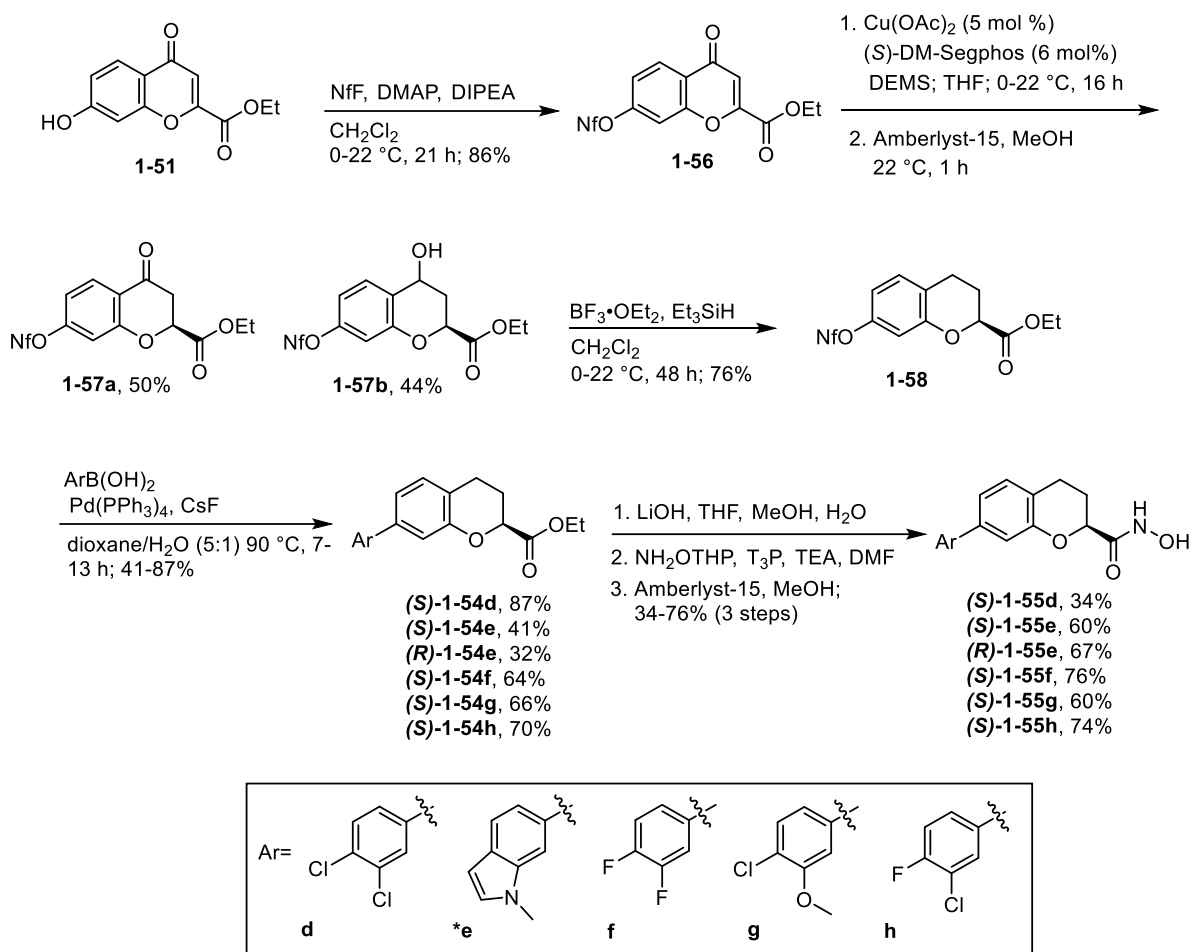




**Figure 17.** Suzuki Cross-coupling Mechanism.

We extended our asymmetric route to the (*S*)-7-arylchromanes (**Scheme 7**). The commercially available **1-51** was sulfonated to the nonaflate chromone **1-56**. Sulfonation with perfluorobutanesulfonyl fluoride (NfF) provided higher yields than when triflic anhydride (Tf<sub>2</sub>O) was used.<sup>67</sup> Asymmetric reduction using the *in situ* generation of the (*S*)-SEGPHOS-CuH species yielded **1-57a** and **1-57b**, the 1,4- and overreduced byproduct, respectively. The mixture was treated with BF<sub>3</sub>•OEt<sub>2</sub> and triethylsilane to afford the chromane **1-58**. The enantioselectivity was determined to be >96% ee by supercritical fluid chromatography (SFC) analysis after derivatization of **1-58** to **1-37**. Transformation to the hydroxamic acids **1-55d-h** was completed in

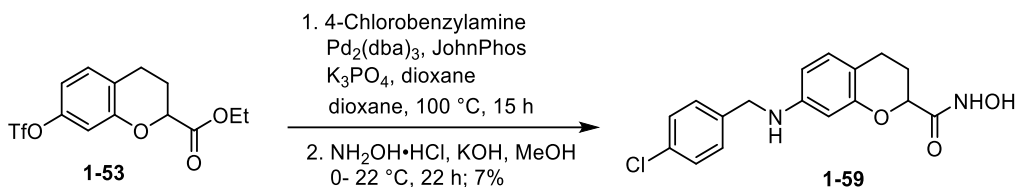
the next four steps. Buchwald coupling of **1-53** with 4-chlorobenzyl amine, followed by aminolysis, provided the hydroxamic acid **1-59** that served as our single example of a 7-amino derivative (**Scheme 8**).



(\*) both R and S enantiomers were synthesized.

R enantiomer intermediate (*R*)-**1-22** (Scheme 7) was obtained from Dr. Prema Iyer

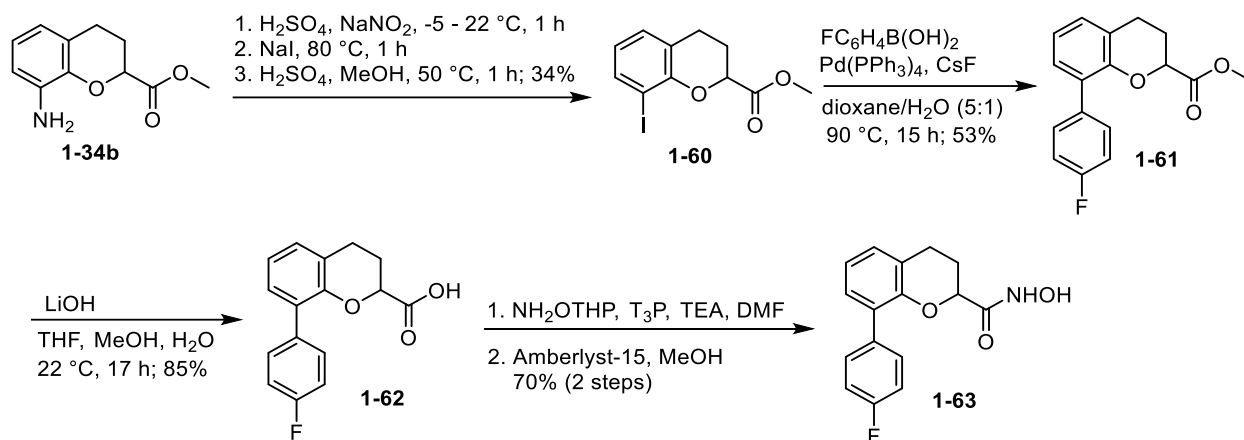
**Scheme 7.** Synthesis of Enantioenriched C(7)-Aryl Chromane Derivatives.



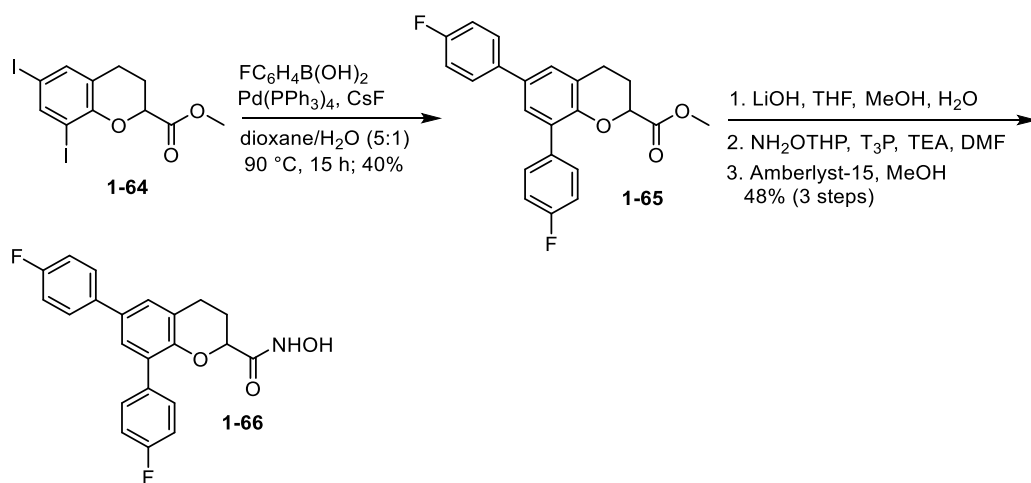
**Scheme 8.** Synthesis of 7-Amino Chromane Derivative **1-59**.

#### 1.2.1.4 Synthesis of 8-Aryl Chromane Derivatives

Since selective iodination at C(8) of **1-37** chromane has not been reported in the literature, the 8-amino chromane methyl ester **1-34b** was converted to the iodochromane **1-60** (**Scheme 9**) using the Sandmeyer reaction conditions. Suzuki coupling to **1-61** followed by saponification, coupling, and deprotection, afforded the hydroxamic acid **1-63**, which showed a significant decrease in activity compared to the C(7) aryl derivatives. Since we were not sure whether the decrease in activity was attributed to the loss of a crucial interaction of the C(6) aryl group or an undesirable interaction the C(8) aryl group might have with the protein, we decided to synthesize the bis-arylated analog **1-66** (**Scheme 10**). The diiodo chromane **1-64** (obtained from Zoe Vaughn) was bis-arylated via a Suzuki arylation, and further conversion afforded the hydroxamic acid **1-66**. The compound displayed a poor inhibition profile.



**Scheme 9.** Synthesis of 8-Aryl Chromane Derivative **1-63**.



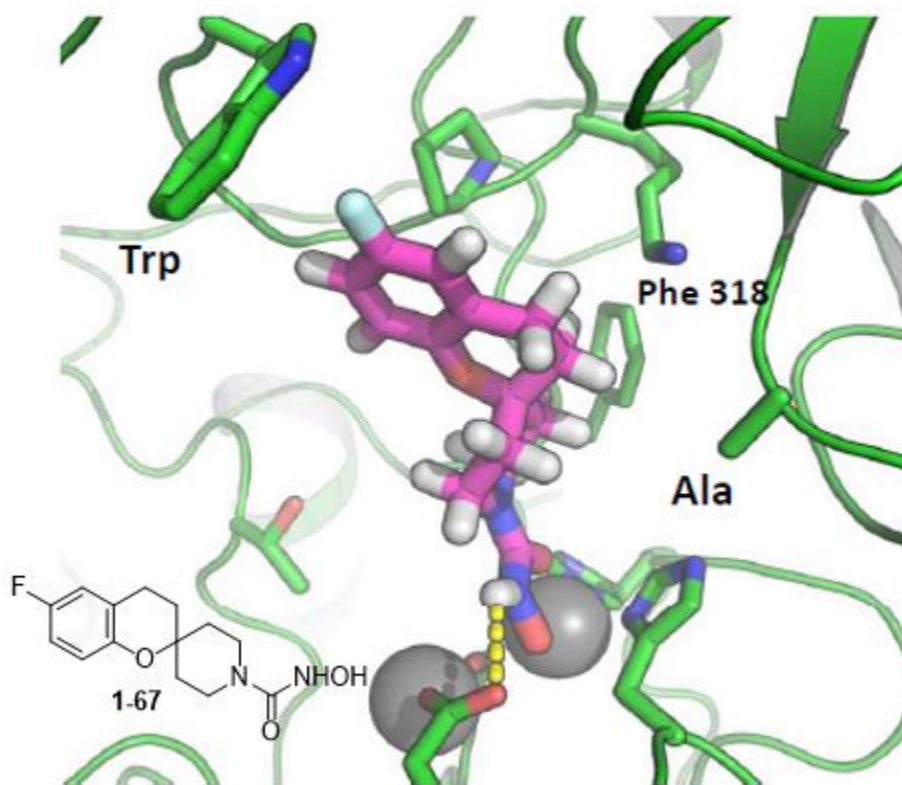
**Scheme 10.** Synthesis of 6,8-Bis(aryl) Chromane Derivative **1-66**.

### 1.2.1.5 Synthesis of 4-(6-Fluorospiro[chromane-2,4'-piperidin]-1'-yl) Hydroxamic Acid

#### Derivatives

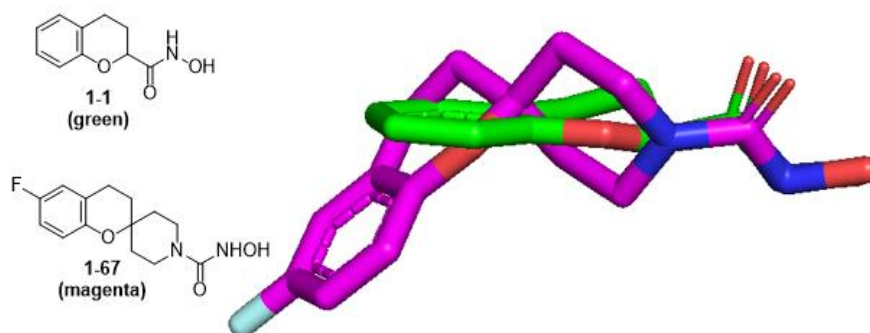
To explore the space near the Artemis catalytic site, we wanted to extend substitution off of the pyranyl group of the chromane. We found literature examples<sup>68</sup> that readily installed

spirocyclic chromanes in a few steps. Docking studies of the spirocyclic derivative **1-67** (**Figure 18**) in the Artemis homology model showed a possible favorable increased occupancy of the space near the catalytic engine of the Artemis active site. The additional space **1-67** may provide can also be visualized from its overlay with the unsubstituted chromane **1-1** (**Figure 19**). In addition to being more elongated than **1-1**, **1-67** contains the pyrrolidine motif that adds three-dimensionality compared to the previously described flat 6-,7-, and 8-aryl substitutions.



**Figure 18.** Docking of **1-67** in the Artemis Homology Model Active Site.

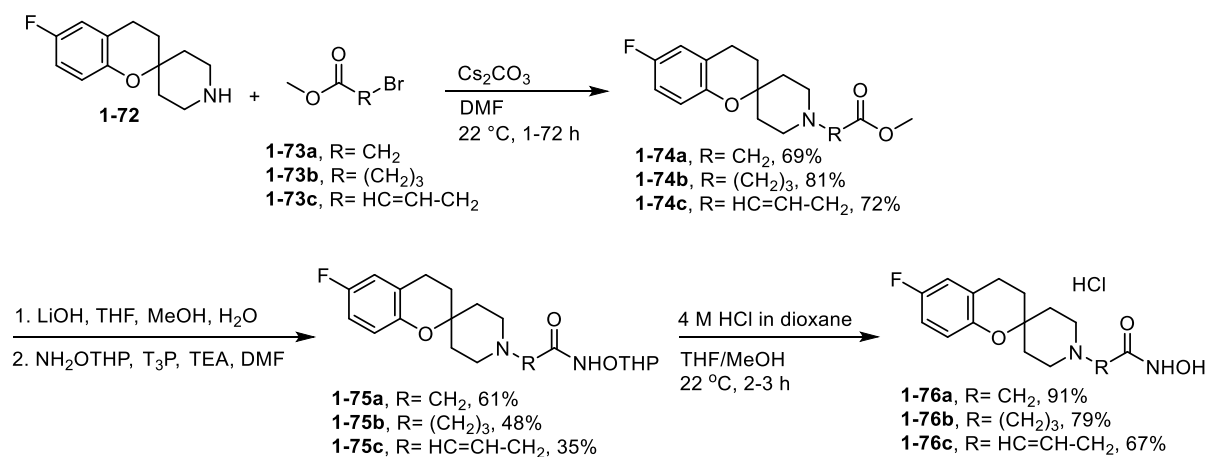
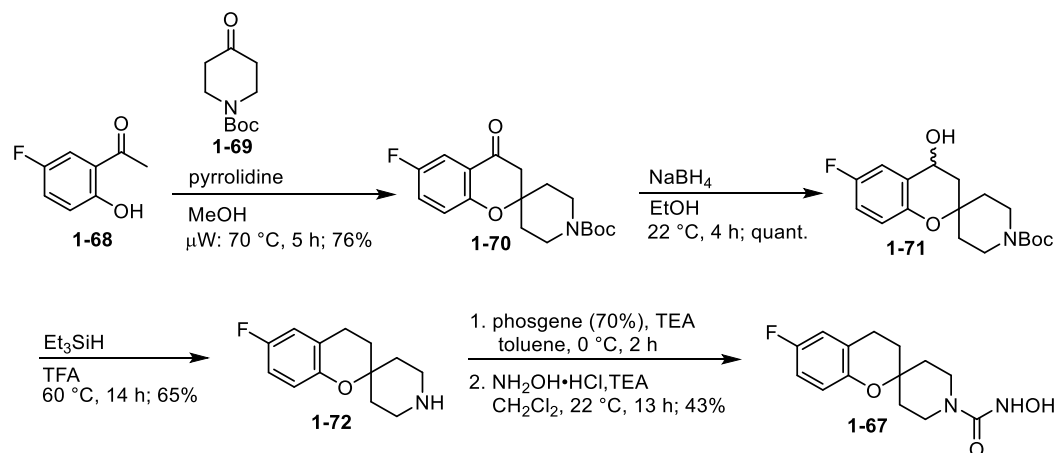
Modelling by Dr. Jim Burnett, University of Pittsburgh Chemical Diversity Center, University of Pittsburgh, Pittsburgh, USA.



**Figure 19.** Overlay of **1-67** and **1-1**.

The compounds were minimized using the Chimera program (2,100 steepest descent steps and 50 conjugate gradient steps), and then overlaid using the Pymol program.

The spirocycle **1-70** was formed upon microwave irradiation of **1-68**, *N*-Boc pyrrolidine-3-one **1-69**, and pyrrolidine, in methanol (**Scheme 11**).<sup>68a</sup> Reduction of the ketone to the chromanol **1-71**, which was subjected to further reduction with triethylsilane in the presence of TFA, formed the chromane amine **1-72**. Acylation of the amine with phosgene (20 wt % in toluene), with subsequent amination of the acyl chloride intermediate, installed the hydroxamic acid **1-67**. Alkylations of **1-72** with bromo acetates, afforded **1-74a–c**, which were converted to the hydroxamic acids **1-76a–c**.



**Scheme 11.** Synthesis of Spirocyclic Chromane Derivatives.

## 1.2.2 Biological Results

*Testing was performed at Sanford Burnham Prebys Medical Discovery Institute.*

Artemis acquires 5' and 3' endonuclease activity when in complex with autophosphorylated DNA-PKcs during NHEJ.<sup>2a</sup> Autophosphorylation of DNA-PKcs occurs in the presence of DNA termini, ATP and Mg<sup>2+</sup>.<sup>3</sup> Ideally, a biochemical assay to evaluate potential inhibitors should include the Artemis enzyme, DNA-PKcs, ATP, and MgCl<sub>2</sub>. However, DNA-

PKcs is not easily obtained, and it was found that Artemis is functional in the presence of Mn as a substitute to DNA-PKcs, ATP, and Mg<sup>2+</sup>. Chang and Lieber<sup>3</sup> suggest that Mn<sup>2+</sup> likely induces a conformational change of Artemis that mimics autophosphorylated DNA-PKcs. For the HTS in vitro data presented in **Table 1 (Section 1.1)**, a fluorescent intensity assay (PubChem Bioassay Record for AID 720701)<sup>69</sup> was used in the presence of Mn<sup>2+</sup> salts. Artemis inhibition activity was measured using the test compounds, the Artemis enzyme, and a hairpin oligomer substrate. The hairpin oligomer was labeled with 6-FAM at the 5'-end and the quencher, BHQ-1, at the 3'-end. The BHQ quencher and 6-FAM are separated upon cleavage near the 5'-end of the oligomer, resulting in the recovered fluorescence of 6-FAM. Artemis activity was measured by fluorescent intensity (Ex. 485 nm, Em. 520 nm). The assay was repeated at varying concentrations to yield a dose-response curve that was used to calculate IC<sub>50</sub> values.

Another assay variation involves C-terminally truncated Artemis lacking the DNA-PKcs interaction domain, in which case Mg<sup>2+</sup> ion alone is sufficient for endonuclease activity. For the evaluation<sup>70</sup> of the chromane derivatives synthesized, the Artemis enzyme and MgCl<sub>2</sub> buffer were used in the fluorescent interference assay described above (**Table 2**).

We synthesized derivatives of the chromane hydroxamic acid **1-1** (IC<sub>50</sub>: 2,403 ± 1,0003 nM) (**Table 2**) for our SAR study. Since previous results indicated that (*S*)-**1-1** had an IC<sub>50</sub> of 1,095 ± 285 nM and (*R*)-**1-1** had an IC<sub>50</sub> of 3,105 ± 469 nM, we were interested in synthesizing (*S*)-derivatives of a select number of racemic chromanes that had an IC<sub>50</sub> <1,000 nM in the assay. Of the compounds synthesized, the only ones that fell within that criteria are the C-7 aryl derivatives, the C-7 amino derivative, and the C-6 benzamides (**Table 2**). The spirocyclic chromane derivatives showed no inhibition under the assay conditions, and derivatives not shown in the table were not further evaluated.



For the C-6 benzamides, the racemic **1-40a**, (*S*)-**1-40a**, and (*R*)-**1-40a** showed an IC<sub>50</sub> of 266 ± 29 nM, 237 ± 82 nM, and 4,400 nM, respectively. While (*S*)-**1-40** was significantly more active than (*R*)-**1-40**, it showed similar potency to its racemic derivative. The biphenyl benzamide **1-40b** and (*S*)-**1-40b** proved to be the most potent Artemis inhibitors, with an IC<sub>50</sub> of 90 ± 13 nM and 71 ± 8 nM, respectively. Overall, the C-7 derivatives all fall within the IC<sub>50</sub> range of 111-450 nM. It turned out that the (*S*)-derivatives were not significantly more potent than the racemic derivatives. For example, compound **1-55d** had an IC<sub>50</sub> of 112 ± 21 nM while (*S*)-**1-55d** had an IC<sub>50</sub> of 175 ± 95 nM. Additionally, the racemic indole derivative **1-55e** displayed an IC<sub>50</sub> of 281 ± 162 nM, while (*S*)-**1-55e** and (*R*)-**1-55e** showed an IC<sub>50</sub> of 326 ± 49 nM and 386 ± 42 nM, respectively. The inhibitory activities of (*S*)-**1-55h** (3-chloro-4-fluorophenyl derivative; 140 ± 49 nM) and (*S*)-**1-55f** (3,4-difluorophenyl derivative; 204 ± 83 nM) were similar to those of racemic compounds **1-55a** (4-methoxyphenyl derivative; 189 ± 25 nM) and **1-55b** (4-isopropylphenyl derivative; 253 ± 40 nM). Substitution at C(8) was much less tolerated, with the C-8 4-fluorophenyl derivative **1-63** displaying an IC<sub>50</sub> of 2,355 ± 205 nM while the 6,8-bisubstituted 4-fluorophenyl derivative **1-66** displayed an IC<sub>50</sub> of 5,265 ± 251 nM.

While these derivatives have shown improvement in activity compared to either of the **1-1** enantiomers, the similar activity between the racemic and (*S*) derivatives may indicate that the *N*-hydroxyacetamide is not the major contributing moiety for inhibition. It may be possible that the added substituents around the chromane heterocycle may switch the hydroxamic acid from a bidentate coordination to a monodentate one. Inhibition may be additionally driven by either the hydroxamate or other substituents engaging in hydrogen bonding with active site residues. While hydroxamic acids typically bind to metalloenzymes through a bidentate chelation mode using the two oxygen atoms of the hydroxamate,<sup>24</sup> they may also inhibit the enzyme through monodentate

coordination. For example, the metal binding group fragment, *N*-hydroxyacetamide, was found to inhibit carbonic anhydrase via monodentate chelation.<sup>24,71</sup> The deprotonated nitrogen atom of the hydroxamate chelates to Zn<sup>2+</sup>. The unusual coordination stems from an extensive hydrogen bonding network of the oxygen atoms with carbonic anhydrase residues.<sup>24,71</sup>

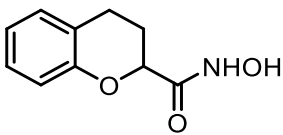
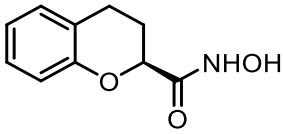
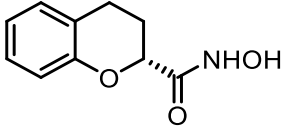
Another possible explanation for why we saw a similar inhibition profile for both enantiomers might be due to the limitations of the assay. At higher concentrations, the assay might provide a more accurate result for the enantiomers, as seen with (*S*)-**1-1** and (*R*)-**1-1**. However, at the relatively low concentrations used (< 500 nM), there might be more variability within the results. This would also apply to the absence of an observed trend between the C-7 aryl derivatives falling within the IC<sub>50</sub> range of 100-450 nM.

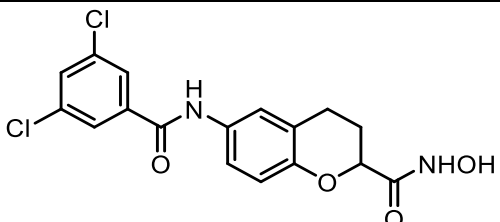
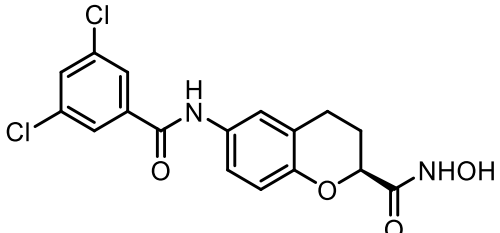
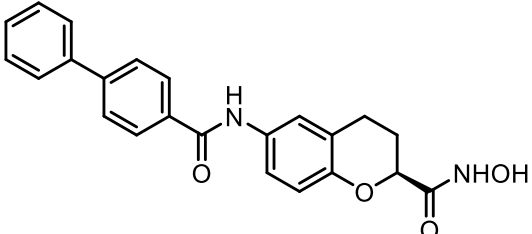
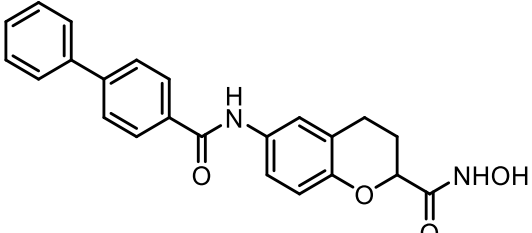
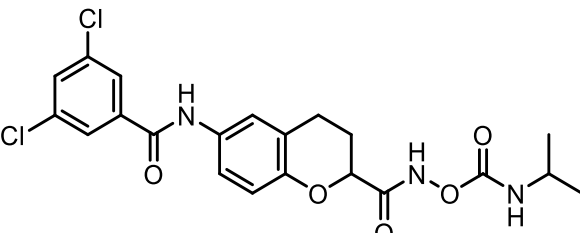
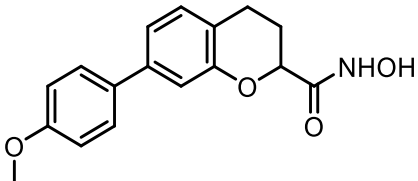
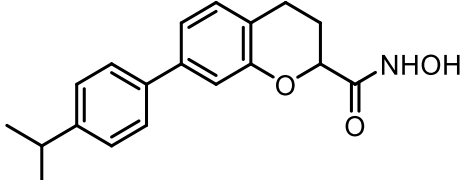
A select number of compounds were evaluated in fluorogenic HDAC assays (see **Appendix A** for protocol). We used a pan-HDAC assay using HeLa nuclear extracts; additionally, we used assays for each of the purified proteins of HDAC 4, 5, 6, 7, and 8. For the pan-HDAC assay, the compounds were mixed with the HeLa Nuclear extract and the HDAC substrate, Boc-Lys(Ac)-AMC (where AMC is 7-aminomethylcoumarin). Upon deacetylation of the lysine, the substrate is prone to proteolysis by a Lysine Developer solution containing the protease trypsin. This results in a fluorophore that is measured in a fluorescence plate reader (Excitation: 365 nm, Emission: 450 nm). The HDAC substrate that remains acetylated does not release the fluorophore upon treatment with the protease. The HDAC 4, 5, 6, 7 and 8 assays follow a similar mechanism as the pan-HDAC assay. However, the identity of the fluorophore and the protease have not been disclosed by the manufacturers.

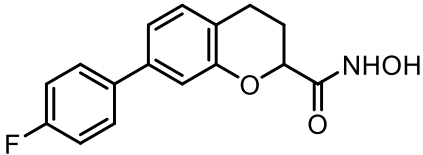
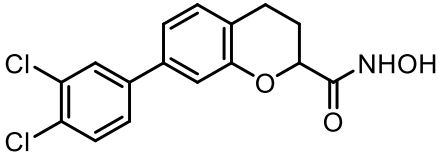
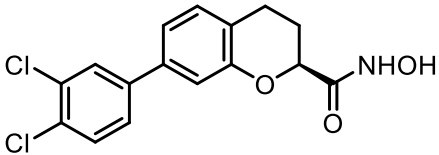
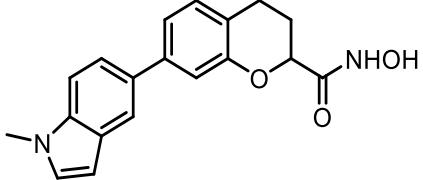
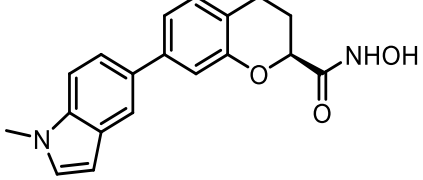
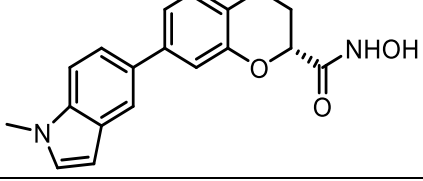
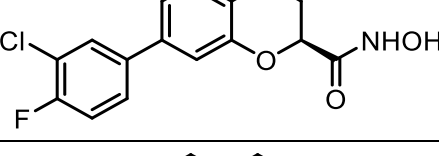
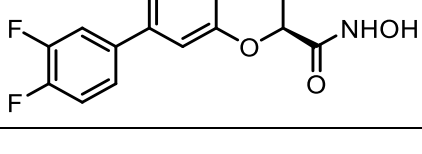
**Table 3** shows the percent inhibition of a select number of compounds in HDACs 6,7, and 8. The hydroxamic acids showed > 56% inhibition of HDAC6 at 200 nM with the 7-aryl

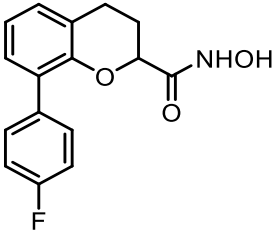
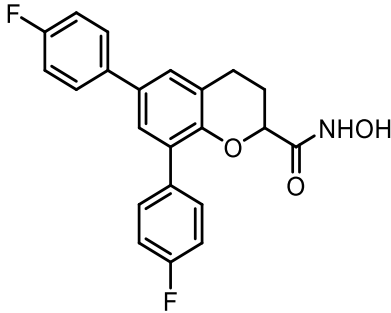
derivatives **1-55d**, **1-55e**, and the 6-benzamide derivative (*S*)-**1-40** displaying > 92% inhibition. The carbamate **1-41b** showed 71% inhibition; carbamates have been previously used as prodrugs for HDAC6 inhibitors where they were found to hydrolyze in cell culture.<sup>72</sup> When they were docked in the HDAC6 CD1, the hydroxamic acids **1-55d** and **1-76a** showed favorable interactions with the HDAC6 protein sidechains (**Figure 20**). For **1-55d**, the hydroxamic acid carbonyl oxygen was hydrogen-bonded with **Tyr363**, and the oxygen ion with **His192** (**Figure 20-top**). For **1-76a**, the hydroxamic acid carbonyl oxygen was hydrogen-bonded to **His193** and the oxygen anion and NH were hydrogen-bonded to **Tyr363** (**Figure 20-bottom**). For HDAC7 at 1  $\mu$ M, the compounds showed < 34% inhibition, except for the 7-aryl derivative **1-55a** that had 89% inhibition. For HDAC 8 at 1  $\mu$ M, the 7-aryl derivatives showed 27-83% inhibition, the benzamide (*S*)-**1-40a** showed 75% inhibition, and the carbamate **1-41b** showed no inhibition. The compounds shown in **Table 3** serve as pertinent examples demonstrating varying degrees of HDAC targeting effects.

**Table 2.** Artemis Inhibition Data.<sup>a</sup>

Entry	ID	Structure	IC <sub>50</sub> (nM)
1	<b>1-1<sup>b</sup></b>		2,403 $\pm$ 1,003 <sup>d</sup>
2	<b>(S)-1-1<sup>b</sup></b>		1,095 $\pm$ 285 <sup>e</sup>
3	<b>(R)-1-1<sup>b</sup></b>		3,105 $\pm$ 469 <sup>e</sup>

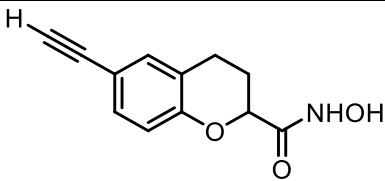
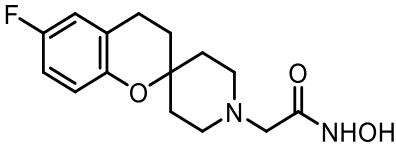
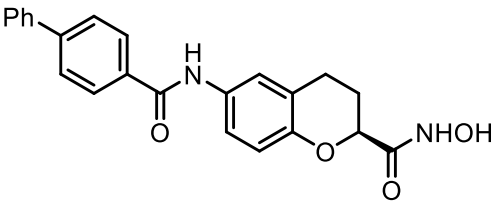
Entry	ID	Structure	IC <sub>50</sub> (nM)
4	1-40a		266 ± 29 <sup>e</sup>
5	(S)-1-40a <sup>b</sup>		237 ± 82 <sup>f</sup>
6	(S)-1-40b		71 ± 8 <sup>e</sup>
7	1-40b <sup>c</sup>		90 ± 13 <sup>e</sup>
8	1-41a		2,228 ± 255 <sup>e</sup>
9	1-55a		189 ± 25 <sup>e</sup>
10	1-55b		253 ± 40 <sup>e</sup>

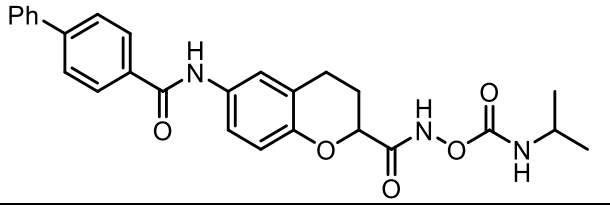
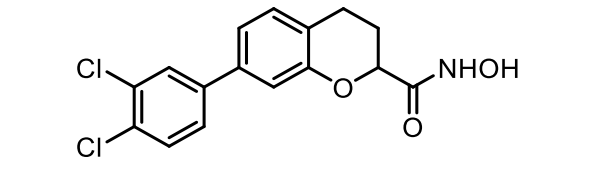
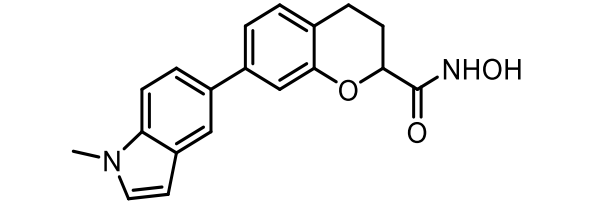
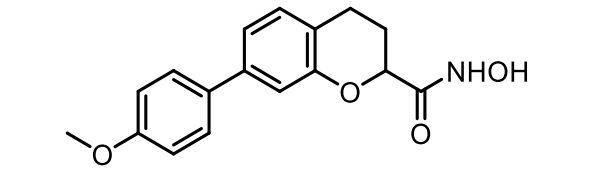
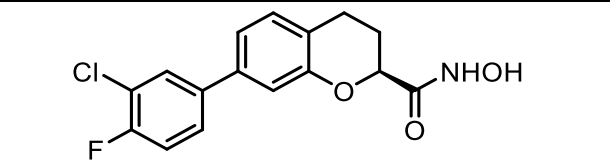
Entry	ID	Structure	IC <sub>50</sub> (nM)
11	1-55c		434 ± 146 <sup>e</sup>
12	1-55d		112 ± 21 <sup>e</sup>
13	(S)-1-55d		175 ± 95 <sup>e</sup>
14	1-55e		281 ± 162 <sup>f</sup>
15	(S)-1-55e		326 ± 49 <sup>f</sup>
16	(R)-1-55e		386 ± 42 <sup>f</sup>
17	(S)-1-55h		140 ± 49 <sup>e</sup>
18	(S)-1-55f		204 ± 83 <sup>e</sup>

Entry	ID	Structure	IC <sub>50</sub> (nM)
19	1-63		2,355 ± 205 <sup>e</sup>
20	1-66		5,265 ± 251 <sup>e</sup>

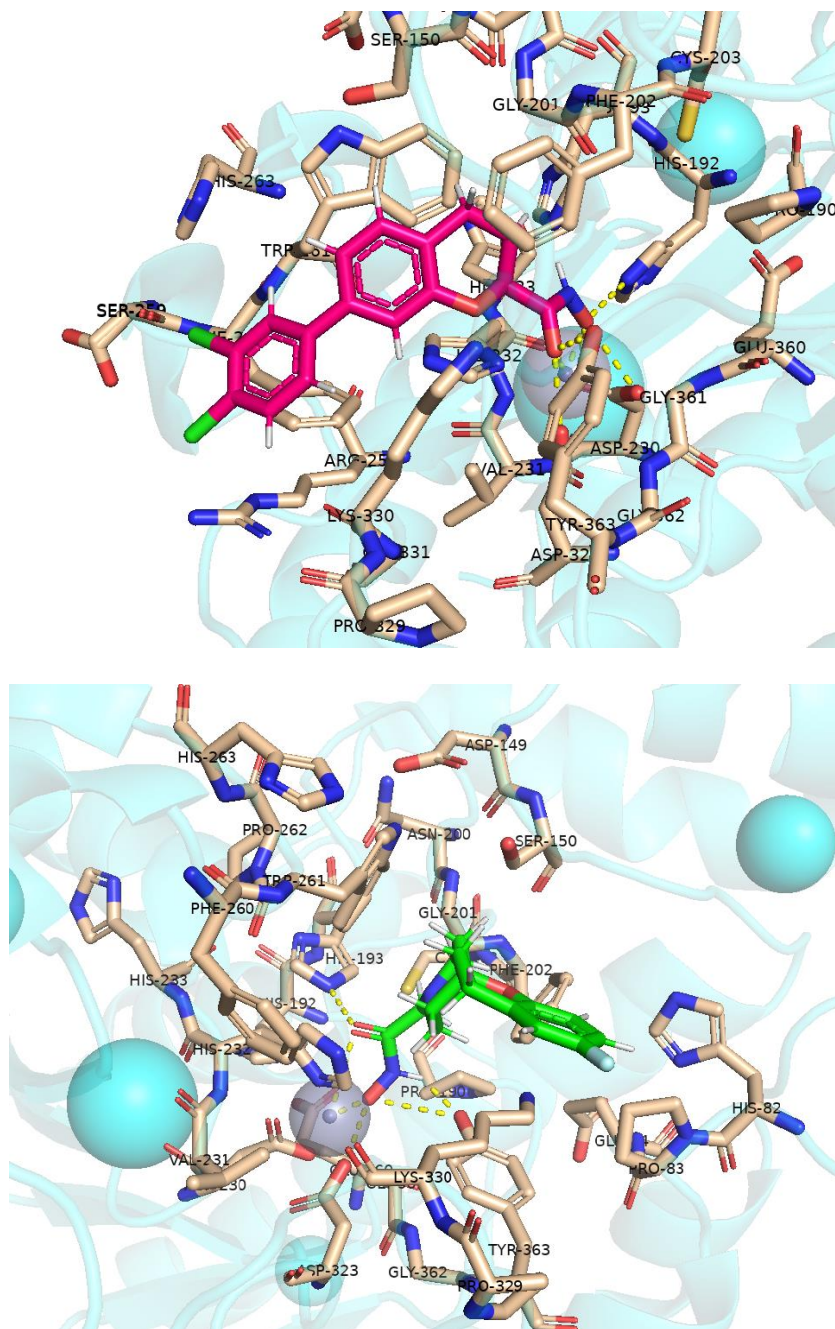
<sup>a</sup>The compounds were evaluated by our collaborators in Sanford Burnham Prebys Medical Discovery Institute (SBP). <sup>b</sup>Synthesized by Dr. Matt Laporte <sup>c</sup>Synthesized by Tyler Kristufek <sup>d</sup>Average from 12 measurements <sup>e</sup>Average from 4 measurements <sup>f</sup>Average from 8 measurements

**Table 3.** HDAC Inhibition Data.<sup>73</sup>

Entry	ID	Structure	HDAC 6 at 200 nM (%)	HDAC 7 at 1 μM (%)	HDAC 8 at 1 μM (%)
1	1-45		65	24	0
2	1-76a		56	22	0
3	(S)-1-40a		92	20	75

Entry	ID	Structure	HDAC 6 at 200 nM (%)	HDAC 7 at 1 $\mu$ M (%)	HDAC 8 at 1 $\mu$ M (%)
4	1-41b		71	19	0
5	1-55d		95	0	83
6	1-55e		92	22	27
7	1-55a		-	89	42
8	(S)-1-55h		-	34	49

<sup>a</sup>The compounds were evaluated by Taber Maskrey and Andrea Topacio (Wipf Group, University of Pittsburgh).

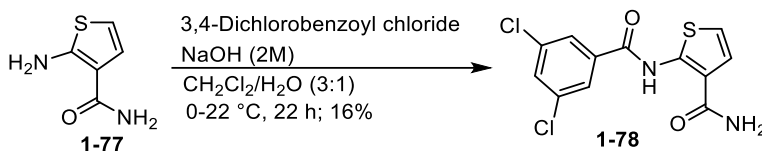


**Figure 20.** Docking of Hydroxamic Acids **1-55d** and **1-76a** in HDAC6-CD1. Docking studies with **1-55d** and **1-76a** on the crystal structure of HDAC6-CD1 (pdb: 5G0G) using the Chimera/Autodock Vina program.<sup>74</sup> Structures were processed using Pymol. Magenta licorice indicates **1-55d** and green licorice indicates **1-76a** docked in the HDAC6 CD1 active site; HDAC6 protein is indicated as a cyan cartoon, with the residues within 6Å of **1-55d** and **1-76a** depicted as wheat-colored licorice; Gray spheres indicates the active site zinc atom; dashes indicated polar contacts: hydrogen bonding or anion chelation to the active site metals.



### 1.2.3 Aminothiophene Series

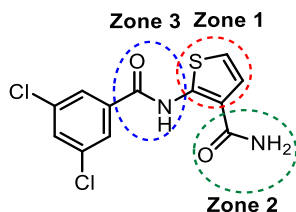
The lead thiophene **B** was synthesized using the Schotten-Baumann reaction conditions in dichloromethane and aqueous NaOH, as an alternative to the monophasic reaction conditions typically used for acylations (**Scheme 12**). Previous studies show that thiophenes unsubstituted at the 5-position are prone to acid-catalyzed polymerization,<sup>75</sup> and we observed decomposition during the reaction and product instability on SiO<sub>2</sub>. With the Schotten-Baumann reaction conditions, the biphasic mixture allows the product to be extracted from the water-soluble hydrogen chloride.



**Scheme 12.** Synthesis of **Hit B**.

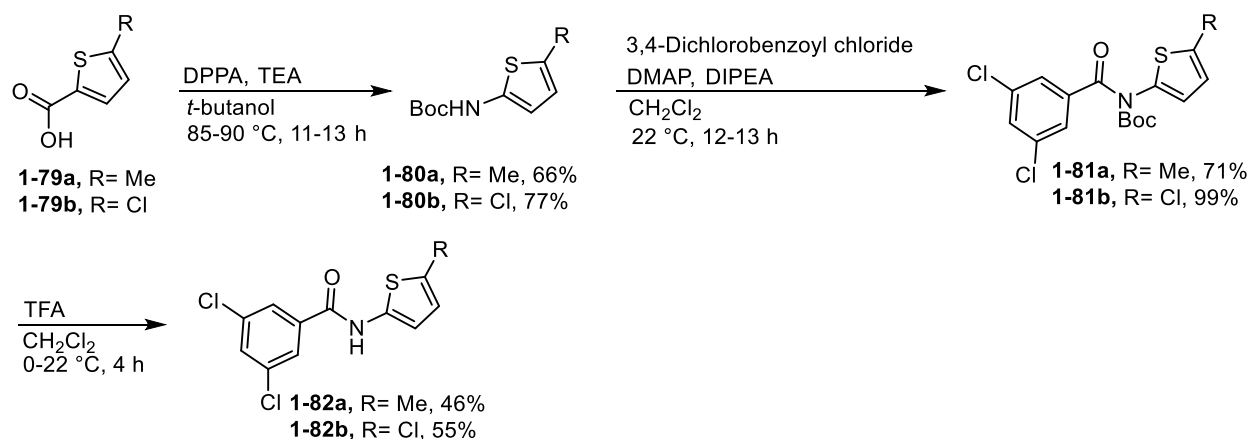
As discussed in **Section 1.1.4**, the thiophene ring is electron rich and is prone to undergo oxidative metabolism. A common way for medicinal chemists to reduce the metabolism of a heteroaromatic ring is by blocking the site of metabolism, changing the electron density of the ring, or reducing the hydrophobicity of the ring.<sup>51a</sup> For example, replacing C(3) of the thiophene with a nitrogen atom lowers the energy levels of the  $\pi$  orbitals, making the thiazole less  $\pi$ -electron rich.<sup>54</sup> Additionally, the thiazole ring (clogP: 0.49) is more polar than the thiophene ring (clogP: 1.79).<sup>51a</sup> We mainly focused on reducing the electron density of the aromatic ring by replacing the thiophene ring with a thiazole, pyridine, and phenyl ring (**Figure 21, zone 1**). Additionally, we

also blocked the thiophene C(5)-site that is prone to oxidative metabolism. For SAR purposes, we additionally looked at removing the **zone 2** and **zone 3** carboxy functionalities.



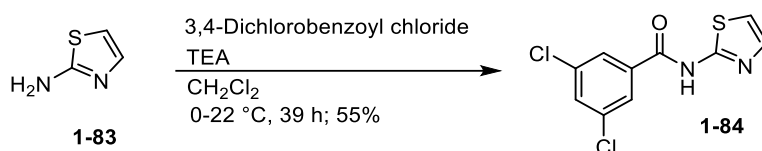
**Figure 21.** Zone Break-down Model of Hit B.

The thiophene carboxylic acids **1-79a,b** were treated with DPPA and triethylamine, forming acyl azides, which underwent a Curtius rearrangement to install the aminothiophenes **1-80a,b** (Scheme 13). Acylation of the aminothiophenes and Boc deprotection of **1-81a,b** provided the thiophenes **1-82a,b**.

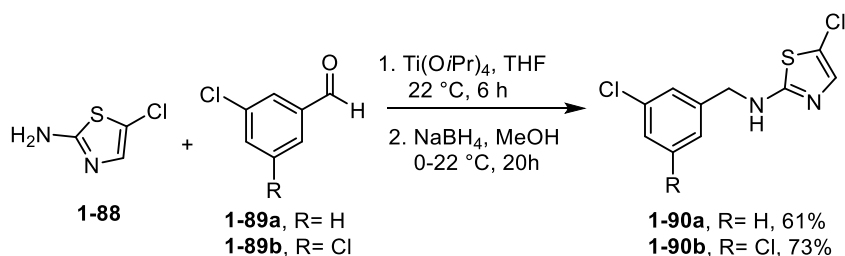
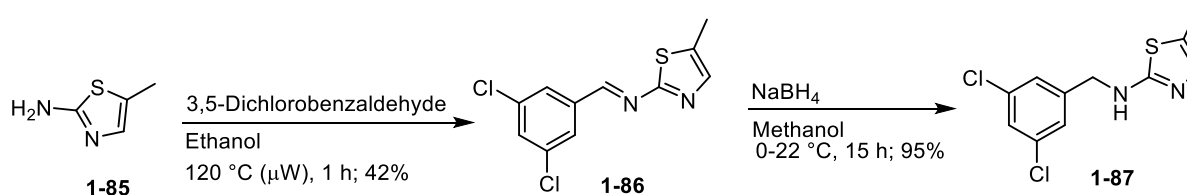


**Scheme 13.** Synthesis of Thiophene Derivatives from the Curtius Rearrangement.

Replacing C(3) of the thiophene with a nitrogen atom lowers the energy levels of the  $\pi$  orbitals, making the thiazole less  $\pi$ -electron rich,<sup>54</sup> and we therefore pursued several thiazole derivatives as a replacement to the zone 2 thiophene ring. The 2-aminothiazole was acylated with 3,5-dichlorobenzoyl chloride, yielding the acylated aminothiazole **1-84** (Scheme 14). Other transformations based on the removal of the carbonyl group of the benzamide via reductive amination are shown in Scheme 15.



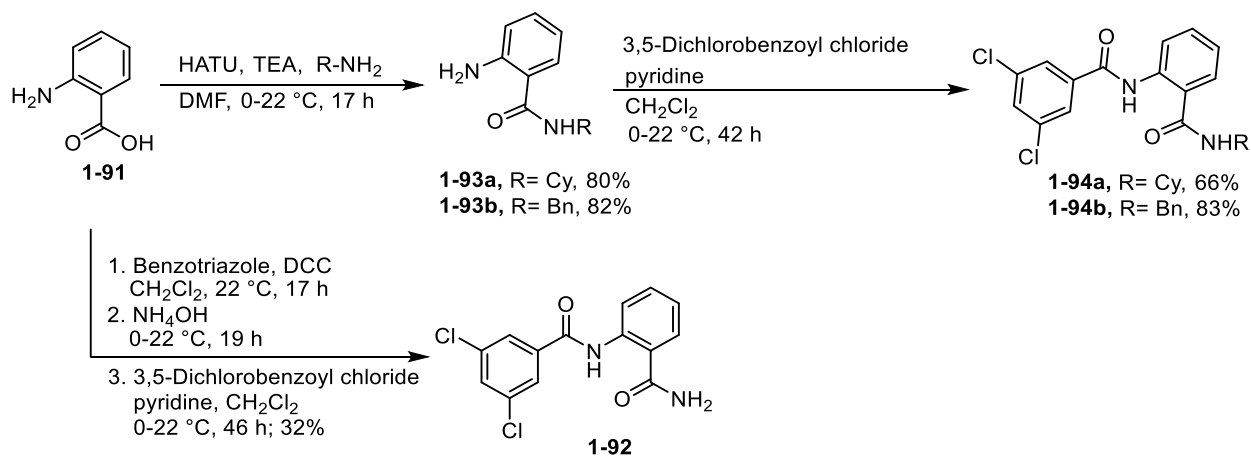
**Scheme 14.** Synthesis of Thiazole **1-84**.



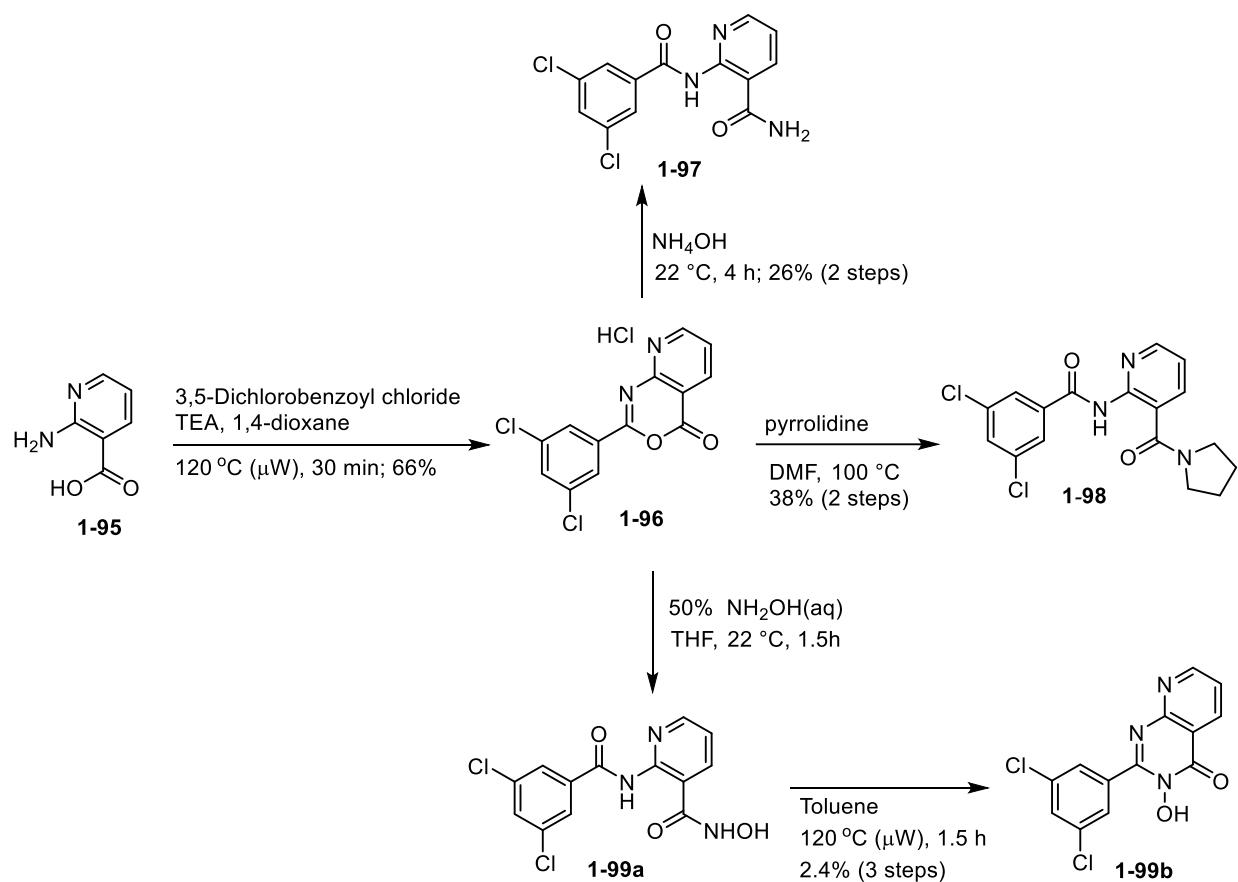
**Scheme 15.** Synthesis of *N*-Benzyl Thiazole Derivatives.

The next analogs that were synthesized involved replacing the **zone 2** thiophene with phenyl (Scheme 16) and pyridine (Scheme 17), while retaining the zone 1 amide. For the synthesis

of the pyridine derivatives, 2-aminonicotinic acid **1-95** was acylated with 3,5-dichlorobenzoyl chloride, which cyclized upon acidification, yielding the oxazolone **1-96**. Treatment of **1-96** with ammonium hydroxide and pyrrolidine opened the ring to form the primary amide **1-97** and the tertiary amide **1-98**, respectively. To introduce a hydroxamic acid, **1-96** was treated with aqueous hydroxylamine. The linear hydroxamic acid **1-99a** was initially observed before its gradual cyclization to **1-99b**. To complete cyclization, microwave irradiation at 120 °C in toluene completed the formation of **1-99b**.



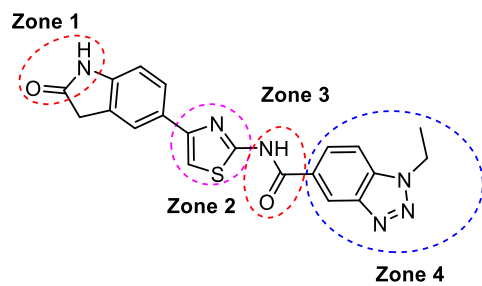
**Scheme 16.** Synthesis of *N*-(2-Carbamoylphenyl)-3,5-dichlorobenzamide Derivatives.



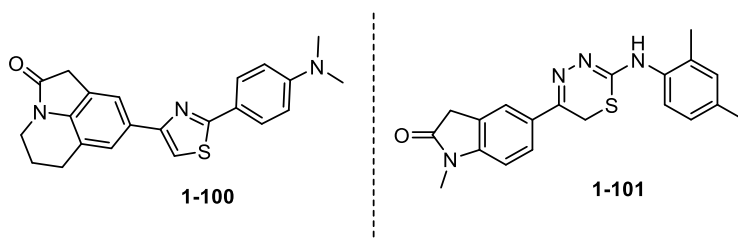
**Scheme 17.** Synthesis of 2-(3,5-Dichlorobenzamido)nicotinamide Derivatives.

### 1.2.4 Aminothiazole Series

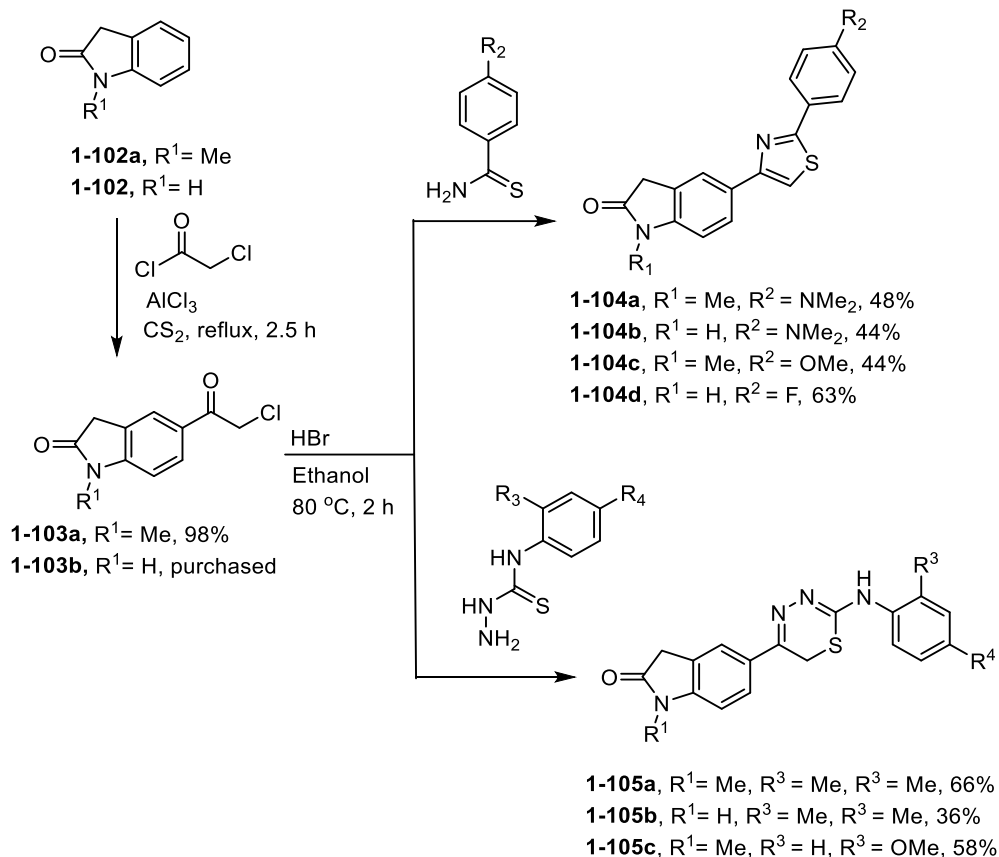
Analogs of hit compound **C** consisted of modifying the **zone 1** and **zone 3** amides; **zone 2** thiazole, and **zone 4** aryl group (**Figure 22**). We chose those zones based on previous work that has shown potency in derivatives **1-100** and **1-101** (**Figure 23**). The reaction between the chloroacetyl indolones **1-103a,b** and either thioamides or thiosemicarbazides produced thiazoles **1-104a-d** or thiadiazines **1-105a-c**, respectively (**Scheme 18**). The chloroacetyl indolones **1-103a,b** were either prepared by Friedel-Crafts acylation of an indolone or were purchased.



**Figure 22.** Zone Break-down Model of **Hit C**.

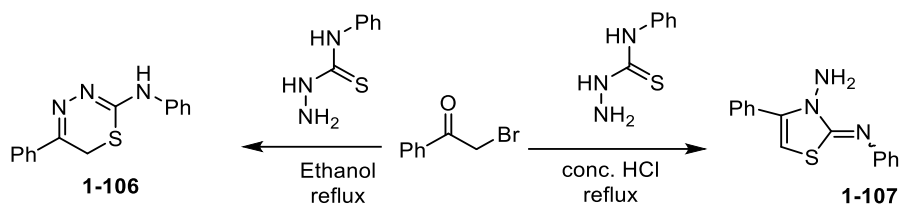


**Figure 23.** Previous Potent Derivatives of **Hit C**.



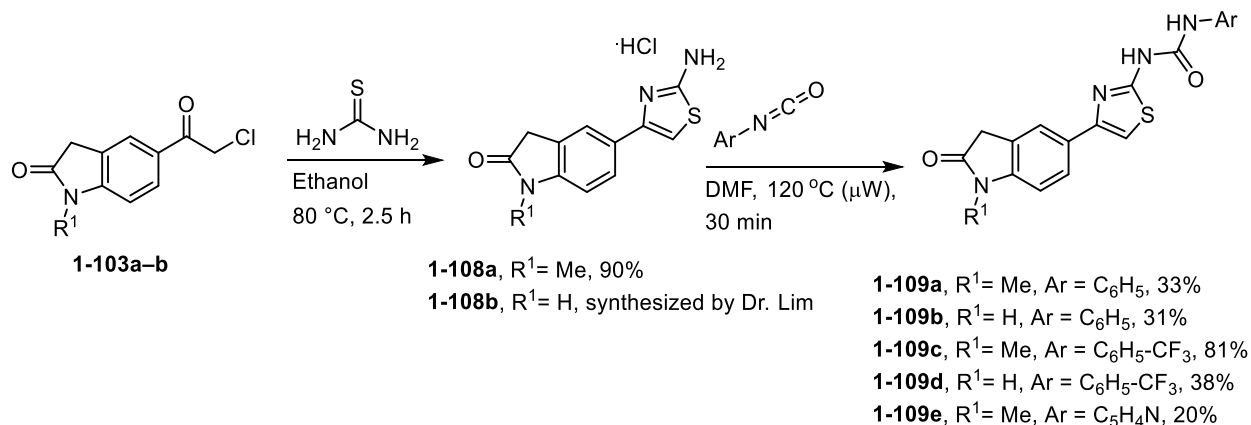
**Scheme 18.** Synthesis of Thiazole and Thiadiazine Derivatives.

A previous report<sup>76</sup> showed that 4-phenylthiosemicarbazide and phenacylbromide heated at reflux in ethanol favored the thiadiazine **1-106** (**Figure 24**) while reflux in concentrated hydrochloric acid favored the thiazolimine **1-107**. While the reaction conditions outlined in **Scheme 19** used 1-3% HBr in ethanol, the possibility of forming a thiazolimine was not ruled out. HSQC analysis of **1-105b** shows the thiadiazine methylene protons,  $\delta$  3.87, correlating to the methylene carbon,  $\delta$  22.95. These shifts match those reported for **1-106**,  $\delta$  3.74 and 22.17, respectively. Additionally, no alkene methine protons of the thiazolimine are detectable for any of the thiadiazine derivatives synthesized.

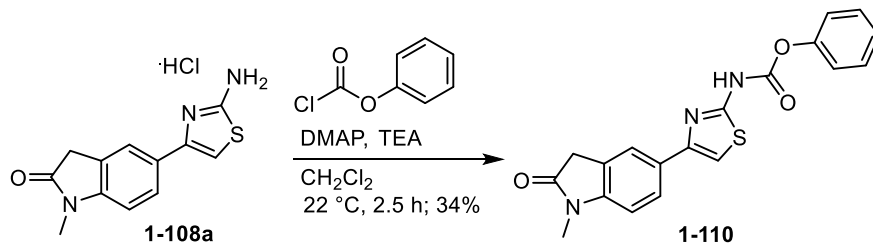


**Figure 24.** Previously Reported<sup>76</sup> Cyclization between Phenylacetyl Bromide and Thiosemicarbazide.

The aminothiazole intermediates **1-108a** and **1-108b** were synthesized from chloroacetylindolone and thiourea (**Scheme 19**). Reaction with the aryl isocyanates formed the ureas **1-109a–e**. The carbamate **1-110** was synthesized by acylation of chloroformate with the aminothiazole **1-108a** (**Scheme 20**).



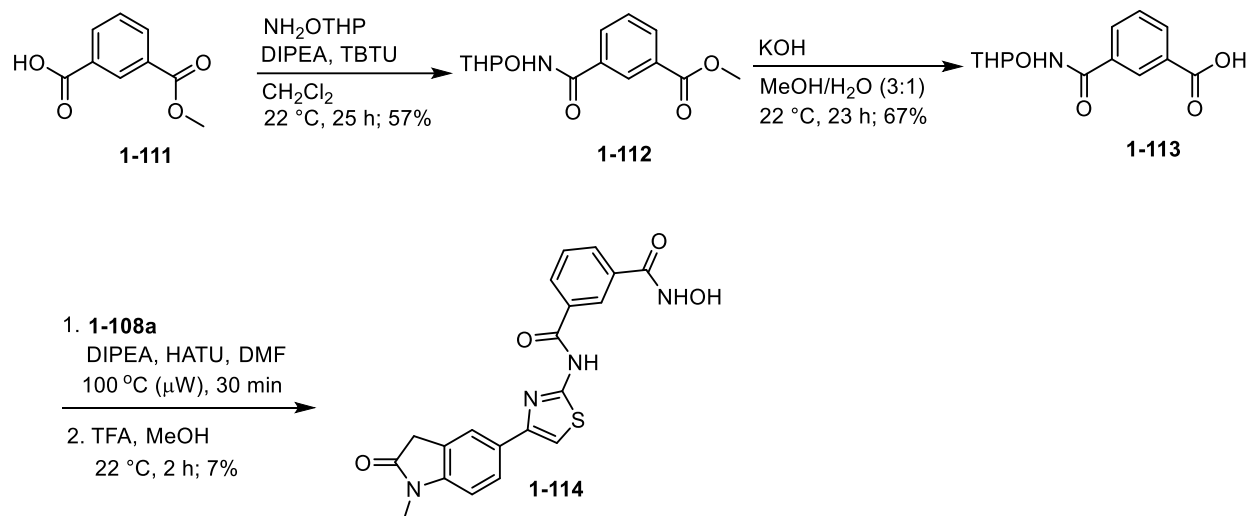
**Scheme 19.** Synthesis of Aminothiazole Derivatives.



**Scheme 20.** Synthesis of Aminothiazole Derivative **1-110**.



3-(Methoxycarbonyl) benzoic acid **1-111** was coupled with THP-protected hydroxylamine, followed by saponification of **1-112** (Scheme 21). The acid **1-113** was coupled to the aminothiazole **1-108a**, and subsequently deprotected with TFA, forming the hydroxamic acid **1-114** in a poor yield of 7%.



**Scheme 21.** Synthesis of Aminothiazole Derivative **1-114**.

### 1.3 Conclusion

We explored multiple substitution patterns of the *N*-hydroxychromane-2-carboxamide scaffold and found that the 7-aryl and 6-amino derivatives revealed our most promising candidates as Artemis inhibitors. These derivatives showed a 5- to 20-fold increase in potency compared to the unsubstituted *N*-hydroxychromane-2-carboxamide. Additionally, we prepared enantioenriched analogs (> 95% ee) that showed no increase in activity compared to the racemic compounds. While these chromane analogs inhibited Artemis, they have also been shown to target histone

deacetylases (HDACs), another family of metalloenzymes. None of the 2-amino-3-carboxythiophene nor 2-aminothiazole analogs have shown significant Artemis inhibition. Nevertheless, most of the compounds synthesized remain novel structures not previously published in the literature.

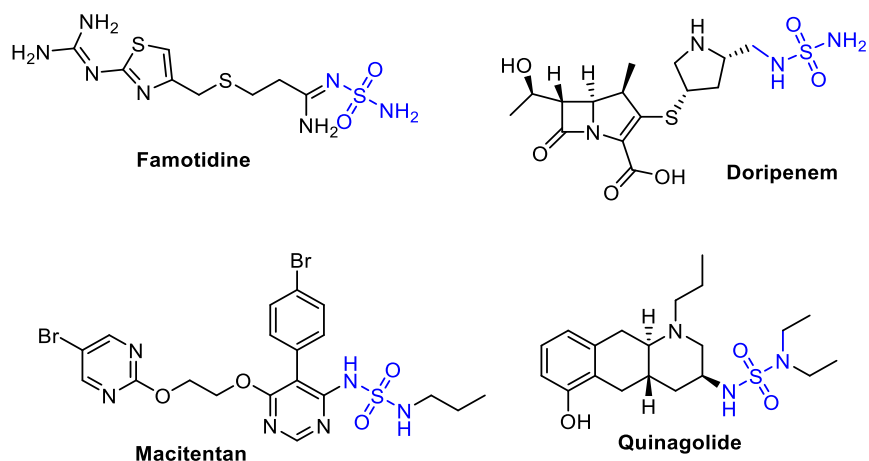
## 2.0 Thiadiazines as HSP70 Agonists

### 2.1 Introduction

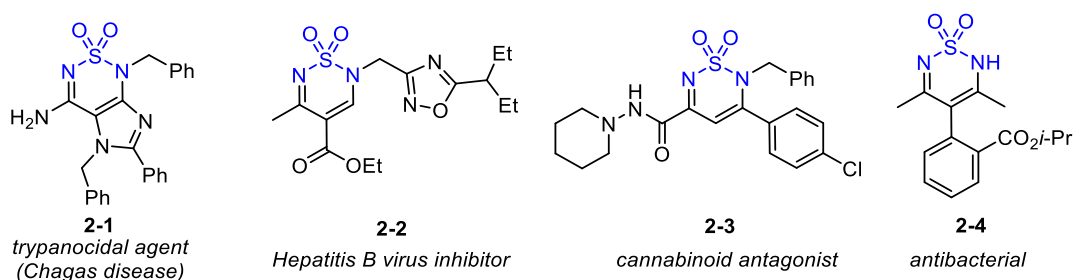
#### 2.1.1 Sulfamides in Medicinal Chemistry

The sulfamide moiety exhibits favorable physicochemical properties, such as enhanced water solubility and bioavailability,<sup>77</sup> and has therefore been incorporated in pharmaceutical compounds. FDA-approved drugs containing the sulfamide functional group are shown in **Figure 25**,<sup>78</sup> none of which include sulfamide-containing heterocycles. Cyclic structures have been used by medicinal chemists for their advantageous molecular properties: scaffold rigidity, 3-dimensionality, and electronic distribution.<sup>79</sup> Each year, on average 28% of new drugs contain one new ring system.<sup>79</sup> Additionally, ring systems can cross therapeutic areas and target classes.<sup>79</sup>

The Wipf Group has previously worked on accessing a subclass of the sulfamide-containing heterocycles, the 1,2,6-thiadiazine 1,1-dioxide scaffold. The thiadiazine scaffold has been shown to exhibit favorable biological properties (**Figure 26**), as trypanocidal agents (Chagas disease) (**2-1**),<sup>80</sup> Hepatitis B virus inhibitors (**2-2**),<sup>81</sup> cannabinoid antagonists (**2-3**),<sup>82</sup> and antibacterial agents (**2-4**).<sup>83</sup>



**Figure 25.** Sulfamide-containing Approved Drugs.

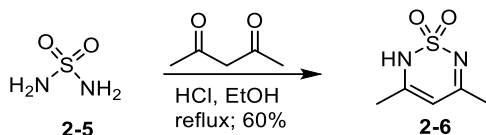


**Figure 26.** Thiadiazine-containing Bioactive Compounds.

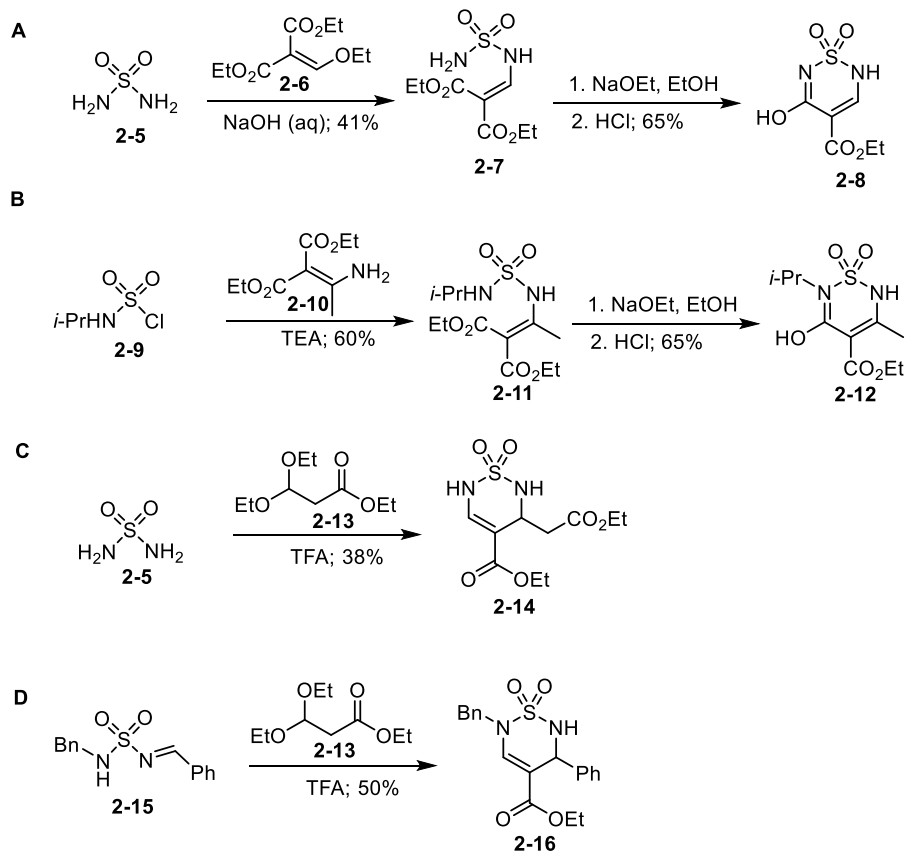
### 2.1.2 The 1,2,6-Thiadiazine 1,1-Dioxide Scaffold

The first synthesis of 1,2,6-thiadiazine 1,1-dioxide was completed in 1952 by condensation of the sulfamide **2-5** and pentanedione (**Scheme 22**).<sup>84</sup> Later reports employed substituted derivatives of pentanedione to establish substitutions around the heterocycle.<sup>85</sup> Over the years, more synthetic efforts have been pursued to establish the addition of more functional groups around the heterocycle, including the C-5 hydroxy thiadiazine **2-8** from the condensation of sulfamide **2-5** and ethoxy methylene **2-6**, followed by base-mediated cyclization

of **2-7** (**Scheme 23A**).<sup>86</sup> Using a modified strategy, a 6-*N*-alkylated C-5 hydroxy thiadiazine **2-9** was prepared from the monoalkylated sulfamoyl chloride **2-10** and amino methylene **2-11** (**Scheme 23B**).<sup>87</sup> When the sulfamide **2-5** was condensed with the 3,3-diethoxy propanoate **2-13**, the thiadiazine **2-14** with substitution at C(3) and C(4) was obtained (**Scheme 23C**).<sup>88</sup> Similar acid-mediated condensations with the sulfamide imine **2-15** and the 3,3-diethoxypropanoate **2-13** afforded the trisubstituted thiadiazine **2-16** (**Scheme 23D**).<sup>89</sup>



**Scheme 22.** The First 1,2,6-Thiadiazine 1,1-Dioxide Synthesis.

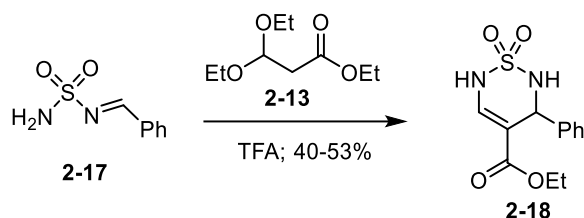


**Scheme 23.** Previous 1,2,6-Thiadiazine 1,1-Dioxide Syntheses.

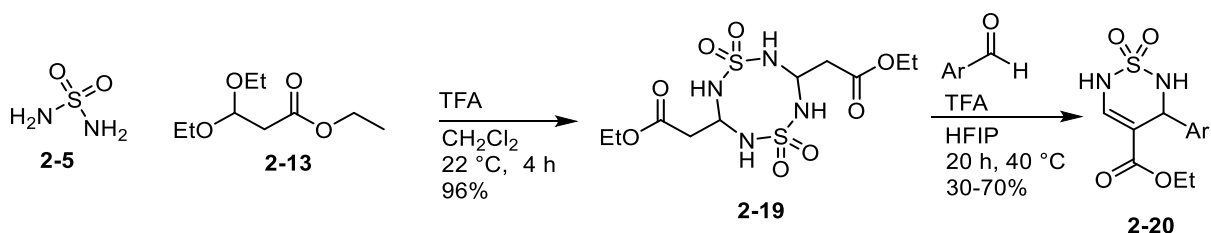
### 2.1.2.1 Wipf Group Strategy: Previous Work

The Wipf group was interested in accessing the thiadiazine scaffold in a convergent manner and in a way that would allow the possibility to functionalize it in several positions. They started by using literature conditions<sup>88-89</sup> for the condensation of the sulfamide imine **2-17** with the 3,3-diethoxy propanoate **2-13** (**Scheme 24**). The reaction times were long and the yields were low. After extensive efforts, they found that condensation of the unsubstituted sulfamide **2-5** with **2-13** produced a 8-membered sulfamide dimer **2-19**<sup>86</sup>

(Scheme 25). The dimer was condensed with benzaldehyde to afford the desired thiadiazine 2-20. A range of acidic conditions were tested before identifying a HFIP and TFA mixture.



Scheme 24. Initial Attempt to Access the 1,2,6-Thiadiazine 1,1-Dioxide Scaffold.



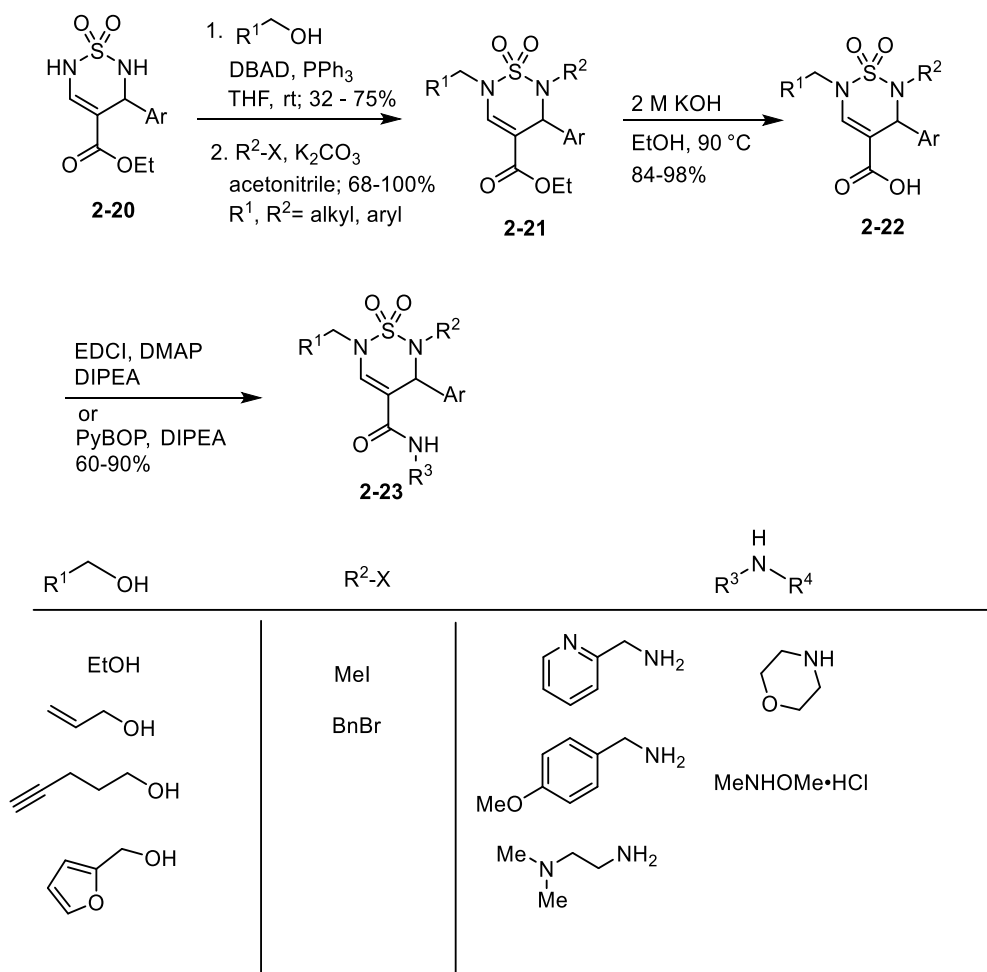
Scheme 25. Wipf Group Strategy to Access the 1,2,6-Thiadiazine 1,1-Dioxide Scaffold.

With the thiadiazine heterocycle in hand, the group worked on selectively functionalizing it by exploiting the difference in acidity of the two protons of the sulfamide, considering that the vinylogous carbamate sulfamide N(6)-H (pK<sub>a</sub><sup>1</sup> ca. 9.2) is more acidic than N(2)-H (pK<sub>a</sub><sup>2</sup> ca. 9.5).<sup>90</sup> Selective alkylation at the more acidic N(6) via a Mitsunobu reaction followed by a base-mediated alkylation at N(2) with an alkyl halide afforded the alkylated derivative 2-21 (Scheme 26). The Mitsunobu reaction (Figure 27) starts with a nucleophilic addition of the triphenyl phosphine to the N=N bond of di-*tert*-butyl azodicarboxylate, generating the zwitterionic adduct 2-20a, which deprotonates the thiadiazine 2-20. The positively charged phosphorous in 2-20b is attacked by the alcohol, forming the 2-20e adduct, which is deprotonated

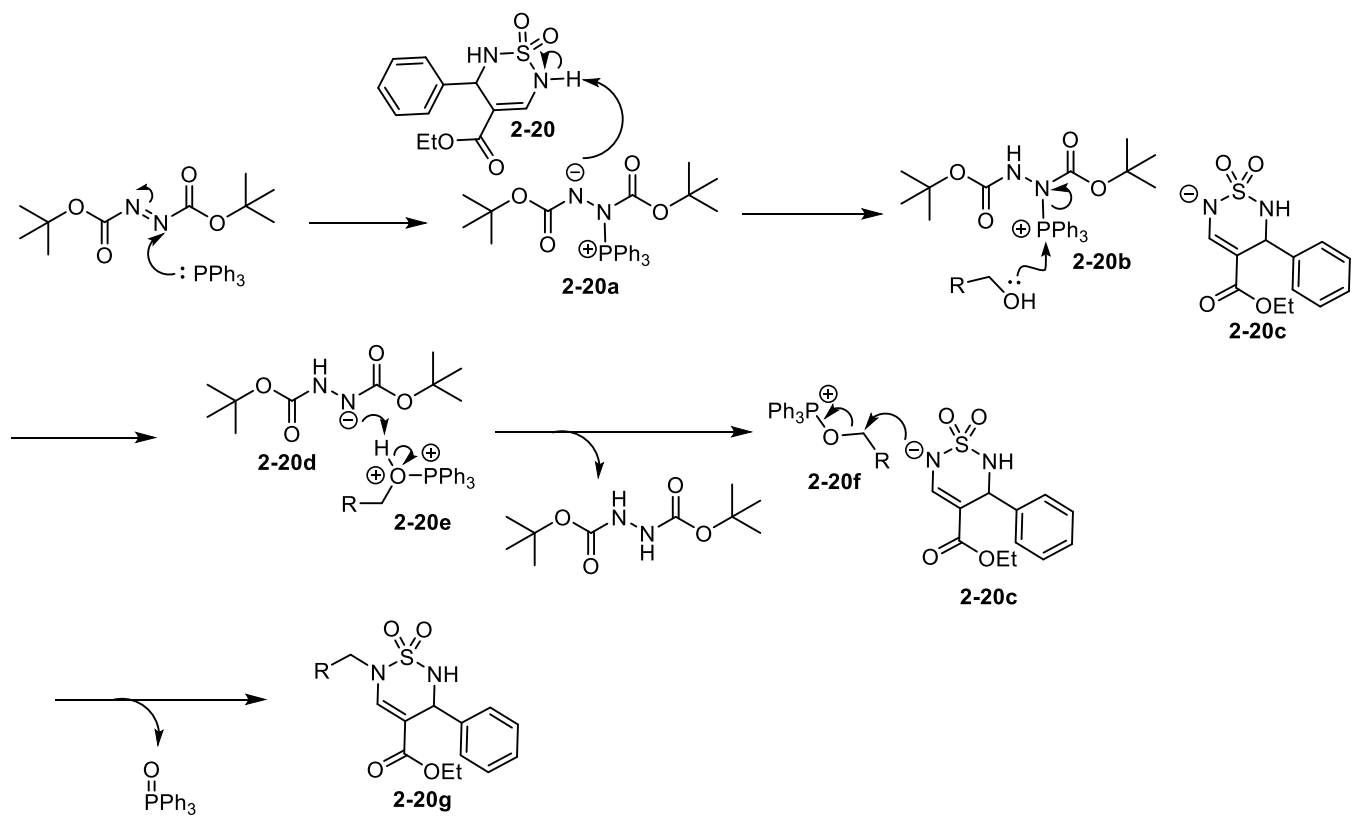
by the nitrogen anion of **2-20d**. Finally, the deprotonated thiadiazine nucleophile **2-20c** attacks the electrophilic oxyphosphonium **2-20f** via an  $S_N2$  reaction at the carbon, forming the desired (N)-6-alkylated thiadiazine **2-20g** and the triphenylphosphine oxide byproduct. The regiochemistry was determined by NOESY correlations between the methylene hydrogens of the allyl derivative and the hydrogen of the thiadiazine alkene. Additionally, N(2)-H coupling with C(3)-H are typically seen by  $^1\text{H}$  NMR. In addition to alkylation at N(6) being driven by the more acidic N(6)-H, it is possible that it may also be driven by sterics. Supposing that the N(2)-deprotonated thiadiazine **2-20h** is the nucleophilic partner of the Mitsunobu, the  $S_N2$  reaction may be hindered by the presence of the phenyl group (**Figure 28**).

Saponification of the ethyl ester **2-21** to **2-22** with subsequent amide coupling resulted in the vinylogous ureas **2-23**. For this work, we were interested in introducing new functional groups to  $R^1$  and  $R^2$  to expand the selective transformations on N(2), N(6), and C(4) while making medically relevant compounds for our HSP70 and HDAC6 projects (**Figure 29**).

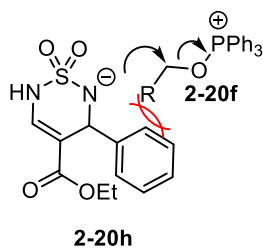




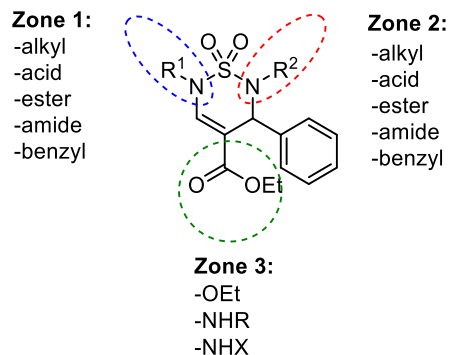
**Scheme 26.** Selective Functionalizations of the Thiadiazine.



**Figure 27.** Mitsunobu Reaction Mechanism.



**Figure 28.**  $\text{S}_{\text{N}}2$  Reaction between **2-20h** and **2-20f**.

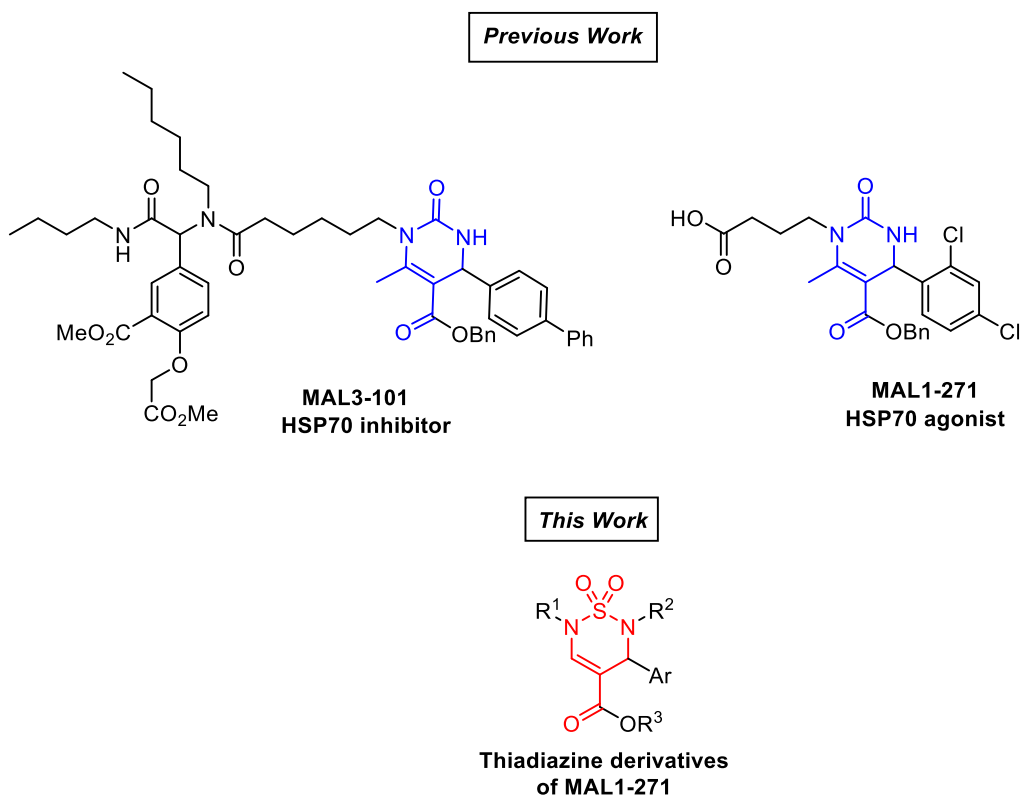


**Figure 29.** Zone Break-down of the Thiadiazine Scaffold.

### 2.1.3 HSP70

Our initial target naturally became HSP70, with the Wipf group's extensive work on bioactive dihydropyrimidones<sup>91</sup> classified as either HSP70 inhibitors or agonists. HSP70 proteins are ATP-dependent molecular chaperones that maintain overall protein homeostasis by regulating protein folding, assembling newly synthesized proteins, refolding misfolded proteins, and transporting proteins across intracellular membranes.<sup>92</sup> HSP70 protects cells from ER stress-induced apoptosis by prolonging XBP1 splicing,<sup>93</sup> is up-regulated in response to protein-homeostasis targeted therapies, and can facilitate tumor cell growth.<sup>92b,94</sup> The J-domain protein class of co-chaperones, HSP40, enhances HSP70's weak ATPase activity.<sup>91b</sup> The HSP70/HSP90 chaperone system is upregulated in cancer cells, and inhibiting the complex is expected to provide anticancer effects.<sup>93,95</sup> HSP70 inhibition by MAL3-101 (**Figure 30**) has been investigated in multiple myeloma (MM),<sup>92b</sup> rhabdomyosarcoma (RMS),<sup>96</sup> and Merkel cell carcinoma (MCC) cells.<sup>92a</sup> In as much as HSP70 inhibition may be promising for cancer and therapy, HSP70 modulation has also shown its therapeutic advantage in neurodegenerative diseases associated with  $\alpha$ -synuclein aggregation. More recently, the HSP70 agonist, MAL1-271 (**Figure 30**), displayed a

decrease in  $\alpha$ -synuclein aggregation in a Parkinson's Disease model.<sup>91e</sup> We wanted to explore the sulfamide of the thiadiazine heterocycle as a bioisosteric replacement of the urea substructure in MAL1-271-type derivatives (**Figure 30**).



**Figure 30.** Dihydropyrimidone Modulators of HSP70.

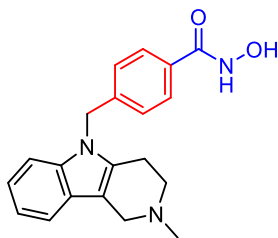
#### 2.1.4 HDAC6

While our initial focus was to synthesize agonists for HSP70, HDAC as a target became of interest due to our experience working with hydroxamic acids. The approved HDAC inhibitors for cancer treatment target multiple HDACs, and their poor selectivity might limit their clinical use.<sup>97</sup> The HDAC inhibitor pharmacophore (**Figure 31**) consists of a cap group that interacts with the

active site amino acid residues and is attached to a linker that directs the zinc-binding-group (ZBG) through a hydrophobic channel.<sup>35a,98</sup> As mentioned in Chapter 1, the ZBG chelates to the active site  $Zn^{2+}$  and is thus a crucial component for enzyme inhibition. Selectivity towards HDAC6 stems from the “cap” group being large and rigid.<sup>35a</sup> With its two catalytic domains, CD1 and CD2, being active,<sup>97</sup> the CD2 domain is comprised of a large basin approximately 14 Å wide,<sup>98-99</sup> and many selective HDAC 6 inhibitors have targeted CD2 by containing a bulky and rigid cap to fit in the broad rim of the pocket.<sup>99</sup> Tubastatin A (**Figure 32**) was one of the earliest HDAC6 selective compounds.<sup>100</sup> The short, bulky *N*-benzyl linker in Tubastatin A and related HDAC6 inhibitors has proven to be beneficial for selectivity.<sup>35a,97,100-101</sup> We wanted to use a synthetic route that can convergently install the three motifs necessary for HDAC6 inhibition, and we decided to use thiadiazine **2-20** as a starting point for HDAC6 inhibitors as well.



**Figure 31.** HDAC Inhibitor Pharmacophore.

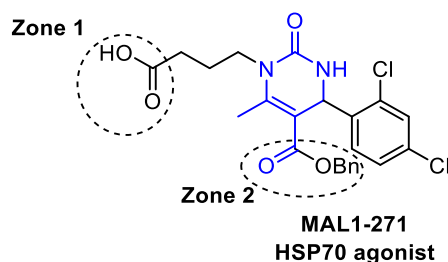


**Figure 32.** Structure of Tubastatin A.

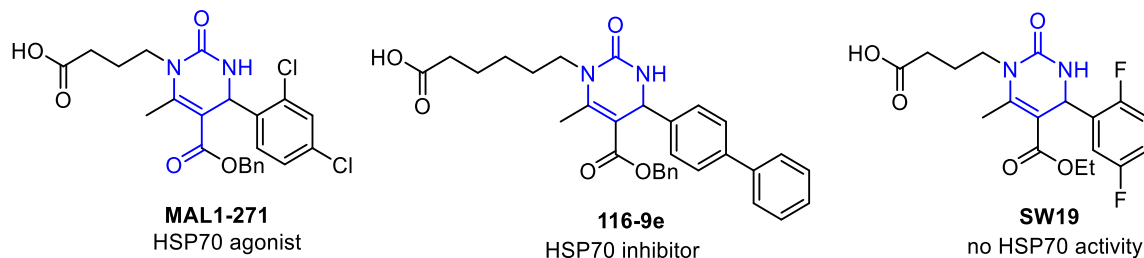
## 2.2 Results and Discussion

### 2.2.1 Compound Design Approach for HSP70

In designing our compounds, we were interested in keeping the 2,4-dichlorophenyl moiety as it was determined to be crucial for HSP70 agonistic activity in the urea derivatives.<sup>91e</sup> We focused on derivatizing the zone 1 and zone 2 carbonyl groups (**Figure 33**) as a good starting point to our chemoselective pursuits. When compared to MAL1-271, replacement of the chlorinated benzene with a diphenyl group (**116-9e**) showed HSP70 inhibition (**Figure 34**), possibly due to the bulky substituent interfering with J-domain interactions.<sup>91c,102</sup> No SAR studies have been done on the benzyl ester replacement, and it is not certain whether a loss in HSP70 modulation in **SW19** might have additionally been caused by the ethyl ester at C(4).<sup>91e</sup>



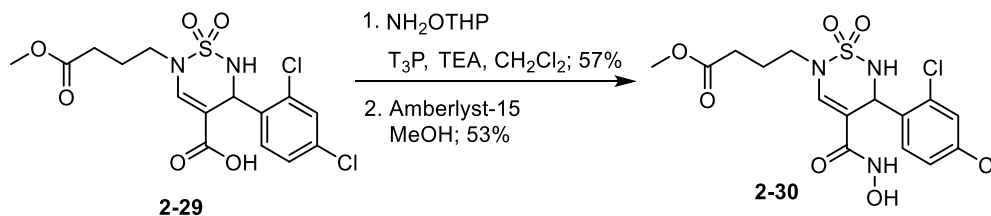
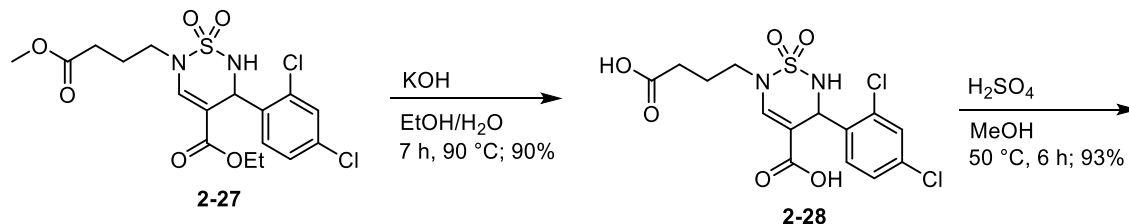
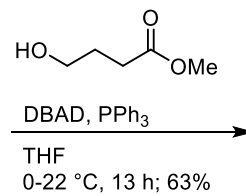
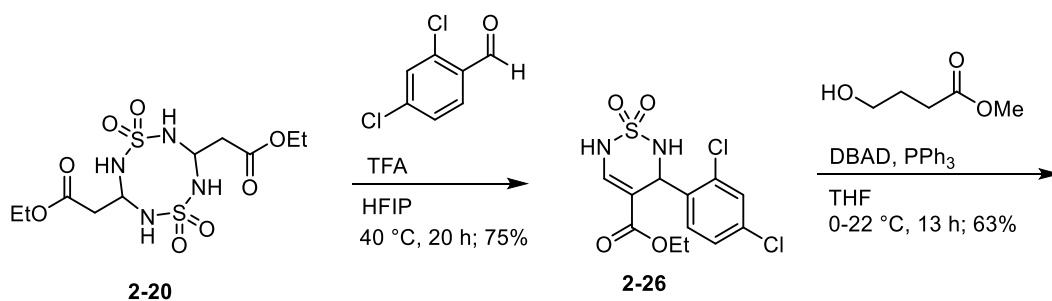
**Figure 33.** Zone Break-down Model of MAL1-271.



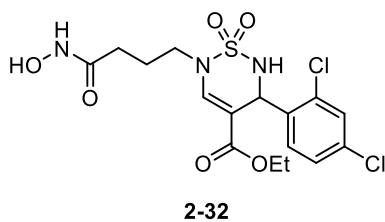
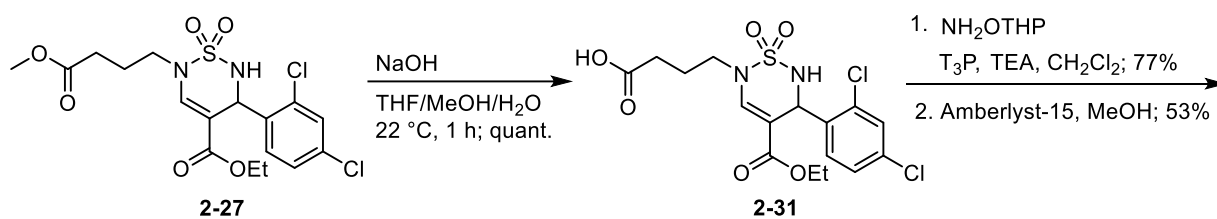
**Figure 34.** Select MAL1-271 Derivatives and their HSP70 Modulation.

### 2.2.2 Selective Functionalization at C(4) and N(6) of the Thiadiazine

We used 2,4-dichlorophenyl benzaldehyde for the condensation with **2-20** to form the heterocycle **2-26** (**Scheme 27**). A Mitsunobu reaction with methyl 4-hydroxybutanoate to install the butyrate, followed by saponification of the nonelectrophilic C(4) carboxylic acid at 90 °C for 7 h yielded the bis-carboxylic acid **2-28**. Selective esterification of the linker carboxylic acid to **2-29** was achieved through H<sub>2</sub>SO<sub>4</sub>/MeOH at 50 °C for 6 h, with no esterification of the conjugated carboxylic acid observed. HMBC correlation between the carbonyl carbon (172.7 ppm) of the methyl ester with both the methyl protons (3.61 ppm) and the  $\alpha$ -methylene protons (2.40 ppm) confirmed the regioselectivity. The N(6) electron donation to the carboxylic acid at C(4) renders it less reactive in Fischer esterifications. Esterifications of vinylogous carbamic acids are more commonly achieved through coupling reagents, and methyl ester formation has been achieved through methylation with dimethyl sulfate.<sup>103</sup> Coupling with T<sub>3</sub>P and Amberlyst-15-mediated deprotection afforded the hydroxamic acid **2-30**. Selective saponification of **2-27** was completed at room temperature, with further conversion of **2-31** to the hydroxamic acid **2-32** (**Scheme 28**). The regioselectivity of the saponification site of **2-27** was easily determined by the remaining ethyl ester as seen from the <sup>1</sup>H NMR. Overall, both the acids in **2-29** and **2-31** were easily converted to the hydroxamic acids under similar reaction conditions with approximately similar yields.



**Scheme 27.** Thiadiazine Derivatives **2-27**, **2-28**, **2-29**, and **2-30** of MAL1-271.

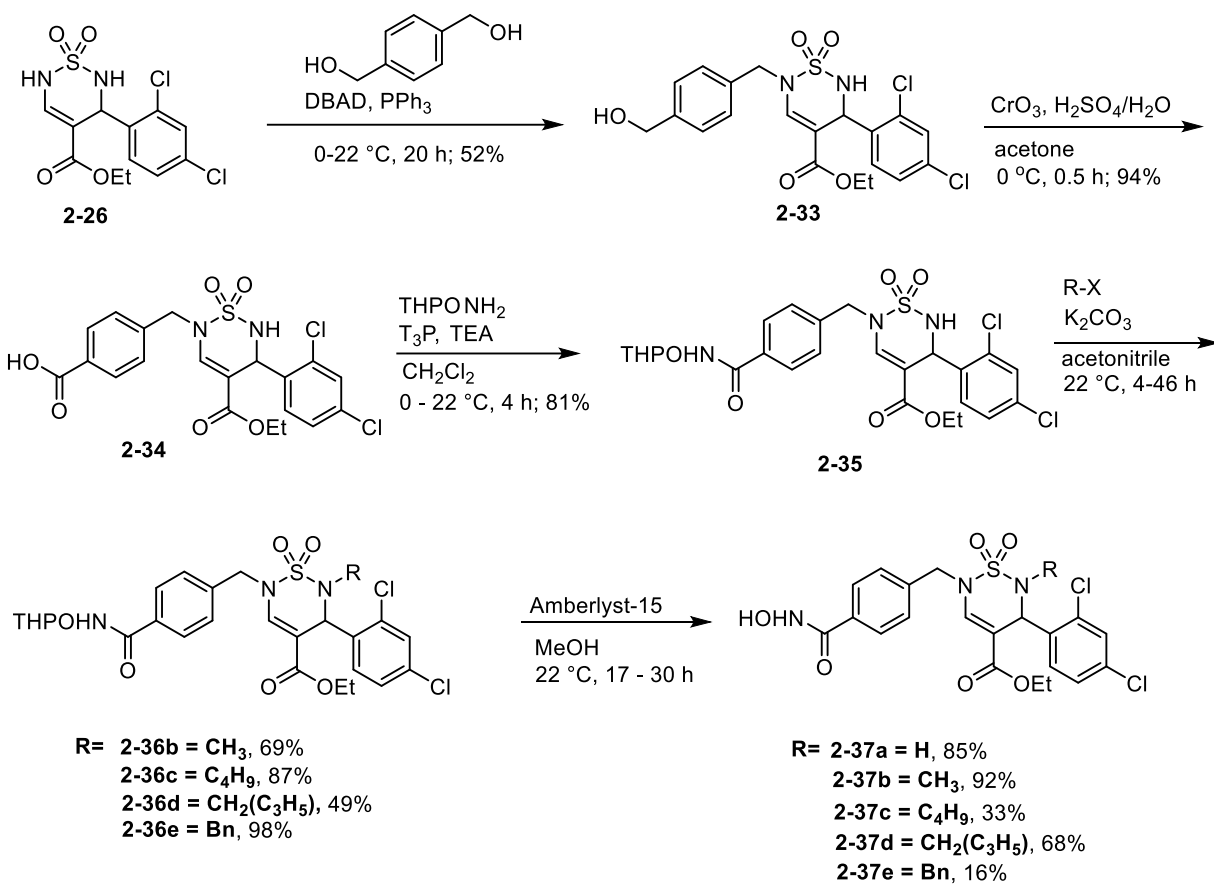


**Scheme 28.** Thiadiazine Derivatives **2-31** and **2-32** of MAL1-271.

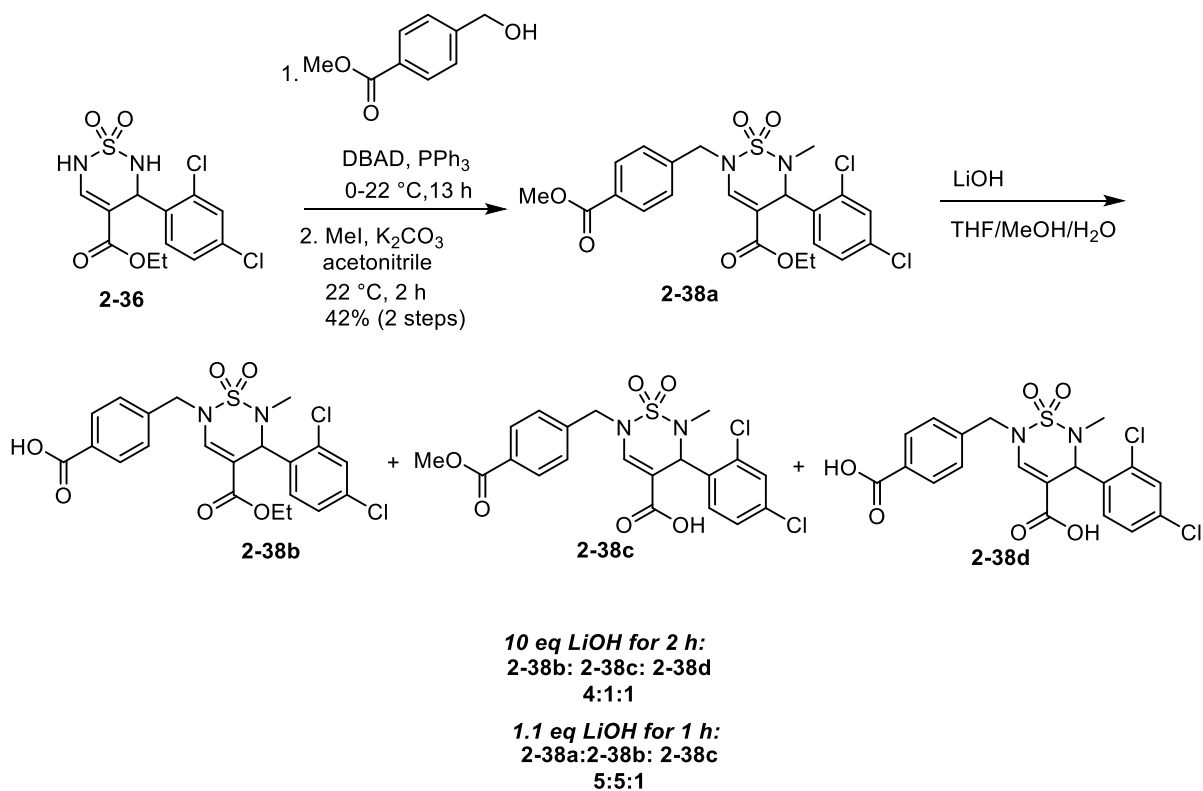


### 2.2.3 Selective Functionalization at N(2) and N(6) of the Thiadiazine

To install the *N*-hydroxybenzamide motif at N(6), selective Mitsunobu reaction at the acidic N(6)-H with 1,4-benzene-dimethanol followed by alcohol oxidation produced the desired *N*-methylbenzoic acid **2-34** (Scheme 29). While a Mitsunobu reaction with methyl 4-(hydroxymethyl)benzoate followed by methylation at N(2) was successful (Scheme 30), the subsequent mild saponification condition to **2-38b** was unselective. Initially, 10 eq of LiOH for 2 h provided the desired acid **2-38b**, undesired acid **2-38c**, and undesired bis-acid **2-38d** in a 4:1:1 ratio. At 1.1 eq of LiOH, the undesired acid **2-38c** was still observed, with **2-38a**, **2-38b**, and **2-38c** formed in a 5:5:1 ratio, respectively. The N(6)'s electron donation to the electron withdrawing benzyl group lowers its conjugation to the zone 2 carbonyl, making the carbonyl more prone to nucleophilic attack by the hydroxide anion. The carboxylic acid **2-34** was transformed to the THP-protected hydroxamate **2-35**, followed by alkylation at N(2) with benzyl-, cyclopropanemethyl-, butyl-, and methyl halides (**b-e**). A small amount of alkylation was observed at the N(H) amide, with <5% for the benzyl derivative and up to 30% for the methyl derivative. The bisalkylated side product was easily separated by chromatography. Deprotection of **2-35** and **2-36b-e** with Amberlyst-15 in methanol yielded the hydroxamic acids **2-37a-e**.



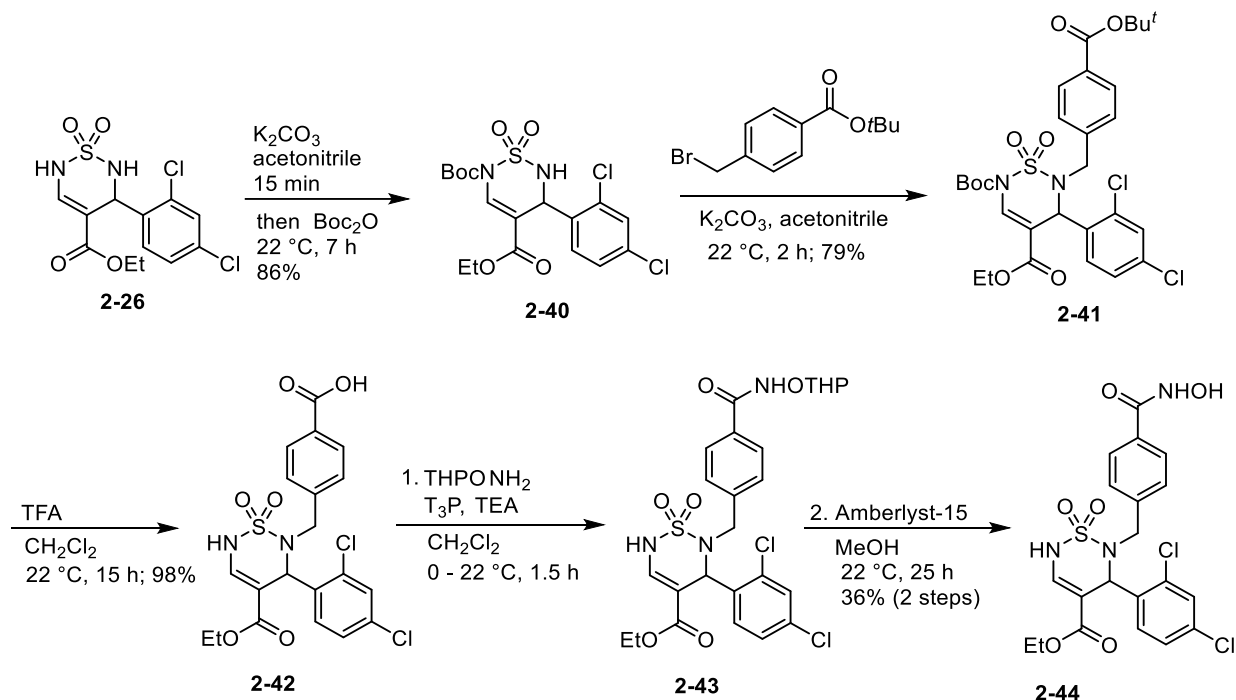
**Scheme 29.** Thiadiazine N(6)-Benzyl Hydroxamic Acids.



**Scheme 30.** Unselective Saponification of Diester **2-38a**.

To install the *N*-hydroxybenzamide motif at the less acidic N(2)-H, it was necessary to protect N(6)-H (**Scheme 31**). We looked for a protecting group that a) could be installed by taking advantage of the more acidic N(6)-H and b) could be removed simultaneously with the *t*-butyl ester of **2-41**. We did not want to use any nucleophilic conditions as to not saponify the C(4) ethyl ester. We therefore found the Boc protecting group to be ideal, and it was selectively added to N(6) (**2-40**) in 86% yield. COSY correlation between N(2)-H (9.37 ppm) and C(3)-H (5.60 ppm) confirmed regioselective addition at N(6). No correlation was observed with the C(5)-H (8.07 ppm). Alkylation of N(2) with *N*-methylbenzoyl *t*-butyl ester followed by global deprotection afforded the carboxylic acid **2-42**. Coupling of **2-42** with THP-protected hydroxylamine resulted in unidentified impurities and poor conversion to the amide. We suspect the acidic N(6)-H might

have been deprotonated by TEA, leading to possible side products. While **2-43** was deprotected to afford the hydroxamic acid **2-44**, alkylation at N(6) was no longer pursued.



**Scheme 31.** Thiadiazine N(2)-Benzyl Hydroxamic Acids.

## 2.2.4 Biological Results and Second-Generation Analogs

### 2.2.4.1 HSP70 Biological Results

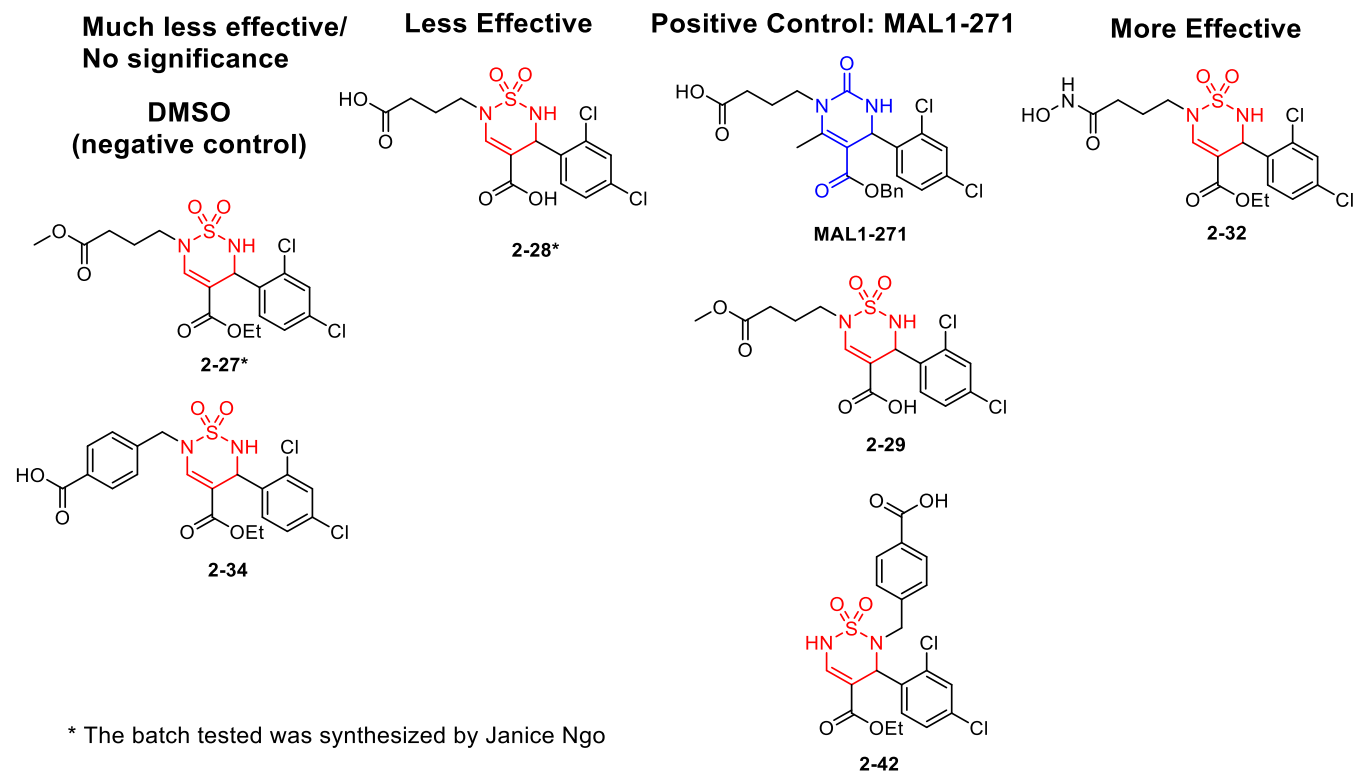
Huntington's Disease (HD) is a neurodegenerative disease caused by polyglutamine (polyQ) expansion in the huntingtin (HTT) disease protein.<sup>104</sup> PolyQ-expanded proteins are misfolded and form aggregates, where the misfolding propensity is proportional to the polyQ repeats.<sup>104</sup> Expression of the molecular chaperone HSP70 suppresses the polyQ neurodegeneration.<sup>105</sup>

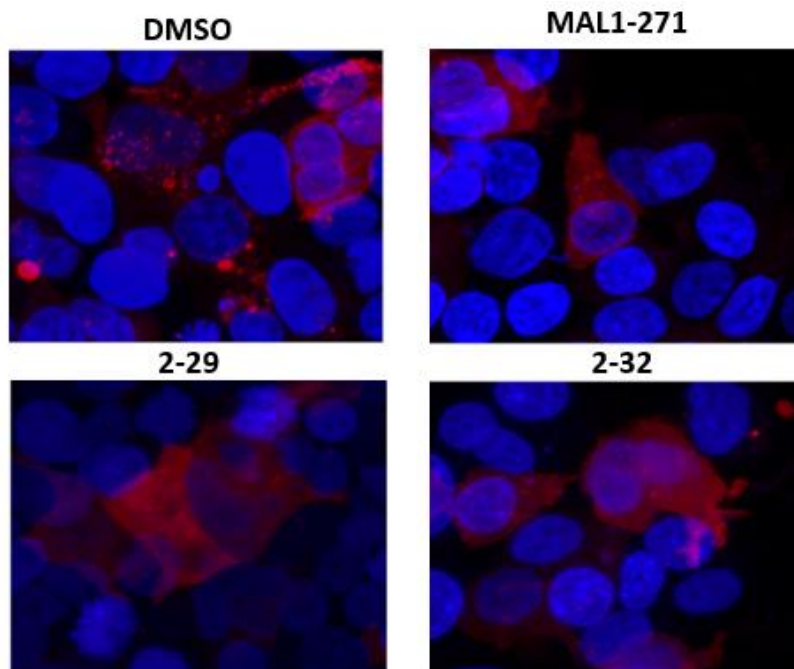
A select number of the synthesized compounds were tested in the lab of Professor Jeff Brodsky at the University of Pittsburgh on their ability to reduce toxic aggregates in HEK293H cells that express an HTT exon containing 17 polyQ aggregates (**Table 4** and **Figure 35**).<sup>106</sup> The HEK293H cells allow the measurement of the aggregation propensity in presence or absence of a specific Hsp70 activity modulator. Using the HTT17Q-mCherry system gave us the possibility of forming distinct aggregates that can be counted. The HTT-17polyQ was fused to mCherry and the HTT-17polyQ-mcherry was cloned between KpnI and BamHI sites in the pcDNA3.1 vector. The mCherry tagged-polyglutamine-expanded (17-polyQ-expanded) huntingtin (HTT) was introduced in the HEK293H cells. HD is characterized by 36 polyQ repeats,<sup>107</sup> but only 17 polyQ repeats were used for this study in order to allow us to visualize the effect of the HSP70 modulators on lowering the growing aggregates instead of their effect on already large HTT aggregates. The limitations of the assay stem from the variability of expression from cell to cell. With the expression factor transfected into cells varying from cell to cell, the results are nonquantitative. Cells were stained with 4',6-diamidino-2-phenylindole (DAPI) for confocal microscope imaging. A bright spot detection tool was used to identify and quantify the number of protein aggregates (“dots”) per cell that decrease upon HSP70 expression.

MAL1-271 was used as a positive control, and compounds **2-29** and **2-42** were found to be as effective as MAL1-271, and **2-32** was found to be more effective than MAL1-271. Compound **2-32** serves as the first hydroxamic acid-containing derivative of MAL1-271. Esters at Zone 1 have already been investigated in MAL1-271 derivatives, and there was no improvement in activity.<sup>91e</sup> Therefore, the presence of a polar group at zone 1 might provide favorable J domain interactions. **Scheme 32** outlines the synthesis of a sulfonamide derivative as a replacement to the hydroxamic acid. We wanted to replace the hydroxamic acid (pKa = 9.10)<sup>90</sup> of **2-32** with another polar group,

and we settled with the sulfonamide (pKa = 12.1)<sup>90</sup>. The benzoic acid **2-42** provides a new substitution pattern not previously explored for HSP70 agonists.

**Table 4.** Relative Ability of Thiadiazine Derivatives to Reduce polyQ Aggregates in a Huntington Disease Model Using MAL1-271 as a Positive Control.

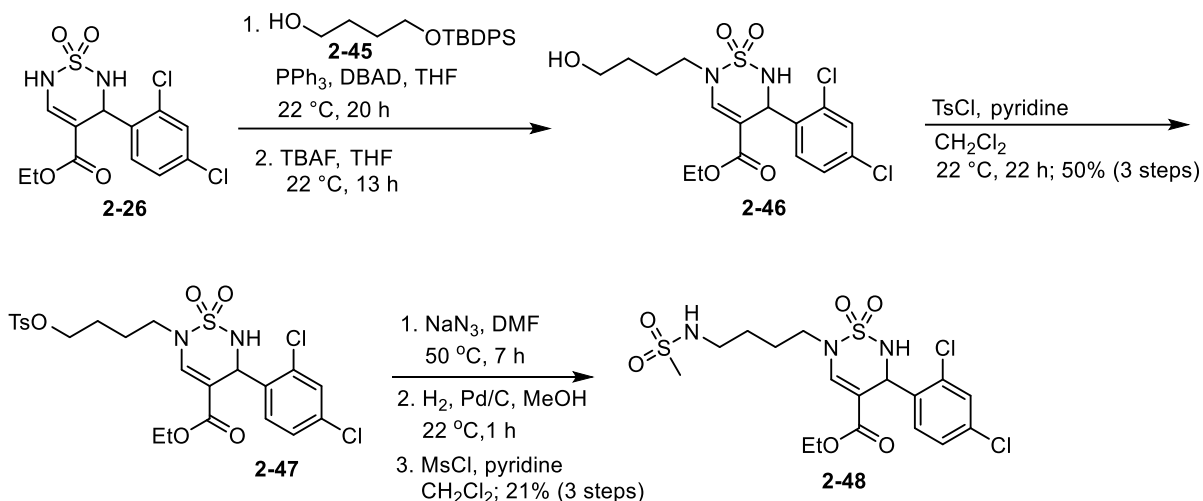




**Figure 35.** HEK293H Cells after Treatment with MAL1-271, **2-29**, and **2-32**.

The positive control, MAL1-271, and its analogs reduced the number of cellular puncta/aggregates compared to the DMSO control. Cells were stained for confocal microscope imaging with 4',6-diamidino-2-phenylindole (DAPI), a fluorescent dye with high affinity to adenine–thymine rich DNA regions. A bright spot detection tool was used to identify and quantify the number of protein aggregates (“dots”) per cell.

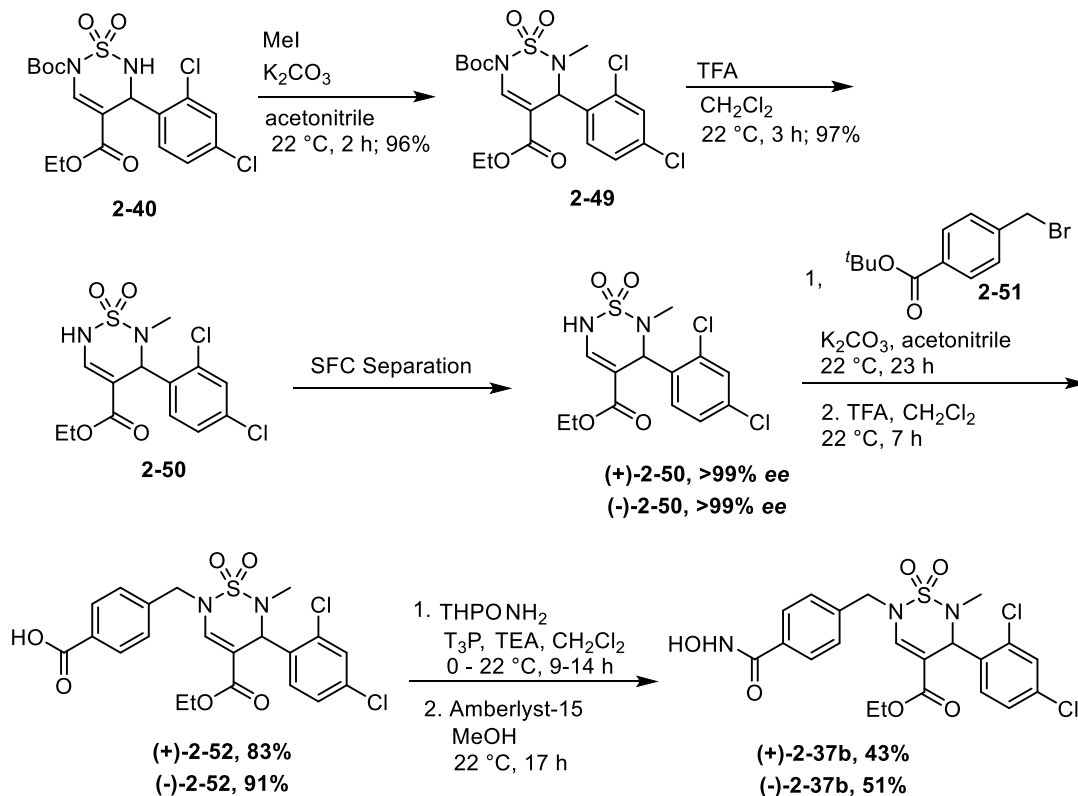
We decided to keep the linker at N(6), and set out to synthesize the sulfonamide analog **2-48** (Scheme 32). We started with a Mitsunobu reaction on the thiadiazine **2-26** with the alcohol **2-45**, followed by TBAF deprotection. The alcohol **2-46** was subsequently tosylated with TsCl to produce the advanced electrophilic intermediate **2-47**. The tosylate **2-55** was treated with sodium azide, and the azide intermediate was carried on crude and subsequently reduced to the primary amine via catalytic hydrogenation. Sulfonylation with methanesulfonyl chloride provided the sulfonamide **2-48**.



**Scheme 32.** Synthesis of Sulfonamide **2-48**.

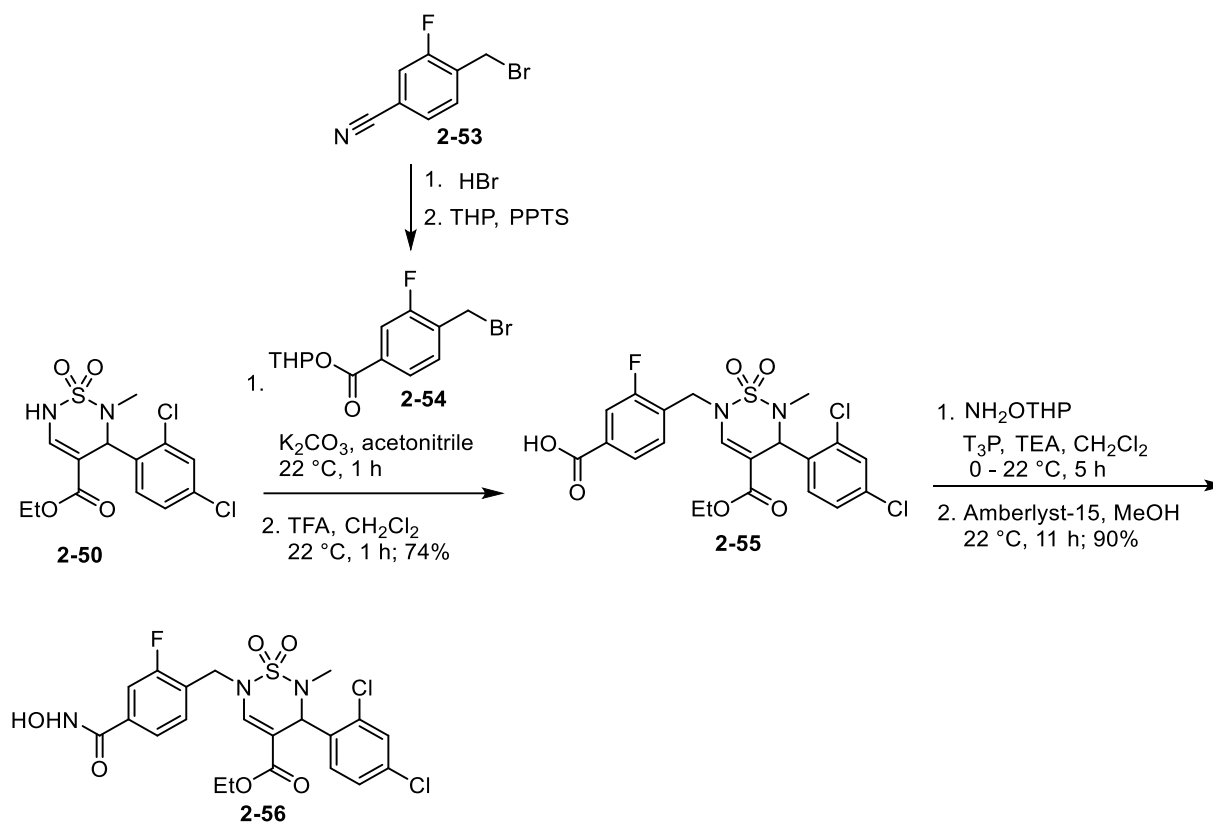
After another round of evaluation of our compounds in the Huntington disease model, we found that the hydroxamic acid **2-37b** among the other compounds tested was more effective than MAL1-271 (**Table 5**). We were interested in resolving the enantiomers of **2-37b**. Attempted resolution of **2-37b** enantiomers by chromatography on chiral stationary phase was unsuccessful, and we proceeded to separating an advanced intermediate that could be further derivatized to (+)-**2-37b** and (-)-**2-37b**. We methylated the N(6)-Boc derivative **2-40** (**Scheme 33**), followed by Boc deprotection to afford **2-50**. After separation on a chiral stationary phase, we collected 65 mg of (-)-**2-50** and 75 mg of (+)-**2-50**, > 99% *ee*. The absolute configurations of the enantiomers were not assigned. However, they could have been assigned by X-ray crystallography or circular dichroism (CD). Each of the enantiomers was alkylated with *tert*-butyl 4-(bromomethyl)benzoate and deprotected to form the acids (+)-**2-52** and (-)-**2-52**. Coupling with THP-protected hydroxylamine and deprotection of the pyranyl group afforded (+)-**2-37b** and (-)-**2-37b**.





**Scheme 33.** Synthesis of (+)-**2-37b** and (-)-**2-37b**.

To explore a fluoro-substitution on the 4-aminomethyl-*N*-hydroxybenzamide group of **2-37b**, we started with the commercially available reagent **2-53** (Scheme 34). Hydrolysis of **2-53** to the acid, followed by protection with the tetrahydropyranyl group, formed **2-54**. Alkylation of the thiazine **2-50** with **2-54** followed by deprotection of the pyranyl group revealed the carboxylic acid **2-55**, which was transformed to the hydroxamic acid **2-56** in the next two steps.



**Scheme 34.** Synthesis of Thiadiazine Fluoro Derivative **2-56**.

The evaluation of these compounds on their ability to reduce polyQ aggregates in the Huntington Disease model is shown in **Table 5** and **Figure 36**. We found that compounds **2-30**, **2-31**, **2-37e**, (-)-**2-37b**, and **2-56** were as effective as MAL1-271; **2-37d** and **2-37b** were more effective; and compounds **2-56**, (+)-**2-37b**, and **2-37c** were less effective. Replacement of the hydroxamic acid of **2-32** with the sulfonamide group in **2-56** showed a decrease in effectiveness as an HSP70 agonist, while the carboxylic acid in **2-31** has proven to be as effective as MAL1-271. Of the three compounds, the sulfonamide **2-56** and the hydroxamic acid **2-32** were less acidic than the carboxylic acid **2-31**. The acidity at the zone 1 carbonyl therefore does not affect the agonistic activity of these compounds. When the zone 1 hydroxamic acid and zone 2 ethyl ester of **2-32** were replaced with the methyl ester and hydroxamic acid, respectively, in **2-30**, the

effectiveness was lower than **2-32** but similar to MAL1-271. Since esters at zone 1 and zone 2, as in compound **2-27**, showed less agonistic activity in HSP70 relative to MAL1-271, having a hydroxamic acid at either zones aids in increasing the agonistic activity of these compounds.

For the N(6)-benzyl hydroxamic acid derivatives, the racemic **2-37b** was more effective than MAL1-271 compared to either of the **2-37b** enantiomers. Compound (+)-**2-37b** was less effective than MAL1-271, and (-)-**2-37b** was as effective. It is not clear yet whether it may be possible that either of the enantioenriched derivatives may interact with other proteins in addition to HSP70, or if the polyQ expansion factor was highly variable in the cells in question. The fluorinated derivative **2-56** was less efficacious than the nonfluorinated derivative. For the other alkylated derivatives, the N(2)-cyclopropane methyl was more effective than the more elongated N(2)-butyl or N(2)-benzyl derivatives.

**Table 5.** Relative Ability of Second-Generation Thiadiazine Derivatives to Reduce polyQ Aggregates in a Huntington Disease Model using MAL1-271 as a Positive Control.

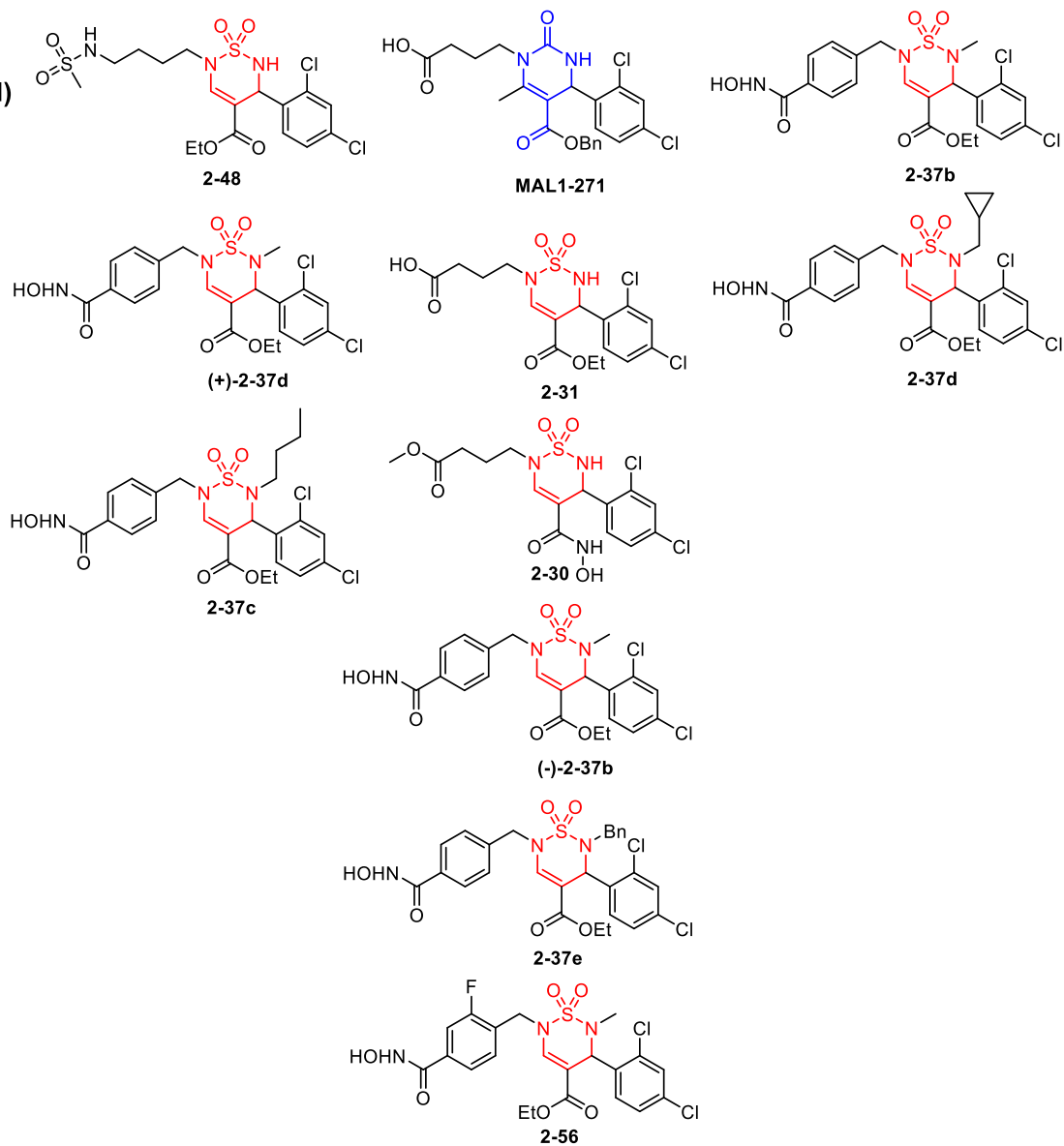
Much less effective/  
No significance

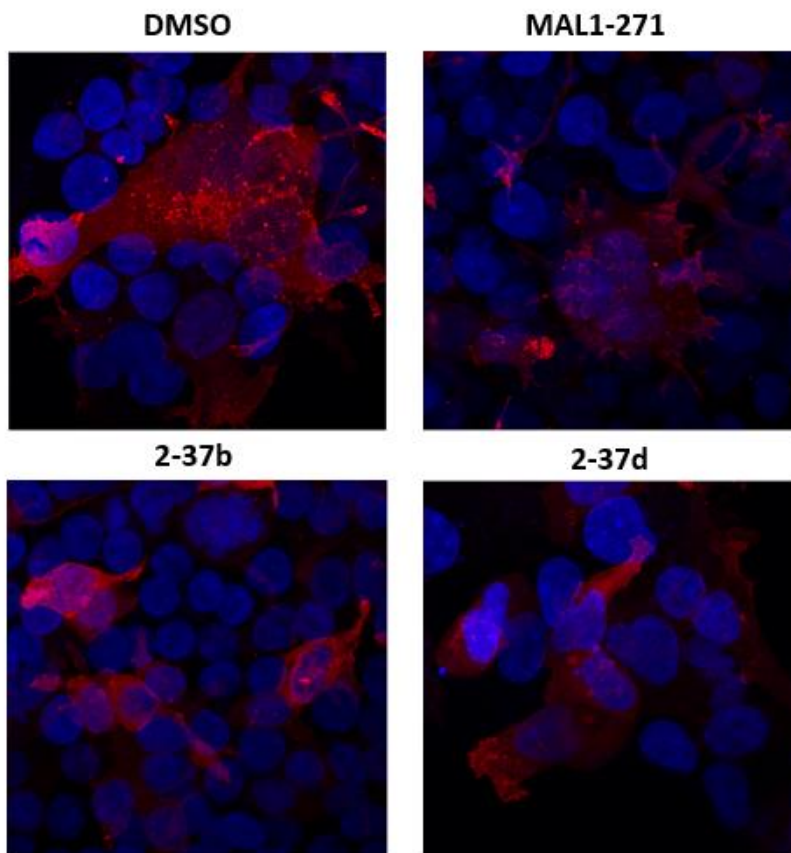
Less Effective

Positive Control: MAL1-271

More Effective

DMSO  
(negative control)



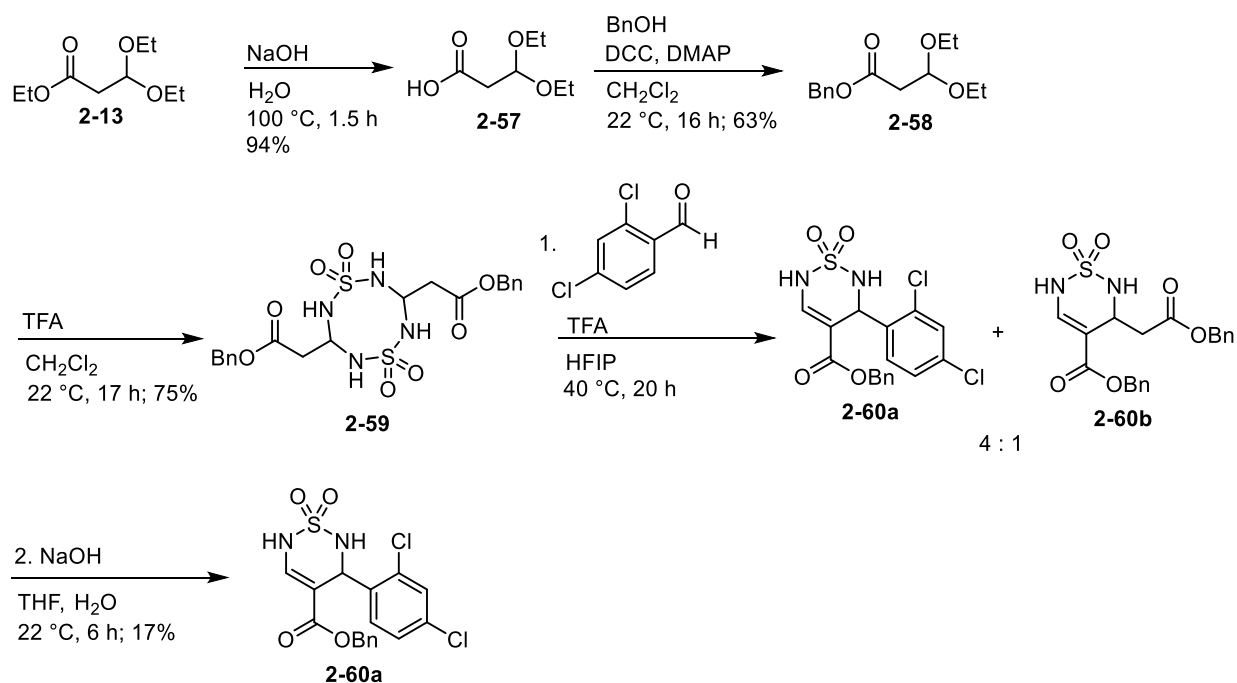


**Figure 36.** HEK293H Cells after Treatment with MAL1-271, 2-37b, and 2-37d.

The positive control, MAL1-271, and its analogs reduced the number of cellular puncta/aggregates compared to the DMSO control. Cells were stained for confocal microscope imaging with 4',6-diamidino-2-phenylindole (DAPI), a fluorescent dye with high affinity to adenine–thymine rich DNA regions. A bright spot detection tool was used to identify and quantify the number of protein aggregates (“dots”) per cell.

From the second set of data, it appears that the thiadiazine heterocycle offered similar effects as the Biginelli dihydropyrimidones. MAL1-271 has served as our standard, and the thiadiazine heterocycle derivatives have shown similar effectiveness as MAL1-271. A limitation of the comparison is the lack of C(5)-methyl at the dihydropyrimidone of MAL1-271 versus the thiadiazine derivatives. This may contribute to a solubility effect that may in turn affect the quantity of these compounds entering the cell and coming in contact with HSP70. One way to quantify the number of transfected gene in the cells would be to run a Western Blot and use an anti-polyQ antibody, such as MW1. What needs to be investigated further is the replacement of

the C(4)-ethyl ester of the thiadiazine derivatives with the C(4)-benzyl ester found in MAL1-271. While such derivatives have not yet been synthesized, formation of the thiadiazine with the C(4)-benzyl ester has been achieved (**Scheme 35**). Steglich esterification of 3,3-diethoxypropanoic acid **2-57** with benzyl alcohol, followed by dimerization of the benzyl ester **2-58** with sulfamide results in the dimer **2-59** in 75% yield. Condensation of the dimer with the 2,4-dichlorobenzaldehyde results in the desired thiadiazine **2-60a** and undesired byproduct **2-60b**, in a 4:1 ratio, respectively. Since the vinylogous carbamate is resistant to saponification, we were able to separate **2-60a** and **2-60b** by saponifying the benzyl ester. The low yield may be attributed to the benzyl ester derivative slowing down the condensation reaction, leaving room to byproduct formation. While **2-60b** was identified, there have been other unidentified byproducts. One way around this would be to increase the aldehyde equivalents. In this case, only two equivalents of the aldehyde were used, similar to the formation of the ethyl ester derivative **2-26** that was obtained in up to 80% yield.



**Scheme 35.** Synthesis of Thiadiazine Benzyl Ester Derivative **2-60a**.

#### 2.2.4.2 HDAC Assay

The compounds were tested in-house on HDAC assays (**Tables 6 and 7, Appendix A**), and they have not shown significant inhibition of HDAC at 0.1 – 1.0  $\mu\text{M}$  concentrations. Compound **2-42** showed 40% inhibition of HDAC7 at 1  $\mu\text{M}$ , and most compounds showed moderate inhibition (35-60%) of HDAC8. This suggests that compounds as effective and more effective than MAL1-271 do not reduce cellular HTT aggregates due to direct HDAC inhibition.

However, a select number of compounds have shown variable results, as can be seen in **Table 7**. For example, **2-32** showed 19% inhibition of HDAC6 at 200 nM, while it showed no inhibition at 100 nM. Acid **2-34** showed 7% inhibition of HDAC8 at 1  $\mu\text{M}$  while it showed 39% inhibition of HDAC8 at 200 nM. We suspected poor aqueous solubility and/or fluorescence interference of our compounds. We determined through a kinetic aqueous solubility test (**Table 9**,

**Appendix B**) that our compounds have low aqueous solubility, in particular compounds **2-29**, **2-37b**, **(+)-2-37b**, **(-)-2-37b**, **2-37d**, **2-37c**, **2-37a**, **2-42**, **2-33**, and **2-41**. Due to the inaccuracy of the assay, it may be possible that these compounds have poor aqueous solubility at 1  $\mu\text{M}$ , however they are likely to be soluble at lower concentrations. Our assay determined that the negative control, Tamoxifen, has an aqueous solubility of  $22 \pm 5.8 \mu\text{M}$ , where literature data<sup>108</sup> show that it is  $<1.6 \mu\text{M}$  aqueous solubility. We expect that our compounds that showed  $< 25 \mu\text{M}$  solubility in our assay may not be soluble at 1  $\mu\text{M}$ . Additionally, we ran a fluorescence assay (**Table 10**, **Appendix C**) and found that our compounds were not fluorescent at excitation  $\lambda = 365 \text{ nm}$  and emission  $\lambda = 450 \text{ nm}$ , as used by the assay.

### 2.3 Chapter 2 Conclusion

Our group previously developed a method to access the 1,2,6-thiadiazine 1,1-dioxide scaffold, and the work presented in this chapter focused on the selective functionalization of the heterocycle to generate medicinally relevant compounds. The syntheses employ chemoselective transformations within the thiadiazine heterocycle's sulfonamide nitrogens and vinylogous carbamate. We synthesized thiadiazine derivatives of the HSP70 agonist MAL1-271. Additionally, we developed a convergent synthetic route to install a library of *N*-hydroxybenzamide-containing thiadiazine heterocycles. We originally used our method for the analog synthesis of HSP70 agonists and HDAC6 inhibitors, but from biological evaluations, the compounds were found to have better efficacy as HSP70 agonists than they have as HDAC inhibitors. Overall, the thiadiazine heterocycle has offered similar if not superior effects versus the Biginelli dihydropyrimidone, MAL1-271. More specifically, three of our compounds showed



greater efficacy than MAL1-271. Two of the three more effective compounds include a new substitution pattern not seen in the dihydropyrimidone HSP70 agonists. These results will undoubtedly allow us to continue working with thiadiazine derivatives for HSP70 modulation.

### 3.0 Experimental Section

#### 3.1 General Experimental

All reactions were performed under a N<sub>2</sub> atmosphere and all glassware was flame dried and cooled in a desiccator prior to use. All chemicals were used as purchased (purity > 95%), unless otherwise noted. THF and Et<sub>2</sub>O were distilled from sodium/benzophenone; CH<sub>2</sub>Cl<sub>2</sub> was distilled from CaH<sub>2</sub>; DIPEA and TEA were distilled from CaH<sub>2</sub> and stored over KOH; *t*-BuOH and DCE were distilled from CaH<sub>2</sub> and stored over 4Å MS; HFIP was distilled from 4Å MS and stored over 4Å MS. Microwave reactions were performed using a Biotage Initiator in glass microwave vials (cap sealed) with continuous magnetic stirring and an external surface temperature sensor. Concentrating under reduced pressure refers to using a rotary evaporator connected to a piab Lab Vac H40 for solvent removal.

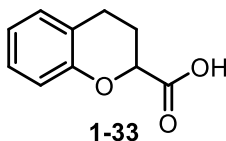
Reactions were monitored by TLC and/or LCMS analysis. TLC was performed using pre-coated silica gel 60 F<sub>254</sub> plates (EMD, 250 µm thickness) and visualization was accomplished with a 254 nm UV light and/or by staining with a KMnO<sub>4</sub> solution (1.5 g of KMnO<sub>4</sub> and 1.5 g of K<sub>2</sub>CO<sub>3</sub> in 100 mL of a 0.1% NaOH solution) or ninhydrin solution (1.5 g ninhydrin in 100 mL of *n*-BuOH and 3 mL AcOH). Low-resolution mass spectra were obtained from Agilent Technologies 1260 Infinity II LCMS. HRMS data were obtained from a Micromass UK Limited, Q-TOF Ultima API or a Thermo Scientific Exactive Orbitrap LCMS. Purity of compounds tested in biological assays was assessed using the Agilent Technologies 1260 Infinity II LC at 220 nm UV absorption (Waters XBridge BEH C<sub>18</sub> 2.1 x 50 mm, 2.5 µm) or an Agilent Technologies 385-ELSD (Microsolv Cogent

2.0 Bidentate C<sub>18</sub> 2.1 x 50 mm, 2.2 μm; ELSD conditions: evaporator and nebulizer set at 45 °C; gas flow set at 1.80 standard liter/min). Melting points were determined using a Laboratory Devices Mel-Temp II in open capillary tubes and are uncorrected. Infrared spectra were determined as neat solids or oils (unless otherwise specified) on a Perkin Elmer Spectrum 100 FT-IR. Flash chromatography on SiO<sub>2</sub> (Silicycle, Silia-P Flash Silica Gel or SiliaFlash® P60, 40-63 μm) was used to purify crude reaction mixtures.

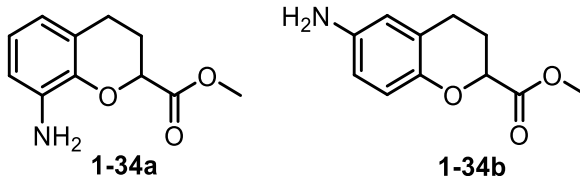
<sup>1</sup>H/<sup>13</sup>C NMR spectra were recorded on a Bruker Avance 300/75 MHz, 400/100 MHz, 500/125, or 600/150 MHz instruments. Chemical shifts were reported in parts per million with the residual solvent peak used as the internal standard: <sup>1</sup>H/<sup>13</sup>C: CDCl<sub>3</sub>, 7.26/77.16 ppm; DMSO-*d*<sub>6</sub>, 2.50/39.52 ppm, acetone-*d*<sub>6</sub>, 2.05/29.84, MeOD, 3.31/49.00 ppm. Chemical shifts were arranged in the following format: chemical shift, multiplicity (s = singlet, bs= broad singlet, d = doublet, t = triplet, q = quartet, sept = septet, dd = doublet of doublet, dt = doublet of triplet, m = multiplet, app =apparent), coupling constants, and integration. All NMR spectra were processed using Bruker TopSpin Software. **The Spectra files are available at the following link: [https://d-scholarship.pitt.edu/40218/1/Terrab\\_Leila\\_Spectra\\_files\\_.pdf](https://d-scholarship.pitt.edu/40218/1/Terrab_Leila_Spectra_files_.pdf).**

Supercritical fluid Chromatography (SFC) (analytical and semi-prep) was completed using a Mettler Toledo AG - Berger SFC™ MiniGram instrument with a Chiralpak IC Column (10 x 250 mm; 5 μ pore size) at 100 bar pressure, oven temp. of 35 °C, detection wavelength of 254 nm, and HPLC-grade iPrOH or MeOH as a modifier. HPLC analyses were completed using a Mettler Toledo Rainin: Dynamax™ HPLC system using a Chiralpak AD-H column (4.6 x 250 mm) with detection wavelength of 254 nm, and hexanes/EtOH as an eluent.

### 3.2 Chapter 1 Experimentals

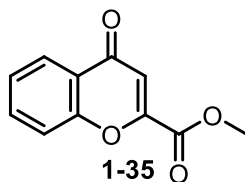


**Chromane-2-carboxylic acid (1-33).**<sup>109</sup> In each of three Parr vials, to a suspension of chromone-carboxylic acid (2.22 g, 11.7 mmol) in MeOH (15 mL) was added H<sub>2</sub>O (0.2 mL) followed by Pd/C (10%, 0.240 g, 0.225 mmol). The mixture was stirred under H<sub>2</sub> (10 bar) using the Parr apparatus. After 5 h, the reaction mixture was recharged with H<sub>2</sub> (10 bar). After 16 h, the combined mixtures were filtered through Celite, rinsed with MeOH, and concentrated to give **1-33** (6.19 g, 97%) as a beige-colored solid: <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 13.01 (s, 1 H), 7.06 (m, 2 H), 6.81 (m, 2 H), 4.90 (dd, *J* = 6.5, 3.8 Hz, 0.15 H), 4.76 (dd, *J* = 6.3, 3.9 Hz, 0.85 H), 2.78 (m, 1 H), 2.68-2.58 (m, 1 H), 2.20-1.99 (m, 2 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>10</sub>H<sub>9</sub>O<sub>3</sub> [M-H]<sup>+</sup> 177.0546, found 177.0537.

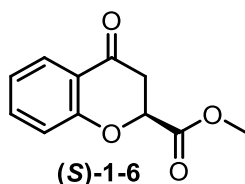


**Methyl 8-aminochromane-2-carboxylate (1-4a) and methyl 6-aminochromane-2-carboxylate (1-34b).**<sup>56</sup> Nitration: An ice cold solution of nitric acid (20.0 mL, 70%) was treated with **1-33** (1.09 g, 6.14 mmol) portionwise. The solution turned green after 30-45 min. The mixture was warmed to room temperature after 45 min, and was stirred for an additional 15 min. The solution was poured into ice, and extracted with chloroform (4 x 110 mL). The combined organic layers were

concentrated to 200 mL, washed with brine (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to give an orange colored residue (1.10 g). Esterification: The residue (1.10 g) was dissolved in MeOH (16 mL) and treated with conc. HCl (3 pasteur pipette drops) at room temperature. The mixture was heated at reflux for 4 h, and concentrated, diluted with EtOAc (125 mL), washed with sat. NaHCO<sub>3</sub> (50 mL), and brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to provide a beige/yellowish powder (970 mg). Reduction: The mixture was diluted with MeOH (15 mL) in a 100 mL RBF. The flask was flushed with N<sub>2</sub>, and Pd/C (10%, 0.215 g, 0.199 mmol) was added. The flask was flushed with H<sub>2</sub> for 10 min, and then kept under H<sub>2</sub> for 18 h. The mixture was filtered through Celite, rinsed with MeOH, and concentrated. The oil was purified by chromatography on SiO<sub>2</sub> (20-100% EtOAc in Hexanes). Aniline **1-34a** was collected as a light pink-colored solid (0.312 g, 25 %), and aniline **1-34b** was collected as a brownish-red oil (0.371 g, 29%). Aniline **1-34a**: Mp 68-70 °C; IR (CDCl<sub>3</sub>) 3464, 3372, 3031, 2952, 2928, 2851, 1745, 1617, 1485, 1438, 1195, 1095 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 6.69 (t, *J* = 7.7 Hz, 1 H), 6.58-6.56 (m, 1 H), 6.45 (dt, *J* = 7.6, 0.7 Hz, 1 H), 4.76 (dd, *J* = 7.8, 3.6 Hz, 1 H), 3.79 (s, 3 H), 3.79 (br, 2 H), 2.81 (1 H), 2.75-2.69 (m, 1 H), 2.31-2.25 (m, 1 H), 2.17 (m, 1 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 141.3, 135.7, 121.4, 121.0, 118.8, 113.4, 74.0, 52.4, 25.0, 23.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>11</sub>H<sub>14</sub>NO<sub>3</sub> [M+H]<sup>+</sup> 208.0968, found 208.0968. Aniline **1-34b**: IR (CDCl<sub>3</sub>) 3431, 3358, 3012, 2952, 2850, 1747, 1629, 1498, 1451, 1203 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 6.76 (d, *J* = 8.6 Hz, 1 H), 6.50 (dd, *J* = 8.6, 2.7 Hz, 1 H), 6.39 (d, *J* = 2.6 Hz, 1 H), 4.65 (dd, *J* = 8.0, 3.3 Hz, 1 H), 3.79 (s, 3 H), 3.37 (s, 2 H), 2.81-2.74 (m, 1 H), 2.70-2.65 (m, 1 H), 2.28-2.22 (m, H), 2.18-2.10 (m, 1 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 171.8, 146.7, 140.1, 122.0, 117.7, 115.9, 115.4, 74.0, 52.5, 25.1, 23.8; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>11</sub>H<sub>14</sub>NO<sub>3</sub> [M+H]<sup>+</sup> 208.0968, found 208.0969.

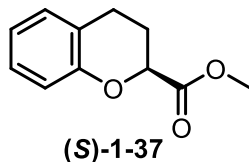


**Methyl 4-oxo-4H-chromene-2-carboxylate (1-35).**<sup>110</sup> A solution of chromone-2-carboxylic acid (45.0 g, 237 mmol) in methanol (700 mL) was treated with conc. sulfuric acid (5.4 mL) and warmed to 50 °C. After 18 h, the reaction mixture was cooled to room temperature, concentrated, transferred to a 2 L flask, and diluted with EtOAc (750 mL) and sat. NaHCO<sub>3</sub> (750 mL). The biphasic layer was stirred for 15 min, transferred to a separatory funnel, and the EtOAc layer was washed with brine (750 mL), and the layers were separated. The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The ester **1-35** (43.8 g, 91%) was collected as a light yellow solid: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 8.19 (dd, *J* = 8.0, 1.7 Hz, 1 H), 7.76-7.71 (m, 1 H), 7.59 (dd, *J* = 8.4, 0.7 Hz, 1 H), 7.47-7.42 (m, 1 H), 7.10 (s, 1 H), 4.00 (s, 3 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>11</sub>H<sub>9</sub>O<sub>4</sub> [M+H]<sup>+</sup> 205.0495, found 205.0493.

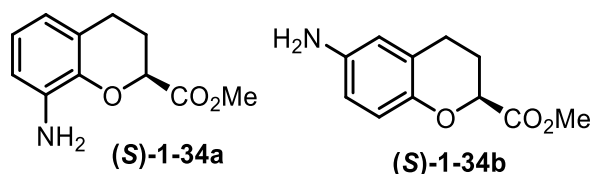


**Methyl (S)-4-oxochromane-2-carboxylate ((S)-1-36).**<sup>57</sup> A Bondi-blue solution of Cu(OAc)<sub>2</sub> (Strem, 0.089 g, 0.490 mmol) in freshly distilled THF (20 mL) was stirred under an atmosphere of N<sub>2</sub> until a homogeneous green-blue solution was obtained (ca. 15 min). Neat (S)-DM-Segphos (390 mg, 0.54 mmol) was added, and the reaction mixture was stirred for 15 min at room temperature, cooled to 0 °C in an ice bath, and treated dropwise with DEMS (990 mg, 7.35 mmol).

The reaction mixture was stirred for an additional 30 min at 0 °C, gradually turning yellow, and a solution of ester **1-35** (1.0 g, 4.90 mmol) in dry THF (10 mL) was added dropwise over 5 min. The solution was stirred at 0 °C for 30 min, and at rt for another 30 min, while it turned light brown. An aliquot was analyzed by LCMS, and no starting material could be detected. The reaction mixture was cooled to 0 °C and quenched with sat. NH<sub>4</sub>Cl (10 mL) under vigorous stirring for 15 min. After addition of EtOAc (50 mL), the solution was transferred into a sep. funnel, sat. NaCl (10 mL) was added, and the layers were separated (a suspension forms after mixing, and takes about 15 min to separate). The organic layer was washed with sat. NaHCO<sub>3</sub> (15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. The oily residue was purified by chromatography on SiO<sub>2</sub> (0-25% EtOAc/hexanes) to give a combined yield of ca. 100% of a 1:1 mixture of ketone:silyl enol ether. A solution of the combined ketone/ enol ether fractions in MeOH (20 mL) was treated with Amberlyst 15 (100 mg) resin and stirred at room temperature for 1 h. TLC (10% EtOAc/hexanes) and LCMS analyses confirmed conversion to the ketone. The mixture was filtered, solvent was evaporated, and the oily residue was dried under high vacuum to provide (**S**)-**1-36** (1.040 g, 93% purity by <sup>1</sup>H NMR, 96%) as a light yellow, waxy solid (HPLC analysis on a chiral stationary phase (Chiralpak AD-H, 0.46 ID x 25 cm; 5 μm); sample prep: 1 mg/mL in 100% absolute EtOH; hexanes/EtOH (90:10), isocratic; 254 nm; 1 mL/min; retention time: 48.6 min) provided > 97% *ee*; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.89 (dd, *J* = 7.9, 1.7 Hz, 1 H), 7.55-7.71 (m, 1 H), 7.11 (d, *J* = 8.4 Hz, 1 H), 7.09-7.06 (m, 1 H), 5.10 (dd, *J* = 8.4, 6.1 Hz, 1 H), 3.82 (s, 3 H), 3.07 (d, *J* = 0.5 Hz, 1 H), 3.06 (d, *J* = 3.2 Hz, 1 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 189.7, 169.3, 160.3, 136.6, 127.1, 122.4, 121.1, 118.3, 75.3, 53.0, 39.7; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>11</sub>H<sub>11</sub>O<sub>4</sub> [M+H]<sup>+</sup> 207.0652, found 207.0650.



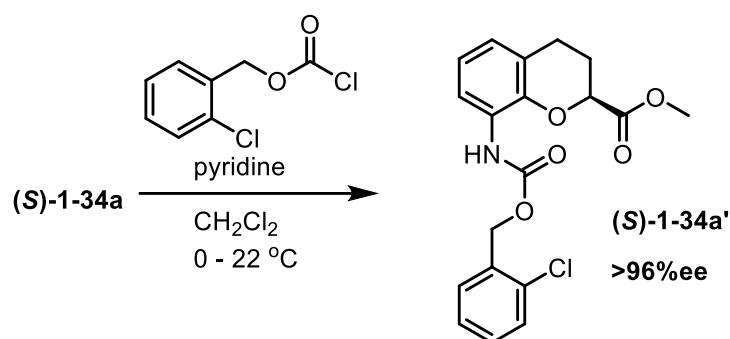
**Methyl (S)-chromane-2-carboxylate ((S)-1-37).**<sup>111</sup> To a suspension of (S)-1-36 (0.094 g, 0.456 mmol) in MeOH (1.20 mL) was added water (0.05 mL) followed by Pd/C (10%, 0.0283 g, 0.0266 mmol). The mixture was stirred under H<sub>2</sub> (balloon). After 40 h, the mixture was filtered through Celite, rinsed with EtOAc (10 mL), and concentrated to provide (S)-1-37 (0.0748 g, 85%) as a colorless oil:  $[\alpha]_D +8.7$  (*c* 2.8, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>)  $\delta$  7.12 (app t, *J* = 7.1 Hz, 1 H), 7.04 (d, *J* = 7.4 Hz, 1 H), 6.93 (dd, *J* = 8.2, 0.8 Hz, 1 H), 6.87 (dt, *J* = 7.4, 1.2 Hz, 1 H), 4.74 (dd, *J* = 7.5, 3.7 Hz, 1 H), 3.80 (s, 3 H), 2.90-2.70 (m, 2 H), 2.34-2.13 (m, 2 H).



**Methyl (S)-8-aminochromane-2-carboxylate ((S)-1-34a) and methyl (S)-6-aminochromane-2-carboxylate ((S)-1-34b).** An ice-cold solution of (S)-1-37 (0.1072 g, 0.558 mmol) was treated with an ice-cold solution of nitric acid (2.50 mL, 70%) portionwise. The solution turned dark blue after 30 min. The mixture was warmed to room temperature and left to stir for 10 min. The solution was poured into ice, and the color change to dark green was observed. The aqueous solution was basified with sat. NaHCO<sub>3</sub> (solid) until pH 8-9. The mixture was extracted with chloroform (4 x 30 mL). The combined organic layers were washed with brine (30 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to give an orange solid. The crude mixture was carried to the reduction with no further purification. The mixture was diluted with methanol (5 mL). The flask was flushed with



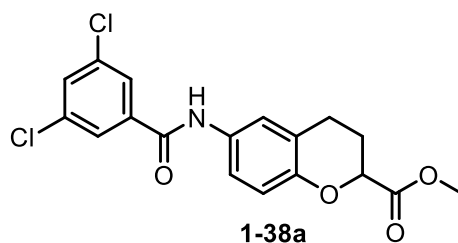
N<sub>2</sub>, and Pd/C (10%, 0.0549 g, 0.0549 mmol) was added. The flask was flushed with H<sub>2</sub> (balloon) for 10 min, and then kept under H<sub>2</sub> (balloon) for 13 h. The mixture was filtered through Celite, rinsed with methanol and EtOAc, and concentrated. The oil was purified by column chromatography on SiO<sub>2</sub> (18-100% EtOAc in hexanes). The *o*-aniline **1-34a** was collected as a light pink-colored oil (0.0263 g, 23 %), and the *p*-aniline **1-34b** was collected as a brownish-red oil (0.0306 g, 26 %). Aniline (**S**)-**1-34a**: [ $\alpha$ ]<sub>D</sub> -18.1 (*c* 0.30, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>)  $\delta$  6.770 (app t, *J*= 7.6 Hz, 1 H), 6.57 (d, *J*= 7.0 Hz, 1 H), 6.45 (d, *J*= 7.5 Hz, 1 H), 4.76 (dd, *J*= 7.8, 3.6 Hz, 1 H), 3.94-3.66 (br, 2 H), 2.85-2.68 (m, 2 H), 2.31-2.14 (m, 2 H); Aniline (**S**)-**1-34b**: [ $\alpha$ ]<sub>D</sub> +39.0 (*c* 0.042, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>)  $\delta$  6.76 (d, *J*= 8.6 Hz, 1 H), 6.50 (dd, *J*= 8.6, 2.8 Hz, 1 H), 6.40 (d, *J*= 2.8 Hz, 1 H), 4.65 (dd, *J*= 8.0, 3.4 Hz, 1 H), 3.79 (s, 3 H), 3.52-3.28 (br, 2 H), 2.82-2.64 (m, 2 H), 2.29-2.09 (m, 2 H).



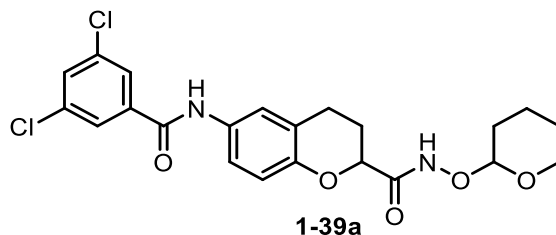
**Methyl (S)-8-(((2-chlorobenzyl)oxy)carbonyl)amino)chromane-2-carboxylate ((S)-1-34a').**

2-Chlorobenzyl carbonochloridate (75%, 0.0450 g, 0.165 mmol) was slowly added dropwise to a mixture of (**S**)-**1-34a** (0.0206 g, 0.0994 mmol), pyridine (0.0400 mL, 0.497 mmol) in anhydrous methylene chloride (0.4 mL) at 0 °C under an atmosphere of nitrogen and stirred for 0.5 h. The reaction mixture was diluted with methylene chloride (2 mL) and water (2 mL) and stirred for 0.5

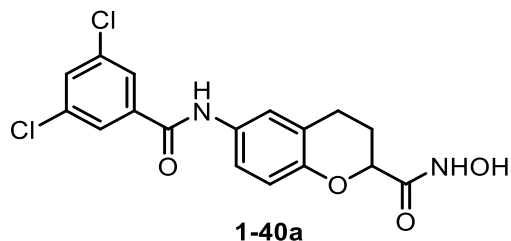
h. The isolated organic solution was sequentially washed with  $\text{KHSO}_4$  (0.5 M, 2 mL), brine (2 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered through a silica pad, providing (*S*)-**1-34a**' (0.0261 g, 70%). 5 mg of the pure material was used for HPLC analysis on a chiral stationary phase (Chiralpak AD-H, 0.46 ID x 25 cm; 5  $\mu\text{m}$ ); sample prep: 1 mg/mL in 100% absolute EtOH; hexanes/EtOH (95:05), isocratic; 254 nm; 1 mL/min; retention time: 26.2 min), and was found to be >96% ee.



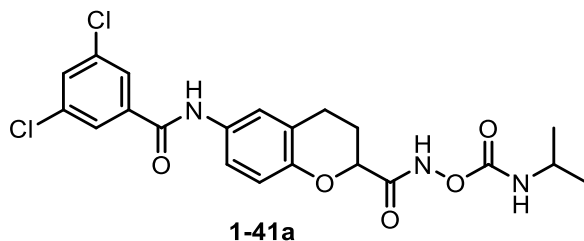
**Methyl 6-(3,5-dichlorobenzamido)chromane-2-carboxylate (1-38a).** A 0 °C solution of aniline **1-34b** in  $\text{CH}_2\text{Cl}_2$  (0.48 M, 1.69 mL, 0.811 mmol) was treated with 3,5-dichlorobenzoyl chloride (0.170 g, 0.813 mmol), DMAP (0.0340 g, 0.278 mmol) and pyridine (0.100 mL, 1.24 mmol). After 5 min, the solution was warmed to rt, and was stirred under  $\text{N}_2$  for 4 h. The mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (30 mL), washed with 0.5 M HCl (1x 30 mL), brine (1x 30 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to provide **1-34b** (93% purity by  $^1\text{H}$  NMR ( $\text{CH}_2\text{Cl}_2$ ), 0.313 g, 94%) as an off-white solid with a slight pink hue:  $^1\text{H}$  NMR (300 MHz;  $\text{DMSO}-d_6$ )  $\delta$  10.27 (s, 1 H), 7.98 (d,  $J = 1.9$  Hz, 2 H), 7.88 (m, 1 H), 7.51 (d,  $J = 2.2$  Hz, 1 H), 7.45 (dd,  $J = 8.7, 2.5$  Hz, 1 H), 6.86 (d,  $J = 8.8$  Hz, 1 H), 4.94 (dd,  $J = 6.5, 3.8$  Hz, 1 H), 3.73 (s, 3 H), 2.89-2.79 (m, 1 H), 2.71-2.61 (m, 1 H), 2.24-2.04 (m, 2 H); HRMS ( $\text{ESI}^+$ )  $m/z$  calcd for  $\text{C}_{18}\text{H}_{16}\text{Cl}_2\text{NO}_4$  [ $\text{M}+\text{H}$ ] $^+$  380.0451, found 380.0449.



**6-(3,5-Dichlorobenzamido)-N-((tetrahydro-2H-pyran-2-yl)oxy)chromane-2-carboxamide (1-39a).** To a solution of the ester **1-38a** (93% purity, 0.302 g, 0.737 mmol) in THF (3.0 mL) and MeOH (1.5 mL) was added LiOH monohydrate (0.0394 g, 0.939 mmol) in H<sub>2</sub>O (2.8 mL) at rt. After 10 h, the solution was concentrated and the residue was azeotroped with toluene (2x 20 mL). The crude residue was dissolved in DMF (2.5 mL) and treated with o-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.195 g, 1.67 mmol) in DMF (1.5 mL), HATU (0.363 g, 0.955 mmol), and DIPEA (0.200 mL, 1.15 mmol). The mixture was stirred under N<sub>2</sub>, and after 22 h, was diluted with EtOAc (50 mL), washed with 0.5 M HCl (50 mL), brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The residue was purified by chromatography on SiO<sub>2</sub> (33-50% hexanes in EtOAc), to give **1-39a** (0.201 g, 59%) as a white solid: <sup>1</sup>H NMR (300 MHz; acetone-*d*<sub>6</sub>) δ 10.39 (s, 1 H), 9.49 (s, 1 H), 7.91 (d, *J* = 1.8 Hz, 2 H), 7.65 (t, *J* = 1.8 Hz, 1 H), 7.51-7.47 (m, 2 H), 6.77 (d, *J* = 8.5 Hz, 1 H), 4.96-4.94 (m, 1 H), 4.61-4.55 (m, 1 H), 4.05-3.97 (m, 1 H), 3.51-3.43 (m, 1 H), 2.87-2.71 (m, 2 H), 2.31-2.22 (m, 1 H), 2.01-2.11 (m, 1 H), 1.73-1.49 (m, 6 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>22</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>Na [M+Na]<sup>+</sup> 487.0798, found 487.0797.



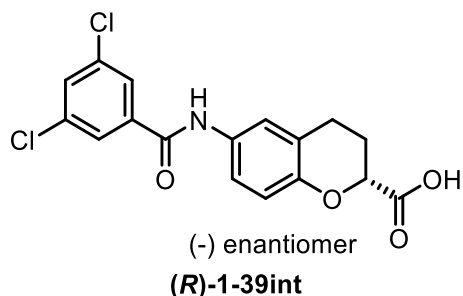
**6-(3,5-Dichlorobenzamido)-N-hydroxychromane-2-carboxamide (1-40a).** A solution of amide **1-39a** (0.200 g, 0.430 mmol) in MeOH (5.0 mL) was treated with TFA (2.39 mL, 32.2 mmol) at rt under N<sub>2</sub>. After 13 h, the mixture was concentrated. The solid residue was slurried with Et<sub>2</sub>O, filtered, and rinsed with Et<sub>2</sub>O (~ 20-30 mL). After drying under vacuum, **1-40a** (0.123 g, 75%) was collected as a white solid: Mp 242-243 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3272, 2961, 2914, 1642, 1569, 1535, 1494, 1263, 1220 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.79 (s, 1 H), 10.23 (s, 1 H), 8.93 (s, 1 H), 7.96 (d, *J* = 1.9 Hz, 2 H), 7.85 (t, *J* = 1.9 Hz, 1 H), 7.47 (d, *J* = 2.3 Hz, 1 H), 7.42 (dd, *J* = 8.8, 2.5 Hz, 1 H), 6.82 (d, *J* = 8.8 Hz, 1 H), 4.51 (dd, *J* = 8.9, 3.1 Hz, 1 H), 2.84-2.78 (m, 1 H), 2.73-2.68 (m, 1 H), 2.15-2.09 (m, 1 H), 2.09-1.91 (m, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 166.4, 162.1, 149.9, 138.1, 134.2, 131.3, 130.7, 126.3, 121.71, 121.67, 120.1, 116.4, 73.8, 24.3, 23.2; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>17</sub>H<sub>13</sub>O<sub>4</sub>N<sub>2</sub>Cl<sub>2</sub> [M-H]<sup>+</sup> 379.0247, found 379.0234; ELS purity 100%.



**6-(3,5-Dichlorobenzamido)-N-((isopropylcarbamoyl)oxy)chromane-2-carboxamide (1-41a).**

A solution of the hydroxamic acid **1-40a** (0.0480 g, 0.126 mmol) in DMF (0.1 mL) and acetone

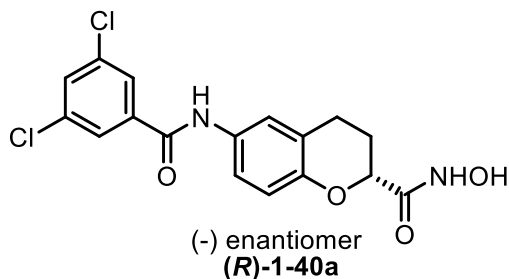
(0.2 mL) was cooled to -15 °C. The solution was treated with isopropyl isocyanate (15.0 μL, 0.153 mmol), and the mixture was warmed to rt. After 30 h, DMF (0.2 mL), acetone (0.4 mL), and isopropyl isocyanate (50.0 μL, 0.509 mmol) were added, and the mixture was stirred for 38 h at room temperature. The acetone in the mixture was evaporated in vacuo, and water (0.4 mL) was added to precipitate out the crude carbamate. Trituration with hexanes/ether (1:1) and concentration in vacuo provided the carbamate **1-41a** (0.0384 g, 65%) as a white solid: Mp 119-121 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3283, 3079, 2975, 1750, 1689, 1534, 1495 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>) δ 11.70 (s, 1 H), 10.26 (s, 1 H), 7.96 (d, *J* = 2.0 Hz, 2 H), 7.86 (t, *J* = 2.0 Hz, 1 H), 7.68-7.67 (m, 1 H), 7.48-7.44 (m, 3 H), 6.84 (d, *J* = 8.8 Hz, 1 H), 4.68 (dd, *J* = 8.4, 3.2 Hz, 1 H), 3.59-3.61 (m, 1 H), 2.82-2.71 (m, 2 H), 2.18-2.13 (m, 1 H), 2.01-1.98 (m, 1 H), 1.10-1.08 (m, 6 H); <sup>13</sup>C NMR (101 MHz; DMSO-*d*<sub>6</sub>) δ 167.4, 162.1, 153.7, 149.7, 138.1, 134.3, 131.5, 130.7, 126.4, 121.8, 121.7, 120.1, 116.5, 73.7, 43.1, 24.4, 23.0, 22.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>21</sub>H<sub>20</sub>O<sub>5</sub>N<sub>3</sub>Cl<sub>2</sub> [M-H]<sup>+</sup> 464.0775, found 464.0782; ELS purity 100%.



**(R)-6-(3,5-Dichlorobenzamido)chromane-2-carboxylic acid ((R)-1-39int)**. To a solution of **(R)-1-38a\*** (0.218 g, 0.573 mmol) in THF (1.5 mL) and MeOH (1.5 mL) was added 1M LiOH (0.70 mL, 0.7 mmol) at rt. After 4 h, the reaction mixture was concentrated and the residue was azeotroped with PhMe (2 x 15 mL). The salt was acidified with 1M HCl (15 mL) and extracted

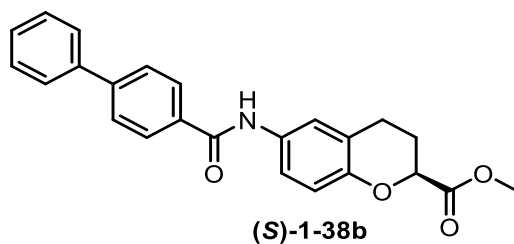
with EtOAc (3 x 60 mL). The combined organic layers were washed with brine (20 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated, yielding **(R)-1-39int** (0.150 g, 71%) as a beige powder: [ $\alpha$ ]<sub>D</sub> -23.5 (*c* 0.13, MeOH); Mp 222-225 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3334, 3092, 2924, 2852, 1724, 1616, 1551, 1493, 1421 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  13.02 (s, 1 H), 10.24 (s, 1 H), 7.96 (d, *J* = 1.9 Hz, 2 H), 7.86 (t, *J* = 1.9 Hz, 1 H), 7.48 (d, *J* = 2.4 Hz, 1 H), 7.41 (dd, *J* = 8.8, 2.5 Hz, 1 H), 6.81 (d, *J* = 8.8 Hz, 1 H), 4.77 (dd, *J* = 6.5, 3.9 Hz, 1 H), 2.84-2.76 (m, 1 H), 2.68-2.61 (m, 1 H), 2.20-2.12 (m, 1 H), 2.10-2.02 (m, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  171.9, 162.1, 150.0, 138.1, 134.2, 131.2, 130.7, 126.3, 121.8, 121.3, 120.2, 116.1, 72.8, 23.8, 22.6; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>17</sub>H<sub>14</sub>O<sub>4</sub>NCl<sub>2</sub> [M+H]<sup>+</sup> 366.0294, found 366.0294; ELS purity 100%.

\***(R)-1-38a** was obtained from separation on a chiral column by Alyssa Thornton.



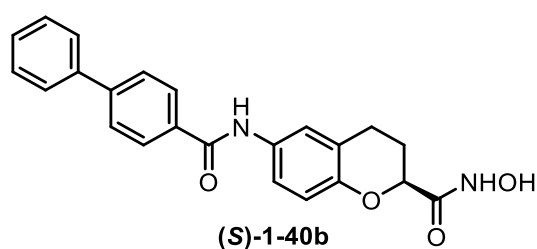
**(R)-6-(3,5-Dichlorobenzamido)-N-hydroxychromane-2-carboxamide ((R)-1-40a).** The carboxylic acid **(R)-1-39int** (0.0875 g, 0.239 mmol) in DMF (0.8 mL) and treated with *O*-(Tetrahydro-2*H*-pyran-2-yl)hydroxylamine (0.0494 g, 0.422 mmol). The mixture was cooled to 0 °C, and treated with TEA (0.0900 mL, 0.646 mmol) and T<sub>3</sub>P (50%, 0.200 mL, 0.336 mmol). The mixture was warmed to rt and stirred under N<sub>2</sub>. After 16 h, the mixture was diluted with EtOAc (20 mL), washed with 0.5 M HCl (10 mL), brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica to remove baseline impurities, and concentrated to provide the amide (0.105 g) as a white solid.

The solid (0.100 g) was dissolved in MeOH (3.0 mL) and treated with Amberlyst-15 (0.0192 g, 90.2 mmol) at rt under N<sub>2</sub>. After 18 h of stirring, the reaction mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc), and **(R)-1-40a** (0.0573 g, 66%) was collected as a pink solid:  $[\alpha]_D -18.2$  (*c* 0.13, MeOH); Mp 187-190 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3256, 3074, 2924, 1642, 1566, 1529, 1495, 1420 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.80 (s, 1 H), 10.24 (s, 1 H), 8.95 (s, 1 H), 7.96 (d, *J* = 1.6 Hz, 2 H), 7.85 (t, *J* = 1.9 Hz, 1 H), 7.47 (d, *J* = 2.0 Hz, 1 H), 7.42 (dd, *J* = 8.6, 2.0 Hz, 1 H), 6.82 (d, *J* = 8.7 Hz, 1 H), 4.51 (dd, *J* = 8.9, 2.9 Hz, 1 H), 2.84-2.78 (m, 1 H), 2.73-2.68 (m, 1 H), 2.14-2.09 (m, 1 H), 1.98-1.91 (m, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 166.4, 162.1, 150.0, 138.1, 134.3, 131.4, 130.7, 126.3, 121.75, 121.72, 120.1, 116.5, 73.8, 24.3, 23.2; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>17</sub>H<sub>15</sub>O<sub>4</sub>N<sub>2</sub>Cl<sub>2</sub> [M+H]<sup>+</sup> 381.0403, found 381.0401; ELS purity 98.3%.



**Methyl (S)-6-([1,1'-biphenyl]-4-carboxamido)chromane-2-carboxylate ((S)-1-38b)**. A 0 °C solution of **(S)-1-34b** (0.0726 g, 0.350 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.8 mL) was treated with [1,1'-biphenyl]-4-carbonyl chloride (0.095 g, 0.438 mmol), pyridine (0.200 mL, 2.47 mmol), and DMAP (0.0170 g, 0.139 mmol). After 5 min, the mixture was warmed to rt, and was stirred under N<sub>2</sub> for 11 h. The mixture was treated with 0.5 M HCl (3 mL) and left to stir for 20 minutes. The mixture was transferred to a separatory funnel and partitioned between CH<sub>2</sub>Cl<sub>2</sub> (15 mL) and 0.5 M HCl (15 mL). The layers were separated, and the organic layer was washed with sat. NaHCO<sub>3</sub> (15 mL) and

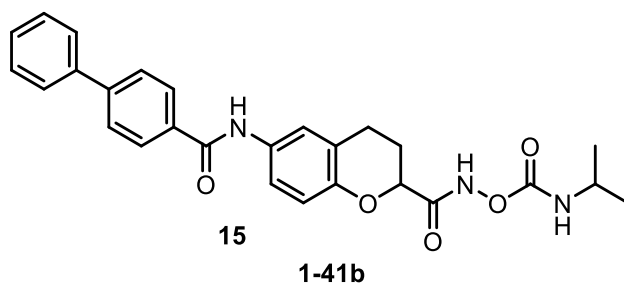
brine (15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to provide **(S)-1-38b** (0.136 g, 98%) as a white solid: <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.97-7.91 (m, 2 H), 7.73-7.68 (m, 3 H), 7.65-7.61 (m, 2 H), 7.56 (d, *J* = 2.5 Hz, 1 H), 7.51-7.45 (m, 2 H), 7.43-7.37 (m, 1 H), 7.21 (dd, *J* = 8.8, 2.9 Hz, 1 H), 6.94 (d, *J* = 8.7 Hz, 1 H), 4.75 (dd, *J* = 7.3, 3.7 Hz, 1 H), 3.81 (s, 3 H), 2.89-2.80 (m, 2 H), 2.31-2.20 (m, 2 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>24</sub>H<sub>22</sub>NO<sub>4</sub> [M+H]<sup>+</sup> 388.1543, found 388.1539.



**(S)-6-([1,1'-Biphenyl]-4-carboxamido)-N-hydroxychromane-2-carboxamide ((S)-1-40b)**. A solution of **(S)-1-38b** (0.125 g, 0.25 mmol) in THF (1.5 mL) and MeOH (1.5 mL) was treated with LiOH monohydrate (0.0135 g, 0.355 mmol) in water (1.0 mL) at room temperature. After 1 h, the mixture was concentrated, and azeotroped with toluene (2 x 10 mL). The residue was dissolved in DMF (1.0 mL) and treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.100 g, 0.854 mmol). The mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 0.280 mL, 0.470 mmol) and TEA (0.200 mL, 1.43 mmol). The mixture was warmed to room temperature after 30 min and stirred under N<sub>2</sub>. After 3 h, the mixture was diluted with EtOAc (10 mL), washed with 0.5 M HCl (10 mL), brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica to remove baseline impurities, and concentrated. The amide (0.118 g) was collected and dissolved in MeOH (5.0 mL). Amberlyst-15 (0.0367 g, 172 mmol) was added at room temperature under N<sub>2</sub>. After 24 h of stirring, the mixture was treated with more Amberlyst-15 (0.0220 g, 103 mmol), and stirred for another 22 h. TLC (1:1 Hex:EtOAc) confirmed consumption of starting material. The mixture was filtered through Celite,

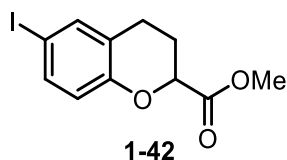


rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc) yielding **(S)-1-40b** (0.0672 g, 54%, 3 steps) as a light grey solid:  $[\alpha]_D +20.1$  ( $c$  0.30, DMSO); Mp 191 °C (dec.); IR (CH<sub>2</sub>Cl<sub>2</sub>) 3259, 1639, 1527, 1494, 1422, 1220 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  10.79 (s, 1 H), 10.11 (s, 1 H), 8.93 (s, 1 H), 8.04 (d,  $J$  = 8.4 Hz, 2 H), 7.78 (d,  $J$  = 8.4 Hz, 2 H), 7.76 (d,  $J$  = 7.3 Hz, 2 H), 7.53-7.49 (m, 3 H), 7.46 (dd,  $J$  = 8.8, 2.4 Hz, 1 H), 7.42 (app t,  $J$  = 7.3 Hz, 1 H), 6.82 (d,  $J$  = 8.8 Hz, 1 H), 4.51 (dd,  $J$  = 9.0, 3.0 Hz, 1 H), 2.87-2.79 (m, 1 H), 2.74-2.69 (m, 1 H), 2.15-2.10 (m, 1 H), 1.99-1.93 (m, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  166.4, 164.6, 149.6, 142.9, 139.1, 133.8, 132.0, 129.0, 128.2, 128.1, 126.9, 126.5, 121.62, 121.57, 120.1, 116.4, 73.8, 24.3, 23.3; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>23</sub>H<sub>21</sub>O<sub>4</sub>N<sub>2</sub> [M+H]<sup>+</sup> 389.1496, found 389.1494; ELS purity 100%.



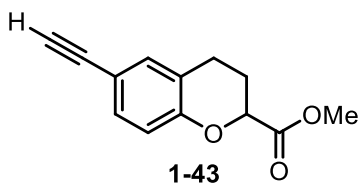
**6-([1,1'-Biphenyl]-4-carboxamido)-N-((isopropylcarbamoyl)oxy)chromane-2-carboxamide (1-41b).** A solution of the hydroxamic acid **1-40b** (0.0720 g, 0.185 mmol) in DMF (0.1 mL) and acetone (0.2 mL) was cooled to -15 °C. The solution was treated with isopropyl isocyanate (20.0  $\mu$ L, 0.204 mmol), and the mixture was warmed to room temperature. After 30 h, DMF (0.2 mL), acetone (0.4 mL), and isopropyl isocyanate (20.0  $\mu$ L, 0.204 mmol) were added, and the mixture was stirred for 15 h at rt. The acetone and isocyanate in the mixture were evaporated in vacuo, and water (2 mL) was added to precipitate out the crude carbamate. Trituration with hexanes/ether

(1:1) and concentration in vacuo provided **1-41b** (0.0483 g, 55%) as a beige solid: Mp 200 °C (dec.); IR (CH<sub>2</sub>Cl<sub>2</sub>) 3300, 2980, 1765, 1676, 1638, 1525, 1495, 1218 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; acetone-*d*<sub>6</sub>) δ 10.72 (s, 1 H), 9.42 (s, 1 H), 8.09 (d, *J* = 8.0 Hz, 2 H), 7.79 (d, *J* = 8.5 Hz, 2 H), 7.73 (d, *J* = 7.5 Hz, 2 H), 7.60-7.52 (m, 2 H), 7.50 (t, *J* = 8.0 Hz, 2 H), 7.42 (t, *J* = 7.0 Hz, 1 H), 6.83 (d, *J* = 8.5 Hz, 1 H), 6.60 (s, 1 H), 4.72 (dd, *J* = 8.5, 3.0 Hz, 1 H), 3.75-3.74 (m, 1 H), 2.90-2.82 (m, 2 H), 2.31-2.29 (m, 1 H), 2.14- 2.09 (m, 1 H), 1.18-1.16 (m, 6 H) ; <sup>13</sup>C NMR (126 MHz; acetone-*d*<sub>6</sub>): δ 168.9, 165.6, 154.7, 150.4 144.7, 140.9, 135.2, 133.7, 129.88, 129.92, 128.88, 127.9, 127.7, 123.1, 122.4, 120.8, 117.5, 76.0, 44.6, 25.6, 24.4, 22.8; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>27</sub>H<sub>26</sub>O<sub>5</sub>N<sub>3</sub> [M-H]<sup>+</sup> 472.1867, found 472.1875; ELS purity 100%.



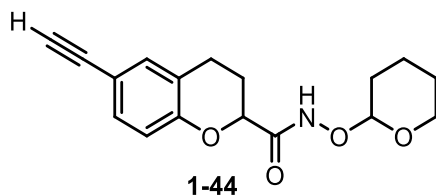
**Methyl 6-iodochromane-2-carboxylate (1-42).**<sup>56</sup> Chromane-2-carboxylic acid (90% purity, 4.29 g, 21.7 mmol) was dissolved in glacial AcOH (70.0 mL) and was treated with ZnCl<sub>2</sub> (4.51 g, 33.1 mmol), followed by Bn(Me<sub>3</sub>)NiCl<sub>2</sub> (8.55 g, 24.5 mmol) at room temperature. After 16 h, the solution was partitioned between CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and H<sub>2</sub>O (50 mL), and the layers were separated. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 x 50 mL) and was washed with H<sub>2</sub>O (2x 60 mL). The organic extractions were concentrated to ~ 150 mL and washed with 5% NaHSO<sub>3</sub> (2x100 mL) and brine (1x150 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure to yield 5.8 g of a light beige/yellow solid. The solid was dissolved in MeOH (55 mL) in a 500 mL round-bottom flask, followed by the addition of conc. H<sub>2</sub>SO<sub>4</sub> (15 drops). The mixture was heated at 50 °C. After 2 h, the solution was concentrated, diluted with EtOAc (200 mL), washed with satd.

NaHCO<sub>3</sub> (1x 100 mL) and brine (1x 100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure to provide a dark yellow/orange residue. A pink colored solution was collected in the receiver of the rotary evaporator. The mixture was diluted with diethyl ether/hexanes and concentrated until the pink color was no longer observed, and **1-42** (5.84 g, 85%) was collected as a beige/yellow solid: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.39 (dd, *J* = 8.5, 2.2 Hz, 1 H), 7.35-7.34 (m, 1 H), 6.69 (d, *J* = 8.5 Hz, 1 H), 4.73 (dd, *J* = 7.0, 4.0 Hz, 1 H), 3.79 (s, 3 H), 2.84-2.65 (m, 2 H), 2.30-2.10 (m, 2 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>11</sub>H<sub>11</sub>IO<sub>3</sub>Na [M+Na]<sup>+</sup> 340.9645, found 340.9643.



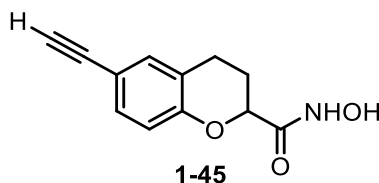
**Methyl 6-ethynylchromane-2-carboxylate (1-43).** Sonogashira: In 2 x 20 mL sealed tubes, a total amount of iodide **1-42** (3.49 g, 11.0 mmol) and alkyne (122.0 mL, 84.9 mmol) in DMF (26 mL) was degassed via Ar sparging for 10 min. PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (0.0680 g, 0.221 mmol) and CuI (0.329 g, 1.73 mmol) were added, and the mixture was sparged for 10 min. TEA (5.0 mL, 35.6 mmol) was added, followed by Ar sparging for 10 min. The mixture was stirred at 80 °C for 12 h. LCMS confirmed reaction completion, and the mixtures were cooled down, combined, and washed with H<sub>2</sub>O (1 x 200 mL) and brine (1 x 200 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through Celite, and concentrated. Deprotection: The intermediate silylalkyne (3.45 g, 11.979 mmol) was dissolved in THF (35 mL) and treated with TBAF (1.0 M in THF, 13.0 mL, 13.0 mmol). After 2 h of stirring, the mixture was filtered through a pad of SiO<sub>2</sub>, and rinsed with EtOAc. After concentrating, the residue was purified by chromatography on SiO<sub>2</sub> (5-20 % EtOAc in Hexanes; product R<sub>f</sub> ~ 0.3 in 10% EtOAc in Hexanes) to provide **1-43** (1.52 g, 64%) was collected as a

yellow solid: Mp 93-96 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3281, 2953, 2103, 1753, 1608, 1578, 1491, 1202, 1123 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.25 (dd, *J* = 8.5, 1.8 Hz, 1 H), 7.19 (s, 1 H), 6.86 (d, *J* = 8.5 Hz, 1 H), 4.76 (dd, *J* = 7.2, 3.7 Hz, 1 H), 3.79 (s, 3 H), 2.97 (s, 1 H), 2.82-2.76 (m, 1 H), 2.74-2.68 (m, 1 H), 2.30-2.23 (m, 1 H), 2.21-2.15 (m, 1 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 171.1, 154.1, 133.6, 131.8, 121.5, 117.2, 114.5, 83.7, 75.8, 74.0, 52.6, 24.4, 23.1; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>13</sub>H<sub>13</sub>O<sub>3</sub> [M+H]<sup>+</sup> 217.0859, found 217.0860.

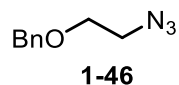


**6-Ethynyl-N-((tetrahydro-2H-pyran-2-yl)oxy)chromane-2-carboxamide (1-44).** To a solution of ester **1-43** (0.351 g, 1.62 mmol) in THF (3.0 mL) and MeOH (3.0 mL) in a 100 mL round-bottom flask was added LiOH monohydrate (0.00880 g, 2.10 mmol) in H<sub>2</sub>O (3.0 mL) at room temperature. After 4 h of stirring under N<sub>2</sub>, the solution was concentrated and the residue was azeotroped with toluene (2x 20 mL). The crude residue was dissolved in DMF (4.0 mL) and treated with o-((tetrahydro-2H-pyran-2-yl)oxy)hydroxylamine (0.223 g, 1.90 mmol). The mixture was cooled to 0 °C, and T<sub>3</sub>P (50%, 0.900 mL, 2.55 mmol) and TEA (0.450 mL, 3.23 mmol) were added. The mixture was stirred under N<sub>2</sub>, and after 12 h, was diluted with EtOAc (50 mL), washed with 0.5 M HCl (50 mL), brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under vacuum. The residue was azeotroped with hexanes multiple times to remove the trapped EtOAc in the thick oil. Drying under vacuum yielded **1-44** (92% purity by <sup>1</sup>H NMR, 0.498 g, 94%) as a thick orange/yellow foam: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 9.10 (s, 1 H), 7.27-7.24 (m, 1 H), 7.23 (bs, 1

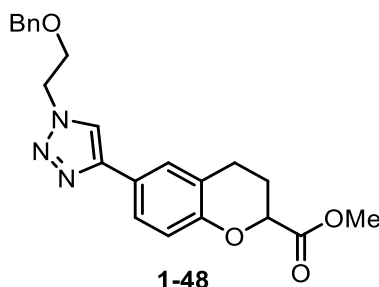
H), 6.82 (dd,  $J = 8.3, 5.0$  Hz, 1 H), 5.02 (app t,  $J = 2.9$  Hz, 0.5 H), 4.96 (app t,  $J = 2.9$  Hz, 0.5 H), 4.65-4.61 (m, 1 H), 4.02-3.94 (m, 1 H), 3.70-3.58 (m, 1 H), 2.99 (s, 1 H), 2.88-2.72 (m, 2 H), 2.50-2.38 (m, 1 H), 2.12-1.98 (m, 2 H), 1.85-1.79 (m, 3 H), 1.64-1.58 (m, 3 H); HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>17</sub>H<sub>19</sub>NO<sub>4</sub>Na [M+Na]<sup>+</sup> 324.1206, found 324.1202.



**6-Ethynyl-N-hydroxychromane-2-carboxamide (1-45).** The amide **1-44** (92% purity) was filtered through a pad of silica, and washed with 100% EtOAc. LCMS indicated pure compound. Upon concentration under vacuum, amide **1-44** (0.153 g, 0.508 mmol) in MeOH (3.0 mL) was added Amberlyst-15 (0.027 g, washed with MeOH) at room temperature. After 20 h of stirring under N<sub>2</sub>, the mixture was filtered through Celite, rinsed with MeOH and concentrated. The residue was purified by chromatography on SiO<sub>2</sub> (0-100 % EtOAc in hexanes, product eluted at 50%), and the collected solid was further purified by trituration (EtOAc/hexanes), yielding **1-45** (0.0350 g, 32%) as a white solid: Mp 161-163 °C; IR (neat) 3335, 3305, 3266, 2981, 2842, 2105, 1662, 1607, 1580, 1491, 1248 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  10.82 (s, 1 H), 8.96 (s, 1 H), 7.22-7.18 (m, 2 H), 6.81 (d,  $J = 9.0$  Hz, 1 H), 4.56 (dd,  $J = 8.7, 3.2$  Hz, 1 H), 3.98 (s, 1 H), 2.80-2.73 (m, 1 H), 2.71-2.66 (m, 1 H), 2.14-2.08 (m, 1 H), 1.96-1.88 (m, 1 H). <sup>13</sup>C NMR (101 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  166.1, 153.9, 133.0, 130.8, 122.5, 117.0, 113.5, 83.6, 79.0, 74.0, 24.0, 22.6; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>12</sub>H<sub>10</sub>O<sub>3</sub>N [M-H]<sup>+</sup> 216.0655, found 216.0668; ELS purity 99.1%.

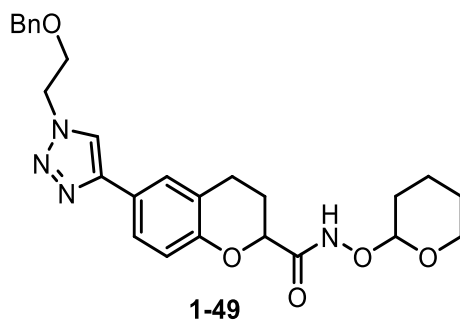


**((2-Azidoethoxy)methyl)benzene (1-46).**<sup>112</sup> To a solution of 2-(benzyloxy)ethyl methanesulfonate (0.482 g, 2.09 mmol) in anhydrous DMF (10.5 mL) was added NaN<sub>3</sub> (0.273 g, 4.20 mmol) under N<sub>2</sub>. The reaction mixture was stirred at 50 °C for 22 h. The reaction mixture was allowed to cool to rt and was partitioned between EtOAc (30 mL) and H<sub>2</sub>O (30 mL). The aq. layer was extracted with EtOAc (3 x 30 mL). The organic layers were combined, washed with brine (5 x 15 mL), dried (MgSO<sub>4</sub>), and concentrated under vacuum to provide **1-46** (0.356 g, 95%) as a light yellow oil: <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.38-7.35 (m, 4 H), 7.33-7.29 (m, 1 H), 4.59 (s, 2 H), 3.67 (t, *J* = 5.1 Hz, 2 H), 3.42 (t, *J* = 5.0 Hz, 2 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 137.9, 128.6, 127.9, 127.8, 73.4, 69.0, 51.0.



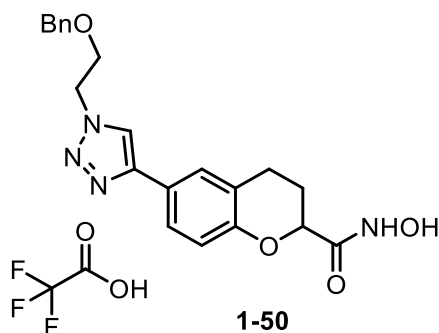
**Methyl 6-(1-(2-(benzyloxy)ethyl)-1H-1,2,3-triazol-4-yl)chromane-2-carboxylate (1-48).** The alkyne **1-45** (0.0699 g, 0.392 mmol), azide **1-46** (85% purity, 0.0980 g, 0.385 mmol), and TEA (0.0500 mL, 0.359 mmol) were dissolved in *t*-BuOH (0.45 mL) and H<sub>2</sub>O (0.45 mL). Copper sulfate pentahydrate (0.0223 g, 0.0913 mmol) and sodium ascorbate (0.0380 g, 0.192 mmol) were added and the mixture was stirred for 4 h N<sub>2</sub>. The solvent was removed under vacuum and the crude residue was partitioned between H<sub>2</sub>O : brine (1:1, 20 mL) and EtOAc (5 mL). The aq. layer was

extracted with EtOAc (3 x 15 mL) and the organics were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated under reduced pressure to provide the crude product which was purified by chromatography on SiO<sub>2</sub> (0-100% EtOAc in hexanes) to provide **1-48** (0.0670 g, 44%) as a yellow solid: Mp 79.0-82.0 °C; IR (neat) 2953, 1752, 1489, 1454, 1206, 1125, 1096, 1044, 1016, 822 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.80 (s, 1 H), 7.60 (m, 1 H), 7.49 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.35-7.28 (m, 3 H), 7.27 (m, 1 H), 7.25 (m, 1 H), 6.98 (d, *J* = 8.5 Hz, 1 H), 4.78 (dd, *J* = 7.5, 3.6 Hz, 1 H), 4.58 (t, *J* = 5.0 Hz, 2 H), 4.52 (s, 2 H), 3.87 (t, *J* = 5.0 Hz, 2 H), 3.81 (s, 3 H), 2.96-2.85 (m, 1 H), 2.85-2.77 (m, 1 H), 2.35-2.29 (m, 1 H), 2.26-2.18 (m, 1 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 171.4, 153.6, 147.7, 137.5, 128.7, 128.2, 127.9, 127.0, 125.4, 123.8, 121.8, 120.2, 117.5, 74.1, 73.6, 68.6, 52.6, 50.6, 24.7, 23.5; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>22</sub>H<sub>24</sub>N<sub>3</sub>O<sub>4</sub> [M+H]<sup>+</sup> 394.1761, found 394.1757.



**6-(1-(2-(Benzyloxy)ethyl)-1H-1,2,3-triazol-4-yl)-N-((tetrahydro-2H-pyran-2-yl)oxy) chromane-2-carboxamide (1-49).** To a solution of ester **1-48** (0.0600 g, 0.153 mmol) in THF (0.6 mL) and MeOH (0.5 mL) was added LiOH monohydrate (0.00870 g, 0.207 mmol) in H<sub>2</sub>O (0.6 mL) at rt. After 2 h of stirring under N<sub>2</sub>, the solution was concentrated under vacuum and the residue was azeotroped with toluene (2 x 5 mL). The crude residue was dissolved in DMF (1.0 mL) and treated with *o*-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.0419 g, 0.358 mmol), HATU (0.0759 g,

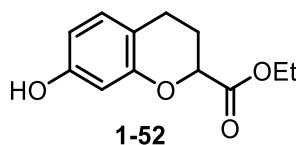
0.200 mmol), and DIPEA (0.0500 mL, 0.287 mmol). The mixture was stirred under N<sub>2</sub>, and after 10 h, was diluted with EtOAc (20 mL), washed with 0.5 M HCl (20 mL), brine (20 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The residue was purified by chromatography on SiO<sub>2</sub> (50% EtOAc in hexanes) to provide **1-49** (0.0582 g, 80%) as a beige solid: <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 9.15 (s, 1 H), 7.81 (s, 1 H), 7.59-7.53 (m, 2 H), 7.34-7.30 (m, 3 H), 7.27-7.25 (m, 2 H), 6.94 (m, 1 H), 5.04-4.98 (m, 1 H), 4.68-4.64 (m, 1 H), 4.59 (t, *J* = 5.0 Hz, 2 H), 4.53 (s, 2 H), 4.03-3.96 (m, 1 H), 3.88 (t, *J* = 5.2 Hz, 2 H), 3.71-3.60 (m, 1 H), 2.96-2.79 (m, 2 H), 2.51-2.44 (m, 1 H), 2.16-2.06 (m, 1 H), 1.91-1.79 (m, 3 H), 1.68-1.63 (m, 3 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>26</sub>H<sub>31</sub>N<sub>4</sub>O<sub>5</sub> [M+H]<sup>+</sup> 479.2289, found 479.2286.



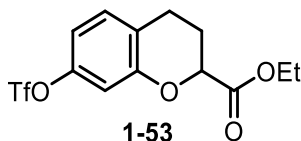
**6-(1-(2-(Benzyloxy)ethyl)-1*H*-1,2,3-triazol-4-yl)-*N*-hydroxychromane-2-carboxamide tri-fluoroacetate (**1-50**).** A solution of **1-49** (0.0580 g, 0.121 mmol) in MeOH (2.4 mL) was treated with TFA (0.700 mL, 9.42 mmol) at rt. After 2 h, the mixture was concentrated. The solid residue was purified by chromatography on SiO<sub>2</sub> (33-100% EtOAc in Hexanes) to provide **1-50** (0.0276 g, 45%) as a beige solid: Mp 150-154 °C; IR (neat) 3142, 2870, 1572, 1489, 1203, 1139, 1106 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.81 (s, 1 H), 8.92 (s, 1 H), 8.39 (s, 1 H), 7.55 (m, 1 H), 7.53 (s, 1 H) 7.33-7.23 (m, 5 H), 6.87 (d, *J* = 8.3 Hz, 1 H), 4.59 (t, *J* = 5.2 Hz, 2 H), 4.53 (m, 1 H), 4.50 (s, 2 H), 3.86 (t, *J* = 5.2 Hz, 2 H), 2.85-2.73 (m, 2 H), 2.18-2.00 (m, 2 H); <sup>19</sup>F NMR (471 MHz,



DMSO-*d*<sub>6</sub>)  $\delta$  -73.53; <sup>13</sup>C NMR (151 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  157.8 (q,  $J_{C-F}$  = 30.2 Hz), 146.2, 138.0, 128.3, 127.54, 127.48, 126.3, 124.2, 122.4, 120.8, 118.3, 117.0, 116.3, 71.8, 68.0, 49.6, 48.6, 24.4, 23.2; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>21</sub>H<sub>23</sub>O<sub>4</sub>N<sub>4</sub> [M+H]<sup>+</sup> 395.1714, found 395.1710; ELS purity 100%.

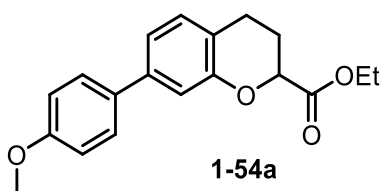


**Ethyl 7-hydroxychromane-2-carboxylate (1-52).**<sup>113</sup> Ethyl 7-hydroxy-4-oxo4H-chromene-2-carboxylate (2.01 g, 8.59 mmol) in EtOH (18 mL) was evacuated and purged with N<sub>2</sub> (2x). The solution was treated with Pd/C (10%, 0.320 g, 0.301 mmol) and evacuated and purged with H<sub>2</sub> (2x) and kept under H<sub>2</sub> balloon (1 atm). After 17 h, the mixture was filtered through Celite and rinsed with EtOAc. The filtrate was concentrated, providing **1-52** (1.86 g, 98%) as a grey solid: <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta$  6.87 (d,  $J$  = 8.2 Hz, 1 H), 6.46 (d,  $J$  = 2.5 Hz, 1 H), 6.39, (dd,  $J$  = 8.2, 2.5 Hz, 1 H), 5.07 (s, 1 H), 4.69 (dd,  $J$  = 7.5, 3.6 Hz, 1 H), 4.26 (q,  $J$  = 7.2 Hz, 2 H), 2.79-2.73 (m, 1 H), 2.69-2.64 (m, 1 H), 2.28-2.22 (m, 1 H), 2.19-2.12 (m, 1 H), 1.29 (t,  $J$  = 7.2 Hz, 3 H).



**Ethyl 7-(((trifluoromethyl)sulfonyl)oxy)chromane-2-carboxylate (1-53).**<sup>89</sup> A 0 °C solution of alcohol **1-52** (1.85 g, 3.32 mmol) and pyridine (1.30 mL, 16.1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was

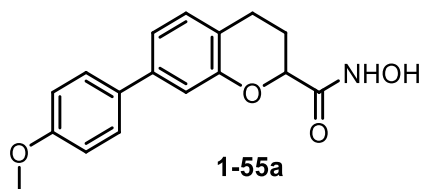
treated with trifluoromethanesulfonic anhydride (2.00 mL, 11.9 mmol) dropwise. After 5 min, the mixture was warmed to room temperature. After 3 h, the mixture was diluted with Et<sub>2</sub>O (50 mL) and quenched with 10% aq HCl (50 mL). The organic layer was washed with sat. NaHCO<sub>3</sub> (50 mL) and brine (50 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through a pad of SiO<sub>2</sub>, and rinsed (2:1, Hexanes: EtOAc) to provide **1-53** (2.64 g, 90%) as a yellow oil: IR (CH<sub>2</sub>Cl<sub>2</sub>) 2942, 1753, 1612, 1597, 1494, 1421, 1206, 1106 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.08 (d, *J* = 8.5 Hz, 1 H), 6.87 (d, *J* = 2.5 Hz, 1 H), 6.79, (dd, *J* = 8.4, 2.5 Hz, 1 H), 4.75 (dd, *J* = 7.1, 5.3 Hz, 1 H), 4.25 (q, *J* = 7.2 Hz, 2 H), 2.86-2.80 (m, 1 H), 2.78-2.72 (m, 1 H), 2.31-2.25 (m, 1 H), 2.24-2.17 (m, 1 H), 1.29 (t, *J* = 7.3 Hz, 3 H); <sup>19</sup>F NMR (471 MHz; CDCl<sub>3</sub>) δ -72.9; <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) 170.3, 154.5, 148.5, 130.6, 121.9, 118.9 (q, *J* = 321 Hz), 113.6, 110.3, 73.9, 61.8, 24.1, 22.9, 14.3. HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>13</sub>H<sub>14</sub>F<sub>3</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 355.0458, found 355.0458.



**7-(4-Methoxyphenyl)-N-((tetrahydro-2H-pyran-2-yl)oxy)chromane-2-carboxamide (1-54a).**

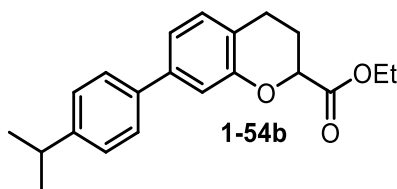
In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0410 g, 0.0355 mmol) and CsF (0.236 g, 1.55 mmol). 4-Methoxyphenylboronic acid (0.164 g, 0.999 mmol) and triflate **1-53** (0.250 g, 0.705 mmol) in degassed dioxane/H<sub>2</sub>O (5:1, 9 mL/1.8 mL) was added, and the mixture was sparged with N<sub>2</sub> for 15 min. The vial was sealed, and stirred at 90 °C for 13 h. The reaction mixture was diluted with EtOAc (75 mL) and 1:1 sat. aq. NaHCO<sub>3</sub> and sat. aq. NaCl (75 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2x 75 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through a pad of SiO<sub>2</sub>, rinsed with EtOAc,

and concentrated to afford ~0.260 g of a crude oil, **1-54a**, that was used without any further purification:  $^1\text{H NMR}$  (300 MHz;  $\text{CDCl}_3$ )  $\delta$  7.51 (d,  $J = 8.8$  Hz, 2 H), 7.16 (bs, 1 H), 7.09-7.07 (m, 2 H), 6.95 (d,  $J = 8.8$  Hz, 2 H), 4.74 (dd,  $J = 7.5, 3.6$  Hz, 1 H), 4.28 (q,  $J = 7.1$  Hz, 2 H), 3.84 (s, 3 H), 2.93-2.73 (m, 2 H), 2.36-2.15 (m, 2 H), 1.31 (t,  $J = 7.1$  Hz, 3 H).

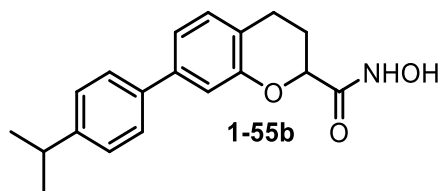


***N*-Hydroxy-7-(4-methoxyphenyl)chromane-2-carboxamide (1-55a)**. The residue from **1-54a** (0.260 g) was dissolved in THF (2.0 mL) and MeOH (2.0 mL). The mixture was treated with LiOH monohydrate (0.0371 g, 0.884 mmol) in water (2.0 mL) at rt. After 1.5 h, the solution was concentrated and the residue was azeotroped with PhMe (2x 20 mL). The salt (0.214 g, 0.736 mmol) was dissolved in DMF (3.5 mL) and treated with *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (0.166 g, 1.42 mmol). After the reaction mixture was cooled to 0 °C, TEA (0.200 mL, 1.43 mmol) and T<sub>3</sub>P (50%, 0.660 mL, 1.11 mmol) were added. The mixture was warmed to rt and stirred under N<sub>2</sub>. After 13 h, the mixture was diluted with EtOAc (30 mL), washed with 0.5 M HCl (15 mL), brine (15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica, and concentrated. The residue was dissolved in MeOH (5.0 mL) and treated with Amberlyst-15 (0.0602 g, 283 mmol) at rt under N<sub>2</sub>. After 16 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc) yielding **1-55a** (0.0780 g, 36%, 4 steps) as a tan-colored solid: Mp 181-183 °C; IR ( $\text{CH}_2\text{Cl}_2$ ) 3321, 2913, 2166, 1679, 1607, 1492, 1248  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (500 MHz;  $\text{DMSO}-d_6$ )  $\delta$  10.79 (s, 1 H), 8.94 (s, 1 H), 7.54 (d,  $J = 8.7$  Hz, 2 H), 7.09-7.06 (m, 3 H), 7.00 (d,  $J = 8.7$  Hz, 2 H), 4.57 (dd,  $J = 8.6, 3.1$  Hz, 1 H), 3.78

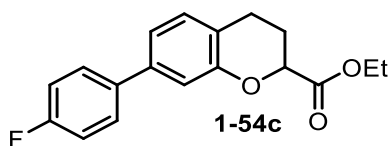
(s, 3 H), 2.82-2.77 (m, 1 H), 2.74-2.68 (m, 1 H), 2.16-2.10 (m, 1 H), 2.01-1.94 (m, 1 H);  $^{13}\text{C}$  NMR (126 MHz; DMSO- $d_6$ )  $\delta$  166.4, 158.8, 153.6, 139.0, 132.2, 129.9, 127.4, 120.4, 118.3, 114.3, 114.1, 73.9, 55.1, 24.3, 22.6; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{17}\text{H}_{18}\text{O}_4\text{N}$   $[\text{M}-\text{H}]^+$  300.1230, found 300.1230; ELS purity 98.6%.



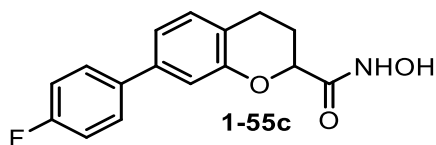
**7-(4-Isopropylphenyl)-N-((tetrahydro-2H-pyran-2-yl)oxy)chromane-2-carboxamide (1-54b).** In a  $\text{N}_2$ -filled glove box, a microwave vial was charged with  $\text{Pd}(\text{PPh}_3)_4$  (0.0410 g, 0.0355 mmol) and CsF (0.236 g, 1.55 mmol). 4-Isopropylphenylboronic acid (0.164 g, 0.999 mmol) and triflate **1-53** (0.250 g, 0.705 mmol) in degassed dioxane/ $\text{H}_2\text{O}$  (5:1, 9 mL/1.8 mL) was added, and the mixture was sparged with  $\text{N}_2$  for 15 min. The vial was sealed, and stirred at 90 °C for 13 h. The reaction mixture was diluted with EtOAc (75 mL) and 1:1 sat. aq.  $\text{NaHCO}_3$  and sat. aq. NaCl (75 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2 x 75 mL). The combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered through a pad of  $\text{SiO}_2$ , rinsed with EtOAc, and concentrated to afford 0.261 g of a crude oil, **1-54b**, that was used without any further purification:  $^1\text{H}$  NMR (300 MHz;  $\text{CDCl}_3$ )  $\delta$  7.51 (d,  $J = 8.2$  Hz, 2 H), 7.28 (d,  $J = 8.2$  Hz, 2 H), 7.19 (d,  $J = 1.4$  Hz, 1 H), 7.12 (dd,  $J = 7.9, 1.6$  Hz, 1 H), 7.08 (d,  $J = 7.9$  Hz, 1 H), 4.75 (dd,  $J = 7.5, 3.6$  Hz, 1 H), 4.28 (q,  $J = 7.1$  Hz, 2 H), 3.02-2.74 (m, 3 H), 2.37-2.15 (m, 2 H), 1.31 (t,  $J = 7.0$  Hz, 3 H), 1.29 (d,  $J = 6.9$  Hz, 6 H).



***N*-Hydroxy-7-(4-isopropylphenyl)chromane-2-carboxamide (1-55b)**. The residue from **1-54b** (0.261 g) was dissolved in THF (2.0 mL) and MeOH (2.0 mL). The mixture was treated with LiOH monohydrate (0.0354 g, 0.843 mmol) in water (2.0 mL) at room temperature. After 1.5 h, the solution was concentrated and the residue was azeotroped with PhMe (2x 20 mL). The salt (0.212 g, 0.703 mmol) was dissolved in DMF (1.5 mL) and treated with *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (0.164 g, 1.40 mmol). After the mixture was cooled to 0 °C, TEA (0.100 mL, 0.717 mmol) and T<sub>3</sub>P (50%, 0.630 mL, 1.06 mmol) were added. The mixture was warmed to room temperature and stirred under N<sub>2</sub>. After 15 h, the mixture was diluted with EtOAc (30 mL), washed with 0.5 M HCl (15 mL), brine (15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica, and concentrated. The residue was dissolved in MeOH (5.0 mL) and treated with Amberlyst-15 (0.0620 g, 291 mmol) at rt under N<sub>2</sub>. After 16 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc) yielding **1-55b** (0.0812 g, 27%, 4 steps) as a peach-colored solid: Mp 165-167 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3188, 3042, 2955, 1648, 1489, 1298 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.80 (s, 1 H), 8.94 (s, 1 H), 7.52 (d, *J* = 8.2 Hz, 2 H), 7.31 (d, *J* = 8.2 Hz, 2 H), 7.13-7.08 (m, 3 H), 4.57 (dd, *J* = 8.6, 3.1 Hz, 1 H), 2.91 (hept, *J* = 6.9 Hz, 1 H); 2.84-2.78 (m, 1 H), 2.75-2.69 (m, 1 H), 2.16-2.11 (m, 1 H), 2.01-1.94 (m, 1 H), 1.23 (d, *J* = 6.9 Hz, 6 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 166.4, 153.6, 147.5, 139.2, 137.4, 129.9, 126.8, 126.3, 120.8, 118.6, 114.4, 73.8, 33.0, 24.2, 23.8, 22.6; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>22</sub>O<sub>3</sub>N [M+H]<sup>+</sup> 312.1594, found 312.1592; ELS purity 100%.

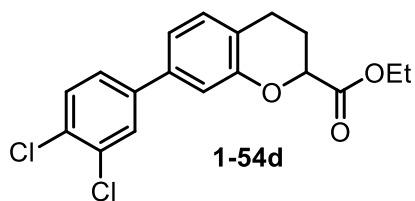


**7-(4-Fluorophenyl)-N-((tetrahydro-2H-pyran-2-yl)oxy)chromane-2-carboxamide (1-54c).** In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0410 g, 0.0355 mmol) and CsF (0.232 g, 1.53 mmol). 4-fluorophenylboronic acid (0.106 g, 0.758 mmol) and triflate **1-53** (0.245 g, 0.692 mmol) in degassed dioxane/H<sub>2</sub>O (5:1, 8.2 mL/1.8 mL) was added, and the mixture was sparged with N<sub>2</sub> for 15 min. The vial was sealed, and stirred at 90 °C for 7 h. The reaction mixture was diluted with EtOAc (75 mL) and 1:1 sat. aq. NaHCO<sub>3</sub> and sat. aq. NaCl (75 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2x 75 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through a pad of SiO<sub>2</sub>, rinsed with EtOAc, and concentrated to afford 0.347 g of a crude yellow semi solid, **1-54c**, that was used without any further purification: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.54-7.50 (m, 2 H), 7.19 (app t, *J* = 8.8 Hz, 2 H), 7.14-7.07 (m, 3 H), 4.75 (dd, *J* = 7.3, 3.7 Hz, 1 H), 4.27 (q, *J* = 7.1 Hz, 2 H), 2.93-2.73 (m, 2 H), 2.37-2.15 (m, 2 H), 1.31 (t, *J* = 7.1 Hz, 3 H).



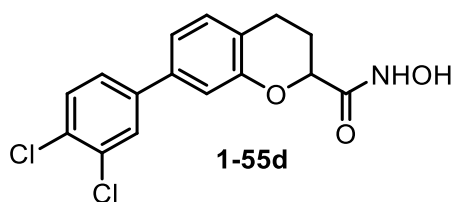
**7-(4-Fluorophenyl)-N-hydroxychromane-2-carboxamide (1-55c).** The residue from **1-54c** (0.347 g) was dissolved in THF (2.0 mL) and MeOH (2.0 mL). The mixture was treated with LiOH monohydrate (0.0370 g, 1.54 mmol) in water (2.0 mL) at rt. After 1.5 h, the solution was concentrated and the residue was azeotroped with PhMe (2x 20 mL). The salt (0.194 g, 0.696

mmol) was dissolved in DMF (1.5 mL) and treated with O-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (0.161 g, 1.37 mmol). After the mixture was cooled to 0 °C, TEA (0.150 mL, 1.08 mmol) and T<sub>3</sub>P (50%, 0.700 mL, 1.18 mmol) were added. The mixture was warmed to rt and stirred under N<sub>2</sub>. After 15 h, the mixture was diluted with EtOAc (30 mL), washed with 0.5 M HCl (15 mL), brine (15mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica, rinsing with EtOAc, and concentrated. The residue was dissolved in MeOH (6.0 mL) was added Amberlyst-15 (0.0496 g, 233 mmol) at rt under N<sub>2</sub>. After 22 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc) yielding **1-55c** (0.0290 g, 17 %, 4 steps) as a tan solid: Mp 157-159 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3200, 2958, 1667, 1485, 1302, 1211 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.79 (s, 1 H), 8.95 (s, 1 H), 7.64 (dd, *J* = 8.6, 5.5 Hz, 2 H), 7.27 (app t, *J* = 8.8 Hz, 2 H), 7.15-7.09 (m, 3 H), 4.58 (dd, *J* = 8.5, 3.0 Hz, 1 H), 2.84-2.78 (m, 1 H), 2.75-2.70 (m, 1 H), 2.16-2.11 (m, 1 H), 2.02-1.95 (m, 1 H); <sup>19</sup>F NMR (471 MHz; DMSO-*d*<sub>6</sub>) δ -115.6; <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 166.3, 161.7 (d, *J*<sub>C-F</sub> = 244.4 Hz), 153.6, 138.2, 136.3, 129.9, 128.3 (d, *J*<sub>C-F</sub> = 8.1 Hz), 121.1, 118.7, 115.6 (d, *J*<sub>C-F</sub> = 21.3 Hz), 114.6, 73.8, 24.2, 22.6; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>16</sub>H<sub>15</sub>O<sub>3</sub>NF [M+H]<sup>+</sup> 288.1030, found 288.1031; ELS purity 99.3%.



**7-(3,4-Dichlorophenyl)-N-((tetrahydro-2*H*-pyran-2-yl)oxy)chromane-2-carboxamide (1-54d)**. In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0410 g, 0.0355

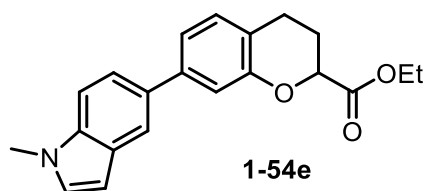
mmol) and CsF (0.232 g, 1.53 mmol). 3,4-Dichlorophenylboronic acid (0.143 g, 0.747 mmol) and triflate **1-53** (0.245 g, 0.692 mmol) in degassed dioxane/H<sub>2</sub>O (5:1, 8.2 mL/1.8 mL) was added, and the mixture was sparged with N<sub>2</sub> for 15 min. The vial was sealed, and stirred at 90 °C for 7 h. The reaction mixture was diluted with EtOAc (75 mL) and 1:1 sat. aq. NaHCO<sub>3</sub> and sat. aq. NaCl (75 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2x 75 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through a pad of SiO<sub>2</sub>, rinsed with EtOAc, and concentrated to afford 0.323 g of a crude yellow oil, **1-54d**, that was used without any further purification: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.65 (d, *J* = 2.0 Hz, 1 H), 7.47 (d, *J* = 8.4 Hz, 1 H), 7.39 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.14-7.10 (m, 2 H), 7.05 (dd, *J* = 7.7, 1.8 Hz, 1 H), 4.76 (dd, *J* = 7.4, 3.8 Hz, 1 H), 4.27 (q, *J* = 7.3 Hz, 2 H), 2.93-2.73 (m, 2 H), 2.37-2.16 (m, 2 H), 1.31 (t, *J* = 7.1 Hz, 3 H).



**7-(3,4-Dichlorophenyl)-N-hydroxychromane-2-carboxamide (1-55d).** The residue from **1-54d** (0.323 g) was dissolved in THF (2.0 mL) and MeOH (2.0 mL). The mixture was treated with LiOH monohydrate (0.0581 g, 1.38 mmol) in water (2.0 mL) at rt. After 1.5 h, the solution was concentrated and the residue was azeotroped with PhMe (2x 20 mL). The salt (0.228 g, 0.692 mmol) was dissolved in DMF (1.5 mL) and treated with O-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (0.146 g, 1.25 mmol). After the mixture was cooled to 0 °C, TEA (0.100 mL, 0.717 mmol) and T<sub>3</sub>P (50%, 0.630 mL, 1.06 mmol) were added. The mixture was warmed to rt

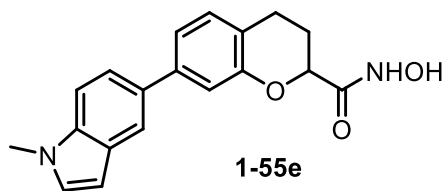


and stirred under N<sub>2</sub>. After 15 h, the mixture was diluted with EtOAc (30 mL), washed with 0.5 M HCl (15 mL), brine (15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica, rinsed with EtOAc, and concentrated. The collected residue was dissolved in MeOH (5.0 mL) and treated with Amberlyst-15 (0.0402 g, 199 mmol) at rt under N<sub>2</sub>. After 44 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1, hexanes: EtOAc) yielding **1-55d** (0.0537 g, 23%, 4 steps) as a tan solid: Mp 167-169 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3167, 2893, 1671, 1550, 1470, 1308 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.78 (s, 1 H), 8.96 (s, 1 H), 7.86 (d, *J* = 1.8 Hz, 1 H), 7.70 (d, *J* = 8.4 Hz, 1 H), 7.62 (dd, *J* = 8.4, 1.8 Hz, 1 H), 7.21-7.16 (m, 3 H), 4.60 (dd, *J* = 8.3, 3.0 Hz, 1 H), 2.85-2.78 (m, 1 H), 2.76-2.70 (m, 1 H), 2.15-2.12 (m, 1 H), 2.02-1.95 (m, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 166.3, 153.6, 140.4, 136.5, 131.6, 130.9, 130.1, 130.0, 128.1, 126.5, 122.3, 118.7, 114.8, 73.8, 24.0, 22.6; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>16</sub>H<sub>12</sub>O<sub>3</sub>NCl<sub>2</sub> [M-H]<sup>+</sup> 336.0189, found 336.0198; ELS purity 100%.



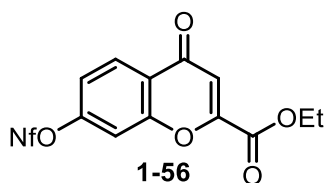
**7-(1-Methyl-1H-indol-5-yl)-N-((tetrahydro-2H-pyran-2-yl)oxy)chromane-2-carboxamide (1-54e).** In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0410 g, 0.0355 mmol) and CsF (0.232 g, 1.53 mmol). *N*-methylindole-5-boronic acid (0.133 g, 0.760 mmol) and triflate **1-53** (0.245 g, 0.692 mmol) in degassed dioxane/H<sub>2</sub>O (5:1, 8.2 mL/1.8 mL) was added, and the mixture was sparged with N<sub>2</sub> for 15 min. The vial was sealed, and stirred at 90 °C for 7 h. The reaction mixture was diluted with EtOAc (75 mL) and 1:1 sat. aq. NaHCO<sub>3</sub> and sat. aq. NaCl (75 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2x 75 mL). The

combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through a pad of SiO<sub>2</sub>, rinsing with EtOAc, and concentrated to afford 0.213 g of a crude light-yellow semi solid, **1-54e**, that was used without any further purification: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.82 (d, *J* = 1.2 Hz, 1 H), 7.47 (dd, *J* = 8.5, 1.6 Hz, 1 H), 7.36 (d, *J* = 8.5 Hz, 1 H), 7.26 (bs, 1 H), 7.19 (dd, *J* = 7.8, 1.8 Hz, 1 H), 7.09 (d, *J* = 7.8 Hz, 1 H), 7.07 (d, *J* = 3.1 Hz, 1 H), 6.52 (d, *J* = 3.1 Hz, 1 H), 4.75 (dd, *J* = 7.6, 3.6 Hz, 1 H), 4.28 (q, *J* = 7.1 Hz, 2 H), 3.81 (s, 3 H), 2.93-2.75 (m, 2 H), 2.36-2.29 (m, 1 H), 2.37-2.17 (m, 1 H), 1.31 (t, *J* = 7.1 Hz, 3 H).



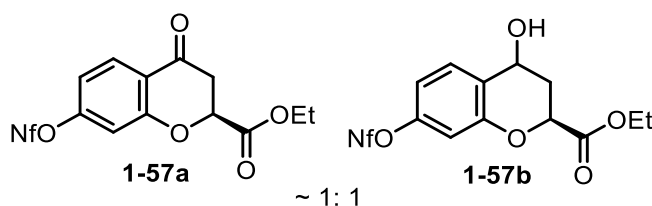
**N-Hydroxy-7-(1-methyl-1H-indol-5-yl)chromane-2-carboxamide (1-55e).** The residue from **1-54e** (0.213 g) was dissolved in THF (2.0 mL) and MeOH (2.0 mL). The mixture was treated with LiOH monohydrate (0.0370 g, 1.54 mmol) in water (2.0 mL) at rt. After 1.5 h, the solution was concentrated and the residue was azeotroped with PhMe (2x 20 mL). The salt (0.217 g, 0.692 mmol) was dissolved in DMF (1.5 mL) and treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.146 g, 1.25 mmol). After the mixture was cooled to 0 °C, TEA (0.300 mL, 1.08 mmol) and T<sub>3</sub>P (50%, 0.600 mL, 1.01 mmol) were added. The mixture was warmed to rt and stirred under N<sub>2</sub>. After 24 h, more T<sub>3</sub>P (50%, 0.700 mL, 2.35 mmol) and O-(Tetrahydro-2H-pyran-2-yl)hydroxylamine (0.125 g, 2.35 mmol). After 40 h, the mixture was diluted with EtOAc (30 mL), washed with 0.5 M HCl (15 mL), brine (15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica, rinsing with EtOAc, and concentrated. The collected residue was dissolved in MeOH (3.0 mL) and treated with Amberlyst-15 (0.0286 g, 134 mmol) at rt under N<sub>2</sub>. After 24 h of stirring, the mixture

was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc) yielding **1-55e** (0.0270 g, 12%, 4 steps) as a grey-brown solid: Mp 155-158 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3207, 2922, 1668, 1618, 1483, 1422, 1212 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.80 (s, 1 H), 8.94 (s, 1 H), 7.76 (s, 1 H), 7.49 (d, *J* = 8.5 Hz, 1 H), 7.40 (dd, *J* = 8.5, 1.2 Hz, 1 H), 7.34 (d, *J* = 2.9 Hz, 1 H), 7.17-7.10 (m, 3 H), 6.46 (d, *J* = 2.8 Hz, 1 H), 4.58 (dd, *J* = 8.5, 2.8 Hz, 1 H), 3.81 (s, 3 H), 2.84-2.78 (m, 1 H), 2.75-2.70 (m, 1 H), 2.17-2.13 (m, 1 H), 2.02-1.95 (m, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 166.5, 153.5, 140.8, 135.9, 131.1, 130.3, 129.7, 128.5, 120.1, 119.8, 118.8, 118.0, 114.6, 110.0, 100.7, 73.9, 32.5, 24.3, 22.6; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>19</sub>O<sub>3</sub>N<sub>2</sub> [M+H]<sup>+</sup> 323.1390, found 323.1390; ELS purity 99.4%.



**Ethyl (S)-4-oxo-7-(((perfluorobutyl)sulfonyl)oxy)chromane-2-carboxylate (1-56).** A solution of ethyl 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylate **1-51** (5.00 g, 21.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (60 mL) was treated with DMAP (0.284 g, 2.33 mmol) and DIPEA (5.20 mL, 29.9 mmol) at rt under N<sub>2</sub>. The solution was cooled to 0 °C in an ice bath and perfluorobutanesulfonyl fluoride (92%, 5.00 mL, 25.6 mmol) was added dropwise. After 30 min, the mixture was warmed to rt. After 21 h, 200 mL of water was added to the reaction mixture and then the mixture was transferred to a separatory funnel and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 x 200 mL). The organic layers were washed with 0.5 M HCl (200 mL), brine (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude mixture was filtered through a pad of silica, rinsing with 2:1 Hexanes: EtOAc. The nonaflate **1-56** (9.44 g, 86%) was collected as a beige solid: Mp 125-128 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3092, 3044, 1745, 1656, 1614, 1429,

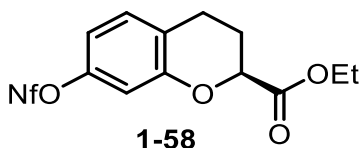
1234, 1191, 1142, 1119  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz;  $\text{CDCl}_3$ )  $\delta$  8.31 (d,  $J = 8.9$  Hz, 1 H), 7.61 (d,  $J = 2.3$  Hz, 1 H), 7.38 (dd,  $J = 8.9, 2.3$  Hz, 1 H), 7.15 (s, 1 H), 4.48 (q,  $J = 7.1$  Hz, 2 H), 1.45 (t,  $J = 7.1$  Hz, 3 H);  $^{19}\text{F}$  NMR (376 MHz;  $\text{CDCl}_3$ )  $\delta$  -80.54 (t,  $J = 9.6$  Hz, 3 F), -108.28 (t,  $J = 13.5$  Hz, 2 F), -120.73 (bs, 2 F), -125.68– -125.76 (m, 2 F);  $^{13}\text{C}$  NMR (101 MHz;  $\text{CDCl}_3$ )  $\delta$  177.1, 160.1, 156.3, 153.2, 153.0, 128.6, 124.1, 119.6, 115.4, 112.3, 63.5, 14.2; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{16}\text{H}_{10}\text{F}_9\text{O}_7\text{S}$   $[\text{M}+\text{H}]^+$  516.9998, found 516.9991.



**Ethyl (*S*)-4-oxo-7-(((perfluorobutyl)sulfonyl)oxy)chromane-2-carboxylate (1-57a) and ethyl (2*S*)-4-hydroxy-7-(((perfluorobutyl)sulfonyl)oxy)chromane-2-carboxylate (1-57b).** In a three-neck flask equipped with an addition funnel,  $\text{N}_2$  sparging line, and an outlet needle, a bondi-blue solution of  $\text{Cu}(\text{OAc})_2$  (Strem, 0.0748 g, 0.412 mmol) in freshly distilled THF (50 mL) was stirred under an atmosphere of  $\text{N}_2$  until a homogeneous green-blue solution was obtained (ca. 15 min). Neat (*S*)-DM-Segphos (0.357 g, 0.494 mmol) was added, and the mixture was stirred for 15 min at rt, cooled to 0  $^\circ\text{C}$  in an ice bath, and treated dropwise with DEMS (1.98 mL, 12.3 mmol) over a period of 5 min. The reaction mixture was stirred for an additional 30 min at 0  $^\circ\text{C}$  at which the solution turned from blue-green to yellow. A solution of ester **1-56** (4.25 g, 8.23 mmol) in dry THF (35 mL) was added dropwise via syringe. The solution was stirred at 0  $^\circ\text{C}$  for 10 min, and at rt for another 1.5 h, while it turned brown. After 5 h, an aliquot was analyzed by LCMS and the reaction was not complete. The reaction mixture was cooled to 0  $^\circ\text{C}$  and quenched with sat.  $\text{NH}_4\text{Cl}$

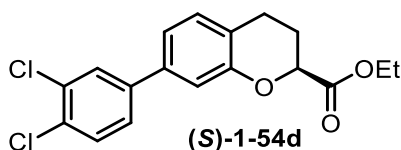
(100 mL) under vigorous stirring for 15 min. After addition of EtOAc (100 mL), the solution was transferred into a sep. funnel, sat. NaCl (50 mL) was added, and the layers were separated. The organic layer was washed with sat. NaHCO<sub>3</sub> (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. The residue was diluted with 25% EtOAc/75% hexanes and filtered through a SiO<sub>2</sub> pad (100% Hexanes, rinsed with 25% EtOAc/ 75% hexanes).

The residue was resubjected to the asymmetric reduction using the procedure from above with the following changes: the residue was dissolved in 75 mL of dry THF for full solvation and no precipitation of the solid. The reaction was stirred for 12 h. A similar workup and silica pad filtration protocol was followed as highlighted above. The crude material (6.5 g) was collected and dissolved in MeOH (100 mL). Amberlyst-15 (0.288 g) were added and the mixture was stirred for 2 h. After filtering the Amberlyst-15 beads and concentrating the filtrate, the crude material was purified by chromatography on SiO<sub>2</sub> (22-66% EtOAc in hexanes) to afford **1-57a**, the chromone (2.28 g, 94% purity/6% SEGPHOS impurity by <sup>1</sup>H NMR, 50%) as a yellow solid and **1-57b**, the chromanol (>99% purity by <sup>1</sup>H NMR, 1.88 g, 44%) as a white solid: **1-57a**: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.98 (d, *J* = 8.7 Hz, 1 H), 7.08 (d, *J* = 2.3 Hz, 1 H), 7.00 (dd, *J* = 8.7, 2.3 Hz, 1 H), 5.14 (dd, *J* = 8.2, 5.6 Hz, 1 H), 4.27 (q, *J* = 7.1 Hz, 2 H), 3.11 (d, *J* = 5.6 Hz, 1 H), 3.10 (d, *J* = 8.2 Hz, 1 H), 1.28 (t, *J* = 7.1 Hz, 3 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>16</sub>H<sub>12</sub>F<sub>9</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 519.0155, found 519.0155; **1-57b**: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.41 (d, *J* = 8.3 Hz, 1 H), 6.93-6.89 (m, 2 H), 4.90 (app t, *J* = 5.0 Hz, 1 H), 4.85 (app t, *J* = 4.5 Hz, 1 H), 4.23 (q, *J* = 7.1 Hz, 2 H), 2.54-2.39 (m, 2 H), 1.28 (t, *J* = 7.1 Hz, 3 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>16</sub>H<sub>13</sub>F<sub>9</sub>O<sub>7</sub>SNa [M+Na]<sup>+</sup> 543.0130, found 543.0131.

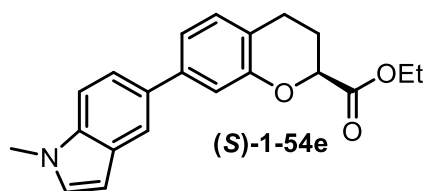


**Ethyl (S)-7-(((perfluorobutyl)sulfonyl)oxy)chromane-2-carboxylate (1-58).** A solution of alcohol **1-57b** (1.70 g, 3.27 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (12 mL) was treated with triethylsilane (1.67 mL, 10.5 mmol) at rt. The reaction mixture was then cooled to 0 °C, and BF<sub>3</sub>•OEt<sub>2</sub> (1.21 mL, 9.80 mmol) was added. After 10 min, the mixture was warmed to rt and left to stir for 48 h. The mixture was treated with H<sub>2</sub>O (40 mL) and CH<sub>2</sub>Cl<sub>2</sub> (40 mL). The suspension was transferred to a separatory funnel and the layers were separated. The organic layer was washed with brine (20 mL) and transferred to an Erlenmeyer. SiO<sub>2</sub> and Na<sub>2</sub>SO<sub>4</sub> were added and the mixture was stirred for 20 min. The suspension was filtered through a pad of silica, and the filtrate was concentrated. The crude mixture was purified by chromatography on SiO<sub>2</sub> (18% EtOAc in Hexanes), providing **1-58** (1.25 g, 76%) as a transparent oil: [α]<sub>D</sub> -7.9 (c 0.34, CH<sub>2</sub>Cl<sub>2</sub>); IR (CH<sub>2</sub>Cl<sub>2</sub>) 2986, 1753, 1612, 1597, 1494, 1422, 1236, 1197, 1143, 1104 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.08 (d, *J* = 8.5 Hz, 1 H), 6.88 (d, *J* = 2.5 Hz, 1 H), 6.81 (dd, *J* = 8.5, 2.5 Hz, 1 H), 4.75 (dd, *J* = 7.1, 3.4 Hz, 1 H), 4.29-4.23 (m, 2 H), 2.87-2.81 (m, 1 H), 2.78-2.72 (m, 1 H), 2.32-2.26 (m, 1 H), 2.24-2.17 (m, 1 H), 1.29 (t, *J* = 7.1 Hz, 3 H); <sup>19</sup>F NMR (471 MHz; CDCl<sub>3</sub>) δ -80.63 (t, *J* = 10.1 Hz, 3 F), -108.95 (t, *J* = 13.6 Hz, 3 F), -120.84– -120.90 (m, 2 F), -125.76– -125.84 (m, 2 F); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 170.3, 154.5, 148.7, 130.6, 121.9, 113.7, 110.3, 74.0, 61.8, 24.1, 22.9, 14.3; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>16</sub>H<sub>13</sub>F<sub>9</sub>O<sub>6</sub>SNa [M+Na]<sup>+</sup> 527.0181, found 527.0182.

The nonaflate chromane **1-58** was derived to chromane **1-37** for SFC analysis on a chiral stationary phase (Chiralpak IC: 7 mL/min; 10% MeOH; 220 nm); sample prep: 1 mg/mL in 100% MeOH; retention time: 4.1 min) indicated > 96% ee.

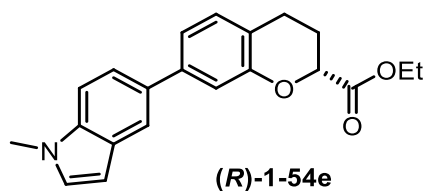


**Ethyl (S)-7-(3,4-dichlorophenyl)chromane-2-carboxylate ((S)-1-54d).** In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0300 g, 0.0260 mmol) and CsF (0.174 g, 1.15 mmol). A solution of nonaflate **1-58** (0.260 g, 0.516 mmol) in dioxane/H<sub>2</sub>O (4.4:1, 5.3 mL/1.2 mL) was sparged with N<sub>2</sub> (15 min) and added to the mixture, followed by 3,4-dichlorophenylboronic acid (0.106 g, 0.556 mmol). After additional sparging with N<sub>2</sub> (5 min), the vial was sealed, and stirred at 90 °C for 16 h. The reaction mixture was diluted with EtOAc (40 mL) and treated with 1:1 sat. aq. NaHCO<sub>3</sub>/ sat. aq. NaCl (40 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2 x 40 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-18% EtOAc in hexanes) provided **(S)-1-54d** (0.158 g, 87%) as a yellow oil: [α]<sub>D</sub> -45.3 (c 0.25, CH<sub>2</sub>Cl<sub>2</sub>); IR (CH<sub>2</sub>Cl<sub>2</sub>) 2979, 2934, 2850, 1751, 1469, 1187, 1130 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.65 (d, *J* = 2.1 Hz, 1 H), 7.47 (d, *J* = 8.3 Hz, 1 H), 7.39 (dd, *J* = 8.3, 2.1 Hz, 1 H), 7.14-7.09 (m, 2 H), 7.05 (dd, *J* = 7.9, 1.8 Hz, 1 H), 4.76 (dd, *J* = 7.2, 3.8 Hz, 1 H), 4.23 (q, *J* = 7.1 Hz, 2 H), 2.93-2.73 (m, 2 H), 2.37-2.16 (m, 2 H), 1.31 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 170.9, 154.0, 140.9, 138.4, 132.9, 131.4, 130.8, 130.2, 128.9, 126.3, 121.5, 119.4, 115.4, 74.0, 61.6, 24.7, 23.2, 14.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>18</sub>H<sub>17</sub>Cl<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup> 351.0549, found 351.0548.

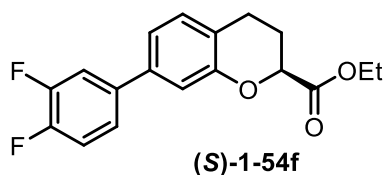


**Ethyl (S)-7-(1-methyl-1H-indol-5-yl)chromane-2-carboxylate ((S)-1-54e).** In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0330 g, 0.0286 mmol) and CsF (0.187 g, 1.23 mmol). A solution of nonaflate **1-58** (0.280 g, 0.555 mmol) in dioxane/H<sub>2</sub>O (5:1, 5.5 mL/1.1 mL) was sparged with N<sub>2</sub> (15 min) and added to the mixture, followed by *N*-methylindole-5-boronic acid (0.107 g, 0.611 mmol). After additional sparging with N<sub>2</sub> (5 min), the vial was sealed, and stirred at 90 °C for 15 h. The reaction mixture was diluted with EtOAc (40 mL) and treated with 1:1 sat. aq. NaHCO<sub>3</sub>/sat. aq. NaCl (40 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2 x 40 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-18% EtOAc in hexanes) provided **(S)-1-54e** (94% purity by <sup>1</sup>H NMR (CH<sub>2</sub>Cl<sub>2</sub>), 0.0816 g, 41%) as a yellow oil: [α]<sub>D</sub> -59.8 (*c* 0.0815, CH<sub>2</sub>Cl<sub>2</sub>); IR (CH<sub>2</sub>Cl<sub>2</sub>) 2932, 1751, 1563, 1483, 1195 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 7.83 (d, *J* = 1.2 Hz, 1 H), 7.47 (dd, *J* = 8.5, 1.7 Hz, 1 H), 7.36 (d, *J* = 8.5 Hz, 1 H), 7.26 (br, 1 H), 7.19 (dd, *J* = 7.8, 1.8 Hz, 1 H), 7.09 (d, *J* = 7.9 Hz, 1 H), 7.07 (d, *J* = 3.1 Hz, 1 H), 6.52 (d, *J* = 3.1 Hz, 1 H), 4.75 (dd, *J* = 7.8, 3.4 Hz, 1 H), 4.28 (q, *J* = 7.1 Hz, 2 H), 3.80 (s, 3 H), 2.93-2.86 (m, 1 H), 2.84-2.77 (m, 1 H), 2.36-2.29 (m, 1 H), 2.26-2.17 (m, 1 H), 1.31 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 171.1, 153.8, 142.4, 136.4, 132.5, 129.7, 129.5, 129.0, 121.4, 120.1, 119.34, 119.32, 115.7, 109.5, 101.5, 74.1, 61.5, 33.0, 25.0, 23.3, 14.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>21</sub>H<sub>22</sub>NO<sub>3</sub> [M+H]<sup>+</sup> 336.1594, found 336.1591.

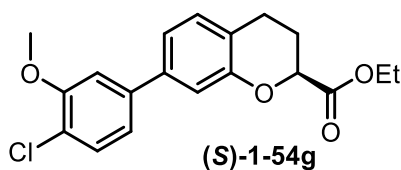




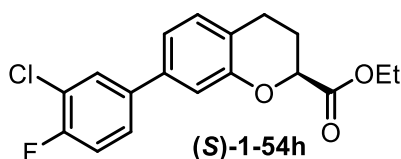
**Ethyl (R)-7-(1-methyl-1H-indol-5-yl)chromane-2-carboxylate ((R)-1-54e).** In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0344 g, 0.0297 mmol) and CsF (0.199 g, 1.31 mmol). A solution of nonaflate **(R)-1-58** (0.300 g, 0.595 mmol) in dioxane/H<sub>2</sub>O (5:1, 5.8 mL/1.2 mL) was sparged with N<sub>2</sub> (15 min) and added to the mixture, followed by *N*-methylindole-5-boronic acid (0.115 g, 0.654 mmol). After additional sparging with N<sub>2</sub> (5 min), the vial was sealed, and stirred at 90 °C for 15 h. The reaction mixture was diluted with EtOAc (40 mL) and treated with 1:1 sat. aq. NaHCO<sub>3</sub>/sat. aq. NaCl (40 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2 x 40 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-18% EtOAc in hexanes) provided **(R)-1-54e** (91% purity by <sup>1</sup>H NMR (CH<sub>2</sub>Cl<sub>2</sub>), 0.0699 g, 32 %) as a yellow oil: [α]<sub>D</sub> +31.3 (c 0.0805, CH<sub>2</sub>Cl<sub>2</sub>); IR (CH<sub>2</sub>Cl<sub>2</sub>) 2935, 1751, 1563, 1483, 1196 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.82 (d, *J* = 1.3 Hz, 1 H), 7.47 (dd, *J* = 8.5, 1.7 Hz, 1 H), 7.36 (d, *J* = 8.6 Hz, 1 H), 7.25 (br, 1 H), 7.19 (dd, *J* = 7.8, 1.8 Hz, 1 H), 7.09 (d, *J* = 7.9 Hz, 1 H), 7.07 (d, *J* = 3.1 Hz, 1 H), 6.52 (d, *J* = 3.1 Hz, 1 H), 4.75 (dd, *J* = 7.8, 3.4 Hz, 1 H), 4.28 (q, *J* = 7.1 Hz, 2 H), 3.81 (s, 3 H), 2.92-2.86 (m, 1 H), 2.83-2.77 (m, 1 H), 2.35-2.30 (m, 1 H), 2.26-2.18 (m, 1 H), 1.31 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 171.1, 153.8, 142.4, 136.4, 132.4, 129.7, 129.5, 129.0, 121.4, 120.1, 119.33, 119.31, 115.7, 109.5, 101.5, 74.1, 61.5, 33.1, 25.0, 23.3, 14.4; HRMS (ESI+) *m/z* calcd for C<sub>21</sub>H<sub>22</sub>NO<sub>3</sub> [M+H]<sup>+</sup> 336.1594, found 336.1593.



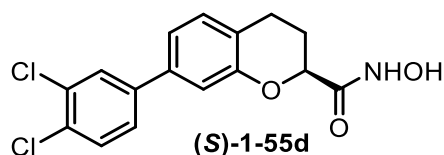
**Ethyl (S)-7-(3,4-difluorophenyl)chromane-2-carboxylate ((S)-1-54f).** In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0332 g, 0.0288 mmol) and CsF (0.192 g, 1.27 mmol). A solution of nonaflate **1-58** (0.290 g, 0.575 mmol) in dioxane/H<sub>2</sub>O (5:1, 5.5 mL/1.1 mL) was sparged with N<sub>2</sub> (15 min) and added to the mixture, followed by 3,4-difluorophenylboronic acid (0.0981 g, 0.621 mmol). After additional sparging with N<sub>2</sub> (5 min), the vial was sealed, and stirred at 90 °C for 15 h. The reaction mixture was diluted with EtOAc (40 mL) and treated with 1:1 sat. aq. NaHCO<sub>3</sub> /sat. aq. NaCl (40 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2 x 40 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-18% EtOAc in hexanes) provided **(S)-1-54f** (0.118 g, 64%) as a colorless oil: [α]<sub>D</sub> -27.4 (c 0.20, CH<sub>2</sub>Cl<sub>2</sub>); IR (CDCl<sub>3</sub>) 2978, 2934, 1752, 1570, 1526, 1497, 1194 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 7.36 (ddd, *J* = 11.6, 7.6, 2.2 Hz, 1 H), 7.28-7.25 (m, 1 H), 7.22-7.15 (m, 1 H), 7.11-7.09 (m, 2 H), 7.03 (dd, *J* = 7.8, 1.7 Hz, 1 H), 4.76 (dd, *J* = 9.2, 4.5 Hz, 1 H), 4.27 (q, *J* = 7.1 Hz, 2 H), 2.91-2.83 (m, 1 H), 2.82-2.75 (m, 1 H), 2.35-2.28 (m, 1 H), 2.26-2.17 (m, 1 H), 1.31 (t, *J* = 7.1 Hz, 3 H); <sup>19</sup>F NMR (376 MHz; CDCl<sub>3</sub>) δ -137.73 (d, *J* = 21.5 Hz, 1 F), -140.40 (d, *J* = 21.5 Hz, 1 F); <sup>13</sup>C NMR (101 MHz; CDCl<sub>3</sub>) δ 170.9, 154.0, 150.60 (dd, *J* = 248.7, 12.7 Hz), 150.01 (dd, *J* = 249.3, 12.7 Hz), 138.8 (dd, *J* = 5.9, 4.0 Hz), 130.1, 122.89 (dd, *J* = 6.3, 3.5 Hz), 121.1, 119.5, 117.56 (d, *J* = 17.3 Hz), 115.91 (d, *J* = 17.8 Hz), 115.4, 74.0, 61.6, 24.7, 23.2, 14.3; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>18</sub>H<sub>16</sub>NaF<sub>2</sub>O<sub>3</sub> [M+Na]<sup>+</sup> 341.0960, found 341.0956.



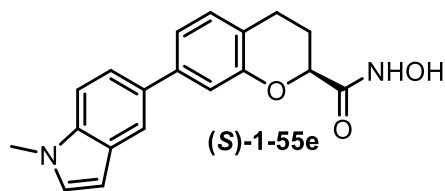
**Ethyl (S)-7-(4-chloro-3-methoxyphenyl)chromane-2-carboxylate ((S)-1-54g).** In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0290 g, 0.0251 mmol) and CsF (0.150 g, 0.987 mmol). A solution of nonaflate **1-58** (0.194 g, 0.385 mmol) in dioxane/H<sub>2</sub>O (5:1, 4.0 mL/0.8 mL) was sparged with N<sub>2</sub> (15 min) and added to the mixture, followed by 4-chloro-3-methoxyphenyl boronic acid (0.0830 g, 0.445 mmol). After additional sparging with N<sub>2</sub> (5 min), the vial was sealed, and stirred at 90 °C for 14 h. The reaction mixture was diluted with EtOAc (40 mL) and 1:1 sat. aq. NaHCO<sub>3</sub>/ sat. aq. NaCl (40 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2 x 40 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-18% EtOAc in hexanes) provided **(S)-1-54g** (0.0890 g, 66%) as a yellow oil: [α]<sub>D</sub> -45.7 (*c* 0.12, CH<sub>2</sub>Cl<sub>2</sub>); IR (CH<sub>2</sub>Cl<sub>2</sub>) 2937, 2847, 1751, 1563, 1484, 1391, 1237, 1188 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.40-7.38 (m, 1 H), 7.17 (d, *J* = 1.3 Hz, 1 H), 7.11-7.07 (m, 4 H), 4.76 (dd, *J* = 7.6, 3.5 Hz, 1 H), 4.28 (q, *J* = 7.1 Hz, 2 H), 3.95 (s, 3 H), 2.91-2.85 (m, 1 H), 2.82-2.77 (m, 1 H), 2.35-2.29 (m, 1 H), 2.25-2.18 (m, 1 H), 1.31 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 170.9, 155.2, 153.9, 140.9, 140.1, 130.4, 130.0, 121.7, 121.0, 119.9, 119.6, 115.5, 111.0, 74.0, 61.6, 56.3, 24.8, 23.3, 14.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>20</sub>ClO<sub>4</sub> [M+H]<sup>+</sup> 347.1045, found 347.1042.



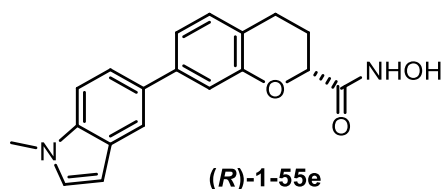
**Ethyl (S)-7-(3-chloro-4-fluorophenyl)chromane-2-carboxylate ((S)-1-54h).** In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0332 g, 0.0288 mmol) and CsF (0.192 g, 1.27 mmol). A solution of nonaflate **1-58** (0.290 g, 0.575 mmol) in dioxane/H<sub>2</sub>O (5:1, 5.5 mL/1.1 mL) was sparged with N<sub>2</sub> (15 min) and added to the mixture, followed by 3-chloro-4-fluorophenyl boronic acid (0.108 g, 0.621 mmol). After additional sparging with N<sub>2</sub> (5 min), the vial was sealed, and stirred at 90 °C for 15 h. The reaction mixture was diluted with EtOAc (40 mL) and treated with 1:1 sat. aq. NaHCO<sub>3</sub>/ sat. aq. NaCl (40 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2 x 40 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-18% EtOAc in hexanes) provided **(S)-1-54h** (97% purity by <sup>1</sup>H NMR (CH<sub>2</sub>Cl<sub>2</sub>), 0.138 g, 70%) as a yellow oil: [α]<sub>D</sub> -28.5 (c 0.14, CH<sub>2</sub>Cl<sub>2</sub>); IR (CH<sub>2</sub>Cl<sub>2</sub>) 2979, 2937, 1752, 1566, 1488, 1190 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.59 (dd, *J* = 7.0, 2.2 Hz, 1 H), 7.41 (ddd, *J* = 8.6, 4.5, 2.3 Hz 1 H), 7.17 (app t, *J* = 8.7 Hz, 1 H), 7.11-7.09 (m, 2 H), 7.03 (dd, *J* = 7.8, 1.7 Hz, 1 H), 4.76 (dd, *J* = 7.4, 7.3 Hz, 1 H), 4.27 (q, *J* = 7.1 Hz, 2 H), 2.90-2.84 (m, 1 H), 2.81-2.76 (m, 1 H), 2.34-2.28 (m, 1 H), 2.25-2.17 (m, 1 H), 1.31 (t, *J* = 7.1 Hz, 3 H); <sup>19</sup>F NMR (471 MHz; CDCl<sub>3</sub>) δ -118.2; <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 170.9, 157.74 (d, *J*<sub>C-F</sub> = 249 Hz), 154.0, 138.7, 138.11 (d, *J*<sub>C-F</sub> = 4.13 Hz), 130.1, 129.2, 126.7 (d, *J*<sub>C-F</sub> = 7.1 Hz), 121.32 (d, *J*<sub>C-F</sub> = 17.7 Hz), 121.1, 119.5, 116.9 (d, *J*<sub>C-F</sub> = 21.2 Hz), 115.5, 74.0, 61.6, 24.7, 23.2, 14.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>18</sub>H<sub>17</sub>ClFO<sub>3</sub> [M+H]<sup>+</sup> 335.0845, found 335.0844.



**(S)-7-(3,4-Dichlorophenyl)-N-hydroxychromane-2-carboxamide ((S)-1-55d).** A solution of the ester **(S)-1-54d** (0.109 g, 0.310 mmol) in THF (2 mL) and MeOH (2 mL) was treated with LiOH monohydrate (0.0143 g, 0.341 mmol) in water (1.5 mL) at room temperature. After 2 h, the solution was concentrated, and azeotroped with toluene (2 x 5 mL). The residue was dissolved in DMF (1.0 mL) and treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.115 g, 0.982 mmol). The mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 0.300 mL, 0.504 mmol) and TEA (0.100 mL, 0.717 mmol). The mixture was warmed to room temperature after 30 min and stirred under N<sub>2</sub>. After 18 h, the mixture was diluted with EtOAc (10 mL), washed with 0.5 M HCl (10 mL), brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica to remove baseline impurities, and concentrated. The amide (0.149 g) was collected as a yellow oil. MeOH (3.0 mL) was added followed by Amberlyst-15 (0.0300 g, 105.8 mmol) at rt under N<sub>2</sub>. After 7 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc) followed by a MeOH (1 mL) rinse, yielding **(S)-1-55d** (0.0290 g, 34%) as a peach/beige solid:  $[\alpha]_D -25.4$  (*c* 0.05, DMSO); Mp 134-136 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3252, 2928, 1651, 1550, 1469, 1130 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.78 (s, 1 H), 8.96 (s, 1 H), 7.87 (d, *J* = 2.1 Hz, 1 H), 7.70 (d, *J* = 8.4 Hz, 1 H), 7.62 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.20 (dd, *J* = 7.9, 1.7 Hz, 1 H), 7.17-7.16 (m, 2 H), 4.60 (dd, *J* = 8.5, 3.2 Hz, 1 H), 2.85-2.78 (m, 1 H), 2.76-2.70 (m, 1 H), 2.16-2.11 (m, 1 H), 2.02-1.95 (m, 1 H); <sup>13</sup>C NMR (101 MHz; DMSO-*d*<sub>6</sub>) δ 166.3, 153.7, 140.4, 136.5, 131.6, 131.0, 130.1, 130.0, 128.1, 126.6, 122.3, 118.8, 114.8, 73.8, 24.1, 22.6; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>16</sub>H<sub>12</sub>O<sub>3</sub>NCl<sub>2</sub> [M-H]<sup>+</sup> 336.0189, found 336.0201; ELS purity 100%.

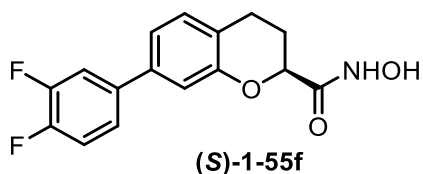


**(S)-N-Hydroxy-7-(1-methyl-1H-indol-5-yl)chromane-2-carboxamide ((S)-1-55e).** A solution of the ester **(S)-1-54e** (0.0760 g, 0.227 mmol) in THF (1.0 mL) and MeOH (1.0 mL) was treated with LiOH monohydrate (0.0117 g, 0.279 mmol) in water (1.0 mL) at rt. After 1 h, the solution was concentrated, and azeotroped with toluene (2 x 5 mL). The residue was dissolved in DMF (0.5 mL) and treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.0923 g, 0.788 mmol). The mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 0.210 mL, 0.353 mmol) and TEA (0.100 mL, 0.717 mmol). The mixture was warmed to rt after 30 min, and stirred under N<sub>2</sub>. After 3 h, the mixture was diluted with EtOAc (8 mL), washed with 0.5 M HCl (8 mL), brine (8 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica to remove baseline impurities, and concentrated. The amide (0.0831 g) was collected as a yellow oil. MeOH (3.0 mL) was added followed by Amberlyst-15 (0.0170 g, 79.9 mmol) at room temperature under N<sub>2</sub>. After 16 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes:EtOAc) yielding **(S)-1-55e** (0.0423 g, 60%) as a light pink solid: [ $\alpha$ ]<sub>D</sub> -25.2 (*c* 0.05, DMSO); Mp 140-144 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3208, 2922, 1665, 1618, 1482, 1422, 1211 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  10.80 (s, 1 H), 8.94 (d, *J* = 1.4 Hz, 1 H), 7.76 (d, *J* = 1.0 Hz, 1 H), 7.49 (d, *J* = 8.6 Hz, 1 H), 7.40 (dd, *J* = 8.6, 1.6 Hz, 1 H), 7.35 (d, *J* = 3.0 Hz, 1 H), 7.18-7.06 (m, 3 H), 6.46 (d, *J* = 2.8 Hz, 1 H), 4.58 (dd, *J* = 8.4, 2.9 Hz, 1 H), 3.81 (s, 3 H), 2.87-2.67 (m, 2 H), 2.18-2.12 (m, 1 H), 2.04-1.92 (m, 1 H); <sup>13</sup>C NMR (75 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  166.5, 153.5, 140.9, 136.0, 131.1, 130.3, 129.8, 128.5, 120.1, 119.8, 118.9, 118.0, 114.7, 110.0, 100.7, 73.9, 32.5, 24.4, 22.6; HRMS (ESI+) *m/z* calcd for C<sub>19</sub>H<sub>19</sub>O<sub>3</sub>N<sub>2</sub> [M+H]<sup>+</sup> 323.1390, found 323.1388; ELS purity 98.3%.



**(R)-N-Hydroxy-7-(1-methyl-1H-indol-5-yl)chromane-2-carboxamide ((R)-1-55e).** A solution of **(R)-1-54e** (0.0660 g, 0.197 mmol) in THF (1.0 mL) and MeOH (1.0 mL) was treated with LiOH monohydrate (0.00991 g, 0.236 mmol) in water (1.0 mL) at room temperature. After 2 h, the solution was concentrated, and azeotroped with toluene (2 x 10 mL). The residue was dissolved in DMF (0.5 mL) was treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.0748 g, 0.639 mmol). The mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 0.180 mL, 0.302 mmol) and TEA (0.0820 mL, 0.590 mmol). The mixture was warmed to room temperature after 30 min and stirred under N<sub>2</sub>. After 3 h, the mixture was diluted with EtOAc (8 mL), washed with 0.5 M HCl (8 mL), brine (8 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica to remove baseline impurities, and concentrated. The amide (0.103 g) was collected as a yellow oil. MeOH (3.0 mL) was added followed by Amberlyst-15 (0.0177 g, 83.0 mmol) at room temperature under N<sub>2</sub>. After 24 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes:EtOAc) yielding **(R)-1-55e** (0.0413 g, 67%) as a light pink solid:  $[\alpha]_D^{25} +23.0$  (*c* 0.05, DMSO); Mp 151-153 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3185, 2918, 1662, 1618, 1482, 1422, 1211 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.80 (s, 1 H), 8.94 (s, 1 H), 7.76 (s, 1 H), 7.49 (d, *J* = 8.5 Hz, 1 H), 7.40 (dd, *J* = 8.5, 1.2 Hz, 1 H), 7.34 (d, *J* = 3.0 Hz, 1 H), 7.17-7.10 (m, 3 H), 6.46 (d, *J* = 2.9 Hz, 1 H), 4.58 (dd, *J* = 8.6, 2.9 Hz, 1 H), 3.80 (s, 3 H), 2.84-2.78 (m, 1 H), 2.75-2.70 (m, 1 H), 2.17-2.13 (m, 1 H), 2.02-1.95 (m, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 166.5, 153.5, 140.9, 136.0, 131.1, 130.3, 129.7, 128.5, 120.1, 119.9, 118.9, 118.0, 114.7, 110.0,

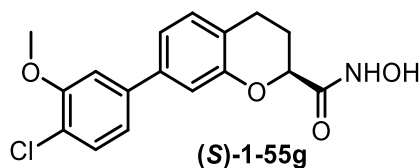
100.7, 73.9, 32.5, 24.4, 22.7; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>19</sub>H<sub>19</sub>O<sub>3</sub>N<sub>2</sub> [M+H]<sup>+</sup> 323.1390, found 323.1389; ELS purity 100%.



**(S)-7-(3,4-Difluorophenyl)-N-hydroxychromane-2-carboxamide ((S)-1-55f).** A solution of ester **(S)-1-54f** (0.104 g, 0.327 mmol) in THF (1.5 mL) and MeOH (1.5 mL) was treated with LiOH monohydrate (0.0151 g, 0.359 mmol) in water (1.0 mL) at rt. After 1 h, the solution was concentrated, and azeotroped with toluene (2 x 10 mL). The residue was dissolved in DMF (0.6 mL) and treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.107 g, 0.915 mmol). The mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 0.300 mL, 0.504 mmol) and TEA (0.100 mL, 0.717 mmol). The mixture was warmed to rt after 30 min and stirred under N<sub>2</sub>. After 4 h, the mixture was diluted with EtOAc (15 mL), washed with 0.5 M HCl (15 mL), brine (15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica to remove baseline impurities, and concentrated. The amide (0.146 g) was collected as a thick orange residue. MeOH (7.0 mL) and Amberlyst-15 (0.0352 g, 165 mmol) were added at rt under N<sub>2</sub>. After 21 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes:EtOAc) yielding **(S)-1-55f** (0.0743 g, 76%) as a white solid:  $[\alpha]_D -18.5$  ( $c$  0.12, DMSO); Mp 152-156 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3260, 2910, 1650, 1527, 1497, 1269 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  10.78 (s, 1 H), 8.95 (s, 1 H), 7.71-7.67 (m, 1 H), 7.53-7.47 (m, 2 H), 7.17-7.13 (m, 3 H), 4.59 (dd,  $J$  = 8.0, 2.3 Hz, 1 H), 2.84-2.78 (m, 1 H), 2.74-2.71 (m, 1 H), 2.14-2.11 (m, 1 H), 2.02-1.95 (m, 1 H); <sup>19</sup>F NMR (471MHz; DMSO-*d*<sub>6</sub>)  $\delta$  -138.22 (d,  $J$ = 22.5 Hz, 1 F), -140.95 (d,  $J$ =

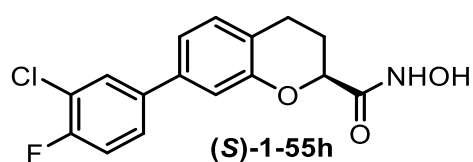


22.5 Hz, 1 F);  $^{13}\text{C}$  NMR (126 MHz; DMSO- $d_6$ )  $\delta$  166.3, 153.6, 149.70 (dd,  $J = 245.4, 12.6$  Hz), 148.95 (dd,  $J = 246.7, 12.9$  Hz), 137.5 (dd,  $J = 6.2, 3.6$  Hz), 137.0, 130.0, 123.05 (dd,  $J = 6.2, 3.1$  Hz), 121.8, 118.8, 117.85 (d,  $J = 17.0$  Hz), 115.37 (d,  $J = 17.8$  Hz), 114.8, 73.8, 24.1, 22.6; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{16}\text{H}_{12}\text{O}_3\text{NF}_2$  [M-H] $^+$  304.0780, found 304.0788; ELS purity 100%.



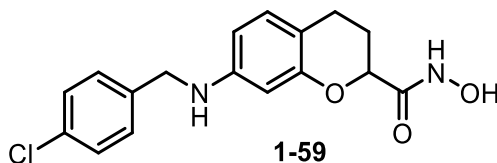
**(S)-7-(4-Chloro-3-methoxyphenyl)-N-hydroxychromane-2-carboxamide ((S)-1-55g).** A solution of ester **(S)-1-54g** (0.0769 g, 0.222 mmol) in THF (1.5 mL) and MeOH (1.5 mL) was treated with LiOH monohydrate (0.0102 g, 0.244 mmol) in water (1.0 mL) at room temperature. After 1 h, the solution was concentrated, and azeotroped with toluene (2 x 5 mL). The residue was dissolved in DMF (0.5 mL) and treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.0730 g, 0.623 mmol). The mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 0.200 mL, 0.336 mmol) and TEA (0.100 mL, 0.717 mmol). The mixture was warmed to room temperature after 30 min and stirred under N<sub>2</sub>. After 4 h, the mixture was diluted with EtOAc (6 mL), washed with 0.5 M HCl (6 mL), brine (6 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica to remove baseline impurities, and concentrated. The amide (0.103 g) was collected as a yellow oil. MeOH (3.0 mL) was added followed by Amberlyst-15 (0.0163 g, 76.4 mmol) at room temperature under N<sub>2</sub>. After 10 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc) yielding **(S)-1-55g** (0.0430 g, 60%) as a white solid:  $[\alpha]_D -11.6$  ( $c$  0.08, DMSO); Mp 172-175 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3203, 2939, 1664, 1562, 1483, 1390, 1235, 1071 cm $^{-1}$ ;  $^1\text{H}$  NMR (500 MHz; DMSO- $d_6$ )  $\delta$  10.80 (d,  $J = 1.2$  Hz, 1 H), 8.95 (d,  $J =$

1.6 Hz, 1 H), 7.47 (d,  $J = 8.2$  Hz, 1 H), 7.30 (d,  $J = 1.9$  Hz, 1 H), 7.20-7.18 (m, 2 H), 7.17-7.14 (m, 2 H), 4.58 (dd,  $J = 8.6, 3.2$  Hz, 1 H), 3.94 (s, 3 H), 2.85-2.79 (m, 1 H), 2.76-2.71 (m, 1 H), 2.16-2.11 (m, 1 H), 2.02-1.95 (m, 1 H);  $^{13}\text{C}$  NMR (126 MHz; DMSO- $d_6$ )  $\delta$  166.4, 154.7, 153.6, 140.3, 138.3, 130.1, 129.9, 121.7, 120.2, 119.3, 118.9, 114.8, 110.8, 73.8, 56.1, 24.2, 22.6; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{17}\text{H}_{17}\text{ClNO}_4$   $[\text{M}+\text{H}]^+$  334.0841, found 334.0842.



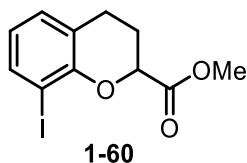
**(S)-7-(3-Chloro-4-fluorophenyl)-N-hydroxychromane-2-carboxamide ((S)-1-55h)**. A solution of **(S)-1-54h** (0.115 g, 0.344 mmol) in THF (1.5 mL) and MeOH (1.5 mL) was treated with LiOH monohydrate (0.0159 g, 0.378 mmol) in water (1.0 mL) at room temperature. After 1 h, the solution was concentrated, and azeotroped with toluene (2 x 10 mL). The residue was dissolved in DMF (0.6 mL) and treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.120 g, 1.02 mmol). The mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 0.300 mL, 0.504 mmol) and TEA (0.100 mL, 0.717 mmol). The mixture was warmed to room temperature after 30 min and stirred under N<sub>2</sub>. After 4 h, the mixture was diluted with EtOAc (15 mL), washed with 0.5 M HCl (15 mL), brine (15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica to remove baseline impurities, and concentrated. The amide (0.127 g) was collected as a thick orange residue. MeOH (6.0 mL) was added followed by Amberlyst-15 (0.0269 g, 126.3 mmol) at room temperature under N<sub>2</sub>. After 20 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc), yielding **(S)-1-55h** (0.0789 g, 74%) as a white solid:  $[\alpha]_{\text{D}}$  -7.7 ( $c$  0.12, DMSO); Mp 173-174 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3261,

2919, 1655, 1488, 1213  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz;  $\text{DMSO-}d_6$ )  $\delta$  10.78 (s, 1 H), 8.96 (s, 1 H) 7.81 (dd,  $J = 7.1, 2.3$  Hz, 1 H), 7.63 (ddd,  $J = 8.6, 4.7, 2.3$  Hz, 1 H), 7.48 (app t,  $J = 8.9$  Hz, 1 H), 7.18-7.13 (m, 3 H), 4.59 (dd,  $J = 8.5, 3.2$  Hz, 1 H), 2.84-2.78 (m, 1 H), 2.75-2.70 (m, 1 H), 2.16-2.10 (m, 1 H), 2.02-1.95 (m, 1 H);  $^{19}\text{F}$  NMR (471 MHz;  $\text{DMSO-}d_6$ )  $\delta$  -118.8;  $^{13}\text{C}$  NMR (126 MHz;  $\text{DMSO-}d_6$ )  $\delta$  166.3, 156.7 (d,  $J_{\text{C-F}} = 247$  Hz), 153.6, 137.7 (d,  $J_{\text{C-F}} = 3.6$  Hz), 136.8, 130.0, 128.3, 126.9 (d,  $J_{\text{C-F}} = 7.3$  Hz), 121.8, 119.9 (d,  $J_{\text{C-F}} = 17.9$  Hz), 118.8, 117.3 (d,  $J_{\text{C-F}} = 20.9$  Hz), 114.8, 73.8, 24.1, 22.6; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{16}\text{H}_{12}\text{O}_3\text{NClF}$   $[\text{M-H}]^+$  320.0484, found 320.0492; ELS purity 100%.



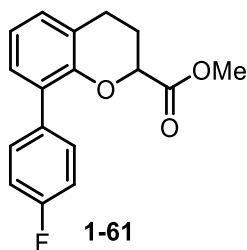
**7-((4-Chlorobenzyl)amino)-N-hydroxychromane-2-carboxamide (1-59).** In a microwave vial, a solution of ethyl 7-(((trifluoromethyl)sulfonyl)oxy)chromane-2-carboxylate (0.311 g, 0.878 mmol) in dioxane (8 mL) was sparged with  $\text{N}_2$  and treated with bis(dibenzylideneacetone)palladium (0.0404 g, 0.0439 mmol), 2-(di-*tert*-butylphosphino)biphenyl (0.0430 g, 0.144 mmol) and  $\text{K}_3\text{PO}_4$  (0.360 g, 1.70 mmol), and 4-chlorobenzylamine (0.120 mL, 0.986 mmol). The flask was sparged for another 5 minutes, sealed, and heated at 100  $^\circ\text{C}$  for 15 h. The mixture was filtered through Celite and rinsed with  $\text{CH}_2\text{Cl}_2$  (60 mL). The filtrate was washed with sat.  $\text{NaHCO}_3$  (40 mL), brine (40 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered through a pad of  $\text{SiO}_2$ , rinsing with EtOAc, and the filtrate was concentrated. A yellow crude oil **1-59i** (0.386 g) was collected, and carried forward to the aminolysis with no further purification. An ice-cooled solution of hydroxylamine hydrochloride (1.22 g, 17.6 mmol) in methanol (25 mL) was treated with KOH

(85%, 1.45 g, 21.9 mmol) portionwise. The mixture was stirred for an additional 1 h, and the precipitate was filtered off. The filtrate was added dropwise to an ice-cooled solution of the **1-59 i** (0.304 g crude) in methanol (5 mL). After 21 h, LCMS confirmed consumption of SM, and the reaction mixture was concentrated in vacuo and treated with water (10 mL). The pH of the solution was adjusted to 8.0 by addition of 1 M HCl. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL), and the combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. Purification by chromatography (C<sub>18</sub> reverse phase, 0-100% acetonitrile in water) provided **1-59** (0.0212 g, 7%, 2 steps) as an orange solid: Mp 105-107 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3216, 2924, 1666, 1627, 1516, 1490 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.69 (s, 1 H), 8.87 (s, 1 H), 7.37-7.33 (m, 4 H), 6.70 (d, *J* = 8.3 Hz, 1 H), 6.16 (t, *J* = 6.2 Hz, 1 H), 6.13 (dd, *J* = 8.2, 2.3 Hz, 1 H), 5.98 (d, *J* = 2.2 Hz, 1 H), 4.34 (dd, *J* = 9.4, 2.8 Hz, 1 H), 4.20 (d, *J* = 6.2 Hz, 1 H), 2.63-2.57 (m, 1 H), 2.53-2.50 (m, 1 H), 2.05-1.99 (m, 1 H), 1.86-1.78 (m, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 166.6, 153.9, 147.8, 139.6, 130.9, 129.5, 128.8, 128.2, 109.1, 106.4, 99.6, 73.8, 45.8, 24.9, 22.5; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>17</sub>H<sub>16</sub>O<sub>3</sub>N<sub>2</sub>Cl [M-H]<sup>+</sup> 331.0844, found 331.0855; ELS purity 99.6%.



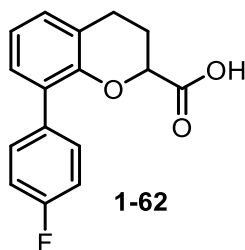
**Methyl 8-iodochromane-2-carboxylate (1-60).** A -5 °C solution of methyl 8-aminochromane-2-carboxylate **1-34b** (0.440 g, 2.12 mmol) in H<sub>2</sub>SO<sub>4</sub> (5.0 mL) was treated dropwise with a solution of NaNO<sub>2</sub> (0.146 g, 2.12 mmol) in H<sub>2</sub>O (4 mL). The mixture was stirred for 30 min at 0 °C and an additional 30 min at rt. A solution of NaI (0.318 g, 2.12 mmol) in H<sub>2</sub>O (2.5 mL) was added, and the mixture was heated to 80 °C for 1 h. After cooling to room temperature, the mixture was

suspended in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and H<sub>2</sub>O (50 mL) in a separatory funnel. The layers were separated and the organic layer was washed with brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The mixture was diluted with MeOH (8 mL) and treated with H<sub>2</sub>SO<sub>4</sub> (0.2 mL). The mixture was heated at 50 °C for 1 h. The MeOH was concentrated, the residue was diluted with EtOAc (10 mL), washed with sat. NaHCO<sub>3</sub> (50 mL) and brine (50 mL). The EtOAc layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-18% EtOAc in hexanes) provided **1-60** (92% purity by <sup>1</sup>H NMR \*, 0.247 g, 34%) as a yellow oil: IR (CH<sub>2</sub>Cl<sub>2</sub>) 2950, 2846, 1755, 1737, 1445, 1210 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.61 (dd, *J* = 7.8, 1.3 Hz, 1 H), 7.00 (dd, *J* = 7.5, 1.2 Hz, 1 H), 6.62 (t, *J* = 7.7 Hz, 1 H), 4.90 (app t, *J* = 5.0 Hz, 1 H), 3.79 (s, 3 H), 2.85-2.67 (m, 2 H), 2.33-2.17 (m, 2 H); <sup>13</sup>C NMR (75 MHz; CDCl<sub>3</sub>) δ 171.0, 152.4, 137.7, 129.8, 122.5, 122.4, 85.3, 74.7, 52.6, 24.5, 23.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>11</sub>H<sub>11</sub>IO<sub>3</sub>Na [M+Na]<sup>+</sup> 340.9645, found 340.9641. \*The impurity is methyl chromane-2-carboxylate that forms as a side product.



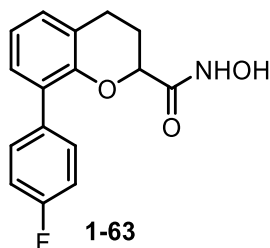
**Methyl 8-(4-fluorophenyl)chromane-2-carboxylate (1-61).** In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0520 g, 0.0450 mmol) and CsF (0.296 g, 1.95 mmol). A solution of iodide **1-60** (92% purity, 0.300 g, 0.877 mmol) in dioxane/H<sub>2</sub>O (5:1, 6 mL/1.2 mL) was sparged with N<sub>2</sub> (15 min) and added to the mixture, followed by 4-fluorophenylboronic acid (0.130 g, 0.930 mmol). After additional sparging with N<sub>2</sub> (5 min), the

vial was sealed, and stirred at 90 °C for 12 h. The reaction mixture was diluted with EtOAc (40 mL) and 1:1 sat. aq. NaHCO<sub>3</sub>/sat. aq. NaCl (40 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2 x 40 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-18% EtOAc in hexanes followed by 10% acetone in hexanes) provided **1-61** (90% purity by <sup>1</sup>H NMR \*, 0.149 g, 53% yield) as a yellow oil: IR (CH<sub>2</sub>Cl<sub>2</sub>) 2952, 2931, 2851, 1753, 1512, 1455, 1198, 1161, 1106 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 7.63-7.58 (m, 2 H), 7.15 (dd, *J* = 7.4, 1.7 Hz, 1 H), 7.12-7.06 (m, 2 H), 7.03-7.01 (m, 1 H), 6.93 (t, *J* = 7.5 Hz, 1 H), 4.81 (app t, *J* = 5.1 Hz, 1 H), 3.78 (s, 3 H), 2.90-2.76 (m, 2 H), 2.29-2.24 (m, 2 H); <sup>19</sup>F NMR (376 MHz; CDCl<sub>3</sub>) δ -116.02; <sup>13</sup>C NMR (101 MHz; CDCl<sub>3</sub>) δ 171.6, 162.18 (d, *J* = 246.6 Hz), 150.2, 134.24 (d, *J* = 3.1 Hz), 131.40 (d, *J* = 8.0 Hz), 129.2, 129.1, 129.0, 121.8, 120.8, 114.9 (d, *J* = 21.3 Hz), 73.8, 52.5, 24.2, 23.3; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>17</sub>H<sub>15</sub>FO<sub>3</sub>Na [M+Na]<sup>+</sup> 309.0897, found 309.0892. \*The impurity is methyl chromane-2-carboxylate.



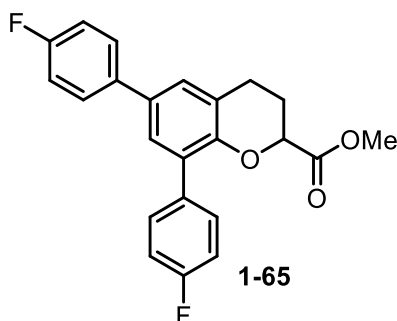
**8-(4-Fluorophenyl)chromane-2-carboxylic acid (1-62).** A solution of the ester **1-61** (90%, 0.145 g, 0.456 mmol) in THF (2.0 mL) and MeOH (2.0 mL) was treated with LiOH monohydrate (0.0235 g, 0.559 mmol) in water (2.0 mL) at room temperature. After 17 h, the solution was concentrated and the residue was diluted with H<sub>2</sub>O (5 mL), acidified with 2M HCl until pH =1, extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>),

filtered and purified by chromatography on C<sub>18</sub>-SiO<sub>2</sub> (5-95% acetonitrile in H<sub>2</sub>O). Product eluted at ~60-70% acetonitrile/H<sub>2</sub>O) to provide **1-62** (0.105 g, 85%) was collected as a white solid: Mp 128-130 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3029, 2932, 1715, 1511, 1455, 1217 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 7.64-7.61 (m, 2 H), 7.23-7.18 (m, 2 H), 7.11 (dd, *J* = 7.5, 1.4 Hz, 1 H), 7.04 (dd, *J* = 7.5, 1.3 Hz), 6.90 (t, *J* = 7.6 Hz, 1 H), 4.86 (app t, *J* = 4.6 Hz, 1 H), 2.84-2.79 (m, 1 H), 2.72-2.66 (m, 1 H), 2.18-2.08 (m, 2 H); <sup>19</sup>F NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ -116.00; <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 172.2, 161.2 (d, *J*<sub>C-F</sub> = 244 Hz), 150.1, 134.4 (d, *J*<sub>C-F</sub> = 3.0 Hz), 131.2 (d, *J*<sub>C-F</sub> = 23.9 Hz), 129.0, 128.3, 127.8, 122.0, 120.2, 114.7 (d, *J*<sub>C-F</sub> = 21.0 Hz), 72.8, 23.3, 22.3; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>16</sub>H<sub>12</sub>FO<sub>3</sub> [M-H]<sup>+</sup> 271.0765, found 271.0766.



**8-(4-Fluorophenyl)-N-hydroxychromane-2-carboxamide (1-63).** The acid **1-62** (0.0960 g, 0.353 mmol) in DMF (0.8 mL) was treated with O-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (0.124 g, 1.06 mmol). The mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 0.330 mL, 0.529 mmol) and TEA (0.147 mL, 1.06 mmol). The mixture was warmed to rt after 30 min and stirred under N<sub>2</sub>. After 3 h, the mixture was diluted with EtOAc (8 mL), washed with 0.5 M HCl (8 mL), brine (8 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica to remove baseline impurities, and concentrated. The amide (0.126 g) was collected as a yellow solid. MeOH (6.0 mL) was added followed by Amberlyst-15 (0.0370 g, 174 mmol) at rt under N<sub>2</sub>. After 22 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by

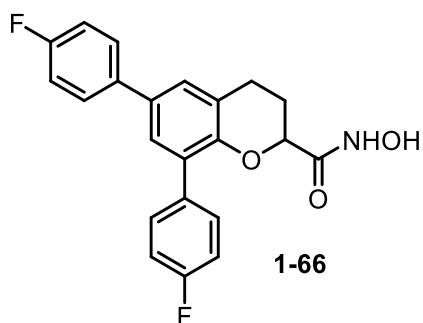
trituration (5:1 hexanes: EtOAc) yielding **1-63** (0.0786 g, 70%, over 2 steps) as a white solid: Mp 163-165 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3412, 3103, 2923, 1671, 1603, 1511, 1455, 1222 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>) δ 10.60 (s, 1 H), 9.01 (s, 1 H), 7.60-7.57 (m, 2 H), 7.22-7.18 (m, 2 H), 7.11-7.06 (m, 2 H), 6.91 (t, *J* = 7.5 Hz, 1 H), 4.48 (dd, *J* = 8.0, 3.6 Hz, 1 H), 2.88-2.76 (m, 2 H), 2.14-1.96 (m, 2 H); <sup>19</sup>F NMR (400 MHz; DMSO-*d*<sub>6</sub>) δ -115.99; <sup>13</sup>C NMR (101 MHz; DMSO-*d*<sub>6</sub>) δ 166.6, 161.21 (d, *J* = 244.5 Hz), 150.4, 134.31 (d, *J* = 3.2 Hz), 131.20 (d, *J* = 8.0 Hz), 128.9, 128.3, 127.9, 122.5, 120.3, 114.63 (d, *J* = 21.2 Hz), 73.7, 24.4, 23.2; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>16</sub>H<sub>13</sub>O<sub>3</sub>NF [M-H]<sup>+</sup> 286.0874, found 286.0883; ELS purity 99.4%.



**Methyl 6,8-bis(4-fluorophenyl)chromane-2-carboxylate (1-65).** In a N<sub>2</sub>-filled glove box, a microwave vial was charged with Pd(PPh<sub>3</sub>)<sub>4</sub> (0.0630 g, 0.0545 mmol) and CsF (0.348 g, 2.29 mmol). A solution of methyl 6,8-diiodochromane-2-carboxylate (0.230 g, 0.518 mmol) in dioxane/H<sub>2</sub>O (5:1, 5.5 mL/1.1 mL) was sparged with N<sub>2</sub> (15 min) and added to the mixture, followed by 4-fluorophenylboronic acid (0.153 g, 1.10 mmol). After additional sparging with N<sub>2</sub> (5 min), the vial was sealed, and stirred at 90 °C for 12 h. The reaction mixture was diluted with EtOAc (50 mL) and 1:1 sat. aq. NaHCO<sub>3</sub>/sat. aq. NaCl (50 mL). The layers were separated, and the aq. phase was extracted with EtOAc (2 x 50 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-18% EtOAc in

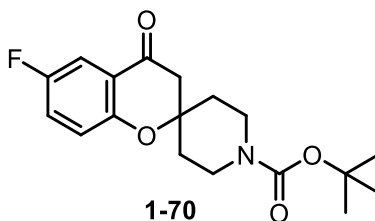


hexanes) provided **1-65** (96% purity by  $^1\text{H}$  NMR (acetone), 82.0 mg, 40% yield) as a yellow oil: IR ( $\text{CH}_2\text{Cl}_2$ ) 2953, 1752, 1603, 1512, 1465, 1219, 1200  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz;  $\text{CDCl}_3$ )  $\delta$  7.66-7.64 (m, 2 H), 7.52-7.49 (m, 2 H), 7.32 (d,  $J = 2.2$  Hz, 1 H), 7.20 (d,  $J = 1.5$  Hz, 1 H), 7.13-7.08 (m, 4 H), 4.86 (app t,  $J = 5.0$  Hz, 1 H), 3.80 (s, 3 H), 2.94-2.82 (m, 2 H), 2.34-2.28 (m, 2 H);  $^{19}\text{F}$  NMR (471 MHz;  $\text{CDCl}_3$ )  $\delta$  -115.58, -116.39;  $^{13}\text{C}$  NMR (126 MHz;  $\text{CDCl}_3$ )  $\delta$  171.5, 162.33 (d,  $J = 246.4$  Hz), 162.29 (d,  $J = 246.6$  Hz), 149.8, 136.90 (d,  $J = 2.9$  Hz), 134.06 (d,  $J = 3.2$  Hz), 133.0, 131.42 (d,  $J = 7.9$  Hz), 129.6, 128.40 (d,  $J = 8.1$  Hz), 127.8, 127.5, 122.2, 115.69 (d,  $J = 21.6$  Hz), 115.04 (d,  $J = 21.2$  Hz), 73.9, 52.5, 24.2, 23.4; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{23}\text{H}_{18}\text{F}_2\text{O}_3\text{Na}$   $[\text{M}+\text{Na}]^+$  403.1116, found 403.1113.



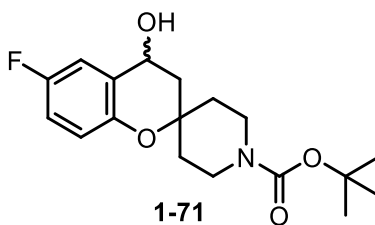
**6,8-Bis(4-fluorophenyl)-N-hydroxychromane-2-carboxamide (1-66).** A solution of **1-65** (96%, 0.0780 g, 0.197 mmol) in THF (1.0 mL) and MeOH (1.0 mL) was treated with LiOH monohydrate (0.0114 g, 0.476 mmol) in water (1.0 mL) at room temperature. After 17 h, the solution was concentrated and azeotroped with toluene (2 x 10 mL). The residue was dissolved in DMF (0.5 mL) and treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.0732 g, 0.625 mmol). The mixture was cooled to 0  $^{\circ}\text{C}$ , and treated with  $\text{T}_3\text{P}$  (50%, 0.200 mL, 0.336 mmol) and TEA (0.0800 mL, 0.574 mmol). The mixture was warmed to room temperature after 30 min and stirred under  $\text{N}_2$ . After 4 h, the mixture was diluted with EtOAc (8 mL), washed with 0.5 M HCl (8 mL), brine

(8 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through silica to remove baseline impurities, and concentrated. The amide (0.0869 g) was collected as an orange oil. MeOH (4.0 mL) was added followed by Amberlyst-15 (0.0194 g, 91.2 mmol) at room temperature under N<sub>2</sub>. After 22 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes:EtOAc) yielding **1-66** (0.0347 g, 48%) as a white solid: Mp 95-98 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3414, 3194, 2897, 1672, 1510, 1463, 1218 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 10.65 (d, *J* = 1.3 Hz, 1 H), 9.03 (d, *J* = 1.5 Hz, 1 H), 7.73-7.67 (m, 4 H), 7.37-7.36 (m, 2 H), 7.28-7.20 (m, 4 H), 4.55 (dd, *J* = 7.4, 3.9 Hz, 1 H), 2.93-2.87 (m, 2 H), 2.16-2.02 (m, 2 H); <sup>19</sup>F NMR (376 MHz; DMSO-*d*<sub>6</sub>) δ -115.71, -116.44; <sup>13</sup>C NMR (101 MHz; DMSO-*d*<sub>6</sub>) δ 166.6, 161.50 (d, *J* = 244.8 Hz), 161.35 (d, *J* = 245.3 Hz), 150.1, 136.28 (d, *J* = 2.8 Hz), 134.14 (d, *J* = 3.6 Hz), 131.40 (d, *J* = 7.8 Hz), 131.3, 128.3, 128.22 (d, *J* = 8.1 Hz), 127.1, 126.6, 123.1, 115.52 (d, *J* = 21.3 Hz), 114.66 (d, *J* = 21.3 Hz), 73.8, 24.4, 23.3; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>22</sub>H<sub>16</sub>O<sub>3</sub>NF<sub>2</sub> [M-H]<sup>+</sup> 380.1093, found 380.1102; ELS purity 100%.

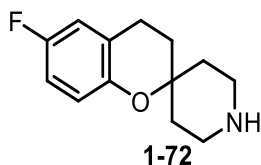


**tert-Butyl 6-fluoro-4-oxospiro[chromane-2,4'-piperidine]-1'-carboxylate (1-70).**<sup>114</sup> A mixture of the acetophenone (0.9923 g, 6.31 mmol), 1-boc-4-piperidinone **1-69** (1.28 g, 6.36 mmol), and pyrrolidine (1.00 mL, 12.2 mmol) in MeOH (4 mL) was irradiated at 70 °C for 5 h. The solution was concentrated, and the mixture was purified by chromatography on SiO<sub>2</sub> (0-100% EtOAc in hexanes) to provide **1-70** (1.62 g, 76%) as a yellow solid: <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.50 (dd,

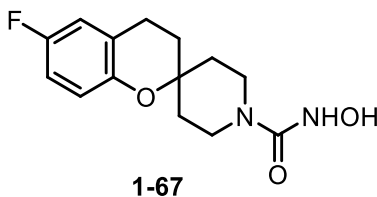
$J = 8.0, 3.0$  Hz, 1 H), 7.21 (ddd,  $J = 9.0, 8.2, 3.0$  Hz, 1 H), 6.95 (dd,  $J = 9.0, 4.0$  Hz, 1 H), 3.86 (bs, 2 H), 3.19 (bs, 2 H), 2.70 (s, 2 H), 2.00 (d,  $J = 13.2$  Hz, 2 H), 1.59 (td,  $J = 13.0, 3.9$  Hz, 2 H), 1.45 (s, 9 H);  $^{19}\text{F}$  NMR (471 MHz;  $\text{CDCl}_3$ )  $\delta$  -121.57;  $^{13}\text{C}$  NMR (126 MHz;  $\text{CDCl}_3$ )  $\delta$  190.9, 157.3 (d,  $J_{\text{C-F}} = 242.7$  Hz), 155.3, 154.8, 123.9 (d,  $J_{\text{C-F}} = 24.4$  Hz), 121.3 (d,  $J_{\text{C-F}} = 6.4$  Hz), 120.0 (d,  $J_{\text{C-F}} = 7.3$  Hz), 111.9 (d,  $J_{\text{C-F}} = 23.9$  Hz) 79.97, 78.26, 77.16, 47.9, 39.2, 34.0. HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{21}\text{H}_{23}\text{O}_4\text{N}_4$   $[\text{M}+\text{H}-\text{Boc}]^+$  236.1081, found 236.1078.



**tert-Butyl-6-fluoro-4-hydroxyspiro[chromane-2,4'-piperidine]-1'-carboxylate (1-71).**<sup>114</sup> A solution of ketone **1-70** (0.310 g, 0.923 mmol), in EtOH (2.5 mL) was treated with  $\text{NaBH}_4$  (0.045 mg, 1.19 mmol) at room temperature, portionwise. The reaction mixture was stirred under  $\text{N}_2$ , diluted with water (20 mL), pH~ 9-10, and extracted with EtOAc (3 x 30 mL). The combined extracts were washed with  $\text{H}_2\text{O}$  (15 mL) and brine (15 mL). The mixture was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to yield **1-71** (0.312 g, 100%) as a white solid:  $^1\text{H}$  NMR (300 MHz;  $\text{CDCl}_3$ )  $\delta$  7.15 (dd,  $J = 9.1, 3.0$  Hz, 1 H), 6.90 (td,  $J = 8.4, 3.1$  Hz, 1 H), 6.79 (dd,  $J = 9.0, 4.7$  Hz, 1 H), 4.84 (m, 1 H), 3.85 (bs, 2 H), 3.28-3.10 (m, 2 H), 2.15 (dd,  $J = 13.6, 6.1$  Hz, 1 H), 1.94-1.60 (m, 5 H), 1.46 (s, 9 H);  $^{19}\text{F}$  NMR (471 MHz;  $\text{CDCl}_3$ )  $\delta$  -123.13; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{18}\text{H}_{24}\text{FNO}_4\text{Na}$   $[\text{M}+\text{Na}]^+$  360.1582, found 360.1581.

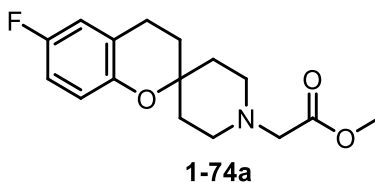


**6-Fluorospiro[chromane-2,4'-piperidine] (1-72).**<sup>114</sup> Triethylsilane (0.700 mL, 4.38 mmol) was added to a solution of alcohol **1-71** (0.259 g, 0.919 mmol) in TFA (3.3 mL). The reaction mixture was heated to 60 °C for 14 h. The mixture was concentrated and the residue was extracted with EtOAc and washed with 0.5 M HCl. The aqueous was treated with 2.5M NaOH to pH 14 and extracted with EtOAc (2x). The combined organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to give **1-72** (0.132 g, 65%) as a beige solid: Mp 119-121.0 °C; IR (neat) 2939, 1554, 1491, 1432, 1218 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 6.81-6.73 (m, 3 H), 3.03 (app t, *J* = 11.0 Hz, 2 H), 2.86-2.84 (m, 2 H), 2.75 (app t, *J* = 6.8 Hz, 2 H), 1.80-1.74 (m, 5 H), 1.53 (app td, *J* = 12.4, 4.2 Hz, 2 H); <sup>19</sup>F NMR (471 MHz; CDCl<sub>3</sub>) δ -125.0; <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 156.7 (d, *J*<sub>C-F</sub> = 238 Hz), 149.5, 122.6 (d, *J*<sub>C-F</sub> = 7.3 Hz), 118.2 (d, *J*<sub>C-F</sub> = 8.0 Hz), 115.3 (d, *J*<sub>C-F</sub> = 22.6 Hz), 114.1 (d, *J*<sub>C-F</sub> = 7.3 Hz), 73.2, 42.2, 35.7, 32.0, 21.7; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>13</sub>H<sub>17</sub>ONF [M+H]<sup>+</sup> 222.1289, found 222.1286; ELS purity 100%.



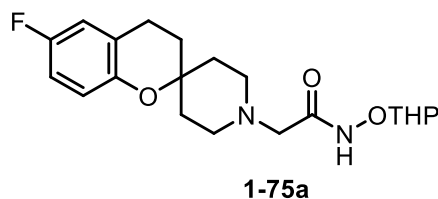
**6-Fluoro-*N*-hydroxyspiro[chromane-2,4'-piperidine]-1'-carboxamide (1-67).** A solution of amine **1-72** (0.103 g, 0.465 mmol) in toluene (4 mL) was cooled to 0 °C, and treated with TEA (0.120 mL, 0.861 mmol) and phosgene (20 wt% in toluene, 0.360 mL, 0.680 mmol). The reaction

mixture was left at 0 °C for 2 h, warmed to rt, and concentrated under vacuum. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) and treated with hydroxylamine hydrochloride (0.0990 g, 1.42 mmol) and TEA (0.200 mL, 1.43 mmol). After 13 h of stirring at rt, the reaction mixture was treated with 0.5 M HCl (15 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 15 mL). The combined organic layers were washed with brine (15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under vacuum. The beige solid was slurried with EtOAc, filtered, and dried under vacuum, to provide **1-67** (0.0560 g, 43%) as a white solid: Mp 199-200 °C; IR (neat) 3167, 2913, 1610, 1576, 1489, 1450, 1219 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 9.04 (d, *J* = 2.0 Hz, 1 H), 7.96 (d, *J* = 2.0 Hz, 1 H), 6.94-6.88 (m, 2 H), 6.78 (dd, *J* = 8.8, 5.0 Hz, 1 H), 3.61 (dt, *J* = 13.4, 3.7 Hz, 2 H), 3.09 (td, *J* = 12.3, 2.6 Hz, 2 H), 2.72 (t, *J* = 6.8 Hz, 2 H), 1.75 (t, *J* = 6.8 Hz, 2 H), 1.62 (app d, *J* = 13.3 Hz, 2 H), 1.52-1.46 (m, 2 H); <sup>19</sup>F NMR (471 MHz; DMSO-*d*<sub>6</sub>) δ -124.6; <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>): δ 159.6, 155.8 (d, *J*<sub>C-F</sub> = 235.7 Hz), 149.0, 122.9 (d, *J*<sub>C-F</sub> = 7.7 Hz), 117.8 (d, *J*<sub>C-F</sub> = 8.0 Hz), 115.2 (d, *J*<sub>C-F</sub> = 22.5 Hz), 113.8 (d, *J*<sub>C-F</sub> = 22.9 Hz), 72.9, 33.6, 30.3, 20.9; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>14</sub>H<sub>16</sub>O<sub>3</sub>N<sub>2</sub>F [M+H]<sup>+</sup> 279.1139, found 279.1133; ELS purity 100%.



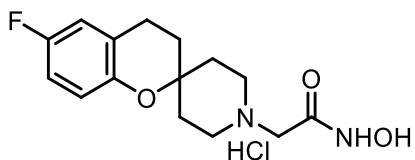
**Methyl 2-(6-fluorospiro[chromane-2,4'-piperidin]-1'-yl)acetate (1-74a).** A solution of amine **1-72** (0.225 g, 1.02 mmol) in anhydrous DMF (4 mL) was treated with Cs<sub>2</sub>CO<sub>3</sub> (0.562 g, 1.73 mmol). The reaction mixture was treated with methylbromoacetate (100.0 μL, 1.03 mmol) dropwise, stirred at room temperature under N<sub>2</sub> for 1 h, treated with brine (90 mL) and extracted with ethyl acetate (2 x 90 mL). After drying (Na<sub>2</sub>SO<sub>4</sub>), filtering, and concentrating under vacuum,

**1-74a** (0.207 g, 69%) was initially collected as a yellow oil that crystallized upon standing:  $^1\text{H}$  NMR (500 MHz;  $\text{CDCl}_3$ )  $\delta$  6.81-6.73 (m, 3 H), 3.73 (s, 3 H), 3.26 (s, 2 H), 2.76-2.71 (m, 4 H), 2.55 (td,  $J = 11.5, 3.0$  Hz, 2 H), 1.83-1.71 (m, 6 H);  $^{19}\text{F}$  NMR (471 MHz;  $\text{CDCl}_3$ )  $\delta$  -124.9; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{16}\text{H}_{21}\text{FNO}_3$   $[\text{M}+\text{H}]^+$  294.1500, found 294.1494.



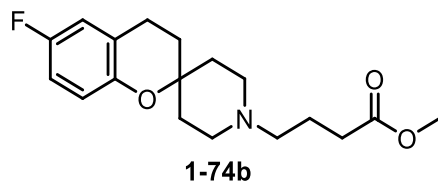
**2-((6-Fluorospiro[chromane-2,4'-piperidin]-1'-yl)-N-((tetrahydro-2H-pyran-2-yl)oxy)acetamide (1-75a).** A solution of ester **1-74a** (0.207 g, 0.706 mmol) in THF (1.5 mL) and MeOH (1.5 mL) was treated with LiOH monohydrate (0.0369 g, 0.879 mmol) in water (1.5 mL) at room temperature. After 1 h, the solution was concentrated and the residue was azeotroped with PhMe (2 x 20 mL). The crude residue was dissolved in DMF (3 mL) and treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.101 g, 0.864 mmol). The mixture was cooled to 0 °C, and T<sub>3</sub>P (50%, 0.650 mL, 0.919 mmol) and TEA (0.200 mL, 1.43 mmol) were added. The mixture was stirred under N<sub>2</sub>, and after 16 h, was diluted with EtOAc (60 mL) and washed with 1 M NaOH (40 mL). The aqueous layer was reextracted with EtOAc (60 mL). The combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. Purification by chromatography on  $\text{SiO}_2$  (100% EtOAc followed by 5% MeOH in  $\text{CH}_2\text{Cl}_2$ ) provided **1-75a** (0.163 g, 61%) as a white foam:  $^1\text{H}$  NMR (500 MHz;  $\text{CDCl}_3$ )  $\delta$  9.50 (s, 1 H), 6.80-6.74 (m, 3 H), 4.97 (s, 1 H), 4.00-3.98 (m, 1 H), 3.66-3.63 (m, 1 H), 3.14 (s, 2 H), 2.75 (app t,  $J = 7.0$  Hz, 2 H), 2.65-2.57 (m, 4 H), 1.84-1.56 (m, 12 H);  $^{19}\text{F}$

NMR (471 MHz; CDCl<sub>3</sub>)  $\delta$  -124.70; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>20</sub>H<sub>28</sub>FN<sub>2</sub>O<sub>4</sub> [M+H]<sup>+</sup> 379.2028, found 379.2027.

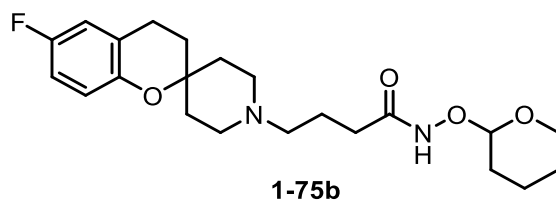


**1-76a**

**2-(6-Fluorospiro[chromane-2,4'-piperidin]-1'-yl)-N-hydroxyacetamide hydrogen chloride salt (1-76a).** The amide **1-75a** (0.160 g, 0.423 mmol) was dissolved in ether (4.55 mL) and MeOH (0.25 mL) and treated with 4M HCl in dioxane (0.250 mL, 1.00 mmol). The reaction mixture was stirred under N<sub>2</sub> for 2 h, with occasional sonication. The white solid was filtered and concentrated under vacuum to provide **1-76a** (0.128 g, 91%) as a white solid: Mp 200-203 °C; IR (neat) 3038, 2852, 1677, 1490, 1432, 1367, 1211 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; 100 °C, DMSO-*d*<sub>6</sub>)  $\delta$  6.93-6.88 (m, 2 H), 6.83-6.81 (m, 1 H), 3.87 (bs, 2 H), 3.38-3.36 (m, 4 H), 2.78 (app t,  $J$  = 6.8 Hz, 2 H), 2.11-2.01 (m, 2 H), 1.97-1.94 (m, 2 H), 1.86 (app t,  $J$  = 6.8 Hz, 2 H); <sup>19</sup>F NMR (376 MHz; 25 °C, rotamers, DMSO-*d*<sub>6</sub>)  $\delta$  -124.92, -123.94, -124.13; <sup>13</sup>C NMR (101 MHz; 25 °C, rotamers, DMSO-*d*<sub>6</sub>):  $\delta$  166.8, 160.7, 156.1 (d,  $J_{C-F}$  = 236.7 Hz), 148.3, 122.9 (d,  $J_{C-F}$  = 7.1 Hz), 118.0 (d,  $J_{C-F}$  = 7.7 Hz), 115.4 (d,  $J_{C-F}$  = 22.8 Hz), 114.0 (d,  $J_{C-F}$  = 23.3 Hz), 70.0, 54.3, 48.4, 47.9, 30.8, 30.4, 20.8; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>15</sub>H<sub>18</sub>O<sub>3</sub>N<sub>2</sub>F [M-H]<sup>+</sup> 293.1296, found 293.1307; ELS purity 100%.



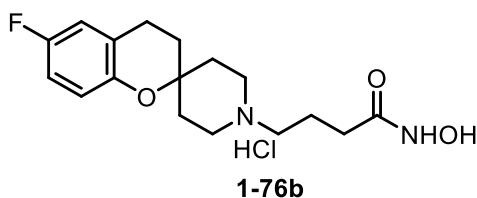
**Methyl 4-(6-fluorospiro[chromane-2,4'-piperidin]-1'-yl)butanoate (1-74b).** A solution of amine **1-72** (0.179 g, 0.810 mmol) in anhydrous DMF (3.5 mL) was treated with Cs<sub>2</sub>CO<sub>3</sub> (0.518 g, 1.59 mmol), and methyl 4-bromobutyrate (105  $\mu$ L, 0.807 mmol) dropwise. The reaction mixture was stirred at rt under N<sub>2</sub> for 72 h, treated with brine (70 mL) and extracted with EtOAc (2 x 70 mL). After drying (Na<sub>2</sub>SO<sub>4</sub>), filtering, and concentrating, **1-74b** (~80% purity by <sup>1</sup>H NMR, 0.211 g, 65%) was collected as an orange oil and was used for the next step without further purification: <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>)  $\delta$  6.81-6.73 (m, 3 H), 3.67 (s, 3 H), 2.74 (t, *J* = 7.2 Hz, 2 H), 2.65-2.62 (m, 2 H), 2.42-2.37 (m, 4 H), 2.35 (t, *J* = 7.4 Hz, 2 H), 1.85-1.75 (m, 6 H), 1.66-1.59 (m, 2 H); <sup>19</sup>F NMR (376 MHz; CDCl<sub>3</sub>)  $\delta$  -125.03; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>18</sub>H<sub>25</sub>FNO<sub>3</sub> [M+H]<sup>+</sup> 322.1813, found 322.1808.



**4-(6-Fluorospiro[chromane-2,4'-piperidin]-1'-yl)-N-((tetrahydro-2H-pyran-2-yl)oxy)butanamide (1-75b).** A solution of ester **1-74b** (~80% purity, 0.210 g, 0.523 mmol) in THF (1.5 mL) and MeOH (1.5 mL) was treated with LiOH monohydrate (0.0611 g, 1.46 mmol) in water (1.5 mL) at room temperature. After 23 h, the solution was concentrated and the residue was azeotroped with PhMe (2 x 20 mL). The crude residue was dissolved in DMF (3 mL) and treated with O-

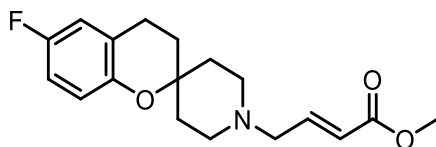


(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (0.119 g, 1.02 mmol). The solution was cooled to 0 °C, and T<sub>3</sub>P (50%, 0.800 mL, 1.13 mmol) and TEA (0.200 mL, 1.43 mmol) were added. The mixture was stirred under N<sub>2</sub>, and after 19 h, was diluted with EtOAc (60 mL) and washed with 1 M NaOH (40 mL). The aqueous layer was reextracted with EtOAc (60 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (5-10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) provided **1-75b** (0.102 g, 48%) as a colorless oil: <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 6.81-6.73 (m, 3 H), 4.93 (bs, 1 H), 3.99 (bs, 1 H), 3.62-3.60 (m, 1 H), 2.77-2.74 (m, 4 H), 2.63 (br, 4 H), 2.52 (br, 2 H), 1.85-1.78 (m, 14 H); <sup>19</sup>F NMR (471 MHz; CDCl<sub>3</sub>) δ -124.70; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>22</sub>H<sub>32</sub>FN<sub>2</sub>O<sub>4</sub> [M+H]<sup>+</sup> 407.2341, found 407.2344.



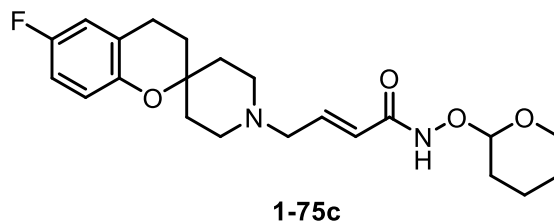
**4-(6-Fluorospiro[chromane-2,4'-piperidin]-1'-yl)-*N*-hydroxybutanamide hydrogen chloride salt (**1-76b**).** A solution of amide **1-75b** (0.100 g, 0.246 mmol) in ether (2.70 mL) and MeOH (0.15 mL) was treated with 4M HCl in dioxane (0.140 mL, 0.560 mmol). The reaction mixture was stirred under N<sub>2</sub> for 2 h, with occasional sonication. The white solid was filtered and concentrated under vacuum to provide **1-76b** (0.0700 g, 79%) as an off-white solid: Mp 210-213 °C; IR (neat) 3263, 2950, 2662, 2574, 2503, 1647, 1498, 1435 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; 25 °C, rotamers, DMSO-*d*<sub>6</sub>) δ 10.69 (bs, 1 H), 10.56 (bs, 1 H), 8.79 (s, 1 H), 6.97-6.77 (m, 3 H), 3.39-3.30 (m, 2 H), 3.09-3.07 (m, 4 H), 2.76 (app t, *J* = 6.8 Hz, 2 H), 2.08 (app t, *J* = 6.8 Hz, 2 H), 2.00-1.88 (m, 6 H), 1.80 (app t, *J* = 6.8 Hz, 2 H); <sup>19</sup>F NMR (376 MHz; 25 °C, rotamers, DMSO-*d*<sub>6</sub>) δ -123.9, -

124.2;  $^{13}\text{C}$  NMR (101 MHz; 25 °C, DMSO- $d_6$ ):  $\delta$  167.9, 156.1 (d,  $J_{\text{C-F}}=237$  Hz), 148.3, 122.9 (d,  $J_{\text{C-F}}=7.7$  Hz), 118.1 (d,  $J_{\text{C-F}}=8.6$  Hz), 115.4 (d,  $J_{\text{C-F}}=22.7$  Hz), 114.0 (d,  $J_{\text{C-F}}=23.2$  Hz), 70.3, 55.4, 47.4, 30.9, 30.4, 29.3, 20.8, 19.5; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{17}\text{H}_{22}\text{O}_3\text{N}_2\text{F}$  [M-H] $^+$  321.1609, found 321.1617; ELS purity 100%.

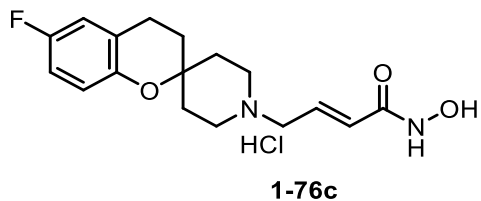


**1-74c**

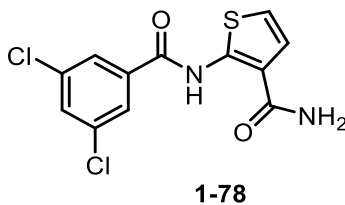
**Methyl (*E*)-4-(6-fluorospiro[chromane-2,4'-piperidin]-1'-yl)but-2-enoate (1-74c).** A solution of amine **1-72** (0.225 g, 1.02 mmol) in anhydrous DMF (4 mL) was treated with  $\text{Cs}_2\text{CO}_3$  (0.573 g, 1.76 mmol) and dropwise addition of methyl 4-bromocrotonate (90%, 0.135 mL, 1.03 mmol). The reaction mixture was stirred at rt under  $\text{N}_2$  for 1 h, treated with brine (70 mL) and extracted with EtOAc (2 x 70 mL). After drying ( $\text{Na}_2\text{SO}_4$ ), filtering, and concentrating under vacuum, **1-74c** (>95% purity by  $^1\text{H}$  NMR, 0.2471 g, 72%) was collected as an orange oil:  $^1\text{H}$  NMR (400 MHz;  $\text{CDCl}_3$ )  $\delta$  2.74 (dt,  $J = 15.6, 6.0$  Hz, 1 H), 6.81-6.74 (m, 3 H), 6.00 (d,  $J = 15.6$  Hz, 1 H), 3.74 (s, 3 H), 3.19 (dd,  $J = 6.4, 1.6$  Hz, 2 H), 2.75 (t,  $J = 6.8$  Hz, 2 H), 2.66-2.63 (m, 2 H), 2.45 (td,  $J = 11.6, 2.4$  Hz, 2 H), 1.83-1.63 (m, 6 H);  $^{19}\text{F}$  NMR (376 MHz;  $\text{CDCl}_3$ )  $\delta$  -124.89; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{18}\text{H}_{23}\text{FNO}_3$  [M+H] $^+$  320.1656, found 320.1654.



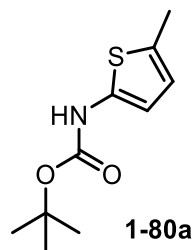
**(E)-4-(6-Fluorospiro[chromane-2,4'-piperidin]-1'-yl)-N-((tetrahydro-2H-pyran-2-yl)oxy)but-2-enamide (1-75c).** A solution of ester **1-74c** (95%, 0.240 g, 0.714 mmol) in THF (2.0 mL) and MeOH (2.0 mL) was treated with LiOH monohydrate (0.0466 g, 1.11 mmol) in water (2.0 mL) at rt. After 3 h, the reaction mixture was concentrated and the residue was azeotroped with PhMe (3 x 20 mL). The crude residue was dissolved in DMF (3 mL) and treated with O-((tetrahydro-2H-pyran-2-yl)hydroxylamine (0.121 g, 1.03 mmol). The mixture was cooled to 0 °C, and T<sub>3</sub>P (50%, 0.800 mL, 1.13 mmol) and TEA (0.250 mL, 1.79 mmol) were added. The mixture was stirred under N<sub>2</sub>, and after 11 h, was diluted with EtOAc (60 mL) and washed with 1 M NaOH (40 mL). The aqueous layer was reextracted with EtOAc (60 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (5% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) provided **1-75c** (86% purity by <sup>1</sup>H NMR, 0.119 g, 35%) as a viscous residue: <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 8.26 (s, 1 H), 6.99-6.97 (m, 1 H), 6.81-6.74 (m, 3 H), 5.94 (br, 1 H), 4.97 (s, 1 H), 3.96-3.92 (m, 1 H), 3.65-3.62 (m, 1 H), 3.19 (d, *J* = 5.6 Hz, 2 H), 2.75 (t, *J* = 6.4 Hz, 2 H), 2.67-2.64 (m, 2 H), 2.44 (t, *J* = 11.6 Hz, 2 H), 1.82-1.59 (m, 12 H); <sup>19</sup>F NMR (471 MHz; CDCl<sub>3</sub>) δ -124.89; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>22</sub>H<sub>30</sub>FN<sub>2</sub>O<sub>4</sub> [M+H]<sup>+</sup> 405.2184, found 405.2181.



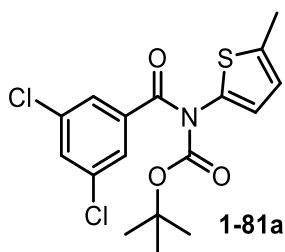
**(E)-4-(6-Fluorospiro[chromane-2,4'-piperidin]-1'-yl)-N-hydroxybut-2-enamide hydrogen chloride salt (1-76c).** The amide **1-75c** (86%, 0.117 g, 0.249 mmol) was dissolved in ether (2.70 mL) and MeOH (0.15 mL) and treated with 4 M HCl in dioxane (0.150 mL, 0.600 mmol). The reaction mixture was stirred under N<sub>2</sub> for 2 h, with occasional sonication. The white solid was filtered and concentrated under vacuum to provide **1-76c** (0.0598 g, 67%) as a white solid: Mp 115 °C (dec.); IR (neat) 3133, 2933, 2574, 1677, 1632, 1491, 1431, 1210 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; 25 °C, DMSO-*d*<sub>6</sub>) δ 10.97 (s, 1 H), 10.81 (bs, 1 H), 9.12 (s, 1 H), 6.98-6.90 (m, 2 H), 6.83-6.81 (m, 1 H), 6.74-6.68 (m, 1 H), 6.13 (d, *J* = 15.5 Hz, 1 H), 3.93 (s, 2 H), 3.34-3.30 (2 H), 3.11 (bs, 2 H), 2.76 (app t, *J* = 6.7 Hz, 2 H), 1.99-1.94 (m, 4 H), 1.79 (app t, *J* = 6.7 Hz, 2 H); <sup>19</sup>F NMR (471 MHz; 25 °C, DMSO-*d*<sub>6</sub>) δ -123.88; <sup>13</sup>C NMR (101 MHz; 25 °C, DMSO-*d*<sub>6</sub>): δ 160.8, 156.2 (d, *J*<sub>C-F</sub> = 236.3 Hz), 148.3, 129.7, 129.1, 122.94 (d, *J*<sub>C-F</sub> = 8.1 Hz), 118.0 (d, *J*<sub>C-F</sub> = 8.1 Hz), 115.4 (d, *J*<sub>C-F</sub> = 22.2 Hz), 114.0 (d, *J*<sub>C-F</sub> = 23.2 Hz), 70.3, 55.8, 47.2, 30.9, 30.4, 20.8; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>17</sub>H<sub>20</sub>O<sub>3</sub>N<sub>2</sub>F [M-H]<sup>+</sup> 319.1452, found 319.1467; ELS purity 98.9%.



**2-(3,5-Dichlorobenzamido)thiophene-3-carboxamide (1-78).** To a solution of 2-aminothiophene-3-carboxamide (0.153 g, 1.06 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.75 mL) and H<sub>2</sub>O (0.5 mL) was added 3,5-dichlorobenzoyl chloride (0.224 g, 1.05 mmol) over a period of 30 min. For the first 2 h, the pH was adjusted to 8-9 with 2M NaOH. After 20 h of stirring under N<sub>2</sub>, the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (25 mL) and washed with 1 M HCl (20 mL). The organic layer was concentrated, diluted with EtOAc, and washed with sat. NaHCO<sub>3</sub> (3x). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude solid was sonicated in distilled hexanes, filtered, sonicated with CH<sub>2</sub>Cl<sub>2</sub>, and filtered. Drying under vacuum afforded **1-78** (0.0527 g, 16%) as a dark beige solid: Mp 259-264 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3498, 3389, 3069, 2927, 1643, 1554, 1503, 1347, 694 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 13.53 (s, 1 H), 8.12 (s, 1 H), 7.97 (t, *J* = 1.9 Hz, 1 H), 7.84 (d, *J* = 1.9 Hz, 2 H), 7.73 (s, 1 H), 7.51 (d, *J* = 5.8 Hz, 1 H), 7.10 (d, *J* = 5.8 Hz, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>): δ 167.3, 159.9, 145.6, 135.7, 134.9, 131.9, 125.8, 123.2, 117.0, 116.3; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>12</sub>H<sub>7</sub>O<sub>2</sub>N<sub>2</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 312.9600, found 312.9601; ELS purity 98.5%.

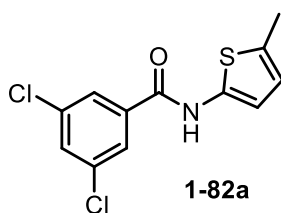


***tert*-Butyl (5-methylthiophen-2-yl)carbamate (1-80a).**<sup>115</sup> To a solution of 5-methyl-2-thiophenecarboxylic acid (0.252 g, 1.74 mmol) in *t*-butanol (3.8 mL) was added TEA (0.380 mL, 2.73 mmol) and diphenylphosphoryl azide (0.460 mL, 2.09 mmol). The reaction mixture was stirred for 13 h at 85-90 °C, cooled to rt and concentrated. The residue was dissolved in EtOAc (10 mL), washed with sat. NaHCO<sub>3</sub> (10 mL), 10% aq. citric acid (10 mL), and brine (10 mL). The organic layer was dried (MgSO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (10% EtOAc in hexanes) afforded **1-80a** (0.246 g, 66%) as a solid: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 6.71 (bs, 1 H), 6.43 (s, 1 H), 6.32 (d, *J* = 3.2 Hz, 1 H), 2.39 (s, 3 H), 1.51 (s, 9 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>10</sub>H<sub>15</sub>NO<sub>2</sub>SNa [M+Na]<sup>+</sup> 236.0716, found 236.0714.

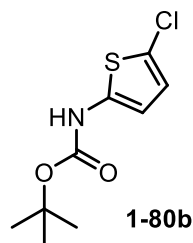


***tert*-Butyl (3,5-dichlorobenzoyl)(5-methylthiophen-2-yl)carbamate (1-81a).** 3,5-Dichlorobenzoyl chloride (0.103 g, 0.481 mmol), DMAP (4.00 mg, 0.0327 mmol), and DIPEA (0.120 mL, 0.683 mmol) were added to a 22 °C solution of amide **1-80a** (0.0507 g, 0.238 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4.5 mL). After 12 h of stirring, the reaction mixture was quenched with sat. aq. NaHCO<sub>3</sub>

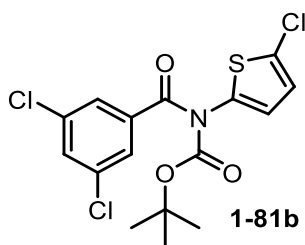
(6 mL), and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 5 mL), dried (MgSO<sub>4</sub>), filtered, and concentrated. The residue was purified by chromatography on SiO<sub>2</sub> (100% EtOAc) to yield **1-81a** (0.0910 g, 99%) as a beige-colored solid: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.55 (d, *J* = 1.8 Hz, 2 H), 7.50 (t, *J* = 1.8 Hz, 1 H), 6.74 (d, *J* = 3.6 Hz, 1 H), 6.62 (dd, *J* = 3.6, 1.0 Hz, 1 H), 2.47 (d, *J* = 0.7 Hz, 3 H), 1.30 (s, 9 H).



**3,5-Dichloro-N-(5-methylthiophen-2-yl)benzamide (1-82a).** TFA (0.262 mL, 3.53 mmol) was added to a 0 °C solution of amide **1-81a** (0.091 g, 0.236 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.10 mL). The reaction mixture was warmed up to rt after 10 min. After 4 h, the reaction mixture was concentrated, and the residue was diluted with EtOAc (5 mL), washed with sat. NaHCO<sub>3</sub>, dried (MgSO<sub>4</sub>), filtered, and concentrated, providing **1-82a** (0.0373 g, 55%) as a light beige solid: Mp 152-156 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3239, 3073, 2917, 1668, 1636, 1565, 1337, 805 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 8.38 (s, 1 H), 7.72 (d, *J* = 1.1 Hz, 2 H), 7.52 (bs, 1 H), 6.62 (d, *J* = 3.6 Hz, 1 H), 6.54 (d, *J* = 2.7 Hz, 1 H), 2.44 (s, 3 H); <sup>13</sup>C NMR (101 MHz; CDCl<sub>3</sub>) δ 161.6, 136.4, 135.87, 135.78, 133.5, 131.9, 125.9, 122.1, 114.0, 15.1; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>12</sub>H<sub>10</sub>ONCl<sub>2</sub>S [M+H]<sup>+</sup> 285.9855, found 285.9853; ELS purity (100%).



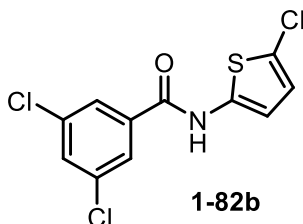
**tert-Butyl (5-chlorothiophen-2-yl)carbamate (1-80b).**<sup>116</sup> To a solution of 5-chlorothiophene-2-carboxylic acid (0.252 g, 1.50 mmol) in *t*-butanol (3.5 mL) was added TEA (0.360 mL, 2.58 mmol) and diphenylphosphoryl azide (0.430 mL, 1.95 mmol). The reaction mixture was stirred for 11 h at 90 °C, concentrated, and the residue was dissolved in EtOAc (10 mL), washed with sat. NaHCO<sub>3</sub> (10 mL), 10% aq. citric acid (10 mL), and brine (10 mL). The organic layer was dried (MgSO<sub>4</sub>), filtered, and concentrated. Chromatography on SiO<sub>2</sub> (10% EtOAc in hexanes) afforded **1-80b** (0.269 g, 77%) as a white solid: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 6.90 (s, 1 H), 6.62 (d, *J* = 4.0 Hz, 1 H), 6.23 (d, *J* = 4.0 Hz, 1 H), 1.51 (s, 9 H).



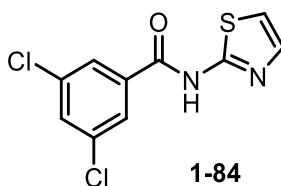
**tert-Butyl (5-chlorothiophen-2-yl)(3,5-dichlorobenzoyl)carbamate (1-81b).** 3,5-Dichlorobenzoyl chloride (0.126 g, 0.592 mmol), DMAP (4.50 mg, 0.0368 mmol), and DIPEA (0.150 mL, 0.862 mmol) were added to a 22 °C solution of amide **1-80b** (0.0689 g, 0.295 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4.5 mL). After 13 h, the mixture was quenched with sat. NaHCO<sub>3</sub> (6 mL), and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 5 mL), dried (MgSO<sub>4</sub>), filtered, and concentrated. The residue was purified by chromatography on SiO<sub>2</sub> (20% EtOAc in hexanes) to afford **1-81b** (0.0847 g, 71%) as a dark



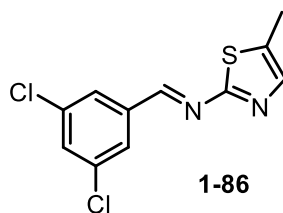
yellow oil:  $^1\text{H NMR}$  (500 MHz;  $\text{CDCl}_3$ )  $\delta$  7.55 (d,  $J = 1.8$  Hz, 2 H), 7.53 (t,  $J = 1.8$  Hz, 1 H), 6.81 (d,  $J = 4.0$  Hz, 1 H), 6.78 (d,  $J = 4.0$  Hz, 1 H), 1.30 (s, 9 H); HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{16}\text{H}_{14}\text{Cl}_3\text{NO}_3\text{NaS}$   $[\text{M}+\text{Na}]^+$  427.9652, found 427.9649.



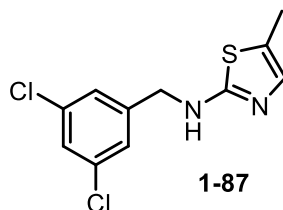
**3,5-Dichloro-*N*-(5-chlorothiophen-2-yl)benzamide (1-82b).** TFA (0.220 mL, 2.96 mmol) was added to a 0 °C solution of amide **1-81b** (0.0806 g, 0.198 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.1 mL). The reaction mixture was warmed up to rt after 10 min. After 10 h, the reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (5 mL), washed with sat.  $\text{NaHCO}_3$ , dried ( $\text{MgSO}_4$ ), filtered, and purified by chromatography on  $\text{SiO}_2$  (10% EtOAc in hexanes) to provide **1-82b** (0.0280 g, 46%) as a grey solid: Mp 168-169 °C; IR ( $\text{CH}_2\text{Cl}_2$ ) 3421, 3227, 3085, 3069, 1618, 1560, 1515, 1292, 873, 774  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (400 MHz;  $\text{DMSO}-d_6$ )  $\delta$  11.94 (s, 1 H), 8.00 (d,  $J = 1.9$  Hz, 2 H), 7.90 (t,  $J = 1.9$  Hz, 1 H), 6.95 (d,  $J = 4.2$  Hz, 1 H), 6.75 (d,  $J = 4.2$  Hz, 1 H);  $^{13}\text{C NMR}$  (126 MHz;  $\text{DMSO}-d_6$ )  $\delta$  160.7, 137.4, 135.6, 134.5, 131.5, 126.5, 123.6, 120.3, 111.4; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{11}\text{H}_7\text{ONCl}_3\text{S}$   $[\text{M}+\text{H}]^+$  305.9308, found 305.9307; ELS purity (100%).



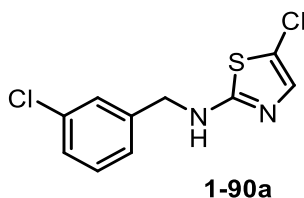
**3,5-Dichloro-*N*-(thiazol-2-yl)benzamide (1-84).** TEA (0.110 mL, 0.789 mmol) was added to a 0 °C solution of 2-aminothiazole (0.0802 g, 0.777 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.50 mL). 3,5-Dichlorobenzoyl chloride (0.0938 g, 0.439 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.60 mL) was added to the solution over a period of 30 min. The reaction mixture was stirred at rt, and after 39 h, it was washed with H<sub>2</sub>O and brine, dried (MgSO<sub>4</sub>), and concentrated. The mixture was purified by chromatography on SiO<sub>2</sub> (50% hexanes in EtOAc) to provide **1-84** (0.0659g, 55%) as a beige solid: Mp 195-198 °C; IR (CDCl<sub>3</sub>) 3146, 3082, 2926, 1672, 1567, 1546, 1290, 805, 735 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 13.21 (s, 1 H), 7.88 (d, *J* = 1.7 Hz, 2 H), 7.60 (t, *J* = 1.6 Hz, 1 H), 7.06-7.03 (m, 2 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 163.7, 160.5, 136.9, 135.93, 135.79, 132.7, 126.9, 114.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>10</sub>H<sub>7</sub>ON<sub>2</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 272.9651, found 272.9652; ELS purity (100%).



**(*E*)-1-(3,5-Dichlorophenyl)-*N*-(5-methylthiazol-2-yl)methanimine (1-86).** To a microwave vial was added 3,5-dichlorobenzaldehyde (0.217 g, 1.21 mmol) and 2-amino-5-methylthiazole (0.352 g, 3.05 mmol) in EtOH (2.5 mL). The mixture was irradiated at 120 °C for 1 h, cooled down, and purified by chromatography on SiO<sub>2</sub> (10% EtOAc in hexanes) to provide **1-86** (0.138 g, 42%) as a yellow solid: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 8.87 (s, 1 H), 7.82 (d, *J* = 1.7 Hz, 2 H), 7.47 (t, *J* = 1.9 Hz, 1 H), 7.35 (s, 1 H), 2.49 (s, 3 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 169.9, 158.6, 139.4, 138.1, 135.8, 132.0, 134.8, 127.7, 12.8.

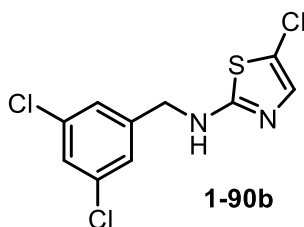


***N*-(3,5-Dichlorobenzyl)-5-methylthiazol-2-amine (1-87)**. A 0 °C solution of the imine **1-86** (0.0701 g, 0.259 mmol) in MeOH (1.8 mL) and EtOH (0.3 mL) was treated with NaBH<sub>4</sub> (0.0601 g, 1.59 mmol) in two portions. The reaction mixture was stirred at rt under N<sub>2</sub> for 15 h, after which it was quenched with dropwise addition of sat. NH<sub>4</sub>Cl. The mixture was stirred for 5 min, concentrated, and the aqueous solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried (MgSO<sub>4</sub>), filtered, and concentrated, to provide **1-87** (0.0673 g, 95%) as a beige solid: Mp 109-113 °C; IR (neat) 3175, 3071, 2970, 2918, 1571, 1501, 796 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.27 (t, *J* = 1.9 Hz, 1 H), 7.25 (d, *J* = 1.8 Hz, 2 H), 6.75 (q, *J* = 1.3 Hz, 1 H), 5.37 (s, 1 H), 4.44 (s, 2 H), 2.28 (d, *J* = 1.3 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 167.9, 141.9, 135.9, 135.4, 127.9, 126.0, 122.3, 48.5, 12.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>11</sub>H<sub>11</sub>N<sub>2</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 273.0015, found 273.0012; ELS purity 100%.

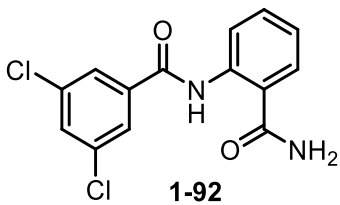


**5-Chloro-*N*-(3-chlorobenzyl)thiazol-2-amine (1-90a)**. A mixture of 3-chlorobenzaldehyde (0.100 mL, 0.856) and 2-amino-5-chlorothiazole (0.0719 g, 0.534 mmol) in anhydrous THF (2 mL) was treated with Ti(O<sup>*i*</sup>Pr)<sub>4</sub> (1.40 mL, 1.10 mmol). After stirring at rt for 6 h, the mixture was cooled to 0 °C and treated with NaBH<sub>4</sub> (0.0484 g, 1.25 mmol), portionwise, and MeOH (2 mL).

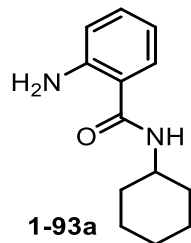
After 14 h of stirring at rt, the solution was concentrated and purified by chromatography on SiO<sub>2</sub> (100% CH<sub>2</sub>Cl<sub>2</sub>) to provide **1-90a** (0.0847, 61%) as a white solid: Mp 99-104 °C; IR (CDCl<sub>3</sub>) 3178, 3078, 2979, 2899, 2855, 1570, 1455, 1344, 796 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.34 (d, *J* = 0.7 Hz, 1 H), 7.29-7.28 (m, 2 H), 7.25-7.22 (m, 1 H), 6.84 (s, 1 H), 6.11 (s, 1 H), 4.40 (s, 2 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 167.6, 139.4, 136.6, 134.9, 130.2, 128.2, 127.8, 125.8, 113.1, 48.8; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>10</sub>H<sub>9</sub>N<sub>2</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 258.9858, found 258.9858; ELS purity 100%.



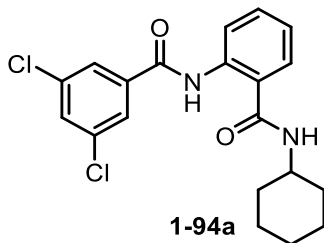
**5-Chloro-N-(3,5-dichlorobenzyl)thiazol-2-amine (1-90b).** A mixture of 3,5-dichlorobenzaldehyde (0.1749 g, 0.979 mmol) and 2-amino-5-chlorothiazole (0.0871 g, 0.647 mmol) in anhydrous THF (2 mL) was treated with Ti(O<sup>*i*</sup>Pr)<sub>4</sub> (0.400 mL, 1.32 mmol). After stirring at rt for 3 h, the reaction mixture was cooled to 0 °C, and treated with NaBH<sub>4</sub> (0.183 g, 4.74 mmol), portionwise, and MeOH (2 mL). After 20 h of stirring at rt, the reaction mixture was concentrated and purified by chromatography on SiO<sub>2</sub> (20% EtOAc in hexanes) to provide **1-90b** (0.139 g, 73%) as a beige solid: Mp 97-100 °C; IR (CDCl<sub>3</sub>) 3199, 3082, 2968, 2901, 1570, 1428, 1140, 799 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 7.29 (t, *J* = 1.9 Hz, 1 H), 7.23 (d, *J* = 1.9 Hz, 2 H), 6.84 (s, 1 H), 6.39 (s, 1 H), 4.39 (s, 2 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 167.5, 140.9, 136.6, 135.5, 128.1, 126.0, 113.4, 48.3; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>10</sub>H<sub>8</sub>Cl<sub>3</sub>N<sub>2</sub>S [M+H]<sup>+</sup> 292.9468, found 292.9469; ELS purity 100%.



***N*-(2-Carbamoylphenyl)-3,5-dichlorobenzamide (1-92)**. A solution of anthranilic acid (0.370 g, 2.69 mmol), benzotriazole (0.275 g, 2.26 mmol), and DCC (0.634 g, 3.08 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5.0 mL) was stirred for 17 h at 22 °C. The reaction mixture was concentrated, and the yellow residue was purified by chromatography on SiO<sub>2</sub> (25% EtOAc in hexanes). The crude material (0.196 g) was dissolved in THF (10 mL), and the solution was cooled to 0 °C and treated with NH<sub>4</sub>OH (4.5 mL, 31.6 mmol) in two portions. The yellow solution gradually turned white over a period of 4 h. After an additional 15 h, the reaction mixture was extracted with EtOAc (3x), washed with brine (1x), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude material (0.121 g) material was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL), and the solution was treated with pyridine (0.0700 mL, 0.865 mmol) and 3,5-dichlorobenzoylchloride (0.186 g, 0.888 mmol). The mixture was stirred for 46 h at 22 °C, washed with NH<sub>4</sub>Cl (1x), brine (1x), dried (MgSO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (50% EtOAc in hexanes) provided **1-92** (0.0845 g, 32%) as a white solid: Mp 240-242 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3376, 3215, 3073, 1662, 1585, 1565, 1522, 1396, 1319, 756 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 13.13 (s, 1 H), 8.61 (dd, *J* = 8.3, 0.9 Hz, 1 H), 8.47 (s, 1 H), 7.99 (s, 1 H), 7.93-7.91 (m, 2 H), 7.88 (d, *J* = 1.9 Hz, 2 H), 7.61-7.57 (m, 1 H), 7.23-7.20 (m, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 171.1, 161.5, 139.6, 138.0, 134.8, 132.7, 131.3, 128.8, 125.8, 123.2, 120.2, 119.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>14</sub>H<sub>9</sub>O<sub>2</sub>N<sub>2</sub>Cl<sub>2</sub> [M+H]<sup>+</sup> 307.0036, found 307.0044; ELS purity 100%.

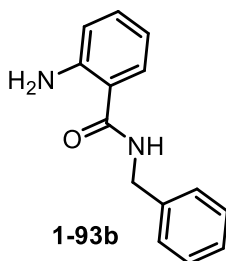


**2-Amino-N-cyclohexylbenzamide (1-93a).**<sup>117</sup> DIPEA (0.800 mL, 5.87 mmol) and HATU (0.718 g, 1.89 mmol) were added to a solution of anthranilic acid (0.213 g, 1.56 mmol) in DMF (3 mL). After 30 min of stirring, cyclohexylamine (0.200 mL, 1.73 mmol) was added. The reaction mixture was stirred for 17 h under N<sub>2</sub>, diluted with EtOAc, washed with H<sub>2</sub>O (1x) and brine (1x), dried (MgSO<sub>4</sub>), and concentrated. The residue was purified by chromatography on SiO<sub>2</sub> (25% EtOAc in hexanes), to provide **1-93a** (92%, 0.305 g, 82 %) as an off-white solid: <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>) δ 7.94 (d, *J* = 7.8 Hz, 1 H), 7.47 (dd, *J* = 7.9, 1.4 Hz, 1 H), 7.13 (ddd, *J* = 8.2, 7.1, 1.3 Hz, 1 H), 6.68 (dd, *J* = 8.2, 1.1 Hz, 1 H), 6.53-6.49 (m, 1 H), 6.32 (s, 2 H), 3.77-3.68 (m, 1 H), 1.81-1.72 (m, 4 H), 1.64-1.60 (m, 1 H), 1.36-1.24 (m, 4 H), 1.17-1.10 (m, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 168.4, 149.9, 131.8, 128.7, 116.6, 115.8, 114.9, 48.3, 32.9, 25.8, 25.5; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>13</sub>H<sub>19</sub>N<sub>2</sub>O [M+H]<sup>+</sup> 219.1492, found 219.1490.



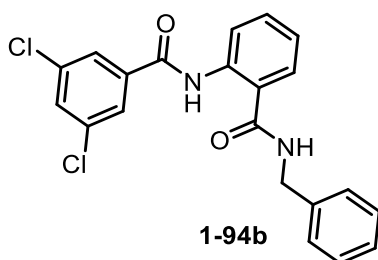
**3,5-Dichloro-N-(2-(cyclohexylcarbamoyl)phenyl)benzamide (1-94a).** A suspension of amide **1-93a** (92%, 0.121 g, 0.511 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (7 mL) was cooled to 0 °C and treated with pyridine (0.0500 mL, 0.618 mmol) and 3,5-dichlorobenzoyl chloride (0.174 g, 0.816 mmol). The reaction

mixture was warmed to rt, stirred for 42 h, diluted with CH<sub>2</sub>Cl<sub>2</sub>, and washed with sat. aq. NH<sub>4</sub>Cl (2x). The organic layer was dried (MgSO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (20% EtOAc in hexanes) provided **1-94a** (0.132 g, 66%) as a white solid: Mp 228-229 °C; IR (CDCl<sub>3</sub>) 3297, 3080, 2932, 2854, 1684, 1622, 1593, 1519, 1314, 757 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 12.28 (s, 1 H), 8.71 (dd, *J* = 8.4, 0.9 Hz, 1 H), 7.90 (d, *J* = 1.9 Hz, 2 H), 7.57-7.50 (m, 3 H), 7.15 (td, *J* = 7.6, 1.0 Hz, 1 H), 6.21 (dd, *J* = 4.2, 3.3 Hz, 1 H), 4.07-3.97 (m, 1 H), 2.09-2.05 (m, 2 H), 1.83-1.78 (m, 2 H), 1.74-1.67 (m, 1 H), 1.54-1.43 (m, 2 H), 1.35-1.22 (m, 3 H); <sup>13</sup>C NMR (101 MHz; CDCl<sub>3</sub>): δ 168.2, 163.1, 139.6, 138.2, 135.7, 132.8, 131.8, 126.5, 126.2, 123.5, 121.9, 121.0, 49.0, 33.2, 25.6, 25.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>21</sub>O<sub>2</sub>N<sub>2</sub>Cl<sub>2</sub> [M+H]<sup>+</sup> 391.0975, found 391.0984; ELS purity 100%.



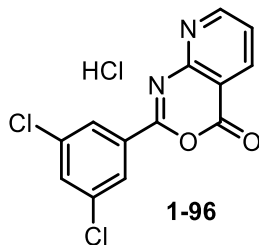
**2-Amino-N-benzylbenzamide (1-93b).**<sup>118</sup> DIPEA (0.800 mL, 4.60 mmol) and HATU (0.729 g, 1.92 mmol) were added to a solution of anthranilic acid (0.204 g, 1.49 mmol) in dry DMF (3 mL). After 30 min of stirring, benzylamine (0.180 mL, 1.65 mmol) was added. The reaction mixture was stirred for 17 h under N<sub>2</sub>, diluted with EtOAc, washed with H<sub>2</sub>O (1x) and brine (1x), dried (MgSO<sub>4</sub>), and concentrated. The residue was purified by chromatography on SiO<sub>2</sub> (25% EtOAc in hexanes), to provide **1-93b** (0.268 g, 80%) as a beige-colored solid: <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 8.78 (t, *J* = 5.2 Hz, 1 H), 7.56 (d, *J* = 7.9 Hz, 1 H), 7.34-7.30 (m, 4 H), 7.25-7.22 (m, 1 H),

7.14 (td,  $J = 7.6, 1.1$  Hz, 1 H), 6.70 (d,  $J = 8.2$  Hz, 1 H), 6.52 (t,  $J = 7.5$  Hz, 1 H), 6.43 (s, 2 H), 4.43 (d,  $J = 6.0$  Hz, 2 H);  $^{13}\text{C}$  NMR (126 MHz; DMSO- $d_6$ )  $\delta$  169.1, 150.1, 140.3, 132.1, 128.54, 128.36, 127.4, 126.9, 116.7, 114.88, 114.71, 42.5; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{14}\text{H}_{15}\text{N}_2\text{O}$   $[\text{M}+\text{H}]^+$  227.1179, found 227.1176.

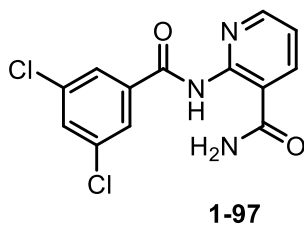


***N*-(2-(Benzylcarbamoyl)phenyl)-3,5-dichlorobenzamide (1-94b)**. A solution of the amide **1-93b** (0.112 g, 0.495 mmol) in  $\text{CH}_2\text{Cl}_2$  (6 mL) was cooled to 0 °C and treated with pyridine (0.04 mL, 0.495 mmol) and 3,5-dichlorobenzoyl chloride (0.110 g, 0.513 mmol). The reaction mixture was warmed to rt, stirred for 42 h, diluted with  $\text{CH}_2\text{Cl}_2$ , and washed with sat. aq.  $\text{NH}_4\text{Cl}$  (2x) and brine (1x). The organic layer was dried ( $\text{MgSO}_4$ ), filtered, and concentrated, providing **1-94b** (>95% purity by  $^1\text{H}$  NMR, 0.165 g, 83%) as a white solid: Mp 207-209 °C; IR ( $\text{CH}_2\text{Cl}_2$ ) 3299, 3069, 2924, 2876, 1685, 1585, 1522, 1446, 756  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz; DMSO- $d_6$ )  $\delta$  12.41 (s, 1 H), 9.42 (t,  $J = 5.9$  Hz, 1 H), 8.46 (dd,  $J = 8.3, 1.0$  Hz, 1 H), 7.91-7.90 (m, 2 H), 7.85 (d,  $J = 1.6$  Hz, 2 H), 7.60-7.57 (m, 1 H), 7.36-7.35 (m, 2 H), 7.33-7.30 (m, 2 H), 7.28-7.22 (m, 2 H), 4.52 (d,  $J = 5.9$  Hz, 2 H);  $^{13}\text{C}$  NMR (126 MHz; DMSO- $d_6$ )  $\delta$  168.4, 161.8, 138.9, 138.5, 138.0, 134.7, 132.2, 131.3, 128.3, 127.8, 127.3, 126.9, 125.9, 123.7, 121.6, 121.1, 42.7; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{21}\text{H}_{17}\text{O}_2\text{N}_2\text{Cl}_2$   $[\text{M}+\text{H}]^+$  399.0662, found 399.0663; ELS purity 100%.

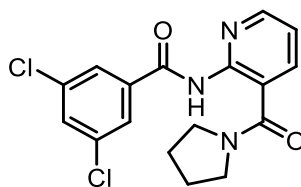




**2-(3,5-Dichlorophenyl)-4H-pyrido[2,3-d][1,3]oxazin-4-one hydrochloride (1-96).** A solution of 2-aminonicotinic acid (0.226 g, 1.60 mmol) in 1,4-dioxane (3.00 mL) was cooled to 0 °C, and treated with TEA (0.650 mL, 4.66 mmol) and 3,5-dichlorobenzoyl chloride (1.07 g, 5.02 mmol). The microwave vial was sealed and the reaction mixture was irradiated at 120 °C for 30 min. The mixture was transferred to a scintillation vial, suspended in EtOH (15 mL), centrifuged, and the solvent decanted. The centrifugation/decant process was repeated with EtOH (15 mL), 1 M HCl (15 mL), and EtOH (15 mL), successively. After concentrating, **1-96** (0.346 g, 66%) was collected as a white solid: Mp 260-264 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3085, 3038, 1769, 1620, 1586, 1563, 1470, 1424, 1322, 792 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>) δ 9.04 (dd, *J* = 4.7, 2.0 Hz, 1 H), 8.58 (dd, *J* = 7.8, 2.0 Hz, 1 H), 8.13 (d, *J* = 1.9 Hz, 2 H), 8.03 (t, *J* = 1.9 Hz, 1 H), 7.69 (dd, *J* = 7.8, 4.7 Hz, 1 H); <sup>13</sup>C NMR (101 MHz; DMSO-*d*<sub>6</sub>): δ 158.6, 157.1, 156.8, 156.4, 137.3, 134.8, 133.2, 132.3, 126.3, 124.4, 113.6; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>13</sub>H<sub>7</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub> [M-HCl+H]<sup>+</sup> 292.9879, found 292.9878.



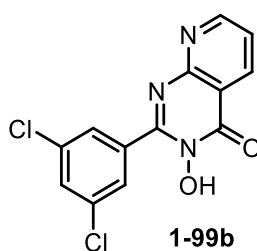
**2-(3,5-Dichlorobenzamido)nicotinamide (1-97).** A solution of oxazinone **1-96** (1.53 mmol) in ammonium hydroxide (28 % solution, 2.6 mL, 27.4 mmol) was stirred at rt under N<sub>2</sub> for 4.5 h. The precipitate was filtered and washed with water and Et<sub>2</sub>O. After drying under vacuum, **1-97** (0.124 g, 26%, 2 steps) was collected as a white solid: Mp 362-366 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3414, 3151, 3084, 1702, 1686, 1667, 1597, 1566, 1496, 1453, 1369, 1262, 794 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 11.87 (s, 1 H), 8.52 (dd, *J* = 4.8, 1.8 Hz, 1 H), 8.18 (s, 1 H), 8.12 (dd, *J* = 7.8, 1.8 Hz, 1 H), 7.94-7.92 (m, 2 H), 7.91 (t, *J* = 1.9 Hz, 1 H), 7.68 (s, 1 H), 7.33 (dd, *J* = 7.7, 4.8 Hz, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 168.7, 162.0, 150.2, 149.6, 137.80, 137.61, 134.5, 131.3, 126.5, 121.3, 120.1; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>13</sub>H<sub>10</sub>O<sub>2</sub>N<sub>3</sub>Cl<sub>2</sub> [M+H]<sup>+</sup> 310.0145, found 310.0142; ELS purity 100%.



**1-98**

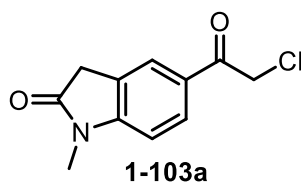
**3,5-Dichloro-N-(3-(pyrrolidine-1-carbonyl)pyridin-2-yl)benzamide (1-98).** A solution of oxazinone **1-96** (0.0900 g, 0.274 mmol) and pyrrolidine (0.0500 mL, 0.603 mmol) in DMF (1.6 mL) was stirred at 110 °C under N<sub>2</sub> for 3 h. The mixture was treated with water (20 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 40 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The mixture was purified via pipette chromatography on SiO<sub>2</sub> (100% EtOAc, then 10% CH<sub>2</sub>Cl<sub>2</sub> in MeOH). DMF was removed by azeotropic distillation with heptane and CH<sub>2</sub>Cl<sub>2</sub>, affording **1-98** (0.0383 g, 38%) as a white solid: Mp 167-170 °C; IR (CDCl<sub>3</sub>) 3176, 3079, 2974, 2871, 1671, 1611, 1595, 1567, 1527, 1427, 1311, 737 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 10.51

(s, 1 H), 8.39 (d,  $J = 4.6$  Hz, 1 H), 7.86 (d,  $J = 1.8$  Hz, 2 H), 7.72 (dd,  $J = 7.6, 1.2$  Hz, 1 H), 7.46 (s, 1 H), 7.09 (dd,  $J = 7.6, 4.9$  Hz, 1 H), 3.67 (t,  $J = 6.7$  Hz, 2 H), 3.57 (t,  $J = 6.3$  Hz, 2 H), 2.02-1.96 (m, 4 H);  $^{13}\text{C}$  NMR (126 MHz;  $\text{CDCl}_3$ )  $\delta$  167.7, 162.9, 149.8, 148.8, 137.0, 136.5, 135.5, 131.9, 126.5, 123.6, 119.5, 50.0, 46.7, 26.5, 24.6; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{16}\text{O}_2\text{N}_3\text{Cl}_2$   $[\text{M}+\text{H}]^+$  364.0614, found 364.0612; ELS purity 100%.



**2-(3,5-Dichlorophenyl)-3-hydroxypyrido[2,3-d]pyrimidin-4(3H)-one (1-99b).** A solution of the oxazinone **1-96** (1.93 mmol) in hydroxylamine (50 % aq. solution, 3.00 mL, 49.0 mmol) was stirred for 15 min. THF (5 mL) was added, and the mixture was stirred at rt for an additional 80 min. The mixture was filtered, washed with  $\text{H}_2\text{O}$  (15 mL) and toluene (15 mL), and a white solid was collected. In solution, the white solid was gradually converting from the uncyclized hydroxamic acid (**1-99a**) to the cyclized hydroxamic acid (**1-99b**). To effect complete cyclization to **1-99b**, the white solid was transferred to a microwave vial, and toluene (3 mL) was added. The mixture was irradiated at 120 °C for 1 h, treated with more toluene (1 mL), and irradiated for an additional 45 min at 120 °C. LCMS showed conversion to the cyclized hydroxamic acid. The mixture was transferred to a scintillation vial, treated with hexanes and EtOH (10 mL and 1 mL, respectively), and centrifuged. The solvent was decanted, and the same centrifuge/decant process was repeated with hexanes (8 mL) and EtOH (2 x 8 mL), subsequently. Upon concentration under vacuum, 0.167 g of crude was collected. Recrystallization: The residue (21.5 mg) was suspended

in 2 mL of *i*-PrOH/THF (1:1) in a 1-dram glass vial and heated at reflux for 5 min. After vigorous shaking, the solution was pipetted off into a 1-dram vial, and another 2 mL of *i*-PrOH/THF (1:1) was added to the remaining solid. This suspension was also heated at reflux for 5 min, and after vigorous shaking a clear solution was obtained. Both vials were kept at room temperature overnight, and then at -20 °C for 36 h. The solutions were pipetted off, and the crystalline precipitates were dried under vacuum, resulting in a combined fraction of ca. 7 mg of solids which were analyzed by <sup>1</sup>H NMR to show a mixture of product and side product. The solutions (mother liquor) were evaporated to dryness, and the residue was concentrated under vacuum, providing **1-99b** (0.0145 g, 2.4%) as a beige solid: Mp 255-259 °C; IR (neat) 3083, 2926, 2851, 2595, 1722, 1561, 1416, 1223, 788 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 12.05 (s, 1 H), 9.03 (dd, *J* = 4.5, 2.0 Hz, 1 H), 8.61 (dd, *J* = 7.9, 1.9 Hz, 1 H), 7.90-7.88 (m, 3 H), 7.62 (dd, *J* = 7.9, 4.5 Hz, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 158.5, 156.1, 155.5, 153.6, 135.83, 135.69, 133.6, 130.0, 128.2, 122.7, 117.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>13</sub>H<sub>8</sub>O<sub>2</sub>N<sub>3</sub>Cl<sub>2</sub> [M+H]<sup>+</sup> 307.9988, found 307.9986; ELS purity 100%.

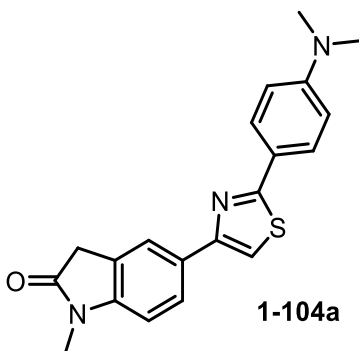


**5-(2-Chloroacetyl)-1-methylindolin-2-one (1-103a).**<sup>119</sup> To a stirred suspension of anhydrous AlCl<sub>3</sub> (4.28 g, 32.1 mmol) in CS<sub>2</sub> (36 mL) was added chloroacetyl chloride (0.730 mL, 9.17 mmol) dropwise. The solution was stirred at rt for 15 min, after which 1-methyl-2-oxindole (0.817 g, 5.55 mmol) was added. The mixture was heated at reflux for 2.5 h, and left to cool on ice. The solvent was decanted, and ice-cold water (20 mL) was added to the remaining thick, brown residue. The

beige/light pink precipitate formed was filtered, washed with hexanes (20 mL), and dried under vacuum to give **1-103a** (1.22 g, 98%) as a beige solid. <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 7.99 (dd, *J* = 8.2, 0.8 Hz, 1 H), 7.86 (s, 1 H), 7.12 (d, *J* = 8.3 Hz, 1 H), 5.11 (s, 2 H), 3.64 (s, 2 H), 3.17 (s, 3 H).

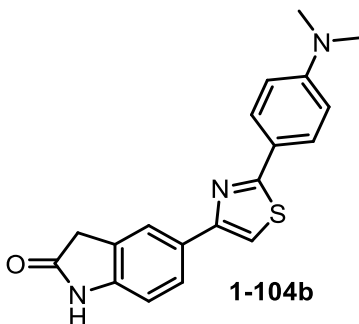
**General Procedure A for the synthesis of the thiazoles (1-104a-d) and thiadiazines (1-105a-c).**

Thiosemicarbazide (1.2 eq) or thiobenzamide (1.2 eq) and hydrobromic acid (0.02-0.04 mL) were added to a suspension of indolone (1.0 eq) in EtOH (0.2 M). After 2 h at reflux, the reaction mixture was cooled to rt and quenched with sat. aqueous NaHCO<sub>3</sub>. The product was extracted with chloroform (3x), washed with brine (1x), and the combined organic layers were dried (MgSO<sub>4</sub>), filtered, and purified as indicated.



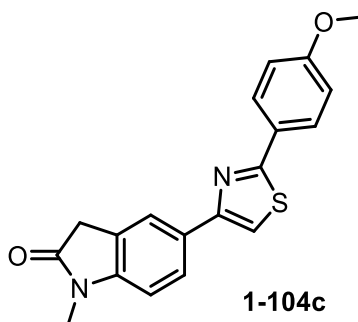
**5-(2-(4-(Dimethylamino)phenyl)thiazol-4-yl)-1-methylindolin-2-one (1-104a).** Prepared by General Procedure A with 4-dimethylaminothiobenzamide (0.0504 g, 0.266 mmol), hydrobromic acid (0.03 mL), **1-103a** (0.0513 g, 0.229 mmol), and EtOH (1.5 mL). The mixture was quenched with sat. aqueous NaHCO<sub>3</sub> (6 mL), extracted with chloroform (3 x 10 mL), washed with brine, and the combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated. The residue was triturated with EtOAc, and the precipitate was filtered, dried under vacuum, providing **1-104a**

(0.0384 g, 48%) as a peach/beige solid: Mp 202-203 °C; IR (neat) 3106, 2912, 2809, 1708, 1607, 1555, 1482, 1365, 1190, 1095, 818, 736 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.90 (m, 4 H), 7.22 (s, 1 H), 6.86 (d, *J* = 8.1 Hz, 1 H), 6.73 (d, *J* = 8.1 Hz, 2 H), 3.59 (s, 2 H), 3.25 (s, 3 H), 3.04 (s, 6 H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 175.3, 168.9, 155.5, 151.7, 145.0, 129.9, 127.9, 126.3, 125.0, 122.8, 122.1, 111.9, 109.4, 108.2, 40.4, 36.0, 26.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>20</sub>ON<sub>3</sub>S [M+H]<sup>+</sup> 350.1322, found 350.1320; ELS purity 100%.

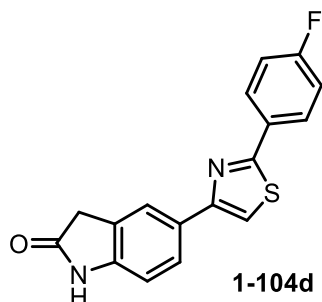


**5-(2-(4-(Dimethylamino)phenyl)thiazol-4-yl)indolin-2-one (1-104b)**. Prepared by General Procedure A with 4-dimethylamino thiobenzamide (0.0746 g, 0.393 mmol), hydrobromic acid (0.03 mL), **1-103b** (0.0693 g, 0.331 mmol), and EtOH (1.5 mL). The mixture was quenched with sat. aqueous NaHCO<sub>3</sub> (10 mL). The product was extracted with chloroform (3 x 15 mL), washed with brine, and the combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated. The residue was triturated with EtOAc, and the precipitate was filtered, dried under vacuum, providing **1-104b** (0.0492 g, 44%) as a light pink solid: Mp 273-277 °C; IR (CDCl<sub>3</sub>) 3141, 3084, 2860, 2210, 1693, 1607, 1480, 1362, 1189, 814, 723 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>) δ 10.50 (s, 1 H), 7.87 (m, 2 H), 7.81 (d, *J* = 8.8 Hz, 2 H), 7.77 (s, 1 H), 6.88 (d, *J* = 8.7 Hz, 1 H), 6.79 (d, *J* = 8.8 Hz, 2 H), 3.56 (s, 2 H), 2.99 (s, 6 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>): δ 176.5, 167.5, 154.9,

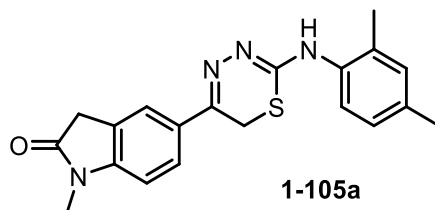
151.5, 143.6, 127.9, 127.3, 126.3, 125.6, 122.3, 120.9, 111.9, 109.8, 109.1, 39.8, 35.8; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>18</sub>ON<sub>3</sub>S [M+H]<sup>+</sup> 336.1165, found 336.1163; ELS purity 100%.



**5-(2-(4-Methoxyphenyl)thiazol-4-yl)-1-methylindolin-2-one (1-104c).** Prepared by General Procedure A with 4-methoxythiobenzamide (0.0511 g, 0.290 mmol), hydrobromic acid (0.02 mL), **1-103a** (0.0553 g, 0.247 mmol), and EtOH (1.5 mL). The mixture was quenched with sat. aqueous NaHCO<sub>3</sub> (6 mL). The product was extracted with chloroform (3 x 10 mL), washed with brine, and the combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated. The residue was triturated with EtOAc, and the precipitate was filtered, dried under vacuum, providing **1-104c** (0.0362 g, 44%) as a light yellow/beige solid: Mp 167-169 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3493, 3106, 2936, 2837, 2246, 1707, 1482, 1253 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.99-7.95 (m, 2 H), 7.93 (s, 1 H), 7.92 (d, *J* = 8.7 Hz, 1 H), 7.30 (s, 1 H), 6.99-6.96 (m, 2 H), 6.87 (d, *J* = 8.0 Hz, 1 H), 3.87 (s, 3 H), 3.60 (s, 2 H), 3.25 (s, 3 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 175.3, 167.9, 161.3, 155.8, 145.2, 129.6, 128.2, 126.8, 126.3, 125.0, 122.8, 114.4, 110.5, 108.3, 55.6, 36.0, 26.5; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>17</sub>O<sub>2</sub>N<sub>2</sub>S [M+H]<sup>+</sup> 337.1005, found 337.1003; ELS purity 100%.



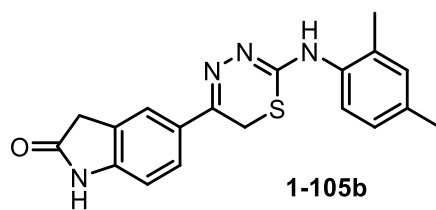
**5-(2-(4-Fluorophenyl)thiazol-4-yl)indolin-2-one (1-104d).** Prepared by General Procedure A with 4-fluorothiobenzamide (0.0643 g, 0.406 mmol), hydrobromic acid (0.03 mL), **1-103b** (0.0711 g, 0.339 mmol), and ethanol (2 mL). The precipitate formed was filtered, dried under vacuum, providing **1-104d** (0.0666 g, 63%) as a brown solid: Mp 264-267 °C; IR (CDCl<sub>3</sub>) 3105, 3079, 2856, 1690, 1620, 1481, 1218, 845, 755 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.52 (s, 1 H), 8.08-8.04 (m, 2 H), 7.99 (s, 1 H), 7.90 (s, 1 H), 7.89 (d, *J* = 7.9 Hz, 1 H), 7.38-7.34 (m, 2 H), 6.90 (d, *J* = 8.0 Hz, 1 H), 3.56 (s, 2 H); <sup>19</sup>F NMR (471 MHz; DMSO-*d*<sub>6</sub>) δ -110.7; <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 176.5, 165.4, 163.20 (d, *J*<sub>C-F</sub> = 248.3 Hz), 155.5, 143.8, 129.76 (d, *J*<sub>C-F</sub> = 3.4 Hz), 128.44 (d, *J*<sub>C-F</sub> = 8.9 Hz), 127.5, 126.4, 125.7, 122.4, 116.27 (d, *J*<sub>C-F</sub> = 21.9 Hz), 112.4, 109.2, 35.8; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>17</sub>H<sub>12</sub>ON<sub>2</sub>FS [M+H]<sup>+</sup> 311.0649, found 311.0644; ELS purity 100%.



**5-(2-((2,4-Dimethylphenyl)amino)-6H-1,3,4-thiadiazin-5-yl)-1-methylindolin-2-one (1-105a).** Prepared by General Procedure A with 4-(2,4-dimethylphenyl)-3-thiosemicarbazide (0.0930 g, 0.467 mmol), hydrobromic acid (0.04 mL), **1-103a** (0.0928 g, 0.388 mmol), and EtOH (2 mL).

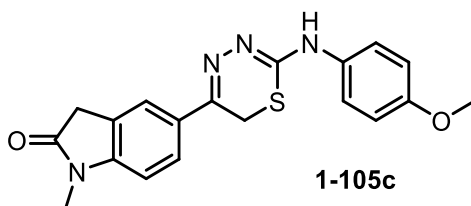


The mixture was quenched with sat. aqueous NaHCO<sub>3</sub> (10 mL). The product was extracted with chloroform (3 x 20 mL), washed with brine, and the combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated. The residue was triturated with EtOAc, and the precipitate was filtered, dried under vacuum, providing **1-105a** (0.0937 g, 66%) as a solid: Mp >410 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 2914, 1714, 1615, 1594, 1566, 1496, 1459, 1370, 1345, 1267, 1193, 1090, 914, 813, 727, 669 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.71 (s, 1 H), 7.62 (d, *J* = 7.8 Hz, 1 H), 7.02 (s, 1 H), 6.96 (d, *J* = 8.3 Hz, 1 H), 6.85 (d, *J* = 8.2 Hz, 1 H), 6.74 (d, *J* = 7.9 Hz, 1 H), 3.71 (s, 2 H), 3.56 (s, 2 H), 3.24 (s, 3 H), 2.30 (s, 3 H), 2.18 (s, 3 H); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 175.1, 152.5, 146.79, 146.72, 144.4, 133.9, 131.3, 130.0, 129.6, 126.9, 126.4, 125.3, 122.3, 121.8, 108.0, 35.7, 26.5, 23.8, 21.0, 18.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>21</sub>ON<sub>4</sub>S [M+H]<sup>+</sup> 365.1431, found 365.1428; ELS purity 100%.



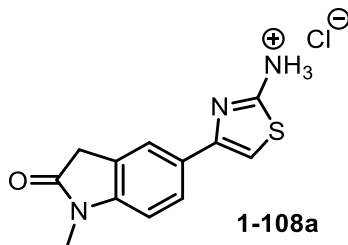
**5-(2-((2,4-Dimethylphenyl)amino)-6H-1,3,4-thiadiazin-5-yl)indolin-2-one (1-105b)**. Prepared by General Procedure A with 4-(2,4-dimethylphenyl)-3-thiosemicarbazide (0.0576 g, 0.289 mmol), hydrobromic acid (0.03 mL), **1-103b** (0.0528 g, 0.247 mmol), and EtOH (1.5 mL). The mixture was quenched with sat. aqueous NaHCO<sub>3</sub> (10 mL). The product was extracted with chloroform (3 x 15 mL), washed with brine, and the combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated. The residue was triturated with EtOAc, and the precipitate was filtered, dried under vacuum, providing **1-105b** (0.0426 g, 49%) as a light yellow solid: Mp > 410 °C; IR

(CH<sub>2</sub>Cl<sub>2</sub>) 3150, 3031, 2915, 1702, 1619, 1599, 1571, 1490, 1316, 1195, 822 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 11.12 (s, 1 H), 10.58 (s, 1 H), 7.67 (s, 1 H), 7.63 (d, *J* = 8.2 Hz, 1 H), 6.98 (s, 1 H), 6.90 (d, *J* = 7.9 Hz, 1 H), 6.86 (d, *J* = 8.2 Hz, 1 H), 6.60 (br, 1 H), 3.86 (s, 2 H), 3.53 (s, 2 H), 2.23 (s, 3 H), 2.08 (s, 3 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 176.5, 151.8, 145.8, 144.9, 132.0, 130.7, 129.1, 128.3, 126.56, 126.37, 125.8, 122.0, 121.6, 109.0, 35.7, 22.5, 20.4, 17.7; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>19</sub>ON<sub>4</sub>S [M+H]<sup>+</sup> 351.1274, found 351.1271; ELS purity 98.1%.



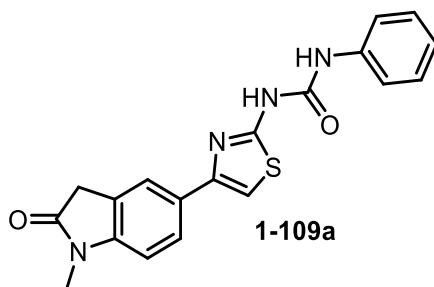
**5-(2-((4-Methoxyphenyl)amino)-6H-1,3,4-thiadiazin-5-yl)-1-methylindolin-2-one (1-105c).**

Prepared by General Procedure A with 4-(4-methoxyphenyl)-3-thiosemicarbazide (0.0458 g, 0.221 mmol), hydrobromic acid (0.0255 mL), **1-103a** (0.0505 g, 0.226 mmol), and EtOH (1.5 mL). The mixture was quenched with sat. aqueous NaHCO<sub>3</sub> (6 mL). The product was extracted with chloroform (3 x 10 mL), washed with brine, and the combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated. The residue was triturated with EtOAc, and the precipitate was filtered, dried under vacuum, providing **1-105c** (0.0480 g, 58%) as a light yellow/beige solid: Mp 194-196 °C; IR (CDCl<sub>3</sub>) 3155, 3048, 2898, 2249, 1695, 1575, 1506, 831 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 8.69 (s, 1 H), 7.73 (s, 1 H), 7.64 (d, *J* = 8.0 Hz, 1 H), 6.89 (m, 5 H), 3.81 (s, 3 H), 3.72 (s, 2 H), 3.57 (s, 2 H), 3.25 (s, 3 H); <sup>13</sup>C NMR (126 MHz; CDCl<sub>3</sub>) δ 175.1, 156.6, 152.4, 146.84, 146.71, 140.9, 129.5, 126.5, 125.3, 123.3, 122.3, 114.2, 108.0, 55.6, 35.7, 26.5, 23.9; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>19</sub>O<sub>2</sub>N<sub>4</sub>S [M+H]<sup>+</sup> 367.1223, found 367.1219; ELS purity 100%.

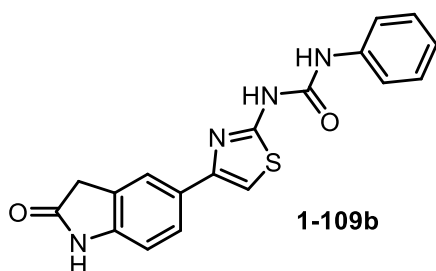


**5-(2-Aminothiazol-4-yl)-1-methylindolin-2-one hydrochloride (1-108a).** To a solution of indolone **1-103a** (0.823 g, 3.68 mmol) in EtOH (4 mL) was added thiourea (0.287 g, 3.77 mmol), and the reaction mixture was heated at reflux for 2.5 h in a sealed tube, transferred to a 20 mL scintillation vial, and centrifuged, and the collected solid was washed with EtOH (3 x 10 mL). After drying under vacuum, **1-108a** (0.936 g, 90%) was collected as a beige solid: Mp (dec.) 301 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3219, 3078, 2804, 2187, 1706, 1616, 1500 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 8.80 (br, 2 H), 7.71 (d, *J* = 8.2 Hz, 1 H), 7.68 (s, 1 H), 7.09 (s, 1 H), 7.07 (s, 1 H), 3.61 (s, 2 H), 3.14 (s, 3 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 174.2, 170.0, 145.8, 125.5, 125.4, 121.5, 108.4, 100.1, 35.0, 26.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>12</sub>H<sub>12</sub>ON<sub>3</sub>S [M+H]<sup>+</sup> 246.0696, found 246.0694.

**General Procedure B for the synthesis of 1-109a-d.** A solution of aminothiazole **1-108** (1 eq) and isocyanate (1.4-3.5 eq) in DMF (0.3-0.4 M) was irradiated at 120 °C for 30 min. The mixture was cooled to rt and purified as indicated.

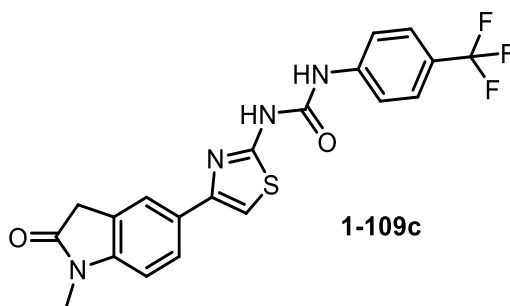


**1-(4-(1-Methyl-2-oxoindolin-5-yl)thiazol-2-yl)-3-phenylurea (1-109a)**. Prepared by General Procedure B with **1-108a** (0.0559 g, 0.198 mmol), phenyl isocyanate (36.0  $\mu\text{L}$ , 0.329 mmol), and DMF (0.6 mL). Upon cooling to rt, the mixture was treated with EtOAc (10 mL), the suspension centrifuged, and the solvent decanted. The centrifuge/decant process was repeated with MeOH (10 mL), EtOAc (10 mL), and EtOAc:MeOH (1:1, 10 mL), successively. Residual DMF was removed by genevac (HT4, 24 h setting), and **1-109a** (0.0242 g, 33 %) was collected as an off-white solid: Mp > 400  $^{\circ}\text{C}$ ; IR ( $\text{CH}_2\text{Cl}_2$ ) 3371, 3203, 2928, 1703, 1672, 1600, 1549, 1366  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz;  $\text{DMSO}-d_6$ )  $\delta$  10.63 (s, 1 H), 8.96 (s, 1 H), 7.84-7.78 (m, 2 H), 7.48 (d,  $J = 7.2$  Hz, 2 H), 7.42 (s, 1 H), 7.33 (t,  $J = 7.3$  Hz, 2 H), 7.03 (t,  $J = 7.4$  Hz, 2 H), 3.61 (s, 2 H), 3.15 (s, 3 H);  $^{13}\text{C}$  NMR (126 MHz;  $\text{DMSO}-d_6$ )  $\delta$  174.4, 158.9, 151.5, 148.8, 144.6, 138.5, 128.9, 128.6, 125.15, 125.10, 122.8, 121.6, 118.6, 108.3, 105.2, 35.2, 26.0; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{19}\text{H}_{17}\text{O}_2\text{N}_4\text{S}$   $[\text{M}+\text{H}]^+$  365.1067, found 365.1064; ELS purity 100%.



**1-(4-(2-Oxoindolin-5-yl)thiazol-2-yl)-3-phenylurea (1-109b)**. Prepared by General Procedure B with **1-108b** (0.0621 g, 0.232 mmol), phenyl isocyanate (90.0  $\mu\text{L}$ , 0.824 mmol), and DMF (0.9 mL). Upon cooling to rt, the mixture was treated with  $\text{H}_2\text{O}$  (10 mL), the suspension centrifuged, and the solvent decanted. The solid was suspended in heptane, and the residual DMF was removed through azeotropic distillation. The solid was treated with hexanes:EtOAc (1:1, 10 mL),

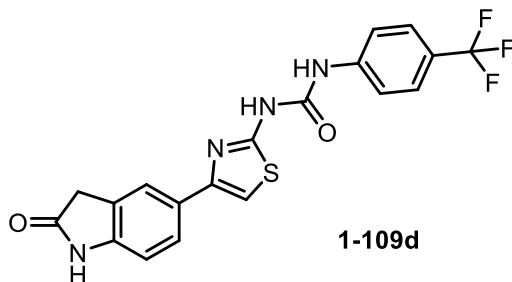
centrifuged, and the solvent decanted. The centrifuge/decant process was repeated with THF (2 x 8 mL). The collected solid was purified by chromatography on SiO<sub>2</sub> (50-100 % EtOAc in Hexanes) to provide **1-109b** (0.0250 g, 31%) as a light orange solid: Mp >400 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3360, 2923, 2854, 1682, 1601, 1554, 1500, 1316, 1246, 1201, 738 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.60 (s, 1 H), 10.47 (s, 1 H), 8.95 (s, 1 H), 7.73 (m, 2 H), 7.48 (d, *J* = 7.6 Hz, 2 H), 7.36 (s, 1 H), 7.33 (t, *J* = 7.7 Hz, 2 H), 7.05 (t, *J* = 7.2 Hz, 1 H), 6.85 (d, *J* = 8.4 Hz, 1 H), 3.53 (s, 2 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 176.4, 158.8, 151.4, 149.0, 143.3, 138.5, 129.02, 128.90, 126.2, 125.1, 122.8, 121.9, 118.6, 109.1, 104.9, 35.8; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>18</sub>H<sub>15</sub>O<sub>2</sub>N<sub>4</sub>S [M+H]<sup>+</sup> 351.0910, found 351.0907; ELS purity 100%.



**1-(4-(1-Methyl-2-oxoindolin-5-yl)thiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)urea (1-109c).**

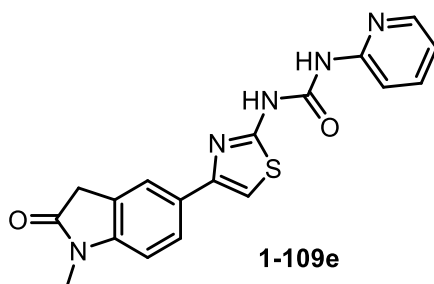
Prepared by General Procedure B with **1-108a** (0.0902 g, 0.320 mmol), 4-(trifluoromethyl)phenyl isocyanate (0.138 mL, 0.966 mmol), and DMF (1.3 mL). The mixture was irradiated for an overall 50 min. Upon cooling to rt, the mixture was treated with H<sub>2</sub>O:EtOH (1:1, 20 mL), the suspension centrifuged, and the solvent decanted. The centrifuge/decant process was repeated with EtOAc:EtOH (3:1, 20 mL) and EtOAc:EtOH (1:1, 10 mL), successively. The solvent was dried under vacuum, and residual DMF was removed by genevac (HT4, 24 h setting) to provide **1-109c** (0.112 g, 81 %) as a yellow/beige powder: Mp 336-339 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3372, 3199, 3104, 3071,

2956, 1672, 1597, 1541, 1324, 1112  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz;  $\text{DMSO-}d_6$ )  $\delta$  10.80 (s, 1 H), 9.36 (s, 1 H), 7.84 (d,  $J = 8.1$  Hz, 1 H), 7.79 (s, 1 H), 7.72-7.67 (m, 4 H), 7.45 (s, 1 H), 7.03 (d,  $J = 8.2$  Hz, 1 H), 3.61 (s, 2 H), 3.15 (s, 3 H);  $^{19}\text{F}$  NMR (471 MHz;  $\text{DMSO-}d_6$ )  $\delta$  -60.2;  $^{13}\text{C}$  NMR (151 MHz;  $\text{DMSO-}d_6$ )  $\delta$  174.3, 158.6, 151.4, 148.93, 148.92, 144.7, 142.3, 128.5, 126.21 (app q,  $J_{\text{C-F}} = 3.3$  Hz), 125.17, 124.42 (q,  $J_{\text{C-F}} = 270.7$  Hz), 122.70 (q,  $J_{\text{C-F}} = 32.5$  Hz), 121.6, 118.4, 108.3, 105.5, 35.2, 26.0; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{20}\text{H}_{16}\text{O}_2\text{N}_4\text{F}_3\text{S}$   $[\text{M}+\text{H}]^+$  433.0941, found 433.0938; ELS purity 100%.



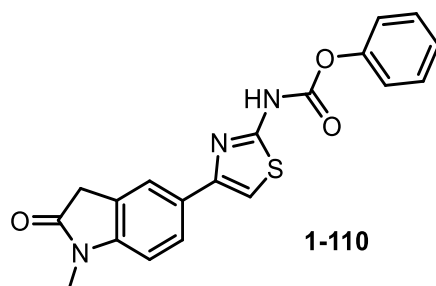
**1-(4-(2-Oxindolin-5-yl)thiazol-2-yl)-3-(4-(trifluoromethyl)phenyl)urea (1-109d).** Prepared by General Procedure B with **1-108b** (0.1028 g, 0.384 mmol), 4-(trifluoromethyl)phenyl isocyanate (0.190 mL, 1.33 mmol), and DMF (1.4 mL). Upon cooling to rt, the mixture was suspended in  $\text{H}_2\text{O}$  (85 mL) and extracted with treated with EtOAc (1 x 125 mL). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The residue was purified by chromatography on  $\text{SiO}_2$  (0-10% MeOH in  $\text{CH}_2\text{Cl}_2$ ). The fractions were dried under vacuum, and residual DMF was removed by genevac (HT4, 24 h setting) to provide **1-109d** (0.0603 g, 38 %) as an orange/brown solid: Mp 330  $^\circ\text{C}$  (dec.); IR ( $\text{CH}_2\text{Cl}_2$ ) 3356, 3069, 2983, 1676, 1604, 1543, 1103, 1667, 1249  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz;  $\text{DMSO-}d_6$ )  $\delta$  10.77 (s, 1 H), 10.47 (s, 1 H), 9.35 (s, 1 H), 7.73-7.67 (m, 6 H), 7.40 (s, 1 H), 6.85 (d,  $J = 8.6$  Hz, 1 H), 3.53 (s, 2 H);  $^{19}\text{F}$  NMR (471 MHz;  $\text{DMSO-}d_6$ )  $\delta$  -60.2;

$^{13}\text{C}$  NMR (126 MHz; DMSO- $d_6$ )  $\delta$  176.4, 158.6, 151.4, 149.1, 143.4, 142.3, 127.86, 126.28, 126.22 (q,  $J_{\text{C-F}} = 3.8$  Hz), 125.2, 124.44, (q,  $J_{\text{C-F}} = 271.5$  Hz) 122.70 (q,  $J_{\text{C-F}} = 32.1$  Hz), 121.9, 118.4, 109.1, 105.2, 35.8; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{19}\text{H}_{12}\text{O}_2\text{N}_4\text{F}_3\text{S}$   $[\text{M-H}]^+$  417.0628, found 417.0614; ELS purity 100%.



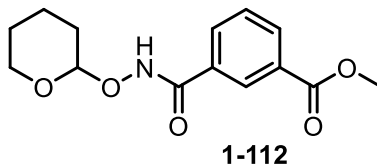
**1-(4-(1-Methyl-2-oxindolin-5-yl)thiazol-2-yl)-3-(pyridin-2-yl)urea (1-109e).** Prepared by General Procedure B with **1-108 a** (0.097 g, 0.395 mmol), pyridine isocyanate (0.170 g, 1.34 mmol), and DMF (1.3 mL). Upon cooling to rt, the mixture was treated with  $\text{H}_2\text{O}$ : EtOH (2:1, 15 mL), the suspension centrifuged, and the solvent decanted. The centrifuge/decant process was repeated with EtOH (15 mL) and EtOAc (15 mL), successively. The product was extracted from the solid mixture with warm MeOH (ca. 40 mL). The impure solid mixture was discarded, and the MeOH solution was concentrated under vacuum. The resulting solid was suspended in ether: hexanes (1:1, 2 x 5 mL), and filtered under vacuum to provide **1-109e** (0.0283 g, 20%) as an orange/brown solid: Mp 250  $^\circ\text{C}$  (dec.); IR ( $\text{CH}_2\text{Cl}_2$ ) 3344, 3054, 2961, 2304, 1699, 1679, 1536, 1433, 1297  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz; DMSO- $d_6$ )  $\delta$  12.13 (s, 1 H), 9.96 (s, 1 H), 8.37 (d,  $J = 4.5$  Hz, 1 H), 7.85 (m, 2 H), 7.81 (s, 1 H), 7.54 (d,  $J = 6.6$  Hz, 1 H), 7.48 (s, 1 H), 7.11 (t,  $J = 6.1$  Hz, 1 H), 7.03 (d,  $J = 8.1$  Hz, 1 H), 3.61 (s, 2 H), 3.15 (s, 3 H);  $^{13}\text{C}$  NMR (151 MHz; DMSO- $d_6$ )  $\delta$  174.4, 158.4, 151.80, 151.62, 148.8, 146.6, 144.8, 139.4, 128.3, 125.2, 121.7, 118.5, 112.5, 108.3,

105.8, 35.2, 26.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>18</sub>H<sub>16</sub>O<sub>2</sub>N<sub>5</sub>S [M+H]<sup>+</sup> 366.1019, found 366.1018; ELS purity 100%.

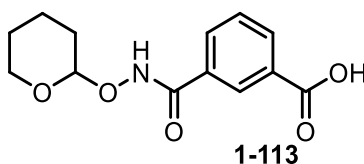


**Phenyl (4-(1-methyl-2-oxoindolin-5-yl)thiazol-2-yl)carbamate (1-110).** To a solution of **1-108a** (0.129 g, 0.457 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL) was added phenylchloroformate (0.0680 mL, 0.531 mmol), DMAP (0.00820 g, 0.0671 mmol), and TEA (0.0900 mL, 0.646 mmol). The mixture was stirred at rt under N<sub>2</sub> for 2.5 h. The reaction mixture was suspended in CH<sub>2</sub>Cl<sub>2</sub> (8 mL), the suspension centrifuged, and the solvent decanted. The centrifuge/decant process was repeated with EtOAc (5 mL), and MeOH at 40 °C (3 x 5 mL). After drying under vacuum, **1-110** (0.0574 g, 34%) was collected as a white solid: Mp 300 °C (dec.); IR (CH<sub>2</sub>Cl<sub>2</sub>) 3162, 3106, 2956, 1729, 1700, 1565, 1229 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 12.41 (s, 1 H), 7.84 (dd, *J* = 8.1, 1.4 Hz, 1 H), 7.80 (s, 1 H), 7.53 (s, 1 H), 7.48-7.44 (m, 2 H), 7.31 (t, *J* = 7.4 Hz, 1 H), 7.28-7.27 (m, 2 H), 7.04 (d, *J* = 8.2 Hz, 1 H), 3.62 (s, 2 H), 3.15 (s, 3 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 174.4, 159.1, 152.4, 150.1, 149.6, 144.8, 129.6, 128.4, 126.0, 125.2, 121.81, 121.69, 108.3, 106.2, 35.2, 26.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>16</sub>O<sub>3</sub>N<sub>3</sub>S [M+H]<sup>+</sup> 366.0907, found 366.0907; ELS purity 100%.



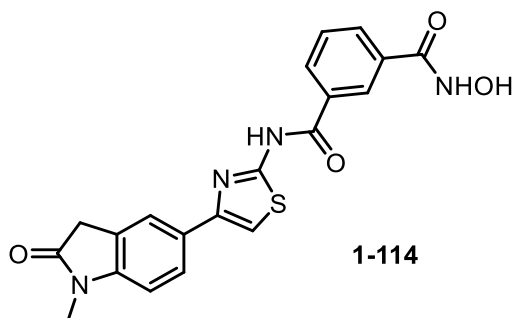


**Methyl-3-(((tetrahydro-2H-pyran-2-yl)oxy)carbamoyl)benzoate (1-112).**<sup>120</sup> To monomethylisophthalate (1.01 g, 5.59 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (17 mL) was added O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.650 g, 5.55 mmol), TBTU (1.41 g, 5.52 mmol), and DIPEA (2.00 mL, 11.5 mmol). The mixture was stirred under N<sub>2</sub> at rt for 25 h. The reaction mixture was concentrated and the residue was purified by chromatography on SiO<sub>2</sub> (50% EtOAc in hexanes) to afford **1-112** (0.877g, 57%) as a white solid: <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 11.86 (s, 1 H), 8.35 (s, 1 H), 8.12 (d, *J* = 7.7 Hz, 1 H), 8.03 (d, *J* = 7.7 Hz, 1 H), 7.64 (t, *J* = 7.7 Hz, 1 H), 5.02 (s, 1 H), 4.07-4.02 (m, 1 H), 3.89 (s, 3 H), 3.55-3.52 (m, 1 H), 1.73 (bs, 3 H), 1.55 (bs, 3 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>14</sub>H<sub>17</sub>NO<sub>5</sub>Na [M+Na]<sup>+</sup> 302.0999, found 302.0997.



**3-(((Tetrahydro-2H-pyran-2-yl)oxy)carbamoyl)benzoic acid (1-113).**<sup>120</sup> The methyl ester **1-112** (0.860g, 3.08 mmol) in MeOH:H<sub>2</sub>O (3:1, 16 mL) was treated with KOH (0.579 g, 10.3 mmol). The reaction mixture was stirred under N<sub>2</sub> at rt for 23 h, concentrated, and the residue was dissolved in H<sub>2</sub>O (10 mL), and washed with CH<sub>2</sub>Cl<sub>2</sub> (3 x 8 mL) and EtOAc (1 x 10 mL). The aqueous layer pH was adjusted to 3 with HCl (1 M), and the precipitate was filtered, washed with hexanes (10 mL), and dried under vacuum to afford **1-113** (0.547 g, 67%) as a white solid: <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 13.24 (s, 1 H), 11.84 (s, 1 H), 8.34 (s, 1 H), 8.10 (d, *J* = 7.8 Hz, 1

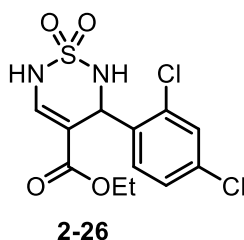
H), 8.00 (d,  $J = 7.9$  Hz, 1 H), 7.61 (t,  $J = 7.8$  Hz, 1 H), 5.02 (s, 1 H), 4.10-4.02 (m, 1 H), 3.53 (m, 1 H), 1.73 (bs, 3 H), 1.55 (bs, 3 H).



***N*<sup>1</sup>-Hydroxy-*N*<sup>3</sup>-(4-(1-methyl-2-oxindolin-5-yl)thiazol-2-yl)isophthalamide (1-114).** DIPEA (0.55 mL, 3.16 mmol) and HATU (0.376 g, 0.989 mmol) were added to a solution of acid **1-113** (0.200 g, 0.754 mmol) in DMF (1.5 mL). The mixture was stirred at rt for 30 min, and **1-108a** (0.213 g, 0.866 mmol) was added, and the reaction mixture was irradiated at 100 °C for 30 min. Upon cooling, TFA (3.40 mL, 37.9 mmol) and MeOH (1.0 mL) were added, and the reaction mixture was stirred at rt for 23 h. The mixture was suspended in MeOH (10 mL), the suspension centrifuged, and the solvent decanted. The centrifuge/decant process was repeated with MeOH (10 mL) and CH<sub>2</sub>Cl<sub>2</sub>: MeOH (1:1, 2 x 12 mL), successively. The remaining solid was concentrated under vacuum and purified by chromatography on Si-C<sub>18</sub> (5-95% acetonitrile in H<sub>2</sub>O) to provide **1-114** (0.0230 g, 7.5%) as a beige solid: Mp 219-225 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3189, 2940, 1653, 1619, 1545, 1492, 1363, 1302, 1273, 1102 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 12.80 (s, 1 H), 11.32 (s, 1 H), 9.19 (s, 1 H), 8.48 (s, 1 H), 8.24 (d,  $J = 7.8$  Hz, 1 H), 7.99 (d,  $J = 7.6$  Hz, 1 H), 7.91 (d,  $J = 8.0$  Hz, 1 H), 7.86 (s, 1 H), 7.65 (t,  $J = 7.8$  Hz, 1 H), 7.59 (s, 1 H), 7.05 (d,  $J = 8.2$  Hz, 1 H), 3.63 (s, 2 H), 3.15 (s, 3 H); <sup>13</sup>C NMR (151 MHz; DMSO-*d*<sub>6</sub>) δ 174.5, 163.6, 158.3, 149.5, 144.98, 144.96, 133.3, 132.4, 130.91, 130.74, 129.0, 127.1, 125.46, 125.41, 121.9, 108.51, 108.48, 106.9,

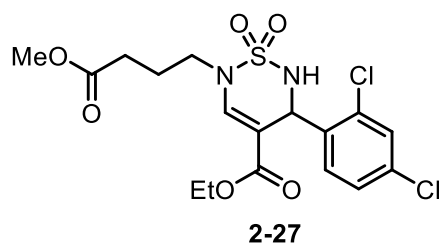
35.3, 26.1; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>17</sub>O<sub>4</sub>N<sub>4</sub>S [M+H]<sup>+</sup> 409.0965, found 409.0964; ELS purity 100%.

### 3.3 Chapter 2 Experimentals



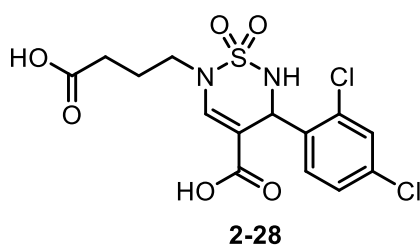
**Ethyl 3-(2,4-dichlorophenyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-26).** A 500 mL 3-neck RBF was charged with dimer **2-20** (20.8 g, 53.7 mmol) and 2,4-dichlorobenzaldehyde (18.6 g, 107 mmol). The flask was evacuated/refilled with N<sub>2</sub> (3x). HFIP (230 mL) was added to the mixture, followed by dropwise addition of TFA (20.0 mL, 268 mmol). The mixture was stirred under N<sub>2</sub> at 40 °C for 20 h, with the mixture gradually changing from a heterogenous white mixture to a homogenous yellow solution. The reaction was cooled to rt, and transferred to a 2 L Erlenmeyer flask. EtOAc (750 mL) and NaHCO<sub>3</sub> (400 mL) were added and the biphasic layer was stirred vigorously for 10 min. The mixture was transferred to a separatory funnel, and the EtOAc layer was washed with water (2 x 400 mL) and brine (2 x 400 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to 100 mL. Hexanes (250 mL) was added and the mixture was azeotroped, and a white solid started precipitating out. More hexanes (200 mL) and CH<sub>2</sub>Cl<sub>2</sub> (150 mL) were added, and the white solid was filtered off. Drying under

vacuum provided **2-26** (14.1 g, 75%) as a white solid: Mp 214-216 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3164, 1666, 1635, 1431, 1352, 1164 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 11.03 (s, 1 H), 8.18 (d, *J* = 7.3 Hz, 1 H), 7.62 (d, *J* = 2.1 Hz, 1 H), 7.59 (s, 1 H), 7.36 (dd, *J* = 8.4, 2.2 Hz, 1 H), 7.22 (d, *J* = 8.4 Hz, 1 H), 5.60 (d, *J* = 7.2 Hz, 1 H), 4.06-3.90 (m, 2 H), 1.05 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (75 MHz; DMSO-*d*<sub>6</sub>) δ 164.7, 139.9, 135.1, 133.9, 133.0, 131.4, 128.7, 126.6, 101.2, 59.7, 54.0, 14.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>12</sub>H<sub>11</sub>O<sub>4</sub>N<sub>2</sub>Cl<sub>2</sub>S [M-H]<sup>+</sup> 348.9811, found 348.9820.

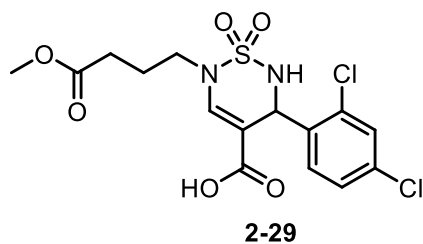


**Ethyl 3-(2,4-dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-27).** To a 500-mL 3-neck flask equipped with a N<sub>2</sub> inlet, septum, and a N<sub>2</sub>-sparge needle, was added thiadiazine **2-26** (5.04 g, 14.3 mmol), THF (80.0 mL), and methyl 4-hydroxybutanoate (1.69 g, 14.3 mmol). The mixture was sparged with N<sub>2</sub> and cooled to 0 °C. After 5 min, PPh<sub>3</sub> (3.78 g, 14.4 mmol) was added, followed by a portionwise addition of DBAD (3.30 g, 14.3 mmol). After 30 min, the solution was warmed to rt, and the N<sub>2</sub>-sparge line was removed. The mixture was stirred for 15 h and was treated with water (200 mL), transferred to a separatory funnel, and extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (200 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-50% EtOAc in hexanes) afforded **2-27** (~98% purity by <sup>1</sup>H NMR, 4.10 g, 63%) as a white solid: Mp 104-107 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3213, 2954, 1697, 1626, 1352, 1169 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>) δ 7.50 (s, 1 H), 7.43 (d, *J* = 2.0 Hz, 1 H), 7.27 (d, *J* = 8.1 Hz, 1 H),

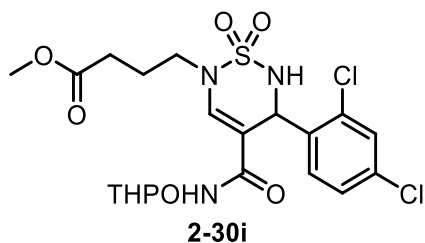
7.21 (dd,  $J = 8.4, 2.0$  Hz, 1 H), 5.90 (d,  $J = 7.9$  Hz, 1 H), 4.94 (d,  $J = 8.0$  Hz, 1 H), 4.12-4.00 (m, 2 H), 3.70 (s, 3 H), 3.68 (t,  $J = 7.2$  Hz, 2 H), 2.46 (t,  $J = 7.0$  Hz, 2 H), 2.14-2.03 (m, 2 H), 1.09 (t,  $J = 7.1$  Hz, 3 H);  $^{13}\text{C}$  NMR (101 MHz;  $\text{CDCl}_3$ )  $\delta$  173.3, 164.7, 142.3, 135.1, 134.7, 133.8, 130.6, 129.8, 127.1, 104.8, 60.8, 55.9, 52.1, 49.6, 30.7, 24.8, 14.2; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{21}\text{O}_6\text{N}_2\text{Cl}_2\text{S}$   $[\text{M} + \text{H}]^+$  451.0492, found 451.0486.



**6-(3-Carboxypropyl)-3-(2,4-dichlorophenyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylic acid 1,1-dioxide (2-28).** A solution of diester **2-27** (0.680 g, 1.51 mmol) in EtOH (6.6 mL) was treated with 2 M KOH (0.746 g in 6.6 mL  $\text{H}_2\text{O}$ , 15.1 mmol). The mixture was stirred at 90 °C for 7 h. Reaction progress was monitored by LCMS. The solution was cooled to 0 °C and acidified with 0.5 M HCl until pH = 2. The aqueous solution was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with water (50 mL) then brine (80 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to provide **2-28** (~98% purity by  $^1\text{H}$  NMR, 0.585 g, 93%) as a beige solid: Mp 198- 202 °C; IR ( $\text{CH}_2\text{Cl}_2$ ) 3167, 1718, 1660, 1633, 1346, 1167  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz;  $\text{DMSO}-d_6$ )  $\delta$  12.23 (bs, 2 H), 8.55 (s, 1 H), 7.70 (s, 1 H), 7.62 (d,  $J = 2.2$  Hz, 1 H), 7.38 (dd,  $J = 8.4, 2.2$  Hz, 1 H), 7.24 (d,  $J = 8.4$  Hz, 1 H), 5.55 (s, 1 H), 3.64-3.58 (m, 2 H), 2.30 (app t,  $J = 7.3$  Hz, 2 H), 1.91-1.81 (m, 2 H);  $^{13}\text{C}$  NMR (126 MHz;  $\text{DMSO}-d_6$ )  $\delta$  173.8, 166.1, 143.0, 135.0, 133.9, 133.1, 131.4, 128.7, 126.6, 102.6, 53.8, 48.5, 30.2, 24.8; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{14}\text{H}_{15}\text{O}_6\text{N}_2\text{Cl}_2\text{S}$   $[\text{M} + \text{H}]^+$  409.0022, found 409.0022.

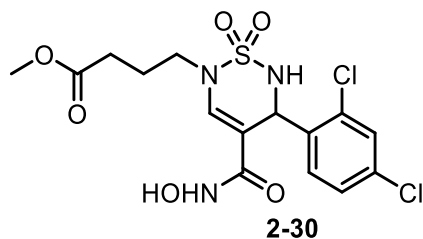


**3-(2,4-Dichlorophenyl)-6-(4-methoxy-4-oxobutyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylic acid 1,1-dioxide (2-29).** A solution of diacid **2-28** (0.102 g, 0.249 mmol) in MeOH (3.5 mL) was treated with 0.2 mL of a H<sub>2</sub>SO<sub>4</sub>/MeOH (0.1 mL/25 mL) solution. The reaction mixture was stirred for 6 h at 50 °C. Analysis by LCMS indicated >95 % conversion to the methyl ester. The mixture was treated with brine (15 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to give **2-29** (0.0981 g, 93%) as an off-white solid: Mp 188-191 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3162, 3129, 1720, 1674, 1609, 1354, 1169 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 12.27 (bs, 1 H), 8.55 (d, *J* = 6.9 Hz, 1 H), 7.70 (s, 1 H), 7.62 (d, *J* = 2.1 Hz, 1 H), 7.38 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.23 (d, *J* = 8.4 Hz, 1 H), 5.55 (d, *J* = 6.4 Hz, 1 H), 3.63 (t, *J* = 7.5 Hz, 2 H), 3.61 (s, 3 H), 2.40 (t, *J* = 7.6 Hz, 2 H), 1.93-1.84 (m, 2 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 172.7, 166.1, 143.0, 134.9, 133.9, 133.1, 131.4, 128.7, 126.6, 102.7, 53.8, 51.4, 48.4, 29.9, 24.7; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>15</sub>H<sub>17</sub>O<sub>6</sub>N<sub>2</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 423.0179, found 423.0177; LCMS-220 nm purity 100%.

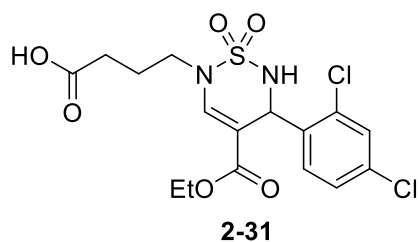


**Methyl 4-(5-(2,4-dichlorophenyl)-1,1-dioxido-4-(((tetrahydro-2H-pyran-2-yl)oxy)carbonyl)-5,6-dihydro-2H-1,2,6-thiadiazin-2-yl)butanoate (2-30i).** A solution of acid **2-29** (0.169 g, 0.399 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) was treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.142 g, 1.21 mmol), cooled to 0 °C and treated with T<sub>3</sub>P (50% in EtOAc, 0.360 mL, 0.605 mmol) and TEA (0.170 mL, 1.22 mmol). The reaction mixture was warmed to rt, and stirred under N<sub>2</sub>. After 2.5 h, the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (30 mL), washed with 0.25 M HCl (30 mL), brine (30 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-100% EtOAc in hexanes) afforded **2-30i** (96% purity by <sup>1</sup>H NMR (MeOH), 0.118 g, 57%, dr ~ 1:1 based on <sup>1</sup>H NMR\*) as a white solid: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 8.29 (s, 1 H, diastereomer A), 8.11 (s, 1 H, diastereomer B), 7.45-7.4 (m, 2 H), 7.35-7.30 (m, 3 H), 7.25-7.21 (m, 3 H), 5.96 (s, 1 H, diastereomer A), 5.94 (s, 1 H, diastereomer B), 4.99 (br s, 2 H), 4.78-4.74 (m, 2 H), 3.90-3.74 (m, 2 H), 3.713 (s, 3 H, diastereomer A), 3.710 (s, 3 H, diastereomer B), 3.68-3.54 (m, 5 H), 2.47 (app t, *J* = 6.8 Hz, 4 H), 2.14-2.03 (m, 4 H), 1.78-1.70 (m, 6 H), 1.25 (br s, 3 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>24</sub>O<sub>7</sub>N<sub>3</sub>Cl<sub>2</sub>S [M-H]<sup>+</sup> 520.07065, found 520.06797.

\*dr ratio based on the methyl ester protons: δ 3.713 integration of 3 H (diastereomer A) and δ 3.710 integration of 3 H (diastereomer B). Characteristic signals of diastereomer A: δ 8.29 (s, 1 H), 5.96 (s, 1 H), 3.713 (s, 3 H); characteristic signals of diastereomer B: 8.11 (s, 1 H), 5.94 (s, 1 H), 3.710 (s, 3 H); All other peaks show overlapping signals between diastereomers A and B.



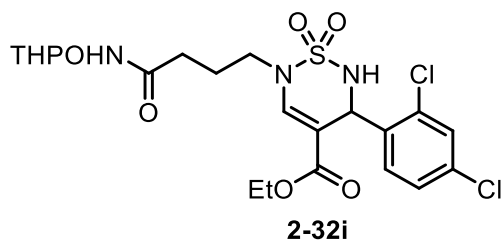
**Methyl 4-(5-(2,4-dichlorophenyl)-4-(hydroxycarbonyl)-1,1-dioxido-5,6-dihydro-2H-1,2,6-thiadiazin-2-yl)butanoate (2-30).** To a solution of **2-30i** (0.115 g, 0.220 mmol) in MeOH (3.0 mL) and CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) was added Amberlyst-15 (0.0375 g, 176 mmol) at rt under N<sub>2</sub>. After 22 h of stirring, the mixture was filtered through Celite®, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: Et<sub>2</sub>O) to afford **2-30** (0.0504 g, 53%) as a white solid: Mp 186-188 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3315, 3234, 3076, 2884, 1714, 1648, 1584, 1175 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 10.67 (s, 1 H), 8.74 (s, 1 H), 8.37 (d, *J* = 7.4 Hz, 1 H), 7.61 (d, *J* = 2.1 Hz, 1 H), 7.37 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.27 (d, *J* = 8.4 Hz, 1 H), 7.20 (s, 1 H), 7.27 (d, *J* = 8.4 Hz, 1 H), 5.73 (d, *J* = 7.2 Hz, 1 H), 3.61 (s, 3 H), 3.55-3.46 (m, 2 H), 2.41 (t, *J* = 7.6 Hz, 2 H), 1.92-1.87 (m, 2 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 172.9, 164.0, 136.4, 134.7, 134.2, 133.2, 131.6, 128.7, 126.5, 106.3, 53.9, 51.4, 48.1, 30.1, 24.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>15</sub>H<sub>18</sub>O<sub>6</sub>N<sub>3</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 438.0288, found 438.0304; LCMS-220 nm purity 100%.



**4-(5-(2,4-Dichlorophenyl)-4-(ethoxycarbonyl)-1,1-dioxido-5,6-dihydro-2H-1,2,6-thiadiazin-**

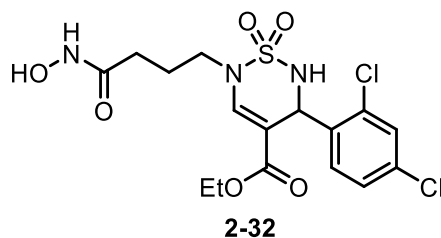


**2-yl)butanoic acid (2-31).** A solution of **2-27** (0.100 g, 0.222 mmol) in THF/MeOH (2 mL/2 mL) was treated with 6 M NaOH (0.370 mL, 2.22 mmol). After stirring at room temperature for 2 h, the mixture was cooled to 0 °C and treated with sat. aq. KHSO<sub>4</sub> (15 mL), pH ~2-3. The aqueous solution was transferred to a separatory funnel and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated, yielding **2-31** (0.0977 g, quant.) as a white solid: Mp 61 °C (dec.); IR (CH<sub>2</sub>Cl<sub>2</sub>) 3220, 2928, 1705, 1626, 1354, 1173 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>) δ 12.19 (s, 1 H), 8.61 (d, *J* = 7.2 Hz, 1 H), 7.74 (s, 1 H), 7.64 (d, *J* = 2.1 Hz, 1 H), 7.38 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.23 (d, *J* = 8.4 Hz, 1 H), 5.60 (d, *J* = 7.2 Hz, 1 H), 4.06-3.93 (m, 2 H), 3.65 (t, *J* = 7.3 Hz, 2 H), 2.31 (t, *J* = 7.4 Hz, 2 H), 1.89-1.82 (m, 2 H), 1.05 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (100 MHz; DMSO-*d*<sub>6</sub>) δ 173.8, 164.5, 143.3, 134.7, 133.9, 133.2, 131.3, 128.7, 126.7, 102.1, 59.8, 53.8, 48.6, 30.2, 24.9, 14.1; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>16</sub>H<sub>19</sub>O<sub>6</sub>N<sub>2</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 437.0335, found 437.0315; LCMS-220 nm purity 100%.



**Ethyl 3-(2,4-dichlorophenyl)-6-(4-oxo-4-(((tetrahydro-2H-pyran-2-yl)oxy)amino) butyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-32i).** A solution of carboxylic acid **2-31** (0.500 g, 1.143 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) was treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.411 g, 3.51 mmol). The reaction mixture was cooled to 0 °C and treated with T<sub>3</sub>P (50% in EtOAc, 1.00 mL, 1.68 mmol) and TEA (0.480 mL, 3.44 mmol). The reaction mixture

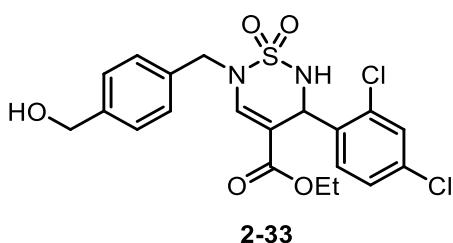
was warmed to rt, and stirred under N<sub>2</sub>. After 14 h, the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (10 mL), washed with 0.25 M HCl (10 mL), brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (100% hexanes to 100% EtOAc), afforded **2-32i** (0.470 g, 77%) as a white solid: <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 8.47 (s, 1 H), 8.40 (s, 1 H), 7.45-7.6 (m, 4 H), 7.37 (d, *J* = 8.2 Hz, 1 H), 7.29-7.26 (m, 1 H), 7.21- 7.18 (m, 2 H), 6.30 (d, *J* = 6.1 Hz, 1 H), 5.89 (d, *J* = 6.8 Hz, 1 H), 5.85 (d, *J* = 7.9 Hz, 1 H), 5.82 (bs, 1 H), 5.00 (s, 2 H), 4.10-4.00 (m, 4 H), 4.00-3.95 (m, 1 H), 3.75-3.63 (m, 4 H), 3.63-3.54 (m, 2 H), 2.28-2.18 (m, 6 H), 2.14-2.04 (m, 2 H), 1.90-1.72 (m, 6 H), 1.52-1.47 (m, 3 H), 1.33-1.25 (m, 2 H), 1.12-1.08 (m, 6 H); HRMS (ESI) *m/z* calcd for C<sub>21</sub>H<sub>26</sub>O<sub>7</sub>N<sub>3</sub>Cl<sub>2</sub>S [M-H]<sup>-</sup> 534.0863, found 534.0859.



**Ethyl 3-(2,4-dichlorophenyl)-6-(4-(hydroxyamino)-4-oxobutyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-32).** To a solution of **2-32i** (0.465 g, 0.867 mmol) in MeOH (5.0 mL) was added Amberlyst-15 (0.174 g, 818 mmol) at rt under N<sub>2</sub>. After 21 h of stirring, the reaction mixture was filtered through Celite®, rinsed with MeOH, and concentrated. Purification by chromatography on SiO<sub>2</sub>\* (100% EtOAc) afforded **2-32** (0.206 g, 53%) as a white solid: Mp 76-78 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3190, 2985, 1622, 1349, 1167 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>) δ 10.43 (s, 1 H), 8.74 (s, 1 H), 8.60 (d, *J* = 6.4 Hz, 1 H), 7.75 (s, 1 H), 7.64 (s, 1 H), 7.37 (d, *J* = 8.4 Hz, 1 H), 7.24 (d, *J* = 8.4 Hz, 1 H), 5.59 (d, *J* = 5.9 Hz, 1 H), 4.06-3.93 (m, 2 H), 3.70-3.57 (m, 2 H), 2.03 (t, *J* = 7.6 Hz, 2 H), 1.90-1.81 (m, 2 H), 1.05 (d, *J* = 7.0 Hz, 3 H); <sup>13</sup>C NMR (101

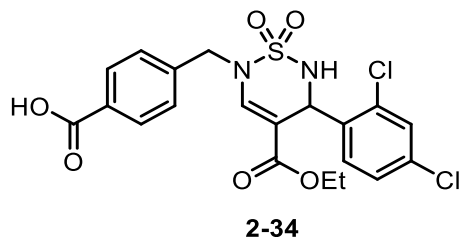
MHz; DMSO-*d*<sub>6</sub>)  $\delta$  168.3, 164.5, 143.4, 134.8, 133.9, 133.2, 131.4, 128.7, 126.7, 102.1, 59.9, 53.8, 49.0, 28.9, 25.6, 14.1; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>16</sub>H<sub>20</sub>O<sub>6</sub>N<sub>3</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 452.0444, found 452.0464; LCMS-220 nm purity 100%.

\*The SiO<sub>2</sub> was washed with aqueous 6 M HCl until colorless, neutralized with distilled water, and dried in an oven at 80-100 °C prior to use.



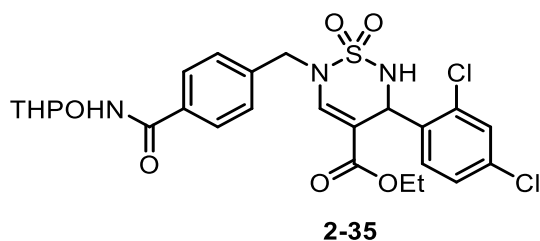
**Ethyl 3-(2,4-dichlorophenyl)-6-(4-(hydroxymethyl)benzyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-33).** To a 500-mL 3-neck flask equipped with a N<sub>2</sub> inlet, septum, and a N<sub>2</sub>-sparge needle, was added thiadiazine **2-26** (1.02 g, 2.90 mmol), THF (16.0 mL), and 1,4-benzenedimethanol (0.398 g, 2.88 mmol). The reaction mixture was sparged with N<sub>2</sub> and cooled to 0 °C. After 5 min, PPh<sub>3</sub> (0.748 g, 2.85 mmol) was added, followed by a portionwise addition of DBAD (0.671 g, 2.91 mmol). After 30 min, the reaction was warmed to rt, and the N<sub>2</sub>-sparge line was removed. The reaction mixture was stirred for 20 h and was treated with water (40 mL), transferred to a separatory funnel, and extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with brine (40 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (100% hexanes to 1:1; EtOAc: hexanes) afforded a mixture of the alkylated product and 1,4-benzenedimethanol. The mixture was dissolved in acetone (4 mL) and triturated with hexanes (20 mL), and the white precipitate was filtered under

vacuum to afford **2-33** (0.714 g, 52%) as a white solid: Mp 75-77 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3467, 3074, 1686, 1625, 1270, 1174 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 8.70 (d, *J* = 7.2 Hz, 1 H) 7.73 (s, 1 H), 7.64 (d, *J* = 2.15 Hz, 1 H), 7.38 (dd, *J* = 8.4, 2.1 Hz, 1 H) 7.36-7.33 (m, 4 H), 7.21 (d, *J* = 8.4 Hz, 1 H), 5.62 (d, *J* = 7.2 Hz, 1 H), 5.20 (t, *J* = 5.7 Hz, 1 H), 4.86 (d, *J* = 15.7 Hz, 1 H), 4.78 (d, *J* = 15.7 Hz, 1 H), 4.50 (d, *J* = 5.7 Hz, 2 H), 4.02-3.91 (m, 2 H), 1.01 (t, *J* = 7.05 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 164.4, 142.9, 142.3, 134.7, 134.6, 133.9, 133.2, 131.3, 128.8, 127.7, 126.75, 126.71, 102.5, 62.6, 59.9, 53.9, 51.4, 14.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>21</sub>O<sub>5</sub>N<sub>2</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 471.0543, found 471.0547; LCMS-220 nm purity 100%.



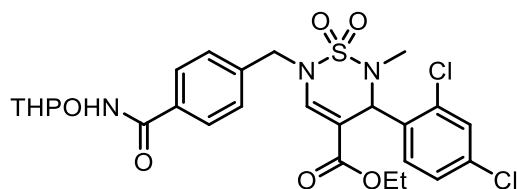
**4-((5-(2,4-Dichlorophenyl)-4-(ethoxycarbonyl)-1,1-dioxido-5,6-dihydro-2H-1,2,6-thiadiazin-2-yl)methyl)benzoic acid (2-34).** A 0 °C solution of **2-33** (2.39 g, 5.07 mmol) in acetone (18 mL) was treated with dropwise addition of the Jones reagent (2.5 M, 5.00 mL, 12.5 mmol). The reaction mixture was stirred at 0 °C for 1.5 h. The dark/brown solution was quenched with a small amount of iPrOH (6 mL) and the reaction mixture was stirred for 5 min. The blue mixture was treated with water (60 mL) and extracted with Et<sub>2</sub>O (3 x 100 mL). The combined organic layers were washed with water (100 mL), brine (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to yield **2-34** (2.32 g, 94%) as a white solid: Mp 101-104 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3183, 2983, 1687, 1614, 1270, 1175 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 12.99 (s, 1 H), 8.76 (d, *J* = 7.2 Hz, 1 H), 7.97 (d, *J* = 8.3 Hz, 2 H), 7.81 (s, 1 H), 7.65 (d, *J* = 2.2 Hz, 1 H), 7.51 (d, *J* = 8.3 Hz, 2 H), 7.40 (dd, *J* = 8.4, 2.2

Hz, 1 H), 7.25 (d,  $J = 8.4$  Hz, 1 H), 5.64 (d,  $J = 7.1$  Hz, 1 H), 4.99 (d,  $J = 16.4$  Hz, 1 H), 4.91 (d,  $J = 16.5$  Hz, 1 H), 4.06-3.90 (m, 2 H), 1.02 (t,  $J = 7.1$  Hz, 3 H);  $^{13}\text{C}$  NMR (75 MHz; DMSO- $d_6$ )  $\delta$  167.1, 164.3, 143.2, 141.5, 134.6, 133.9, 133.3, 131.3, 130.3, 129.6, 128.8, 127.8, 126.8, 102.8, 60.0, 53.9, 51.4, 14.0; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{20}\text{H}_{19}\text{O}_6\text{N}_2\text{Cl}_2\text{S}$   $[\text{M}+\text{H}]^+$  485.0335, found 485.0360; LCMS-220 nm purity 100%.



**Ethyl 3-(2,4-dichlorophenyl)-6-(4-(((tetrahydro-2H-pyran-2-yl)oxy)carbamoyl)benzyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-35).** A solution of carboxylic acid **2-34** (1.80 g, 3.71 mmol) in  $\text{CH}_2\text{Cl}_2$  (12 mL) was treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (1.24 g, 10.6 mmol). The mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 3.30 mL, 5.54 mmol) and TEA (1.60 mL, 11.5 mmol). The reaction mixture was warmed to rt, and stirred under  $\text{N}_2$ . After 4 h, the mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (150 mL), washed with 0.25 M HCl (100 mL), brine (100 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. Purification by chromatography on  $\text{SiO}_2$  (100% hexanes to 100% EtOAc), afforded **2-35** (1.76 g, 81%, dr ~ 1:1 based on  $^1\text{H}$  NMR) as a white solid: Mp 115-117 °C (dec, hexanes); IR ( $\text{CH}_2\text{Cl}_2$ ) 3183, 2949, 2871, 1627, 1269, 1176  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz;  $\text{CDCl}_3$ )  $\delta$  9.35 (s, 1 H), 7.64 (app d,  $J = 8.0$  Hz, 2 H), 7.63 (app d,  $J = 7.9$  Hz, 2 H), 7.43 (app d,  $J = 5.5$  Hz, 2 H), 7.41 (app d,  $J = 2.1$  Hz, 2 H), 7.36 (app dd,  $J = 8.2, 2.1$  Hz, 4 H), 7.24 (app dd,  $J = 8.4, 1.5$  Hz, 2 H), 7.17 (app dd,  $J = 8.4, 2.0$  Hz, 2

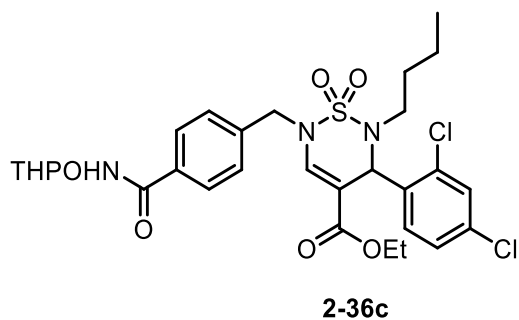
H), 6.24-6.20 (m, 2 H), 5.91 (app d,  $J = 7.7$  Hz, 2 H), 5.02 (s, 2 H), 4.80 (d,  $J = 15.9$  Hz, 1 H), 4.78 (d,  $J = 15.8$  Hz, 1 H), 4.63 (d,  $J = 15.8$  Hz, 1 H), 4.62 (d,  $J = 15.9$  Hz, 1 H), 4.03-3.93 (m, 6 H), 3.65-3.63 (m, 2 H), 1.88-1.81 (m, 8 H), 1.66-1.57 (m, 2 H), 1.016 (t,  $J = 7.1$  Hz, 3 H), 1.014 (t, 3 H,  $J = 7.1$  Hz);  $^{13}\text{C}$  NMR (500 MHz;  $\text{CDCl}_3$ )  $\delta$  165.7, 164.9, 141.96, 141.93, 139.47, 139.46, 135.0, 134.7, 133.8, 131.98, 131.97, 130.7, 129.7, 128.37, 128.35, 128.1, 127.0, 105.59, 105.56, 102.9, 62.95, 62.91, 55.5, 52.2, 52.1, 28.2, 25.1, 18.7, 14.1; HRMS (ESI $^-$ )  $m/z$  calcd for  $\text{C}_{25}\text{H}_{26}\text{O}_7\text{N}_3\text{Cl}_2\text{S}$  [M-H] $^-$  582.0863, found 582.0860.



**2-36b**

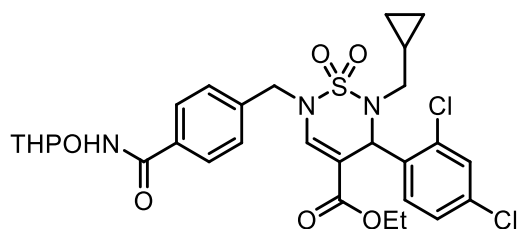
**Ethyl 3-(2,4-dichlorophenyl)-2-methyl-6-(4-(((tetrahydro-2H-pyran-2-yl)oxy)carbamoyl)benzyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-36b).** A solution of carboxylic acid **2-35** (0.205 g, 0.351 mmol) in acetonitrile (3.0 mL) was treated with  $\text{K}_2\text{CO}_3$  (0.145 g, 1.05 mmol) and methyl iodide (65.0  $\mu\text{L}$ , 1.04 mmol) at rt under  $\text{N}_2$ . After 3 h, LCMS indicated an incomplete reaction, and more  $\text{K}_2\text{CO}_3$  (0.149 g, 1.08 mmol) was added to the reaction mixture. After an additional 2 h of stirring, the reaction mixture was complete and was treated with  $\text{H}_2\text{O}$  (15 mL) and EtOAc (15 mL). The layers were transferred to a separatory funnel and separated. The aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (1 x 15 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. Purification by chromatography on  $\text{SiO}_2$  (0-100 % EtOAc in hexanes) afforded **2-36b** (92 % purity by  $^1\text{H}$  NMR (MeOH), 0.157 g, 69%) as a yellow oil:  $^1\text{H}$  NMR (500 MHz;  $\text{CDCl}_3$ )  $\delta$  8.82 (s, 1 H), 7.80 (d,  $J =$

8.1 Hz, 2 H), 7.50 (s, 1 H), 7.47 (d,  $J = 8.1$  Hz, 2 H), 7.42 (bs, 1 H), 7.19-7.15 (m, 2 H), 5.51 (s, 1 H), 5.09 (s, 1 H), 4.82 (app dd,  $J = 15.6, 1.7$  Hz, 1 H), 4.71 (app dd,  $J = 15.6, 2.2$  Hz, 1 H), 4.12-3.98 (m, 3 H), 3.69-3.65 (m, 1 H), 2.96 (s, 3 H), 1.93-1.85 (m, 3 H), 1.72-1.63 (m, 3 H), 1.10 (t,  $J = 7.1$  Hz, 3 H); HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>26</sub>H<sub>30</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 598.1176, found 598.1177.



**Ethyl 2-butyl-3-(2,4-dichlorophenyl)-6-(4-((tetrahydro-2H-pyran-2-yl)oxy)carbamoyl)benzyl-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-36c).** A solution of carboxylic acid **2-35** (0.201 g, 0.344 mmol) in acetonitrile (3.0 mL) was treated with K<sub>2</sub>CO<sub>3</sub> (0.140 g, 1.01 mmol) and bromobutane (38.0  $\mu$ L, 0.352 mmol) in acetonitrile (0.1 mL) at rt under N<sub>2</sub>. After 5 h, LCMS indicated an incomplete reaction, and more K<sub>2</sub>CO<sub>3</sub> (0.145 g, 1.05 mmol) was added to the reaction mixture. After an additional 2 h, more bromobutane (0.300 mL, 2.78 mmol) was added. After another 39 h of stirring, the reaction mixture was complete and was treated with H<sub>2</sub>O (15 mL) and EtOAc (15 mL). The layers were transferred to a separatory funnel and separated. The aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (1 x 15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-100 % EtOAc in hexanes) afforded **2-36c** (93 % purity by <sup>1</sup>H NMR (MeOH), 0.206 g, 87%) as a yellow oil: <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)  $\delta$  8.82 (s, 1 H), 7.80 (d,  $J = 8.1$  Hz, 2 H), 7.48 (d,  $J = 8.1$  Hz, 2 H), 7.47 (s, 1 H), 7.40 (d,  $J = 2.1$  Hz, 1 H), 7.23 (d,  $J = 8.4$  Hz,

1 H), 7.16 (dd,  $J = 8.4, 2.1$  Hz, 1 H), 5.65 (s, 1 H), 5.09 (s, 1 H), 4.79 (d,  $J = 15.6$  Hz, 1 H), 4.74 (d,  $J = 15.6$  Hz, 1 H), 4.12-3.98 (m, 3 H), 3.68-3.66 (m, 1 H), 3.42-3.35 (m, 1 H), 3.15-3.09 (m, 1 H), 1.93-1.82 (m, 3 H), 1.82-1.72 (m, 2 H), 1.70-1.63 (m, 3 H), 1.41-1.26 (m, 2 H), 1.10 (t,  $J = 7.1$  Hz, 3 H), 0.92 (t,  $J = 7.4$  Hz, 3 H); HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>29</sub>H<sub>36</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 640.1646, found 640.1637.

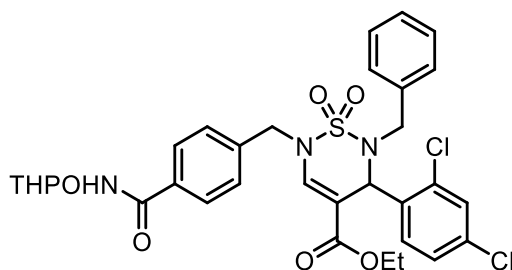


**2-36d**

**Ethyl 2-(cyclopropylmethyl)-3-(2,4-dichlorophenyl)-6-(4-(((tetrahydro-2H-pyran-2-yl)oxy)carbamoyl)benzyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-36d).** A solution of carboxylic acid **2-35** (0.2060 g, 0.352 mmol) in acetonitrile (3.0 mL) was treated with K<sub>2</sub>CO<sub>3</sub> (0.147 g, 1.06 mmol) and (bromomethyl)cyclopropane (35.0 μL, 0.361 mmol) at rt under N<sub>2</sub>. After 5 h, LCMS indicated an incomplete reaction, and more K<sub>2</sub>CO<sub>3</sub> (0.146 g, 1.06 mmol) was added to the reaction mixture. After an additional 2 h, more (bromomethyl)cyclopropane (0.240 mL, 2.47 mmol) was added. After another 24 h of stirring, the reaction mixture was complete and was treated with H<sub>2</sub>O (15 mL) and EtOAc (15 mL). The layers were transferred to a separatory funnel and separated. The aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (1 x 15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-100 % EtOAc in hexanes) afforded **2-36d** (6% MeOH impurity, 0.117 g, 49%) as a yellow oil: <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>)



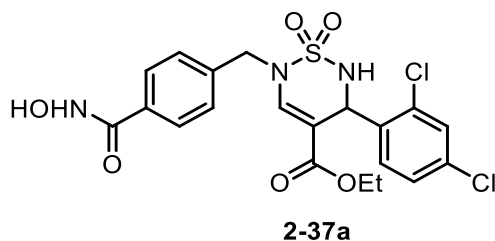
$\delta$  8.78 (s, 1 H), 7.80 (d,  $J = 8.1$  Hz, 2 H), 7.48 (d,  $J = 8.2$  Hz, 2 H), 7.46 (s, 1 H), 7.40 (d,  $J = 2.0$  Hz, 1 H), 7.23 (d,  $J = 8.4$  Hz, 1 H), 7.17 (dd,  $J = 8.4, 2.0$  Hz, 1 H), 5.91 (s, 1 H), 5.09 (s, 1 H), 4.80 (d,  $J = 15.9$  Hz, 1 H), 4.72 (d,  $J = 15.7$  Hz, 1 H), 4.07 (q,  $J = 7.1$  Hz, 2 H), 4.03-3.99 (m, 1 H), 3.69-3.67 (m, 1 H), 3.42 (dd,  $J = 14.4, 6.8$  Hz, 1 H), 3.18 (dd,  $J = 14.4, 7.3$  Hz, 1 H), 1.93-1.85 (m, 3 H), 1.71-1.57 (m, 3 H), 1.21-1.14 (m, 1 H), 1.11 (t,  $J = 7.1$  Hz, 3 H), 0.62-0.54 (m, 2 H), 0.31-0.25 (m, 2 H); HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>29</sub>H<sub>34</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 638.1489, found 638.1486.



**2-36e**

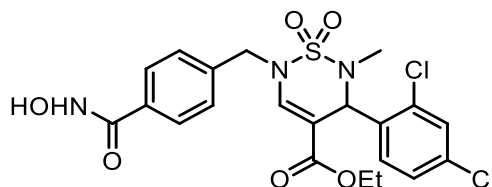
**Ethyl 2-benzyl-3-(2,4-dichlorophenyl)-6-(4-(((tetrahydro-2H-pyran-2-yl)oxy) carbamoyl) benzyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-36e).** A solution of carboxylic acid **2-35** (0.199 g, 0.341 mmol) in acetonitrile (3.0 mL) was treated with K<sub>2</sub>CO<sub>3</sub> (0.143 g, 1.04 mmol) and benzyl bromide (44.0  $\mu$ L, 0.370 mmol) at rt under N<sub>2</sub>. After 2 h, LCMS indicated an incomplete reaction, and more K<sub>2</sub>CO<sub>3</sub> (0.141 g, 1.02 mmol) was added to the reaction mixture. After an additional 2 h of stirring, the reaction was complete and the reaction mixture was treated with H<sub>2</sub>O (15 mL) and EtOAc (15 mL). The layers were transferred to a separatory funnel and separated. The aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (1 x 15 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification

by chromatography on SiO<sub>2</sub> (0-100 % EtOAc in hexanes) afforded **2-36e** (91% purity by <sup>1</sup>H NMR (MeOH), 0.249 g, 98%) as a yellow oil: <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ 8.82 (s, 1 H), 7.78 (d, *J* = 8.1 Hz, 2 H), 7.42 (d, *J* = 8.1 Hz, 2 H), 7.40-7.38 (m, 2 H), 7.36-7.30 (m, 4 H), 7.19-7.17 (m, 2 H), 7.13 (dd, *J* = 8.5, 2.0 Hz, 1 H), 5.82 (s, 1 H), 5.09 (s, 1 H), 4.81 (d, *J* = 15.8 Hz, 1 H), 4.63 (d, *J* = 14.3 Hz, 1 H), 4.53 (app dd, *J* = 15.7, 1.8 Hz, 1 H), 4.35 (d, *J* = 14.3 Hz, 1 H), 4.03 (q, *J* = 7.1 Hz, 2 H), 4.01-3.98 (m, 1 H), 3.69-3.65 (m, 1 H), 1.92-1.85 (m, 3 H), 1.69-1.65 (m, 3 H), 1.08 (t, *J* = 7.1 Hz, 3 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>32</sub>H<sub>34</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 674.1489, found 674.1489.



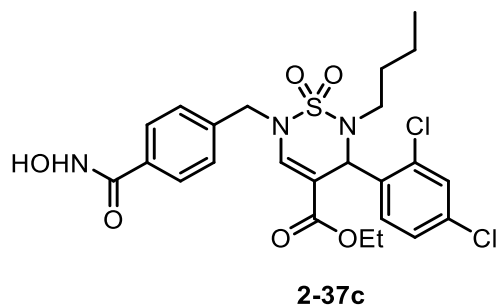
**Ethyl 3-(2,4-dichlorophenyl)-6-(4-(hydroxycarbonyl)benzyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-37a).** To a solution of **2-35** (0.190 g, 0.325 mmol) in MeOH (5.0 mL) and CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) was added Amberlyst-15 (0.0517 g, 253 mmol) at rt under N<sub>2</sub>. After 17 h of stirring, the reaction mixture was filtered through Celite®, rinsed with MeOH, and concentrated. The residue was purified by trituration (4:1; hexanes: EtOAc) to afford **2-37a** (0.133 g, 82%) as a white solid: Mp 97 °C (dec.); IR (CH<sub>2</sub>Cl<sub>2</sub>) 3188, 2862, 1627, 1265, 1175 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 11.24 (s, 1 H), 9.06 (s, 1 H), 8.76 (d, *J* = 7.3 Hz, 1 H), 7.79 (s, 1 H), 7.77 (d, *J* = 8.2 Hz, 2 H), 7.65 (d, *J* = 2.2 Hz, 1 H), 7.46 (d, *J* = 8.3 Hz, 2 H), 7.39 (dd, *J* = 8.5, 2.1 Hz, 1 H), 7.24 (d, *J* = 8.5 Hz, 1 H), 5.64 (d, *J* = 7.2 Hz, 1 H), 4.95 (d, *J* = 16.3 Hz, 1 H), 4.88 (d, *J* = 16.3 Hz, 1 H), 4.03-3.92 (m, 2 H), 1.02 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 164.4, 164.0, 143.2, 139.7, 134.7, 133.9, 133.3, 132.3, 131.3, 128.8, 127.7, 127.2,

126.8, 102.8, 60.0, 54.0, 51.4, 14.0; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>20</sub>H<sub>20</sub>O<sub>6</sub>N<sub>3</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 500.0444, found 500.0469; LCMS-220 nm purity 100%.

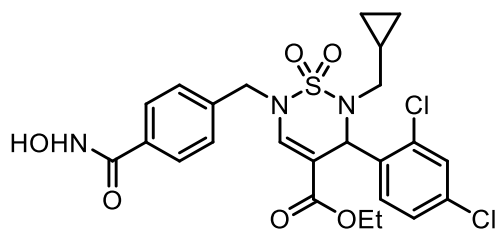


**2-37b**

**Ethyl 3-(2,4-dichlorophenyl)-6-(4-(hydroxycarbonyl)benzyl)-2-methyl-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-37b).** To a solution of **2-36b** (92% purity (MeOH), 0.155 g, 0.239 mmol) in MeOH (3.0 mL) and CH<sub>2</sub>Cl<sub>2</sub> (0.8 mL) was added Amberlyst-15 (0.0439 g, 206 mmol) at room temperature under N<sub>2</sub>. After 30 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes; EtOAc) to afford **2-37b** (0.109 g, 89%) as an off-white solid: Mp 93 °C (dec.); IR (CH<sub>2</sub>Cl<sub>2</sub>) 3234, 3060, 2984, 1697, 1626, 1372, 1167 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 11.23 (s, 1 H), 9.06 (s, 1 H), 7.95 (s, 1 H), 7.77 (d, *J* = 8.3 Hz, 2 H), 7.65 (d, *J* = 2.2 Hz, 1 H), 7.47 (d, *J* = 8.3 Hz, 2 H), 7.37 (dd, *J* = 8.4, 2.2 Hz, 1 H), 7.18 (d, *J* = 8.4 Hz, 1 H), 5.48 (s, 1 H), 4.98 (d, *J* = 16.0 Hz, 1 H), 4.88 (d, *J* = 16.0 Hz, 1 H), 4.09-3.97 (m, 2 H), 2.87 (s, 3 H), 1.07 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 164.6, 163.9, 142.3, 139.4, 134.6, 134.1, 133.2, 132.5, 131.5, 128.7, 127.8, 127.3, 126.6, 100.1, 62.9, 60.1, 52.1, 14.0; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>21</sub>H<sub>22</sub>O<sub>6</sub>N<sub>3</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 514.0601, found 514.0626; LCMS-220 nm purity 100%.

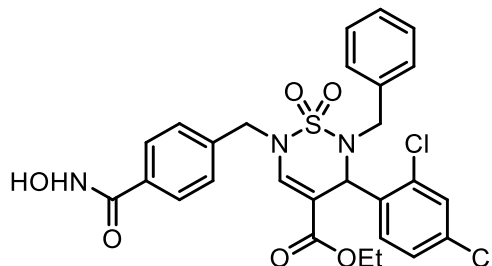


**Ethyl 2-butyl-3-(2,4-dichlorophenyl)-6-(4-(hydroxycarbamoyl)benzyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-37c).** To a solution of **2-36c** (93% purity (MeOH), 0.206 g, 0.299 mmol) in MeOH (5.0 mL) and CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL) was added Amberlyst-15 (0.0533 g, 251 mmol) at rt under N<sub>2</sub>. After 24 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. Purification by chromatography on SiO<sub>2</sub>\* (9% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) afforded **2-37c** (0.0549 g, 33%) as a pink solid: Mp 78-82 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3222, 2960, 2933, 2874, 1696, 1625, 1370, 1179 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 11.23 (s, 1 H), 9.06 (s, 1 H), 7.94 (s, 1 H), 7.77 (d, *J* = 8.2 Hz, 2 H), 7.63 (d, *J* = 2.1 Hz, 1 H), 7.47 (d, *J* = 8.2 Hz, 2 H), 7.37 (dd, *J* = 8.5, 2.1 Hz, 1 H), 7.22 (d, *J* = 8.5 Hz, 1 H), 5.59 (s, 1 H), 4.98 (d, *J* = 15.9 Hz, 1 H), 4.89 (d, *J* = 16.0 Hz, 1 H), 4.11-3.93 (m, 2 H), 3.26-3.05 (m, 2 H), 1.71-1.60 (m, 2 H), 1.33-1.15 (m, 2 H), 1.06 (t, *J* = 7.1 Hz, 3 H), 0.83 (t, *J* = 7.4 Hz, 3 H); <sup>13</sup>C NMR (75 MHz; DMSO-*d*<sub>6</sub>) δ 164.4, 163.8, 142.5, 139.4, 134.6, 133.8, 133.3, 132.5, 132.2, 128.7, 128.0, 127.3, 126.6, 101.6, 62.2, 60.1, 53.3, 52.2, 29.4, 19.4, 14.0, 13.5; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>24</sub>H<sub>28</sub>O<sub>6</sub>N<sub>3</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 556.10704, found 556.10974; LCMS-220 nm purity 100%.



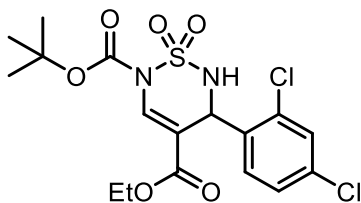
**2-37d**

**Ethyl 2-(cyclopropylmethyl)-3-(2,4-dichlorophenyl)-6-(4-(hydroxycarbonyl)benzyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-37d).** To a solution of **2-36d** (94 % purity (MeOH), 0.115 g, 0.170 mmol) in MeOH (3.0 mL) and CH<sub>2</sub>Cl<sub>2</sub> (0.8 mL) was added Amberlyst-15 (0.0340 g, 160 mmol) at rt under N<sub>2</sub>. After 24 h of stirring, the mixture was filtered through Celite®, rinsed with MeOH, and concentrated. The residue was purified by trituration (10:1; Hexanes: EtOAc) to afford **2-37d** (0.0631 g, 67%) as a beige solid: Mp 89 °C (dec.); IR (CH<sub>2</sub>Cl<sub>2</sub>) 3253, 3066, 2981, 1695, 1626, 1371, 1184, 1168 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 11.23 (s, 1 H), 9.06 (s, 1 H), 7.91 (s, 1 H), 7.76 (d, *J* = 8.2 Hz, 2 H), 7.63 (d, *J* = 2.2 Hz, 1 H), 7.46 (d, *J* = 8.2 Hz, 2 H), 7.46 (dd, *J* = 8.5, 2.2 Hz, 1 H), 7.23 (d, *J* = 8.5 Hz, 1 H), 5.82 (s, 1 H), 4.96 (d, *J* = 16.0 Hz, 1 H), 4.90 (d, *J* = 16.0 Hz, 1 H), 4.06-4.02 (m, 2 H), 3.28 (dd, *J* = 14.4, 6.8 Hz, 1 H), 3.05 (dd, *J* = 14.4, 7.4 Hz, 1 H), 1.15-1.09 (m, 1 H) 1.07 (t, *J* = 7.0 Hz, 3 H), 0.58-0.53 (m, 1 H), 0.49-0.45 (m, 1 H), 0.26-0.19 (m, 2 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 164.5, 163.8, 142.6, 139.3, 134.9, 133.9, 133.2, 132.5, 132.0, 128.7, 127.9, 127.2, 126.6, 101.4, 60.3, 60.1, 57.5, 52.3, 14.1, 8.4, 4.4, 3.4; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>24</sub>H<sub>26</sub>O<sub>6</sub>N<sub>3</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 554.0914, found 554.0940; LCMS-220 nm purity 98.7%.



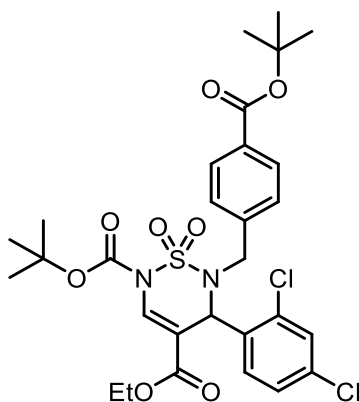
**2-37e**

**Ethyl 2-benzyl-3-(2,4-dichlorophenyl)-6-(4-(hydroxycarbonyl)benzyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-37e).** To a solution of **2-36e** (91% purity (MeOH), 0.246 g, 0.331 mmol) in MeOH (4.0 mL) and CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) was added Amberlyst-15 (0.0580 g, 273 mmol) at rt under N<sub>2</sub>. After 20 h of stirring, the reaction mixture was filtered through Celite®, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1; hexanes: Et<sub>2</sub>O) to afford **2-37e** (0.0305 g, 16%) as a white solid: Mp 80 °C (dec.); IR (CH<sub>2</sub>Cl<sub>2</sub>) 3228, 3067, 2983, 1698, 1628, 1375, 1175 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 11.24 (s, 1 H), 9.07 (s, 1 H), 7.78 (d, *J* = 8.1 Hz, 2 H), 7.77 (s, 1 H), 7.53 (d, *J* = 2.2 Hz, 1 H), 7.48 (d, *J* = 8.2 Hz, 2 H), 7.36-7.33 (m, 6 H), 7.22 (d, *J* = 8.5 Hz, 1 H), 5.69 (s, 1 H), 4.92 (s, 2 H), 4.48 (d, *J* = 14.5 Hz, 1 H), 4.27 (d, *J* = 14.5 Hz, 1 H), 4.05-3.96 (m, 2 H), 1.05 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 164.3, 163.9, 142.4, 139.2, 134.5, 133.9, 133.3, 132.5, 132.3, 129.7, 128.6, 128.2, 128.1, 128.0, 127.3, 126.5, 101.1, 60.5, 60.0, 55.9, 52.3, 14.1; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>27</sub>H<sub>26</sub>O<sub>6</sub>N<sub>3</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 590.0914, found 590.0942; LCMS-220 nm purity 93.6%.



**2-40**

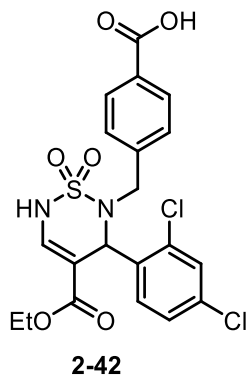
**2-(tert-Butyl) 4-ethyl 5-(2,4-dichlorophenyl)-5,6-dihydro-2H-1,2,6-thiadiazine-2,4-dicarboxylate 1,1-dioxide (2-40).** A solution of the thiadiazine **2-26** (5.06 g, 14.4 mmol) in acetonitrile (120 mL) was treated with  $K_2CO_3$  (4.45 g, 13.2 mmol). After stirring at rt for 25 min,  $Boc_2O$  (2.8 g, 13.0 mmol) was added and the reaction mixture was stirred for 7 h. The mixture was treated with  $H_2O$  (300 mL), transferred to a separatory funnel, and extracted with EtOAc (3 x 300 mL). The combined organic layers were washed with brine (200 mL), dried ( $Na_2SO_4$ ), filtered, and concentrated. The residue was purified by chromatography on  $SiO_2$  (100% hexanes to 1:1; EtOAc: hexanes) to afford **2-40** (5.08 g, 86%) as a white solid: Mp 56-58 °C (dec.); IR ( $CH_2Cl_2$ ) 3246, 2985, 1745, 1709, 1372, 1254, 1141  $cm^{-1}$ ;  $^1H$  NMR (500 MHz;  $DMSO-d_6$ )  $\delta$  9.36 (d,  $J = 5.5$  Hz, 1 H), 8.07 (d,  $J = 0.6$  Hz, 1 H), 7.69 (d,  $J = 2.2$  Hz, 1 H), 7.38 (dd,  $J = 8.4, 2.2$  Hz, 1 H), 7.27 (d,  $J = 8.4$  Hz, 1 H), 5.59 (d,  $J = 5.2$  Hz, 1 H), 4.12-4.01 (m, 2 H), 1.52 (m, 9 H), 1.09 (t,  $J = 7.1$  Hz, 3 H);  $^{13}C$  NMR (126 MHz;  $DMSO-d_6$ )  $\delta$  163.7, 147.5, 135.9, 133.9, 133.7, 133.3, 131.4, 128.9, 127.0, 108.2, 86.1, 60.7, 53.1, 27.4, 13.8; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $C_{17}H_{19}O_6N_2Cl_2S$  [M-H]<sup>+</sup> 449.0335, found 449.0333.



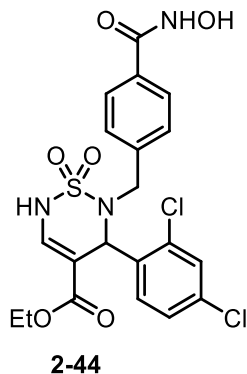
**2-41**

**2-(tert-Butyl) 4-ethyl 6-(4-(tert-butoxycarbonyl)benzyl)-5-(2,4-dichlorophenyl)-5,6-dihydro-2H-1,2,6-thiadiazine-2,4-dicarboxylate 1,1-dioxide (2-41).** To a suspension of **2-40** (4.43 g, 9.81 mmol) and  $K_2CO_3$  (7.49 g, 54.2 mmol) in MeCN (125 mL) was added *tert*-butyl 4-(bromomethyl)benzoate (2.80 g, 10.3 mmol). The reaction mixture was stirred at rt for 2 h, diluted with  $H_2O$  (150 mL)/brine (150 mL) and EtOAc (200 mL). The layers were transferred to a separatory funnel and separated. The aqueous layer was extracted with EtOAc (2 x 300 mL). The combined organic layers were dried ( $Na_2SO_4$ ), filtered, and concentrated. Purification by chromatography on  $SiO_2$  (100% hexanes to 1:5; EtOAc: hexanes) afforded **2-41** (5.00 g, 79%) as a white solid: Mp 99-102 °C; IR ( $CH_2Cl_2$ ) 2981, 1746, 1709, 1396, 1242, 1142  $cm^{-1}$ ;  $^1H$  NMR (300 MHz;  $CDCl_3$ )  $\delta$  7.933 (d, 2 H,  $J = 8.2$  Hz), 7.927 (s, 1 H), 7.45 (d,  $J = 8.2$  Hz, 2 H), 7.37 (s, 1 H), 7.16-7.14 (m, 2 H), 5.77 (s, 1 H), 4.75 (d,  $J = 14.8$  Hz, 1 H), 4.61 (d,  $J = 14.8$  Hz, 1 H), 4.20-4.01 (m, 2 H), 1.60 (s, 9 H), 1.59 (s, 9 H), 1.16 (t,  $J = 7.1$  Hz, 3 H);  $^{13}C$  NMR (75 MHz;  $CDCl_3$ )  $\delta$  165.3, 164.2, 148.0, 138.1, 136.1, 135.3, 134.9, 133.0, 132.5, 131.4, 129.7, 129.65, 129.61, 126.8, 107.3, 87.1, 81.5, 61.3, 60.2, 57.4, 28.3, 28.0, 14.3; HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $C_{29}H_{35}O_8N_2Cl_2S$   $[M+H]^+$  641.1486, found 641.1513; LCMS-220 nm purity 100%.



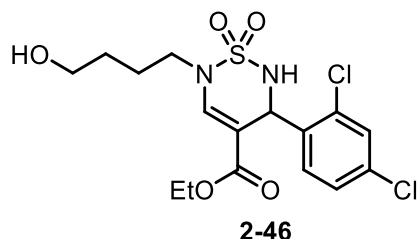


**4-((3-(2,4-Dichlorophenyl)-4-(ethoxycarbonyl)-1,1-dioxido-3,6-dihydro-2H-1,2,6-thiadiazin-2-yl)methyl)benzoic acid (2-42).** A solution of the di-*tert*-butyl ester **2-41** (4.80 g, 7.48 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL) was treated with TFA (11.1 mL, 150 mmol), and the reaction mixture was stirred at rt under N<sub>2</sub>. After 1.5 h, TLC (2:1; CH<sub>2</sub>Cl<sub>2</sub>: EtOAc) indicated reaction completion. The reaction mixture was treated with H<sub>2</sub>O (~80 mL), and the precipitate was filtered in vacuo to give **2-42** (3.56 g, 98%) as a white solid: Mp 213-215 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3185, 1287, 1662, 1634, 1286, 1166 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 12.99 (s, 1 H), 11.42 (s, 1 H), 7.91 (d, *J* = 8.1 Hz, 2 H), 7.54-7.51(m, 4 H), 7.33 (dd, *J* = 8.5, 2.1 Hz, 1 H), 7.20 (d, *J* = 8.5 Hz, 1 H), 5.64 (s, 1 H), 4.55 (d, *J* = 15.1 Hz, 1 H), 4.37 (d, *J* = 15.1 Hz, 1 H), 4.06-3.94 (m, 2 H), 1.07 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 167.1, 164.5, 140.3, 139.1, 134.8, 133.9, 133.2, 132.3, 130.2, 129.5, 129.0, 128.5, 126.4, 99.9, 61.3, 59.9, 55.5, 14.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>19</sub>O<sub>6</sub>N<sub>2</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 485.0335, found 485.0357; LCMS-220 nm purity 100%.



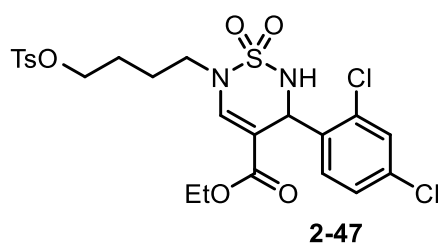
**Ethyl 3-(2,4-dichlorophenyl)-2-(4-(hydroxycarbonyl)benzyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-44).** A solution of **2-42** (0.406 g, 0.837 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) was cooled to 0 °C. TEA (0.170 mL, 1.22 mmol), T<sub>3</sub>P (50%, 0.540 mL, 0.907 mmol), and O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.202 g, 1.72 mmol) were added subsequently. The reaction was monitored by TLC (1:3 Hexanes: EtOAc), and after 1.5 h, the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (40 mL), washed with 0.25 M HCl (40 mL), brine (40 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-75% EtOAc in hexanes), afforded the THP-protected amide (0.250 g crude). The crude amide (0.0600g, 0.103 mmol) in MeOH (3.0 mL) and CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) was treated with Amberlyst-15 (0.0107 g, 50.3 mmol) at rt under N<sub>2</sub>. After 25 h of stirring, the reaction mixture was filtered through Celite®, rinsed with MeOH, and concentrated. The residue was purified by trituration (4:1 hexanes: EtOAc) to afford **2-44** (0.0365 g, 36%, 2 steps) as a white solid: Mp 105 °C (dec.); IR (CH<sub>2</sub>Cl<sub>2</sub>) 3170, 2847, 2352, 1629, 1365, 1284, 1158 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 11.42 (s, 1 H), 11.24 (s, 1 H), 9.03 (s, 1 H), 7.73 (d, *J* = 8.1 Hz, 2 H), 7.54 (s, 1 H), 7.52 (d, *J* = 1.9 Hz, 1 H), 7.47 (d, *J* = 8.1 Hz, 2 H), 7.32 (dd, *J* = 8.5, 2.0 Hz, 1 H), 7.21 (d, *J* = 8.5 Hz, 1 H), 5.62 (s, 1 H), 4.52 (d, *J* = 14.8 Hz, 1 H), 4.29 (d, *J* = 14.8 Hz, 1 H), 4.05-3.95 (m, 2 H), 1.07 (t, *J* = 7.0 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 164.5, 163.8, 139.2, 138.5, 134.8, 133.9, 133.2, 132.3, 132.2, 129.3, 128.5, 126.6,

126.4, 99.7, 61.2, 59.9, 55.5, 14.1; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>20</sub>O<sub>6</sub>N<sub>3</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 500.04444, found 500.04679; LCMS-220 nm purity 100%.



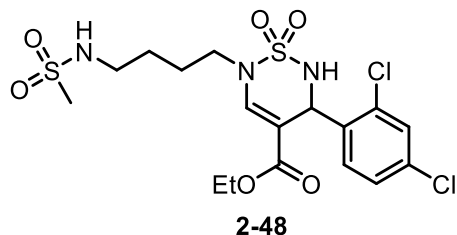
**Ethyl 3-(2,4-dichlorophenyl)-6-(4-hydroxybutyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-46).** The thiadiazine **2-26** (1.52 g, 4.32 mmol) in THF (15 mL) was treated with the 4-((*tert*-butyldiphenylsilyl)oxy)butan-1-ol (1.43 g, 4.35 mmol) in THF (15 mL) under N<sub>2</sub>. After 5 min, PPh<sub>3</sub> (1.20 g, 4.56 mmol) was added, followed by a portionwise addition of DBAD (0.994 g, 4.32 mmol). The reaction mixture and was stirred for 24 h under N<sub>2</sub> at rt, and was partitioned between EtOAc (100 mL) and brine (100 mL). The layers were transferred to a separatory funnel and separated. The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-33% EtOAc in hexanes) afforded **2-54i**, ethyl 6-(4-((*tert*-butyldiphenylsilyl)oxy)butyl)-3-(2,4-dichlorophenyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (3.78 g), as a yellow oil that was used without further purification; **2-46i**: HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>32</sub>H<sub>39</sub>O<sub>5</sub>N<sub>2</sub>Cl<sub>2</sub>SSi [M+H]<sup>+</sup> 661.1720, found 661.1727. A 0 °C solution of **2-46i** (2.86 g, 4.32 mmol) in THF (40 mL) was treated with TBAF (1 M in THF, 6.47 mL, 6.47 mmol). The reaction was warmed to rt and left to stir under N<sub>2</sub>. After 13 h, the reaction mixture was quenched with sat. NH<sub>4</sub>Cl (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) afforded **2-54** (2.6 g) that was used with no

further purification:  $^1\text{H NMR}$  (400 MHz;  $\text{DMSO-}d_6$ )  $\delta$  8.57 (d,  $J = 7.3$  Hz, 1 H), 7.76 (s, 1 H), 7.63 (d,  $J = 2.1$  Hz, 1 H), 7.38 (dd,  $J = 8.4, 2.2$  Hz, 1 H), 7.22 (d,  $J = 8.4$  Hz, 1 H), 5.59 (d,  $J = 7.0$  Hz, 1 H), 4.49 (t,  $J = 5.1$  Hz, 1 H), 4.06 – 3.93 (m, 2 H), 3.63 (t,  $J = 7.2$  Hz, 2 H), 3.42 (q,  $J = 5.2$  Hz, 2 H), 1.68 (quint,  $J = 7.2$  Hz, 2 H), 1.45 (quint,  $J = 7.9$  Hz, 2 H), 1.05 (t,  $J = 7.0$  Hz, 3 H); HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{16}\text{H}_{19}\text{O}_5\text{N}_2\text{Cl}_2\text{S}$   $[\text{M}+\text{H}]^+$  421.0386, found 421.0396.



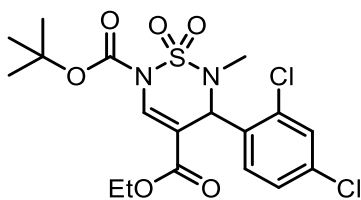
**Ethyl 3-(2,4-dichlorophenyl)-6-(4-(tosyloxy)butyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-47).** A solution of the crude alcohol **2-46** mixture (1.65 g) in  $\text{CH}_2\text{Cl}_2$  (12 mL) was treated with pyridine (0.420 mL, 5.19 mmol) followed by  $\text{TsCl}$  (0.589 g, 3.09 mmol). After 15 h, LCMS indicated SM, and more  $\text{TsCl}$  (0.178 g, 0.934 mmol) and pyridine (0.150 mL, 1.85 mmol) were added. After 6 h of stirring under  $\text{N}_2$ , LCMS indicated >95% conversion, and the mixture was partitioned between water (100 mL) and  $\text{EtOAc}$  (100 mL) in a separatory funnel. The layers were separated, and the organic layer was washed with brine (100 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. Purification by chromatography on  $\text{SiO}_2$  (0-33%  $\text{EtOAc}$  in hexanes), afforded **2-47** (1.12 g, 50% in 3 steps) as a white solid: Mp 37-39  $^\circ\text{C}$ ; IR ( $\text{CH}_2\text{Cl}_2$ ) 3253, 2978, 1693, 1626, 1350, 1171  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (500 MHz;  $\text{CDCl}_3$ )  $\delta$  7.80 (d,  $J = 8.3$  Hz, 2 H), 7.49 (d,  $J = 0.4$  Hz, 1 H), 7.43 (d,  $J = 2.0$  Hz, 1 H), 7.37 (d,  $J = 8.1$  Hz, 2 H), 7.28 (d,  $J = 2.6$  Hz, 1 H), 7.20 (dd,  $J = 8.4, 2.0$  Hz, 1 H), 5.90 (d,  $J = 7.9$  Hz, 1 H), 5.26 (d,  $J = 7.9$  Hz, 1 H), 4.14-4.02 (m, 4 H), 3.65-3.54 (m, 2 H), 2.47 (s, 3 H), 1.87-1.81 (m, 2 H), 1.80-1.75 (m, 2 H), 1.11 (t,  $J = 7.1$  Hz, 3 H);

$^{13}\text{C}$  NMR (126 MHz;  $\text{CDCl}_3$ )  $\delta$  164.8, 145.2, 142.3, 135.0, 134.7, 133.8, 132.8, 130.6, 130.1, 129.7, 128.0, 127.0, 104.7, 69.7, 60.8, 55.6, 49.7, 26.0, 25.7, 21.8, 14.2; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{23}\text{H}_{27}\text{O}_7\text{N}_2\text{Cl}_2\text{S}_2$   $[\text{M}+\text{H}]^+$  577.0631, found 577.0636.



**Ethyl 3-(2,4-dichlorophenyl)-6-(4-(methylsulfonamido)butyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-48).** To a solution of tosylate **2-47** (0.800 g, 1.39 mmol) in anhydrous DMF (0.25 M, 5.5 mL) was added  $\text{NaN}_3$  (0.180 g, 2.77 mmol) under  $\text{N}_2$ . The reaction mixture was stirred at 50  $^\circ\text{C}$  for 7 h until the tosylate was consumed, as monitored by LCMS. The reaction mixture was allowed to cool to rt and partitioned between EtOAc (100 mL) and  $\text{H}_2\text{O}$  (100 mL). The aq. layer was extracted with EtOAc (3 x 75 mL). The organic layers were combined, washed with brine (3 x 75 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to provide the azide, ethyl 6-(4-azidobutyl)-3-(2,4-dichlorophenyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide **2-48i** (~5% DMF impurity, 0.528 g, 81%) as a thick, colorless oil that was used without further purification:  $^1\text{H}$  NMR (300 MHz;  $\text{CDCl}_3$ )  $\delta$  7.50 (s, 1 H), 7.43 (d,  $J = 1.9$  Hz, 1 H), 7.26 (d,  $J = 8.3$  Hz, 1 H), 7.21 (dd,  $J = 8.3, 1.9$  Hz, 1 H), 5.90 (d,  $J = 8.2$  Hz, 1 H), 4.83 (d,  $J = 8.1$  Hz, 1 H), 4.13 - 3.98 (m, 2 H), 3.72 - 3.54 (m, 2 H), 3.37 (t,  $J = 6.5$  Hz, 2 H), 1.92 - 1.82 (m, 2 H), 1.72 - 1.63 (m, 2 H), 1.09 (t,  $J = 7.1$  Hz, 3 H); HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{16}\text{H}_{18}\text{O}_4\text{N}_5\text{Cl}_2\text{S}$   $[\text{M}-\text{H}]^+$  446.0451, found 446.0460. A solution of the azide **2-48i** (95% purity by  $^1\text{H}$  NMR, 0.515 g, 1.09 mmol) in MeOH (6.0 mL) was treated with Pd/C (10%, 0.0581 g, 0.0546 mmol) under  $\text{N}_2$ .

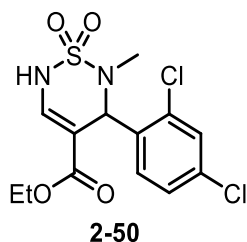
The atmosphere was replaced with H<sub>2</sub> (1 atm), and the reaction mixture was stirred under H<sub>2</sub> for 1 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated and azeotroped with CH<sub>2</sub>Cl<sub>2</sub>. The oily residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3.0 mL) and treated with MsCl (0.422 mL, 5.46 mmol) and pyridine (0.0880 mL, 1.13 mmol). After 1 h, LCMS indicated ~ 1:1 SM: desired product. The mixture was partitioned between H<sub>2</sub>O (50 mL) and EtOAc (50 mL). The layers were separated, and the organic layer was washed with brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-66% EtOAc in hexanes) afforded **2-48** (0.128 g, 24%) as a white solid: Mp 62 °C (dec.); IR (CDCl<sub>3</sub>) 3257, 2936, 1691, 1626, 1315, 1175, 1146 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.50 (s, 1 H), 7.43 (d, *J* = 2.0 Hz, 1 H), 7.27 (d, *J* = 8.1 Hz, 1 H), 7.20 (dd, *J* = 8.4, 2.0 Hz, 1 H), 5.90 (d, *J* = 8.1 Hz, 1 H), 5.30 (d, *J* = 8.0 Hz, 1 H), 4.54 (t, *J* = 6.1 Hz, 1 H), 4.13-3.97 (m, 2 H), 3.64 (t, *J* = 6.8 Hz, 2 H), 3.19 (q, *J* = 6.7 Hz, 2 H), 2.97 (s, 3 H), 1.90-1.80 (m, 2 H), 1.74-1.65 (m, 2 H), 1.08 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (75 MHz; CDCl<sub>3</sub>) δ 165.1, 142.5, 134.9, 134.7, 133.9, 130.7, 130.0, 127.0, 104.6, 60.9, 55.4, 49.6, 42.6, 40.2, 26.9, 26.5, 14.1; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>17</sub>H<sub>22</sub>O<sub>6</sub>N<sub>3</sub>Cl<sub>2</sub>S<sub>2</sub> [M-H]<sup>+</sup> 498.0322, found 498.0322; LCMS-220 nm purity 100%.



**2-49**

**2-(tert-Butyl) 4-ethyl 5-(2,4-dichlorophenyl)-6-methyl-5,6-dihydro-2H-1,2,6-thiadiazine-2,4-dicarboxylate 1,1-dioxide (2-49).** To a suspension of carbamate **2-40** (3.40 g, 7.53 mmol) and

K<sub>2</sub>CO<sub>3</sub> (6.25 g, 45.2 mmol) in acetonitrile (30 mL) was added iodomethane (2.81 mL, 45.2 mmol). The reaction mixture was stirred at rt under N<sub>2</sub> for 2 h, diluted with water (100 mL) and EtOAc (100 mL). The layers were transferred to a separatory funnel and separated. The aqueous layer was extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with sat. NaHCO<sub>3</sub> (1 x 10 mL) and brine (1 x 10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to provide **2-49** (3.37 g, 96%) as a white solid: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 8.12 (s, 1 H), 7.69 (d, *J* = 2.1 Hz, 1 H), 7.36 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.23 (d, *J* = 8.4 Hz, 1 H), 5.55 (s, 1 H), 4.19-4.04 (m, 2 H), 3.08 (s, 3 H), 1.51 (s, 9 H), 1.13 (t, *J* = 7.1 Hz, 3 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>18</sub>H<sub>22</sub>O<sub>6</sub>N<sub>2</sub>Cl<sub>2</sub>NaS [M+Na]<sup>+</sup> 487.0468, found 487.0457.



**Ethyl 3-(2,4-dichlorophenyl)-2-methyl-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-50).** A solution of the carbamate **2-49** (2.48 g, 5.33 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (13 mL) was treated with TFA (4.00 mL, 53.9 mmol). The reaction mixture was stirred at rt under N<sub>2</sub>, and after 3 h, TLC (2:1 Hex: EtOAc) indicated reaction completion. The reaction mixture was treated with water (80 mL) and sat. NaHCO<sub>3</sub> (80 mL), pH ~ 7-8, transferred to a separatory funnel, and the organic layer was washed with brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under vacuum to provide **2-50** (1.89 g, 97%) as a beige solid: Mp 187-189 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3191, 1665, 1626, 1417, 1368, 1287, 1156, 1147 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 11.29 (s, 1 H), 7.65 (s, 1 H), 7.63 (d, *J* = 2.2 Hz, 1 H), 7.34 (dd, *J* = 8.4, 2.2 Hz, 1 H), 7.17 (d, *J* = 8.4 Hz, 1 H), 5.44

(s, 1 H), 4.08-3.96 (m, 2 H), 2.88 (s, 3 H), 1.09 (t,  $J = 7.1$  Hz, 3 H);  $^{13}\text{C}$  NMR (500 MHz; DMSO- $d_6$ )  $\delta$  164.9, 139.1, 135.0, 134.2, 133.0, 131.5, 128.6, 126.5, 98.6, 63.0, 60.0, 39.42, 14.0; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{13}\text{H}_{15}\text{O}_4\text{N}_2\text{Cl}_2\text{S}$   $[\text{M}+\text{H}]^+$  365.0124, found 365.0120.

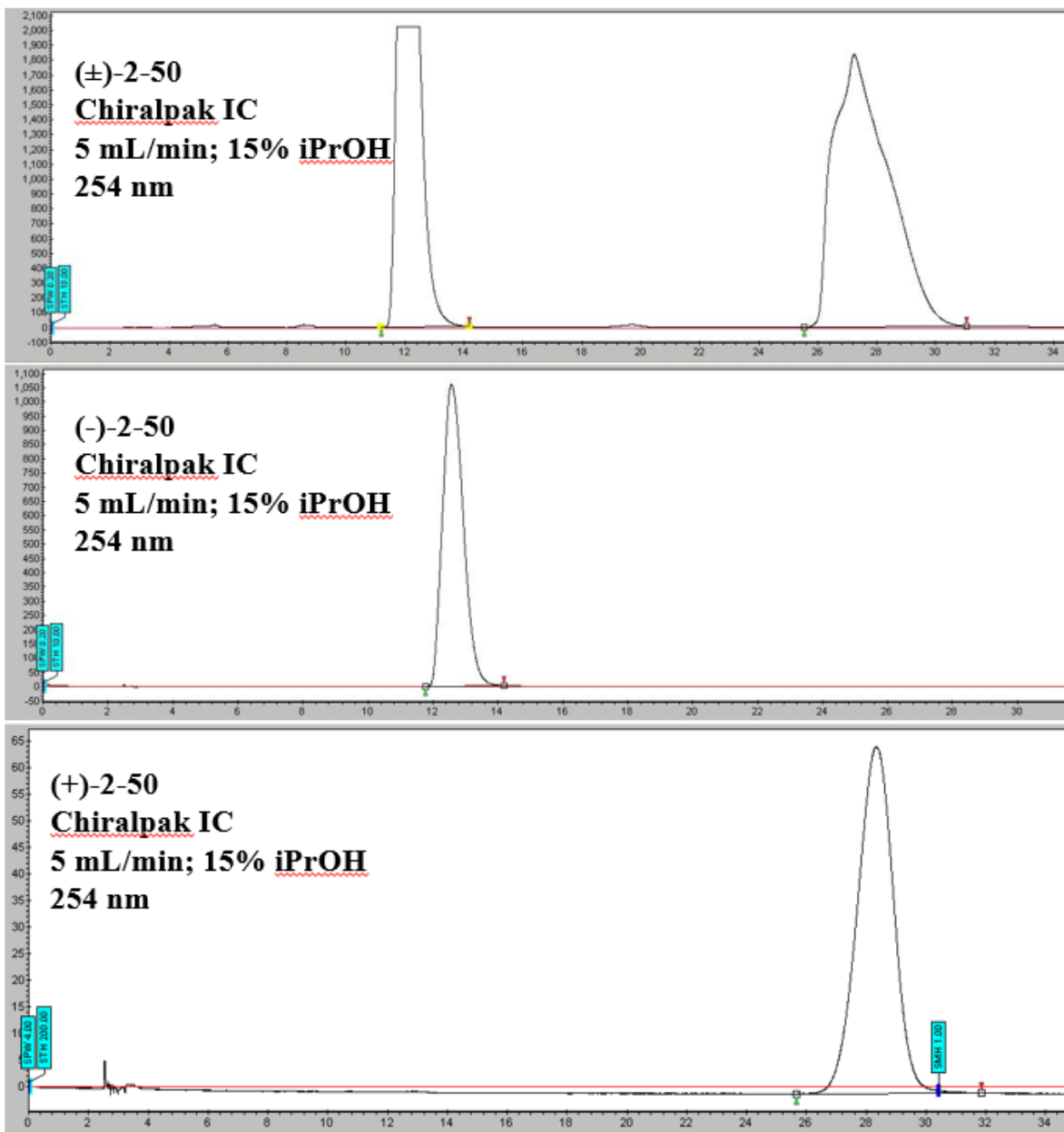
*Note:* HSQC analysis shows the methyl peak overlapping with DMSO- $d_6$  carbon shifts. Methyl carbon of **2-50** and its derivatives is only seen in  $^{13}\text{C}$  NMR in a concentrated NMR sample. For **2-50**, the methyl peak is at 39.42.

The enantiomers of **2-50** were separated by **SFC semi-prep (Chiralpak IC: 5 mL/min; 15% iPrOH; 254 nm)**.

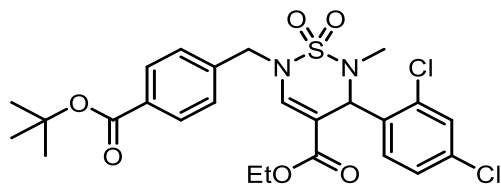
Peak 1 (RT: 12 min)  $[\alpha]_{\text{D}}$  -179.1 ( $c$  0.16,  $\text{CH}_2\text{Cl}_2$ ); Mp 210-212  $^{\circ}\text{C}$ ;  $^1\text{H}$  NMR (300 MHz; DMSO- $d_6$ )  $\delta$  11.28 (s, 1 H), 7.65 (s, 1 H), 7.63 (d,  $J = 2.1$  Hz, 1 H), 7.34 (dd,  $J = 8.4, 2.1$  Hz, 1 H), 7.17 (d,  $J = 8.4$  Hz, 1 H), 5.43 (s, 1 H), 4.11-3.94 (m, 2 H), 2.88 (s, 3 H), 1.09 (t,  $J = 7.1$  Hz, 3 H); HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{13}\text{H}_{15}\text{O}_4\text{N}_2\text{Cl}_2\text{S}$   $[\text{M}+\text{H}]^+$  365.0124, found 365.0119.

Peak 2 (RT: 29 min)  $[\alpha]_{\text{D}}$  +135.6 ( $c$  0.16,  $\text{CH}_2\text{Cl}_2$ ); Mp 178-180  $^{\circ}\text{C}$ ;  $^1\text{H}$  NMR (300 MHz; DMSO- $d_6$ )  $\delta$  11.28 (s, 1 H), 7.65 (s, 1 H), 7.63 (d,  $J = 2.1$  Hz, 1 H), 7.34 (dd,  $J = 8.4, 2.1$  Hz, 1 H), 7.17 (d,  $J = 8.4$  Hz, 1 H), 5.43 (s, 1 H), 4.10-3.93 (m, 2 H), 2.87 (s, 3 H), 1.09 (t,  $J = 7.1$  Hz, 3 H); HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{13}\text{H}_{15}\text{O}_4\text{N}_2\text{Cl}_2\text{S}$   $[\text{M}+\text{H}]^+$  365.0124, found 365.0120.



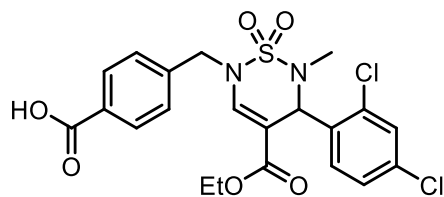


**Figure 37.** SFC Chromatograms of **2-50** and Separated Enantiomers.



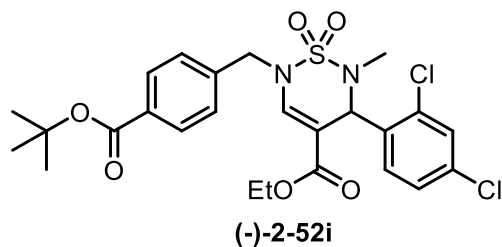
**(+)-2-52i**

**Ethyl 6-(4-(*tert*-butoxycarbonyl)benzyl)-3-(2,4-dichlorophenyl)-2-methyl-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide ((+)-2-52i).** To a suspension of (+)-2-50 (0.0705 g, 0.193 mmol) and  $K_2CO_3$  (0.0819 g, 0.593 mmol) in acetonitrile (2.5 mL) was added 4-bromomethyl benzoic acid mono *tert*-butyl ester (0.0513 g, 0.89 mmol). The reaction mixture was stirred at rt under  $N_2$ . After 6 h, LCMS indicated SM, and more  $K_2CO_3$  (0.0750 g, 0.547 mmol) was added. After an additional 17 h, LCMS indicated reaction completion, and the mixture was suspended in  $H_2O$  (15 mL) and EtOAc (15 mL). The layers were transferred to a separatory funnel and separated, and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with sat.  $NaHCO_3$  (10 mL) and brine (10 mL), dried ( $Na_2SO_4$ ), filtered, and concentrated to provide (+)-2-52i (0.106 g, 99%) as a white solid:  $^1H$  NMR (300 MHz;  $CDCl_3$ )  $\delta$  8.03 (d,  $J = 8.3$  Hz, 2 H), 7.50 (s, 1 H), 7.44 (d,  $J = 8.4$  Hz, 2 H), 7.42-7.41 (m, 1 H), 7.19-7.16 (m, 2 H), 5.51 (s, 1 H), 4.84 (d,  $J = 15.4$  Hz, 1 H), 4.69 (d,  $J = 15.6$  Hz, 1 H), 4.12-4.00 (m, 2 H), 2.96 (s, 3 H), 1.60 (s, 9 H), 1.10 (t,  $J = 7.1$  Hz, 3 H); HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $C_{25}H_{32}O_6N_3Cl_2S$   $[M+H]^+$  572.13834, found 572.13967.

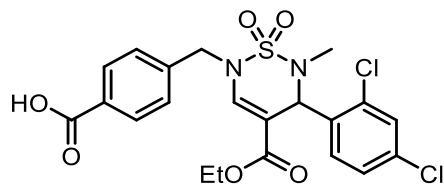


**(+)-2-52**

**4-((5-(2,4-Dichlorophenyl)-4-(ethoxycarbonyl)-6-methyl-1,1-dioxido-5,6-dihydro-2H- 1,2,6-thiadiazin-2-yl)methyl)benzoic acid ((+)-2-52).** A solution of *tert*-butyl ester (+)-2-52i (0.102 g, 0.184 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3.5 mL) was treated with TFA (0.136 mL, 1.84 mmol). The reaction mixture was stirred at rt under N<sub>2</sub>. After 7 h, TLC (2:1 Hex: EtOAc) indicated reaction completion. The mixture was treated with H<sub>2</sub>O (5 mL) and sat. NaHCO<sub>3</sub> (5 mL), pH ~ 7-8, transferred to a separatory funnel, and the organic layer was washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under vacuum to provide (+)-2-52 (0.0758 g, 83%) as a beige solid: [α]<sub>D</sub> +40.9 (*c* 0.085, MeOH); Mp 92-94 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 2929, 1697, 1629, 1378, 1281, 1169 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 12.99 (s, 1 H), 7.97 (d, *J* = 8.4 Hz, 2 H), 7.96 (s, 1 H), 7.64 (d, *J* = 2.1 Hz, 1 H), 7.52 (d, *J* = 8.2 Hz, 2 H), 7.37 (dd, *J* = 8.5, 2.1 Hz, 1 H), 7.19 (d, *J* = 8.5 Hz, 1 H), 5.49 (s, 1 H), 5.02 (d, *J* = 15.9 Hz, 1 H), 4.91 (d, *J* = 16.0 Hz, 1 H), 4.12-3.94 (m, 2 H), 2.87 (s, 3 H), 1.07 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 167.0, 164.6, 142.4, 141.3, 134.6, 134.1, 133.2, 131.5, 130.4, 129.7, 128.6, 128.0, 126.6, 100.1, 62.9, 60.1, 52.1, 14.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>21</sub>H<sub>21</sub>O<sub>6</sub>N<sub>2</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 499.04919, found 499.04870.

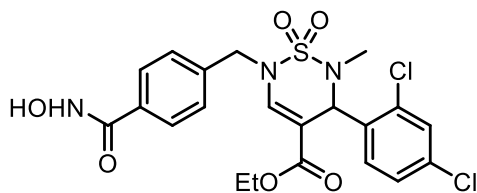


**Ethyl 6-(4-(*tert*-butoxycarbonyl)benzyl)-3-(2,4-dichlorophenyl)-2-methyl-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide ((-)-2-52i).** To a suspension of (-)-2-50 (0.0580 g, 0.159 mmol) and  $K_2CO_3$  (0.132 g, 0.953 mmol) in acetonitrile (2.5 mL) was added 4-bromomethyl benzoic acid mono *tert*-butyl ester (0.0431 g, 0.159 mmol). The reaction mixture was stirred at rt under  $N_2$ . After 23 h, TLC (5:1 Hex:EtOAc) indicated consumption of SM, and the mixture was suspended in  $H_2O$  (15 mL) and EtOAc (15 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with sat.  $NaHCO_3$  (10 mL) and brine (10 mL), dried ( $Na_2SO_4$ ), filtered, and concentrated to provide (-)-2-52i (0.0880 g, 100%) as a white solid:  $^1H$  NMR (300 MHz;  $CDCl_3$ )  $\delta$  8.03 (d,  $J = 8.2$  Hz, 2 H), 7.50 (s, 1 H), 7.44 (d,  $J = 8.2$  Hz, 2 H), 7.42-7.41 (m, 1 H), 7.19-7.17 (m, 2 H), 5.51 (s, 1 H), 4.84 (d,  $J = 15.5$  Hz, 1 H), 4.69 (d,  $J = 15.5$  Hz, 1 H), 4.13-3.98 (m, 2 H), 2.96 (s, 3 H), 1.60 (s, 9 H), 1.10 (t,  $J = 7.1$  Hz, 3 H); HRMS (ESI $^+$ )  $m/z$  calcd for  $C_{25}H_{32}O_6N_3Cl_2S$   $[M+H]^+$  572.13834, found 572.13947.



**(-)-2-52**

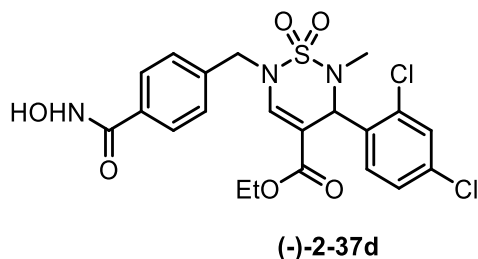
**4-((5-(2,4-Dichlorophenyl)-4-(ethoxycarbonyl)-6-methyl-1,1-dioxido-5,6-dihydro-2H- 1,2,6-thiadiazin-2-yl)methyl)benzoic acid ((-)-2-52).** A solution of *tert*-butyl ester (-)-2-52i (0.0850 g, 0.153 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3.0 mL) was treated with TFA (0.114 mL, 1.53 mmol). The reaction mixture was stirred at rt under N<sub>2</sub>. After 7 h, TLC (2:1 Hex: EtOAc) indicated reaction completion. The mixture was treated with H<sub>2</sub>O (5 mL) and sat. NaHCO<sub>3</sub> (5 mL), pH ~ 7-8, and the organic layer was washed with brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under vacuum to provide (-)-2-52 (0.0697 g, 91%) as a beige solid: [α]<sub>D</sub> -40.7 (*c* 0.086, MeOH); Mp 92-94 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 2924, 1695, 1628, 1376, 1280, 1168 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 12.99 (s, 1 H), 7.97 (d, *J* = 8.4 Hz, 2 H), 7.96 (s, 1 H), 7.64 (d, *J* = 2.1 Hz, 1 H), 7.52 (d, *J* = 8.2 Hz, 2 H), 7.37 (dd, *J* = 8.5, 2.1 Hz, 1 H), 7.19 (d, *J* = 8.5 Hz, 1 H), 5.49 (s, 1 H), 5.02 (d, *J* = 16.0 Hz, 1 H), 4.91 (d, *J* = 16.0 Hz, 1 H), 4.12-3.94 (m, 2 H), 2.87 (s, 3 H), 1.07 (t, *J* = 7.1 Hz, 3 H); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>21</sub>H<sub>21</sub>O<sub>6</sub>N<sub>2</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 499.04919, found 499.04860.



**(+)-2-37d**

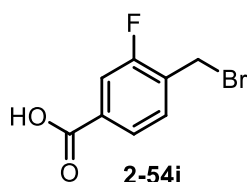
**(+)-Ethyl 3-(2,4-dichlorophenyl)-6-(4-(hydroxycarbonyl)benzyl)-2-methyl-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide ((+)-2-37d).** A solution of carboxylic acid (+)-**2-52** (0.0758 g, 0.152 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.8 mL) was treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.0533 g, 0.456 mmol). The reaction mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 0.136 mL, 0.228 mmol) and TEA (0.0635 mL, 0.455 mmol). After warming to rt and stirring under N<sub>2</sub> for 9 h, the reaction mixture was diluted with EtOAc (8 mL), washed with 0.5 M HCl (8 mL), brine (8 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-100 % EtOAc in hexanes) afforded the amide (0.0616 g, 68%) as a white solid. Characterization was consistent with that of the racemic material. The white solid (0.0600 g, 0.100 mmol) in MeOH (3.0 mL) and CH<sub>2</sub>Cl<sub>2</sub> (0.8 mL) was treated with Amberlyst-15 (0.0430 g, 202 mmol) at rt under N<sub>2</sub>. After 17 h of stirring, the mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc) to afford (+)-**2-37d** (0.0330 g, 64%) as an off-white solid: [ $\alpha$ ]<sub>D</sub> +46.0 (*c* 0.12, MeOH); Mp 105-109 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3241, 3061, 2983, 1670, 1626, 1373, 1167 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  11.23 (s, 1 H), 9.05 (s, 1 H), 7.95 (s, 1 H), 7.77 (d, *J* = 8.1 Hz, 2 H), 7.64 (d, *J* = 1.8 Hz, 1 H), 7.47 (d, *J* = 8.1 Hz, 2 H), 7.37 (dd, *J* = 8.4, 1.8 Hz, 1 H), 7.18 (d, *J* = 8.4 Hz, 1 H), 5.48 (s, 1 H), 4.98 (d, *J* = 15.9 Hz, 1 H), 4.88 (d, *J* = 15.9 Hz, 1 H), 4.09-3.97 (m, 2 H), 2.87 (s, 3 H), 1.07 (t, *J* = 7.1 Hz, 3 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>)  $\delta$  164.6, 163.8, 142.3, 139.4, 134.6, 134.1,

133.2, 132.5, 131.5, 128.7, 127.8, 127.3, 126.6, 100.1, 62.9, 60.1, 52.1, 14.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>21</sub>H<sub>22</sub>O<sub>6</sub>N<sub>3</sub>Cl<sub>2</sub>S [M+H]<sup>+</sup> 514.0601, found 514.0578; LCMS-220 nm purity 97.4%.



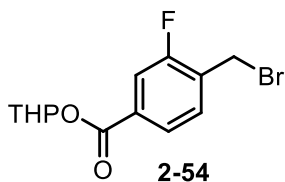
**(-)-Ethyl 3-(2,4-dichlorophenyl)-6-(4-(hydroxycarbonyl)benzyl)-2-methyl-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide ((-)-2-37d).** A solution of carboxylic acid **(-)-2-52** (0.0700 g, 0.140 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.2 mL) was treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.0980 g, 0.837 mmol). The reaction mixture was cooled to 0 °C, and treated with T<sub>3</sub>P (50%, 0.125 mL, 0.210 mmol) and TEA (0.0586 mL, 0.421 mmol). After warming to rt and stirring under N<sub>2</sub> for 14 h, the reaction mixture was diluted with EtOAc (8 mL), washed with 0.5 M HCl (8 mL), brine (8 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-100% EtOAc in hexanes) afforded the amide (0.0510 g, 61%) as a white solid. Characterization was consistent with that of the racemic material. To a solution of the white solid (0.0510 g, 0.0852 mmol) in MeOH (2.5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (0.8 mL) was added Amberlyst-15 (0.0310 g, 146 mmol) at rt under N<sub>2</sub>. After 17 h of stirring, the reaction mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (5:1 hexanes: EtOAc) to afford **(-)-2-37d** (0.0370 g, 84%) as an off-white solid: [α]<sub>D</sub> -43.0 (c 0.12, MeOH); Mp 126-129 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3221, 2983, 1698, 1627, 1375, 1168 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz; DMSO-*d*<sub>6</sub>) δ 11.24 (s, 1 H), 9.07 (s, 1 H), 7.96 (s, 1 H), 7.78 (d, *J* = 8.2 Hz, 2

H), 7.65 (d,  $J = 2.1$  Hz, 1 H), 7.48 (d,  $J = 8.2$  Hz, 2 H), 7.38 (dd,  $J = 8.4, 2.1$  Hz, 1 H), 7.19 (d,  $J = 8.4$  Hz, 1 H), 5.49 (s, 1 H), 4.98 (d,  $J = 15.9$  Hz, 1 H), 4.89 (d,  $J = 15.9$  Hz, 1 H), 4.10-3.97 (m, 2 H), 2.88 (s, 3 H), 1.07 (t,  $J = 7.1$  Hz, 3 H);  $^{13}\text{C}$  NMR (126 MHz; DMSO- $d_6$ )  $\delta$  164.6, 163.9, 142.3, 139.4, 134.6, 134.1, 133.2, 132.5, 131.5, 128.7, 127.8, 127.3, 126.6, 100.1, 62.9, 60.1, 52.1, 14.0; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{21}\text{H}_{22}\text{O}_6\text{N}_3\text{Cl}_2\text{S}$   $[\text{M}+\text{H}]^+$  514.0601, found 514.0581; LCMS-220 nm purity 98.4%.

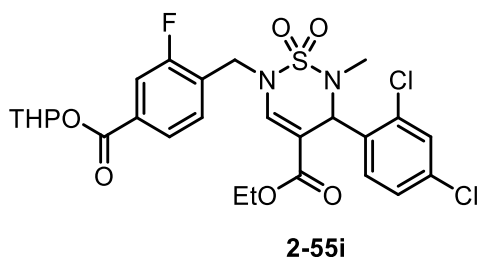


**4-(Bromomethyl)-3-fluorobenzoic acid (2-54i).**<sup>121</sup> A pressure vial was charged with 4-cyano-2-fluorobenzyl bromide (1.13 g, 5.26 mmol) and 48% HBr (18 mL). The vial was flushed with  $\text{N}_2$ , sealed, and heated to 100 °C. After stirring for 15 h, the reaction mixture was cooled to rt, transferred to a separatory funnel, treated with  $\text{H}_2\text{O}$  (100 mL), and extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine/sat.  $\text{NaHCO}_3$  (50 mL/50 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The carboxylic acid **2-54i** (1.12 g, 91%) was collected as a white solid:  $^1\text{H}$  NMR (400 MHz; DMSO- $d_6$ )  $\delta$  13.37 (s, 1 H), 7.77 (dd,  $J = 7.8, 1.5$  Hz, 1 H), 7.69-7.65 (m, 2 H), 4.74 (s, 2 H).



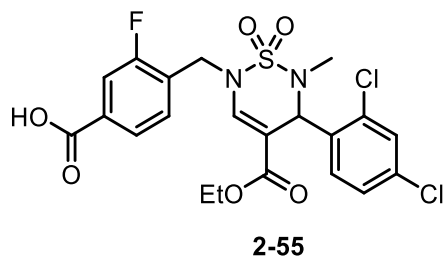


**Tetrahydro-2*H*-pyran-2-yl 4-(bromomethyl)-3-fluorobenzoate (2-54).** To a solution of **2-54i** (0.102 g, 0.430 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) were added 3,4-dihydropyran (0.900 g, 10.7 mmol) and PPTS (0.0510 g, 0.203 mmol). The reaction mixture was stirred at rt under N<sub>2</sub> for 11 h, after which it was treated with sat. NaHCO<sub>3</sub> (10 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to provide **2-54** (quant.) that was used in the next step with no further purification: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.86 (dd, *J* = 8.0, 1.5 Hz, 1 H), 7.76 (dd, *J* = 10.1, 1.5 Hz, 1 H), 7.49 (app t, *J* = 7.7 Hz, 1 H), 6.24 (s, 1 H), 4.52 (s, 2 H), 4.03-3.84 (m, 1 H), 3.80-3.73 (m, 1 H), 2.04-1.54 (m, 6 H); <sup>19</sup>F NMR (300 MHz; CDCl<sub>3</sub>) δ -115.96 (s, 1 F).



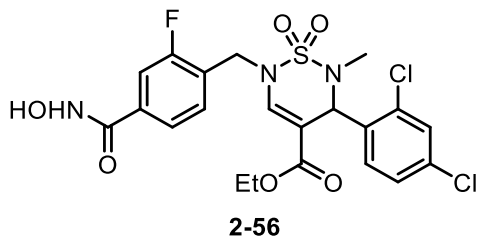
**Ethyl 3-(2,4-dichlorophenyl)-6-(2-fluoro-4-(((tetrahydro-2*H*-pyran-2-yl)oxy)carbonyl)benzyl)-2-methyl-3,6-dihydro-2*H*-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-55i).** To a suspension of thiadiazine **2-50** (0.135 g, 0.370 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.540 g, 3.91 mmol) in acetonitrile (4 mL) was added bromide **2-54** (0.130 g, 0.410 mmol). The reaction mixture was stirred at rt under N<sub>2</sub> for 3 h, diluted with H<sub>2</sub>O (10 mL) and EtOAc (10 mL). The layers were

transferred to a separatory funnel and separated. The aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with sat. NaHCO<sub>3</sub> (1 x 10 mL) and brine (1 x 10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to provide **2-55i** (quant.) that was used with no further purification: <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 8.01 (s, 1 H), 7.92 (dd, *J* = 7.9, 1.6 Hz, 1 H), 7.81 (dd, *J* = 10.1, 1.5 Hz, 1 H), 7.67-7.62 (m, 2 H), 7.37 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.18 (d, *J* = 8.4 Hz, 1 H), 6.15 (s, 1 H), 5.50 (s, 1 H), 5.11 (d, *J* = 16.2 Hz, 1 H), 5.01 (d, *J* = 16.2 Hz, 1 H), 4.14-3.96 (m, 2 H), 3.92-3.81 (m, 1 H), 3.71-3.65 (m, 1 H), 2.88 (s, 3 H), 1.96-1.40 (m, 6 H), 1.09 (t, *J* = 7.1 Hz, 3 H); <sup>19</sup>F NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ -115.64; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>26</sub>H<sub>27</sub>O<sub>7</sub>N<sub>2</sub>Cl<sub>2</sub>FNaS [M+Na]<sup>+</sup> 623.0792, found 623.0804.



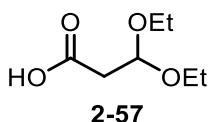
**4-((5-(2,4-Dichlorophenyl)-4-(ethoxycarbonyl)-6-methyl-1,1-dioxido-5,6-dihydro-2H-1,2,6-thiadiazin-2-yl)methyl)-3-fluorobenzoic acid (2-55).** A solution of ester **2-55i** (0.222 g, 0.369 mmol) in MeOH/CH<sub>2</sub>Cl<sub>2</sub> (3 mL/3 mL) was treated with TFA (0.411 mL, 5.54 mmol). The reaction mixture was stirred at rt under N<sub>2</sub> for 1 h, quenched with brine/sat. NaHCO<sub>3</sub> (20 mL/20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated, providing **2-55** (0.142 g, 74%) as a white solid: Mp 99-101 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 2927, 2854, 1699, 1625, 1562, 1377, 1281, 1262, 1168 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 7.92 (s, 1 H), 7.70 (d, *J* = 7.2 Hz, 1 H), 7.63-7.58 (m, 1 H), 7.42 (d, *J* = 7.3 Hz, 1 H), 7.36 (dd, *J* = 8.5, 2.0 Hz, 1 H), 7.17 (d, *J* = 8.4 Hz, 1 H), 5.47 (s, 1 H), 5.00 (d, *J* = 15.7 Hz, 1 H), 4.90 (d, *J* =

15.7 Hz, 1 H), 4.12-3.95 (m, 2 H), 2.85 (s, 3 H), 1.07 (t,  $J = 7.0$  Hz, 3 H);  $^{19}\text{F}$  NMR (300 MHz; DMSO- $d_6$ )  $\delta$  -118.21; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{21}\text{H}_{20}\text{O}_6\text{N}_2\text{Cl}_2\text{FS}$   $[\text{M}+\text{H}]^+$  517.0398, found 517.0386.

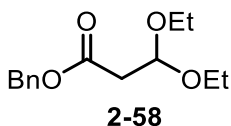


**Ethyl 3-(2,4-dichlorophenyl)-6-(2-fluoro-4-(hydroxycarbonyl)benzyl)-2-methyl-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-56).** A solution of carboxylic acid **2-55** (0.110 g, 0.213 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) was treated with O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.124 g, 1.06 mmol). The mixture was cooled to 0 °C and treated with T<sub>3</sub>P (50% in EtOAc, 0.250 mL, 0.420 mmol) and TEA (0.129 mL, 1.28 mmol). The reaction mixture was warmed to rt, and stirred under  $\text{N}_2$ . After 5 h, the mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (10 mL), washed with 0.25 M HCl (10 mL), brine (10 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered through a pad of Celite, and concentrated. The residue was dissolved in MeOH (3.0 mL) and  $\text{CH}_2\text{Cl}_2$  (0.5 mL), and treated with Amberlyst-15 (0.123 g, 578 mmol) at rt under  $\text{N}_2$ . After 11 h of stirring, the reaction mixture was filtered through Celite, rinsed with MeOH, and concentrated. The residue was purified by trituration (3:1 hexanes: EtOAc) to afford **2-56** (0.102 g, 90%) as a white solid: Mp 95-98 °C; IR ( $\text{CH}_2\text{Cl}_2$ ) 3218, 3069, 2983, 2935, 1695, 1625, 1373, 1167  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz; DMSO- $d_6$ )  $\delta$  11.34 (s, 1 H), 9.18 (s, 1 H), 7.97 (s, 1 H), 7.634 (s, 1 H), 7.625 (d,  $J = 10.9$  Hz, 1 H), 7.58 (d,  $J = 10.9$  Hz, 1 H), 7.54 (app t,  $J = 7.6$  Hz, 1 H), 7.37 (d,  $J = 8.2$  Hz, 1 H), 7.17 (d,  $J = 8.3$  Hz, 1 H), 5.49 (s, 1 H), 5.05 (d,  $J = 15.9$  Hz, 1 H), 4.95 (d,  $J = 15.9$  Hz, 1 H), 4.11-3.98 (m, 2 H), 2.87 (s, 3

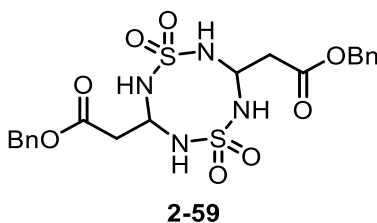
H), 1.08 (t,  $J = 7.0$  Hz, 3 H);  $^{19}\text{F}$  NMR (300 MHz; DMSO- $d_6$ )  $\delta$  -116.21;  $^{13}\text{C}$  NMR (126 MHz; DMSO- $d_6$ )  $\delta$  164.6, 162.4, 160.0 (d,  $J = 247.9$  Hz), 142.6, 134.94 (d,  $J = 6.7$  Hz), 134.5, 134.1, 133.2, 131.5, 130.74 (d,  $J = 3.5$  Hz), 128.6, 126.6, 126.19 (d,  $J = 14.9$  Hz), 123.1, 114.01 (d,  $J = 23.2$  Hz), 99.9, 62.9, 60.1, 47.4, 14.0; HRMS (ESI $^+$ )  $m/z$  calcd for  $\text{C}_{21}\text{H}_{21}\text{O}_6\text{N}_3\text{Cl}_2\text{FS}$   $[\text{M}+\text{H}]^+$  532.0498, found 532.0507; LCMS-220 nm purity 100%.



**3,3-Diethoxypropanoic acid (2-57).**<sup>122</sup> A suspension of ethyl 3,3-diethoxypropanoate (6.61 g, 34.7 mmol) and NaOH (1.42 g, 35.6 mmol) in  $\text{H}_2\text{O}$  (15 mL) was stirred in a sealed tube at 100 °C for 1.5 h. After cooling to 0 °C, HCl (aq., 37%) was added dropwise until the solution reached pH 2-3. The product was extracted with EtOAc (4 x 80 mL). After each extraction, the aqueous fraction was acidified to pH 3 using HCl (aq., 37%). The combined organic layers were washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated to provide 3,3-Diethoxypropanoic acid **2-57** (5.24 g, 93%) as a yellow oil:  $^1\text{H}$  NMR (300 MHz; DMSO- $d_6$ )  $\delta$  12.23 (s, 1 H), 4.82 (t,  $J = 5.9$  Hz, 1 H), 3.64-3.54 (m, 2 H), 3.52-3.42 (m, 2 H), 2.51 (d,  $J = 5.9$  Hz, 2 H), 1.10 (t,  $J = 7.1$  Hz, 6 H).

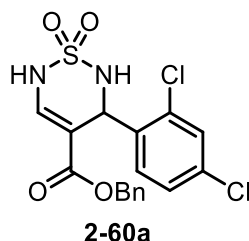


**Benzyl 3,3-diethoxypropanoate (2-58).**<sup>123</sup> DMAP (1.01 g, 8.29 mmol) and benzyl alcohol (6.00 mL, 57.7 mmol) were added to a solution of acid **2-57** (5.20 g, 32.1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (75 mL). The reaction mixture was cooled down to 0 °C, treated with DCC (6.96 g, 33.7 mmol), and warmed to rt. After 16 h of stirring, the resulting precipitate was filtered out under Celite, and the filtrate was diluted with more CH<sub>2</sub>Cl<sub>2</sub> (overall volume of ~ 150 mL). The organic layer was washed with sat. NaHCO<sub>3</sub> (150 mL) and brine (150 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered through a pad of Celite, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-18% EtOAc in hexanes) afforded the ester **2-58** (5.10 g, 63%) as a yellow oil: <sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ 7.36-7.31 (m, 5 H), 5.14 (s, 2 H), 4.96 (t, *J* = 5.9 Hz, 1 H), 3.73-3.61 (m, 2 H), 3.57-3.47 (m, 2 H), 2.72 (d, *J* = 5.9 Hz, 2 H), 1.17 (t, *J* = 7.1 Hz, 6 H).



**Dibenzy 2,2'-(1,1,5,5-tetraoxido-1,5,2,4,6,8-dithiatetrazocane-3,7-diyl)diacetate (2-59).** A solution of sulfamide (1.77 g, 18.4 mmol) and acetal **2-59** (5.10 g, 20.2 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (34 mL) was treated with TFA (6.80 mL, 91.5 mmol) dropwise. After 17 h of stirring at 22 °C under N<sub>2</sub>, the suspension containing the white precipitate was treated with CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and diethyl ether (40 mL), in which more precipitate formed. The suspension was filtered, and drying under vacuum afforded the dithiatetrazocane (3.52 g, 75%) as a white solid: Mp 166-167 °C; IR (CH<sub>2</sub>Cl<sub>2</sub>) 3315, 1720, 1635, 1448 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz; DMSO-*d*<sub>6</sub>) δ 7.62 (d, *J* = 8.2 Hz, 4 H), 7.42-7.30 (m, 10 H), 5.24 (bs, 2 H), 5.11 (s, 4 H), 2.75 (d, *J* = 7.1 Hz, 4 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ

168.5, 135.9, 128.4, 127.9, 127.8, 65.8, 62.0, 40.9; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>20</sub>H<sub>23</sub>O<sub>8</sub>N<sub>4</sub>S<sub>2</sub> [M-H]<sup>+</sup> 511.0952, found 511.0963.



**Benzyl 3-(2,4-dichlorophenyl)-3,6-dihydro-2H-1,2,6-thiadiazine-4-carboxylate 1,1-dioxide (2-60a).** A 250 mL 3-neck RBF was charged with dimer **2-59** (3.43 g, 6.68 mmol) and 2,4-dichlorobenzaldehyde (2.35 g, 13.4 mmol). The flask was evacuated/refilled with N<sub>2</sub> (3x). HFIP (35 mL) was added to the mixture, followed by dropwise addition of TFA (2.50 mL, 33.7 mmol). The mixture was stirred under N<sub>2</sub> at 40 °C for 20 h, with the mixture gradually changing from a heterogenous white mixture to a homogenous yellow solution. The reaction mixture was cooled to rt, and transferred to an Erlenmeyer flask. EtOAc (75 mL) and NaHCO<sub>3</sub> (75 mL) were added and the biphasic layer was stirred vigorously for 10 min. The mixture was transferred to a separatory funnel, and the EtOAc layer was washed with water (2 x 40 mL) and brine (2 x 40 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. Purification by chromatography on SiO<sub>2</sub> (0-33% EtOAc in hexanes) provided a mixture of desired product **2-60a** and byproduct **2-60b** in a 4:1 ratio (778 mg). A solution of **2-60a** and **2-60b** (4: 1, 670 mg) in THF (10 mL) was treated with 1 M NaOH (1.60 mL, 1.60 mmol). The reaction mixture was stirred for 6 h at rt, cooled to 0 °C, and acidified with 6 M HCl until pH = 2. The aqueous layer was extracted with EtOAc (3 x 50 mL), and the combined organic layers were washed with brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The residue was filtered through a pad of SiO<sub>2</sub> (50% EtOAc

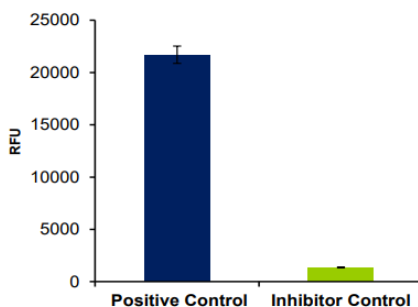
in hexanes) to provide **2-60a** (0.470 g, 17%, 2 steps) as a white solid: Mp 63 °C (dec.); IR (CH<sub>2</sub>Cl<sub>2</sub>) 3276, 1691, 1634, 1431 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz; DMSO-*d*<sub>6</sub>) δ 11.1 (s, 1 H), 8.23 (d, *J* = 7.2 Hz, 1 H), 7.67 (s, 1 H), 7.61 (s, 1 H), 7.35-7.23 (m, 5 H), 7.10 (br s, 2 H), 5.63 (d, *J* = 7.1 Hz, 1 H), 5.10 (d, *J* = 12.8 Hz, 1 H), 4.97 (d, *J* = 12.8 Hz, 1 H); <sup>13</sup>C NMR (126 MHz; DMSO-*d*<sub>6</sub>) δ 164.5, 140.4, 136.2, 135.0, 133.9, 133.1, 131.5, 128.7, 128.2, 127.8, 127.2, 126.6, 100.8, 65.1, 54.0; HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>17</sub>H<sub>13</sub>O<sub>4</sub>N<sub>2</sub>Cl<sub>2</sub>S [M-H]<sup>+</sup> 410.9968, found 410.9980.

## Appendix A HDAC Assays

The HDAC Assays were conducted by Andrea Topacio, following the protocol below.

For the pan HDAC assay, kits from BioVision Incorporated (<https://www.biovision.com/>) were used and their recommended protocols followed: “HDAC Assay Protocol: **1)** Screen compounds, Inhibitor Control and Positive Control Preparations: Dissolve candidate inhibitors into proper solvent. Dilute to 2X the desired test concentration with ddH<sub>2</sub>O. Add 50 µl of diluted candidate inhibitor into well(s). For Positive Control, add 50 µl ddH<sub>2</sub>O only. For Negative Control, add 48 µl of ddH<sub>2</sub>O and 2 µl of Trichostatin A. **2)** Reaction Mix Preparation: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing: 10X HDAC Assay Buffer (10 µl), HeLa Nuclear Extract (2 µl), HDAC Substrate (5 µl), ddH<sub>2</sub>O (33 µl). Mix well. Add 50 µl of the Reaction Mix into each well. Mix well. Incubate plate at 37 °C for 30 min (or longer if desired). **3)** Stop the reaction by adding 10 µl of Lysine Developer and mix well. Incubate the plate at 37°C for 30 min. **4)** Measurement: Read sample in a fluorescence plate reader with Ex. = 350-380 nm and Em. = 440-460 nm. Signal should be stable for several hours at RT.

$$\text{Activity remaining with candidate compounds} = \frac{\text{RFU of Candidate}}{\text{RFU of Positive Control}} \times 100\%$$





5) Calculation: Set the RFU of Positive Control as the 100%, and calculate the relative activity remains with candidate compounds as follow.”

For HDAC 4-8 assays, kits from BPS Bioscience (<https://bpsbioscience.com/>) were used and their recommended protocols followed: “**ASSAY PROTOCOL: Immediately prior to assay: 1)** Dilute Trichostatin A 1 mM stock 10-fold with HDAC Assay Buffer to make a 100  $\mu$ M solution. Make only sufficient quantity needed for the assay; store remaining 1 mM Trichostatin A stock solution in aliquots at  $-80^{\circ}\text{C}$ . **2)** Dilute HDAC substrate 5 mM stock 250-fold with HDAC Assay Buffer to make a 20  $\mu$ M solution. (Make only sufficient quantity needed for the assay; store remaining 5 mM stock solution in aliquots at  $-80^{\circ}\text{C}$ ). **3)** Dilute HDAC4 in HDAC Assay Buffer to 12 pg/ $\mu$ l (60 pg/reaction)\*. Aliquot any remaining enzyme and store undiluted at  $-80^{\circ}\text{C}$ . Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use. \*Note: optimal enzyme concentration may vary with the specific activity of the enzyme.

**Step 1:** In duplicate, add the reaction mixtures (below) to the microtiter black plate as follows: **1)** Prepare the master mixture: N wells  $\times$  (5  $\mu$ l HDAC substrate (20  $\mu$ M) + 5  $\mu$ l BSA (1 mg/ml) + 30  $\mu$ l HDAC Assay Buffer). Add 40  $\mu$ l of master mixture to all wells. **2)** Add 5  $\mu$ l of inhibitor solution of each well designated “Test Inhibitor.” For the “Positive Control” and “Blank,” add 5  $\mu$ l of 10% DMSO in water (inhibitor buffer). Add 5  $\mu$ l of diluted Trichostatin A (100  $\mu$ M) to the wells designated "Inhibitor Control." Keep final DMSO concentration at or below 1%. **3)** Add 5  $\mu$ l of HDAC Assay Buffer to the wells designated "Blank." **4)** Initiate reaction by adding 5  $\mu$ l of diluted HDAC4 enzyme to the wells designated “Positive Control,” "Test Inhibitor," and "Inhibitor Control." Incubate at  $37^{\circ}\text{C}$  for 30 min.

	“Blank”	Positive Control	Test Inhibitor	Inhibitor Control
HDAC substrate (20 $\mu$ M)	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l
BSA (1 mg/ml)	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l
HDAC Assay Buffer	35 $\mu$ l	30 $\mu$ l	30 $\mu$ l	30 $\mu$ l
Diluted Trichostatin A (100 $\mu$ M)	-	-	-	5 $\mu$ l
Test Inhibitor	-	-	5 $\mu$ l	-
10% DMSO in water (Inhibitor buffer)	5 $\mu$ l	5 $\mu$ l	-	-
Diluted HDAC4 (0.012 ng/ $\mu$ l)	-	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l
<b>Total</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>

**Step 2:** Add 50  $\mu$ l of undiluted HDAC Developer (2x) to each well. Incubate the plate at room temperature for 15 minutes. **Step 3:** Read sample in a microtiter plate-reading fluorimeter capable of excitation at a wavelength in the range of 350-380 nm and detection of emitted light in the range of 440-460 nm. “Blank” value is subtracted from all other values.”

For a summary of results, see **Tables 6** and **7**. Each compound was dissolved in DMSO to generate a 100  $\mu$ M stock solution, then diluted using HPLC-grade water to prepare 10  $\mu$ M, 2  $\mu$ M, and 1  $\mu$ M solutions. These solutions were used for the assays. For the pan HDAC assay, samples were subjected to an additional 2x dilution and for HDAC 4, 5, 6, 7, and 8 assays the additional dilution was 10x. The standards Trichostatin A (TSA) and Vorinostat (SAHA) were measured each time an HDAC assay was performed (**Table 8**). Assays utilized a BioTek Synergy H1 microplate reader and black Nunc MicroWell 96-well optical-bottom plates with polymer base.

Pan HDAC: 10  $\mu$ L of each diluted compound (10  $\mu$ M) and 40  $\mu$ L of HPLC-grade water were mixed and added into a well on the plate. Different concentrations of TSA and SAHA (for standard curves) were added to their respective wells. 50  $\mu$ L of HPLC-grade water was added to each positive control well. Then, 50  $\mu$ L of the reaction mixture was added to each well and the the solution was mixed thoroughly. The reaction mixture consisted of 500  $\mu$ L of 10x HDAC Assay

buffer, 100  $\mu\text{L}$  of HeLa nuclear extract, 250  $\mu\text{L}$  of HDAC substrate, and 1.65 mL of HPLC-grade water. The plate was then warmed in an incubator at 37  $^{\circ}\text{C}$  with a rocker platform and was incubated for 30 min. After the incubation, 10  $\mu\text{L}$  of Lysine Developer solution was added to each well. The plate was kept in the incubator for an additional 30 min. Afterwards, the plate was analyzed using a BioTek Synergy H1 microplate reader, taking two independent readings per well that were subsequently averaged.

HDAC 4 (and, by analogy, HDAC 5, 6, 7, and 8): 40  $\mu\text{L}$  of the parent solution was added to each well on the plate. The parent solution was prepared from a fluorogenic HDAC substrate, a 1 mg/mL solution of bovine serum albumin (BSA) in water, and HDAC assay buffer. 5  $\mu\text{L}$  of the inhibitor buffer (10% DMSO in water) was added to the wells designated as “Blank” and “Positive Control” (no inhibitor). 5  $\mu\text{L}$  of the test compound was added to each well designated as “Test Inhibitor”. Different concentrations of TSA (for a standard curve) was added to each well designated as “Standard.” 5  $\mu\text{L}$  of HDAC assay buffer was added to the “Blank” wells. Then, 5  $\mu\text{L}$  of HDAC 4 human recombinant enzyme was added to the wells designated as “Positive Control”, “Test Inhibitor”, and “Standard.” The plate was then warmed in an incubator at 37  $^{\circ}\text{C}$  with a rocker platform for 30 min. After the incubation, 50  $\mu\text{L}$  of 2x HDAC Developer solution was added to each well. The plate was returned to the incubator and was shaken on the rocker for an additional 15 min. at room temperature. Afterwards, the plate was analyzed using a BioTek Synergy H1 microplate reader, taking two independent readings per well that were subsequently averaged.

**Table 6.** Percent Inhibition in pan HDAC, HDAC 4, and HDAC 7 Assays.

<b>Entry</b>	<b>ID</b>	<b>Pan HDAC at 1 <math>\mu</math>M</b>	<b>HDAC 4 at 1 <math>\mu</math>M</b>	<b>HDAC 7 at 1 <math>\mu</math>M</b>
1	<b>2-29</b>	NIA	NIA	1%
2	<b>2-42</b>	NIA	NIA	NIA
3	<b>2-32</b>	1%	5%	NIA
4	<b>2-37a</b>	22%	23%	40%
5	<b>2-30</b>	NIA	NIA	NIA
6	<b>2-37b</b>	18%	0%	0%
7	<b>(+)-2-37b</b>	15%	13%	32%
8	<b>(-)-2-37b</b>	13%	11%	NIA
9	<b>2-37d</b>	NIA	9%	4%
10	<b>2-37c</b>	NIA	0%	1%
11	<b>2-37e</b>	11%	NIA	3%
12	<b>2-34</b>	NIA	NIA	6%
13	<b>2-33</b>	NIA	9%	4%
14	<b>2-44</b>	0%	3%	NIA
15	<b>2-41</b>	NIA	10%	NIA

**Table 7.** Percent Inhibition in HDAC 5, HDAC 6, and HDAC 8 Assays.

<b>Entry</b>	<b>ID</b>	<b>HDAC 5 at 200 nM</b>	<b>HDAC 6 at 200 nM</b>	<b>HDAC 6 at 100 nM</b>	<b>HDAC 8 at 1 <math>\mu</math>M</b>	<b>HDAC 8 at 200 nM</b>
1	<b>2-29</b>	NIA	NIA	NIA	58%	63%
2	<b>2-42</b>	9%	83%	NIA	35%	40%
3	<b>2-32</b>	NIA	19%	NIA	50%	42%
4	<b>2-37a</b>	19%	8%	33%	66%	50%
5	<b>2-30</b>	0%	NIA	NIA	64%	50%
6	<b>2-37b</b>	NIA	NIA	NIA	62%	51%
7	<b>(+)-2-37b</b>	NIA	11%	17%	NIA	48%
8	<b>(-)-2-37b</b>	27%	8%	22%	24%	64%
9	<b>2-37d</b>	NIA	NIA	6%	72%	56%
10	<b>2-37c</b>	NIA	NIA	13%	50%	44%
11	<b>2-37e</b>	24%	NIA	NIA	48%	48%
12	<b>2-34</b>	11%	1%	NIA	7%	39%
13	<b>2-33</b>	21%	NIA	NIA	22%	36%
14	<b>2-44</b>	NIA	3%	20%	NIA	28%
15	<b>2-41</b>	1%	26%	NIA	31%	31%

**Table 8.** Percent HDAC Inhibition of the Positive Controls, Trichostatin A (TSA) and Vorinostat (SAHA).

NIA = no inhibitory activity noted.

Entry	HDAC	Standard	Concentration (uM)	Percent Inhibition	
1	Pan	TSA	1	68%	
			0.1	49%	
			0.05	33%	
			0.005	NIA	
			0.0025	NIA	
			SAHA	1	29%
				0.1	3%
				0.05	NIA
				0.005	NIA
				0.0025	NIA
2	4	TSA	10	77%	
			7	67%	
			3	35%	
			1	17%	
			0.5	NIA	
3	5	TSA	10	85%	
			5	73%	
			2	63%	
			1	41%	
			0.5	20%	
			0.2	NIA	
4	6	SAHA	0.5	94%	
			0.1	82%	
			0.01	47%	
			0.005	25%	
			0.001	6%	
5	7	TSA	5	83%	
			1	37%	
			0.5	NIA	

Entry	HDAC	Standard	Concentration (uM)	Percent Inhibition
6	8	TSA	0.1	NIA
			0.01	9%
			10	97%
			5	87%
			1	80%
			0.1	67%
			0.01	51%
		SAHA	5	96%
			2	86%
			1	70%
			0.2	67%
			0.05	54%

In Vitro Biological Activity: **Trichostatin:** 1) inhibits HDAC activity in multiple breast cancer cell lines (IC<sub>50</sub> 0.6-2.6 nM, mean 2.4 nM), and results in increased H4 hyperacetylation.<sup>124</sup> 2) HDAC1(6.0 ±2.5 nM); HDAC4 (38 ± 4 nM) ; HDAC6 (8.6 ± 1.4 nM)<sup>125</sup>; **SAHA:** 1) HDAC1 (10 nM); HDAC3 (20 nM)<sup>126</sup>

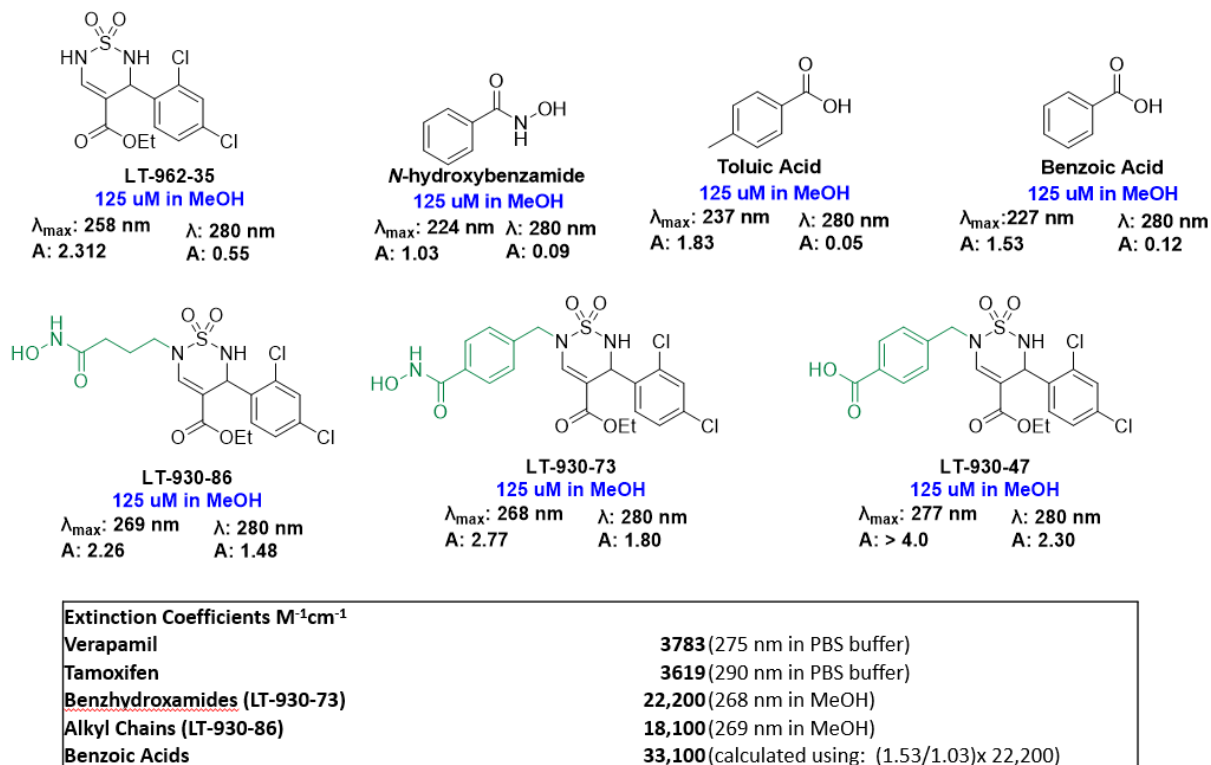
## Appendix B Kinetic Aqueous Solubility

The assay was run following the protocol in *J. Med. Chem.* **2019**, *62*, 5470-5500:<sup>108</sup> Kinetic Aqueous Solubility in PBS (pH 7.4); UV detection: 280 nm; Positive Control: Verapamil HCl; Negative Control: Tamoxifen. **Method: 1)** The test compounds were prepared as 10 mM stock solutions in DMSO. **2)** The stock solutions were diluted 100x with PBS (0.01 M) pH 7.4 to make 100  $\mu$ M solutions. **3)** The suspensions were shaken at 150 rpm for 1 hour at room temperature. **4)** The suspensions were then transferred to a 0.45  $\mu$ m hydrophilic PVDF 96 - well filter plate mounted on a fresh 2 mL 96 - well plate and were filtered by centrifugation at 2,150 rpm for 2 min using the Genevac centrifuge option. **4)** 150  $\mu$ L of filtrates were then transferred to a 96 - well UV plate for absorbance measurement at 280 nm.

The assay results for the controls were as follows: Positive control: Verapamil HCl, purchased from BIOMOL – (UV at 280 nm) = 132  $\mu$ M (Reference<sup>108</sup>: 94  $\mu$ M; theoretical: 100  $\mu$ M); Negative Control: Tamoxifen, purchased from TOCRIS (biotechnne brand) – (UV at 280 nm) = 22  $\mu$ M (Reference<sup>108</sup>: <1.6  $\mu$ M).

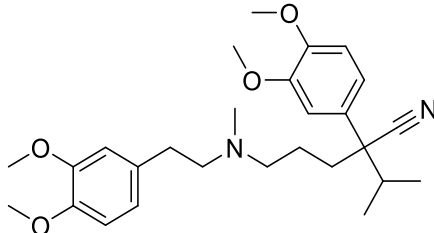
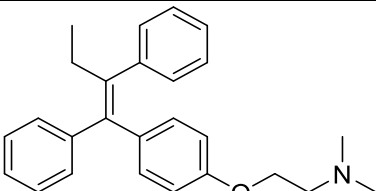
The calculations were completed using Beer's law:  $A = \epsilon \times c \times l$ , with the Absorbance (A) obtained from the plate reader, and the path length (l) of the well measured to be 0.39 cm, we determined the extinction coefficient ( $\epsilon$ ) of fragments of our samples in MeOH using the UV-Vis spectrophotometer. For Verapamil, the extinction coefficient ( $\epsilon$ ) was calculated in PBS buffer using the UV-Vis spectrophotometer. Verapamil  $\epsilon = 3,783 \text{ M}^{-1}\text{cm}^{-1}$  at 280 nm. (Reference<sup>127</sup> : 3,400  $\text{M}^{-1}\text{cm}^{-1}$  at 275 nm). For Tamoxifen, the extinction coefficient ( $\epsilon$ ) was obtained from the literature<sup>128</sup>, 3619  $\text{M}^{-1}\text{cm}^{-1}$  at 290 nm.

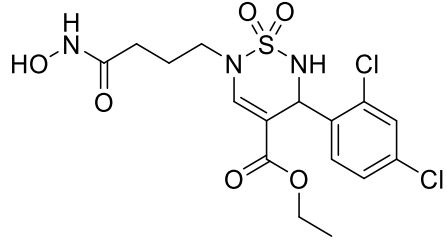
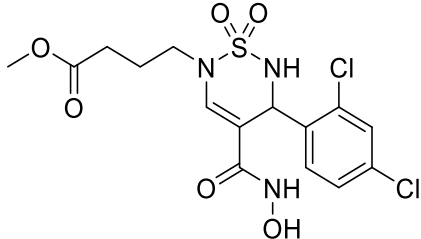
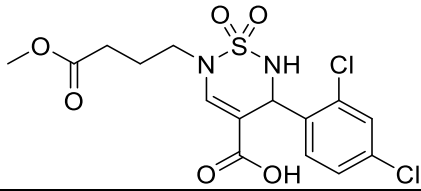
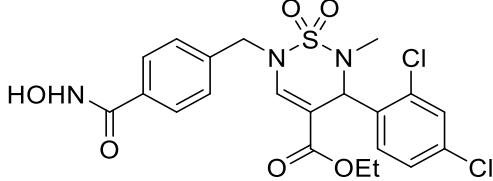
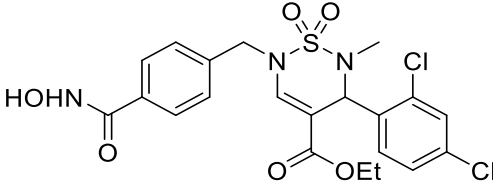
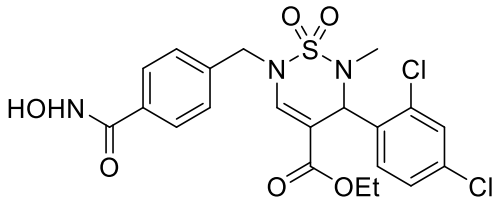
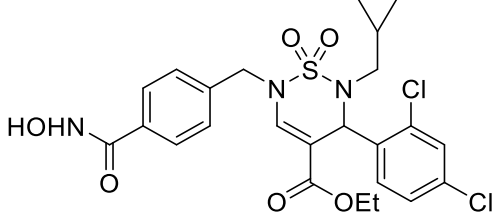


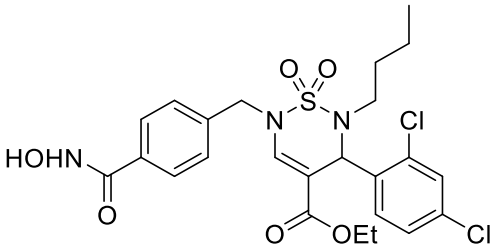
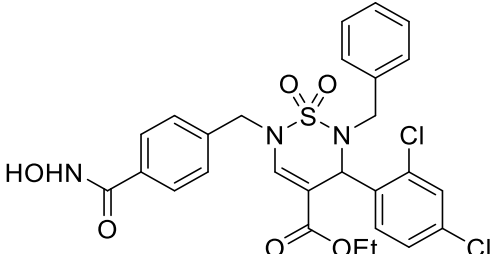
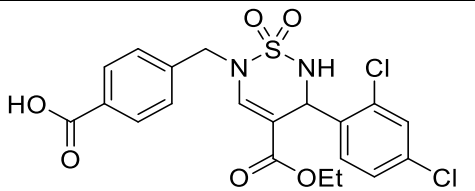
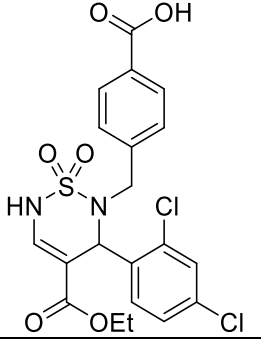
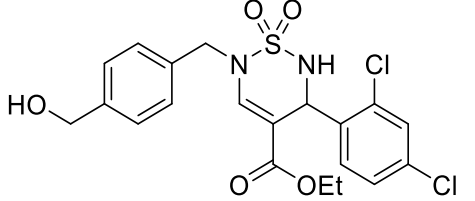
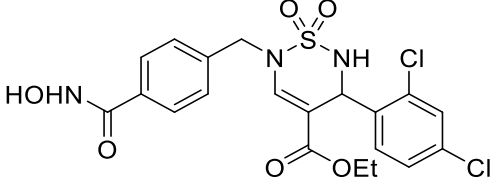


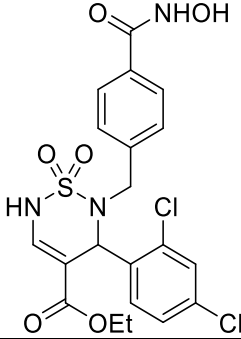
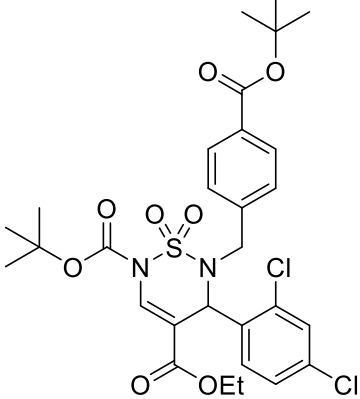
**Figure 38.** Determination of Molar Extinction Coefficients.

**Table 9.** Kinetic Aqueous Solubility Assay Results.

Entry	ID	Structure	Calculated Kinetic Aqueous Solubility ( $\mu$ M) <sup>a</sup>
1	<b>Verapamil (+ control)</b>		132 $\pm$ 3.8
2	<b>Tamoxifen (- control)</b>		22 $\pm$ 5.8

Entry	ID	Structure	Calculated Kinetic Aqueous Solubility ( $\mu\text{M}$ ) <sup>a</sup>
3	2-32		$77 \pm 7.8$
4	2-30		$46 \pm 16.0$
5	2-29		$21 \pm 7.1$
6	2-37b		$9 \pm 0.5$
7	(+)-2-37b		$14 \pm 2.4$
8	(-)-2-37b		$10 \pm 1.1$
9	2-37d		$0 \pm 0.1$

Entry	ID	Structure	Calculated Kinetic Aqueous Solubility ( $\mu\text{M}$ ) <sup>a</sup>
10	2-37c		$0 \pm 0.2$
11	2-37e		$1.0 \pm 0.4$
12	2-34		$49 \pm 11.0$
13	2-42		$36 \pm 3.7^b$
14	2-33		$18 \pm 5.6$
15	2-37a		$60 \pm 3.7$

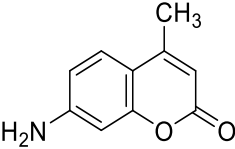
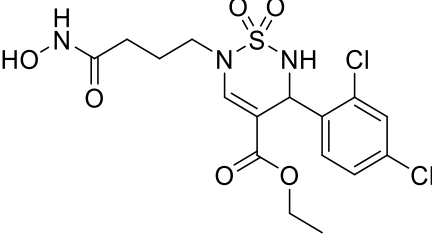
Entry	ID	Structure	Calculated Kinetic Aqueous Solubility ( $\mu\text{M}$ ) <sup>a</sup>
16	2-44		71 ± 11.5
17	2-41		11 ± 9.1

<sup>a</sup> Calculations are from an average of triplicate measurements ( $\pm$  SD), unless otherwise stated. <sup>b</sup> Calculations are from an average of duplicate measurements.

## Appendix C Fluorescence Measurements

The protocol for the fluorescence measurements was as follows: **1)** 100  $\mu\text{M}$  stock solutions of the samples were prepared in 100% DMSO. **2)** The stock solutions were diluted (100x) with PBS buffer to make 1  $\mu\text{M}$  solutions (1%DMSO/99% PBS Buffer). 7-Amino-4-methyl coumarin (AMC) was prepared to serve as a positive control. A blank sample was prepared (1%DMSO/99% PBS Buffer) was prepared to serve as a negative control. **3)** The samples were shaken at 200 rpm for 30 min. **4)** 200  $\mu\text{L}$  of the samples were transferred to a black Nunc MicroWell 96-well optical-bottom plates with polymer base. The blank (negative control) and AMC (positive control) were transferred into three wells for triplicate measurements. The test compounds were transferred into two wells for duplicate measurements. **5)** The BioTek Synergy H1 microplate reader was used for the fluorescence measurement (Excitation: 365 nm, Emission: 450 nm). **6)** The RFU values of samples were subtracted from the RFU value of the blank, and the results are shown in **Table 10**.

**Table 10.** Fluorescence Assay Results.

Entry	ID	Structure	Fluorescence (RFU) <sup>a</sup>
1	<b>7-Amino-4-methylcoumarin</b>		$8315 \pm 203^b$
2	<b>2-32</b>		$3.5 \pm 2.1$

Entry	ID	Structure	Fluorescence (RFU) <sup>a</sup>
3	2-30		13 ± 8.5
4	2-29		3.0 ± 4.2
5	2-37b		< 1
6	2-37e		0
7	2-37d		< 1
8	2-37c		< 1
9	2-34		0

Entry	ID	Structure	Fluorescence (RFU) <sup>a</sup>
10	2-42		0
11	2-33		16.5 ± 12.0
12	2-37e		0
13	2-44		5.0 ± 5.6
14	2-41	<p style="text-align: center;"><b>2-41</b></p>	10.5 ± 21.9

<sup>a</sup> Calculations are from an average of duplicate measurements (± SD). <sup>b</sup> Calculations are from an average of triplicate measurements.

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