Structure–Activity Relationship Studies of Sphingosine Kinase Inhibitors and Mitochondrial Uncouplers

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Chemistry

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June 20, 2017
Blacksburg, VA

Keywords: Structure–Activity Relationship, Sphingosine Kinase, Sphingosine 1-Phosphate, Mitochondrial Uncoupler, Protonophore, Oxygen Consumption Rate

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Abstract

Sphingosine 1-phosphate (S1P) is a cellular signaling molecule that has been implicated in a variety of diseases including cancer, fibrosis, Alzheimer’s, and sickle cell disease. It is formed from the phosphorylation of sphingosine (Sph) by sphingosine kinase (SphK) and SphK exists as two isoforms—SphK1 and SphK2, which differ with respect to their cellular activity and localization. As the key mediators in the synthesis of S1P, SphKs have attracted attention as viable targets for pharmaceutical inhibition. To validate their potential as therapeutic targets, we aimed to develop potent, selective, and in vivo active inhibitors of SphK.

Herein, we describe the design, synthesis and biological evaluation of SphK2 inhibitors. We first describe the development of six SphK2 inhibitors that assess the utility of replacing lipophilic tail groups with heterocyclic rings. These six compounds demonstrate that the lipid binding pocket for SphK2 cannot accommodate compounds with tail groups that are conformationally restricted or positively charged. We then describe the development of aminothiazole-based analogues of an SphK1-selective inhibitor. A library of 37 aryl-substituted aminothiazole tail groups were synthesized, revealing a structure–activity relationship study that examines electronic effects on the aryl-substituted aminothiazoles and the effect of modifying the amino portion of the aminothiazole. These molecules show surprisingly good potency and selectivity for SphK2. In particular, we highlight 3.20dd (SLC4101431), a biphenyl aminothiazole that is the most potent and selective SphK2 inhibitor to date, with an SphK2 $K_i$ of 90 nM and 100-fold selectivity for SphK2. This molecule’s in vivo activity will also be discussed.
Mitochondrial uncouplers are small molecules that shuttle protons from the intermembrane space to the mitochondrial matrix independent of ATP synthase, which disrupts oxidative phosphorylation and promotes increased nutrient metabolism for homeostasis to be maintained. Consequently, small molecule mitochondrial uncouplers have been pursued as probes for mitochondrial function and as potential therapeutics for the treatment of obesity and type 2 diabetes.

Herein, we describe the design, synthesis, and biological evaluation of small molecule mitochondrial uncouplers. We report a library of 52 compounds that have good mitochondrial uncoupling activity over a wide therapeutic range, including 5.16t (SHC4111522) and 5.17i (SHC4091665), which have EC_{50} values of 0.63 µM and 1.53 µM, respectively, and achieve at least 2-fold increase in oxygen consumption rates relative to basal levels. With these molecules, we demonstrate that pK_{a} and cLogP significantly contribute to uncoupling activity and must be accounted for when developing new generation small molecule mitochondrial uncouplers.
Sphingosine kinase 1 and 2 (SphK1 and SphK2) are enzymes that facilitate the production of the biomolecule sphingosine 1-phosphate (S1P), which plays an essential role in cell growth and survival. However, overproduction of S1P has been linked to a number of diseases including cancer, Alzheimer’s, and sickle cell disease. Therefore, because S1P is involved in these diseases, the amount of available S1P must be controlled. This work describes the design, development, and biological study of over 40 compounds that could be used as potential inhibitors of SphK2 to help control S1P levels and, therefore, hopefully alleviate the effects of disease. In particular, this work describes molecules that probe the SphK2 binding pocket and demonstrates that the molecules cannot be rigid or positively charged when binding to the hydrophobic portion of the SphK2 binding pocket. Additionally, this work describes the most potent and selective reported SphK2 inhibitor to date, $3.20_{dd}$ (SLC4101431).

Mitochondrial uncouplers are compounds that target our body's mitochondria and aim to make ATP production challenging, causing the mitochondria to burn extra energy in the form of glucose and fatty acids to allow normal levels of ATP to be produced. By making the mitochondria burn extra energy, mitochondrial uncouplers have the potential to be treatments for diseases such as obesity and diabetes. This work describes the design, development, and biological study of over 50 mitochondrial uncouplers that are capable of increasing mitochondrial activity over a wide concentration range, including $5.16_{lt}$ (SHC4111522) and $5.17_{lt}$ (SHC4091665), which are very potent and effective uncouplers.
Dedication

For my grandma, Barbara Blanton, the truest Hokie I have ever known.

Acknowledgements

I would first like to thank my advisor Dr. Webster Santos, for all of his support, advice, patience, and guidance. He has been such a wonderful role model and mentor throughout the four-and-a-half years that I have worked for him. I clearly remember him coming to visit my hood one day to discuss the progress of my research. I was having a difficult time purifying one of my compounds. He chuckled and said, “You’ll figure it out.” He then walked away. His response frustrated me at the time but that moment helped me develop my problem-solving skills and become an independent scientist.

I would next like to thank my committee members Dr. Paul Carlier, Dr. Richard Gandour, and Dr. David Kingston for their insights and constructive criticisms. You have helped improve my critical thinking and communication skills and cultivated my scientific curiosity.

In addition, I would like to thank my collaborators Dr. Kevin Lynch and Yugesh Kharel for conducting all of the biological studies for my sphingosine kinase inhibitors. I would also like to thank my collaborators Dr. Kyle Hoehn and Stefan Hargett for conducting all of the biological studies for my mitochondrial uncouplers. I would also like to thank the Santos group—Cheryl Peck, Russell Snead, Hao Li, Ashley Peralta, Yumin Dai, Jacob Murray, Eric Medici, Chris Sibley, Russell Fritzemeier, Ashley Gates, Jose Santiago-Rivera, and Laura Wonilowicz—for all of their support and for providing me with such a fun and wonderful research environment. It has made the tedious and frustrating days bearable. I would like to thank Karen Iannaccone for her patience and help, especially with scheduling meetings and dealing with travel documentation. I would also like to thank Dr. Molly Congdon and Dr. Jessica
Wynn for their guidance and friendship over the years. I am also grateful for Ashley Gates for being the editor-in-chief for several of my dissertation’s chapters and for all of the walks to Starbucks.

I would like to thank Christopher Presley for his unwavering love and support. I am so thankful that graduate school brought you into my life. You are my number one. I would also like to thank my family for their encouragement, faith, and unconditional love. They have always believed in me, especially when I didn’t believe in myself. To my brother Michael, thank you for pushing me to be the best version of myself and for always knowing how to make me laugh. To my parents Floyd and Mary, thank you for your constant love and support and for all of the sacrifices that you have made over the years to provide me with the opportunities that I have now. Everything that I am and have accomplished is because of you. Finally, I would like to thank my grandparents Bob and Barbara Blanton for showing me how to live a life full of kindness, grace, and generosity.
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1 The Chemical Biology of Sphingosine Kinase 2

1.1 Introduction

A key regulator in the production of the sphingolipid sphingosine 1-phosphate (S1P) is sphingosine kinase (SphK). This enzyme exists as two isoforms in humans: sphingosine kinase 1 (SphK1) and sphingosine kinase 2 (SphK2). A multitude of studies have demonstrated SphK1’s role in cell proliferation, growth, and survival pathways.\(^1,2\) Less is known about SphK2’s role in cellular processes, while much of the literature indicates that SphK2 is involved in pro-apoptotic pathways.\(^1,2\) Inhibition or even knockdown of SphK2 in disease-state models has demonstrated that SphK2 may also be proliferative.\(^1,2\) These contradictory roles make SphK2 an attractive enzyme for study. This review will focus on the known chemical biology of SphK2, its role in disease, as well as selective inhibitors for this enzyme.

1.2 Sphingolipids

Sphingolipids are biomolecules associated with the cell membrane that participate in a variety of cellular processes including lipid bilayer regulation, cellular growth and migration, as well as angiogenesis and programmed cell death (apoptosis).\(^3-5\) Common examples of sphingolipids include ceramide (Cer), sphingosine (Sph), and S1P. These three sphingolipids belong to a proposed reversible rheostat (Figure 1.1) that is finely controlled by a set of enzymes that determine cell proliferation or apoptosis.\(^6\) Synthesis of Cer and Sph has been implicated in pro-apoptotic pathways, while the production of S1P leads to pro-survival and growth pathways.
S1P has generated a great deal of interest for study due to its involvement in various cellular pathways that include cell proliferation and survival, as well as inflammation and immune responses.\textsuperscript{1, 7} As seen in Figure 1.1, S1P is produced from the SphK-catalyzed phosphorylation of the primary hydroxyl group of Sph. Once produced, S1P can act either inside or outside of the cell to elicit various cellular responses until it is dephosphorylated by S1P phosphatases (Figure 1.1) to regenerate Sph or irreversibly metabolized into phosphoethanolamine and hexadecenal via S1P lyase (Figure 1.2).\textsuperscript{8}
S1P acts as an extracellular ligand for its G-protein coupled receptors, S1PR1–5, once transported outside of the cell by the spinster 2 transporter. The binding of S1P to any one of its receptors elicits a series of second messenger signals that eventually activate a variety of targets involved in pro-survival processes, including the proteins Rho, Rac, Ras, and JNK along with the MAPK/ERK pathway (Figure 1.3). More recently, S1P receptor signaling has been implicated in insulin signaling, energy homeostasis, and as promoter of chromosome segregation and spindle checkpoint relaxation during mitosis.

Less is known about the intracellular signaling pathways of S1P (Figure 1.3), though S1P is known to bind to and inhibit histone deacetylases 1 and 2 (HDAC 1/2), promoting epigenetic modifications. S1P also stimulates Ca$^{2+}$ release from the endoplasmic reticulum (ER), triggering pro-apoptotic pathways. In addition, S1P can bind to the mitochondrial protein prohibitin 2 to regulate cellular respiration, and it can also bind to the tumor necrosis factor TRAF2, which stimulates E3 ubiquitin ligase activity, promoting proliferative and pro-inflammatory pathways. More recently, intracellular S1P was shown to increase erythrocyte glycolysis and O$_2$ release under hypoxic conditions.
Figure 1.3. Intracellular and extracellular S1P signaling pathways.

Due to its role in a variety of inflammatory and proliferative processes, S1P plays a key role in a variety of diseases, most notably cancer, fibrosis, Alzheimer’s disease, and sickle cell disease.\textsuperscript{8, 25-38} High levels of S1P have been reported in cancer cell lines, mouse models, and cancer patient samples, implicating S1P as a potential mediator for cancer.\textsuperscript{1, 8, 27, 29} The role of S1P in fibrosis is more complex than its role in cancer. Studies have indicated that S1P is a “Janus-faced” participant in fibrosis, having contradictory roles depending on whether it is acting as an intracellular or extracellular ligand.\textsuperscript{3, 37, 38} Intracellular S1P has been implicated as a promoter of anti-fibrotic processes whereas extracellular S1P has been associated with profibrotic pathways.\textsuperscript{3, 37-39} S1P has also been shown to regulate the transmembrane protein BACE-1, which is involved in the production of amyloid-β peptide, a senile plaque commonly associated with Alzheimer’s disease,\textsuperscript{25} and decreased S1P production has been implicated in the progression of Alzheimer’s disease.\textsuperscript{35, 36} Furthermore, elevated S1P levels have been linked to sickle cell disease.\textsuperscript{32, 34} Interestingly, these disease states were commonly reliant upon the expression levels and activity of the S1P-producing enzymes SphK1 and SphK2.\textsuperscript{1, 3, 8, 27, 32, 34, 35} Therefore, controlling S1P synthesis via SphK inhibition may be crucial for the treatment of these respective diseases.
1.4 Sphingosine Kinase

The levels of pro-apoptotic Sph and pro-survival S1P are tightly regulated in the proposed Cer-S1P rheostat. Two key regulators of this rheostat are the highly conserved enzymes SphK1 and SphK2. These two isoforms have an approximate 47% amino acid sequence identity and 83% amino acid sequence similarity. Additionally, both contain five conserved domains (C1–C5) in their sequences, including a catalytic domain in the C1–C3 regions that contains the ATP binding site (Figure 1.4). Although SphK1 and SphK2 share amino-acid-sequence identity and similarity, they differ in their sequence makeup. SphK1 is shorter in sequence, comprising 384 amino acid residues compared to 618 for SphK2. A proline-rich domain and four transmembrane domains contribute to the longer sequence length in SphK2.

![Figure 1.4. SphK1 and SphK2 sequence composition.](image)

Enzymatic activity studies found that SphK1 is substrate specific for D-erythro-sphingosine, the native ligand form of Sph. On the other hand, these same studies found that SphK2 is less substrate-specific than SphK1. D-erythro-sphingosine is not the only substrate for SphK2; D-erythro-dihydrosphingosine, D,L-threo-dihydrosphingosine, and phytosphingosine can also be phosphorylated by SphK2. Not only do SphK1 and SphK2 differ in their substrate affinities, they also differ in their binding affinity for Sph (Kₘ). SphK1 has a Kₘ of 10 µM for Sph, while SphK2 has a Kₘ of 5 µM for Sph.

Knockout experiments in mice have illustrated the relevance of both SphK isoforms despite both sharing the same substrate and function. Double knockout of SphK1 and SphK2 in mice
was found to be embryonically lethal, as this caused vascular and neuronal developmental issues; however, single knockout of either SphK1 or SphK2 in mice was not physiologically detrimental. These results demonstrate that normal cellular function is maintained as long as one isoform is functional.

1.5 **Sphingosine Kinase 1**

1.5.1 **Localization and Regulation**

SphK1 is a cytosolic enzyme that promotes cell proliferation and survival. Post-translational modification studies of SphK1 have shown that phosphorylation by the kinases ERK 1/2 causes SphK1 to transfer from the cytosol to the plasma membrane. This relocation allows SphK1 to synthesize S1P that can be transported out of the cell and bind S1PRs. In addition, phosphorylation of SphK1 by ERK 1/2 causes its activity to increase to 10 times higher than normal. The kinases Lyn and Fyn, epidermal growth factor (EGF), and eukaryotic elongation factor 1A (eEF1A) are also known to elevate SphK1 activity. Upregulation of SphK1 activity, though, has been linked to numerous diseases such as cancer, fibrosis, and sickle cell disease.

1.5.2 **Crystal Structure**

In 2013, Wang et al. published the first crystal structure of human SphK1 as an apoprotein and as a haloprotein (PDB ID 3VZB and 3VZC, respectively). The crystal structure of SphK1 bound to Sph revealed that the binding pocket is J-shaped (Figure 1.5) and that SphK1 substrates likely enter the binding pocket tail-first through a “tunneling mechanism.” The crystal structure also revealed how ATP binds to SphK1, allowing molecular modeling of SphK1 catalytic activity. The proposed model suggests that SphK1’s aspartic acid residue 81 activates Sph by deprotonating the Sph primary hydroxyl group to allow nucleophilic attack on the γ-phosphate.
group of ATP, forming S1P.\textsuperscript{54} Scientists at the pharmaceutical company Pfizer corroborated this report by publishing a crystal structure (PDB ID 4V24) of SphK1 co-crystallized with the SphK1-selective inhibitor 1.1 (PF-543 (Figure 1.6)).\textsuperscript{55} Information gathered from these crystal structures has helped guide the development of SphK inhibitors.\textsuperscript{56-58}

**Figure 1.5.** Crystal structure of SphK1 with Sph docked in the J-shaped binding pocket. Reprinted with permission from reference 54. Copyright 2017 Elsevier.

![Figure 1.5](image1.png)

**Figure 1.6.** SphK1-selective inhibitor 1.1

\[ \text{1.1 (PF-543)} \]

\[ \text{SphK1 } K_i = 3.6 \text{ nM} \]

1.6 **Sphingosine Kinase 2**

1.6.1 **Structure–Function Relationship**

Despite sharing five conserved domains, SphK2 deviates from SphK1 with respect to its structural makeup. Several domains and amino acid sequences (Figure 1.7) contribute to the SphK2 sequence length, which helps dictate its cellular function. The proline-rich region of the SphK2 sequence binds the cytokine receptor IL-12R\(\beta1\), activating IFN-\(\gamma\) to promote immune responses.\textsuperscript{59} Another component of the SphK2 sequence is a Bcl-2 homology 3 (BH3)-like domain, which is found in the N-terminus of the enzyme.\textsuperscript{60} Within this domain, SphK2 interacts
with the pro-survival mitochondrial transmembrane molecule Bcl-xL; this interaction promotes the release of pro-apoptotic factors BAK and BAX.\textsuperscript{60, 61}

In addition to a BH3 domain, SphK2 also contains a nuclear localization sequence (NLS) in the N-terminus of the enzyme. Studies demonstrated that SphK2 could trigger cell cycle arrest by preventing the cell from entering the \( S_1 \) phase of interphase in the cell cycle.\textsuperscript{20} The NLS in SphK2 was found to be directly linked to this event as SphK2s lacking an NLS failed to prevent cells from entering the \( S_1 \) phase.\textsuperscript{20}

While SphK2 contains an NLS to allow its localization to the nucleus, it also contains at least one nuclear export sequence (NES) to allow its transport out of the nucleus.\textsuperscript{62} The identified NES is located between residues 416 and 425 in the proline-rich domain.\textsuperscript{62} Phosphorylation of the serine residues 419 and 421 in the NES causes SphK2 to enter the cytoplasm,\textsuperscript{62} after which SphK2 can catalyze the formation of S1P for receptor signaling.

The SphK2 sequence also contains a lipid-binding domain. Although its exact location and amino acid makeup is unknown, studies determined that the lipid-binding domain is located within the first 172 amino acid residues of the SphK2 sequence.\textsuperscript{63} Studies have implicated this domain in SphK2 localization, as SphK2 was reported to bind sulfatide, a component of cell membranes, and SphK2 lacking the first 172 residues failed to translocate from the cytosol.\textsuperscript{63}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{sphk2-domains.png}
\caption{Location of SphK2 localization sequences and binding domains.}
\end{figure}

1.6.2 Isoforms

In humans, SphK2 exists as two isoforms—SphK2-S,\textsuperscript{40} consisting of 618 residues, and SphK2-L, consisting of 654 residues.\textsuperscript{40, 64} The amino acid sequencing of SphK2-L is the same as
that for SphK2-S, which includes the BH3 domain, lipid-binding domain, NLS sequence, and NES sequence; the exact role of the extra 36 N-terminal amino acid residues in SphK2-L remains to be determined.

Polymerase chain reaction (PCR) experiments suggest that SphK2-L is the commonly expressed isoform of SphK2 in the human body, except in the brain and kidneys (Figure 1.). The PCR findings were further supported by immunoprecipitation experiments, which specifically isolated SphK2-L from lysed cells and showed that the loss of SphK2-L resulted in a significant loss of SphK2 activity.

![Figure 1.8. SphK2 isoform distribution in human tissue. Reused with permission from reference 64. Copyright 2017 American Society for Biochemistry and Molecular Biology.](image)

1.6.3 Localization

Cellular imaging studies have shown that SphK2 can be found within the nucleus as well as in the cytosol. The presence of an NLS in the SphK2 sequence further suggests SphK2 localization to the nucleus, since removal of the NLS resulted in an accumulation of tagged SphK2 in the cytosol. Additionally, high concentrations of SphK2 were observed in the nucleus when the serine residues in the SphK2 NES were mutated, preventing NES phosphorylation and SphK2 export. In contrast, wild-type SphK2 could be found in the cytosol, implying phosphorylation of the NES.
Besides acting as a nucleic and cytosolic enzyme, SphK2 has also been associated with the plasma membrane, ER, mitochondria, and extracellular space. Expression of SphK2 at the plasma membrane has been demonstrated at basal conditions, synthesizing S1P that can be exported out of the cell to engage S1PRs.\textsuperscript{65} Yet under cellular stress, SphK2 levels at the plasma membrane were much lower, preventing activation of S1PR-mediated processes.\textsuperscript{65}

SphK2 activity in the mitochondria, specifically the inner mitochondrial membrane, has been documented.\textsuperscript{22, 66} Direct interaction between the pro-apoptotic mitochondrial protein Bcl-x\textsubscript{L} and the SphK2 BH3 domain further supports this localization.\textsuperscript{60} How SphK2 localizes to the mitochondria from the nucleus or cytoplasm remains unclear, though cellular stress is known to not be a trigger.\textsuperscript{65} However, cellular stress does promote ER localization.\textsuperscript{65}

SphK2 localization to the extracellular space has been observed.\textsuperscript{67} Studies found that during apoptosis, SphK2 localized to the extracellular space following the caspase-1-promoted removal of the SphK2 N-terminus.\textsuperscript{67}

### 1.6.4 Localization–Function Relationship

SphK2’s role within the cell is influenced by its localization (Figure 1.9). As stated earlier, nuclear SphK2 can prevent the cell from entering the S\textsubscript{1} phase of the cell cycle, which then prevents DNA synthesis.\textsuperscript{20} Furthermore, S1P synthesized by nuclear SphK2 can prevent HDAC 1/2 activity, promoting gene transcription and expression of p\textsubscript{21}, a protein associated with cell cycle arrest.\textsuperscript{19, 68} A pair of studies, though, have reported that nuclear SphK2 can be proliferative. S1P synthesized by overexpressed nuclear SphK2 prevented the anti-proliferative activities of retinoic acid receptor beta (RAR\textbeta).\textsuperscript{69} S1P synthesized by nuclear SphK2 has been shown to stabilize the catalytic portion of telomerase, thereby promoting telomerase stability and cell proliferation.\textsuperscript{70}
Mitochondrial SphK2 binds Bcl-xL, inducing the apoptotic BAK/BAX-mediated emission of cytochrome c from mitochondria.\textsuperscript{60, 61} SphK2 in the ER can promote Ca\textsuperscript{2+} release during cellular stress, and the Ca\textsuperscript{2+} activates pro-apoptotic mediators such as cytochrome C.\textsuperscript{21, 65} In addition, SphK2 in the ER can also increase intracellular levels of pro-apoptotic Cer.\textsuperscript{65} Extracellular SphK2 is proposed to have anti-inflammatory functions because S1P synthesized extracellularly promoted lymphocyte localization to damaged cells.\textsuperscript{67}

Multiple studies have reported elevated blood S1P levels in SphK2-null mice or mice treated with SphK2-selective inhibitors, a phenomenon not observed with SphK1-null mice or SphK1-selective inhibitors.\textsuperscript{42, 65, 71-73} Recent studies have elucidated these observations by implicating SphK2 in the clearance of blood S1P. In particular, mass-labeled exogenous S1P was observed to have slower clearance rates in SphK2-null mice and mice treated with SphK2-selective inhibitors, suggesting SphK2 functions not only as a generator of S1P but also as aids in the removal of blood S1P.\textsuperscript{72} The exact mechanism for how SphK2 contributes to the turnover of blood S1P is unclear, and the localization of SphK2 during this process is currently unknown.
1.6.5 Regulation

Regulation of SphK2 activity and localization is still being elucidated; however, several proteins and mediators have already been implicated in SphK2 regulation (Table 1.1). Due to the homology between SphK1 and SphK2, it is not surprising that SphK2 is also phosphorylated by ERK1/2 in the cytosol. ERK1 specifically phosphorylates the SphK2 residues serine 351 and threonine 578, and this phosphorylation causes SphK2 activity to be 7 times faster than normal SphK2 activity. Unlike SphK1, ERK-mediated phosphorylation of SphK2 does not cause it to relocate to a different site in the cell; however, phosphorylation of the SphK2 NES at the serine residues 419 and 421 by protein kinase D (PKD) triggers SphK2 translocation from the nucleus to the cytosol. Epidermal growth factor (EGF) has also been shown to increase SphK2 activity by more than 2-fold, but it does not affect SphK2 localization. The kinases Lyn and Fyn have also been shown to promote movement of SphK2 to the cell membrane to promote inflammatory pathways by interacting with the immunoglobulin E receptor FceR1. Notably, Lyn and Fyn are
unable to increase SphK2 activity.\textsuperscript{52} SphK2 activity, though, increases by almost 3-fold when interacting with eEF1A.\textsuperscript{51} Although it is still unknown how eEF1A promotes SphK2 activity, eEF1A is hypothesized to make the Sph/ATP-SphK2 binding interactions more efficient.\textsuperscript{51} Besides proteins and mediator molecules, cellular stress can also regulate SphK2 activity, as hypoxia was found to increase SphK2 activity by almost 2-fold during hypoxia, resulting in an increase in extracellular S1P.\textsuperscript{76}

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERK1/2</td>
<td>Phosphorylates SphK2; increases SphK2 activity by 7-fold.</td>
</tr>
<tr>
<td>PKD</td>
<td>Phosphorylates the SphK2 NES</td>
</tr>
<tr>
<td>EFG</td>
<td>Increases SphK2 activity by &gt; 2-fold</td>
</tr>
<tr>
<td>Lyn and Fyn</td>
<td>Promotes SphK2 localization to the cell membrane; promotes SphK2 interaction with FceR1</td>
</tr>
<tr>
<td>eEF1A</td>
<td>Increases SphK2 activity by 3-fold</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Increases SphK2 activity by 2-fold</td>
</tr>
</tbody>
</table>

Table 1.1. Regulators of SphK2

1.6.6 Activity in Disease

Reports on SphK2 activity in disease have been conflicting. In some cases SphK2 seems to lessen or even prevent disease while in other cases SphK2 has shown to promote the progression of disease. One such disease is cancer. Due to reports implicating SphK2 in pro-apoptotic activity, it would seem that expression and even up-regulation of SphK2 would help alleviate the progression of cancer. However, several studies\textsuperscript{2, 77-84} have reported that this may not be the case. Overexpression of SphK2 has been observed in numerous cancer cell lines,
including non-small cell lung carcinoma, multiple myeloma, breast cancer, melanoma, and leukemia.\textsuperscript{82, 85-87} Interestingly, in cases of SphK2 overexpression in cancer cell lines, SphK2 was found to be highly localized to the cytosol and plasma membrane, a localization associated with cell proliferation.\textsuperscript{82} Complete knockdown or down-regulation of SphK2 in kidney, breast, and cerebral cell lines suppressed cancer cell proliferation and migration.\textsuperscript{80} The extent to which this suppression occurred was greater than that observed for SphK1.\textsuperscript{2, 80} Breast cancer xenograft mouse models have also demonstrated that knockdown of SphK2 can suppress the progression of cancer by eliciting pro-inflammatory and anti-tumor activity.\textsuperscript{81} Inhibition of SphK2 in mice models and cancer cell lines by SphK2-specific inhibitors have also shown anti-tumor activity.\textsuperscript{2, 78, 79} Additionally, breast cancer cell lines became susceptible to chemotherapy treatment with the synergistic administration of chemotherapy and an SphK2-selective inhibitor.\textsuperscript{77} Inhibition of SphK1 occurred concomitantly with SphK2 in this study, suggesting tumor cell chemoresistance might not be solely SphK2-derived. Despite a growing body of evidence implicating SphK2 (as well as SphK1) involvement in cancer progression, one study found that inhibition of SphK2 (and SphK1) activity does not cause tumor cell death, suggesting that pharmacologically targeting SphK2 may not be the most feasible route for cancer treatment.\textsuperscript{88} However, more studies are needed to corroborate this report.

SphK2 activity in inflammatory diseases and responses has also been documented.\textsuperscript{1, 79, 89-92} Inhibition of SphK2 was shown to reduce the extent of colitis and arthritis in mice models.\textsuperscript{79, 89, 93} Inhibition of SphK2 has also been found to lessen inflammation in rat liver cell grafts following liver transplants, demonstrating SphK2 facilitation of inflammation.\textsuperscript{91} However, SphK2 activity can also be anti-inflammatory as demonstrated by either knockdown or inhibition of SphK2 in murine arthritis.\textsuperscript{1, 90, 94} These conflicting reports appear to be disease model-specific,
suggesting more information is needed to truly understand SphK2 activity in inflammatory diseases and responses.

SphK2’s role in fibrosis, specifically kidney fibrosis, is emerging. One recent study showed that SphK2-null mice were less susceptible to kidney fibrosis upon folic acid treatment as wild-type and SphK1-null mice. The SphK2 knockout mice also showed reduced fibrotic and inflammatory markers. These observations were recapitulated when wild-type mice treated with folic acid to induce kidney fibrosis showed reduced kidney fibrosis and fibrotic markers upon administration of an SphK2-selective inhibitor. These results suggest that SphK2 inhibition may be a viable route for controlling kidney fibrosis.

SphK2 has also been implicated in ischemic reperfusion (IR) injury. Reports on its role in IR are also conflicting. Inhibition of SphK2 in mice liver models protected against IR, whereas knockout of SphK2 in mouse myocardia promoted IR, leading to tissue injury. These results suggest that the method of preventing SphK2 activity and the tissue type influence the outcome in IR studies.

SphK2 may also be involved in neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease. High concentrations of SphK2 were observed in autopsy cerebral samples of Alzheimer patients. Additionally, inhibition of SphK2 in mice resulted in a decrease in the BACE-1-mediated production of amyloid-β (Aβ), a protein often found in Alzheimer’s patient brains. In comparison, SphK2 was discovered to be down-regulated in cerebral samples of Parkinson’s patients. Knockdown studies of SphK2 showed decreased S1P levels in dopaminergic neurons, which was accompanied by a reduction in ATP levels and an increase in radical oxygen species. SphK2 Cellular localization studies of dopaminergic neuronal cells indicate that SphK2 is primarily localized to the mitochondria, suggesting down-regulation of
SphK2 in the Parkinson’s disease cerebral samples is associated with mitochondrial dysfunction.⁹⁸

1.7 Sphingosine Kinase 2 Inhibitors

As a mediator in the formation of S1P and implicated role in disease, SphK2 is emerging as a potential therapeutic target for pharmaceutical inhibition. In comparison to SphK1-selective inhibitors, there is a paucity of reported potent and selective SphK2 inhibitors. Nevertheless, a number of SphK2 inhibitors have been developed, with some showing promising results in disease models (Figure 1.10).

![Figure 1.10. Structures of Select SphK2 inhibitors](image)

**1.7.1 (R)-FTY720-OMe**

Compound 1.2 ((R)-FTY720-OMe) is a derivative of an S1P pro-drug agonist that inhibits SphK2 activity rather than acting as an agonist for S1PRs like FTY720.⁹⁹ Compound 1.2 significantly inhibits SphK2 activity by 60% at 50 µM without affecting SphK1 activity and has
an inhibition constant ($K_i$) of 16.5 µM for SphK2 (Figure 1.10). Studies have demonstrated that 1.2 promotes apoptosis in HEK 293 cells and reduces DNA synthesis in MCF-7 breast cancer cells. Recently, 1.2 was shown to enhance pulmonary vascular barrier function to reduce and prevent vascular leak in acute respiratory distress syndrome models.

1.7.2 **SKI-II**

Compound 1.3 (SKI-II) is an aminothiazole-based SphK inhibitor that was discovered from a library screen. It is a non-selective SphK inhibitor, having $K_i$’s of 16 µM and 7.9 µM for SphK1 and SphK2, respectively (Figure 1.10). Notably, it was utilized in the creation and isolation of the first reported SphK1 crystal structure, as mentioned earlier in this review. Information derived from this work has led to the development of more potent SphK2 inhibitors, including aminothiazole-based compounds reported by Vogt et al., Gustin et al., and Aurelio et al. Multiple studies have demonstrated its efficacy in promoting apoptosis in cancer cell lines such as T24 cells. However, 1.3 was recently shown to also inhibit dihydroceramide desaturase (Des1), which facilitates the production of ceramide. Inhibition of Des1 has been shown to promote cell cycle arrest. Thus, the apoptosis observed in cancer cell lines from 1.3 treatment result from a concomitant inhibition of SphK1/2 and Des1.

1.7.3 **ABC294640**

As a compound discovered from a high-throughput screening of a series of compounds, 1.4 (ABC294640) is an SphK2-selective inhibitor with a $K_i$ of 9.8 µM (Figure 1.10). Studies indicate its good oral bioavailability and low toxicity. Its anticancer potential as a suppressor of tumor proliferation has been documented in numerous reports. Additionally, it has shown promise in alleviating the effects of inflammatory diseases such as arthritis and colitis. Interestingly, 1.4 can also serve as an estrogen receptor antagonist, preventing
breast cancer proliferation and survival in MCF-7 breast cancer cell lines and preventing tumor cell growth in mice models.\textsuperscript{109} Additionally, it has also been implicated as an inhibitor of Des1.\textsuperscript{110} Regardless, it was recently tested in phase I clinical trials (clinicaltrials.gov registry identifier NCT01488515). Twenty-two patients with various cancer types were dosed twice daily for 3 years with an average dose of 500 mg 1.4, which resulted in reduced detected plasma S1P levels within the first 12 h of treatment.\textsuperscript{111} Of the enrolled patients, one showed partial response to treatment and 6 showed a stable response by the end of the trial, suggesting a therapeutic potential for this molecule.\textsuperscript{111}

1.7.4 VT-ME6

Developed from structure-activity relationship (SAR) studies conducted on an S1P pro-drug agonist, 1.5 (VT-ME6) is an SphK2-selective inhibitor with a K\textsubscript{i} of 8 µM (Figure 1.10).\textsuperscript{6} Tail length modification studies\textsuperscript{112} demonstrated that the tail length of 1.5 influences its selectivity for SphK2 over SphK1. Tail groups containing alkyl chain lengths of 8 and 14 carbon atoms imparted a 3-fold selectivity for SphK2 over SphK1, while all other tested tail lengths (6, 9, 10, 11, 12, 16) showed little to no selectivity for SphK2 over SphK1.\textsuperscript{112} Studies have shown that 1.5 affects S1P signaling by preventing the phosphorylation of ERK; however, treatment of leukemia U937 cells with this inhibitor did not cause a drop in S1P levels.\textsuperscript{6}

1.7.5 Amgen 82

As a product of structure-based analysis of the SphK1 crystal structure, 1.6 (Amgen 82) is a non-selective SphK inhibitor that targets the Sph binding pocket rather than the ATP binding site.\textsuperscript{56} Assays found that this inhibitor has an average half maximal inhibitory concentration (IC\textsubscript{50}) of 0.02 µM and 0.10 µM for SphK1 and SphK2, respectively.\textsuperscript{56} The SphK K\textsubscript{i}’s for 1.6 were not determined. Compound 1.6 can inhibit SphK activity in multiple cancer cell lines,
including breast cancer and skin cancer cell lines.\textsuperscript{56, 88} Surprisingly, administration of the inhibitor at low concentrations (0.001–1 \( \mu \text{M} \)) inhibited SphK activity without affecting tumor cell viability.\textsuperscript{88} However, higher concentrations of 1.6 (> 1 \( \mu \text{M} \)) were found to concomitantly inhibit SphK activity and reduce tumor cell viability.\textsuperscript{88} This decreased tumor cell viability was not linked to SphK inhibition; rather, it was attributed to a concentration-promoted, surfactant-like behavior of 1.6, causing cell lysis.\textsuperscript{88} Nevertheless, 1.6 was shown to be orally bioavailable with the ability to lower blood S1P by 72\% at 100 mg/kg compared to controls.\textsuperscript{88} In a tumor xenograph mouse model, 1.6 was unable to reduce tumor cell growth despite reducing blood S1P levels,\textsuperscript{88} suggesting that reducing tumor cell growth in vivo requires an approach more complex than solely reducing blood S1P levels.

1.7.6 K145

Another SphK2-selective inhibitor is 1.7 (K145), which has a \( K_i \) of 6.4 \( \mu \text{M} \) (Figure 1.10).\textsuperscript{113} In vitro studies found that 1.7 decreased S1P levels and reduced cell proliferation in U937 cells.\textsuperscript{113} In addition, decreased ERK activity was also observed, indicating that SphK2 may not be the only target of 1.7.\textsuperscript{113} In vivo studies found that 1.7 is orally bioavailable and can prevent leukemia and breast cancer cell growth.\textsuperscript{113}

1.7.7 SLR080811

Derived from the SphK2-selective inhibitor 1.5, 1.8 (SLR080811) is a 10-fold SphK2-selective inhibitor with a \( K_i \) of 1.3 \( \mu \text{M} \) (Figure 1.8).\textsuperscript{73} Various cell studies demonstrated that 1.8 can decrease the levels of intracellular S1P.\textsuperscript{73} Treatment of SphK1-null mice with 1.8 caused a decrease in blood S1P levels 2 to 4 hours after injection, demonstrating its effect on SphK2; however, treatment of wild-type mice with 1.8 resulted in elevated levels of blood S1P following injection,\textsuperscript{73} a phenotype commonly observed in SphK2-null mice.\textsuperscript{42, 65, 71-73} Interestingly,
insertion of a methylene unit between 1.8’s guanylated pyrrolidine ring and oxadiazole ring generates a potent SphK1-selective inhibitor that is capable of lowering S1P levels in vitro and in vivo.\textsuperscript{114}

1.7.8  \textit{SLP120701}

As the azetidine analogue of 1.8, 1.9 (SLP120701) is a > 10-fold SphK2-selective inhibitor with a \(K_i\) of 1.2 \(\mu M\).\textsuperscript{114} Like 1.8, 1.9 decreases S1P levels in cultured cells and elevates blood S1P in mice. Pharmacokinetic studies of 1.9 found that it has an improved half-life (t\(_{1/2}\)) to 1.8, with a t\(_{1/2}\) of 8 h in comparison to 4h.\textsuperscript{114} Compound 1.9 was recently tested in a kidney fibrosis mouse model, showing promise as an antifibrotic treatment, as treatment of mice with induced kidney injury with 10 mg/kg of 1.9 showed reduced fibrotic markers and fibrosis of the kidneys.\textsuperscript{95}

1.7.9  \textit{SLM6031434}

Developed from an SAR study of 1.8, 1.10 (SLM6031434) is a potent SphK2-selective inhibitor with a \(K_i\) of 0.4 \(\mu M\) and 50-fold selectivity for SphK2. It has been shown to decrease S1P levels in cultured cells in a dose dependent manor, and as observed with 1.8 and 1.9, causes an elevation in blood S1P levels when given to wild-type mice. It has recently been utilized as a chemical biology tool to probe SphK2 function, specifically to elucidate the elevated blood S1P phenomenon observed when wild-type mice are treated with SphK2-selective inhibitors. 1.10 helped implicate SphK2 as a mediator in blood S1P clearance because wild-type mice treated with 1.10 showed a remarkably decreased clearance rate of mass-labeled S1P from the blood.

1.8  \textit{Conclusions}

The current understanding of the roles and regulation of SphK2 in normal cellular function and in disease states will continue to evolve as more studies are conducted using
different types of cell lines, animal models, and SphK2 inhibitors. Although the crystal structure of SphK2 has yet to be solved, current efforts to develop new and potent SphK2-selective inhibitors have benefited from multiple publications of the SphK1 crystal structure and information derived from the SphK2 inhibitors discussed herein.

1.9 Dissertation Overview For Developing Inhibitors of Sphingosine Kinase 2

Chapter 1 discussed the chemical biology of SphK2, disease states associated with its activity, and select SphK2-selective inhibitors. Chapter 2 will disclose an SAR of the tail group region of 1.8, revealing that the lipid binding pocket of SphK2 cannot accommodate charged tail groups and is much larger than SphK1. Chapter 3 will disclose an SAR of aminothiazole derivatives of an SphK1-selective inhibitor derived from 1.8. These derivatives show surprisingly good potency and selectivity for SphK2. Chapter 6 provides the supplemental information for the compounds synthesized and characterized by the author of this dissertation. NMR spectra for the synthesized compounds can be found online (http://www.sciencedirect.com/science/article/pii/S0960894X15002474 for compounds synthesized in Chapter 2 and http://pubs.acs.org/doi/suppl/10.1021/acs.jmedchem.7b00233 for compounds synthesized in Chapter 3).
1.10 References


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103. Aurelio, L.; Scullino, C. V.; Pitman, M. R.; Sexton, A.; Oliver, V.; Davies, L.; Rebello, R. J.; Furic, L.; Creek, D. J.; Pitson, S. M.; Flynn, B. L. From Sphingosine Kinase to


2 Structure-Activity Relationship Studies of the Lipophilic Tail Region of Sphingosine Kinase 2 Inhibitors

2.1 Contributions

Multiple members of the Santos group contributed to this work. The reported compounds were synthesized by the author, Dr. Molly Congdon, Dr. Neeraj Patwardhan, James Grumkowski, and Emily Morris. Biological analyses of the reported compounds were conducted by Dr. Yugesh Kharel of the University of Virginia Department of Pharmacology. The final manuscript was written by Dr. Molly Congdon and Dr. Webster Santos. The author synthesized and characterized final compounds 2.13d, 2.13e, and 2.16a-d and their corresponding intermediates and contributed to the experimental write-up and revision of the manuscript. This chapter is an adapted reprint with permission from Elsevier [Congdon, M.D.; Childress, E.S.; Patwardhan, N.N.; Gumkowski, J.; Morris, E.A.; Kharel, Y.; Lynch, K.R. and Santos, W.L., Structure-Activity Relationship Studies of the Lipophilic Tail Region of Sphingosine Kinase 2 Inhibitors, Bioorg. Med. Chem. Lett. 2015, 25, 4956–4960, © 2015 Elsevier].

2.2 Abstract

Sphingosine 1-phosphate (S1P) is a ubiquitous, endogenous signaling molecule that is synthesized by the isozymes sphingosine kinase 1 and 2. Alteration of S1P levels is linked to several disease states including cancer, fibrosis, Alzheimer’s disease, and sickle cell disease, making the S1P signaling pathway an attractive target for therapeutic intervention. While much attention has been placed on developing SphK1-selective inhibitors, there is a dearth of reported SphK2-selective agents. Herein, we report our investigations on the structure-activity relationship studies on the lipophilic tail region of 2.4 (SLR080811), a SphK2-selective inhibitor. Our studies demonstrate that the internal phenyl ring is a key structural feature in the scaffold of
2.4. Our studies also indicate that the SphK2 lipophilic binding pocket is not amenable for charged heteroatom-bearing tailgroups. Further, we show the dependence of SphK2 activity and selectivity on alkyl tail length, suggesting a larger lipid binding pocket in SphK2 compared to SphK1.

2.3 Introduction

Sphingosine 1-phosphate (S1P) is a pleotropic sphingolipid that is synthesized from the phosphorylation of sphingosine (Sph) by the two isoforms of sphingosine kinase: SphK1 and SphK2. S1P functions as both an intracellular and extracellular signaling molecule, with S1P’s primary function being an extracellular ligand for its G-protein coupled receptors S1P1-5. S1P signaling has been associated with a variety of diseases including cancer, fibrosis, multiple sclerosis, Alzheimer’s disease, and sickle cell disease.1-6 As a result of their key role in the synthesis of S1P, regulation of SphKs has attracted an increasing amount of attention as a therapeutic target. The ability to control SphKs function would also aid in the understanding of their in vivo function, as well as their effects in the sphingolipid signaling pathway.

Many differences exist between SphK1 and SphK2 including size, cellular localization, and function.7 While double knockout studies in mice suggest that SphKs are the sole source of S1P, some functional redundancy exists as SphK1 or SphK2 null mice are viable and fertile. Although inhibitor development towards SphK1 has been a focus of intense studies,8,9 inhibitors of SphK2 are emerging (Figure 2.1). For example, 2.1 (ABC294640 (Ki = 10 µM)) was the first inhibitor with SphK2 activity that has been deployed in a variety of disease models including lupus nephritis, diabetic nephropathy, Crohn’s disease, ulcerative colitis, and osteoarthritis.9,10 However, it was recently shown to be a tamoxifen-like partial agonist of estrogen receptors in breast cancer cells.11 Another inhibitor, thiazolidine-2,4-dione 2.2 (K145 (Ki = 6.4 µM)), which
is an analog of sphingosine was recently reported as a selective SphK2 inhibitor.\textsuperscript{12} \textbf{2.2} was shown to inhibit leukemia cell growth in vitro as well as in a xenograph mouse model.

\textbf{Figure 2.1.} Structure of SphK2 Inhibitors

Due to our interest in understanding the in vivo function of SphK2 and a paucity of highly potent and selective inhibitors,\textsuperscript{12} we focused our studies in developing unique scaffolds to achieve our goals. Our first generation inhibitor, \textbf{2.3} (VT-ME6), contained a quaternary ammonium head group as a warhead and established that a positively charged moiety is necessary for engaging key amino acid residues in the enzyme binding pocket.\textsuperscript{13, 14} This compound is moderately potent ($K_i = 8 \ \mu M$) and displays three-fold selectivity for SphK2 over SphK1. Subsequent improvements produced \textbf{2.4} (SLR080811), which features a 1,2,4-oxadiazole linker and a guanylated pyrrolidine head group and possesses a $K_i$ of 13.3 $\mu M$ and 1.3 $\mu M$ for SphK1 and SphK2, respectively.\textsuperscript{15} A significant discovery from these studies is that pharmacological inhibition of SphK2 elevates blood S1P levels in mice. Further structure–activity relationship studies on the guanidine head group revealed that the azetidine-containing derivative \textbf{2.5} (SLP120701) has an improved half-life of 8 h in mice (in comparison to 4 h).\textsuperscript{16} In this report, we detail our investigations on the tail region of the guanidine-based scaffold (Figure 2.2). Our studies demonstrate that the internal phenyl ring is essential to maintain inhibitory
activity for SphK2, the alkyl tail length has a significant effect on the potency and selectivity towards SphK2, and that the SphK2 binding pocket cannot accommodate charged heteroatom-bearing tail groups.

2.4 Results and Discussion

2.4.1 Inhibitor Development

The synthesis of derivatives of 2.4 with varying alkyl length as well as heterocycles attached to the phenyl ring is shown in Schemes 2.1 and 2.2. In Scheme 2.1, 4-iodobenzonitrile (2.6a) was cross-coupled to a series of alkynes or hydroborated intermediates under standard Sonogashira or Suzuki–Miyaura conditions. Subsequent reaction with hydroxylamine afforded amidoximes 2.7a–e, which were cyclized to 1,2,4-oxadiazoles 2.8a–f in the presence of HCTU and Boc-L-proline. Deprotection with HCl and reduction of alkynyl groups with tosylhydrazine at refluxing conditions yielded amines 2.9a–h. To install the guanidine moiety, the amines were treated with DIEA and N,N’-Di-Boc-1H-pyrazole-1-carboxamidine for several days at room temperature and deprotected with HCl to produce the desired derivatives 2.10a,d,f–h. A similar synthetic strategy was employed to access the remaining phenyl/alkyl derivatives (2.13c and 2.13f–g); however, heterocycles 2.13d–e were obtained via Buchwald–Hartwig coupling conditions as shown in Scheme 2.2. Similarly, Scheme 2.3 illustrates the synthesis of various amidopiperazine tail surrogates 2.16a–d via Buchwald–Hartwig and acetylation conditions.
Scheme 2.1. Synthesis of derivatives of 2.4

Reagents and conditions: (a) alkyne, TEA, DMF, PdCl$_2$(PPh$_3$)$_2$, Cul, 80 °C, 18 h, 72–93%; (b) i. alkene, 0.5 M 9-BBN, in THF, rt, 12 h; ii. Pd(dppf)Cl$_2$, Cs$_2$CO$_3$, DMF, 70 °C, 18 h, 75–93%; (c) NH$_2$OH-HCl, TEA, EtOH, 80 °C, 6 h, 43–95%; (d) Boc-L-proline, DIEA, HCTU, DMF, 110 °C, 18 h, 25–65%; (e) 20% DME, 4-toluenesulfonyl hydrazide, TEA, reflux, 67–71%; (f) HCl (g), MeOH, 35–100%; (g) DIEA, N,N'-di-Boc-1H-pyrazole-1-carboxamidine, CH$_3$CN, rt, 3 days, 27–76%.

Scheme 2.2. Synthesis of derivatives of 2.5$^a$

Reagents and conditions: (c) Boc-L-azetidine, DIEA, HCTU, DMF, 110 °C, 18 h, 63%; (b) alkyne, TEA, DMF, PdCl$_2$(PPh$_3$)$_2$, Cul, 80 °C, 18 h, 33–57%; (c) phenylboronic acid, Cs$_2$CO$_3$, DMF, PdCl$_2$(dpff), 80 °C, 18 h, 91%; (d) amine, Pd(dba)$_3$, Cs$_2$CO$_3$, PtBu$_3$, toluene, 120 °C, 6 d, 81–83%; (e) 20% DME, 4-toluenesulfonyl hydrazide, TEA, reflux, 60–71%; (f) HCl (g), MeOH, 78–96%; (g) DIEA, N,N'-di-Boc-1H-pyrazole-1-carboxamidine, CH$_3$CN, rt, 3 days, 43–66%.
Reagents and conditions: (a) piperazine, Pd\(_2\)(dba)\(_3\), PtBu\(_3\), Cs\(_2\)CO\(_3\), toluene, 120 °C, 3 days, 52%; (b) acid chloride, CH\(_2\)Cl\(_2\), 0 °C to rt, 2 h, 66–88%; (c) HCl (g), MeOH, 76–95%; (d) DIEA, N,N'-di-Boc-1H-pyrazole-1-carboxamidine, CH\(_3\)CN, rt, 3 days, 23–74%.

Compounds 2.20 and 2.23 were synthesized as shown in Schemes 2.4 and 2.5, respectively. 4-(3-ethoxymethyl)-5-methylbenzylbenzonitrile 2.19 was formed in two steps via mono-substitution of 1,3-bis(bromomethyl)-5-methylbenzene 2.17 and subsequent palladium-catalyzed cross coupling reaction with 4-cyanophenylboronic acid to afford 2.19. Alternatively, benzonitrile 2.22 was achieved using sodium benzenesulfonate and 2.21. Standard oxadiazole formation, guanidylation, and deprotection afforded 2.20 and 2.23, respectively. Finally, a series of alkyl tails directly linked to the oxadiazole ring were synthesized (Scheme 2.5). Treatment of alkylbromides with potassium cyanide gave alkylnitriles 2.25a-c, which were converted to amidoximes 2.26a-c. Transformation to oxadiazoles 2.27a-c was effected either by HCTU-mediated cyclization at 110 °C or by two-step coupling/TBAF-catalyzed cyclization, which eventually led to 2.28a-c.

Scheme 2.4. Synthesis of 2.20a

Reagents and conditions: (a) NaH, EtOH, 0 °C to rt, 46%; (b) 4-cyanophenylboronic acid, Pd(PPh\(_3\))\(_4\), Na\(_2\)CO\(_3\), 1:1 THF:H\(_2\)O, 94%; (c) NH\(_2\)OH·HCl, TEA, EtOH, 80 °C, 6 h, 71–93%; (d) Boc-L-proline, DIEA, HCTU, DMF, 110 °C, 18 h, 46–82%; (e) HCl (g), MeOH, 33–91%; (f) DIEA, N,N'-di-Boc-1H-pyrazole-1-carboxamidine, CH\(_3\)CN, rt, 3 days, 53–83%.
Scheme 2.5. Synthesis of 2.23

\[
\begin{align*}
\text{Br} & \quad \text{SO}_2 \quad \text{Ph} & \quad \text{N} & \quad \text{HCl} \\
2.21 & \quad \text{a} & \quad 2.22 & \quad \text{b-e, d} & \quad 2.23 \\
\end{align*}
\]

\(^a\)Reagents and conditions: (a) sodium benzenesulfonate, DMF, 60 °C, 2 h, 89%; (b) NH\(_2\)OH·HCl, TEA, EtOH, 80 °C, 6 h, 71–93%; (d) Boc-L-proline, DIEA, HCTU, DMF, 110 °C, 18 h, 46–82%; (e) HCl (g), MeOH, 33–91%; (f) DIEA, \(N,N'\)-Di-Boc-1H-pyrazole-1-carboxamidine, CH\(_3\)CN, rt, 3 days, 53–83%.

Scheme 2.6. Synthesis of derivatives of 2.4 lacking an internal phenyl ring

\[
\begin{align*}
\text{R} & \quad \text{Br} & \quad \text{R} & \quad \text{N} & \quad \text{HCl} \\
2.24a & = \text{C}_8\text{H}_{17} & 2.25a & = \text{C}_9\text{H}_{17} & 2.26a & = \text{C}_9\text{H}_{17} & 2.27a & = \text{C}_9\text{H}_{17} \\
2.24b & = \text{C}_{12}\text{H}_{25} & 2.25b & = \text{C}_{13}\text{H}_{25} & 2.26b & = \text{C}_{13}\text{H}_{25} & 2.27b & = \text{C}_{13}\text{H}_{25} \\
2.24c & = \text{C}_{16}\text{H}_{33} & 2.25c & = \text{C}_{17}\text{H}_{33} & 2.26c & = \text{C}_{17}\text{H}_{33} & 2.27c & = \text{C}_{17}\text{H}_{33} \\
2.28a & = \text{C}_8\text{H}_{17} & 2.28b & = \text{C}_{12}\text{H}_{25} & 2.28c & = \text{C}_{16}\text{H}_{33} \\
\end{align*}
\]

\(^a\)Reagents and conditions: (a) KCN, 9:1 EtOH:H\(_2\)O, 80 °C, 18 h, 20–93%; (b) NH\(_2\)OH·HCl, TEA, EtOH, 80 °C, 12 h, 53–69%; (c) Boc-L-proline, DIEA, HCTU, DMF, 110 °C, 18 h, 50%; (d) Boc-L-proline, DIEA, HCTU, CH\(_2\)Cl\(_2\), rt, 4 h, 57–80%; (e) 1 M TBAF, THF, rt, 1 h, 93–95%; (f) HCl (g), MeOH, 66–100%; (g) DIEA, \(N,N'\)-di-Boc-1H-pyrazole-1-carboxamidine, CH\(_3\)CN, rt, 3 days, 51–71%.

2.4.2 Structure–Activity Relationship Studies and Biological Evaluation of Derivatives

With the library of putative inhibitors synthesized, the inhibitory effects of the compounds were determined for hSphK1 and mSphK2 using a previously published protocol (Table 1). Briefly, Sph and cell lysate containing recombinant SphK1 or SphK2 were incubated with or without inhibitor in the presence of \(\gamma\text{-[}^{32}\text{P}\text{]}\text{ATP}. After 20 minutes, the reaction mixtures were extracted, separated using thin layer chromatography, and quantified using liquid scintillation counting. The kinase inhibition was determined as the amount of \(^{32}\text{P}\)-S1P produced as a function of inhibitor concentration. Compounds were screened at 10 µM inhibitor concentrations.
As shown in Table 2.1, replacement of the octyl chain of 2.4 with iodide, phenyl or phenethyl groups did not improve inhibitory activity (entries 1-3). Decreasing or increasing the lipophilic alkyl tail length from hexyl to tetradecyl in two-carbon increments resulted in compounds with similar inhibitory activity as 2.4 (entries 4-10), although the hexyl chain was slightly less active. In cases where the kinase activity was similar to 2.4 at 10 µM, rescreening at a more stringent inhibitor concentration (1 µM) was performed: the results indicated that none of these analogs had improved activity compared to 2.4. We also note that the pyrrolidine and azetidine rings have been shown to have similar potency, but with the advantage of improved in vivo half-life for the azetidine derivatives. Interestingly, as the alkyl tail increased to a decyl group, SphK2 selectivity decreased as SphK1 inhibition increased. However, as the chain length increased further to a dodecyl and tetradecyl, inhibition of SphK1 decreased while maintaining SphK2 activity. These results suggest that the lipid binding pocket in SphK2 is much larger than that of SphK1 and is consistent with the prediction based on a crystal structure of SphK1 bound to a SphK1-selective inhibitor.\(^{17}\) We next investigated the effect of the phenyl substituent next to the 1,2,4-oxadiazole ring. Removal of this ring while maintaining the overall length of the molecule resulted not only in diminished SphK2 selectivity but also inhibitory activity (entries 11-13). Our data indicate that the phenyl ring is necessary for selectivity and potency using this scaffold.
Table 2.1. Inhibitory Effects of Derivatives of 2.4 and 2.5 on SphK1 and SphK2<sup>a</sup>

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R</th>
<th>n</th>
<th>SphK1</th>
<th>SphK2</th>
<th>Entry</th>
<th>Compound</th>
<th>R</th>
<th>n</th>
<th>SphK1</th>
<th>SphK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.10a</td>
<td></td>
<td>1</td>
<td>100 ± 2</td>
<td>63 ± 1</td>
<td>12</td>
<td>2.28b</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;25&lt;/sub&gt;</td>
<td>1</td>
<td>60 ± 1</td>
<td>57 ± 1</td>
</tr>
<tr>
<td>2</td>
<td>2.13c</td>
<td></td>
<td>0</td>
<td>101 ± 1</td>
<td>91 ± 1</td>
<td>13</td>
<td>2.28c</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;53&lt;/sub&gt;</td>
<td>1</td>
<td>37 ± 5</td>
<td>52 ± 2</td>
</tr>
<tr>
<td>3</td>
<td>2.10e</td>
<td></td>
<td>1</td>
<td>78 ± 1</td>
<td>70 ± 7</td>
<td>14</td>
<td>2.13d</td>
<td></td>
<td>0</td>
<td>90 ± 4</td>
<td>94 ± 2</td>
</tr>
<tr>
<td>4</td>
<td>2.13f</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;</td>
<td>1</td>
<td>103 ± 2</td>
<td>35 ± 3</td>
<td>(76 ± 6)</td>
<td>15</td>
<td>2.13e</td>
<td></td>
<td>0</td>
<td>97 ± 2</td>
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<tr>
<td>5</td>
<td>2.10f</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;</td>
<td>0</td>
<td>94 ± 2</td>
<td>45 ± 2</td>
<td>(86 ± 6)</td>
<td>16</td>
<td>2.16d</td>
<td></td>
<td>1</td>
<td>89 ± 2</td>
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<td>6</td>
<td>2.4</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;</td>
<td>1</td>
<td>60 ± 1</td>
<td>9 ± 4</td>
<td>(44 ± 4)</td>
<td>17</td>
<td>2.16a</td>
<td></td>
<td>1</td>
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<tr>
<td>7</td>
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<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;21&lt;/sub&gt;</td>
<td>1</td>
<td>18 ± 9</td>
<td>9 ± 7</td>
<td>(64 ± 4</td>
<td>46 ± 5)</td>
<td>18</td>
<td>2.16c</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>2.13g</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;21&lt;/sub&gt;</td>
<td>0</td>
<td>11 ± 4</td>
<td>12 ± 2</td>
<td></td>
<td>19</td>
<td>2.16b</td>
<td></td>
<td>1</td>
<td>81 ± 28</td>
</tr>
<tr>
<td>9</td>
<td>2.10h</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;25&lt;/sub&gt;</td>
<td>1</td>
<td>37 ± 2</td>
<td>15 ± 1</td>
<td>(56 ± 1)</td>
<td>20</td>
<td>2.20</td>
<td>ElO</td>
<td>1</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>10</td>
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<td>C&lt;sub&gt;14&lt;/sub&gt;H&lt;sub&gt;29&lt;/sub&gt;</td>
<td>1</td>
<td>75 ± 1</td>
<td>16 ± 10</td>
<td>(52 ± 4)</td>
<td>21</td>
<td>2.23</td>
<td>O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>11</td>
<td>2.28a</td>
<td>C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;</td>
<td>1</td>
<td>96 ± 1</td>
<td>88 ± 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Values represent % activity of human SphK1 or mouse SphK2 with 10 and 5 µM Sph,
respectively, in the presence of 10 μM inhibitor. Each value is an average of two experiments. Values in parenthesis indicate compounds also assayed at 1 μM.

As there is no published crystal structure of SphK2, further modifications were necessary for elucidation of the lipid-binding pocket. Morpholine and a series of heterocyclic ring derivatives were synthesized to probe for potential non-covalent interactions that were not van der Waals interactions (entries 14-19). A piperazine ring was an attractive selection because of increased conformational rigidity and ability to function as an anchor point in which various groups can be appended. The morpholine, N-methyl, and N-benzyl piperazine derivatives were all found to be inactive toward SphK1 and SphK2, despite an increase in respective tail length. As the N-methyl and N-benzyl piperazine are positively charged, their lack of inhibitory activity can be attributed to the high likelihood that the lipid binding pocket is lined with hydrophobic groups, as observed in published SphK1 crystal structures, \(^17, 18\) which would prevent effective inhibitor binding. In an effort to avoid the creation of positively charged substituents, neutral amide versions with increasing steric bulk were tested. Isovaleryl-, phenacetyl-, and adamantylcarbonyl-substituted analogues were also inactive. As the SphK1 binding pocket is “J-shaped”\(^18\) and the SphK2 binding pocket is also likely this conformation, the tested amides were probably too rigid to adopt the necessary conformation needed for enzyme binding. Finally, the trisubstituted aryl (2.20) and sulfonate (2.23) bearing groups featured in a SphK1-selective inhibitor developed by Pfizer (PF-543) were tested but were also found to be poor inhibitors (entries 20-21).\(^19\)

2.5 Conclusions

In summary, a focused library of SphK2-selective inhibitor derivatives of 2.4 that interrogated the lipophilic tail region of the pharmacophore were synthesized. Our studies demonstrate the dependence of SphK2 inhibitory activity on alkyl chain length; the most optimal
length includes octyl and decyl substituents, which suggests an ideal ‘head-to-tail’ (positive charge to terminal methyl group) length of approximately 18-21 atoms. Furthermore, our studies provide evidence for the much larger lipophilic binding cavity in SphK2 over SphK1, and neither enzyme’s binding cavity can accommodate positively charged or conformationally restricted tail groups. In the scaffold of 2.4, the internal phenyl ring appears to be essential for activity and is likely interacting with residues in the kinase binding pocket. These predictions can be aided by a SphK2 crystal structure, which is currently unavailable.

2.6 Funding Sources

We acknowledge financial support by NIH (Grants R01 GM104366 and R01 GM067958).
2.7 References


Transforming Sphingosine Kinase 1 Inhibitors into Dual and Sphingosine Kinase 2 Selective Inhibitors: Design, Synthesis, and in Vivo Activity

3.1 Contributions

The work in this chapter was done primarily by the author. The author synthesized and characterized all final compounds and their corresponding intermediates. Dr. Yugesh Kharel of the University of Virginia Department of Pharmacology conducted biological analyses of the reported compounds. Dr. Anne M. Brown conducted the molecular modeling experiments. The final manuscript was written by the author and Dr. Webster Santos. This chapter is a reprint with permission from the Journal of Medicinal Chemistry [Childress, E. S.; Kharel, Y.; Brown, A. M.; Bevan, D. R.; Lynch, K. R.; Santos, W. L. Transforming Sphingosine Kinase 1 Inhibitors into Dual and Sphingosine Kinase 2 Selective Inhibitors: Design, Synthesis, and in Vivo Activity. J. Med. Chem. 2017, 60, 33933–3957, © 2015 American Chemical Society].

3.2 Abstract

Sphingosine 1-phosphate (S1P) is a pleiotropic signaling molecule that interacts with its five G-protein coupled receptors S1PR1–5 to regulate cell growth and survival and has been implicated in a variety of diseases including cancer and sickle cell disease. As the key mediators in the synthesis of S1P, sphingosine kinase (SphK) isoforms 1 and 2 have attracted attention as viable targets for pharmaceutical inhibition. In this article, we describe the design, synthesis, and biological evaluation of aminothiazole-based guanidine inhibitors of SphK. Surprisingly, combining features of reported SphK1 inhibitors generated SphK1/2 dual inhibitor 3.20l (SLC4011540) (hSphK1 $K_i = 120$ nM, hSphK2 $K_i = 90$ nM) and SphK2 inhibitor 3.20dd (SLC4101431) ($K_i = 90$ nM, 100-fold SphK2 selectivity). These compounds effectively decrease
S1P levels in vitro. In vivo administration of 3.20dd validated that inhibition of SphK2 increases blood S1P levels.

3.3 Introduction

Sphingosine 1-phosphate (S1P) is a ubiquitous cellular signaling molecule that has been implicated in a variety of diseases including cancer, fibrosis, Alzheimer’s disease, sickle cell disease, and viral infections such as Chikungunya virus. S1P interacts with proteins both within and outside of the cell. As an extracellular ligand, S1P promotes cell migration and survival by binding to five G-protein coupled receptors, S1PR1-5. Although its function as an intracellular ligand is less well defined, S1P is reported to control epigenetic modifications through regulation of HDAC1/2 activity via sphingosine kinase isotype 2 (SphK2) and alter erythrocyte glycolysis to increase O2 release under hypoxic conditions. S1P is synthesized by the phosphorylation of sphingosine (Sph) by SphK, which exists as two isotypes: SphK1 and SphK2. SphK1 and SphK2 share approximately 50% sequence identity but differ with respect to their function, in part due to their differing localization within the cell. SphK1 is a cytosolic enzyme that promotes cell survival and proliferation, whereas some SphK2 is found in the nucleus but can relocate to the cytosol on phosphorylation. Depending on this localization, SphK2 can promote either apoptosis or cell proliferation. Although isotype-specific SphK null mice are viable, fertile, and phenotypically unremarkable, SphK1-null mice have about a 2-fold reduction in blood S1P levels, whereas SphK2-null mice have 2-4 fold increased blood S1P levels. However, ablation of both SphK isotype genes in the mouse is embryonically lethal in mid gestation as a consequence of complications during vascular and neurological development.
The therapeutic potential of drugging the S1P pathway was realized by the approval of fingolimod by the U. S. Food and Drug Administration for the treatment of multiple sclerosis.\textsuperscript{33}\textsuperscript{,34} Fingolimod is a pro-drug that is phosphorylated by SphK2 to act as a functional antagonist of S1P receptors, S1PR1/3. Other approaches have also been employed to manipulate S1P activity including targeting SphKs to control S1P synthesis.\textsuperscript{3,35-37} There are currently multiple reports of potent SphK1-selective inhibitors and SphK1/SphK2-dual inhibitors (Figure 3.1). Inhibitors such as SphK1-selective inhibitor 3.1 (PF-543)\textsuperscript{38,39} and SphK1/SphK2-dual inhibitor 3.5 (SKI-II)\textsuperscript{40-42} have been co-crystallized with SphK1 and have been useful in developing next generation inhibitors.\textsuperscript{43,44} Indeed, medicinal chemists at Amgen improved 3.5 to realize a selective SphK1 inhibitor, 3.2 (Amgen 23),\textsuperscript{45} while preserving the aminothiazole region. While 3.5 is recently reported to have off-target activity against dihydroceramide desaturase,\textsuperscript{46} 3.1 has shown promise for disease states such as sickle cell disease, where inflammation, cell sickling, and hemolysis were reduced in treated mouse models.\textsuperscript{11}

In contrast with SphK1 inhibitors, highly potent SphK2-selective inhibitors are lacking. Early SphK2 inhibitors (3.8 (SLR080811),\textsuperscript{29,47} 3.9 (ABC294640),\textsuperscript{48} and 3.10 (K145)\textsuperscript{49,50}), which have low micromolar potency and are modestly selective vs SphK1, are active in cultured cells, as measured by their ability to lower cellular S1P levels. Among these, 3.9 has been employed in a variety of animal disease models, including ulcerative colitis,\textsuperscript{51} Crohn’s disease,\textsuperscript{52} ischemia/reperfusion injury,\textsuperscript{53} osteoarthritis,\textsuperscript{54} and colon cancer.\textsuperscript{55} However, 3.9 has recently been reported to have an off-target effect of acting as a tamoxifen-like molecule with the estrogen receptor.\textsuperscript{56} The development of improved inhibitors that are SphK2 specific will help in understanding the physiological function of SphK2 in vivo. Recently, SphK2 was shown to play a role in endothelial cell barrier integrity as well as attenuation of kidney fibrosis through...
interferon gamma using the azetidine analogue of \textbf{3.8}. In addition, an oncogenic role for SphK2 is emerging where high-level overexpression is associated with reduced cell survival and proliferation. \textsuperscript{59}

**Figure 3.1.** Select SphK inhibitors

The paucity of small molecule chemical biology tools for investigating SphK2 function prompted our interest in developing better selective SphK2 inhibitors. We previously reported two new guanidine-based inhibitors: SphK1/SphK2 dual-inhibitor \textbf{3.7} (SLC5111312)\textsuperscript{28, 60} and SphK2-selective inhibitor \textbf{3.11} (SLM6031434)\textsuperscript{28} (>50-fold selective). Treatment of mice with these inhibitors slows the clearance of mass-labeled S1P from the bloodstream, which implicates
SphK2 in the disposal of circulating S1P. Although the mechanism whereby SphK2 influences S1P clearance from blood is currently unclear, these results highlight the need for more potent and selective SphK2 and SphK1/SphK2 dual inhibitors to explore this phenomenon further. Herein, we report the synthesis, structure-activity relationship study, and biological evaluation of guanidine-based aminothiazoles with \textit{in vivo} activity as selective SphK2 inhibitors.

### 3.4 Results and Discussion

#### 3.4.1 Inhibitor Design and Development

Our group reported the synthesis of 3.3 (SLP7111228),\textsuperscript{47} an SphK1-selective inhibitor with a $K_i$ of 48 nM and >100-fold selectivity for SphK1 over SphK2 for both the rat and human enzymes. This compound was found to reduce S1P levels in both cultured human U937 leukemia cells and in the bloodstream of rats. The latter result recapitulates the phenotype of SphK1 null mice.\textsuperscript{32, 47} In developing this molecule, the addition of a methylene unit between the 1,2,4-oxadiazole and pyrrolidine rings was key in converting a 10-fold SphK2-selective inhibitor (3.8) into a >100-fold SphK1-selective inhibitor. In this work, we sought to improve 3.3’s potency and selectivity further by replacing the octyl chain with an aminothiazole group, as present in SphK1 inhibitors such as 3.2 (Figure 3.2). Docking of 3.2 into the binding pocket of a homology model of

![Figure 3.2. Tail group modifications toward the development of new “J-shaped” SphK1 inhibitors.](image-url)
SphK2 and superimposition with 3.2’s “aminothiazole tail” linked to an oxadiazole phenyl ring suggested binding interactions mimicking 3.2 (Figure 3). We hypothesized that these altered “tail” groups, represented by the 14-18 carbon aliphatic groups of sphingoid bases, would create inhibitors that can adopt the necessary “J-shape” for strong binding interactions with the sphingosine binding pocket, as established by published co-crystal structures of SphK1 with aminothiazole-based inhibitors 3.2 and 3.5. Gustin et al. recently developed potent aminothiazole-based SphK1/SphK2 dual inhibitors derived from 3.5, including 3.2. Although most compounds reported were dual inhibitors, some compounds in their series show a bias toward SphK1 inhibition with attractive pharmacokinetic properties. Vogt and co-workers subsequently reported the synthesis of aminothiazole-based derivatives of 3.5 that are SphK1/SphK2-dual inhibitors with several inhibitors showing a slight bias for SphK2 selectivity. However, these molecules show only micromolar potency, and extensive biological

![Figure 3.3. Superimposition of the lowest energy docked poses of 3.2 (purple sticks, colored by atom) and an aminothiazole analogue of 3.3 (teal sticks, color by atom) in a homology model of SphK2. Key residues in the binding cavity are shown as gray sticks, ATP is shown in green, and the overall protein structure is shown as a gray cartoon for perspective.](image-url)
studies have yet to be reported. Aurelio et al. also recently reported the synthesis of derivatives of 3.5 that show limited success as SphK1/SphK2 dual inhibitors and SphK1- and SphK2-selective inhibitors.\textsuperscript{62} Because of the prominence of aminothiazole moieties in SphK inhibitor scaffolds, we incorporated aminothiazole moieties into our guanidine-based scaffold, specifically to achieve SphK1-selective inhibitors.

3.4.2 Inhibitor Synthesis

In prior studies,\textsuperscript{47, 63} we established that the guanidine, 1,2,4-oxadiazole, and internal phenyl ring moieties were key features of the sphingosine kinase inhibitor scaffold. Therefore, we focused our attention on the “tail” region by appending aminothiazoles decorated with diverse aryl structures. The synthesis of aryl-substituted aminothiazoles is shown in Scheme 3.1. 4-Trifluoromethylacetamide benzonitrile 3.13 was synthesized by the acetylation of 4-aminobenzonitrile 3.12 with trifluoroacetic anhydride. Benzonitrile 3.13 was then reacted with hydroxylamine hydrochloride and triethylamine in ethanol in a microwave reactor to yield amidoxime 3.14, which was reacted with homoproline using HCTU and Hunig’s base at 100 °C to afford 1,2,4-oxadiazole 3.15. The trifluoroacetate group was removed using lithium hydroxide to afford amine 3.16. Treatment of 3.16 with thiocarbodiimidazole followed by ammonia provided the key intermediate thiourea 3.17. Aminothiazoles 3.18a–ee were produced by reacting the required α-bromoketone with thiourea 3.17 and Hunig’s base in ethanol in a microwave reactor. The Boc group was removed with trifluoroacetic acid and immediately reacted with $N,N'$-di-Boc-1H-pyrazole-1-carboxamidine in a microwave reactor to afford bis-Boc-protected guanidines 3.19a–ee. Bubbling HCl gas subsequently provided the desired guanidine derivatives 3.20a–ee (see Tables 3.1 and 3.2 for structures).
Scheme 3.1. Synthesis of substituted aminothiazole derivatives 3.20a–ee\(^a\)

\[ \text{Reagents and conditions: (a) trifluoroacetic anhydride, DCM, 0 °C to rt, 19 h, 88%; (b) NH}_2\text{OH.HCl, TEA, ACN, 150 °C microwave, 6 min, 57%; (c) Boc-L-homoproline, HCTU, DIEA, DMF, rt to 100 °C, 4 h, 67%; (d) 1:1 M LiOH:MeOH, 100 °C, 3 h, 82%; (e) Thio-CDI, THF, rt, 4 h; (f) NH}_3\text{(g), 1 min, 86%; (g) α-bromoketone, DIEA, EtOH, 100 °C, microwave, 5 min, 30–90%; (h) 1:1 TFA:DCM; (i) N, N'-di-Boc-1H-pyrazole-1-carboxamidine, DIEA, ACN, 50 °C, microwave, 2 h, 26–87%; (j) HCl (g), MeOH, 90–100%.}

Biphenyl derivatives were synthesized as outlined in Scheme 3.2. Using either compound 3.18f or 3.18i, Suzuki-Miyaura cross-coupling with various aryl boronic acids yielded biaryl aminothiazoles 3.21a–d. The standard synthetic sequence of deprotection, guanylation, and deprotection was then followed to yield the desired biaryl aminothiazole derivatives 3.23a–d.

Scheme 3.2. Synthesis of biphenyl aminothiazole derivatives 3.23a–d\(^a\)

\[ \text{Reagents and conditions: (a) aryl boronic acid, PdCl}_2\text{(dppf), Cs}_2\text{CO}_3, \text{DMF, 150 °C microwave, 90 min; (b) 1:1 TFA:DCM; (c) N, N'-di-Boc-1H-pyrazole-1-carboxamidine, DIEA, ACN, 50 °C, microwave, 2 h; (d) HCl (g), MeOH.} \]
To assess the significance of the hydrogen bond effect in this series of aminothiazole, a comparison of activity between a representative aminothiazole example of the series (3.20k, Table 3.1) and its analogous oxathiazole was done. The corresponding oxathiazole derivative was synthesized as presented in Scheme 3.3. 4-tert-Butoxy benzonitrile 3.25 was synthesized via nucleophilic aromatic substitution of 4-fluorobenzonitrile 3.24 with 1 M potassium tert-butoxide. Benzonitrile 3.25 was then reacted with hydroxylamine hydrochloride and triethylamine in ethanol using a microwave reactor to yield its amidoxime, which was then reacted with homoproline using HCTU and Hunig’s base at 100 °C to afford 1,2,4-oxadiazole 3.26. Phenol derivative 3.27 was achieved via treatment of 1,2,4-oxadiazole 3.26 with trifluoroacetic acid followed by reprotection with Boc-anhydride. Nucleophilic aromatic substitution with phenol derivative 3.27 on 2,4-dibromothiazole afforded oxathiazole 3.28. Suzuki–Miyaura cross-coupling with 4-trifluoromethylphenyl boronic acid yielded the desired oxathiazole 3.29. The standard synthetic sequence of deprotection, guanylation, and deprotection was followed for compound 3.29 to yield the desired oxathiazole derivative 3.31.
Scheme 3.3. Synthesis of the oxathiazole analogue of inhibitor 3.20k<sup>a</sup>

Reagents and conditions: (a) 1 M KOtBu, THF, reflux, 15 h; (b) NH₂OH.HCl, TEA, EtOH, 150 °C microwave, 6 min; (c) Boc-L-homoproline, HCTU, DIEA, DMF, rt to 100 °C, 4 h; (d) 1:1 TFA:DCM; (e) di-tert-butyl dicarbonate, TEA, dioxane, 0 °C, 1 h; (f) 2,5-dibromothiazole, K₂CO₃, DMF, 135 °C, 1 h; (g) 4-trifluoromethylphenyl boronic acid, PdCl₂(dppf), Cs₂CO₃, DMF, 150 °C, microwave, 90 min; (h) N, N'-di-Boc-1H-pyrazole-1-carboxamidine, DIEA, ACN, 50 °C, microwave, 2 h; (i) HCl (g), MeOH.

To further probe the significance of the hydrogen bond effect of the amino moiety in 3.20dd, its methylated derivative was synthesized as illustrated in Scheme 3.4. Biaryl aminothiazole 3.18dd was methylated with methyl iodine in refluxing acetone to yield 32. The standard synthetic sequence of deprotection, guanylation, and deprotection was then followed to yield the desired methylated aminothiazole derivative 3.34.

Scheme 3.4. Synthesis of the methylated analogue of 3.20dd<sup>a</sup>

Reagents and conditions: (a) MeI, K₂CO₃, acetone, reflux, 17 h; (b) 1:1 TFA:DCM; (c) N, N'-di-Boc-1H-pyrazole-1-carboxamidine, DIEA, ACN, 50 °C, microwave, 2 h; (d) HCl (g), MeOH.
3.4.3  Structure–Activity Relationship Studies and Biological Evaluation of Derivatives

With the goal of defining the structure-activity profile of SphKs, a focused library of aminothiazoles bearing a guanidine group was synthesized. These analogues were assayed using a previously described protocol.\textsuperscript{47, 64} In particular, synthesized inhibitors were tested at 0.3 µM with human recombinant enzymes (Tables 1 & 2). All aminothiazoles vary with respect to the number and type of substituents on the appended aryl ring as well as the substitution pattern on this ring.

As shown in Table 3.1, substituting the thiazole ring with an unsubstituted phenyl ring (3.20a) resulted in poor inhibition of either SphK isotype in our assay. Replacement of the phenyl ring with either a 4’ (3.20b) or 3’ (3.20c) pyridyl ring also produced compounds that were inactive with both SphK isotypes. Halogen substituents at the para position of the phenyl ring inhibited both enzymes but with slight selectivity for SphK2. The selectivity and potency for SphK2 was unanticipated because these compounds were expected to inhibit SphK1, as previous work from our laboratories demonstrated that the homologated guanidine-pyrrolidine headgroup\textsuperscript{47} generates an SphK1 inhibitor when the “tail” is an unsubstituted octyl group. Furthermore, aminothiazoles in the Amgen series are also selective for SphK1,\textsuperscript{45} although their scaffold differed in other aspects also. The identity of the halogen atom—fluorine, chlorine, or bromine—did not greatly affect the compounds’ (3.20d–f) SphK2 potency reducing the enzyme activity to ~50%. Moving the halogen group to the meta or ortho position (3.20g–j) resulted in a retention of SphK2 potency, but with a loss in SphK2 selectivity, producing only moderately SphK2-selective inhibitors.
Table 3.1. SphK1 and SphK2 Inhibitory Activity for Monosubstituted Aminothiazole Derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>hSphK1</th>
<th>hSphK2</th>
<th>Compound</th>
<th>R</th>
<th>hSphK1</th>
<th>hSphK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.20a</td>
<td></td>
<td>100 ± 6</td>
<td>83 ± 4</td>
<td>3.20k</td>
<td></td>
<td>57 ± 0.4</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>3.20b</td>
<td></td>
<td>100 ± 5</td>
<td>94 ± 4</td>
<td>3.20l</td>
<td></td>
<td>31 ± 12</td>
<td>28 ± 0.7</td>
</tr>
<tr>
<td>3.20c</td>
<td></td>
<td>95 ± 10</td>
<td>94 ± 7</td>
<td>3.20m</td>
<td></td>
<td>69 ± 4</td>
<td>72 ± 6</td>
</tr>
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<td>3.20d</td>
<td></td>
<td>100 ± 13</td>
<td>55 ± 1</td>
<td>3.20n</td>
<td></td>
<td>89 ± 0.7</td>
<td>44 ± 12</td>
</tr>
<tr>
<td>3.20e</td>
<td></td>
<td>70 ± 3</td>
<td>51 ± 0</td>
<td>3.20o</td>
<td></td>
<td>58 ± 4</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>3.20f</td>
<td></td>
<td>88 ± 4</td>
<td>46 ± 6</td>
<td>3.20p</td>
<td></td>
<td>75 ± 0.2</td>
<td>80 ± 7</td>
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<tr>
<td>3.20g</td>
<td></td>
<td>60 ± 1</td>
<td>45 ± 2</td>
<td>3.20q</td>
<td></td>
<td>91 ± 2</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>3.20h</td>
<td></td>
<td>64 ± 1</td>
<td>45 ± 10</td>
<td>3.20r</td>
<td>EtO-</td>
<td>100 ± 9</td>
<td>84 ± 2</td>
</tr>
<tr>
<td>3.20i</td>
<td></td>
<td>79 ± 12</td>
<td>44 ± 8</td>
<td>3.20s</td>
<td></td>
<td>90 ± 1</td>
<td>100 ± 2</td>
</tr>
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<td>3.20j</td>
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<td>50 ± 4</td>
<td>44 ± 9</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

SphK activity is presented as % control (no inhibitor added). Recombinant human SphK1 or SphK2 was isolated from a cell lysate, and enzyme activity was measured with 5 μM (SphK1) or 10 μM (SphK2) sphingosine and 250 μM γ-[32P]ATP. Compounds were assayed at 0.3 μM in triplicate.

Because of the SphK2 selectivity observed with halogen moieties at the para position, a bulkier electron-withdrawing trifluoromethyl group at the para position (3.20k (SLC4071411)) was synthesized. 3.20k was more potent but less selective for SphK2. Interestingly, placement of
the trifluoromethyl group at the meta position (3.20l) produced a more potent SphK inhibitor (28% inhibition). However, this molecule showed no selectivity for SphK1 vs SphK2. The ortho trifluoromethyl analogue (3.20m) did not improve potency or selectivity. These results suggest that the key binding interactions were lost with the ortho-substituted derivative likely due to a loss of CF₃ interactions with residues Cys533, His556, and Tyr566 that are found at the end of the hydrophobic tunnel of the SphK2 binding pocket, which was shown by our group to be important for strong inhibitor binding.

Replacement of the electron-withdrawing groups with electron-donating groups—methyl (3.20n), methyl ether (3.20o), and ethyl ether (3.20p)—at the para position markedly diminished SphK2 inhibition (<30%) (Table 3.1). Likewise, positioning the methyl ether to the meta position (3.20q) did not improve inhibitory activity at either enzyme isotype. A trifluoromethyl ether group was then tested at the para position for increased lipophilicity and to reestablish deactivating electronics to the aryl ring. The resulting molecule (3.20r (SLC4081418)) decreased SphK2 activity to ~40%, which is likely due to a reestablishment of the fluorine bonding interactions mentioned (vide supra). Good selectivity was also observed with this molecule, as SphK1 activity was diminished only by ~10%. However, shifting the trifluoromethyl ether moiety from the para position to the meta position (3.20s) led to a switch in SphK potency and selectivity. Collectively, these results indicate that placement of electron-donating groups on the aryl ring is unfavorable for SphK2 inhibition.

The effects of disubstitution on the aryl ring of the aminothiazole were also explored (Table 3.2). Although poor inhibition activities were observed with monosubstituted aryl rings containing traditional electron-donating groups, we were curious about the effects of having
Table 3.2. SphK1 and SphK2 Inhibitory Activity for Disubstituted and Bulky Aminothiazole Derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>hSphK1</th>
<th>hSphK2</th>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>hSphK1</th>
<th>hSphK2</th>
</tr>
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<tbody>
<tr>
<td>3.20t</td>
<td>H₃C</td>
<td>NH</td>
<td>91 ± 4</td>
<td>67 ± 12</td>
<td>3.20cc</td>
<td>F₃C</td>
<td>NH</td>
<td>65 ± 2</td>
<td>48 ± 1</td>
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<td>3.20u</td>
<td>H₂CO</td>
<td>NH</td>
<td>100 ± 7</td>
<td>100 ± 7</td>
<td>3.20dd</td>
<td>F</td>
<td>NH</td>
<td>100 ± 2</td>
<td>27 ± 12</td>
</tr>
<tr>
<td>3.20v</td>
<td>H₂CO</td>
<td>NH</td>
<td>100 ± 0.2</td>
<td>71 ± 11</td>
<td>3.23a</td>
<td>F₃C</td>
<td>NH</td>
<td>92 ± 8</td>
<td>73 ± 7</td>
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<td>3.20w</td>
<td>F</td>
<td>NH</td>
<td>51 ± 5</td>
<td>51 ± 5</td>
<td>3.23b</td>
<td>F</td>
<td>NH</td>
<td>82 ± 9</td>
<td>31 ± 7</td>
</tr>
<tr>
<td>3.20x</td>
<td>Cl</td>
<td>NH</td>
<td>51 ± 4</td>
<td>46 ± 11</td>
<td>3.23c</td>
<td>F₃C</td>
<td>NH</td>
<td>100 ± 2</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>3.20y</td>
<td>F</td>
<td>NH</td>
<td>58 ± 5</td>
<td>33 ± 5</td>
<td>3.23d</td>
<td>F₃C</td>
<td>NH</td>
<td>99 ± 6</td>
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<td>NH</td>
<td>68 ± 7</td>
<td>67 ± 5</td>
</tr>
<tr>
<td>3.20aa</td>
<td>F</td>
<td>NH</td>
<td>51 ± 4</td>
<td>30 ± 15</td>
<td>3.31</td>
<td>F₃C</td>
<td>O</td>
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<td>55 ± 2</td>
</tr>
<tr>
<td>3.20bb</td>
<td>F</td>
<td>NH</td>
<td>100 ± 4</td>
<td>63 ± 4</td>
<td>3.34</td>
<td>NCH₃</td>
<td>NCH₃</td>
<td>100 ± 5</td>
<td>100 ± 5</td>
</tr>
</tbody>
</table>

*SphK activity is presented as % control (no inhibitor added). Recombinant human SphK1 or SphK2 was isolated from a cell lysate, and enzyme activity was measured with 5 µM (SphK1) or 10 µM (SphK2) sphingosine and 250 µM γ-[³²P]ATP. Compounds were assayed at 0.3 µM in triplicate.*

Disubstituted aryl rings. Compounds with either two methyl (3.20t) or two methyl ether moieties (3.20u) showed minimal activity. Placement of a methyl ether at the para position and the chlorine moiety at the meta position (3.20v) led to modest SphK2 activity (~70%) and no SphK1 inhibition. In contrast, disubstitution with electron-withdrawing groups reestablished SphK2
potency, particularly fluorines (3.20w, 3.20z), chlorine (3.20x), or a combination of fluorine with a trifluoromethyl group (3.20y (SLC4091423), 3.20aa (SLC4091424), 3.20cc). The substitution pattern for these molecules did not significantly affect their SphK2 potency; however, they did show SphK1/SphK2 dual inhibitor activity, affording good SphK2 inhibition (50–70%) and modest to good SphK1 inhibition (30–50%). Disubstitution of the aryl ring with two trifluoromethyl moieties (3.20bb) led to a loss in compound potency, but this loss in potency was met with a gain in selectivity, as it was inactive toward SphK1 at concentrations up to 1 µM. Together, these results reinforce the preference for an electron-deficient aryl ring.

Our previous studies63, 67 suggest that the SphK2 lipid-binding pocket is larger than that of SphK1. To explore the effects of bulky moieties on the aminothiazole ring, biphenyl derivatives were synthesized and tested (Table 3.2). We discovered that the para-substituted biphenyl derivative (3.20dd) was not only potent toward SphK2 but also selective for SphK2. The meta-substituted biphenyl derivative (3.23a) essentially lost activity. However, a fluorine on the 4-position (3.23b) maintained the potency and selectivity observed with 3.20dd. Attempts to substitute 3.20dd with a trifluoromethyl moiety at either the 4- (3.23c) or 3-position (3.23d) on the biphenyl ring led to compounds inactive in both SphKs, suggesting that the molecules may either be too large for the binding pocket or have poor solubility (cLogP = 6.78). In an effort to introduce additional bulk onto the aminothiazole, a benzofuran moiety (3.20ee) was synthesized but was found to be ineffective as an inhibitor. Collectively, these results suggest that the biphenyl moiety has the optimal binding interaction as well as bend to fit in the J-shaped hydrophobic tunnel.

In addition to investigating the effects of various aryl groups on the aminothiazoles, we also explored the effects of modifying the exocyclic nitrogen of the aminothiazoles (Table 3.2).
The co-crystal structure of SphK1 with 3.5 reported by Wang et al. notes a key hydrogen bonding interaction between the aminothiazole NH and SphK1 threonine-196. Although our scaffold would most likely fit slightly deeper into the SphK1 binding pocket, we probed the significance of this hydrogen bonding capability by either replacing the NH with an ether linkage (3.31) or methylating (3.34) the amino group. N-Me derivative 3.34 was found to be completely inactive in both SphKs up to 1 µM, whereas the ether derivative 3.31 showed moderate activity towards both SphKs, decreasing SphK1 and SphK2 activity by ~ 45% at 0.3 µM. These results suggest that the amino moiety plays a key role in enzyme binding likely through hydrogen bonding (compare 3.34 with 3.20dd) although we cannot rule out the possibility that the loss in SphK2 potency may also be due to steric effects created from methylation. Comparison of 3.31 with amino moiety 3.20k shows a loss in SphK2 potency with equipotent SphK1 inhibition, indicating that the molecules’ hydrogen bonding role (donor vs. acceptor) at this position is more significant in SphK2 than SphK1.

To quantify the aminothiazoles’ activity toward SphK1 and SphK2, we determined the inhibitory constant (Ki) of the most potent compounds in the library (Table 3.3). Consistent with the results of the initial screen (Tables 3.1 and 3.2), fluoro and trifluoromethyl positional isomers of disubstituted aryls 3.20y and 3.20aa had good activity with both enzymes and partial selectivity toward SphK2 (~5 fold) (entries 1 and 2). Fortunately, monosubstitution with a meta-
trifluoromethyl group (3.20l) by removal of the fluorine atom from 3.20y or 3.20aa resulted in improved binding inhibition (entry 3). 3.20l is a dual inhibitor with Ki values of 120 nM and 90 nM for SphK1 and SphK2, respectively. Moving the trifluoromethyl group to the para position had a negative inhibitory effect as expected (entry 4). However, switching to a trifluoromethyl ether on the para position (3.20r) improved the Ki towards SphK2 (250 nM) while decreasing the inhibitory activity against SphK1 (Ki 5 µM), affording 20-fold selectivity toward SphK2 (entry 5). The introduction of a more lipophilic and larger substituent such as a phenyl ring on the para position (3.20dd) resulted in a potent and selective SphK2 inhibitor (Ki 90 nM). To the best of our knowledge, 3.20dd is the most potent SphK2 reported to date; this compound is 100-fold selective toward SphK2 vs SphK1. In comparing compounds 3.20k, 3.20r, and 3.20dd, it is

Table 3.3. Ki and cLogP\textsuperscript{a} Values of Selected Inhibitors of SphK1 and SphK2

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Structure</th>
<th>hSphK1</th>
<th>hSphK2</th>
<th>hSphK2 Selectivity</th>
<th>cLogP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.20y</td>
<td><img src="image" alt="Image" /></td>
<td>0.7 ± 0.05</td>
<td>0.17 ± 0.15</td>
<td>4</td>
<td>5.33</td>
</tr>
<tr>
<td>2</td>
<td>3.20aa</td>
<td><img src="image" alt="Image" /></td>
<td>0.8 ± 0.07</td>
<td>0.17 ± 0.17</td>
<td>5</td>
<td>5.33</td>
</tr>
<tr>
<td>3</td>
<td>3.20l</td>
<td><img src="image" alt="Image" /></td>
<td>0.12 ± 0.04</td>
<td>0.09 ± 0.01</td>
<td>1</td>
<td>5.18</td>
</tr>
<tr>
<td>4</td>
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<td>0.82 ± 0.25</td>
<td>0.39 ± 0.09</td>
<td>2</td>
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<tr>
<td>5</td>
<td>3.20r</td>
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<td>5 ± 0.22</td>
<td>0.25 ± 0.10</td>
<td>20</td>
<td>5.42</td>
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<tr>
<td>6</td>
<td>3.20dd</td>
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<td>9 ± 2</td>
<td>0.09 ± 0.02</td>
<td>100</td>
<td>6.18</td>
</tr>
</tbody>
</table>

\textsuperscript{a}cLogP was calculated for the protonated inhibitor with Chemdraw Professional 13.0.
interesting to note that the inhibition constant and selectivity improved as the steric bulk and lipophilicity of the molecules increased (cLogP 5.18, 5.42 and 6.18, respectively). These results are consistent with the observation that the hydrophobic binding pocket of SphK2 is larger than that of SphK1.

We selected the most potent SphK2-selective (3.20dd) and SphK1/2 dual inhibitors (3.20i) to evaluate their effect on S1P synthesis. U937 cells, a histiocytic lymphoma myeloid cell line that expresses both SphK isotypes, were incubated with either compound. Following incubation, the cells were lysed and sphingosine and S1P levels were determined by LC–MS–MS. Both inhibitors showed a concentration-dependent decrease in S1P levels (Figure 3.4 A,C),

![Figure 3.4](image)

**Figure 3.4.** Effect of 3.20i and 3.20dd on sphingolipids in U937 cells. Following a 2 h incubation U937 cells were harvested by centrifugation, and lysed, and the levels of (A, C) S1P and (B, D) sphingosine were measured using LC-MS/MS. Amounts associated with cells are expressed as the number of picomoles per 10^6 cells. The experiment was performed in duplicate. The level of significance is indicated for each experiment (**P<0.005, ***P<0.001) using an unpaired t-test (compared to control).
whereas Sph levels remained constant (Figure 3.4 B,D), indicating that the compounds are cell permeable and capable of inhibiting SphK activity in whole cells. Further, the selectivity of 3.20dd toward SphK2 in vitro was observed as its effect on the level of S1P is lower than that of 3.20l—S1P is still generated by functional SphK1. To assess the in vivo properties of 3.20dd, C57BL/6 mice were treated with a single 10 mg/kg intraperitoneal dose. The concentration of S1P in blood was monitored over a course of 24 h via LC-MS/MS. As shown in Figure 5, blood S1P levels increased on treatment with SphK2-selective inhibitor 3.20dd at 2 and a 6 h time points and returned to basal levels after 24 h. Such an observation is in accordance with three SphK2-selective inhibitors28, 29, 47 and supports the notion that SphK2-selective inhibitors drive elevated S1P levels in whole blood. To date, 3.20dd is the most potent and selective SphK2 inhibitor reported with in vivo activity.

**Figure 3.5.** Detected S1P blood levels in mice following injection with 3.20dd. Wild-type mice were treated with a single 10 mg/kg ip dose of 3.20dd, and blood samples were collected at the indicated time points. S1P levels from the blood samples were measured via LC-MS/MS. The standard deviations are values from a group of three to four mice. The level of significance is indicated for each experiment (**P < 0.01) using one-way analysis of variance with the Bonferroni multiple comparison test.
3.5 Conclusions

Herein, we disclose the discovery and development of the most potent and selective SphK2 inhibitor reported to date. The scaffold contains guanidine head and aminothiazole tail groups. A surprising result of our studies is that principles obtained from earlier SphK1 inhibitors—insertion of a methylene unit between the oxadiazole and pyrrolidine ring of 3.3 and aminothiazole from 3.2—generated a potent and selective SphK2 inhibitor. Unfortunately, the X-ray crystal structure of SphK2 is not yet available. Thus, docking of inhibitors in the binding site using a validated homology model of SphK2 has been utilized to facilitate the development of inhibitors. Structure-activity relationship studies of these inhibitors indicate that potent inhibition of both SphK1 and SphK2 necessitates an electron-deficient phenyl ring, but these substituents likely benefit from interacting with residues Cys533, His556 and Tyr566 at the end of the binding pocket. Our investigations also support the importance of hydrogen bonding with the exocyclic NH of the aminothiazole ring; removal of hydrogen bonding capacity resulted in a significant loss in activity.

Biological analysis of aminothiazole inhibitors 3.20l and 3.20dd revealed that they effectively lower S1P levels in U937 cells. In vivo study demonstrated that SphK2-selective 3.20dd caused elevated blood S1P levels in wild type mice, recapitulating our previous findings\textsuperscript{28, 29, 47, 60} with SphK2 selective inhibitors. Collectively, our work provides a novel chemical biology approach toward selective SphK2 and dual SphK inhibition. We expect that these studies will aid in elucidating the \textit{in vivo} function of SphK2 as well as the development of improved SphK inhibitors.
3.6 Funding Sources

This work was supported by NIH (grants R01 GM104366 and R01 GM121075) and Alzheimer’s & Related Diseases Research Award Fund.
3.7 References


Antiestrogenic Effects of the Novel Sphingosine Kinase-2 Inhibitor ABC294640.


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Small Molecule Mitochondrial Uncouplers and Their Therapeutic Potential

4.1 Contributions

This chapter is an adaptation of a manuscript currently being written by the author, Stephanie J. Alexopoulos of the University of New South Wales, Dr. Kyle Hoehn of the University of New South Wales, and Dr. Webster Santos for publication in a peer-reviewed journal.

4.2 Introduction

Cellular respiration is a physiological process with a fundamental goal of producing energy in the form of ATP. It is comprised of four main steps: glycolysis, pyruvate oxidation, the citric acid cycle, and oxidative phosphorylation. In glycolysis, glucose derived from carbohydrate catabolism and glycogenolysis is metabolized in the cytosol into two molecules of pyruvate, which are translocated into the mitochondrial matrix (MM) to be converted into acetyl coenzyme A (acetyl CoA) via pyruvate dehydrogenase. Acetyl CoA generated from pyruvate oxidation and fatty acid oxidation is carried forward into the citric acid cycle, which proceeds as a series of redox reactions to produce the reduced electron carriers NADH and FADH₂.

In the final step of cellular respiration, oxidative phosphorylation, NADH and FADH₂, which are primarily produced during the citric acid cycle, transfer their electrons to complexes I and II in the electron transport chain, respectively.¹ This converts the cofactors into their oxidized forms NAD⁺ and FAD, which are recycled in other steps of cellular respiration to regenerate NADH and FADH₂. Complexes I and II then pass their electrons along the electron transport chain (ETC) to further complexes, ultimately resulting in the reduction of molecular oxygen into water at complex IV.¹ The movement of electrons along the ETC from complex I to complex IV is an exergonic process that releases energy by pumping protons from the MM into
the mitochondrial inner membrane (MIM) space, generating an electrochemical gradient known as a proton motive force (pmf).\textsuperscript{2, 3} ATP synthase harnesses the pmf to generate ATP by transporting protons in the MIM space back into the MM.\textsuperscript{4}

### 4.3 Mitochondrial Uncoupling

Transport of protons from the MIM space into the MM independent of ATP synthase is known as mitochondrial uncoupling (Figure 4.1).\textsuperscript{5, 6} It is hypothesized that this physiological process is a protective adaptation to prevent the production of reactive oxygen species (ROS) during oxidative phosphorylation.\textsuperscript{7} Mitochondrial uncoupling can occur in both plants and animals in either a passive or active fashion. Passive uncoupling is associated with proton leak via the adenine nucleotide translocase (ANT) protein complexes, while active uncoupling is known to occur via uncoupling proteins (UCPs) that are found along the mitochondrial inner membrane.\textsuperscript{8} There are five known UCPs in mammals, UCP1–5,\textsuperscript{9} and of the five UCPs, the literature is the richest on UCP1–3.\textsuperscript{8–11} UCP1 has been established as a promoter of non-shivering thermogenesis\textsuperscript{12} while UCP2 and UCP3 are implicated\textsuperscript{7} in a protective role by minimizing oxidative damage from ROS.
4.4 Pharmacological Mimicry of Uncoupling Proteins

Pharmacological mimicry of UCP activity with small molecule mitochondrial uncouplers has been reported. These molecules are typically lipophilic weak acids that rely on the pH gradient between the MIM space (pH = 6.8) and the MM (pH = 8.0–8.1) to facilitate uncoupling activity. As protonophores, mitochondrial uncouplers shuttle protons from the MIM to the MM independent of ATP synthase, disrupting the pmf generated by the ETC. The change in the pmf stimulates an increase in nutrient metabolism and energy expenditure via glycolysis, gluconeogenesis, and fatty acid oxidation to help reestablish the pmf, which is necessary for homeostasis. Increased metabolism also reduces the time that electrons spend in the electron transport chain, which decreases the formation of ROS in the mitochondria, reducing the likelihood of oxidative stress and injury. Consequently, the therapeutic potential of mitochondrial uncouplers have been investigated for the treatment of metabolic diseases such as...
obesity\textsuperscript{17, 19, 23, 32, 33} and type 2 diabetes (T2D),\textsuperscript{24, 32-35} as well as for neurodegenerative diseases including Alzheimer’s disease\textsuperscript{36, 37} and Parkinson’s disease.\textsuperscript{37, 38} This perspective will review the mitochondrial uncouplers reported to date and explore their potential as therapeutics.

4.5 Uncouplers of Oxidative Phosphorylation

Mitochondrial uncouplers with the ability to promote proton leakage from the MIM space to the MM are listed in Table 4.1. For some, their mechanism of action is unclear but most are hypothesized to have protonophoric activity. Early development of mitochondrial uncouplers appears to emphasize compound acidity (pK\textsubscript{a}), as many of the early reported uncouplers such as 4.1 and 4.5 have pK\textsubscript{a} values below 6.8. These compounds are notorious for off-target effects and toxicity, which has fostered general reluctance to use and develop mitochondrial uncouplers as therapeutics. Whereas more recent reported mitochondrial uncouplers have pK\textsubscript{a}s within the range of 6.8–8.1 including 4.8 and 4.19, a shift towards the development of more weakly acidic uncouplers to mitigate off-target effects and minimize toxicity is emerging. Indeed, greater emphasis has been placed towards the development of uncouplers that are selective for the mitochondria, as highlighted with the synthesis of 4.3, 4.14, and 4.19. However, the need for potent, selective, and nontoxic mitochondrial uncouplers is evident, as there is a dearth of reported compounds within the past 10 years. Reexamining approved clinical drugs for mitochondrial uncoupling activity has partially met this need with the discovery that 4.12 is an active mammalian mitochondrial uncoupler. However, phenotypic screens for mitochondrial selectivity will also need to be generated to ensure that library hits are viable leads for structure–activity relationship (SAR) studies in the discovery of next generation mitochondrial uncouplers. Only once new mitochondrial uncouplers are shown to be selective for the mitochondria and to
have low toxicity will these small molecules garner significant interest as therapeutic strategies for the treatment of metabolic diseases and neurodegenerative diseases.

**Table 4.1. Reported Mitochondrial Uncouplers**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>Structure</th>
<th>pKₐ</th>
<th>cLogP</th>
<th>Minimum Concentration Required for Uncoupling Activity</th>
<th>Toxicity</th>
<th>Comments</th>
</tr>
</thead>
</table>
| 4.1      | DNP        | ![DNP Structure](image) | 4.1  | 1.82  | 5 µM | Human LD = 1-3 g  
White rat LD₅₀ = 30 mg/kg  
Dog LD₅₀ = 30 mg/kg | Depolarizes plasma membrane  
-FDA-banned drug |
| 4.2      | MitoDNP    | ![MitoDNP Structure](image) | 4.48 | 8.29  | 50 µM | NR | Mitochondria targeted  
Inactive below 50 µM |
| 4.3      | MitoPhotoDNP | ![MitoPhotoDNP Structure](image) | N/A  | 9.82  | 0.2 µM | Cytotoxicity = > 5 µM | Mitochondria targeted  
Produces active species, DNP, upon 355 nm UV irradiation |
| 4.4      | DNPME      | ![DNPME Structure](image) | N/A  | 1.67  | NR | Rat LD₅₀ = > 500 mg/kg  
Mouse LD₅₀ = 8 mg/kg | Depolarizes plasma membrane  
Commonly used in mitochondrial function studies |
| 4.5      | FCCP       | ![FCCP Structure](image) | 6.2  | 3.71  | 100 nM | Mouse LD₅₀ = 8 mg/kg  
Mouse LD₅₀ = 8 mg/kg | Depolarizes plasma membrane  
Commonly used in mitochondrial function studies |
| 4.6      | Bupivicaine | ![Bupivicaine Structure](image) | 6.1  | 3.69  | ~0.125 µM | Rat LD₅₀ = 12.7 mg/kg | General aesthetic  
Causes myotoxicity and neurotoxicity |
| 4.7      | TTFB       | ![TTFB Structure](image) | 5.04-5.8 | 5.16  | 100 nM | Rat LD₅₀ = 1.6 mg/kg | Preferentially uncouples brain mitochondria |
| 4.8      | C4R1       | ![C4R1 Structure](image) | 7.3  | 6.52  | 1 µM | Cytotoxicity = > 60 µM | Wide therapeutic range  
Confers neuroprotection  
Suppresses appetite and energy expenditure in C57Bl/6 HFD mice |
| 4.9      | SR4        | ![SR4 Structure](image) | NR   | 6.32  | 3 µM | Cytotoxicity = > 25 µM | Activates AMPK  
Reduces body weight and body fat mass in C57Bl/6 HFD mice |
| 4.10     | Ppc-1      | ![Ppc-1 Structure](image) | NR   | 5.77  | 5 µM | Cytotoxicity = > 20 µM | Uncoupling mechanism unknown  
Stimulates fatty acid release from adipocytes |
<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
<th>Structure</th>
<th>pKₐ</th>
<th>cLogP</th>
<th>Minimum Concentration Required for Uncoupling Activity</th>
<th>Toxicity</th>
<th>Comments</th>
</tr>
</thead>
</table>
| 4.11     | ES9  | ![ES9 Structure](image1) | NR  | 3.44  | 1 µM | Cytotoxicity = > 10 µM | • Inhibits CME via cellular acidification  
• Depolarizes plasma membrane |
| 4.12     | Niclosamide ethanolamine | ![Niclosamide Structure](image2) | NR  | 4.34  | 0.5 µM | Rat LD₅₀ = 500 mg/kg | • Salt form of niclosamide, a treatment of human tapeworm infections  
• Orally bioavailable  
• Improves glycemic control and insulin sensitivity in C57Bl/6 HFD mice  
• Slowed development of hyperphagia in diabetic mouse models |
| 4.13     | Ellipticine | ![Ellipticine Structure](image3) | 7.4 | 4.37  | 0.1 µM | Cytotoxicity = > 50 µM | • Antitumor activity via topoisomerase II-β inhibition  
• Induces endoplasmic reticulum stress |
| 4.14     | Cr₇TPP | ![Cr₇TPP Structure](image4) | N/A | 11.95 | ~ 5.5 µM | Cytotoxicity = > 40 µM | • Co-administration with DNP or FCCP allows lower dosages of the respective compounds  
• Reduced appetite, body mass, and body fat in C57Bl/6 HFD mice |
| 4.15     | S13  | ![S13 Structure](image5) | 5.8 | 6.07  | 32 nM | Cytotoxicity = > 5.10 µM | • Forms intramolecular 6-membered ring in deprotonated form |
| 4.16     | SF-6647/  
Tyrphostin A9/AG17 | ![SF-6647 Structure](image6) | 6.7 | 4.22  | 20 nM | Cytotoxicity = > 0.2 µM  
Hepatotoxicity = > 0.97 µM  
Mouse LD₅₀ = 29 mg/kg | • Most potent reported mitochondrial uncoupler  
• Inhibits tyrosine kinase  
• Documented neuroprotective and antiproliferative activity  
• Reported hepatotoxicity |
| 4.17     | CZ5  | ![CZ5 Structure](image7) | NR  | 7.36  | 1 µM | Cytotoxicity = > 10 µM  
Mouse LD₅₀ = 12.7 mg/kg | • Dose-dependent mitochondrial uncoupling activity  
• Orally bioavailable (58%)  
• Lacks acute toxicity up to 500 mg/kg  
• Reduced appetite and weight of HFD mice  
• Improved metabolic panels of HFD mice |
| 4.18     | MitoQ10 | ![MitoQ10 Structure](image8) | N/A | 6.23  | 2.5 µM | Cytotoxicity = > 10 µM  
In vivo Toxicity = > 230 mg/kg | • Most mitochondria targeted  
• Mitochondrial uncoupling activity via ANT rather than as a protonophore  
• Reduced total body fat levels and liver triacylglycerol content in mice  
• Behaves more as an antioxidant than uncoupler |
| 4.19     | BAM15 | ![BAM15 Structure](image9) | 7.5 | 6.52  | 0.27 µM | Cytotoxicity = > 50 µM | • Selectively depolarizes mitochondrial membrane  
• Protective against ischemia reperfusion injury  
• Reduced total body fat and liver triacylglycerol content in mice  
• Lacks cell-type selectivity |

<sup>a</sup>cLogP values calculated with ChemDraw Professional 16.0.
4.5.1 DNP (2,4-Dinitrophenol)

Of the published mitochondrial uncouplers, 4.1 ((2,4-Dinitrophenol, DNP) is the most well known. It was originally used during World War I in the preparation of munitions, but many workers exposed to the chemical experienced weight loss, fever, nausea, and death.\(^{39}\) Due to its weight loss inducing effects, DNP was explored as a treatment for obesity. Early studies found that regulated doses of 4.1 successfully promoted weight loss in obese patients.\(^{40, 41}\) However, cases of cataracts, blindness\(^{42-44}\) or death from drug-induced hyperthermia were reported.\(^{45-47}\) Consequently, the Food and Drug Administration (FDA) removed the drug from the market in 1938.\(^{48}\) Later studies found that the toxicity issues associated with 4.1 treatment result from a narrow drug therapeutic window and a concomitant depolarization of the plasma membrane and the MIM.\(^{49-52}\) Today, it is considered an illicit drug, but it is still used as an uncontrolled weight loss supplement, particularly by body builders.\(^{53}\)

Although 4.1’s therapeutic potential for the treatment of obesity and T2D is emerging, a major obstacle as a pharmaceutical is its narrow therapeutic window. Caldeira da Silva et al. and Goldgof et al. both found that under controlled dosing regimens, mice fed on a high fat diet (HFD) showed improved blood glucose, insulin, and triglyceride levels and reduced body mass.\(^{17, 54}\) Interestingly, mice receiving a controlled dosage of 4.1 lived longer,\(^{54}\) a common observation in calorie-restricted diet studies.\(^{55, 56}\)

A controlled release version of 4.1, CRMP, was more recently developed.\(^{34}\) This orally bioavailable formulation facilitates a wide therapeutic window for 4.1, eliciting an effect up to 100 mg/kg without causing toxicity (4.1 began to show toxicity issues at 1 mg/kg). This formulation also achieves a minimum effective dose of 0.5 mg/kg to lower blood, liver, and
muscle triacylglycerol content in rats, a dose 10-fold lower than that required for 4.1 (5 mg/kg). Additionally, CRMP improved glucose tolerance and insulin sensitivity in T2D rats.

In an alternative approach to reduce toxicity due to off-target effects, Blaikie et al. tethered 4.1 to triphenylphosphonium (TPP) (4.2, MitoDNP) to selectively target 4.1 to the mitochondria.\textsuperscript{57} The dispersed positive charge in TPP affords selective targeting to mitochondria. Unfortunately, this maneuver lacks uncoupling activity, failing to increase mitochondrial respiration rates at concentrations below 50 µM (4.1 increases respiration rates at 5 µM).\textsuperscript{57} To circumvent this failure, Chalmers and coworkers modified 4.2 by inserting a photocleavable linker between 4.1 and the TPP moiety, creating 4.3 (MitoPhotoDNP).\textsuperscript{58} The TPP moiety delivers 4.1 to the mitochondria and releases 4.1 upon 355 nm UV irradiation.\textsuperscript{58} 4.3 is active at concentrations as low as 0.2 µM and lacks cytotoxicity up to 5 µM.\textsuperscript{58} However, it has yet to be tested for its efficacy in vivo.

Tissue targeted delivery of 4.1 also offers a promising strategy. For example, Perry et al. developed a prodrug of 4.1 to produce 4.4 (DNPME).\textsuperscript{33} This methyl ether analogue of 4.1 has no uncoupling activity, as it reduced circulating plasma levels of 4.1, with the majority of 4.1 accumulating in white adipose tissue. Bioactivation of 4.4 in the liver via P450-mediated demethylation reveals 4.1 locally. Studies have shown that 4.4 improved glucose tolerance and insulin sensitivity in T2D rats. This prodrug was also shown to have reduced toxicity, having a ~10-fold higher single dose LD\textsubscript{50} (~350 mg/kg) than 4.1 (~45 mg/kg).\textsuperscript{33}

The therapeutic potential of 4.1 is expanding, for example, toward treatment for spinal cord injury and neurodegenerative diseases. Excitotoxic forces and physical trauma have both been shown\textsuperscript{36, 37, 59} to lead to mitochondrial dysfunction, which includes increased ROS production and extensive Ca\textsuperscript{2+} mitochondrial sequestration. In particular, over-activation of the
$N$-methyl-$d$-aspartate (NMDA)-sensitive glutamate receptor by the neurotransmitter glutamate has been linked\textsuperscript{59} to neurodegenerative diseases like Alzheimer’s,\textsuperscript{36, 60} Parkinson’s,\textsuperscript{38, 61} and Huntington’s diseases.\textsuperscript{62} \textsuperscript{4.1}’s ability to reduce mitochondrial Ca\textsuperscript{2+} levels and ROS production has been well documented,\textsuperscript{37, 63-67} which highlights the therapeutic utility of mitochondrial uncouplers as a treatment for mitochondrial dysfunction.

\textbf{4.5.2 \textit{FCCP (Carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone)}}

The other mitochondrial uncoupler that has been extensively studied is the hydrazone \textbf{4.5} (Carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone, FCCP). Its development and discovery was first reported by Heytler et al. in 1962, along with its analogues such as carbonyl cyanide \textit{m}-chlorophenylhydrazone (CCCP).\textsuperscript{68} Compound \textbf{4.5}’s and CCCP’s uncoupling activity in mitochondria\textsuperscript{68, 69} and chloroplasts\textsuperscript{68-71} were noted early on, with \textbf{4.5} proving to be the more potent uncoupler ($\textbf{4.5} \text{IC}_{50} = 0.04 \mu\text{M}, \text{CCCP IC}_{50} = 0.1 \mu\text{M}$).\textsuperscript{72} Unfortunately, \textbf{4.5} also has a narrow therapeutic window and causes off-target effects,\textsuperscript{51, 52, 73-75} such as depolarization of the mitochondria and plasma membrane. Nevertheless, \textbf{4.5} serves as a valuable as a chemical probe for mitochondrial and cellular function, as well as for studying neurodegenerative diseases.\textsuperscript{37, 52, 74, 76-87}

\textbf{4.5.3 \textit{Bupivacaine}}

The effect of general aesthetics on oxidative phosphorylation have long been studied for their ability to disrupt ATP synthesis, affect electron and Ca\textsuperscript{2+} transport, or act as mitochondrial uncouplers has been reported.\textsuperscript{88} Compound \textbf{4.6} (Bupivacaine) has emerged\textsuperscript{89-93} as a general aesthetic that uncouples oxidative phosphorylation. Dabadie et al. showed that it can increase mitochondrial respiration by acting as a protonophore,\textsuperscript{89} which was confirmed by mitochondrial swelling experiments.\textsuperscript{94, 95} Compound \textbf{4.6}’s mitochondrial uncoupling activity is hypothesized to
arise from due to its liposolubility and p$K_a$ (8.1), which allows it to passively transfer between the MIM space and MM. However, Terada et al. and van Dam et al. discovered, the protonophoric mitochondrial uncoupling occurs only in the presence of hydrophobic anions such as picrate; otherwise, it acts as a decoupler by inducing a slip in the proton pumps during oxidative phosphorylation. Sztark et al. later showed that 4.6 can be either a mitochondrial uncoupler or decoupler, depending upon the respiration state of the mitochondria and the membrane potential.

Unfortunately, 4.6 has been shown to have myotoxicity and neurotoxicity. Weinberg and coworkers found that 4.6’s myotoxicity is in part due to the inhibition of carnitine metabolism in cardiac mitochondria. Lirk et al. established that 4.6’s neurotoxicity is linked to ROS activation of mitogen activated protein kinase (MAPK) pathway. Further, 4.6 has been shown to promote ROS generation, which is thought to contribute to its toxicity. Although the exact mechanism of the enhanced ROS generation is unclear, disruption of the electron transport via reversible binding to complexes I and III plays a role. 4.6-induced cardiotoxicity in rats could be reversed with lipid infusion using Intralipid. More recently, Chen and coworkers showed that a lipid emulsion version of 4.6 helped to minimize myocardial toxicity.

4.5.4 TTFB (4,5,6,7-Tetrachloro-2-trifluoromethylbenzimidazole)

Compound 4.7 (TTFB) was as a constituent of a benzimidazole library intended for herbicidal use. Jones and Watson and Büchel et al. concurrently reported that 4.7 is a mitochondrial uncoupler, showing that 0.03–0.08 µM was necessary to elicit the uncoupling. The observed uncoupling activity is attributed to the compound’s p$K_a$ (NH) of 5.04–5.6, suggesting that the molecule’s active form is its anionic form, which is stabilized by the aromatic imidazole ring. However, due to reported mammalian toxicity and an uncoupling
preference for brain mitochondria over liver mitochondria,\textsuperscript{110} \textbf{4.7} has not been pursued as a therapy for metabolic or neurodegenerative diseases.

\textbf{4.5.5 C4R1}

Developed from the fluorescent dye rhodamine 19,\textsuperscript{14} \textbf{4.8} (C4R1) is a mitochondrial uncoupler (IC$_{50}$ of $\sim 1 \mu$M) that can promote mitochondrial respiration up to $\sim 60 \mu$M, highlighting the molecule’s wide therapeutic range.\textsuperscript{111} This mild uncoupling activity is attributed to its measured pK$_a$ of 7.3.\textsuperscript{14} Studies found that \textbf{4.8} is a cationic protonophore that is selective for the mitochondria with activity that is dependent upon membrane potential.\textsuperscript{14, 111}

Compound \textbf{4.8} is orally bioavailable and offers neuroprotection by reducing brain swelling and cognitive decline in a stroke model.\textsuperscript{19, 111} It also showed promise as a lead molecule for the treatment of obesity by suppressing appetite and increasing resting metabolism in C57Bl/6 HFD mice over a 30-day period.\textsuperscript{19} With a daily dosage of 30 $\mu$mol/kg/day of \textbf{4.8}, overall body mass and body fat decreased by 19\% and 20\%, respectively, without observed toxicity effects.\textsuperscript{19} Interestingly, 27 $\mu$mol/kg/day of \textbf{4.1} did not alter the metabolism of C57Bl/6 HFD mice.\textsuperscript{17}

\textbf{4.5.6 SR4}

A library screen for anti-cancer activity led to the discovery of the dichlorophenylurea \textbf{4.9} (SR4).\textsuperscript{112} Treatment of HL-60 leukemia cells with \textbf{4.9} resulted in a mitochondria-induced apoptosis, which was associated with a depolarization of the mitochondrial membrane.\textsuperscript{112} Further investigation in melanoma cell lines and mouse models led to the discovery that it activates AMP-activated kinase (AMPK) by promoting phosphorylation and induction of anti-tumor mechanisms.\textsuperscript{113} Further study revealed that this process occurs in adipocytes and inhibits adipogenesis, suggesting \textbf{4.9} has anti-obesity potential.\textsuperscript{114} C57Bl/6 HFD mice dosed with 5
mg/kg three times a week experienced a 16% reduction in body weight and ~ 35% body fat mass compared to controls.\textsuperscript{32} Treatment with \textbf{4.9} also improved insulin sensitivity and glucose metabolism and reduced liver lipid content as a direct consequence of AMPK activation\textsuperscript{32} and mitochondrial uncoupling activity.\textsuperscript{16} Compound \textbf{4.9} increases mitochondrial respiration at concentrations as low as 3 µM and up to 25 µM independent of the ANT and UCPs, indicating protonophoric uncoupling activity.\textsuperscript{16} However, selectivity for the mitochondria has not been established, and \textbf{4.9} exhibits similar liver toxicity to \textbf{4.5}.\textsuperscript{16}

\textbf{4.5.7} \textit{Ppc-1}

Discovered from a natural product library screen, \textbf{4.10} (Ppc-1) is a secondary metabolite of the slime mold \textit{Polysphondylium pseudo-candidum}.\textsuperscript{115} Kikuchi and coworkers discovered\textsuperscript{116} that this molecule has mitochondrial uncoupling activity, increasing oxygen consumption at concentrations as high 20 µM without causing toxicity or inhibiting mitochondrial respiration.\textsuperscript{117} It is currently unclear how \textbf{4.10} functions as a mitochondrial uncoupler, but studies have established that its mitochondrial uncoupling activity is not from the formation of a permeability transition pore (PTP), which improves ion permeability into the mitochondrial matrix.\textsuperscript{23, 118}

Compound \textbf{4.10}’s potential as a weight loss therapy has been explored. ICR mice fed on a normal diet and dosed with 0.8 mg/kg/week maintained a normal appetite and constant weight over an 8-week period versus control.\textsuperscript{23} Analysis of tissue and blood serum samples revealed high concentrations of \textbf{4.10} in adipocytes and higher than normal blood fatty acid levels, which is believed to be a consequence of \textbf{4.10}-stimulated fatty acid release from adipocytes.\textsuperscript{23} Surprisingly, higher (4 mg/kg/week and 10 mg/kg/week) and lower doses (0.16 mg/kg/week) of \textbf{4.10} were ineffective in preventing weight gain.\textsuperscript{23}
4.5.8 **ES9 (Endosin 9)**

Discovered from a small molecule library screen for inhibitors of tobacco pollen germination and growth, 4.11 (ES9)\textsuperscript{119} was found to also inhibit clathrin-mediated endocytosis (CME) with an IC\textsubscript{50} of 5 µM.\textsuperscript{15} Because CME is dependent on ATP stores, 4.11’s impact on ATP production was assessed, revealing mitochondrial uncoupling activity.\textsuperscript{15} Compound 4.11 increased mitochondrial respiration at concentrations as low as 1 µM\textsuperscript{15} but depleted ATP stores above 10 µM, which was attributed to depolarization of the plasma membrane.\textsuperscript{15} Dejonghe et al. discovered that 4.11 inhibits CME via cellular acidification through mitochondrial uncoupling and depolarization of the plasma membrane.\textsuperscript{15} Compound 4.11 has yet to be explored as a chemical probe mitochondrial function.

4.5.9 **Niclosamide Ethanolamine**

Compound 4.12 (niclosamide ethanolamine, NEN) is the salt form of niclosamide, an FDA approved anthelmintic drug whose mode of action is mitochondrial uncoupling of the parasite’s mitochondria.\textsuperscript{120} Compound 4.12 is orally bioavailable and has an excellent safety profile.\textsuperscript{121, 122} Because of its mitochondrial activity, Tao et al. assessed 4.12 as a potential treatment for T2D.\textsuperscript{24} Compound 4.12 uncouples mammalian mitochondria at concentrations as low as 0.5 µM, and dosing mice fed on a HFD with 40 mg/kg improved glycemic control, increased insulin sensitivity, reduced liver fat content, and limited weight gain versus control.\textsuperscript{24} In a mouse model for diabetes, treatment of db/db mice with 4.12 improved glycemic control and slowed the development of hyperphagia.\textsuperscript{24} It also improved glucose metabolism without increasing insulin sensitivity, which was later found to be a consequence of 4.12 functioning as a blocker of the glucagon PKA signaling pathway.\textsuperscript{123}
4.5.10 Ellipticine

Compound 4.13 (Ellipticine) is an alkaloid isolated from the plant *Ochrosia elliptica* with antitumor activity and noted toxicity. Its toxicity results from mitochondrial uncoupling activity, which increases respiration and highly depolarizes the mitochondrial membrane in both plant and mammalian cells at concentrations as low as 0.1 µM. However, concentrations above 50 µM were found to inhibit mitochondrial respiration. Compound 4.13’s cationic (pKₐ = 7.4) and neutral forms are implicated in its mitochondrial uncoupling activity, and its membrane permeability was demonstrated to be pH independent. Unfortunately, 4.13 is cytotoxic, inducing endoplasmic reticulum stress and inhibiting of topoisomerase II-β. There are no current reports of 4.13 as a chemical probe for mitochondrial function.

4.5.11 C₁₂TPP

Because cationic TPPs accumulate in the mitochondria, they are commonly used as a handle for mitochondrial drug delivery. Severin et al. and Sukhanova et al. discovered that 4.14 (C₁₂TPP) in the presence of the fatty acid palmitate can induce mild mitochondrial uncoupling in rat-heart mitochondria and decrease the amount of mitochondrial H₂O₂. In the absence of fatty acid additives, 4.14 can uncouple yeast mitochondria at concentrations as low as ~5.5 µM, but this likely occurs due to the presence of endogenous fatty acids in the yeast cells. Although 4.14 lacks pro-oxidant activity at high concentrations, it does inhibit mitochondrial respiration above 40 µM, a consequence of mitochondrial swelling induced by detergent behavior.

Compound 4.14’s therapeutic potential has been explored. Synergistic administration of 4.14 with either 4.5 or 4.1 reduced the concentration of uncoupler needed to increase mitochondrial respiration (0.4 µM vs. 0.6 µM and 10 µM vs. 55 µM, respectively).
hypothesized\textsuperscript{13} that 4.14 interacts with the anionic forms of 4.5 and 4.1 to facilitate mitochondrial localization, minimizing off-target depolarization of the plasma membrane. This system has yet to be tested in animals. Interestingly, dosing mice fed a HFD with 50 \(\mu\)mol/kg/day reduced food intake, body mass, and body fat without noticeable toxicity.\textsuperscript{142} These results are a consequence of fatty acid-associated mitochondrial uncoupling and an UCP-1 independent up-regulation of brown adipose tissue.\textsuperscript{142}

4.5.12 \(S\)-13

The salicylanilide 4.15 (\(S\)-13)\textsuperscript{143} increases mitochondrial respiration at concentrations as low as 0.032 \(\mu\)M.\textsuperscript{144} Its anionic form (p\(K_a\) = 5.8) is suggested to be the active form during mitochondrial uncoupling activity.\textsuperscript{145} It is proposed that in its anionic state, 4.15 forms an internal 6-membered ring, which retains the neutral state’s lipophilicity, permitting passive diffusion through the mitochondrial membrane.\textsuperscript{144} However, 4.15 inhibits mitochondrial respiration above 0.10 \(\mu\)M.\textsuperscript{146}

4.5.13 SF-6847/Tyrphostin A9/AG17

Originally synthesized for agricultural fungicidal and acaricidal usage,\textsuperscript{147} 4.16 (SF-6847/Tyrphostin A9/AG17) increases mitochondrial respiration at concentrations as low as 0.020 \(\mu\)M.\textsuperscript{148} Its anionic state (p\(K_a\) = 6.70) is hypothesized to be the active structure during the uncoupling process.\textsuperscript{149} NMR studies and energy barrier calculations indicate\textsuperscript{150, 151} that a restricted rotational barrier localizes the negative charge, which is also shielded by the two tert-butyl moieties on the benzene ring, allowing 4.16 to retain its lipophilicity when deprotonated.\textsuperscript{152}

Compound 4.16’s therapeutic potential has only been explored to a limited extent. Concentrations as low as \(\sim 0.5 \mu\)M 4.16 have been shown to be protective against glutamate-induced oxidative damage in neuronal cells.\textsuperscript{153} Additionally, 4.16 was shown to reduce the
impact of insulin on the white fat tissue cells; however, it is unclear if this observation is associated with 4.16’s mitochondrial uncoupling activity.\textsuperscript{154} Unfortunately, 4.16 has reported cytotoxicity above 0.20 µM,\textsuperscript{152} hepatotoxicity above 0.97 µM\textsuperscript{152} and a mouse LD\textsubscript{50} of 29 mg/kg.\textsuperscript{147} In addition to uncoupling the mitochondria, 4.16 inhibits tyrosine kinase\textsuperscript{155, 156} and Ca\textsuperscript{2+} release.\textsuperscript{157}

4.5.14 CZ5

Discovered from a high-throughput screen, 4.17 (CZ5) is a protonophoric mitochondrial uncoupler\textsuperscript{158} that increases mitochondrial respiration dose-dependently and preferentially uncouples myocyte and adipocyte mitochondria at concentrations as low as 1 µM.\textsuperscript{158} Higher concentrations were required to increase mitochondrial respiration in hepatocytes.\textsuperscript{158} Additionally, 4.17 has minimal cytotoxicity up to 10 µM.

Compound 4.17 has good pharmacokinetics, is orally bioavailable (58%), and can be administered up to 500 mg/kg in a single dose without causing acute toxicity.\textsuperscript{158} HFD fed mice dosed with 30 mg/kg/day over a 5-week period consumed less food and had reduced body weight and body fat content in comparison to controls.\textsuperscript{158} Improved metabolic panels were also observed, including reduced cholesterol, triacylglycerol, and fasting blood glucose levels, and improved insulin levels.\textsuperscript{158}

4.5.15 Cationic Triphenylphosphoniums

In addition to 4.14, numerous TPP mitochondrial uncouplers have been reported, some of which have shown success in vitro and in vivo. These compounds include MitoQ,\textsuperscript{159} SKQ1,\textsuperscript{13, 160} MitoFluo,\textsuperscript{161, 162} FF16-TPP,\textsuperscript{163} and MitoBHT.\textsuperscript{21} Of the TPPs reported, the literature is the richest on 4.18 (MitoQ10).\textsuperscript{164} Initially designed as a method for delivering ubiquinone to the mitochondria for antioxidant purposes,\textsuperscript{159} 4.18 was found to have mitochondrial uncoupling
properties, stimulating mitochondrial respiration at concentrations as low as 2.5 µM via the ANT;\textsuperscript{21} concentrations above 10 µM led to decreased membrane potential and cell death.\textsuperscript{159} However, later studies discovered that 4.18 has nanomolar uncoupling activity, although exact concentrations were not specified. 4.18 displays antioxidant properties in addition to mitochondrial uncoupling activity, as it was able to prevent H$_2$O$_2$ oxidation of the fatty acid cis-parinaric acid.\textsuperscript{159}

Compound 4.18 is orally bioavailable and lacks toxicity up to 230 mg/kg/day.\textsuperscript{165} Long-term (28 weeks) dosing of 3.2 µmol/day/mouse decreased total mouse body fat and liver triacylglycerol content without affecting mouse body mass.\textsuperscript{166} Other mouse studies with 4.18 have explored its effects on models of obesity,\textsuperscript{167} cardiac ischemia reperfusion,\textsuperscript{168} hypertension and stroke,\textsuperscript{169} and sepsis.\textsuperscript{170} Two human phase II studies have also been conducted with 4.18. One study explored its ability to slow the progression of Parkinson’s disease in newly diagnosed patients;\textsuperscript{171} however, it produced no improvements in comparison to the placebo.\textsuperscript{171} The other study explored its ability to reduce blood serum hepatitis C virus (HCV) RNA levels in patients with chronic HCV, but instead of reducing HCV RNA levels, 4.18 reduced liver damage associated with HCV.\textsuperscript{172} For all studies mentioned above, 4.18’s therapeutic use was as an antioxidant rather than as a mitochondrial uncoupler.

4.5.16 $\text{BAM15}$

Discovered from a screen of a known library of compounds,\textsuperscript{20} 4.19 (BAM15) was found to have mitochondrial uncoupling activity with an EC$_{50}$ of 0.27 µM.\textsuperscript{173} Unlike 4.1 and 4.5, 4.19 is selective for the mitochondria over the plasma membrane, which was confirmed by electrophysiology studies.\textsuperscript{20} Kenwood et al. hypothesized\textsuperscript{20} that the selectivity for the mitochondria is due to the molecule’s pK$_a$, (7.5). SAR studies established\textsuperscript{173} that the furazan and
pyrazine rings were essential for uncoupling.\textsuperscript{173} However, \textbf{4.19} lacks cell-type selectivity, as it was capable of uncoupling, muscle, liver, heart, and connective tissue cells.\textsuperscript{20} Nonetheless, \textbf{4.19} showed protective effects in kidney ischemia reperfusion injury.\textsuperscript{20} More recent studies have established\textsuperscript{174-176} its utility for the study of basic mitochondrial function, particularly due to its limited off-target effects and wide therapeutic window.\textsuperscript{20}

\textbf{4.6 Conclusions}

Mitochondrial uncoupling is a key physiological mechanism in mammals that has evolved to provide endogenous heat and to counteract ROS production during oxidative phosphorylation, as indicated by the presence of multiple UCP isoforms. Pharmacological mitochondrial uncoupling has evolved as a therapeutic for various metabolic and neurodegenerative diseases. Cell toxicity from off-target effects such as depolarization of the plasma membrane has generated reluctance towards the use of mitochondrial uncouplers in medicine. However, fine-tuning of molecular electronics, pro-drug strategies, and mitochondrial selective handles have produced mitochondrial uncouplers with excellent therapeutic potential. Future mitochondrial uncouplers will need to be developed with pK\textsubscript{a} and lipophilicity in consideration to promote mitochondrial selectivity and to maintain drug-likeness. Further SAR studies will aid in the elucidation of mitochondrial function in disease states and will help bring to fruition the development of mitochondrial uncoupler clinical candidates.

\textbf{4.7 Dissertation Overview for Developing Mitochondrial Uncouplers}

Chapter 4 discussed the process of mitochondrial uncoupling, small molecule mitochondrial uncouplers, and the therapeutic potential of small molecule mitochondrial uncouplers for the treatment of metabolic diseases and mitochondrial dysfunction. Chapter 5 will disclose an SAR on the aniline portion of \textbf{4.19}. These derivatives show good mitochondrial
uncoupling activity over a wide concentration range and reveal that $pK_a$ and cLogP contribute equally to mitochondrial uncoupling activity. Chapter 6 provides the supplemental information for the compounds synthesized and characterized by the author of this dissertation.
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5 Structure-Activity Relationship Studies of Uncouplers of Oxidative Phosphorylation

5.1 Contributions

The work in this chapter was done primarily by the author. The author synthesized and characterized all final compounds and their corresponding intermediates. Stefan Hargett of the University of Virginia Department of Pharmacology conducted all biological analyses of the reported compounds. Dr. Yumin Dai conducted all pK_a determinations of the reported compounds. This chapter is an adaptation of a manuscript currently being written by the author and Dr. Webster Santos for publication in a peer-reviewed journal.

5.2 Abstract

Mitochondrial uncouplers are small molecules that leak protons from the mitochondrial membrane space into the mitochondrial matrix independent of ATP synthase, causing a disruption in the proton motive force generated during oxidative phosphorylation. In response, nutrient metabolism increases to reestablish the proton gradient necessary for normal cellular function. Consequently, mitochondrial uncouplers have generated interest over the years for their therapeutic potential for the treatment of obesity and type 2 diabetes. In this article, we describe the synthesis and biological evaluation of small molecule mitochondrial uncouplers. Altering the electronics on the aniline ring of the mitochondrial uncoupler 5.13 (BAM15) generated analogues with good mitochondrial uncoupling activity across a wide therapeutic range. Evaluation of pK_a and cLogP of analogues of 5.13 suggest the best uncoupling activity is observed from molecules that have a pK_a between 6.8 and 8.0 and a cLogP > 6.
5.3 Introduction

Oxidative phosphorylation is an essential aerobic process that utilizes the potential energy in the form of a proton motive force (pmf) generated from the electron transport chain to produce the high-energy biomolecule adenosine triphosphate (ATP) via ATP synthase. Once formed, ATP can be used to fuel various biological reactions to maintain cellular function, or it can be used as a signaling molecule. Leakage of protons from the mitochondrial inner membrane space into the mitochondrial matrix independent of ATP synthase to disrupt the pmf used to generate ATP is known as mitochondrial uncoupling. The process of mitochondrial uncoupling occurs naturally in mammals, particularly in brown adipose tissue, which use uncoupling proteins (UCP) that are situated throughout the mitochondrial inner membrane. Mammals have five known UCPs, UCP1–5. Of the five UCPs, UCP1–3 are the most studied and well understood. UCP1 has long been established as a promoter of non-shivering thermogenesis, while UCP2–3 are thought to play a protective role by minimizing oxidative damage from radical oxygen species.

Small molecule mitochondrial uncouplers have been developed to pharmacologically mimic UCP activity (Figure 5.1). These uncouplers are typically lipophilic weak acids that can transport protons from the mitochondrial inner membrane space into the mitochondrial matrix independent of ATP synthase. Their therapeutic potential for the treatment of various disease states have been explored, particularly for the treatment of obesity and type 2 diabetes (T2D) due to their ability to promote increased nutrient metabolism and energy expenditure. Multiple reports have shown that mice fed a high fat diet supplemented with small molecule mitochondrial uncouplers gained less weight than controls and also had reduced blood glucose and lipid levels, in addition to improved insulin sensitivity,
highlighting the therapeutic benefits of mitochondrial uncouplers in the treatment of obesity and T2D.

![Image of Mitochondrial Uncouplers](image)

**Figure 5.1.** Select Mitochondrial Uncouplers

Over the past 80 years, numerous mitochondrial uncouplers have been developed and reported. Of the published mitochondrial uncouplers, 5.1 (DNP) is the most well-known. Originally used as an explosive, 5.1 was found to have weight-loss-inducing effects, promoting upwards of 50% increase in metabolism, which led this drug to be marketed as a weight-loss supplement. However, 5.1 caused adverse side effects including drastic increases in body temperature, cataracts, and blindness, which led the U. S. Food and Drug Administration to remove this drug from the market in 1938. Studies now indicate that the toxicity issues associated with 5.1 are due to a narrow therapeutic index and off-target effects resulting from dual depolarization of the mitochondrial inner membrane and plasma membrane. Unfortunately, a mitochondria-targeted analogue of 5.1 tethered to a triphenylphosphonium cation (5.2, MitoDNP) severely lost uncoupling activity below 50 μM.
A modified version of 5.2 caged with a photocleavable linker (5.3, MitoPhotoDNP) successfully released 5.1 in the mitochondria upon 355 nm UV irradiation. In vivo application of this molecule has yet to be reported. More recently, Perry et al. reported a controlled-release derivative of 5.1 that is orally bioavailable with an increased therapeutic range, reduced toxicity, promoted insulin sensitivity, and improved glucose tolerance in T2D rats. Further, a prodrug version of 5.1, 5.4 (DNPME,) was found to be non-toxic and liver specific while improving glucose tolerance in T2D rats and increasing insulin sensitivity. Another notable potent mitochondrial uncoupler is 5.5 (FCCP). Unfortunately, its very narrow therapeutic window and off-target effects at the plasma membrane severely limit therapeutic applications in vivo. Nonetheless, the continued search for mitochondrial uncouplers has generated 5.6 (niclosamide ethanolamine), 5.7 (Ppc-1), 5.8 (SR4), 5.9 (C4R1), 5.10 (ES9), 5.11 (CZ5), and 5.12 (C12TPP). Despite this recent progress, the field has suffered from a paucity of mitochondrial selective agents with a wide therapeutic window.

Recently, we reported the discovery and synthesis of a novel mitochondrial uncoupler, 5.13 (BAM15), which is selective for the mitochondria and has a wide therapeutic window. Preliminary studies revealed that the furazan, pyrazine, and aniline moieties on 5.13 are necessary for mitochondrial uncoupling activity (Figure 5.2). Although the aniline moiety cannot be replaced with a phenol moiety, our studies highlighted the importance of the aniline rings in mitochondrial respiration studies, as measured by oxygen consumption rate (OCR). Herein, we report the synthesis, structure–activity relationship study (SAR), and biological evaluation of derivatives of 5.13 that explore electronic effects of substituents on the aniline ring. These analogues show good mitochondrial uncoupling activity with a wide therapeutic window.
5.4 Results and Discussion

5.4.1 Inhibitor Development

Studies have established that mitochondrial uncouplers are typically lipophilic, weak acids. An issue most commonly associated with reported mitochondrial uncouplers is selectivity for the mitochondria. Uncouplers such as 5.1 and 5.5 are notoriously nonselective for the mitochondria with off target effects of depolarizing the plasma membrane. Our recently reported mitochondrial uncoupler 5.13 was demonstrated to be selective for the mitochondria over the plasma membrane. We hypothesize that its selectivity is due to its measured pKa, which was found to be 7.56. This pKa value allows 5.13 to easily shuttle a proton between the mitochondrial inner membrane space (pH = 6.8) and mitochondrial matrix (pH = 8.0–8.1). In an effort to explore the effect of uncoupler pKa on uncoupling activity, we made modifications to the aniline moiety of 5.13 by varying the electronics on the ring by having either electron-donating or electron-withdrawing groups at different positions on the ring.

5.4.2 Chemical Synthesis

The synthesis of symmetrical and unsymmetrical derivatives of 5.13 is shown in Scheme 5.1. Dichlorofurazan 5.15 was synthesized from the reaction of diaminofurazan (5.14) with oxalic acid in a 10% HCl solution, followed by treatment PCl5/POCl3. Compound 5.15 was then reacted with various alkyl or aryl amines in THF to afford the desired symmetrical
derivatives 5.16a–w. To synthesize the unsymmetrical derivatives, compound 5.15 was reacted with 2-fluoroaniline and triethylamine in THF followed by the addition of the desired aryl amine and triethylamine to yield the unsymmetrical derivatives 5.17a–x.

Scheme 5.1. Synthesis of Symmetrical and Unsymmetrical Derivatives of 5.13

Reagents and conditions: (a) oxalic acid, 10% HCl, reflux, 3 h, quantitative; (b) PCl$_5$, POCl$_3$, reflux, 2 h, 30%; (c) amine, THF, reflux, 19 h, 19–95%; (d) 2-fluoroaniline, TEA, THF, 0 ºC, 0.25 to 1 h, then aryl amine, TEA, THF, 0 ºC to rt, 19 h, 13–59%.

The synthesis of amide derivatives of 5.13 is shown in Scheme 5.2. The diamine 5.18 was synthesized from the reaction between 5.15 and ammonium hydroxide. Compound 5.18 was then reacted with either an anhydride or sulfonyl chloride and triethylamine in DCM to afford compounds 5.19a–e.

Scheme 5.2. Synthesis of Amide Derivatives of 5.13

Reagents and conditions: (a) NH$_4$OH, ACN, 0 ºC to rt, 3 h, quantitative; (b) anhydride, TEA, DCM, 0 ºC to rt, 19 h, 40%; (c) sulfonyl chloride, TEA, DCM, 0 ºC to rt, 19 h, 8–29%.
5.4.3 Structure–Activity Relationship Studies and Biological Characterization of 5.13

Derivatives

We synthesized a series of derivatives of 5.13 that focused on modifications to the aniline rings. Symmetrical derivatives maintain the aniline ring with either electron rich or electron deficient substituents at varying positions. For unsymmetrical derivatives, the 2-fluoroaniline was maintained while the electronics and sterics on the neighboring aniline ring was investigated. Further, the 2-fluoroaniline moiety of 5.13 was replaced with amides or sulfonamides to determine their effect on mitochondrial uncoupling. All compounds were tested at concentrations of 0.1, 0.25, 0.5, 1, 5, 10, 20, and 40 μM for mitochondrial uncoupling activity as a function of oxygen consumption rate (OCR). OCR serves as a gauge for mitochondrial respiration because molecular oxygen is required for the production of ATP via oxidative phosphorylation. The maximum percentage of basal (100%) respiration that can be stimulated at 40 μM is reported in Tables 5.1–5.3. We focused our attention to developing compounds that have good efficacy over a wide therapeutic range. Therefore, to effectively identify compounds that maintain uncoupling activity at high concentrations, we used 40 μM as a filter to prevent championing compounds that may have good potency but lack efficacy and to highlight compounds with poor potency but good efficacy.
### Table 5.1. Activity of Symmetrical Derivatives of 5.13

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Max% Basal Activity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Compound</th>
<th>R</th>
<th>Max% Basal Activity&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>5.13</td>
<td></td>
<td>329 ± 11</td>
<td>5.16i</td>
<td></td>
<td>110 ± 4</td>
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<tr>
<td>5.16a</td>
<td></td>
<td>105 ± 2</td>
<td>5.16m</td>
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<td>149 ± 27</td>
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<tr>
<td>5.16b</td>
<td></td>
<td>99 ± 1</td>
<td>5.16n</td>
<td></td>
<td>115 ± 27</td>
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<tr>
<td>5.16c</td>
<td></td>
<td>198 ± 4</td>
<td>5.16o</td>
<td></td>
<td>88 ± 1</td>
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<tr>
<td>5.16d</td>
<td></td>
<td>140 ± 4</td>
<td>5.16p</td>
<td></td>
<td>121 ± 4</td>
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<tr>
<td>5.16e</td>
<td></td>
<td>100 ± 1</td>
<td>5.16q</td>
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<td>131 ± 16</td>
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<tr>
<td>5.16f</td>
<td></td>
<td>249 ± 13</td>
<td>5.16r</td>
<td></td>
<td>127 ± 22</td>
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<tr>
<td>5.16g</td>
<td></td>
<td>97 ± 3</td>
<td>5.16s</td>
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<td>5.16i</td>
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<td>100 ± 1</td>
<td>5.16v</td>
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<td>9 ± 27</td>
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<tr>
<td>5.16k</td>
<td></td>
<td>135 ± 2</td>
<td>5.16w</td>
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<td>86 ± 2</td>
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</table>

<sup>a</sup>The maximum percentage of basal (100%) respiration that could be stimulated using 40 µM uncoupler.
Using 5.13 as the standard, the effect of replacing the 2-fluoroaniline aniline moiety with alkylamines was initially explored (Table 5.1). Substitution with the bulky tert-butylamine (5.16a) or a cyclopropylamine (5.16b) did not increase OCR. Increasing the cyclic amine ring size to five- (5.16c) and six- (5.16d, SHC4041644) membered resulted in a modest (> 2-fold) increase in OCR. Replacement of the cyclic amines with a heterocyclic morpholine analogue (5.16e) produced an inactive molecule (no increase in respiration).

We next switched our focus to altering the electronics of the aniline ring portion of 5.13 (Table 5.1). Installation of methyl substituents on the anline rings (5.16f–h) resulted in increased OCR values relative to basal levels. While the meta-substituted 5.16g (SHC4051652) had no effect on respiration, the ortho- (5.16f) and para-substituted (5.16h, SHC4081662) derivatives increased OCR to 250%. Encouraged by these results, electron rich methyl (5.16i–k) and ethyl (5.16l–n) ether derivatives were synthesized and tested. Unfortunately, all had minimal effect in increasing OCR. To further confirm that strongly electron-donating moieties have deleterious effects on respiration, phenol 16o and trifluoroacetamide 5.16p were tested and results indicate that both compounds lack uncoupling activity. These studies suggest that increasing electron density on the aniline ring of the 5.13 scaffold has a negligible effect on uncoupling activity.

To investigate the effect of increased lipophilicity and decreased ring electron density, trifluoromethyl ether (5.16q–s) and trifluoromethyl (5.16t–v) derivatives at the ortho, meta, and para positions were then synthesized.\textsuperscript{196, 197} 5.16q–s equally had minimal effects on OCR, increasing OCR up to 130%. Interestingly, the position of the trifluoromethyl group had significant influence on respiration rates (ortho $>$ meta $>$ para) with 5.16t (SHC4111522) stimulating 2-fold basal activity. Replacing the trifluoromethyl group with a nitrile (5.16w) also produced an inactive uncoupler.
Among symmetrical derivatives tested, **5.13** remained as the best in class. Therefore, unsymmetrical analogues were synthesized wherein the 2-fluoroaniline moiety was maintained. As shown in Table 5.2, an unsymmetrical derivative bearing an aniline ring (**5.17a**) modestly stimulated respiration (176%) up to 40 µM, which was similarly observed with an isoelectronic replacement with a 2-aminopyridine ring (**5.17b**). Transposition of the fluorine atom in **5.13** to either the meta (**5.17c**) or para position (**5.17d**) slightly diminished uncoupling activity. Subsequent addition of an extra fluorine atom to the 2-fluoroaniline at either the 3- (**5.17e**) or 4-position of the ring (**5.17f**) decreased the activity further.
Table 5.2. Activity of Unsymmetrical Derivatives of 5.13

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Max% Basal Activity&lt;sup&gt;a&lt;/sup&gt;</th>
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<th>R</th>
<th>Max% Basal Activity&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>5.17m</td>
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<td>165 ± 7</td>
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<td>5.17n</td>
<td></td>
<td>113 ± 15</td>
</tr>
<tr>
<td>5.17c</td>
<td></td>
<td>204 ± 17</td>
<td>5.17o</td>
<td></td>
<td>188 ± 22</td>
</tr>
<tr>
<td>5.17d</td>
<td></td>
<td>252 ± 13</td>
<td>5.17p</td>
<td></td>
<td>131 ± 2</td>
</tr>
<tr>
<td>5.17e</td>
<td></td>
<td>156 ± 9</td>
<td>5.17q</td>
<td></td>
<td>200 ± 24</td>
</tr>
<tr>
<td>5.17f</td>
<td></td>
<td>137 ± 21</td>
<td>5.17r</td>
<td></td>
<td>165 ± 35</td>
</tr>
<tr>
<td>5.17g</td>
<td></td>
<td>253 ± 14</td>
<td>5.17s</td>
<td></td>
<td>58 ± 11</td>
</tr>
<tr>
<td>5.17h</td>
<td></td>
<td>288 ± 47</td>
<td>5.17t</td>
<td></td>
<td>112 ± 34</td>
</tr>
<tr>
<td>5.17i</td>
<td></td>
<td>302 ± 35</td>
<td>5.17u</td>
<td></td>
<td>153 ± 8</td>
</tr>
<tr>
<td>5.17j</td>
<td></td>
<td>121 ± 2</td>
<td>5.17v</td>
<td></td>
<td>93 ± 17</td>
</tr>
<tr>
<td>5.17k</td>
<td></td>
<td>160 ± 17</td>
<td>5.17w</td>
<td></td>
<td>29 ± 16</td>
</tr>
<tr>
<td>5.17l</td>
<td></td>
<td>58 ± 13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>The maximum percentage of basal (100%) respiration that could be stimulated using 40 μM uncoupler.
In contrast, unsymmetrical derivatives with a methyl group at the ortho (5.17g), meta (5.17h, SHC4081663), or para (5.17i, SHC4091665) position were found to have good efficacy and produced OCRs comparable to 5.13. Replacement with the stronger electron-donating methyl (5.17j-1) and ethyl (5.17m-o) ethers afforded moderate uncouplers, with the meta-substituted methyl ether (5.17k) and the ortho- (5.17m) and para-substituted ethyl (5.17n) ethers being the most efficacious and potent of their respective series. Phenol 5.17p and trifluoroacetamide 5.17q also achieved moderate uncoupling activity. The position of the electron-withdrawing trifluoromethyl ether and trifluoromethyl substituents significantly influenced respiration rates with the ortho-substituted analogues (5.17r and 5.17u, SHC4111523) producing the highest respiration rates of their respective series. The meta and para positions (5.17s-t and 5.17v-w) were found to be relatively inactive.

To expand structural features on the current scaffold, the aniline moiety was replaced with amide moieties (Table 5.3). Our previous work established that 5.15 lacked mitochondrial uncoupling activity. Conversion of 5.15 into the diamine 5.18 did not afford an active uncoupler. Acetylation of 5.18 with a trifluoromethylacetyl group (5.19a) also resulted in an inactive uncoupler. Because sulfonamides have been shown to have mitochondrial uncoupling activity, sulfonamides 5.19b-e were synthesized. The tosyl (19c, SHC4031633) and 4-fluorobenzenesulfonamide (5.19d) were unable to significantly stimulate an increase in respiration levels. Increasing the lipophilicity of the sulfonamide library with a 3-trifluoromethylbenzenesulfonamide (5.19e) achieved moderate uncoupling activity (195%); however, replacement of the trifluoromethyl moiety with a stronger deactivating nitro group (5.19f) eliminated uncoupling activity. The general lack of mitochondrial uncoupling activity
with the amide and sulfonamide derivatives is most likely due to the compounds’ decreased $pK_a$ ($< 6.8$), making protonophoric uncoupling challenging.

**Table 5.3. Activity of Amide Derivatives of 5.13**

<table>
<thead>
<tr>
<th>Compound #</th>
<th>R</th>
<th>Max% Basal Activity</th>
<th>Compound #</th>
<th>R</th>
<th>Max% Basal Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.18</td>
<td>H</td>
<td>99 ± 2</td>
<td>5.19c</td>
<td></td>
<td>111 ± 3</td>
</tr>
<tr>
<td>5.19a</td>
<td>CF₃</td>
<td>99 ± 2</td>
<td>5.19d</td>
<td></td>
<td>195 ± 7</td>
</tr>
<tr>
<td>5.19b</td>
<td>SO₂</td>
<td>120 ± 6</td>
<td>5.19e</td>
<td></td>
<td>102 ± 2</td>
</tr>
</tbody>
</table>

*The maximum percentage of basal (100%) respiration that could be stimulated using 40 µM uncoupler.

Because mitochondrial uncouplers are typically lipophilic weak acids, we decided to determine the $pK_a$ and cLogP of select compounds (Table 5.4). We selected compounds across the spectrum of activity: active ($> 2$-fold increase in OCR at 40 µM vs basal), moderately active (1.3 $≤$ 2-fold increase in OCR at 40 µM vs basal), and inactive ($≤ 1.2$-fold increase in OCR at 40 µM vs basal). To understand the link between uncoupling activity and physiochemical properties of compounds, we measured the $pK_a$ of select compounds and related them to calculated cLogP values. Evaluation of the calculated cLogP values revealed that moderately active (5.16d, 5.16r, and 5.17u) and active (5.16h, 5.16t, 5.17h, 5.17i) compounds in the set have cLogP values $> 6$, which is not surprising because these compounds must be membrane permeable to achieve any uncoupling activity. Interestingly, moderately active uncouplers were found to have $pK_a$s around 6.8 and between 8.0 and 10 while a majority of active uncouplers were found to have $pK_a$s within the ideal range of 6.8–8.0 with the exception of 5.16h, which was found to have a $pK_a$ of 10.75.
While there are examples of compounds in our library with cLogP > 6 that are inactive (e.g. 5.16g, 5.16n, and 5.17s), 5.16g and 5.16n have high pKₐs far from the ideal range (> 10) and 5.17s appears to be cytotoxic. Analogues with cLogP < 5.5 (e.g. 5.19c) were found to be inactive as uncouplers; their measured pKₐs were also found to be below 6.8 (e.g. 5.19c), which would collectively make uncoupling challenging. Therefore, these results suggest that compound lipophilicity (cLogP values) and pKₐs equally contribute to uncoupling activity, as corroborated by the OCR data provided in Tables 5.1–5.3, and must be taken into consideration when evaluating the development of future uncouplers.
Table 5.4. \( pK_a \) and cLogP\(^a\) Values of Select Uncouplers

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Structure</th>
<th>Max% Basal Activity (40 ( \mu )M)</th>
<th>( pK_a )</th>
<th>cLogP</th>
<th>Entry</th>
<th>Compound</th>
<th>Structure</th>
<th>Max% Basal Activity (40 ( \mu )M)</th>
<th>( pK_a )</th>
<th>cLogP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.13</td>
<td><img src="image1.png" alt="Image" /></td>
<td>329 ± 11</td>
<td>7.56</td>
<td>6.52</td>
<td>7</td>
<td>5.16t</td>
<td><img src="image2.png" alt="Image" /></td>
<td>212 ± 26</td>
<td>6.93 ( \pm ) 0.06</td>
<td>8.01</td>
</tr>
<tr>
<td>2</td>
<td>5.16d</td>
<td><img src="image3.png" alt="Image" /></td>
<td>140 ± 4 ( \geq ) 9(^b)</td>
<td>6.6</td>
<td>8</td>
<td>5.17h</td>
<td><img src="image4.png" alt="Image" /></td>
<td>288 ± 47</td>
<td>7.92 ( \pm ) 0.05</td>
<td>6.87</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.16g</td>
<td><img src="image5.png" alt="Image" /></td>
<td>97 ± 3</td>
<td>10.07</td>
<td>7.22</td>
<td>9</td>
<td>5.17i</td>
<td><img src="image6.png" alt="Image" /></td>
<td>302 ± 35</td>
<td>7.97 ( \pm ) 0.04</td>
<td>6.87</td>
</tr>
<tr>
<td>4</td>
<td>5.16h</td>
<td><img src="image7.png" alt="Image" /></td>
<td>252 ± 18</td>
<td>10.75</td>
<td>7.22</td>
<td>10</td>
<td>5.17s</td>
<td><img src="image8.png" alt="Image" /></td>
<td>58 ± 11</td>
<td>7.88 ( \pm ) 0.05</td>
<td>7.40</td>
</tr>
<tr>
<td>5</td>
<td>5.16n</td>
<td><img src="image9.png" alt="Image" /></td>
<td>115 ± 27 ( \geq ) 12(^c)</td>
<td>7.13</td>
<td>11</td>
<td>5.17u</td>
<td><img src="image10.png" alt="Image" /></td>
<td>153 ± 8</td>
<td>6.86 ( \pm ) 0.05</td>
<td>7.27</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.16r</td>
<td><img src="image11.png" alt="Image" /></td>
<td>127 ± 22</td>
<td>8.03 ( \pm ) 0.03</td>
<td>8.29</td>
<td>12</td>
<td>5.19b</td>
<td><img src="image12.png" alt="Image" /></td>
<td>120 ± 6</td>
<td>6.64(^d)</td>
<td>4.46</td>
</tr>
</tbody>
</table>

\(^a\) cLogP was calculated for the uncoupler with Chemdraw Professional 16.0. \(^b\) Above pH 9, compound began precipitating out of solution, occluding \( pK_a \) determination. \(^c\) \( pK_a \) outside of measurable pH range. \(^d\) Only one \( pK_a \) measurement was made.
To quantify the uncoupling activity of derivatives of 5.13, we determined the half maximal effective concentration ($EC_{50}$) values of select compounds classified as active uncouplers (Table 5.5) because of their ability to increase OCR $> 200\%$ up to 40 $\mu$M (Figure 5.3). The para methyl (5.16h) symmetrical derivative was found to be the least potent of the selected active compounds, which is consistent with OCR data presented in Figure 5.3, as it stimulated $\sim 2$-fold increase in OCR up to 40 $\mu$M. Interestingly, its has the highest $pK_a$ of the set (Table 5.4). Conversely, the ortho trifluoromethyl (5.16t, SHC4111522) symmetrical derivative, an isoelectronic analogue of 5.13, was found to be the most potent uncoupler of the set, having an $EC_{50}$ of 0.63, which is consistent with its measured $pK_a$ and cLogP (Table 5.4). All active uncouplers with $\sim 3$-fold or higher OCR at 40 $\mu$M were found to be unsymmetrical derivatives (5.17h and 5.17i). Consequently, their $EC_{50}$ values were found to be low micromolar, and they show similar efficacy to 5.13 (Figure 5.3).
**Table 5.5.** EC$_{50}$ Values of Select Uncouplers with Activity at 40 µM

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Structure</th>
<th>Max% Basal Activity (40 µM)</th>
<th>EC$_{50}$ (µM)</th>
<th>Entry</th>
<th>Compound</th>
<th>Structure</th>
<th>Max% Basal Activity (40 µM)</th>
<th>EC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.13</td>
<td><img src="image" alt="Structure" /></td>
<td>329 ± 11</td>
<td>0.27</td>
<td>4</td>
<td>5.17h</td>
<td><img src="image" alt="Structure" /></td>
<td>288 ± 47</td>
<td>1.34</td>
</tr>
<tr>
<td>2</td>
<td>5.16h</td>
<td><img src="image" alt="Structure" /></td>
<td>252 ± 18</td>
<td>24.43</td>
<td>5</td>
<td>5.17i</td>
<td><img src="image" alt="Structure" /></td>
<td>302 ± 35</td>
<td>1.53</td>
</tr>
<tr>
<td>3</td>
<td>5.16t</td>
<td><img src="image" alt="Structure" /></td>
<td>212 ± 26</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*EC$_{50}$ values calculated from a best fit curve for OCR values between 0.25 and 40 µM.

**Figure 5.3.** Changes in oxygen consumption rate relative to DMSO control for 5.5, 5.13, 5.16h, 5.16t, 5.17h, and 5.17i. Each data point is from 2 to 3 replicates. Lines are present as an eye guide rather than as a trendline.
5.5 **Current Efforts and Future Directions**

From the data presented in Table 5.4, the most active uncouplers in our library have cLogP values > 6 and have $pK_a$ values between 6.8 and 8.0. Because **5.13** has a $pK_a$ near the median of this ideal range, we decided to select compounds that have similar potency to **5.13** but with $pK_a$s abutting the two extremes of the ideal $pK_a$ range to test in vivo. Current efforts involve dosing mice with **5.16t** ($EC_{50} = 0.63 \mu M$, $pK_a = 6.93$) and **5.17h** ($EC_{50} = 1.34 \mu M$, $pK_a = 7.92$) to determine if these analogues have good oral bioavailability and improved pharmacokinetics to **5.13**. These studies will also determine if **5.16t** and **5.17h** have similar or improved efficacy to **5.13** by assessing their ability to increase OCR in mice. Positive feedback from these studies will lead to future studies to evaluate **5.16t** and **5.17h**’s ability to promote leanness in mouse models of obesity.

5.6 **Conclusions**

Herein, we report the design and synthesis of potent and efficacious derivatives of **5.13**, a mitochondrial uncoupler that does not depolarize the plasma membrane. Modifications were made to the aniline moiety of **5.13**. Symmetrical, unsymmetrical, and amide derivatives were synthesized. Symmetrical and unsymmetrical derivatives that increased respiration at least 2-fold higher than normal basal levels were found to have cLogP values > 6 and $pK_a$ values between 6.8 and 8.0. Collectively, our work provides greater insight into the chemical property requirements for developing new mitochondrial uncouplers and expands upon a novel chemical scaffold that selectively uncouples the mitochondria. This work should help elucidate the mechanism of pharmaceutical mitochondrial uncoupling and pave the way for the use of this therapeutic strategy for the treatment of metabolic-related diseases.
5.7 References


6 Experimental Section

6.1 Structure–Activity Relationship Studies of the Lipophilic Tail Region of Sphingosine Kinase 2 Inhibitors

6.1.1 Sphingosine Kinase Assays

The inhibitory activity of the synthesized compounds on human SphK1 and SphK2 was determined using a previously published method. Recombinant human SphK1 or mouse SphK2 isolated from a cell lysate was briefly incubated with (10 µM) or without compound, sphingosine, and γ-[³²P]ATP. The radiolabeled sphingosine 1-phosphate was isolated via extraction and thin-layer chromatography and then quantified via scintillation counting.

6.1.2 General Material and Synthetic Procedures

All chemical reagents were purchased from commercial sources and used without further purification. Thin layer chromatography (TLC) was performed on aluminum-backed silica gel, 200 µm, F254, and column chromatography was performed on flash grade silica gel, 40-63 µm, using a Combiflash Rf purification system. ¹H NMR spectra were recorded at 500 or 400 MHz; the corresponding ¹³C NMR resonant frequencies were 126 and 101 MHz, respectively. ¹H NMR chemical shifts are reported in ppm with the solvent resonance as an internal standard (CDCl₃: 7.26 ppm; CD₃OD: 4.87 ppm). ¹³C NMR, chemical shifts are reported in ppm with the solvent resonance as the internal standard (CDCl₃: 77.16 ppm; CD₃OD: 49.00 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m= multiplet), coupling constants (Hz), and integration. Rotamers are denoted by an asterisk (*). High resolution mass spectroscopy (HRMS) was performed on an LC/MS time–of–flight mass spectrometer using electrospray ionization (ESI). HPLC analyses were performed with a Thermo Electron TSQ triple quadrupole mass spectrometer equipped with an ESI source. All compounds
tested in biological assays are >95% pure by $^1\text{H}$ NMR and HPLC analyses unless noted otherwise. NMR spectra for the synthesized compounds can be found online (http://www.sciencedirect.com/science/article/pii/S0960894X15002474).

General Procedure 2A: Synthesis of amide-oxime derivatives. TEA (2.7 equiv) was added to a solution of benzonitrile 2.6a (1 equiv) with hydroxylamine hydrochloride (2.6 equiv) in ethanol (0.47 M solution). The mixture was stirred at 80 °C for 6 h. The organic solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography to yield the desired product.

General Procedure 2B: Coupling of amide-oxime derivatives with ((S)-1-(tert-butoxycarbonyl)azetidine-2-carboxylic acid or (S)-1-(tert-butoxycarbonyl)pyrrolidine-2-carboxylic acid. DIEA (1.80 equiv) was added to a solution of amide-oxime 2.7a (1 equiv) and ((S)-1-(tert-butoxycarbonyl)azetidine-2-carboxylic acid or (S)-1-(tert-butoxycarbonyl)pyrrolidine-2-carboxylic acid in DMF (0.2 M solution). HCTU (1.5 equiv) was then added to the resulting mixture at rt and stirred at 100 °C for 4–8 h. The reaction progress was followed by TLC. The solution was partitioned between EtOAc and LiBr aqueous solution. The aqueous solution was washed with EtOAc three times, and the combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated via vacuum. The resulting residue was purified by silica gel column chromatography.

General Procedure 2C: Buchwald–Hartwig coupling of aryl iodides with amines. The desired amine (1.2 equiv) was added to a round bottom containing 2.6a (1 equiv) in toluene (0.120 M). The reaction mixture was degassed for 10 min by bubbling N$_2$ through the solution. Cs$_2$CO$_3$ (1.2 equiv), tri-tert-butylphosphine (0.4 equiv), and Pd$_2$(dba)$_3$ (0.1 equiv) were added together. The resulting reaction mixture was then stirred at 100 °C for 24 h, after which it was poured into
water and extracted three times with EtOAc. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting brown residue was purified by flash chromatography over silica gel.

**General Procedure 2D: Deprotection of t-Boc protecting groups with TFA and guanylation of amines.** To a solution of t-Boc protected intermediate in DCM, a 1N TFA solution in DCM was added. The reaction mixture was then stirred at rt for 4 h. At this time, TLC showed complete conversion of starting material. The organic solvent was removed under reduced pressure. The residue was then dissolved in ACN (0.02 M solution). Diisopropylethylamine (10 equiv) and (Z)-tert-butyl (((tert-butoxycarbonyl)imino)(1H-pyrazol-1-yl)methyl)carbamate (1.05 equiv) were added to the solution, and the resulting reaction mixture was left to mix at rt for 3 days after which the organic solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography.

**General Procedure 2E: Deprotection of t-Boc protecting groups with HCl (g).** Hydrochloric acid gas was bubbled through a solution of the N-Boc protected compound in methanol for 2-5 minutes, until complete consumption of starting material was observed by TLC. The reaction mixture was concentrated under reduced pressure and triturated with diethyl ether to yield the corresponding free amine hydrochloride salt.

**General Procedure 2F: Acylation of amines.** The desired acyl chloride (1.8 equiv) was added to a round bottom containing tert-butyl (S)-2-(3-(4-(piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (1 equiv) and TEA (1.6 equiv) in DCM (0.045 M) cooled to 0 ºC. The reaction mixture was then warmed to room temperature and stirred for 17 h. The organic solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography.
*(Z)-N′-hydroxy-4-iodobenzimidamide (2.7a).* Synthesized by General Procedure 2A. 88%, white solid. \(^1^H\) NMR (400 MHz, CD\(_2\)OD) \(\delta\) 7.78 (dt, \(J=7.8, 3.6 \text{ Hz}, 2H\)), 7.44 (dt, \(J=7.4, 0.1 \text{ Hz}, 2H\)); \(^{13}\)C NMR (101 MHz, CD\(_2\)OD) \(\delta\) 154.5, 138.7, 133.8, 129.0, 96.2. HRMS (ESI\(^+\)): calcd for C\(_7\)H\(_8\)N\(_2\)OI \([\text{M+H}]^+\): 262.9681, found: 262.9695.

*(S)-tert-butyl-2-(3-(4-iodophenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (2.8a).*

Synthesized by General Procedure 2B. 53%, yellow oil. \(^1^H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.85-7.76 (m, 4H), 5.22-4.99 (m, 1H), 3.75-3.43 (m, 2H), 2.46-2.30 (m, 1H), 2.20-1.94 (m, 3H), 1.45 (s, 3H), 1.28 (s, 6H); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 181.0, 167.9, 153.6, 138.2, 129.0, 126.3, 98.1, 80.6, 53.9, 46.5, 32.5, 28.3, 23.8; HRMS (ESI\(^+\)): Calcd for C\(_{17}\)H\(_{20}\)N\(_3\)O\(_3\)INa \([\text{M+Na}]^+\): 464.0447, Found: 464.0405.

*(S)-tert-butyl 2-(3-(4-iodophenyl)-1,2,4-oxadiazol-5-yl)azetidine-1-carboxylate (2.11).*

Synthesized by General Procedure 2B. 11%, yellow oil. \(^1^H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.83 (s, 4H), 5.41 (dd, \(J=8, 4 \text{ Hz}, 1H\)), 4.23-4.17 (m, 1H), 4.08-4.02 (m, 1H) 2.76-2.67 (m, 1H), 2.58-2.52 (m, 1H), 1.35 (bs, 9H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 178.76, 168.12, 138.26, 129.12, 126.30, 98.13, 80.81, 55.29, 47.66, 28.31, 22.00; HRMS (ESI\(^+\)): calcd for C\(_{16}\)H\(_{18}\)IN\(_3\)NaO\(_3\) \([\text{M+Na}]^+\): 450.0291, found: 450.0314.

*(S)-tert-butyl 2-(3-(4-morpholinophenyl)-1,2,4-oxadiazol-5-yl)azetidine-1-carboxylate (2.12d).*

Synthesized by General Procedure 2C. 81%, white-yellow solid. \(^1^H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.98 (d, \(J=8.4 \text{ Hz}, 2H\)), 6.95 (d, \(J=8.5 \text{ Hz}, 2H\)), 5.40 (dd, \(J=8.8, 5.6 \text{ Hz}, 1H\)), 4.20 (td, \(J=8.7, 5.8 \text{ Hz}, 1H\)), 4.04 (td, \(J=8.6, 6.2 \text{ Hz}, 4H\)), 3.89 – 3.85 (m, 4H), 3.29 – 3.24 (m, 4H), 2.75 – 2.65 (m, 1H), 2.58 – 2.48 (m, 1H), 1.34 (bs, 9H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 178.02, 168.44, 153.26, 128.86, 117.41, 114.77, 80.69, 66.84, 48.31, 28.31, 22.02; HRMS (ESI\(^+\)): calcd for C\(_{20}\)H\(_{27}\)N\(_4\)O\(_4\) \([\text{M+H}]^+\): 387.2032, found: 387.2022.
(S)-tert-butyl 2-(3-(4-(4-methylpiperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)azetidine-1-carboxylate (2.12e). Synthesized by General Procedure 2C. 47%, white-yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.94 (d, $J = 8.4$ Hz, 2H), 6.93 (d, $J = 8.5$ Hz, 2H), 5.37 (dd, $J = 8.6$, 5.5 Hz, 1H), 4.17 (dd, $J = 8.6$, 5.5 Hz, 1H), 4.05 – 3.96 (m, 1H), 3.37 – 3.24 (m, 4H), 2.66 (m, 1H), 2.60 – 2.44 (m, 5H), 2.33 (s, 3H), 1.34 (br s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.86, 168.38, 153.06, 128.72, 116.74, 114.88, 80.56, 77.48, 77.16, 76.84, 54.89, 47.87, 46.16, 29.13, 28.22, 21.92; HRMS (ESI+): calcd for C$_{21}$H$_{30}$N$_5$O$_3$ [M+H]$^+$: 400.2349, found: 400.2376.

(S)-tert-butyl (((tert-butoxycarbonylimino)(2-(3-(4-morpholinophenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-yl)methyl)carbamate. Synthesized by General Procedure 2D. 66%, clear oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.98 (d, $J = 8.4$ Hz, 2H), 6.95 (d, $J = 8.5$ Hz, 2H), 6.08 – 5.94 (m, 1H), 4.63 (dd, $J = 8.6$ Hz, 1H), 4.19 (td, $J = 9.4$, 5.5 Hz, 1H), 3.92 – 3.83 (m, 4H), 3.33 – 3.22 (m, 4H), 2.90 – 2.77 (m, 1H), 2.63 – 2.50 (m, 1H), 1.47 (s, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 176.86, 168.43, 159.88, 153.30, 128.94, 117.24, 114.74, 80.10, 66.85, 48.30, 28.22, 22.53; HRMS (ESI+): calcd for C$_{26}$H$_{37}$N$_6$O$_6$ [M+H]$^+$: 529.2775, found: 529.2795.

(S)-tert-butyl (((tert-butoxycarbonylimino)(2-(3-(4-(4-methylpiperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-yl)methyl)carbamate. Synthesized by General Procedure 2D. 43%, yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.84 (s, 1H), 7.99 – 7.92 (m, 2H), 6.99 – 6.93 (m, 2H), 6.02 (s, 1H), 4.67 – 4.56 (m, 1H), 4.18 (td, $J = 9.4$, 5.6 Hz, 1H), 3.36 – 3.30 (m, 4H), 2.90 – 2.77 (m, 1H), 2.61 – 2.51 (m, 5H), 2.36 (s, 3H), 1.54 – 1.37 (m, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 191.21, 176.61, 162.69, 153.40, 128.91, 128.80, 125.54, 114.96, 114.92, 81.77, 78.93, 58.17, 54.97, 47.97, 46.24, 41.05, 31.39, 29.85, 29.22, 28.15, 22.53, 20.86, 20.52; HRMS (ESI+): calcd for C$_{27}$H$_{40}$N$_7$O$_5$ [M+H]$^+$: 542.3091, found: 542.3108.
(S)-amino(2-(3-(4-morpholinophenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-yl)methaniminium chloride (2.13d). Synthesized by General Procedure 2E. 78%, white solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.95 (d, $J$ = 8.9 Hz, 2H), 7.06 (d, $J$ = 8.9 Hz, 2H), 5.80 (dd, $J$ = 9.4, 5.2 Hz, 1H), 4.37 (m, 1H), 4.26 (m, 1H), 3.86 – 3.82 (m, 4H), 3.30 – 3.26 (m, 4H), 3.10 – 3.00 (m, 1H), 2.62 (m, 1H); $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 177.78, 169.72, 158.56, 155.09, 129.63, 117.55, 115.78, 67.79, 58.43, 50.82, 22.94; HRMS (ESI+): calcd for C$_{16}$H$_{21}$N$_6$O$_2$ [M+H]$^+$: 329.1729, found: 329.1719.

(S)-4-(4-(1-amino(iminio)methyl)azetidin-2-yl)-1,2,4-oxadiazol-3-yl)phenyl)1-methylpiperazine-1-ium chloride (2.13e). Synthesized by General Procedure 2E. 83%, white solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.94 (d, $J$ = 8.9 Hz, 2H), 7.07 (d, $J$ = 8.9 Hz, 2H), 5.80 (m, 1H), 4.37 (m, 1H), 4.26 (m, 1H), 3.38 – 3.34 (m, 9H), 3.05 (m, 1H), 2.67 – 2.61 (m, 5H), 2.38 (s, 3H); $^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 177.31, 167.46, 157.18, 146.52, 128.99, 125.00, 120.04, 64.64, 57.08, 53.10, 49.60, 21.64; HRMS (ESI+): calcd for C$_{17}$H$_{25}$N$_7$O [M+H]$^+$: 343.2121, found: 343.2027.

tert-butyl (S)-2-(3-(4-(piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)azetidine-1-carboxylate. 

Synthesized by General Procedure 2C. 40%, beige solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.97 (d, $J$ = 8.8 Hz, 2H), 6.96 (d, $J$ = 8.8 Hz, 2H), 5.40 (dd, $J$ = 8.8, 5.7 Hz, 1H), 4.25 – 4.15 (m, 1H), 4.08 – 3.99 (m, 1H), 3.29 – 3.25 (m, 4H), 3.06 – 3.01 (m, 4H), 2.75 – 2.65 (m, 1H), 2.59 – 2.47 (m, 1H), 1.35 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.83, 168.28, 153.19, 128.69, 117.12, 115.07, 80.54, 55.25, 48.53, 45.45, 29.68, 28.15, 21.85; HRMS (ESI+): calcd for C$_{20}$H$_{28}$N$_5$O$_3$ [M+H]$^+$: 386.2192, found: 386.2202.

tert-butyl (S)-2-(3-(4-(piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (2.14). Synthesized by General Procedure 2C. 50%, off-white solid. $^1$H NMR (400 MHz, CDCl$_3$)
δ 7.93 (d, 2H), 6.94 (d, J = 8.5 Hz, 2H), 5.19 – 4.96 (m, 1H), 4.59 (s, 1H), 3.79 – 3.59 (m, 1H), 3.56 – 3.40 (m, 1H), 3.37 – 3.28 (m, 4H), 3.14 – 3.06 (m, 4H), 2.43 – 2.26 (m, 1H), 2.20 – 2.05 (m, 2H), 2.02 – 1.90 (m, 1H), 1.43<sup>+</sup> (s, 3H), 1.27<sup>+</sup> (s, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 180.19, 168.13, 153.11, 128.73, 117.39, 115.24, 80.45, 53.88, 48.33, 46.41, 45.32, 32.44, 29.76, 28.46, 28.22, 23.77; HRMS (ESI+): calcd for C<sub>21</sub>H<sub>30</sub>N<sub>5</sub>O<sub>3</sub> [M+H]<sup>+</sup>: 400.2349, found: 400.2352.

<sup>tert</sup>-butyl (S)-2-(3-(4-(4-(3-methylbutanoyl)piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (2.15a). Synthesized by General Procedure 2F. 86%, beige amorphous solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.97 (d, J = 8.5 Hz, 2H), 6.95 (d, J = 8.3 Hz, 2H), 5.21 – 5.01 (m, 1H), 3.85 – 3.75 (m, 2H), 3.77 – 3.61 (m, 3H), 3.60 – 3.43 (m, 1H), 3.34 – 3.24 (m, 4H), 2.47 – 2.30 (m, 1H), 2.26 (d, J = 7.0 Hz, 2H), 2.22 – 2.06 (m, 4H), 2.05 – 1.92 (m, 1H), 1.46 (s, 3H), 1.29 (s, 6H), 0.99 (d, J = 6.5 Hz, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 180.34, 171.26, 168.19, 152.81, 128.88, 117.81, 115.45, 80.54, 53.97, 48.63, 48.37, 46.49, 45.60, 42.18, 41.32, 32.52, 28.55, 28.31, 25.97, 24.49, 23.85, 22.91; HRMS (ESI+): calcd for C<sub>26</sub>H<sub>38</sub>N<sub>5</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 484.2924, found: 484.2887.

<sup>tert</sup>-butyl (S)-2-(3-(4-((3r,5r,7r)-adamantane-1-carbonyl)piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (2.15b). Synthesized by General Procedure 2F. 68%, yellow amorphous solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.96 (d, 2H), 6.95 (d, J = 8.2 Hz, 2H), 5.21 – 5.00 (m, 1H), 3.92 – 3.81 (m, 4H), 3.75 – 3.61 (m, 1H), 3.60 – 3.42 (m, 1H), 3.33 – 3.23 (m, 4H), 2.45 – 2.28 (m, 1H), 2.21 – 2.11 (m, 2H), 2.07 – 1.95 (m, 9H), 1.80 – 1.68 (m, 7H), 1.46 (s, 3H), 1.29 (s, 6H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 191.60, 180.30, 175.99, 168.20, 152.91, 128.83, 117.64, 115.23, 80.53, 53.96, 48.55, 46.47, 45.08, 41.87, 39.27, 36.78, 36.66, 32.51, 28.61, 28.29, 28.10, 23.83; HRMS (ESI+): calcd for C<sub>32</sub>H<sub>44</sub>N<sub>5</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 562.3393, found: 562.3384.
**tert-butyl (S)-2-(3-(4-(2-phenylacetyl)piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (2.15c).** Synthesized by General Procedure 2F. 67 %, beige amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.92 (d, $J = 8.4$ Hz, 2H), 7.34 – 7.27 (m, 2H), 7.27 – 7.20 (m, 3H), 6.88 (d, $J = 8.3$ Hz, 2H), 5.22 – 4.97 (m, 1H), 3.85 – 3.75 (m, 3H), 3.73 – 3.40 (m, 4H), 3.29 – 3.19 (m, 2H), 3.12 – 3.02 (m, 2H), 2.44 – 2.24 (m, 1H), 2.20 – 2.04 (m, 2H), 2.02 – 1.91 (m, 1H), 1.44 (s, 3H), 1.27 (s, 6H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 180.32, 169.80, 168.14, 153.73, 152.66, 134.91, 129.48, 128.98, 128.83, 128.69, 128.65, 128.51, 127.27, 127.09, 117.76, 115.40, 80.55, 53.95, 48.27, 48.07, 46.47, 45.85, 41.59, 41.21, 32.49, 28.53, 28.28, 23.82; HRMS (ESI+): calcd for C$_{29}$H$_{36}$N$_5$O$_4$ [M+H]$^+$: 518.2767, found: 518.2812.

**tert-butyl (S)-2-(3-(4-(benzylpiperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)azetidine-1-carboxylate (2.15d).** Benzyl bromide (0.01 mL, 0.057 mmol) was added to a round bottom containing tert-butyl (S)-2-(3-(4-(piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)azetidine-1-carboxylate (33 mg, 0.0.086 mmol) in DCM (0.18 mL). The reaction mixture was stirred for 17 h, after which it was poured into water and extracted three times with DCM. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting brown residue was purified by flash chromatography over silica gel (50 – 70% EtOAc in hexanes) to give the title compound (20 mg, 49%) as an off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.96 (d, $J = 8.4$ Hz, 2H), 7.38 – 7.31 (m, 4H), 7.30 – 7.27 (m, 1H), 6.94 (d, $J = 8.5$ Hz, 2H), 5.39 (dd, $J = 8.8$, 5.6 Hz, 1H), 4.25 – 4.15 (m, 1H), 4.08 – 3.98 (m, 1H), 3.58 (s, 2H), 3.36 – 3.29 (m, 4H), 2.75 – 2.47 (m, 6H), 1.35 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.93, 168.53, 153.29, 129.32, 128.82, 128.47, 127.37, 114.93, 80.70, 63.17, 52.99, 48.08, 28.33, 22.03; HRMS (ESI+): calcd for C$_{27}$H$_{34}$N$_5$O$_3$ [M+H]$^+$: 476.2662, found: 476.2644.
tert-butyl (S,E)-(((tert-butoxycarbonyl)imino)(2-(3-(4-(4-(3-methylbutanoyl)piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate. Synthesized by General Procedure 2D. 43%, off-white amorphous solid. $^1$H NMR (500 MHz, CDCl$_3$) δ 7.90 (d, $J$ = 8.4 Hz, 2H), 6.87 (d, 2H), 5.50 (dd, $J$ = 7.6, 4.5 Hz, 1H), 3.84 – 3.77 (m, 1H), 3.76 – 3.68 (m, 3H), 3.62 – 3.57 (m, 2H), 3.26 – 3.19 (m, 4H), 2.41 – 2.32 (m, 1H), 2.20 (d, $J$ = 7.0 Hz, 2H), 2.14 – 2.04 (m, 2H), 2.01 – 1.92 (m, 1H), 1.39 (s, 18H), 0.93 (d, $J$ = 6.6 Hz, 6H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 178.37, 171.38, 168.20, 153.49, 152.83, 131.98, 128.98, 128.76, 128.41, 125.42, 117.64, 115.41, 81.36, 55.61, 49.77, 48.63, 48.34, 45.62, 42.17, 41.35, 35.14, 31.46, 28.25, 28.11, 26.01, 24.10, 22.91; HRMS (ESI+): calcd for C$_{32}$H$_{48}$N$_7$O$_6$ [M+H]$^+$: 626.3666, found: 626.3685.

tert-butyl ((E)-(S)-2-(3-(4-((3r,5r,7r)-adamantane-1-carbonyl)piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)((tert-butoxycarbonyl)imino)methyl)carbamate. Synthesized by General Procedure 2D. 26%, yellow amorphous solid. $^1$H NMR (500 MHz, CDCl$_3$) δ 7.96 (d, $J$ = 8.4 Hz, 2H), 6.94 (d, $J$ = 8.4 Hz, 2H), 5.58 (dd, $J$ = 7.5, 4.4 Hz, 1H), 3.87 (dd, 5H), 3.81 (q, $J$ = 6.4, 5.7 Hz, 1H), 3.29 (dd, $J$ = 5.0 Hz, 4H), 2.47 – 2.38 (m, 1H), 2.29 – 2.22 (m, 1H), 2.21 – 2.12 (m, 1H), 2.09 – 2.00 (m, 10H), 1.78 – 1.68 (m, 6H), 1.46 (s, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 178.59, 171.38, 168.20, 153.49, 152.83, 131.98, 128.98, 128.76, 128.41, 125.42, 117.64, 115.41, 81.36, 55.61, 49.77, 48.63, 48.34, 45.62, 42.17, 41.35, 35.14, 31.46, 28.25, 28.11, 26.01, 24.10, 22.91; HRMS (ESI+): calcd for C$_{38}$H$_{54}$N$_7$O$_6$ [M+H]$^+$: 704.4136, found: 704.4191.

tert-butyl (S,E)-(((tert-butoxycarbonyl)imino)(2-(3-(4-(4-(2-phenylacetetyl)piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate. Synthesized by General Procedure 2D. 74%, off-white amorphous solid. $^1$H NMR (500 MHz, CDCl$_3$) δ 10.10 (s, 1H), 7.93 (d, $J$ = 8.9 Hz, 2H), 7.50 (d, 2H), 6.80 (d, 2H), 5.50 (dd, $J$ = 7.6, 4.5 Hz, 1H), 3.84 – 3.77 (m, 1H), 3.76 – 3.68 (m, 3H), 3.62 – 3.54 (m, 2H), 3.26 – 3.19 (m, 4H), 2.41 – 2.32 (m, 1H), 2.19 (d, $J$ = 7.0 Hz, 2H), 2.14 – 2.04 (m, 2H), 2.01 – 1.92 (m, 1H), 1.39 (s, 18H), 0.93 (d, $J$ = 6.6 Hz, 6H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 178.37, 171.38, 168.20, 153.49, 152.83, 131.98, 128.98, 128.76, 128.41, 125.42, 117.64, 115.41, 81.36, 55.61, 49.77, 48.63, 48.34, 45.62, 42.17, 41.35, 35.14, 31.46, 28.25, 28.11, 26.01, 24.10, 22.91; HRMS (ESI+): calcd for C$_{32}$H$_{48}$N$_7$O$_6$ [M+H]$^+$: 626.3666, found: 626.3685.
Hz, 2H), 7.36 – 7.31 (m, 2H), 7.28 – 7.27 (m, 3H), 6.89 (d, J = 8.8 Hz, 2H), 5.56 (dd, J = 6.1 Hz, 1H), 3.91 – 3.73 (m, 6H), 3.61 (dd, J = 5.2 Hz, 2H), 3.27 (dd, J = 5.3 Hz, 2H), 3.10 (dd, J = 5.1 Hz, 2H), 2.47 – 2.38 (m, 1H), 2.29 – 2.11 (m, 2H), 2.07 – 1.98 (m, 1H), 1.46 (s, 18H); 13C NMR (126 MHz, CDCl3) δ 178.60, 169.70, 168.15, 153.81, 152.69, 134.96, 128.97, 128.67, 128.49, 127.78, 127.11, 117.73, 115.65, 115.36, 115.03, 114.82, 100.42, 100.12, 82.28, 79.81, 63.22, 55.41, 49.56, 48.29, 48.08, 45.86, 41.91, 41.57, 41.26, 31.70, 31.48, 28.26, 27.95; HRMS (ESI+): calcd for C35H46N7O6 [M+H]+: 660.3510, found: 660.3520.

tert-butyl (S,Z)-((2-3-(4-(4-benzylpiperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-yl)((tert-butoxycarbonylimino)methyl)carbamate. Synthesized by General Procedure 2D. 44%, yellow amorphous solid. 1H NMR (400 MHz, CDCl3) δ 10.82 (s, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.39 – 7.31 (m, 4H), 7.30 – 7.27 (m, 1H), 6.94 (d, J = 8.5 Hz, 2H), 6.07 – 5.92 (m, 1H), 4.68 – 4.56 (m, 1H), 4.23 – 4.10 (m, 1H), 3.58 (s, 2H), 3.32 (dd, J = 5.0 Hz, 4H), 2.89 – 2.74 (m, 1H), 2.66 – 2.49 (m, 5H), 1.45 (s, 18H); 13C NMR (101 MHz, CDCl3) δ 176.70, 168.50, 162.79, 153.32, 138.02, 129.31, 129.17, 128.87, 128.72, 128.45, 127.35, 116.56, 114.88, 82.51, 79.89, 63.15, 52.97, 48.07, 28.22, 28.14, 22.52; HRMS (ESI+): calcd for C33H44N7O5 [M+H]+: 618.3404, found: 618.3431.

(S)-amino(2-(3-(4-(3-methylbutanoyl)piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl) methaniminium chloride (2.16a). Synthesized by General Procedure 2E. 88%, white solid. 1H NMR (400 MHz, CD3OD) δ 8.03 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 5.47 (d, J = 7.4 Hz, 1H), 3.91 – 3.77 (m, 6H), 3.65 (q, J = 9.3 Hz, 1H), 3.48 (dt, J = 18.2, 4.2 Hz, 4H), 3.34 (dt, J = 3.3, 1.7 Hz, 4H), 2.66 – 2.45 (m, 2H), 2.39 (d, J = 7.0 Hz, 2H), 2.26 (q, J = 6.3 Hz, 1H), 2.19 – 2.06 (m, 1H), 1.03 (d, J = 6.5 Hz, 6H); 13C NMR (101 MHz, CD3OD) δ 175.78, 173.68,
HRMS (ESI+): calcd for C\textsubscript{22}H\textsubscript{32}N\textsubscript{7}O\textsubscript{2} \[M+H^+\]: 426.2617, found: 426.2617.

\((S)-2-(3-(4-(3r,5r,7r)-adamantane-1-carbonyl)piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)(amino)methaniminium chloride (2.16b). Synthesized by General Procedure 2E. 91%, off-white solid. \(^1\)H NMR (500 MHz, CD\textsubscript{3}OD) \(\delta\) 7.94 – 7.89 (m, 2H), 7.09 – 7.04 (m, 2H), 5.40 (dd, \(J = 8.0, 1.9\) Hz, 1H), 3.88 (t, \(J = 5.1\) Hz, 4H), 3.80 – 3.73 (m, 1H), 3.60 (td, \(J = 9.7, 7.2\) Hz, 1H), 3.34 – 3.31 (m, 4H), 2.59 – 2.43 (m, 2H), 2.22 (dddt, \(J = 9.6, 7.1, 5.0, 2.6\) Hz, 1H), 2.05 (s, 10H), 1.80 (s, 6H); \(^{13}\)C NMR (126 MHz, CD\textsubscript{3}OD) \(\delta\) 178.44, 177.95, 169.55, 157.08, 154.64, 129.63, 117.65, 116.13, 56.44, 46.33, 43.01, 40.13, 37.60, 32.69, 29.98, 24.33; HRMS (ESI+): calcd for C\textsubscript{28}H\textsubscript{38}N\textsubscript{7}O\textsubscript{2} \[M+H^+\]: 504.3087, found: 504.3059.

\((S)-amino(2-(3-(4-(2-phenylacetyl)piperazin-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (2.16c). Synthesized by General Procedure 2E. 92%, white solid. \(^1\)H NMR (500 MHz, CD\textsubscript{3}OD) \(\delta\) 7.94 (d, \(J = 8.6\) Hz, 2H), 7.36 – 7.21 (m, 5H), 7.12 (d, \(J = 8.6\) Hz, 2H), 5.40 (d, \(J = 7.4\) Hz, 1H), 3.84 (s, 2H), 3.81 (t, \(J = 5.2\) Hz, 2H), 3.75 (d, \(J = 6.7\) Hz, 3H), 3.58 (ddd, \(J = 16.4, 9.8, 6.6\) Hz, 2H), 3.35 (q, \(J = 6.0, 5.2\) Hz, 3H), 3.22 (d, \(J = 4.9\) Hz, 2H), 2.53 (dd, \(J = 12.8, 6.6\) Hz, 1H), 2.44 (dd, \(J = 13.2, 6.4\) Hz, 1H), 2.25 – 2.16 (m, 1H), 2.11 – 2.02 (m, 1H); \(^{13}\)C NMR (126 MHz, CD\textsubscript{3}OD) \(\delta\) 178.61, 172.29, 169.36, 154.64, 129.85, 129.83, 129.76, 127.99, 119.26, 119.05, 117.21, 117.03, 56.46, 49.97, 49.63, 46.68, 46.55, 44.62, 42.49, 41.30, 32.71, 29.98, 24.33; HRMS (ESI+): calcd for C\textsubscript{28}H\textsubscript{38}N\textsubscript{7}O\textsubscript{2} \[M+H^+\]: 460.2461, found: 460.2477.

\((S)-4-(4-(5-(1-(amino(iminio)methyl)azetidin-2-yl)-1,2,4-oxadiazol-3-yl)phenyl)-1-benzylpiperazin-1-ium chloride (2.16d). Synthesized by General Procedure 2E. 97 %, off-white solid. \(^1\)H NMR (400 MHz, CD\textsubscript{3}OD) \(\delta\) 8.05 – 7.99 (m, 2H), 7.65 (dd, \(J = 6.5, 3.0\) Hz, 2H), 7.58 –
7.52 (m, 3H), 7.18 (d, J = 8.7 Hz, 2H), 5.85 (dd, J = 9.4, 5.2 Hz, 1H), 4.48 (s, 2H), 4.40 (td, J = 8.9, 6.1 Hz, 1H), 4.30 (ddd, J = 9.4, 8.2, 5.8 Hz, 1H), 4.08 (d, J = 13.2 Hz, 2H), 3.68 – 3.58 (m, 2H), 3.31 – 3.22 (m, 2H), 3.18 – 3.04 (m, 1H), 2.76 – 2.60 (m, 1H); 13C NMR (101 MHz, CD3OD) δ 177.97, 169.51, 158.55, 153.28, 132.59, 131.42, 130.44, 129.99, 129.82, 119.06, 117.04, 61.58, 58.48, 52.57, 50.94, 46.56, 23.00; HRMS (ESI+): calcd for C_{23}H_{29}N_7O_2^{2+} [M+2H]^{2+}: 209.62115, Found: 209.6207.

6.2 Transforming Sphingosine Kinase 1 Inhibitors into Dual and Sphingosine Kinase 2 Selective Inhibitors: Design, Synthesis, and in Vivo Activity

6.2.1 Sphingosine kinase assays

The inhibitory activity of the synthesized compounds on human SphK1 and SphK2 was determined using a previously published method. Recombinant human SphK1 or SphK2 isolated from a cell lysate was briefly incubated with (0.3 μM) or without compound, sphingosine, and γ-[32P]ATP. The radiolabeled sphingosine 1-phosphate was isolated via extraction and thin-layer chromatography and then quantified via scintillation counting.

6.2.2 Sample Preparation and LC–MS–MS Analysis

Sample preparation protocols were from our previous publication with minor modifications. Cell pellets (2–3 × 10^6 cells), whole blood (10 μL) or plasma (10 μL) was mixed with 2 mL of a methanol:chloroform solution (3:1) and transferred to a capped glass vial. Suspensions were supplemented with 10 μL of internal standard solution containing 10 pmol of deuterated (D7) S1P and deuterated (D7) sphingosine. The mixture was placed in a bath sonicator for 10 min and incubated at 48 °C for 16 h. The mixture was then cooled to ambient temperature and mixed with 200 μL of 1M KOH in methanol. The samples were again sonicated and incubated a further 2 h at 37 °C. Samples were then neutralized by the addition of 20 μL of glacial acetic acid and
transferred to 2 mL microcentrifuge tubes. Samples were then centrifuged at 12,000 x g for 12 min at 4 °C. The supernatant fluid was collected in a separate glass vial and evaporated under a stream of nitrogen gas. Immediately prior to LC–MS analysis, the dried material was dissolved in 0.3 ml of methanol and centrifuged at 12,000 x g for 12 min at 4 °C. Fifty µL of the resulting supernatant fluid were analyzed by Liquid Chromatography-ESI Mass Spectrometry (LC–MS) using a triple quadrupole mass spectrometer (AB-Sciex 4000 Q-Trap) coupled to a Shimadzu LC-20AD LC system. A binary solvent gradient with a flow rate of 1 mL/min was used to separate sphingolipids and drugs by reverse phase chromatography using a Supelco Discovery C18 column (50 mm × 2.1 mm, 5 µm bead size). Mobile phase A consisted of water:methanol:formic acid (79:20:1) while mobile phase B was methanol : formic acid (99:1). The run started with 100% A for 0.5 minutes. Solvent B was then increased linearly to 100% B in 5.1 minutes and held at 100% for 4.3 min. The column was finally re-equilibrated to 100% A for 1 min. Natural sphingolipids were detected using multiple reaction monitoring (MRM) as follows: S1P (380.4 → 264.4); deuterated (D7)C18S1P (387.4 → 271.3); sphingosine (300.5 → 264.4); deuterated (D7) sphingosine (307.5 → 271.3).

6.2.3 Pharmacokinetic Analysis

Mouse studies were conducted using a previously reported method. 3.20dd (10 mg/kg) was administered intraperitoneally to groups of 3 to 4 mice (strain: C57BL6/j) or an equal volume of vehicle (2% solution of hydroxypropyl-β-cyclodextrin (Cargill Cavitron 82004)). Blood samples were then collected at the specified time points (ASAP time points were collected 1-2 min following drug addition). The blood samples were analyzed via LC–MS, as described (vide supra). Animal protocols were approved prior to experimentation by the University of Virginia’s School of Medicine Animal Care and Use Committee.
6.2.4 Molecular Docking

Molecular docking was performed using compounds (3.2, 3.20x) to assess potential difference in position in the binding pocket of SphK2. The SphK2 model, with ATP and Mg$^{2+}$ bound, was generated with Molecular Operating Environment (MOE) and energy minimized as previously described. Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions for preparation in docking programs, Marvin 17.3.13, 2017, ChemAxon (http://www.chemaxon.com). AutoDock Tools was used to prepare the protein and ligand files, while AutoDock Vina was used to perform the docking for pose prediction.

The grid box was set to 20 x 20 x 28 Angstrom, with a 1.000 Å grid spacing was used. The center of the box was placed at the approximate center of the ligand-binding cavity, with a part of the ATP binding cavity included as previously performed and to ensure coverage and interaction with key Asp residues. Up to ten docked poses were predicted for each compound. The number of predicted poses is dependent on the fitness of the sampled compound orientations. The lowest energy pose for each docked ligand in SphK2 was then used for analysis of interactions with key residues in the SphK2 binding pocket. Free energy of binding scores were cataloged for each docked compound and used as one level of comparison between compounds.

6.2.5 General Material and Synthetic Procedures

All reactions conducted in a microwave were conducted in a Discover SP microwave synthesizer (CEM Corporation). All solvents were dried using the PureSolv solvent purification system prior to use. All chemical reagents were purchased from commercial sources and used without further purification. Thin layer chromatography (TLC) was performed on aluminum-backed silica gel, 200 µm, F254, and column chromatography was performed on flash grade silica gel, 40-63 µm,
using a Combiflash Rf purification system. $^1$H NMR spectra were recorded at 500 or 400 MHz; the corresponding $^{13}$C NMR resonant frequencies were 126 and 101 MHz, respectively; the corresponding $^{19}$F NMR resonant frequencies were 471 and 376 MHz, respectively. $^1$H NMR chemical shifts are reported in ppm with the solvent resonance as an internal standard (CDCl$_3$: 7.26 ppm; CD$_3$OD: 4.87 ppm; acetone-$d_6$: 2.05 ppm). $^{13}$C NMR, chemical shifts are reported in ppm with the solvent resonance as the internal standard (CDCl$_3$: 77.16 ppm; CD$_3$OD: 49.00 ppm; acetone-$d_6$: 206.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m= multiplet), coupling constants (Hz), and integration. Rotamers are denoted by an asterisk (*). High resolution mass spectroscopy (HRMS) was performed on an LC–MS time–of–flight mass spectrometer using electrospray ionization (ESI). HPLC analyses were performed with a Thermo Electron TSQ triple quadrupole mass spectrometer equipped with an ESI source. All compounds tested in biological assays are >95% pure by $^1$H NMR and HPLC analyses unless noted otherwise. NMR spectra for the synthesized compounds can be found online (http://pubs.acs.org/doi/suppl/10.1021/acs.jmedchem.7b00233).

**General Procedure 3A: Synthesis of amide-oxime derivatives.** TEA (2.7 equiv) was added to a solution of the appropriate benzonitrile (1 equiv) with hydroxylamine hydrochloride (2.6 equiv) in ethanol (0.47 M solution). The mixture was reacted in a microwave for 6 min at 150 °C. The organic solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography to yield the desired product.

**General Procedure 3B: Coupling of amide-oxime derivatives with (S)-2-(1-(tert-butoxycarbonyl)pyrrolidin-2-yl)acetic acid.** DIEA (1.80 equiv) was added to a solution of the appropriate amidoxime (1 equiv) and (S)-2-(1-(tert-butoxycarbonyl)pyrrolidin-2-yl)acetic acid in DMF (0.2 M solution). HCTU (1.5 equiv) was then added to the resulting mixture at rt and
stirred at 100 °C for 4 to 8 h. The reaction progress was followed by TLC. The solution was partitioned between EtOAc and LiBr aqueous solution. The aqueous solution was washed with EtOAc three times, and the combined organic layers were washed with brine, dried over Na2SO4, filtered, and concentrated via vacuum. The resulting residue was purified by silica gel column chromatography.

*General Procedure 3C: Coupling of 3.17 with alpha-bromoketones.* DIEA (2 equiv) was added to a solution of tert-butyl (S)-2-((3-(4-thioureidophenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate 3.17 (1 equiv) and alpha-bromoketone (1 equiv) in ethanol (0.2 M solution). The resulting reaction mixture was then reacted in a microwave at 100 °C for 5 min. The organic solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography.

*General Procedure 3D: Suzuki coupling of aryl bromides with phenyl boronic acids.* Cs2CO3 (2 equiv) was added to a solution of of t-Boc protected aryl bromide intermediate (1 equiv) and the appropriate phenyl boronic acid derivative (3 equiv) in DMF (0.045 M solution). The resulting mixture was degassed for 10 min by bubbling N2 through the solution. PdCl2(dppf) (0.03 equiv) was then added to the mixture, and heated in a microwave reactor at 150 °C for 90 min. The solution was partitioned between EtOAc and LiBr aqueous solution. The aqueous solution was washed with EtOAc three times, and the combined organic layers were washed with brine, dried over Na2SO4, filtered, and concentrated via vacuum. The resulting residue was purified by silica gel column chromatography.

*General Procedure 3E: Deprotection of t-Boc protecting groups with TFA and guanylation of amines.* To a solution of t-Boc protected intermediate in DCM, a 1N TFA solution in DCM was added. The reaction mixture was then stirred at rt for 4 h. At this time, TLC showed complete
conversion of starting material. The organic solvent was removed under reduced pressure. The residue was then dissolved in ACN (0.02 M solution). Diisopropylethylamine (10 equiv) and (Z)-tert-butyl (((tert-butoxycarbonyl)imino)(1H-pyrazol-1-yl)methyl)carbamate (1.05 equiv) were added to the solution, and the resulting reaction mixture was reacted in a microwave reactor at 50 °C for 2 h. The organic solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography.

**General Procedure 3F: Deprotection of t-Boc protecting groups with HCl (g).** Hydrochloric acid gas was bubbled through a solution of the N-Boc protected compound in methanol for 2 to 5 minutes, until complete consumption of starting material was observed by TLC. The reaction mixture was concentrated under reduced pressure and triturated with diethyl ether to yield the corresponding free amine hydrochloride salt.

*N-(4-cyanophenyl)-2,2,2-trifluoroacetamide (3.13).* 4-aminobenzonitrile 3.12 (1.0 g, 8.46 mmol) was dissolved in DCM (8.5 mL) and cooled to 0 °C. Triethylamine (1.3 mL, 9.31 mmol) was added dropwise and allowed to stir at 0 °C for 10 min. Trifluoroacetic anhydride (1.8 mL, 9.31 mmol) was then added dropwise. The reaction mixture was allowed to warm up to rt and stirred for 19 h. The resulting reaction mixture was quenched with sat. aq. NH₄Cl, and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over Na₂SO₄, and concentrated *via* vacuum. The residue was purified by silica gel column chromatography (30% EtOAc/ hexanes) to yield 3.13 (1.0 g, 88%) as white solid. ¹H NMR (400 MHz, CD₃OD) δ 7.89 (d, *J* = 8.7 Hz, 2H), 7.77 (d, *J* = 8.8 Hz, 2H); ¹³C NMR (101 MHz, CD₃OD) δ 157.14 (q, *J*<sub>CF</sub> = 38.4 Hz), 156.76 (q, *J*<sub>CF</sub> = 38.4 Hz), 142.17, 122.19, 121.26 (q, *J*<sub>CF</sub> = 281.3 Hz), 119.35, 118.57 (q, *J*<sub>CF</sub> = 281.3 Hz), 115.70 (q, *J*<sub>CF</sub> = 281.3 Hz), 112.85 (q, *J*<sub>CF</sub> = 281.3 Hz), 109.84; ¹⁹F
NMR (376 MHz, CD$_3$OD) $\delta$ -75.64 (s, 3F); HRMS (ESI-): calcd for C$_9$H$_4$F$_3$N$_2$O [M-H]: 213.0275, found: 213.0273.

(Z)-2,2,2-trifluoro-N-(4-(N'-hydroxycarbamimidoyl)phenyl)acetamide (3.14). Synthesized by General Procedure 3A. 57%, white solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.39 (d, $J$ = 8.9 Hz, 2H), 6.70 (d, $J$ = 8.9 Hz, 2H); $^{13}$C NMR (101 MHz, acetone-$d_6$) $\delta$ 156.22 (q, $^2J_{CF}$ = 38.4 Hz), 155.85 (q, $^2J_{CF}$ = 38.4 Hz), 155.48 (q, $^2J_{CF}$ = 38.4 Hz), 155.11 (q, $^2J_{CF}$ = 38.4 Hz), 152.17, 138.14, 131.54, 127.11, 121.23, 120.91 (q, $^1J_{CF}$ = 280.2 Hz), 118.29 (q, $^1J_{CF}$ = 280.2 Hz), 115.42 (q, $^1J_{CF}$ = 280.2 Hz), 112.56 (q, $^1J_{CF}$ = 280.2 Hz); $^{19}$F NMR (471 MHz, acetone-$d_6$) $\delta$ -76.14 (s, 3F); HRMS (ESI+): calcd for C$_9$H$_4$F$_3$N$_2$O$_2$ [M+H]$^+$: 248.0659, found: 248.1822.

tert-butyl (S)-2-((3-(2,2,2-trifluoroacetamido)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.15). Synthesized by General Procedure B. 67%, yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.19 (s, 1H), 8.12 – 7.93 (m, 2H), 7.75 (dd, $J$ = 14.9, 8.2 Hz, 2H), 4.35 – 4.19 (m, 1H), 3.46 – 3.21 (m, 3H), 3.07 (dt, $J$ = 15.3, 7.3 Hz, 1H), 2.06 (q, $J$ = 8.0, 6.9 Hz, 1H), 1.94 – 1.73 (m, 3H), 1.43 (s, 9H); $^{13}$C NMR (101 MHz, cdcl$_3$) $\delta$ 177.39, 167.67, 155.56, 155.19 (q, $^2J_{CF}$ = 53.5 Hz), 154.79 (q, $^2J_{CF}$ = 53.5 Hz), 154.47 (q, $^2J_{CF}$ = 53.5 Hz), 153.94 (q, $^2J_{CF}$ = 53.5 Hz) 138.54, 128.45, 124.30, 120.89, 120.11 (q, $^1J_{CF}$ = 289.9 Hz), 117.24 (q, $^1J_{CF}$ = 289.9 Hz), 114.37 (q, $^1J_{CF}$ = 289.9 Hz), 111.51 (q, $^1J_{CF}$ = 289.9 Hz), 80.54$^*$, 80.00$^*$, 55.32$^*$, 55.18$^*$, 46.82$^*$, 46.37$^*$, 31.68$^*$, 31.05$^*$, 30.27, 28.47, 23.53$^*$, 22.81$^*$; $^{19}$F NMR (470 MHz, CDCl$_3$) $\delta$ -74.47 (s, 3F); HRMS (ESI+): calcd for C$_{20}$H$_{23}$F$_3$N$_4$NaO$_4$ [M+Na]$^+$: 463.1569, found: 463.1546.

tert-butyl (S)-2-((3-(4-aminophenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.16). (S)-tert-butyl-2-(3-(4-iodophenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate 3.15 (54 mg, 0.123 mmol) was dissolved in MeOH (5 mL) and then 1 M LiOH (5 mL) was added.
The reaction mixture was refluxed for 3 h. At this time, TLC showed complete conversion of starting material. The resulting product was extracted with EtOAc, and the combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated via vacuum to provide 3.16 (45 mg, 82%) as a clear solid without further purification. ^1^H NMR (400 MHz, CDCl₃) δ 7.83 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 8.1 Hz, 2H), 4.34 – 4.17 (m, 1H), 4.04 (s, 2H), 3.49 – 3.19 (m, 3H), 3.13 – 2.90 (m, 1H), 2.02 (s, 1H), 1.93 – 1.69 (m, 3H), 1.45 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 177.14, 177.01, 168.62, 154.81, 154.59, 149.68, 129.26, 116.93, 116.78, 115.03, 114.71, 80.34*, 79.92*, 55.55, 47.11*, 46.70*, 32.16*, 31.35*, 31.14*, 30.38*, 28.85, 23.91*, 23.13*; HRMS (ESI+): calcd for C₁₈H₂₄N₄O₃ [M+Na]^+: 367.1746, found: 367.1743.

**tert-butyl (S)-2-((3-(4-thioureidophenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.17).** tert-butyl (S)-2-((3-(4-aminophenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate 3.16 (100 mg, 0.290 mmol) was dissolved in THF (4 mL). Di(1H-imidazol-1-yl)methanethione (70 mg, 0.392 mmol) was added to the reaction mixture and allowed to stir at rt until TLC showed complete conversion of starting material. Ammonia gas was then passed through the solution for 1 min. The organic solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (35%-80% EtOAc/hexane) to yield 3.17 (101 mg, 86%) as an off-white solid. ^1^H NMR (400 MHz, CDCl₃) δ 8.48 (d, J = 13.6 Hz, 1H), 8.12 (d, J = 8.1 Hz, 2H), 7.42 – 7.32 (m, 2H), 6.33 (s, 2H), 4.29 (d, J = 30.3 Hz, 1H), 3.52 – 3.25 (m, 3H), 3.08 (t, J = 7.6 Hz, 1H), 2.07 (s, 0H), 1.93 – 1.76 (m, 3H), 1.45 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 181.73, 177.91, 167.46, 154.36, 151.61, 139.13, 129.40, 125.85, 124.54, 80.28*, 79.89*, 55.28, 46.88*, 46.53*, 31.90*, 31.15*, 30.34, 28.62, 23.69*, 22.92,*; HRMS (ESI+): calcd for C₁₉H₂₅N₅O₃S [M+H]^+: 404.1756, found: 404.1751.
tert-butyl (S)-2-((3-(4-((4-phenylthiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18a). Synthesized by General Procedure 3C. 56%, yellow amorphous solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.10 – 8.04 (m, 2H), 7.91 – 7.84 (m, 2H), 7.71 – 7.61 (m, 1H), 7.59 – 7.51 (m, 2H), 7.42 (dd, \(J = 8.4, 6.9\) Hz, 2H), 7.37 – 7.30 (m, 1H), 6.91 (s, 1H), 4.30 (m, 1H), 3.40 (m, 3H), 3.07 (m, 1H), 2.08 (m, 1H), 1.93 – 1.81 (m, 3H), 1.48 (s, 9H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 177.22, 168.00, 162.90, 154.37, 151.71, 142.84, 134.53, 128.96, 128.83, 128.19, 126.28, 120.75, 117.33, 102.84, 80.21, 55.28, 46.90 \(^*\), 46.51 \(^*\), 31.95 \(^*\), 31.17 \(^*\), 30.24 \(^*\), 28.64, 23.72 \(^*\), 22.93 \(^*\); HRMS (ESI+): calcd for C\(_{27}\)H\(_{29}\)N\(_5\)O\(_3\)S [M+H]\(^+\): 504.2069, found: 504.2024.

tert-butyl (S)-2-((3-(4-((4-pyridin-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18b). Synthesized by General Procedure 3C. 39%, off-white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.67 (s, 2H), 8.09 (d, \(J = 8.3\) Hz, 2H), 7.85 – 7.69 (m, 2H), 7.60 (t, \(J = 7.8\) Hz, 2H), 7.15 (s, 1H), 4.41 – 4.21 (m, 1H), 3.51 – 3.27 (m, 3H), 3.15 – 2.99 (m, 1H), 2.15 – 2.01 (m, 1H), 1.96 – 1.78 (m, 3H), 1.48 (s, 9H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 177.33, 169.03, 167.92, 163.43, 150.29, 149.18, 142.56, 141.56, 131.98, 129.01, 121.16, 120.56, 117.59, 106.73, 80.22, 55.32, 46.90 \(^*\), 46.53 \(^*\), 31.94 \(^*\), 31.18 \(^*\), 30.28 \(^*\), 29.85 \(^*\), 28.65, 23.72 \(^*\), 22.95 \(^*\); HRMS (ESI+): calcd for C\(_{26}\)H\(_{29}\)N\(_6\)O\(_3\)S [M+H]\(^+\): 505.2022, found: 505.2016.

tert-butyl (S)-2-((3-(4-((4-pyridin-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18c). Synthesized by General Procedure 3C. 75%, off-white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.20 (d, \(J = 1.9\) Hz, 1H), 8.57 (dd, \(J = 4.8, 1.7\) Hz, 1H), 8.23 – 8.13 (m, 2H), 8.08 (d, \(J = 8.2\) Hz, 2H), 7.65 – 7.54 (m, 2H), 7.36 (dd, \(J = 7.9, 4.7\) Hz, 1H), 7.00 (s, 1H), 4.40 – 4.21 (m, 1H), 3.52 – 3.26 (m, 3H), 3.15 – 2.98 (m, 1H), 2.13 – 2.01 (m, 1H), 1.95 – 1.75 (m, 3H), 1.48 (s, 9H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 177.24, 167.95, 163.59,
154.40, 148.85, 148.62, 147.73, 142.84, 133.54, 130.48, 128.98, 123.73, 120.88, 117.47, 104.04, 80.23, 79.81, 55.29, 46.91, 46.50, 31.92, 31.15, 30.23, 29.85, 28.63, 23.71, 22.93; HRMS (ESI+): calcd for C_{26}H_{29}N_{6}O_{3}S [M+H]^+: 505.2022, found: 505.2012.

tert-butyl (S)-2-(((3-(4-((4-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18d). Synthesized by General Procedure C. 66%, light yellow solid. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.05 (ap t, $J = 10$, 2H), 7.95 (br s, 1H), 7.86 – 7.81 (m, 2H), 7.58 – 7.50 (m, 2H), 7.09 (ap t, $J = 10$, 2H), 6.82 (s, 1H), 4.38 – 4.23 (m, 1H), 3.50 – 3.26 (m, 3H), 3.14 – 3.00 (m, 1H), 2.12 – 2.00 (m, 1H), 1.94 – 1.77 (m, 3H), 1.48 (br s, 9H); $^3$C NMR (101 MHz, CDCl$_3$) δ 177.23, 167.97, 164.00 (d, $^1$J$_{CF} = 248.5$ Hz), 163.13, 161.54 (d, $^1$J$_{CF} = 248.5$ Hz), 158.61, 154.41, 151.31, 150.70, 142.80, 130.86 (d, $^4$J$_{CF} = 3.0$ Hz), 130.83 (d, $^4$J$_{CF} = 3.0$ Hz), 129.23, 128.95, 128.03 (d, $^3$J$_{CF} = 8.1$ Hz), 127.95 (d, $^3$J$_{CF} = 8.1$ Hz), 125.54, 120.95, 117.37, 115.83 (d, $^2$J$_{CF} = 22.2$), 115.6 (d, $^2$J$_{CF} = 22.2$ Hz), 102.34, 80.24, 79.82, 55.34, 46.90, 46.52, 31.94, 31.17, 31.01, 30.26, 28.64, 23.71, 22.94; $^{19}$F NMR (376 MHz, CDCl$_3$) δ -109.71 to -116.29 (m, 1F); HRMS (ESI+): calcd for C$_{27}$H$_{28}$FN$_{5}$O$_{3}$S [M+H]$^+$: 522.1975, found: 522.1966.

tert-butyl (S)-2-(((3-(4-((4-chlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18e). Synthesized by General Procedure 3C. 77%, yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.05 (ap t, $J = 10$ Hz, 2H), 7.98 (br s, 1H), 7.79 (dt, $J = 10$, 2.5 Hz, 2H), 7.59 – 7.50 (m, 2H), 7.37 (dt, $J = 10$, 2.5 Hz, 2H), 6.87 (s, 1H), 4.40 – 4.23 (m, 1H), 3.50 – 3.26 (m, 3H), 3.14 - 3.00 (m, 1H), 2.12 – 2.03 (m, 1H), 1.94 – 1.77 (m, 3H), 1.48 (br s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.05, 167.80, 162.99, 154.50, 150.34, 142.63, 133.70, 132.87, 128.80, 128.77, 127.34, 120.62, 117.23, 102.96, 80.10, 79.67, 55.14, 46.74.
tert-butyl (S)-2-((3-((4-((4-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18f). Synthesized by General Procedure 3C. 84%, off-white amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.07 (d, $J = 8.2$ Hz, 2H), 7.77 – 7.70 (m, 2H), 7.53 (dd, $J = 9.2$, 2.7 Hz, 3H), 6.90 (s, 1H), 4.40 – 4.22 (m, 1H), 3.50 – 3.26 (m, 3H), 3.15 – 2.99 (m, 1H), 2.13 – 2.03 (m, 1H), 1.95-178 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.12, 168.01, 163.27, 154.44, 150.39, 142.98, 142.86, 133.45, 131.84, 128.82, 127.76, 121.95, 120.50, 117.35, 103.14, 80.33, 79.87, 55.34, 55.22, 46.86, 46.51, 31.84, 31.13, 31.05, 30.99, 30.25, 29.80, 28.61, 23.64, 22.88; HRMS (ESI+): calcd for C$_{27}$H$_{28}$Cl$_2$N$_5$NaO$_3$S [M+Na]$^+$: 560.1499, found 560.1502.

tert-butyl (S)-2-((3-((4-((3-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18g). Synthesized by General Procedure 3C. 90%, off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.07 (d, $J = 8.2$ Hz, 2H), 7.74 (br. s, 1H), 7.63 (d, $J = 7.9$ Hz, 1H), 7.60 – 7.51 (m, 3H), 7.37 (td, $J = 8.0$, 5.9 Hz, 1H), 7.01 (q, $J = 8.5$, 1H), 6.92 (s, 1H), 4.40 – 4.40 – 4.21 (m, 1H), 3.3.57 – 3.26 (m, 3H), 3.16 – 2.96 (m, 1H), 2.15 – 2.01 (m, 1H), 1.94 – 1.78 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.28, 167.98, 164.52 (d, $^1$J$_{CF}$ = 246.4 Hz), 163.06, 162.08 (d, $^1$J$_{CF}$ = 246.4 Hz), 154.42, 150.44, 142.71, 136.72 (d, $^3$J$_{CF}$ = 8.1 Hz), 136.64 (d, $^3$J$_{CF}$ = 8.1 Hz), 130.35 (d, $^3$J$_{CF}$ = 9.1 Hz), 130.26 (d, $^3$J$_{CF}$ = 9.1 Hz), 128.97, 121.80 (d, $^4$J$_{CF}$ = 2.0 Hz), 121.78 (d, $^4$J$_{CF}$ = 2.0 Hz), 121.11, 120.88 117.44, 115.05 (d, $^2$J$_{CF}$ = 21.2 Hz), 114.84 (d, $^2$J$_{CF}$ = 21.2 Hz), 113.36 (d, $^2$J$_{CF}$ = 23.2 Hz), 113.13 (d, $^2$J$_{CF}$ = 21.2 Hz), 103.77, 80.26, 79.84, 55.32, 46.91, 46.52, 31.94, 31.16, 30.27, 29.85, 28.64, 23.72, 23.54, 22.76; HRMS (ESI+): calcd for C$_{27}$H$_{28}$BrN$_3$NaO$_3$S [M+Na]$^+$: 604.0993, found: 604.0967.
19F NMR (376 MHz, CDCl₃) δ -112.92 to -113.24 (m, 1F); HRMS (ESI+): calcd for C₂₇H₂₈FN₃NaO₃S [M+Na]⁺: 544.1795, found: 544.1784.

**tert-butyl (S)-2-((3-(4-((3-chlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18h).** Synthesized by General Procedure 3C. 63%, white solid. ¹H NMR (400 MHz, CDCl₃) δ 8.04 (d, J = 7.7 Hz, 3H), 7.90 – 7.79 (m, 1H), 7.76 – 7.68 (m, 1H), 7.56 (t, J = 9.6 Hz, 2H), 7.40 – 7.21 (m, 2H), 6.90 (s, 1H), 4.45 – 4.19 (m, 1H), 3.54 – 3.24 (m, 3H), 3.07 (dd, J = 14.7, 8.6 Hz, 1H), 2.16 – 1.99 (m, 1H), 1.87 (dd, J = 14.3, 7.1 Hz, 3H), 1.48 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 177.19, 168.01, 163.18, 154.70, 154.44, 150.21, 142.87, 136.28, 134.76, 130.02, 128.90, 128.03, 126.36, 124.29, 120.69, 117.41, 103.78, 80.30*, 79.86*, 55.35*, 55.24*, 50.92*, 46.89*, 46.52*, 31.89*, 31.15*, 30.27*, 28.63*, 23.68*, 22.91*; HRMS (ESI+): calcd for C₂₇H₂₈ClN₃O₃S [M+H]⁺: 538.1680, found: 538.1679.

**tert-butyl (S)-2-((3-(4-((3-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18i).** Synthesized by General Procedure 3C. 78%, yellow amorphous solid. ¹H NMR (400 MHz, CDCl₃) δ 8.11 – 8.04 (m, 2H), 8.01 (d, J = 1.9 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.55 (t, J = 8.9 Hz, 1H), 7.47 – 7.41 (m, 1H), 7.27 (d, J = 5.5 Hz, 1H), 6.91 (s, 1H), 4.30 – 4.22 (m, 1H), 3.40 – 3.16 – 2.98 (m, 3H), 3.07 (m, 1H), 2.20 – 1.97 (m, 1H), 1.97 – 1.77 (m, 2H), 1.48 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ 177.22, 167.99, 163.11, 154.39, 150.10, 142.70, 136.48, 131.01, 130.33, 129.28, 128.96, 128.63, 124.75, 122.99, 120.83, 117.40, 103.84, 80.25*, 79.80*, 55.34, 46.90*, 46.51*, 31.92*, 31.16*, 30.24*, 28.63*, 23.71*, 22.92*; HRMS (ESI+): calcd for C₂₇H₂₈BrN₃NaO₃S [M+Na]⁺: 604.0993, found: 604.0988.

**tert-butyl (S)-2-((3-(4-((2-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18j).** Synthesized by General Procedure 3C. 25 mg, 56%, off-white amorphous solid. ¹H NMR (400 MHz, CDCl₃) δ 8.16 (td, J = 7.7, 2.0 Hz, 1H), 8.06 (d,
$J = 7.2 \text{ Hz, 2H}$, $7.75 \text{ (s, 1H)}$, $7.58 \text{ (d, } J = 8.5 \text{ Hz, 2H)}$, $7.32 – 7.18 \text{ (m, 3H)}$, $7.13 \text{ (ddd, } J = 12.0, 7.9, 1.5 \text{ Hz, 1H)}$, $4.40 – 4.22 \text{ (m, 1H)}$, $3.54 – 3.24 \text{ (m, 3H)}$, $3.16 – 2.99 \text{ (m, 1H)}$, $2.14 – 2.01 \text{ (m, 1H)}$, $1.96 – 1.77 \text{ (m, 3H)}$, $1.48 \text{ (s, 9H)}$; $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 191.63, 177.19, 168.00, 161.95, 161.71 (d, $^1J_{CF} = 251.5 \text{ Hz}$), 159.22 (d, $^1J_{CF} = 251.5 \text{ Hz}$), 154.41, 145.30, 142.90, 130.07 (d, $^4J_{CF} = 3.0 \text{ Hz}$), 130.04 (d, $^4J_{CF} = 3.0 \text{ Hz}$), 129.17 (d, $^3J_{CF} = 9.1 \text{ Hz}$), 129.08 (d, $^3J_{CF} = 9.1 \text{ Hz}$), 128.92, 124.54, 124.50, 122.39 (d, $^3J_{CF} = 11.1 \text{ Hz}$), 122.28 (d, $^3J_{CF} = 11.1 \text{ Hz}$), 120.69, 117.34, 117.24, 116.15 (d, $^2J_{CF} = 22.2 \text{ Hz}$), 115.93 (d, $^2J_{CF} = 22.2 \text{ Hz}$), 108.05 (d, $^3J_{CF} = 16.2 \text{ Hz}$), 107.89 (d, $^3J_{CF} = 16.2 \text{ Hz}$), 80.25*, 79.80*, 55.35, 46.91*, 46.52*, 31.93*, 31.16*, 30.26, 28.65, 23.71*, 22.93*; $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -114.11 (s, 1F); HRMS (ESI+): calcd for C$_{27}$H$_{28}$FN$_3$NaO$_3$S [M+Na]$^+$: 544.1795, found: 544.1782.

tert-butyl (S)-2-((3-(4-((4-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18k). Synthesized by General Procedure 3C. 70%, off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.17 (br s, 1H), 8.10 – 8.00 (m, 2H), 7.96 (ap d, $J = 8, 2H$), 7.64 (ap d, $J = 8, 2H$), 7.62 – 7.53 (m, 2H), 6.98 (br s, 1H), 4.41 – 4.23 (m, 1H), 3.51 – 3.25 (m, 3H), 3.14 – 3.01 (m, 1H), 2.14 – 2.02 (m, 1H), 1.95 – 1.78 (m, 3H), 1.48 (br s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.19, 167.99, 163.35, 154.44, 150.17, 142.83, 137.74, 130.26 (q, $^2J_{CF} = 32.3 \text{ Hz}$), 129.94 (q, $^2J_{CF} = 32.3 \text{ Hz}$), 129.62 (q, $^2J_{CF} = 32.3 \text{ Hz}$), 129.30 (q, $^2J_{CF} = 32.3 \text{ Hz}$), 128.89, 126.38, 125.82 (q, $^3J_{CF} = 4.0 \text{ Hz}$), 125.78 (q, $^3J_{CF} = 4.0 \text{ Hz}$), 125.74 (q, $^3J_{CF} = 4.0 \text{ Hz}$), 125.70 (q, $^3J_{CF} = 4.0 \text{ Hz}$), 122.97 (q, $^1J_{CF} = 224.2 \text{ Hz}$), 120.90, 120.75 (q, $^1J_{CF} = 224.2 \text{ Hz}$), 120.26, 117.44, 104.67, 104.65, 80.34*, 79.89*, 55.25, 46.89*, 46.53*, 31.86*, 31.13*, 30.27*, 29.84*, 28.62*, 23.67*, 22.90*; $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -62.49 (S, 3F); HRMS (ESI+): calcd for C$_{28}$H$_{29}$F$_3$N$_5$O$_3$S [M+H]$^+$: 572.1943, found: 572.1958.
tert-butyl (S)-2-((3-(4-((4-(3-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18l). Synthesized by General Procedure 3C. 79%, yellow amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.43 (d, $J = 12.3$ Hz, 1H), 8.10 (s, 1H), 8.03 (dt, $J = 14.0$, 7.3 Hz, 3H), 7.54 (ddt, $J = 23.4$, 15.4, 8.1 Hz, 4H), 6.95 (s, 1H), 4.42 – 4.23 (m, 1H), 3.54 – 3.25 (m, 3H), 3.13 – 3.02 (m, 1H), 2.13 – 2.02 (m, 1H), 1.96 – 1.77 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.12, 168.02, 163.39, 154.49, 150.03, 142.94, 135.24, 131.58 (q, $^2$J$_{CF}$ = 32.3 Hz), 131.25 (q, $^2$J$_{CF}$ = 32.3 Hz), 130.93 (q, $^2$J$_{CF}$ = 32.3 Hz), 130.61 (q, $^2$J$_{CF}$ = 32.3 Hz), 129.32, 129.22, 128.82, 125.58 (q, $^1$J$_{CF}$ = 165.2 Hz), 124.56 (q, $^3$J$_{CF}$ = 4.0 Hz), 124.52(q, $^3$J$_{CF}$ = 4.0 Hz), 124.49 (q, $^3$J$_{CF}$ = 4.0 Hz; q, $^1$J$_{CF}$ = 165.2 Hz)), 124.45 (q, $^3$J$_{CF}$ = 4.0 Hz), 122.95, 122.91 (q, $^1$J$_{CF}$ = 165.2 Hz), 122.87, 120.65 (q, $^1$J$_{CF}$ = 165.2 Hz), 120.50, 117.37, 103.95, 80.40*, 79.90*, 55.35*, 55.20*, 46.86*, 46.51*, 31.81*, 31.10*, 30.99*, 30.25*, 28.59, 23.62*, 22.86*; $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -62.71 (s, 3F). HRMS (ESI+): calcd for C$_{28}$H$_{28}$F$_{3}$N$_{5}$O$_{3}$S [M+Na]$^+$: 594.1763, found: 594.1767.

tert-butyl (S)-2-((3-(4-((2-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18m). Synthesized by General Procedure 3C. 79%, yellow amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.30 (br s, 1H), 7.98 (d, $J = 8.5$ Hz, 2H), 7.78 – 7.73 (m, 1H), 7.68 (d, $J = 7.7$ Hz, 1H), 7.56 (td, $J = 7.7$, 1.5 Hz, 1H), 7.46 (t, $J = 7.7$ Hz, 1H), 7.40 (d, $J = 8.4$ Hz, 2H), 6.76 (s, 1H), 4.38 – 4.22 (m, 1H), 3.49 – 3.27 (m, 3H), 3.16 – 2.97 (m, 1H), 2.13 – 2.00 (m, 1H), 1.95 – 1.77 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 191.63, 177.20, 167.97, 162.95, 154.38, 148.62, 142.83, 134.47, 132.09, 131.72, 129.01 (q, $^2$J$_{CF}$ = 30.3 Hz), 128.86, 128.71 (q, $^2$J$_{CF}$ = 30.3 Hz), 128.40, 128.38, 128.32 (q, $^2$J$_{CF}$ = 30.3 Hz), 128.10 (q, $^1$J$_{CF}$ = 275.7 Hz), 126.66 (q, $^3$J$_{CF}$ = 6.1 Hz), 126.60 (q, $^3$J$_{CF}$ = 6.1 Hz), 126.55 (q, $^3$J$_{CF}$ = 6.1 Hz), 126.49 (q, $^3$J$_{CF}$ = 6.1 Hz), 125.59 (q, $^1$J$_{CF}$ = 275.7 Hz), 122.87 (q, $^1$J$_{CF}$
= 275.7 Hz), 120.93, 120.73, 120.14 (q, $^1J_{CF} = 275.7$ Hz), 117.48, 106.95, 106.91, 80.22*, 79.77*, 55.34, 46.90*, 46.51*, 31.95*, 31.16*, 30.98*, 30.23*, 29.84, 28.63*, 28.52*, 23.70*, 22.92*; $^{19}$F NMR (376 MHz, CDCl$_3$) δ -57.78 (s, 3F); HRMS (ESI+): calcd for C$_{28}$H$_{32}$F$_3$N$_5$NaO$_3$S [M+H]$^+$: 572.1943, found: 572.1942.

tert-butyl (S)-2-((3-(4-((4-(p-tolyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18n). Synthesized by General Procedure 3C. 47%, off-white amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.07 (d, $J = 8.2$ Hz, 2H), 7.75 (d, $J = 7.8$ Hz, 2H), 7.58 – 7.47 (m, 2H), 7.22 (d, $J = 7.9$ Hz, 2H), 6.84 (s, 1H), 4.41 – 4.21 (m, 1H), 3.42 (t, $J = 20.8$ Hz, 3H), 3.18 – 2.97 (m, 1H), 2.38 (s, 3H), 2.16 – 2.01 (m, 1H), 1.87 (s, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.30, 167.99, 162.98, 151.64, 142.84, 138.08, 131.75, 129.52, 128.96, 126.20, 120.69, 117.32, 102.00, 80.22*, 79.80*, 55.35, 46.91*, 46.51*, 31.97*, 31.17*, 30.24*, 29.85*, 28.64, 23.72*, 22.93*, 21.43; HRMS (ESI+): calcd for C$_{28}$H$_{32}$N$_5$O$_3$S [M+H]$^+$: 518.2226, found: 518.2225.

tert-butyl (S)-2-((3-(4-((4-(4-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18o). Synthesized by General Procedure 3C. 75%, clear amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.15 – 7.96 (m, 3H), 7.80 (d, 2H), 7.61 – 7.44 (m, 2H), 6.93 (d, $J = 8.7$ Hz, 2H), 6.75 (s, 1H), 4.40 – 4.20 (m, 1H), 3.83 (s, 3H), 3.50 – 3.24 (m, 3H), 3.21 – 2.97 (m, 1H), 2.13 – 1.99 (m, 1H), 1.96 – 1.76 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.14, 168.03, 162.96, 159.63, 154.64, 154.39, 151.36, 143.01, 128.85, 127.65, 127.55, 127.53, 120.60, 120.41, 117.23, 114.16, 100.95, 80.24*, 79.78*, 55.45*, 55.34, 55.21*, 46.88*, 46.50*, 31.89*, 31.13*, 30.96*, 30.21*, 28.62, 23.68*, 22.90*; HRMS (ESI+): calcd for C$_{28}$H$_{32}$N$_5$O$_3$S [M+H]$^+$: 534.2175, found: 534.2160.
tert-butyl (S)-2-((3-(4-((4-ethoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18p). Synthesized by General Procedure 3C. 92%, light yellow amorphous solid. $^1$H NMR (400 MHz, CDCl₃) $\delta$ 8.05 (d, $J = 8.3$ Hz, 2H), 7.82 (s, 1H), 7.79 (d, $J = 8.5$ Hz, 2H), 7.54 (d, $J = 7.7$ Hz, 2H), 6.93 (d, $J = 8.4$ Hz, 2H), 6.75 (s, 1H), 4.39 – 4.21 (m, 1H), 4.07 (q, $J = 7.0$ Hz, 2H), 3.51 – 3.26 (m, 3H), 3.07 (m, 1H), 2.13 – 2.02 (m, 1H), 1.95 – 1.78 (m, 4H), 1.48 (s, 9H), 1.43 (t, $J = 7.0$ Hz, 3H); $^{13}$C NMR (101 MHz, CDCl₃) $\delta$ 177.09, 167.84, 162.73, 158.91, 154.25, 151.25, 142.74, 128.75, 127.38, 127.16, 120.41, 117.11, 114.58, 100.72, 80.06*, 79.63*, 63.49, 55.14, 46.72*, 46.34*, 31.77*, 30.99*, 30.05, 28.47, 23.54*, 22.75*, 14.80; HRMS (ESI+): calcd for C$_{29}$H$_{34}$N$_5$O$_4$S [M+H]$^+$: 548.2332, found: 548.2308.

tert-butyl (S)-2-((3-(4-((3-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18q). Synthesized by General Procedure 3C. 70%, yellow amorphous solid. $^1$H NMR (400 MHz, CDCl₃) $\delta$ 8.09 – 7.97 (m, 3H), 7.58 – 7.51 (m, 2H), 7.49 – 7.40 (m, 2H), 7.32 (t, $J = 8.1$ Hz, 1H), 6.96 – 6.81 (m, 2H), 4.43 – 4.18 (m, 1H), 3.86 (s, 3H), 3.54 – 3.24 (m, 4H), 3.19 – 2.97 (m, 1H), 2.17 – 2.00 (m, 1H), 1.97 – 1.74 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl₃) $\delta$ 177.18, 167.97, 162.95, 160.02, 154.64, 154.40, 151.45, 142.89, 135.90, 129.82, 129.16, 128.88, 120.55, 118.71, 117.30, 113.91, 111.80, 103.11, 80.24*, 79.79*, 55.44*, 55.35*, 55.22*, 46.89*, 46.50*, 31.91*, 31.14*, 30.99*, 30.22*, 28.62, 23.69*, 22.91*; HRMS (ESI+): calcd for C$_{28}$H$_{32}$N$_5$O$_4$S [M+H]$^+$: 534.2175, found: 534.2182.

tert-butyl (S)-2-((3-(4-((4-(trifluoromethoxy)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18r). Synthesized by General Procedure 3C. 30%, yellow solid. $^1$H NMR (400 MHz, CDCl₃) $\delta$ 8.07 (ap. t, $J = 7.5$ Hz, 2H), 7.90 (s, 1H), 7.88 (d, $J = 8.5$ Hz, 2H), 7.59 – 7.51 (m, 2H), 7.28 – 7.23 (m, 2H), 6.88 (s, 1H), 4.39 – 4.22 (m, 1H),
3.54 – 3.27 (m, 2H), 3.14 – 2.99 (m, 1H), 2.13 – 1.99 (m, 1H), 1.95 – 1.77 (m, 3H), 1.48 (s, 9H); 
\( ^{13}\text{C} \text{NMR} \) (101 MHz, CDCl\(_3\)) \( \delta \) 177.04, 167.73, 163.03, 154.22, 150.12, 148.80, 142.54, 133.08, 128.76, 127.46, 121.72 (q, \( ^{1}J_{CF} = 258.6 \text{ Hz} \)), 121.09, 119.16 (q, \( ^{1}J_{CF} = 258.6 \text{ Hz} \)), 117.22, 103.12, 80.09, 79.64, 55.18, 46.73, 46.34, 31.73, 30.98, 30.83, 30.06, 29.68, 28.46, 23.53, 22.74; \( ^{19}\text{F} \text{NMR} \) (376 MHz, CDCl\(_3\)) \( \delta \) -57.82 (s, 3F); HRMS (ESI+): calcd for C\(_{28}\)H\(_{28}\)F\(_3\)N\(_5\)O\(_4\)S [M+H\(^+\)]: 588.1892, found: 588.1916.

\textit{tert-butyl} (S)-2-((3-(4-((4-(3,4-dimethylphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18s). Synthesized by General Procedure 3C. 30%, yellow solid. \( ^{1}\text{H} \text{NMR} \) (400 MHz, CDCl\(_3\)) \( \delta \) 8.08 (d, \( J = 8.3 \text{ Hz} \), 2H), 7.78 (dt, \( J = 7.9 \text{, } 1.3 \text{ Hz} \), 1H), 7.73 (dt, \( J = 2.9 \text{, } 1.4 \text{ Hz} \), 1H), 7.64 (s, 1H), 7.57 (d, \( J = 7.7 \text{ Hz} \), 2H), 7.43 (t, \( J = 8.0 \text{ Hz} \), 1H), 7.17 (ddt, \( J = 8.1 \text{, } 2.3 \text{, } 1.1 \text{ Hz} \), 1H), 6.95 (s, 1H), 4.40 – 4.20 (m, 1H), 3.51 – 3.25 (m, 3H), 3.16 – 2.98 (m, 1H), 2.16 – 2.00 (m, 1H), 1.96 – 1.76 (m, 3H), 1.48 (s, 9H); \( ^{13}\text{C} \text{NMR} \) (101 MHz, CDCl\(_3\)) \( \delta \) 177.24, 167.96, 163.09, 154.40, 150.19, 149.84, 142.65, 136.56, 130.14, 128.98, 124.42, 121.97 (q, \( ^{1}J_{CF} = 156.6 \text{ Hz} \)), 120.96, 120.32 (q, \( ^{1}J_{CF} = 156.6 \text{ Hz} \)), 119.41, 118.87 (q, \( ^{1}J_{CF} = 156.6 \text{ Hz} \)), 117.44, 104.04, 80.23, 79.82, 55.33, 46.89, 46.52, 31.94, 31.17, 30.26, 28.64, 23.71, 22.93; \( ^{19}\text{F} \text{NMR} \) (376 MHz, CDCl\(_3\)) \( \delta \) -57.82 (s, 3F); HRMS (ESI+): calcd for C\(_{28}\)H\(_{28}\)F\(_3\)N\(_5\)O\(_4\)S [M+Na\(^{+}\)]: 610.1712, found: 610.1721.

\textit{tert-butyl} (S)-2-((3-(4-((3,4-dimethylphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18t). Synthesized by General Procedure 3C. 75%, yellow amorphous solid. \( ^{1}\text{H} \text{NMR} \) (400 MHz, CDCl\(_3\)) \( \delta \) 8.30 – 8.08 (m, 1H), 8.03 (d, \( J = 8.1 \text{ Hz} \), 2H), 7.63 (s, 1H), 7.57 (d, \( J = 7.7 \text{, } 2.1 \text{ Hz} \), 1H), 7.54 – 7.46 (m, 2H), 7.15 (d, \( J = 7.9 \text{ Hz} \), 1H), 6.82 (s, 1H), 4.30 (d, \( J = 29.1 \text{ Hz} \), 1H), 3.55 – 3.26 (m, 4H), 3.20 – 2.93 (m, 1H), 2.29 (s, 3H), 2.27 (s, 3H), 2.13 – 2.00 (m, 1H), 1.94 – 1.77 (m, 4H), 1.54 – 1.40 (m, 9H); \( ^{13}\text{C} \text{NMR} \) (101 MHz, CDCl\(_3\))
δ 177.15, 168.03, 163.03, 154.64, 154.39, 151.76, 143.01, 136.92, 136.69, 132.19, 130.04, 128.84, 128.29, 127.48, 123.71, 120.40, 117.27, 101.87, 80.25, 79.80, 55.34, 55.21, 46.88, 46.49, 31.89, 31.12, 30.96, 30.20, 28.61, 23.67, 22.89, 20.07, 19.99, 19.72; HRMS (ESI+): calcd for C_{29}H_{34}N_{5}O_{3}S [M+H]^+: 532.2382, found: 532.2398.

tert-butyl (S)-2-((3-(4-(4-(3,4-dimethoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18u). Synthesized by General Procedure 3C. 63%, yellow amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.23 – 7.98 (m, 3H), 7.58 – 7.48 (m, 2H), 7.46 – 7.37 (m, 2H), 6.90 (d, J = 8.6 Hz, 1H), 6.78 (s, 1H), 4.30 (d, J = 30.3 Hz, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.53 – 3.25 (m, 3H), 3.19 – 2.95 (m, 1H), 2.15 – 1.99 (m, 1H), 1.97 – 1.75 (m, 3H), 1.47 (s, 9H); $^{13}$C NMR (101 MHz, cdcl$_3$) δ 177.18, 167.99, 162.95, 154.37, 151.37, 149.16, 149.11, 142.96, 128.87, 128.32, 127.78, 120.50, 118.79, 117.22, 111.36, 109.63, 101.28, 80.23, 79.78, 56.18, 56.08, 56.06, 56.02, 55.31, 46.87, 46.48, 31.91, 31.13, 30.98, 30.20, 28.61, 23.68, 22.89; HRMS (ESI+): calcd for C_{29}H_{34}N_{5}O_{3}S [M+H]^+: 564.2281, found: 564.2271.

tert-butyl (S)-2-((3-(4-(4-chloro-3-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18v). Synthesized by General Procedure 3C. 91%, light yellow amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.07 (d, J = 8.3 Hz, 2H), 7.85 (d, J = 2.2 Hz, 1H), 7.73 (dd, J = 8.6, 2.2 Hz, 1H), 7.59 – 7.50 (m, 2H), 6.95 (d, J = 8.6 Hz, 1H), 6.76 (s, 1H), 4.40 – 4.22 (m, 1H), 3.93 (s, 3H), 3.47 – 3.29 (m, 3H), 3.07 (ddd, J = 30.3, 14.3, 8.2 Hz, 1H), 2.14 – 2.02 (m, 1H), 1.94 – 1.78 (m, 4H), 1.47 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.24, 167.90, 163.26, 155.01, 149.42, 142.47, 128.97, 128.11, 127.80, 125.73, 122.84, 117.56, 112.20, 101.65, 80.23, 79.79, 56.38, 55.34, 55.23, 46.90, 46.50, 31.93, 31.15, 30.99, 30.23, 28.63, 23.72, 22.92; HRMS (ESI+): calcd for C_{28}H_{31}ClN_{5}O_{4}S [M+H]^+: 568.1785, found: 568.1780.
tert-butyl (S)-2-((3-(4-((4-(3,4-difluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18w). Synthesized by General Procedure 3C. 58%, off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.11 – 8.03 (m, 2H), 7.72 – 7.62 (m, 2H), 7.61 – 7.50 (m, 3H), 7.19 (dd, $J = 10.1, 8.3$ Hz, 1H), 6.84 (s, 1H), 4.29 (t, $J = 18.7$ Hz, 1H), 3.50 – 3.26 (m, 3H), 3.15 – 2.99 (m, 1H), 2.16 – 2.01 (m, 1H), 1.96 – 1.77 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.25, 167.94, 163.13, 154.42, 151.93 (dd, $^2$J$_{CF} = 36.4$ Hz, $^3$J$_{CF} = 13.1$ Hz), 151.80 (dd, $^2$J$_{CF} = 36.4$ Hz, $^3$J$_{CF} = 13.1$ Hz), 149.65, 149.47 (dd, $^2$J$_{CF} = 37.4$ Hz, $^3$J$_{CF} = 13.1$ Hz), 149.34 (dd, $^2$J$_{CF} = 37.4$ Hz, $^3$J$_{CF} = 13.1$ Hz), 149.10 (dd, $^2$J$_{CF} = 37.4$ Hz, $^3$J$_{CF} = 13.1$ Hz), 148.97 (dd, $^2$J$_{CF} = 37.4$ Hz, $^3$J$_{CF} = 13.1$ Hz), 142.64, 131.80 (d, $^3$J$_{CF} = 4.0$ Hz), 131.76 (d, $^3$J$_{CF} = 4.0$ Hz), 131.74 (d, $^3$J$_{CF} = 4.0$ Hz), 131.70 (d, $^3$J$_{CF} = 4.0$ Hz), 128.97, 122.21 (d, $^3$J$_{CF} = 4.0$ Hz), 122.17 (d, $^3$J$_{CF} = 4.0$ Hz), 122.15 (d, $^3$J$_{CF} = 4.0$ Hz), 122.11 (d, $^3$J$_{CF} = 4.0$ Hz), 120.97, 117.68 80 (dd, $^1$J$_{CF} = 227.8$ Hz, $^2$J$_{CF} = 18.2$ Hz), 117.50 (dd, $^1$J$_{CF} = 227.8$ Hz, $^2$J$_{CF} = 18.2$ Hz), 117.45, 115.43 (dd, $^1$J$_{CF} = 227.8$ Hz, $^2$J$_{CF} = 19.2$ Hz), 115.24 (dd, $^1$J$_{CF} = 227.8$ Hz, $^2$J$_{CF} = 19.2$ Hz), 103.26, 80.26*; 79.85*, 55.31, 46.91*, 46.53*, 31.93*, 31.17*, 30.28, 28.64, 23.71*, 22.94*; $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -137.57 (s, 1F), -138.63 (s, 1F); HRMS (ESI+): calcd for C$_{27}$H$_{27}$F$_2$N$_5$NaO$_3$S [M+Na]$^+$: 562.1695, found: 562.1666.

tert-butyl (S)-2-((3-(4-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18x). Synthesized by General Procedure 3C. 59%, yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.09 – 7.98 (m, 2H), 7.91 (d, $J = 8.5$ Hz, 1H), 7.56 – 7.44 (m, 2H), 7.33 – 7.23 (m, 2H), 4.41 – 4.23 (m, 1H), 3.53 – 3.29 (m, 3H), 3.16 – 2.99 (m, 1H), 2.14 – 2.02 (m, 1H), 1.95 – 1.74 (m, 3H), 1.49 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.25, 167.95, 162.20, 154.67, 146.89, 142.77, 133.94, 132.53, 132.30, 131.74, 130.36, 128.90, 127.38,
117.45, 108.45, 80.27*, 79.84*, 55.34, 46.90*, 46.52*, 31.91*, 31.14*, 30.26, 28.64, 23.69*, 22.92*; HRMS (ESI+): calcd for C_{27}H_{28}Cl_{2}N_{5}O_{3}S [M+H]^+: 572.1290, found: 572.1244.

tert-butyl (S)-2-((3-(4-((4-(4-fluoro-3-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18y). Synthesized by General Procedure 3C. 24%, clear yellow solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.15 – 7.98 (m, 4H), 7.82 (s, 1H), 7.60 – 7.51 (m, 2H), 7.31 – 7.17 (m, 1H), 6.90 (s, 1H), 4.40 – 4.23 (m, 1H), 3.52 – 3.27 (m, 3H), 3.15 – 3.01 (m, 1H), 2.14 – 2.03 (m, 1H), 1.94 – 1.81 (m, 3H), 1.48 (s, 9H); \(^13\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 177.27, 167.92, 163.40, 160.64 (d, \(^1J_{CF} = 257.6\) Hz), 158.09 (d, \(^1J_{CF} = 257.6\) Hz), 154.42, 149.25, 142.64, 131.46 (d, \(^3J_{CF} = 8.1\) Hz), 131.38 (d, \(^3J_{CF} = 8.1\) Hz), 131.14 (d, \(^4J_{CF} = 4.0\) Hz), 131.10 (d, \(^4J_{CF} = 4.0\) Hz), 128.96, 126.76 (q, \(^1J_{CF} = 272.7\) Hz), 125.04 (qd, \(^3J_{CF} = 5.1\) Hz, \(^4J_{CF} = 1.0\) Hz), 125.03 (qd, \(^3J_{CF} = 5.1\) Hz, \(^4J_{CF} = 1.0\) Hz), 125.00 (qd, \(^3J_{CF} = 5.1\) Hz, \(^4J_{CF} = 1.0\) Hz), 124.98 (qd, \(^3J_{CF} = 5.1\) Hz, \(^4J_{CF} = 1.0\) Hz), 124.95, 124.94, 124.05 (q, \(^1J_{CF} = 272.7\) Hz), 121.35 (q, \(^1J_{CF} = 272.7\) Hz), 120.99, 118.96 (d, \(^3J_{CF} = 13.1\) Hz), 118.83 (d, \(^3J_{CF} = 13.1\) Hz), 118.63 (q, \(^1J_{CF} = 272.7\) Hz), 118.50, 117.48, 117.43, 117.38, 117.22, 103.56, 80.30*, 79.88*, 55.33, 46.90*, 46.54*, 31.91*, 31.17*, 30.29, 28.63, 23.69*, 22.93*; \(^19\)F NMR (376 MHz, CDCl\(_3\)) \(\delta\) -61.47 (d, \(J = 12.6\) Hz 3F), -115.74 (br. s, 1F); HRMS (ESI+): calcd for C_{28}H_{27}F_{4}N_{5}NaO_{3}S [M+Na]^+: 612.1668, found: 612.1663.

tert-butyl (S)-2-((3-(4-((3,5-difluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18z). Synthesized by General Procedure 3C. 76%, yellow amorphous solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.06 (t, \(J = 10.4\) Hz, 2H), 7.94 (s, 1H), 7.62 – 7.52 (m, 2H), 7.40 – 7.32 (m, 1H), 6.92 (s, 1H), 6.75 (tt, \(J = 8.9, 2.5\) Hz, 1H), 4.44 – 4.21 (m, 1H), 3.53 – 3.24 (m, 3H), 3.15 – 3.00 (m, 1H), 2.15 – 2.01 (m, 1H), 1.95 – 1.75 (m, 3H), 1.48 (s, 9H); \(^13\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 177.21, 168.01, 164.70 (dd, \(^1J_{CF} = 248.5\) Hz, \(^3J_{CF} = 13.1\) Hz, \(^4J_{CF} = 1.0\) Hz), 121.37, 120.99, 118.96 (d, \(^3J_{CF} = 13.1\) Hz), 118.83 (d, \(^3J_{CF} = 13.1\) Hz), 118.63 (q, \(^1J_{CF} = 272.7\) Hz), 118.50, 117.48, 117.43, 117.38, 117.22, 103.56, 80.30*, 79.88*, 55.33, 46.90*, 46.54*, 31.91*, 31.17*, 30.29, 28.63, 23.69*, 22.93*; HRMS (ESI+): calcd for C_{28}H_{27}F_{4}N_{5}NaO_{3}S [M+Na]^+: 612.1668, found: 612.1663.
Hz), 164.57 (dd, $J_{CF} = 248.5$ Hz, $J_{CF} = 13.1$ Hz), 163.17, 162.24 (dd, $J_{CF} = 248.5$ Hz, $J_{CF} = 13.1$ Hz), 162.11 (dd, $J_{CF} = 248.5$ Hz, $J_{CF} = 13.1$ Hz), 154.47, 149.46, 149.42, 142.70, 137.75 (d, $J_{CF} = 20.2$ Hz), 137.65, 137.55 (d, $J_{CF} = 20.2$ Hz), 128.93, 120.87, 117.48, 109.16 (d, $J_{CF} = 27.3$ Hz), 109.08 (d, $J_{CF} = 11.1$ Hz), 108.97 (d, $J_{CF} = 11.1$ Hz), 108.89 (d, $J_{CF} = 27.3$ Hz), 104.66, 103.48 (d, $J_{CF} = 51.5$ Hz), 103.23, 102.97 (d, $J_{CF} = 51.5$ Hz), 80.34*, 79.89*, 55.34, 46.90*, 46.54*, 31.88*, 31.16*, 30.30, 28.74*, 28.63*, 23.68*, 22.92*; $^{19}$F NMR (376 MHz, CDCl$_3$) δ -109.78 (br. S, 2F); HRMS (ESI+): calcd for C$_{27}$H$_{28}$F$_{5}$N$_{5}$O$_{3}$S [M+H]$^+$: 540.1881, found: 540.1845.

tert-butyl (S)-2-((3-((4-(4-fluoro-5-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18aa). Synthesized by General Procedure 3C. 44%, yellow amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.12 – 8.02 (m, 2H), 7.91 (d, $J = 12.9$ Hz, 2H), 7.75 (d, $J = 9.6$ Hz, 1H), 7.64 – 7.51 (m, 2H), 7.28 – 7.23 (m, 1H), 7.00 (s, 1H), 4.41 – 4.23 (m, 1H), 3.52 – 3.27 (m, 3H), 3.14 – 3.01 (m, 1H), 2.14 – 2.02 (m, 1H), 1.97 – 1.75 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.24, 167.98, 164.16 (d, $J_{CF} = 248.5$ Hz), 163.39, 161.70 (d, $J_{CF} = 248.5$ Hz), 154.47, 149.04, 142.61, 137.75 (d, $J_{CF} = 8.1$ Hz), 137.67 (d, $J_{CF} = 8.1$ Hz), 133.44 (qd, $J_{CF} = 33.3$ Hz, $J_{CF} = 8.1$ Hz), 133.36 (qd, $J_{CF} = 33.3$ Hz, $J_{CF} = 8.1$ Hz), 133.11 (qd, $J_{CF} = 33.3$ Hz, $J_{CF} = 8.1$ Hz), 133.03, 132.78 (qd, $J_{CF} = 33.3$ Hz, $J_{CF} = 8.1$ Hz), 132.70 (qd, $J_{CF} = 33.3$ Hz, $J_{CF} = 8.1$ Hz), 132.45 (qd, $J_{CF} = 33.3$ Hz, $J_{CF} = 8.1$ Hz), 132.37 (qd, $J_{CF} = 33.3$ Hz, $J_{CF} = 8.1$ Hz), 128.96, 127.50 (q, $J_{CF} = 272.7$ Hz), 124.81 (q, $J_{CF} = 272.7$ Hz), 122.10 (q, $J_{CF} = 272.7$ Hz), 121.00, 119.36 (q, $J_{CF} = 272.7$ Hz), 118.70, 118.66, 118.63, 117.53, 116.59 (d, $J_{CF} = 23.2$ Hz), 116.36 (d, $J_{CF} = 23.2$ Hz), 112.11 (dq, $J_{CF} = 23.2$ Hz, $J_{CF} = 4.0$ Hz), 112.08 (dq, $J_{CF} = 23.2$ Hz, $J_{CF} = 4.0$ Hz), 111.87 (dq, $J_{CF} = 23.2$ Hz, $J_{CF} = 4.0$ Hz), 111.83 (dq, $J_{CF} = 23.2$ Hz, $J_{CF} = 4.0$ Hz), 105.06, 80.35*, 79.91*, 55.33, 46.90*,
46.54\textsuperscript{\circ}, 31.89\textsuperscript{\circ}, 31.16\textsuperscript{\circ}, 30.31, 28.63, 23.69\textsuperscript{\circ}, 22.93\textsuperscript{\circ}; \textsuperscript{19}F NMR (376 MHz, CDCl\textsubscript{3}) \(\delta\) \textsuperscript{19}F NMR (376 MHz, CDCl\textsubscript{3}) \(\delta\) -62.87 (s, 3F), -110.68 (q, \(J = 8.2\), 7.6 Hz, 1F); HRMS (ESI\textsuperscript{+}): calcd for C\textsubscript{29}H\textsubscript{27}F\textsubscript{6}N\textsubscript{5}S\textsubscript{3} [M+H\textsuperscript{+}]: 662.1636, found: 662.1627.

tert-butyl (S)-2-((3-(4-(4-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18bb). Synthesized by General Procedure 3C. 49%, yellow amorphous solid. \(^1\)H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.29 (s, 2H), 8.08 (dd, \(J = 13.4, 8.2\) Hz, 2H), 7.89 (s, 1H), 7.80 (s, 1H), 7.57 (t, \(J = 9.0\) Hz, 2H), 7.08 (s, 1H), 4.41 – 4.23 (m, 1H), 3.52 – 3.26 (m, 3H), 3.16 – 3.00 (m, 1H), 2.15 – 2.03 (m, 1H), 1.97 – 1.80 (m, 3H), 1.48 (s, 9H); \(^{13}\)C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 177.28, 167.93, 163.64, 154.46, 148.68, 142.53, 136.44, 132.65 (d, \(^2J_{CF} = 33.3\) Hz), 132.32 (q, \(^2J_{CF} = 33.3\) Hz), 131.99 (q, \(^2J_{CF} = 33.3\) Hz), 131.66 (d, \(^2J_{CF} = 33.3\) Hz), 129.00, 127.56 (q, \(^1J_{CF} = 273.7\) Hz), 126.12, 124.85 (q, \(^1J_{CF} = 273.7\) Hz), 122.14 (q, \(^1J_{CF} = 273.7\) Hz), 121.35, 119.42 (q, \(^1J_{CF} = 273.7\) Hz), 117.59, 105.45, 80.36\textsuperscript{\circ}, 79.91\textsuperscript{\circ}, 55.31, 46.90\textsuperscript{\circ}, 46.55\textsuperscript{\circ}, 31.91\textsuperscript{\circ}, 31.18\textsuperscript{\circ}, 30.32, 28.64, 23.69\textsuperscript{\circ}, 22.94\textsuperscript{\circ}; \textsuperscript{19}F NMR (376 MHz, CDCl\textsubscript{3}) \(\delta\) -62.96 (s, 6F); HRMS (ESI\textsuperscript{+}): calcd for C\textsubscript{29}H\textsubscript{27}F\textsubscript{6}NaO\textsubscript{3}S [M+Na\textsuperscript{+}]: 662.1636, found: 662.1627.

tert-butyl (S)-2-((3-(4-((2-fluoro-5-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18cc). Synthesized by General Procedure 3C. 78%, yellow amorphous solid. \(^1\)H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.47 (dd, \(J = 7.1, 2.5\) Hz, 1H), 8.07 (ap. t, \(J = 9.6\) Hz, 2H), 7.91 (s, 1H), 7.63 – 7.48 (m, 3H), 7.32 – 7.15 (m, 2H), 4.42 – 4.21 (m, 1H), 3.52 – 3.27 (m, 3H), 3.16 – 3.02 (m, 1H), 2.15 – 2.02 (m, 1H), 1.97 – 1.77 (m, 3H), 1.48 (s, 9H); \(^{13}\)C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 177.24, 167.94, 163.19 (d, \(^1J_{CF} = 256.5\) Hz), 162.29, 160.65 (d, \(^1J_{CF} = 256.6\) Hz), 154.46, 143.80, 142.71, 128.95, 128.00 (q, \(^1J_{CF} = 272.7\) Hz), 127.72, 127.67, 127.61, 127.38 (q, \(^2J_{CF} = 33.3\) Hz), 127.05 (q, \(^2J_{CF} = 33.3\) Hz), 126.72 (q, \(^2J_{CF} = 33.3\) Hz).
Hz), 126.00, 125.30 (q, $J_{CF} = 272.7$ Hz), 123.11 (d, $J_{CF} = 12.1$ Hz), 122.99 (d, $J_{CF} = 12.1$ Hz), 122.59 (q, $J_{CF} = 272.7$ Hz), 120.89, 119.89 (q, $J_{CF} = 272.7$ Hz), 117.43, 117.08, 116.81 (d, $J_{CF} = 24.2$ Hz), 116.57 (d, $J_{CF} = 24.2$ Hz), 109.27 (d, $J_{CF} = 16.2$ Hz), 109.11 (d, $J_{CF} = 16.2$ Hz), 80.34*, 79.88*, 55.31, 46.89*, 46.54*, 31.91*, 31.17*, 31.04*, 30.29*, 28.63, 23.69*, 22.92*; $^{19}$F NMR (376 MHz, CDCl$_3$) δ -62.08 (s, 3F), -109.18 (br. s, 1F); HRMS (ESI+): calcd for C$_{28}$H$_{27}$F$_{4}$N$_{5}$O$_{3}$NaO$_{3}$ [M+Na]$^{+}$: 612.1668, found: 612.1621.

tert-butyl (S)-2-((3-(4-((1,1'-biphenyl)-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18dd). Synthesized by General Procedure 3C. 19%, light yellow amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.08 (d, $J = 8.3$ Hz, 2H), 7.95 (d, $J = 8.0$ Hz, 2H), 7.74 – 7.53 (m, 3H), 7.46 (t, $J = 7.6$ Hz, 2H), 7.36 (t, $J = 7.4$ Hz, 1H), 6.95 (s, 1H), 4.40 – 4.22 (m, 1H), 3.52 – 3.27 (m, 3H), 3.17 – 2.98 (m, 1H), 2.14 – 2.02 (m, 1H), 1.95 – 1.78 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.22, 167.98, 162.92, 154.64, 151.40, 142.85, 140.86, 140.81, 133.55, 129.00, 128.97, 128.95, 127.51, 127.31, 127.13, 126.67, 117.35, 102.90, 80.21*, 79.79*, 55.32, 46.52, 31.95*, 31.17*, 30.23, 28.65, 23.71*, 22.93*; HRMS (ESI+): calcd for C$_{33}$H$_{34}$N$_{5}$O$_{3}$S [M+H]$^{+}$: 580.2382, found: 580.2377.

tert-butyl (S)-2-((3-(4-((benzofuran-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.18ee). Synthesized by General Procedure 3C. 71%, tan amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.12 (s, 1H), 8.07 (d, $J = 8.3$ Hz, 2H), 7.99 – 7.93 (m, 1H), 7.77 (s, 1H), 7.61 – 7.51 (m, 3H), 7.40 – 7.31 (m, 2H), 6.94 (s, 1H), 4.40 – 4.22 (m, 1H), 3.53 – 3.27 (m, 3H), 3.17 – 2.98 (m, 1H), 2.15 – 2.01 (m, 1H), 1.97 – 1.77 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.25, 167.98, 163.02, 155.87, 154.39, 143.94, 143.76, 142.75, 128.96, 125.51, 124.82, 123.32, 120.99, 120.90, 117.43, 117.29, 117.13, 111.95,
103.23, 80.25*, 79.81*, 55.33, 46.90, 46.52, 31.94*, 31.18*, 31.02*, 30.26*, 28.65, 23.71*, 22.94*;

HRMS (ESI+): calcd for C_{29}H_{29}N_{5}NaO_{4}S [M+Na]^+: 566.1838, found: 566.1813.

**tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-phenylthiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19a).** Synthesized by General Procedure 3E. 82%, off-white oily solid. ¹H NMR (500 MHz, CDCl₃) δ 8.07 (d, J = 8.3 Hz, 2H), 7.87 (d, J = 8.3 Hz, 2H), 7.56 (d, J = 8.3 Hz, 2H), 7.43 (t, J = 7.6 Hz, 2H), 7.34 (dd, J = 7.4 Hz, 1H), 6.90 (s, 1H), 4.79 (dd, J = 8.3, 4.9 Hz, 1H), 3.72 – 3.61 (m, 2H), 3.50 (s, 1H), 3.14 (dd, J = 15.2, 8.5 Hz, 1H), 2.32 – 2.25 (m, 1H), 1.93 (dt, J = 10.2, 5.1 Hz, 1H), 1.82 (dd, J = 14.0, 7.5 Hz, 2H), 1.48 (s, 18H); ¹³C NMR (126 MHz, CDCl₃) δ 177.01, 167.91, 163.01, 154.20, 151.38, 142.61, 134.29, 129.06, 128.87, 128.28, 126.27, 117.38, 102.66, 77.73, 56.66, 50.25, 30.81, 30.45, 28.31; HRMS (ESI+): calcd for C_{33}H_{40}N_{7}O_{5}S [M+H]^+: 646.2812, found: 646.2805.

**tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((pyridin-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19b).** Synthesized by General Procedure 3E. 11%, off-white oily solid. ¹H NMR (500 MHz, CDCl₃) δ 10.41 (s, 1H), 8.65 (ap. s, 2H), 8.09 (s, 1H), 8.01 (d, J = 7.8 Hz, 2H), 7.74 (ap. s, 2H), 7.60 (d, J = 7.9 Hz, 2H), 7.13 (s, 1H), 4.81 – 4.73 (s, 1H), 3.81 – 3.62 (m, 2H), 3.59 – 3.40 (m, 1H), 3.08 (dd, J = 15.2, 8.8 Hz, 1H), 2.36 – 2.26 (m, 1H), 1.98 – 1.89 (m, 1H), 1.84 – 1.75 (m, 2H), 1.48 (s, 18H); ¹³C NMR (126 MHz, CDCl₃) δ 176.85, 167.83, 163.35, 162.48, 159.25, 156.79, 154.30, 150.41, 150.01, 149.24, 142.56, 141.49, 128.97, 127.21, 122.29, 121.09, 120.50, 117.42, 106.53, 82.01, 79.68, 56.60, 50.32, 30.94, 30.46, 28.32, 24.52; HRMS (ESI+): calcd for C_{32}H_{39}N_{8}O_{5}S [M+H]^+: 647.2764, found: 647.2772.

**tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((pyridin-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19c).**
Synthesized by General Procedure 3E. 78%, yellow oily solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.42 (s, 1H), 9.18 (s, 1H), 8.90 (s, 1H), 8.56 (d, $J$ = 4.8 Hz, 1H), 8.17 (d, $J$ = 8.0 Hz, 1H), 7.95 (d, $J$ = 8.3 Hz, 2H), 7.62 (d, $J$ = 8.5 Hz, 2H), 7.35 (dd, $J$ = 8.0, 4.8 Hz, 1H), 6.96 (s, 1H), 4.81 – 4.71 (m, 1H), 3.85 – 3.62 (m, 2H), 3.59 – 3.42 (m, 1H), 3.04 (dd, $J$ = 15.7, 9.0 Hz, 1H), 2.37 – 2.26 (m, 1H), 1.96 – 1.87 (m, 1H), 1.82 – 1.71 (m, 2H), 1.57 – 1.36 (m, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 176.57, 167.78, 163.53, 162.51, 154.41, 148.70, 148.51, 147.62, 142.92, 133.59, 130.59, 128.80, 123.71, 120.40, 117.17, 103.81, 82.25, 79.85, 56.54, 50.41, 31.02, 30.43, 28.29, 24.64; HRMS (ESI+): calcd for C$_{32}$H$_{38}$N$_8$NaO$_5$S $[M+Na]^+$: 669.2584, found: 669.2589.

tert-butyl (S,E)-(((tert-butoxycarbonyl)amino)(2-((3-(4-((4-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (3.19d).

Synthesized by General Procedure 3E. 43%, light yellow oily solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.40 (s, 1H), 8.01 (d, $J$ = 8.7 Hz, 2H), 7.88 – 7.81 (m, 2H), 7.56 (d, $J$ = 8.7 Hz, 2H), 7.14 – 7.06 (m, 2H), 6.81 (s, 1H), 4.82 – 4.73 (m, 1H), 3.79 – 3.62 (m, 2H), 3.57 – 3.46 (m, 1H), 3.09 (dd, $J$ = 15.5, 8.8 Hz, 1H), 2.35 – 2.25 (m, 1H), 1.96 – 1.88 (m, 1H), 1.85 – 1.74 (m, 2H), 1.48 (s, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 176.57, 167.78, 163.53, 162.51, 154.41, 148.70, 148.51, 147.62, 142.92, 133.59, 130.59, 128.80, 123.71, 120.40, 117.17, 103.81, 82.25, 79.85, 56.54, 50.41, 31.02, 30.43, 28.29, 24.64; HRMS (ESI+): calcd for C$_{33}$H$_{39}$FN$_7$O$_5$S $[M+H]^+$: 664.2717, found: 664.2710.

tert-butyl (S,E)-(((tert-butoxycarbonyl)amino)(2-((3-(4-((4-chlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (3.19e).

Synthesized by General Procedure 3E. 57%, light yellow oily solid. $^1$H NMR (400 MHz, CDCl$_3$)
δ 10.36 (s, 1H), 8.02 (d, $J = 8.3$ Hz, 2H), 7.85 (s, 1H), 7.81 (d, $J = 8.2$ Hz, 2H), 7.56 (d, $J = 8.3$ Hz, 2H), 7.38 (d, $J = 8.2$ Hz, 2H), 6.88 (s, 1H), 4.77 (qd, $J = 7.2$, 3.9 Hz, 1H), 3.79 – 3.62 (m, 2H), 3.57 – 3.45 (m, 1H), 3.09 (dd, $J = 15.5$, 8.8 Hz, 1H), 2.31 (dt, $J = 12.3$, 6.5 Hz, 1H), 1.96 – 1.88 (m, 1H), 1.86 – 1.74 (m, 1H), 1.72 – 1.59 (m, 1H), 1.48 (s, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 176.84, 167.84, 163.03, 154.28, 153.95, 151.48, 150.56, 142.69, 133.84, 133.34, 133.10, 128.96, 127.52, 120.83, 117.25, 103.04, 102.80, 82.17, 81.67, 56.61, 50.28, 30.93, 30.46, 28.32; HRMS (ESI+): calcd for C$_{33}$H$_{38}$ClN$_7$O$_5$S [M+H]$^+$: 680.2422, found: 680.2425.

tert-butyl (S,Z)-((2-((3-(4-((4-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)((tert-butoxycarbonyl)imino)methyl)carbamate (3.19f). Synthesized by General Procedure 3E. 83%, off-white oily solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.56 – 10.37 (m, 1H), 8.65 – 8.42 (m, 1H), 7.90 (d, $J = 8.2$ Hz, 2H), 7.58 (d, $J = 8.4$ Hz, 2H), 7.52 (d, $J = 8.2$ Hz, 2H), 6.86 (s, 1H), 4.75 (dq, $J = 11.1$, 6.7, 6.0 Hz, 1H), 3.90 – 3.72 (m, 1H), 3.72 – 3.61 (m, 1H), 3.57 – 3.43 (m, 1H), 3.00 (dd, $J = 15.9$, 9.2 Hz, 1H), 2.39 – 2.27 (m, 1H), 1.97 – 1.85 (m, 1H), 1.82 – 1.70 (m, 1H), 1.48 (d, $J = 5.7$ Hz, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 176.42, 167.73, 162.96, 154.49, 150.48, 142.82, 133.57, 131.86, 128.73, 127.78, 121.90, 120.23, 116.99, 102.96, 82.26, 79.99, 56.52, 50.47, 31.13, 30.44, 28.63, 28.29, 28.10, 24.60; HRMS (ESI+): calcd for C$_{33}$H$_{39}$BrN$_7$O$_5$S [M+H]$^+$: 724.1917, found: 724.1912.

tert-butyl (S,E)-(((tert-butoxycarbonyl)amino)(2-((3-(4-((4-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (3.19g). Synthesized by General Procedure 3E. 57%, yellow oily solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.43 (s, 1H), 8.21 – 8.04 (m, 0H), 7.98 (d, $J = 8.3$ Hz, 2H), 7.65 (d, $J = 8.0$ Hz, 1H), 7.58 (d, $J = 8.6$ Hz, 3H), 7.37 (ap q, $J = 8.0$, 5.8 Hz, 1H), 7.01 (td, $J = 8.5$, 2.7 Hz, 1H), 6.91 (s, 1H), 4.81 – 4.72 (m, 1H), 3.82 – 3.63 (m, 2H), 3.57 – 3.44 (m, 1H), 3.06 (dd, $J = 15.6$, 8.9 Hz, 1H), 2.38 –
2.27 (m, 1H), 1.96 – 1.87 (m, 1H), 1.84 – 1.72 (m, 2H), 1.49 (s, 18H); \(^{13}\text{C}\) NMR (101 MHz, CDCl\(_3\)) \(\delta\) 176.61, 167.80, 164.50 (d, \(^1J_{\text{CF}} = 245.4\) Hz), 162.93, 162.55, 162.07 (d, \(^1J_{\text{CF}} = 245.4\) Hz), 154.39, 150.44, 142.75, 136.84 (d, \(^3J_{\text{CF}} = 8.1\) Hz), 136.76 (d, \(^3J_{\text{CF}} = 8.1\) Hz), 130.30 (d, \(^3J_{\text{CF}} = 8.1\) Hz), 130.22 (d, \(^3J_{\text{CF}} = 8.1\) Hz), 128.85, 121.76, 120.52, 117.11, 114.93 (d, \(^2J_{\text{CF}} = 21.2\) Hz), 114.72 (d, \(^2J_{\text{CF}} = 21.2\) Hz), 113.31 (d, \(^2J_{\text{CF}} = 23.2\) Hz), 113.08 (d, \(^2J_{\text{CF}} = 23.2\) Hz), 105.33, 103.58, 82.25, 79.80, 56.57, 50.45, 31.04, 30.43, 28.35, 28.25, 28.13; \(^{19}\text{F}\) NMR (376 MHz, CDCl\(_3\)) \(\delta\) -113.07 to -113.17 (m, 1F); HRMS (ESI+): calcd for C\(_{33}\)H\(_{39}\)FN\(_7\)O\(_5\)S [M+H\(^+\)]: 664.2717, found: 664.2727.

**tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-((4-((4-((3-chlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19h).**

Synthesized by General Procedure 3E. 77%, light yellow solid. \(^1\text{H}\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 10.43 (s, 1H), 8.35 (s, 1H), 7.93 (d, \(J = 8.3\) Hz, 2H), 7.86 (s, 1H), 7.76 (d, \(J = 7.6\) Hz, 1H), 7.58 (d, \(J = 8.3\) Hz, 2H), 7.37 – 7.24 (m, 2H), 6.89 (s, 1H), 4.80 – 4.71 (m, 1H), 3.86 – 3.73 (m, 1H), 3.72 – 3.62 (m, 1H), 3.57 – 3.45 (m, 1H), 3.02 (dd, \(J = 15.8, 9.1\) Hz, 1H), 2.38 – 2.30 (m, 1H), 1.96 – 1.87 (m, 1H), 1.84 – 1.71 (m, 2H), 1.48 (ap d. rot, 18H); \(^{13}\text{C}\) NMR (101 MHz, CDCl\(_3\)) \(\delta\) 176.51, 167.78, 162.95, 162.52, 154.47, 150.43, 150.27, 142.77, 136.40, 134.73, 130.02, 128.80, 127.97, 126.32, 124.34, 120.42, 117.09, 103.61, 82.28, 79.89, 56.55, 50.43, 31.10, 30.46, 28.36, 28.25; HRMS (ESI+): calcd for C\(_{33}\)H\(_{38}\)ClN\(_7\)O\(_5\)S [M+H\(^+\)]: 680.2422, found: 680.2435.

**tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-((4-((3-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19i).** Synthesized by General Procedure 3E. 57%, off-white oily solid. \(^1\text{H}\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 10.39 (s, 1H), 8.03 – 8.00 (m, 2H), 7.98 (s, 1H), 7.79 (dt, \(J = 7.8, 1.3\) Hz, 1H), 7.66 (s, 1H), 7.58 – 7.54 (m, 1H), 7.46 – 7.42 (m, 1H), 7.29 (d, \(J = 7.9\) Hz, 1H), 6.90 (s, 1H), 4.82 – 4.73 (m, 1H), 3.79 – 3.62
(m, 2H), 3.58 – 3.46 (m, 1H), 3.07 (dd, $J = 15.6$, 8.8 Hz, 1H), 2.37 – 2.26 (m, 1H), 1.97 – 1.87 (m, 1H), 1.86 – 1.73 (m, 2H), 1.48 (s, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 176.60, 167.81, 163.23, 154.59, 154.32, 150.03, 142.82, 136.65, 130.91, 130.32, 129.23, 128.84, 124.80, 122.96, 120.53, 117.15, 103.57, 83.05, 56.54, 50.33, 31.02, 30.45, 28.30, 24.45; HRMS (ESI+): calcd for C$_{33}$H$_{39}$BrN$_7$O$_5$S [M+H$^+$]: 724.1917, found: 724.1909.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(2-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19j).

Synthesized by General Procedure 3E. 86%, light yellow oily solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.42 (s, 1H), 8.18 (td, $J = 7.8$, 2.0 Hz, 1H), 8.09 (s, 1H), 7.95 (d, $J = 8.3$ Hz, 2H), 7.63 – 7.56 (m, 2H), 7.25 – 7.18 (m, 2H), 7.17 – 7.09 (m, 1.5 Hz, 1H), 4.82 – 4.72 (m, 1H), 3.86 – 3.62 (m, 2H), 3.57 – 3.47 (d, $J = 11.3$ Hz, 1H), 3.04 (dd, $J = 15.7$, 9.0 Hz, 1H), 2.38 – 2.29 (m, 1H), 1.96 – 1.87 (m, 1H), 1.84 – 1.74 (m, 2H), 1.48 (ap. d., rot., 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 176.57, 167.83, 162.58, 161.80, 161.73 (d, $^1$J$_{CF}$ = 250.5 Hz), 159.25 (d, $^1$J$_{CF}$ = 250.5 Hz), 154.46, 150.45, 145.33 (d, $^4$J$_{CF}$ = 3.0 Hz), 145.30 (d, $^4$J$_{CF}$ = 3.0 Hz), 142.87, 130.15 (d, $^4$J$_{CF}$ = 4.0 Hz), 130.11 (d, $^4$J$_{CF}$ = 4.0 Hz), 129.05 (d, $^3$J$_{CF}$ = 8.1 Hz), 128.97 (d, $^3$J$_{CF}$ = 8.1 Hz), 128.83, 124.55, 124.52, 122.48 (d, $^3$J$_{CF}$ = 11.1 Hz), 122.37 (d, $^3$J$_{CF}$ = 11.1Hz), 120.46, 117.06, 116.11 (d, $^2$J$_{CF}$ = 23.2 Hz), 115.88 (d, $^2$J$_{CF}$ = 23.2 Hz), 107.92(d, $^3$J$_{CF}$ = 16.2 Hz), 107.76 (d, $^3$J$_{CF}$ = 16.2 Hz), 82.24, 79.79, 77.36, 56.56, 50.42, 31.07, 30.46, 28.38, 28.25, 28.13; $^{19}$F NMR (376 MHz, CDCl$_3$) δ - 114.04 to -114.17 (m, 1F); HRMS (ESI+): calcd for C$_{33}$H$_{38}$FN$_7$NaO$_5$S [M+Na$^+$]: 686.2537, found: 686.2550.

tert-butyl (S,E)-(((tert-butoxycarbonyl)amino)(2-((3-(4-((4-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (3.19k).

Synthesized by General Procedure 3E. 45%, off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) δ
10.45 (br s, 1H), 8.54 (br s, 1H), 7.98 (d, J = 8 Hz, 2H), 7.93 (d, J = 8 Hz, 2H), 7.65 (d, J = 8 Hz, 2H), 7.61 (dt, J = 8, 4 Hz, 2H), 6.98 (br s, 1H), 4.81 – 4.72 (m, 1H), 3.85 – 3.62 (m, 2H), 3.58 – 3.43 (m, 1H). 3.02 (dd, J = 16, 8 Hz, 1H), 2.39 – 2.27 (m, 2H), 1.97 – 1.87 (m, 1H), 1.82 – 1.70 (m, 2H), 1.52-1.44 (m, 18H); 13C NMR (101 MHz, CDCl3) δ 176.49, 167.75, 163.18, 154.41, 150.11, 149.03, 142.73, 137.81, 130.19 (q, 2JCF = 32.8 Hz), 129.86 (q, 2JCF = 32.8 Hz), 129.54 (q, 2JCF = 32.8 Hz), 129.22 (q, 2JCF = 32.8 Hz), 128.77, 128.39, 126.38, 125.80 (q, 4JCF = 4.0 Hz), 125.75 (q, 4JCF = 4.0 Hz), 125.71 (q, 4JCF = 4.0 Hz), 125.68 (q, 4JCF = 4.0 Hz), 122.98, 120.45, 117.13, 104.45, 83.52, 66.00, 56.54, 50.45, 31.08, 30.42, 28.28, 28.10; 19F NMR(376 MHZ, CDCl3) δ -62.53 (s, 3F); HRMS (ESI+): calcd for C34H39F3N7O5S [M+H]+: 714.2685, found: 714.2703.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((3-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.191).

Synthesized by General Procedure 3E. 6%, off-white oily solid. 1H NMR (400 MHz, CDCl3) δ 10.46 (s, 1H), 8.60 (s, 1H), 8.11 (s, 1H), 8.07 (d, J = 7.5 Hz, 1H), 7.89 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.57 – 7.48 (m, 2H), 6.95 (s, 1H), 4.81 – 4.71 (m, 1H), 3.87 – 3.74 (m, 1H), 3.73 – 3.62 (m, 1H), 3.57 – 3.46 (m, 1H), 2.99 (dd, J = 16.0, 9.3 Hz, 1H), 2.41 – 2.30 (m, 1H), 1.96 – 1.86 (m, 1H), 1.82 – 1.71 (m, 2H), 1.48 (d, rot., J = 22.4 Hz, 18H); 13C NMR (101 MHz, CDCl3) δ 176.38, 167.74, 163.12, 162.49, 154.55, 150.41, 150.18, 142.79, 135.41, 131.61 (q, 2JCF = 32.3 Hz), 131.29 (q, 2JCF = 32.3 Hz), 130.97 (q, 2JCF = 32.3 Hz), 130.65 (q, 2JCF = 32.3 Hz), 129.45, 129.24, 128.88, 128.73, 125.66, 124.53 (q, 4JCF = 3.0 Hz), 124.48 (q, 4JCF = 3.0 Hz), 124.45 (q, 4JCF = 3.0 Hz), 124.41 (q, 4JCF = 3.0 Hz), 122.96 (q, 4JCF = 3.0 Hz), 122.92 (q, 4JCF = 3.0 Hz), 122.89 (q, 4JCF = 3.0 Hz), 122.85 (q, 4JCF = 3.0 Hz), 120.34, 117.04, 103.79, 82.32,
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79.98, 56.50, 50.48, 31.17, 30.47, 28.35, 28.22, 28.09; $^1$F NMR (376 MHz, CDCl$_3$) $\delta$ -62.66 (s, 3F); HRMS (ESI+): calcd for C$_{34}$H$_{39}$F$_3$N$_7$O$_5$S [M+H]$^+$: 714.2685, found: 714.2682.

$\text{tert-butyl} \ (S,Z)-(((\text{tert-butoxycarbonyl})\text{imino})((\text{2-((3-((4-((4-((4-((\text{p-tolyl})\text{thiazol}-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate})3.19m).}$

Synthesized by General Procedure 3E. 37%, yellow oily solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.33 (s, 1H), 8.01 (d, $J$ = 8.2 Hz, 2H), 7.84 – 7.74 (m, 2H), 7.70 (d, $J$ = 7.7 Hz, 1H), 7.58 (t, $J$ = 7.5 Hz, 1H), 7.52 – 7.44 (m, 3H), 6.77 (s, 1H), 4.78 (qd, $J$ = 7.3, 4.1 Hz, 1H), 3.74 – 3.61 (m, 2H), 3.56 – 3.44 (m, 1H), 3.12 (dd, $J$ = 15.4, 8.5 Hz, 1H), 2.33 – 2.23 (m, 1H), 1.92 (dq, $J$ = 10.4, 4.8 Hz, 1H), 1.87 – 1.75 (m, 2H), 1.48 (d, $J$ = 9.0 Hz, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 191.61, 176.91, 167.90, 162.66 48 (q, $^3J_{CF}$ = 19.2 Hz), 162.47 48 (q, $^2J_{CF}$ = 319.2 Hz), 154.20, 150.48, 148.79, 142.69, 134.55 48 (q, $^1J_{CF}$ = 285.8 Hz), 132.10, 131.72 (q, $^1J_{CF}$ = 285.8 Hz), 128.97 48 (q, $^1J_{CF}$ = 285.8 Hz), 128.65, 128.32, 126.58 48 (q, $^1J_{CF}$ = 285.8 Hz), 120.91, 117.31, 107.12, 82.14, 79.54, 56.61, 50.27, 30.83, 30.46, 28.39*, 28.24*, 24.56; $^1$F NMR (376 MHz, CDCl$_3$) $\delta$ -57.78 (s, 3F); HRMS (ESI+): calcd for C$_{34}$H$_{38}$F$_3$N$_7$NaO$_5$S [M+Na]$^+$: 736.2505, found: 714.2500.

$\text{tert-butyl} \ (S,Z)-(((\text{tert-butoxycarbonyl})\text{imino})((\text{2-((3-((4-((\text{p-tolyl})\text{thiazol}-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate})3.19n).}$

Synthesized by General Procedure 3E. 82%, off-white oily solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.39 (s, 1H), 8.00 (d, $J$ = 8.3 Hz, 2H), 7.76 (d, $J$ = 7.8 Hz, 2H), 7.56 (d, $J$ = 8.3 Hz, 2H), 7.22 (d, $J$ = 7.9 Hz, 2H), 6.83 (s, 1H), 4.81 – 4.73 (m, 1H), 3.80 – 3.60 (m, 2H), 3.56 – 3.45 (m, 1H), 3.08 (dd, $J$ = 15.4, 8.8 Hz, 1H), 2.38 (s, 3H), 2.35 – 2.25 (m, 1H), 1.98 – 1.87 (m, 1H), 1.85 – 1.74 (m, 2H), 1.48 (s, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 176.75, 167.87, 162.80, 154.28, 151.70, 142.87, 137.94, 131.88, 129.49, 129.22, 128.90, 126.16, 120.47, 117.09, 101.88, 81.88, 79.77, 56.61, 50.32,

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19o).

Synthesized by General Procedure 3E. 61%, clear oily solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.40 (s, 1H), 7.94 (d, $J$ = 8.3 Hz, 1H), 7.84 – 7.75 (m, 2H), 7.62 (d, $J$ = 2.1 Hz, 1H), 7.59 – 7.55 (m, 1H), 6.96 – 6.91 (m, 2H), 6.72 (s, 1H), 4.82 – 4.69 (m, 1H), 3.83 (s, 3H), 3.74 – 3.58 (m, 2H), 3.55 – 3.42 (m, 1H), 3.03 (dd, $J$ = 15.7, 8.9 Hz, 1H), 2.36 – 2.23 (m, 1H), 1.96 – 1.83 (m, 1H), 1.81 – 1.71 (m, 1H), 1.58 – 1.35 (m, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 176.75, 167.87, 162.80, 154.28, 151.70, 142.87, 137.94, 131.88, 129.49, 129.22, 128.90, 126.16, 120.47, 117.09, 101.88, 100.89, 81.88, 56.61, 50.32, 29.81, 28.30, 24.53, 21.44; HRMS (ESI+): calcd for C$_{34}$H$_{42}$N$_7$O$_5$S [M+H]$^+$: 676.2917, found: 676.2903.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-ethoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19p).

Synthesized by General Procedure 3E. 68%, light yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.35 (s, 1H), 8.04 (d, $J$ = 8.4 Hz, 2H), 7.79 (d, $J$ = 8.6 Hz, 2H), 7.55 (d, $J$ = 8.4 Hz, 2H), 6.94 (d, $J$ = 8.4 Hz, 2H), 6.74 (s, 1H), 4.82 – 4.73 (m, 1H), 4.08 (q, $J$ = 7.0 Hz, 2H), 3.77 – 3.62 (m, 2H), 3.56 – 3.45 (m, 1H), 3.11 (dd, $J$ = 15.4, 8.6 Hz, 1H), 2.34 – 2.24 (m, 1H), 1.97 – 1.88 (m, 2H), 1.86 – 1.75 (m, 2H), 1.52 – 1.40 (m, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 176.96, 167.84, 162.78, 159.07, 153.98, 152.88, 151.53, 142.85, 134.03, 129.00, 127.54, 127.43, 117.19, 114.75, 105.35, 100.89, 83.18, 77.73, 63.67, 56.64, 50.25, 30.79, 30.45, 29.85, 28.31, 28.19, 15.00; HRMS (ESI+): calcd for C$_{35}$H$_{44}$N$_7$O$_6$S [M+H]$^+$: 690.3074, found: 690.3084.
**tert-butyl (S,Z)-(((tert-butoxycarbonylimino)(2-((3-(4-((4-(3-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19q).**

Synthesized by General Procedure 3E. 51%, clear amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.41 (s, 1H), 7.96 (d, $J = 8.2$ Hz, 2H), 7.63 (s, 1H), 7.57 (d, $J = 8.3$ Hz, 2H), 7.48 – 7.41 (m, 2H), 7.32 (t, $J = 8.0$ Hz, 1H), 6.92 – 6.83 (m, 2H), 6.36 (s, 1H), 4.82 – 4.71 (m, 1H), 3.86 (s, 3H), 3.80 – 3.61 (m, 2H), 3.58 – 3.44 (m, 1H), 3.05 (dd, $J = 15.6$, 8.9 Hz, 1H), 2.39 – 2.22 (m, 0H), 1.97 – 1.87 (m, 1H), 1.84 – 1.70 (m, 2H), 1.55 – 1.39 (m, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 176.65, 167.84, 163.00, 162.58, 160.02, 154.31, 153.02, 151.37, 150.40, 142.95, 136.02, 133.88, 129.80, 128.83, 120.35, 118.71, 117.07, 113.79, 111.79, 105.30, 102.87, 82.19, 79.75, 56.56, 55.45, 50.33, 30.43, 29.83, 28.29, 28.10, 24.50; HRMS (ESI+): calcd for C$_{34}$H$_{42}$N$_7$O$_6$S [M+H]$^+$: 676.2917, found: 676.2900.

**tert-butyl (S,E)-(((tert-butoxycarbonylamino)(2-((3-(4-((4-(3-trifluoromethoxy)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (3.19r).**

Synthesized by General Procedure 3E. 70%, yellow amorphous solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 10.41 (s, 1H), 8.02 (d, $J = 8.3$ Hz, 2H), 7.89 (d, $J = 8.0$ Hz, 2H), 7.85 (s, 1H), 7.57 (d, $J = 8.1$ Hz, 2H), 6.88 (s, 1H), 4.81 – 4.71 (m, 1H), 3.79 – 3.61 (m, 2H), 3.56 – 3.44 (m, 1H), 3.14 – 3.04 (dd, $J = 15.5$, 8.6 Hz, 1H), 2.34 – 2.25 (m, 1H), 1.95 – 1.88 (m, 1H), 1.85 – 1.74 (m, 2H), 1.48 (s, 18H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 176.70, 168.89, 167.68, 162.92, 154.13, 150.83, 150.19, 148.84, 142.49, 133.15, 128.82, 127.48, 121.10, 120.75, 119.45, 117.11, 103.06, 81.60, 79.46, 56.46, 50.14, 30.75, 30.29, 29.67, 28.16, 24.33; $^{19}$F NMR (470 MHz, CDCl$_3$) $\delta$ -57.82 (s, 3F); HRMS (ESI+): calcd for C$_{34}$H$_{38}$F$_3$N$_7$O$_6$S [M+H]$^+$: 730.2634, found: 730.2672.
tert-butyl (S,Z)-(((tert-butoxycarbonylimino)(2-((3-(4-((4-(3-( trifluoromethoxy)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19s).

Synthesized by General Procedure 3E. 83%, yellow amorphous solid. \(^\text{1H} \text{NMR} (400 \text{ MHz, CDCl}_3) \delta 10.38 (s, 1H), 8.04 – 8.00 (m, 2H), 7.80 (ddd, \(J = 7.8, 1.6, 1.0 \text{ Hz, 1H}), 7.73 (dt, \(J = 2.5, 1.3 \text{ Hz, 1H}), 7.60 – 7.54 (m, 2H), 7.43 (t, \(J = 8.0 \text{ Hz, 1H}), 7.19 – 7.15 (m, 1H), 6.94 (s, 1H), 4.82 – 4.73 (m, 1H), 3.82 – 3.67 (m, 2H), 3.57 – 3.45 (m, 1H), 3.09 (dd, \(J = 15.5, 8.8 \text{ Hz, 1H}), 2.32 (d, \(J = 11.6 \text{ Hz, 1H}), 1.97 – 1.88 (m, 1H), 1.86 – 1.75 (m, 1H), 1.67 – 1.56 (m, 1H), 0.83 (s rot., 9H), 1.46 (s rot., 9H); \(^{13}\text{C NMR} (101 \text{ MHz, CDCl}_3) \delta 176.81, 163.00, 150.47 (q, \(^3J_{\text{CF}} = 32.8 \text{ Hz}), 150.20 (q, \(^3J_{\text{CF}} = 32.8 \text{ Hz}), 149.82 (q, \(^3J_{\text{CF}} = 32.8 \text{ Hz}), 142.60, 136.61, 130.13, 128.96, 124.46, 120.90, 120.28, 118.85, 117.26, 103.93, 82.20, 79.66, 56.59, 50.34, 30.95, 30.47, 28.38^\ast, 28.24^\ast, 28.14; \(^{19}\text{F NMR} (376 \text{ MHz, CDCl}_3) \delta -57.66 (s, 3F); \text{HRMS (ESI+): calcd for C}_{34}\text{H}_{38}\text{F}_3\text{N}_7\text{O}_5\text{S} [\text{M+H}^+]^\ast: 730.2634, \text{found: 730.2635.}

tert-butyl (S,Z)-(((tert-butoxycarbonylimino)(2-((3-(4-(4-(3,4-dimethylphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19t).

Synthesized by General Procedure 3E. 92%, clear amorphous solid. \(^\text{1H} \text{NMR} (400 \text{ MHz, CDCl}_3) \delta 10.38 (s, 1H), 7.99 (d, \(J = 8.3 \text{ Hz, 2H}), 7.67 – 7.51 (m, 6H), 7.17 (d, \(J = 7.8 \text{ Hz, 1H}), 6.81 (s, 1H), 6.36 (d, \(J = 2.4 \text{ Hz, 1H}), 4.82 – 4.72 (m, 1H), 3.81 – 3.60 (m, 2H), 3.50 (s, 1H), 3.07 (dd, \(J = 15.5, 8.7 \text{ Hz, 1H}), 2.30 (d, \(J = 11.3 \text{ Hz, 6H}), 1.98 – 1.85 (m, 1H), 1.85 – 1.72 (m, 2H), 1.47 (s, 18H); \(^{13}\text{C NMR} (101 \text{ MHz, CDCl}_3) \delta 176.70, 167.88, 163.10, 162.57, 154.72, 154.24, 153.05, 151.67, 150.44, 143.05, 136.92, 136.63, 133.77, 132.34, 130.07, 128.85, 127.44, 123.69, 120.31, 117.05, 105.24, 101.66, 82.97, 82.15, 79.69, 56.57, 50.32, 30.90, 30.42, 28.29, 28.11, 24.55, 20.04, 19.74; \text{HRMS (ESI+): calcd for C}_{35}\text{H}_{44}\text{N}_7\text{O}_5\text{S} [\text{M+H}^+]^\ast: 674.3125, \text{found: 674.3134.}
tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((3,4-dimethoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19u).

Synthesized by General Procedure 3E. 78%, clear amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.41 (s, 1H), 8.56 – 8.24 (m, 1H), 7.95 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.47 – 7.39 (m, 2H), 7.22 (s, 1H), 6.76 (s, 1H), 4.76 (dq, J = 11.7, 7.1, 5.1 Hz, 1H), 3.93 (d, J = 18.3 Hz, 6H), 3.83 – 3.60 (m, 3H), 3.56 – 3.43 (m, 1H), 3.04 (dd, J = 15.6, 9.0 Hz, 1H), 2.38 – 2.23 (m, 1H), 1.97 – 1.84 (m, 1H), 1.82 – 1.70 (m, 2H), 1.54 – 1.38 (m, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 176.63, 167.81, 162.82, 162.56, 154.36, 151.37, 150.41, 149.11, 142.93, 128.79, 127.90, 120.32, 118.80, 117.01, 111.35, 109.61, 101.06, 83.08, 82.20, 79.74, 56.54, 56.08, 56.06, 50.36, 30.98, 30.46, 28.26, 28.09; HRMS (ESI+): calcd for C$_{35}$H$_{44}$N$_7$O$_7$S [M+H]$^+$: 706.3023, found: 706.3028.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-chloro-4-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19v).

Synthesized by General Procedure 3E. 34%, light yellow oil. $^1$H NMR (500 MHz, CDCl$_3$) δ 10.38 (s, 1H), 8.03 (d, J = 8.8 Hz, 2H), 7.87 (d, J = 2.2 Hz, 1H), 7.75 (dd, J = 8.5, 2.2 Hz, 1H), 7.59 – 7.51 (m, 2H), 6.97 (d, J = 8.6 Hz, 1H), 6.78 (s, 1H), 4.82 – 4.72 (m, 1H), 3.94 (s, 3H), 3.84 – 3.62 (m, 2H), 3.58 – 3.42 (m, 1H), 3.10 (dd, J = 15.4, 8.7 Hz, 1H), 2.36 – 2.23 (m, 1H), 1.97 – 1.87 (m, 1H), 1.88 – 1.74 (m, 1H), 1.74 – 1.56 (m, 1H), 1.54 – 1.40 (m, 18H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 176.89, 167.87, 162.97, 154.91, 150.17, 142.71, 128.98, 128.46, 128.11, 125.70, 122.84, 117.23, 112.21, 101.84, 100.14, 83.18, 77.73, 56.62, 56.40, 30.88, 30.46, 29.85, 28.31, 28.18; HRMS (ESI+): calcd for C$_{34}$H$_{41}$ClN$_7$O$_6$S [M+H]$^+$: 710.2528, found: 710.2542.
**tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(3-(4-((4,3,5-dichlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19w).**

Synthesized by General Procedure 3E. 43%, off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.42 (s, 1H), 7.99 (d, $J = 8.3$ Hz, 2H), 7.96 – 7.87 (m, 1H), 7.74 – 7.65 (m, 1H), 7.62 – 7.52 (m, 3H), 7.23 – 7.15 (m, 1H), 6.84 (s, 1H), 4.81 – 4.72 (m, 1H), 3.86 – 3.61 (m, 2H), 3.57 – 3.43 (m, 1H), 3.07 (dd, $J = 15.6$, 8.8 Hz, 1H), 2.38 – 2.26 (m, 1H), 1.97 – 1.88 (m, 1H), 1.84 – 1.72 (m, 2H), 1.48 (d, $J = 17.1$ Hz, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 176.74, 167.79, 162.99, 162.59, 150.44, 149.63, 142.58, 128.91, 122.12, 120.79, (dd, $^1$J$_{CF} = 228.3$ Hz, $^3$J$_{CF} = 17.2$ Hz), 117.49 (dd, $^1$J$_{CF} = 228.3$ Hz, $^3$J$_{CF} = 17.2$ Hz), 117.19, 115.40 (dd, $^1$J$_{CF} = 228.3$ Hz, $^3$J$_{CF} = 17.2$ Hz), 115.22 (dd, $^1$J$_{CF} = 228.3$ Hz, $^3$J$_{CF} = 17.2$ Hz), 103.12, 82.23, 79.74, 56.57, 50.43, 31.01, 30.44, 28.36, 28.24; $^{19}$F NMR (376 MHz, CDCl$_3$) δ -137.54 to -137.74 (m, 1F), -138.64 – -138.84 (m, 1F); HRMS (ESI+): calcd for C$_{33}$H$_{38}$F$_2$N$_7$O$_5$S [M+H]$^+$: 714.2032, found: 714.2042.

**tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4,3,5-dichlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19x).**

Synthesized by General Procedure 3E. 45%, clear yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.36 (s, 1H), 7.94 (d, $J = 8.5$ Hz, 3H), 7.55 – 7.49 (m, 2H), 7.45 (d, $J = 2.1$ Hz, 1H), 7.29 (dd, $J$ = 8.5, 2.2 Hz, 1H), 7.24 (s, 1H), 4.79 – 4.70 (m, 1H), 3.82 – 3.60 (m, 2H), 3.55 – 3.41 (m, 1H), 3.04 (dd, $J = 15.6$, 8.9 Hz, 1H), 2.33 – 2.23 (m, 1H), 1.95 – 1.85 (m, 1H), 1.82 – 1.68 (m, 2H), 1.48 (s, 12H), 1.44 (s, 6H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 176.68, 167.81, 162.57, 161.89, 150.45, 146.89, 142.75, 133.80, 132.44, 132.39, 131.76, 130.38, 128.87, 127.40, 120.65, 117.18, 108.44, 82.23, 79.76, 56.58, 50.34, 30.99, 30.45, 28.38, 28.24, 28.13; HRMS (ESI+): calcd for C$_{33}$H$_{38}$Cl$_2$N$_7$O$_5$S [M+H]$^+$: 714.2032, found: 714.2042.
tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-fluoro-3-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19y). Synthesized by General Procedure 3E. 48%, off-white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 10.38 (s, 1H), 8.13 – 7.91 (m, 5H), 7.56 (d, \(J = 8.4\) Hz, 2H), 7.23 (d, \(J = 9.4\) Hz, 1H), 6.90 (s, 1H), 4.84 – 4.71 (m, 1H), 3.83 – 3.63 (m, 2H), 3.57 – 3.46 (m, 1H), 3.07 (dd, \(J = 15.6, 8.9\) Hz, 1H), 2.37 – 2.27 (m, 1H), 1.98 – 1.88 (m, 1H), 1.85 – 1.72 (m, 2H), 1.55 – 1.42 (m, 18H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 176.79, 167.79, 163.28, 162.59, 160.66, 158.10, 154.35, 150.46, 149.30, 142.55, 140.47, 137.78, 131.52 (d, \(^3\)J\(_{CF}\) = 9.1 Hz), 131.43 (d, \(^3\)J\(_{CF}\) = 9.1 Hz), 131.19, 128.94, 126.81 (q, \(^1\)J\(_{CF}\) = 273.7 Hz), 124.94, 124.09 (q, \(^1\)J\(_{CF}\) = 273.7 Hz), 121.38 (q, \(^1\)J\(_{CF}\) = 273.7 Hz), 120.93, 119.32, 118.64 (q, \(^1\)J\(_{CF}\) = 273.7 Hz), 118.51, 117.43, 117.29, 117.22, 103.45, 82.24, 79.76, 56.58, 50.34, 30.99, 30.49, 28.36, 28.26; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)) \(\delta\) -61.44 (d, \(J = 12.7\) Hz, 3F), -115.54 to -116.19 (m, 1F); HRMS (ESI+): calcd for C\(_{34}\)H\(_{38}\)F\(_4\)N\(_7\)O\(_5\)S [M+H]\(^+\): 732.2591, found: 732.2542.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((3,5-difluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19z).

Synthesized by General Procedure 3E. 79%, yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 10.83 (s, 1H), 8.92 (s, 1H), 8.26 (d, \(J = 8.3\) Hz, 2H), 7.95 (d, \(J = 8.4\) Hz, 2H), 7.75 (d, \(J = 7.5\) Hz, 2H), 7.62 (s, 1H), 7.15 – 7.07 (m, 1H), 5.16 – 5.05 (m, 1H), 4.24 – 4.09 (m, 1H), 4.09 – 3.98 (m, 1H), 3.93 – 3.80 (m, 1H), 3.36 (dd, \(J = 15.9, 9.2\) Hz, 1H), 2.78 – 2.65 (m, 1H), 2.34 – 2.23 (m, 1H), 2.18 – 2.05 (m, 2H), 1.92 – 1.74 (m, 18H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 176.41, 167.72, 164.69 (dd, \(^1\)J\(_{CF}\) = 248.5 Hz, \(^3\)J\(_{CF}\) = 13.1 Hz), 164.56 (dd, \(^1\)J\(_{CF}\) = 248.5 Hz, \(^3\)J\(_{CF}\) = 13.1 Hz), 162.95, 162.49, 162.23 (dd, \(^1\)J\(_{CF}\) = 248.5 Hz, \(^3\)J\(_{CF}\) = 13.1 Hz), 162.10 (dd, \(^1\)J\(_{CF}\) = 248.5 Hz, \(^3\)J\(_{CF}\) = 13.1 Hz), 154.55, 150.38, 149.46, 142.68, 137.86 (dd, \(^3\)J\(_{CF}\) = 10.1 Hz), 137.76 (dd, \(^3\)J\(_{CF}\) = 10.1 Hz).
Hz), 137.66 (dd, \( ^3J_{CF} = 10.1 \) Hz), 128.77, 120.40, 117.07, 109.14 (dd, \( ^3J_{CF} = 19.2 \) Hz, \( ^4J_{CF} = 7.1 \) Hz), 109.07 (dd, \( ^3J_{CF} = 19.2 \) Hz, \( ^4J_{CF} = 7.1 \) Hz), 108.95 (dd, \( ^3J_{CF} = 19.2 \) Hz, \( ^4J_{CF} = 7.1 \) Hz), 108.88 (dd, \( ^3J_{CF} = 19.2 \) Hz, \( ^4J_{CF} = 7.1 \) Hz), 104.44, 103.40 (dd, \( ^2J_{CF} = 25.8 \) Hz), 103.14 (dd, \( ^2J_{CF} = 25.8 \) Hz), 102.89 (dd, \( ^2J_{CF} = 25.8 \) Hz), 82.33, 79.97, 56.53, 50.56, 31.17, 30.44, 28.34

\(^1^9F\) NMR (376 MHz, CDCl\(_3\)) \( \delta \) -109.87 (t, \( J = 8.2 \) Hz, 2F); HRMS (ESI+): calcd for C\(_{33}\)H\(_{38}\)F\(_2\)N\(_7\)O\(_5\)S [M+H]\(^+\): 682.2623, found: 682.2615.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-((3-fluoro-5-( trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)carbamate (3.19aa). Synthesized by General Procedure 3E. 51%, clear yellow solid.

\(^1^H\) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 10.43 (s, 1H), 8.34 (s, 1H), 7.94 (d, \( J = 8.4 \) Hz, 2H), 7.89 (s, 1H), 7.78 (dt, \( J = 9.6 \) Hz, 1H), 7.61 – 7.55 (m, 2H), 7.25 (d, \( J = 6.2 \) Hz, 2H), 6.99 (s, 1H), 4.81 – 4.72 (m, 1H), 3.86 – 3.62 (m, 2H), 3.59 – 3.44 (m, 1H), 3.02 (dd, \( J = 15.7 \) Hz, 9.0 Hz, 1H), 2.41 – 2.29 (m, 1H), 1.98 – 1.87 (m, 1H), 1.83 – 1.71 (m, 2H), 1.51 (s, 8H), 1.45 (s, 10H); \(^1^3C\) NMR (101 MHz, CDCl\(_3\)) \( \delta \) 176.54, 167.74, 164.19 (d, \( ^1J_{CF} = 248.5 \) Hz), 163.20, 162.52, 161.73 (d, \( ^1J_{CF} = 248.5 \) Hz), 154.49, 150.43, 149.10, 142.57, 137.89 (q, \( ^3J_{CF} = 9.1 \) Hz), 137.80 (d, \( ^3J_{CF} = 9.1 \) Hz), 133.40 (qd, \( ^2J_{CF} = 34.3 \) Hz, \( ^3J_{CF} = 9.1 \) Hz), 133.32 (qd, \( ^2J_{CF} = 34.3 \) Hz, \( ^3J_{CF} = 9.1 \) Hz), 133.08 (qd, \( ^2J_{CF} = 34.3 \) Hz, \( ^3J_{CF} = 9.1 \) Hz), 132.99 (qd, \( ^2J_{CF} = 34.3 \) Hz, \( ^3J_{CF} = 9.1 \) Hz), 132.74 (qd, \( ^2J_{CF} = 34.3 \) Hz, \( ^3J_{CF} = 9.1 \) Hz), 132.66 (qd, \( ^2J_{CF} = 34.3 \) Hz, \( ^3J_{CF} = 9.1 \) Hz), 132.41 (qd, \( ^2J_{CF} = 34.3 \) Hz, \( ^3J_{CF} = 9.1 \) Hz), 132.33 (qd, \( ^2J_{CF} = 34.3 \) Hz, \( ^3J_{CF} = 9.1 \) Hz), 128.84, 127.56 (q, \( ^1J_{CF} = 274.7 \) Hz), 124.85 (q, \( ^1J_{CF} = 274.7 \) Hz), 122.13 (q, \( ^1J_{CF} = 274.7 \) Hz), 120.72, 119.41 (q, \( ^1J_{CF} = 274.7 \) Hz), 118.61, 117.20, 116.68 (d, \( ^2J_{CF} = 23.2 \) Hz), 116.45 (d, \( ^2J_{CF} = 23.2 \) Hz), 112.02 (d, \( ^2J_{CF} = 25.3 \) Hz), 111.77 (d, \( ^2J_{CF} = 25.3 \) Hz), 104.90, 82.32, 79.92, 56.54, 50.46, 31.13, 30.49, 28.37.
28.24*, 24.62; 19F NMR (376 MHz, CDCl3) δ -62.84 (s, 3F) , -110.73 (t, J = 9.0 Hz, 1F); HRMS (ESI+): calcd for C34H38F4N7O5S [M+H]⁺: 732.2591, found: 732.2602.

tert-butyl (S,Z)-((2-((3-(3,5-bis(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)((tert-butoxycarbonyl)imino)methyl)carbamate (3.19bb).

Synthesized by General Procedure 3E. 84%, off-white solid. 1H NMR (400 MHz, CDCl3) δ 10.40 (s, 1H), 8.30 (s, 2H), 8.11 (d, J = 19.0 Hz, 1H), 7.99 (d, J = 8.3 Hz, 2H), 7.80 (s, 1H), 7.56 (d, J = 8.6 Hz, 2H), 7.07 (s, 1H), 4.82 – 4.73 (m, 1H), 3.84 – 3.63 (m, 2H), 3.58 – 3.47 (m, 1H), 3.05 (dd, J = 15.6, 9.0 Hz, 1H), 2.39 – 2.28 (m, 1H), 1.99 – 1.88 (m, 1H), 1.85 – 1.74 (m, 2H), 1.48 (d, J = 18.2 Hz, 18H); 13C NMR (101 MHz, CDCl3) δ 176.75, 167.74, 163.51, 162.55, 150.45, 148.74, 142.42, 136.53, 132.64 (q, JCF = 33.3 Hz), 132.31 (q, JCF = 33.3 Hz), 131.98 (q, JCF = 33.3 Hz), 131.66 (q, JCF = 33.3 Hz), 128.94, 127.60(q, JCF = 274.7 Hz), 126.15, 124.88 (q, JCF = 274.7 Hz), 122.16 (q, JCF = 274.7 Hz), 121.35, 121.05, 119.45 (q, JCF = 274.7 Hz), 117.35, 105.34, 82.27, 79.83, 56.56, 50.40, 31.04, 30.53, 28.37*, 28.24*; 19F NMR (376 MHz, CDCl3) δ -62.94 (s, 6F); HRMS (ESI+): calcd for C35H38F6N7O5S [M+H]⁺: 782.2559, found: 782.2541.


tert-butyl (S,Z)-((((tert-butoxycarbonyl)imino)(2-((3-(4-(4-(2-fluoro-5-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.19cc). Synthesized by General Procedure 3E. 51%, clear yellow solid. 1H NMR (400 MHz, CDCl3) δ 10.34 (s, 1H), 8.48 (dd, J = 7.1, 2.5 Hz, 1H), 8.04 (d, J = 8.3 Hz, 2H), 7.83 (s, 1H), 7.55 (d, J = 8.4 Hz, 3H), 7.25 – 7.18 (m, 1H), 4.83 – 4.73 (m, 1H), 3.80 – 3.62 (m, 2H), 3.58 – 3.45 (m, 1H), 3.09 (dd, J = 16.9, 8.4 Hz, 1H), 2.36 – 2.24 (m, 1H), 1.99 – 1.89 (m, 1H), 1.86 – 1.72 (m, 2H), 1.49 (d, J = 9.5 Hz, 18H); 13C NMR (101 MHz, CDCl3) δ 176.91, 167.83, 163.22 (d, JCF = 256.5 Hz), 162.25, 160.68 (d, JCF = 256.5 Hz), 158.33, 154.26, 148.80,
143.85, 142.54, 129.01, 127.72, 126.04, 123.36, 123.26 (q, $^3J_{CF} = 12.1$ Hz), 123.15 (q, $^3J_{CF} = 12.1$ Hz), 123.03 (q, $^3J_{CF} = 12.1$ Hz), 122.90 (q, $^3J_{CF} = 12.1$ Hz), 122.61, 121.09, 117.33, 116.82, 116.58, 109.22 (d, $^3J_{CF} = 16.2$ Hz), 109.06 (d, $^3J_{CF} = 16.2$ Hz), 83.56, 56.61, 50.26, 30.92, 30.50, 29.85, 28.31, 28.14; $^{19}$F NMR (376 MHz, CDCl$_3$) δ -62.05 (s, 3F), -109.15 to -109.28 (m, 1F); HRMS (ESI+): calcd for C$_{34}$H$_{38}$F$_4$N$_7$O$_5$S [M+H]$^+$: 732.2591, found: 732.2605.

tert-butyl (S,Z)-((2-(3-(4-((3-((4-((1,1'-biphenyl)-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)((tert-butoxycarbonylimino)methyl)carbamate (3.19dd).

Synthesized by General Procedure 3E. 83%, light yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.43 – 10.31 (m, 1H), 8.05 (d, $J = 8.3$ Hz, 2H), 7.99 – 7.92 (m, 2H), 7.79 – 7.69 (m, 1H), 7.65 (dd, $J = 8.5$, 6.9 Hz, 4H), 7.61 – 7.56 (m, 2H), 7.45 (t, $J = 7.6$ Hz, 2H), 7.35 (t, $J = 7.3$ Hz, 1H), 6.94 (s, 1H), 4.84 – 4.74 (m, 1H), 3.78 – 3.62 (m, 2H), 3.59 – 3.44 (m, 1H), 3.11 (dd, $J = 15.5$, 8.7 Hz, 1H), 2.36 – 2.25 (m, 1H), 1.97 – 1.88 (m, 1H), 1.87 – 1.75 (m, 2H), 1.53 – 1.44 (m, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 176.82, 167.87, 162.83, 162.58, 154.31, 151.36, 150.33, 142.77, 140.80, 133.58, 128.95, 128.67, 127.49, 127.27, 127.12, 126.93, 126.65, 120.68, 117.18, 113.25, 102.79, 82.17, 79.67, 56.60, 50.33, 30.91, 30.45, 29.86, 28.31, 24.60; HRMS (ESI+): calcd for C$_{39}$H$_{44}$N$_7$O$_5$S [M+H]$^+$: 722.3125, found: 722.3107.

tert-butyl (S,Z)-((2-((3-((4-((benzofuran-2-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)((tert-butoxycarbonylimino)methyl)carbamate (3.19ee). Synthesized by General Procedure 3E. 69%, off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.39 (s, 1H), 8.13 (br. s, 2H), 8.05 – 7.92 (m, 3H), 7.62 – 7.50 (m, 3H), 7.40 – 7.31 (m, 2H), 6.92 (s, 1H), 4.83 – 4.73 (m, 1H), 3.83 – 3.62 (m, 1H), 3.57 – 3.45 (m, 1H), 3.08 (dd, $J = 15.3$, 8.6 Hz, 1H), 2.36 – 2.27 (m, 1H), 1.97 – 1.87 (m, 1H), 1.85 – 1.71 (m, 2H), 1.49$^*$ (ap. d, $J = 5.9$ Hz, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 176.75, 167.84, 163.01, 155.88, 154.34, 148.93, 143.88, 143.82, 142.76,
128.91, 125.53, 124.78, 123.31, 120.92, 120.74, 117.26, 117.16, 111.95, 103.02, 83.55, 82.15, 79.75, 56.58, 50.33, 36.83, 30.97, 30.48, 29.85, 28.32, 28.13, 24.84, 24.61; HRMS (ESI+): calcd for C_{35}H_{40}N_{7}O_{6}S [M+H]^+: 686.2761, found: 686.2760.

(S)-amino(2-(((3-((4-phenylthiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl) methaniminium chloride (3.20a). Synthesized by General Procedure 3E. 85%, yellow solid. ¹H NMR (500 MHz, CD_{3}OD) δ 8.10 (d, J = 8.3 Hz, 2H), 7.85 (dd, J = 16.1, 8.0 Hz, 4H), 7.46 (t, J = 7.6 Hz, 2H), 7.38 (t, J = 7.4 Hz, 1H), 7.19 (s, 1H), 4.56 – 4.50 (m, 1H), 3.56 – 4.50 (m, 1H), 3.51 – 3.42 (m, 1H), 3.33 (s, 2H), 2.33 – 2.24 (m, 1H), 2.17 – 1.97 (m, 3H); ¹³C NMR (126 MHz, CD_{3}OD) δ 177.97, 169.20, 166.40, 156.47, 144.36, 129.88, 129.72, 129.63, 127.27, 122.36, 119.87, 57.22, 31.61, 30.08, 23.59; HRMS (ESI+): calcd for C_{23}H_{24}N_{7}OS [M+H]^+: 446.1763, found: 446.1755.

(S)-amino(2-(((3-((4-pyridin-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl) methaniminium trifluoroacetate (3.20b). Synthesized by General Procedure 3E. 100%, yellow solid. ¹H NMR (400 MHz, CD_{3}OD) δ 8.75 (d, J = 6.0 Hz, 2H), 8.47 – 8.41 (m, 2H), 8.10 – 8.02 (m, 3H), 7.95 – 7.89 (m, 2H), 4.58 – 4.49 (m, 1H), 3.59 – 3.52 (m, 1H), 3.51 – 3.42 (m, 1H), 3.34 – 3.31 (m, 2H), 2.36 – 2.23 (m, 1H), 2.19 – 1.96 (m, 3H); ¹³C NMR (101 MHz, CD_{3}OD) δ 176.41, 169.20, 166.40, 156.47, 144.36, 129.88, 129.72, 129.63, 128.02, 122.18, 119.62, 117.04, 114.31, 55.77, 30.14, 28.63, 22.15; HRMS (ESI+): calcd for C_{22}H_{23}N_{8}OS [M+H]^+: 447.1716, found: 447.1704. HPLC purity: 87%.

(S)-amino(2-(((3-((4-pyridin-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl) methaniminium chloride (3.20c). Synthesized by General Procedure 3E. 85%, yellow solid. ¹H NMR (400 MHz, CD_{3}OD) δ 9.31 (s, 1H), 8.96 (dt, J = 8.4, 1.7 Hz, 1H), 8.69 (d, J = 5.6 Hz, 1H), 8.05 – 7.97 (m, 3H), 7.91 – 7.82 (m, 2H), 7.66 (s, 1H), 4.55 – 4.48
(m, 1H), 3.57 – 3.50 (m, 1H), 3.48 – 3.40 (m, 1H), 3.31 – 3.29 (m, 1H), 2.33 – 2.21 (m, 1H), 2.17 – 1.95 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 177.82, 169.29, 165.60, 156.45, 145.97, 145.00, 142.24, 142.04, 141.36, 135.43, 129.44, 128.13, 120.86, 118.37, 109.98, 57.18, 31.57, 30.06, 23.58; HRMS (ESI+): calcd for C$_{22}$H$_{23}$N$_8$OS [M+H]$^+$: 447.1716, found: 447.1698.

(S)-amino(2-((3-(4-((4-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20d). Synthesized by General Procedure 3E. 100%, yellow solid. $^1$H NMR (500 MHz, CD$_3$OD) δ 8.09 (d, $J = 8.3$ Hz, 2H), 7.89 (dd, $J = 8.4$, 5.3 Hz, 2H), 7.19 (t, $J = 8.6$ Hz, 2H), 7.15 (s, 1H), 4.57 – 4.51 (m, 1H), 3.51 – 3.41 (m, 1H), 3.36 – 3.33 (m, 2H), 2.29 (ddd, $J = 18.0$, 10.1, 5.4 Hz, 1H), 3.39 – 3.36 (m, 0H), 2.20 – 1.96 (m, 3H); $^{13}$C NMR (126 MHz, CD$_3$OD) δ 176.54, 167.77, 165.04, 163.86 (d, $^1$J$_{CF} = 199.0$ Hz), 161.89 (d, $^1$J$_{CF} = 199.0$ Hz), 155.04, 146.83, 142.85, 129.13, 128.29, 127.95 (d, $^4$J$_{CF} = 6.1$ Hz), 127.89 (d, $^4$J$_{CF} = 6.1$ Hz), 120.97, 118.47, 115.30 (d, $^3$J$_{CF} = 18.2$ Hz), 115.12 (d, $^3$J$_{CF} = 18.2$ Hz), 55.80, 36.84, 30.19, 28.67, 22.18; $^{19}$F NMR (376 MHz, CD$_3$OD) δ -116.45 to -116.59 (m, 1F); HRMS (ESI+): calcd for C$_{23}$H$_{23}$FNO$_7$S [M+H]$^+$: 464.1669, found: 464.1681.

(S)-amino(2-((3-(4-((4-chlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20e). Synthesized by General Procedure 3E. 100%, off-white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ 8.12 – 8.05 (m, 2H), 7.92 – 7.80 (m, 4H), 7.50 – 7.42 (m, 2H), 7.22 (d, $J = 4.4$ Hz, 1H), 4.55 (q, $J = 6.6$ Hz, 1H), 3.60 – 3.52 (m, 1H), 3.52 – 3.43 (m, 1H), 2.35 – 2.24 (m, 1H), 2.19 – 1.98 (m, 3H); $^{13}$C NMR (151 MHz, CD$_3$OD) δ 177.90, 169.24, 156.48, 148.86, 144.56, 135.11, 133.30, 129.92, 129.63, 128.72, 121.91, 119.47, 64.45, 57.24, 31.62, 30.10, 23.60; HRMS (ESI+): calcd for C$_{23}$H$_{23}$ClN$_7$OS [M+H]$^+$: 481.1452, found: 481.1456.
(S)-amino(2-((3-(4-((4-(4-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20f). Synthesized by General Procedure 3E. 100% light yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.06 (d, $J = 8.7$ Hz, 2H), 7.84 (dd, $J = 10.2$, 8.6 Hz, 4H), 7.59 (d, $J = 8.5$ Hz, 2H), 7.22 (s, 1H), 4.59 – 4.50 (m, 1H), 3.59 – 3.51 (m, 1H), 3.51 – 3.43 (m, 1H), 3.30 – 3.28 (m, 2H), 2.35 – 2.23 (m, 1H), 2.19 – 1.98 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 177.71, 169.35, 164.59, 164.52, 156.43, 151.28, 151.16, 145.44, 135.19, 134.57, 132.73, 129.38, 128.78, 123.98, 122.39, 120.31, 118.08, 104.75, 57.20, 31.60, 30.06, 23.58; HRMS (ESI+): calcd for C$_{23}$H$_{23}$BrN$_7$OS [M+H]$^+$: 524.0868, found: 524.0873.

(S)-amino(2-((3-(4-((4-(3-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.9g). Synthesized by General Procedure 3E. 100%, yellow solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 8.08 (d, $J = 8.7$ Hz, 2H), 7.81 (d, $J = 7.8$ Hz, 1H), 7.60 (d, $J = 10.1$ Hz, 1H), 7.49 – 7.41 (m, 1H), 7.26 (s, 1H), 7.09 (td, $J = 8.4$, 2.1 Hz, 1H), 4.56 – 4.48 (m, 1H), 3.56 – 3.50 (m, 1H), 3.48 – 3.41 (m, 1H), 3.32 – 3.30 (m, 2H), 2.33 – 2.22 (m, 1H), 2.17 – 1.97 (m, 3H); $^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 177.96, 169.19, 166.35, 165.52 (d, $^1$J$_{CF}$ = 195.9 Hz), 163.58 (d, $^1$J$_{CF}$ = 195.9 Hz), 156.46, 148.11, 144.28, 136.47 (d, $^4$J$_{CF}$ = 6.1 Hz), 136.41 (d, $^4$J$_{CF}$ = 6.1 Hz), 131.74 (d, $^4$J$_{CF}$ = 7.1 Hz), 131.67 (d, $^4$J$_{CF}$ = 7.1 Hz), 129.71, 123.04, 123.02, 122.35, 119.82, 116.20 (d, $^3$J$_{CF}$ = 17.2 Hz), 116.03 (d, $^3$J$_{CF}$ = 17.2 Hz), 114.06 (d, $^3$J$_{CF}$ = 18.2 Hz), 113.88 (d, $^3$J$_{CF}$ = 18.2 Hz), 105.55, 57.22, 31.62, 30.08, 23.60; $^{19}$F NMR (376 MHz, CD$_3$OD) $\delta$ -77.20 (s, 1F); HRMS (ESI+): calcd for C$_{23}$H$_{23}$FN$_7$OS [M+H]$^+$: 465.1747, found: 465.1724.

(S)-amino(2-((3-(4-((4-(3-chlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20h). Synthesized by General Procedure 3E. 100%, light yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.13 (d, $J = 8.2$ Hz, 2H), 7.89 (s,
1H), 7.80 (t, J = 8.2 Hz, 3H), 7.51 – 7.39 (m, 2H), 7.31 (s, 1H), 4.61 – 4.52 (m, 1H), 3.63 – 3.53 (m, 1H), 3.53 – 3.44 (m, 1H), 2.37 – 2.25 (m, 1H), 2.20 – 2.00 (m, 3H); \(^{13}\)C NMR (101 MHz, CD\(_{3}\)OD) δ 178.03, 169.05, 167.18, 156.42, 143.65, 135.88, 135.09, 131.50, 129.81, 129.73, 127.24, 125.65, 123.22, 120.58, 57.19, 31.60, 30.09, 23.59; HRMS (ESI+) calcd for C\(_{23}\)H\(_{23}\)ClN\(_{7}\)O\(_{3}\)[M+H]\(^+\): 480.1373, found: 480.1370.

\((S)-\text{amino}(2-((3-((4-((3-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20i)\). Synthesized by General Procedure 3E. 100%, off-white solid. \(^1\)H NMR (400 MHz, CD\(_{3}\)OD) δ 8.09 – 8.05 (m, 1H), 8.04 – 7.98 (m, 2H), 7.91 – 7.82 (m, 3H), 7.47 – 7.42 (m, 1H), 7.31 (t, J = 7.9 Hz, 1H), 7.21 (s, 1H), 4.56 – 4.48 (m, 1H), 3.58 – 3.50 (m, 1H), 3.48 – 3.40 (m, 1H), 3.29 – 3.27 (m, 2H), 2.33 – 2.21 (m, 1H), 2.16 – 1.97 (m, 3H); \(^{13}\)C NMR (101 MHz, CD\(_{3}\)OD) δ 180.23, 171.87, 167.12, 158.95, 153.37, 147.95, 140.81, 134.06, 133.94, 132.42, 131.90, 128.20, 126.20, 122.87, 120.59, 107.96, 59.72, 34.12, 32.58, 26.10; HRMS (ESI+) calcd for C\(_{23}\)H\(_{22}\)BrN\(_{7}\)O\(_{3}\)[M+H]\(^+\): 524.0868, found: 524.0869.

\((S)-\text{amino}(2-((3-((4-((2-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20j)\). Synthesized by General Procedure 3E. 100%, light yellow solid. \(^1\)H NMR (400 MHz, CD\(_{3}\)OD) δ 8.17 – 8.06 (m, 3H), 7.87 (d, J = 8.3 Hz, 2H), 7.47 – 7.39 (m, 1H), 7.38 – 7.21 (m, 3H), 4.62 – 4.54 (m, 1H), 3.66 – 3.56 (m, 1H), 3.55 – 3.44 (m, 1H), 3.39 – 3.35 (m, 2H), 2.40 – 2.27 (m, 1H), 2.22 – 2.02 (m, 3H); \(^{13}\)C NMR (101 MHz, CD\(_{3}\)OD) δ 177.92, 169.20, 165.30, 162.76 (d, \(^1\)J\(_{CF}\) = 250.5 Hz), 160.28 (d, \(^1\)J\(_{CF}\) = 250.5 Hz), 156.45, 156.01, 152.88, 148.68, 144.41, 143.41, 131.07, 130.97, 130.93, 129.66, 125.73 (d, \(^4\)J\(_{CF}\) = 3.0 Hz), 125.70 (d, \(^4\)J\(_{CF}\) = 3.0 Hz), 122.10, 121.98, 119.61, 117.13 (d, \(^2\)J\(_{CF}\) = 23.2 Hz), 116.90 (d, \(^2\)J\(_{CF}\) = 23.2 Hz), 57.22, 31.62, 30.09, 23.60; \(^{19}\)F NMR (471 MHz, CD\(_{3}\)OD) δ
-116.05 to -116.22 (m, 1F); HRMS (ESI+): calcd for C$_{23}$H$_{23}$FN$_7$OS [M+H]$^+$: 464.1669, found: 464.1658.

(S)-amino(2-((3-(4-((4-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20k). Synthesized by General Procedure 3E. 100%, yellow solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 8.10 (d, $J$ = 8.1 Hz, 2H), 8.06 (d, $J$ = 8.6 Hz, 2H), 7.88 (d, $J$ = 8.3 Hz, 2H), 7.73 (d, $J$ = 8.0 Hz, 2H), 7.37 (s, 1H), 4.57 – 4.50 (m, 1H), 3.60 – 3.51 (m, 1H), 3.51 – 3.42 (m, 1H), 3.32 (s, 1H), 2.34 – 2.24 (m, 1H), 2.19 – 1.98 (m, 3H); $^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 177.81, 169.32, 165.27, 156.46, 149.94, 145.07, 139.07, 130.70 (q, $^2$J$_{CF}$ = 25.3 Hz), 130.45 (q, $^2$J$_{CF}$ = 25.3 Hz), 129.51, 127.53, 126.84, 126.70 (q, $^4$J$_{CF}$ = 3.0 Hz), 126.67 (q, $^4$J$_{CF}$ = 3.0 Hz), 126.64 (q, $^4$J$_{CF}$ = 3.0 Hz), 126.60 (q, $^4$J$_{CF}$ = 3.0 Hz), 124.69, 121.06, 118.72, 106.64, 57.24, 31.63, 30.09, 23.60; $^{19}$F NMR (471 MHz, CD$_3$OD) $\delta$ -64.02 (s, 3F); HRMS (ESI+): calcd for C$_{24}$H$_{23}$F$_3$N$_7$OS [M+H]$^+$: 515.1715, found: 515.1718.

(S)-amino(2-((3-(4-((3-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20l). Synthesized by General Procedure 3E. 100%, light yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.21 (s, 1H), 8.19 – 8.15 (m, 1H), 8.06 – 7.98 (m, 2H), 7.89 – 7.84 (m, 2H), 7.63 – 7.56 (m, 2H), 7.32 (s, 1H), 4.56 – 4.49 (m, 1H), 3.63 – 3.59 (m, 1H), 3.53 – 3.45 (m, 1H), 2.34 – 2.22 (m, 1H), 2.18 – 1.96 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 177.72, 169.34, 164.73, 156.46, 150.89, 145.42, 137.08, 134.58, 132.49 (q, $^2$J$_{CF}$ = 31.3 Hz), 132.17 (q, $^2$J$_{CF}$ = 31.3 Hz), 131.86 (q, $^2$J$_{CF}$ = 31.3 Hz), 131.54 (q, $^2$J$_{CF}$ = 31.3 Hz), 130.49, 130.43, 129.95, 129.80 (q, $^1$J$_{CF}$ = 272.7 Hz), 129.37, 127.10, 126.52 (q, $^1$J$_{CF}$ = 272.7 Hz), 125.14 (q, $^4$J$_{CF}$ = 4.0 Hz), 125.11 (q, $^4$J$_{CF}$ = 4.0 Hz), 125.06 (q, $^4$J$_{CF}$ = 4.0 Hz), 125.03 (q, $^4$J$_{CF}$ = 4.0 Hz), 124.40 (q, $^1$J$_{CF}$ = 272.7 Hz), 123.98, 123.59 (q, $^4$J$_{CF}$ = 4.0 Hz), 123.56 (q, $^4$J$_{CF}$ = 4.0 Hz), 123.51 (q, $^4$J$_{CF}$ = 4.0 Hz), 123.48 (q, $^4$J$_{CF}$ = 4.0 Hz), 121.70 (q, $^1$J$_{CF}$ = 272.7 Hz), 120.38, 118.08,
$^{19}$F NMR (376 MHz, CD$_3$OD) δ -64.27 (s, 3F); HRMS (ESI+): calcd for C$_{24}$H$_{23}$F$_3$N$_7$OS [M+H]$^+$: 514.1637, found: 514.1625.

**(S)-amino(2-((3-(4-((4-(2-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20m)**. Synthesized by General Procedure 3E. 93%, off-white solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.15 – 8.09 (m, 2H), 7.89 (d, $J = 7.8$ Hz, 1H), 7.83 – 7.65 (m, 5H), 7.04 (s, 1H), 4.61 – 4.53 (m, 1H), 3.62 – 3.55 (m, 1H), 3.54 – 3.46 (m, 1H), 3.40 – 3.35 (m, 2H), 2.37 – 2.26 (m, 1H), 2.21 – 2.00 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 178.06, 169.06, 166.70, 156.45, 148.60, 144.53, 133.49, 133.34, 132.47, 130.78, 129.82, 129.34 (q, $^2$J$_{CF} = 29.3$ Hz), 129.05 (q, $^2$J$_{CF} = 29.3$ Hz), 127.68 (q, $^3$J$_{CF} = 5.1$ Hz), 127.63 (q, $^4$J$_{CF} = 5.1$ Hz), 127.57 (q, $^4$J$_{CF} = 5.1$ Hz), 127.52 (q, $^4$J$_{CF} = 5.1$ Hz), 123.47, 120.86, 108.89, 57.20, 31.60, 30.08, 23.59; $^{19}$F NMR (376 MHz, CD$_3$OD) δ -59.48 (s, 3F); HRMS (ESI+): calcd for C$_{24}$H$_{23}$F$_3$N$_7$OS [M+H]$^+$: 515.1715, found: 515.1663.

**(S)-amino(2-((3-(4-((p-tolyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20n)**. Synthesized by General Procedure 3E. 85%, light yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.02 (d, $J = 8.7$ Hz, 2H), 7.88 (d, $J = 8.8$ Hz, 2H), 7.81 (d, $J = 7.8$ Hz, 2H), 7.23 (d, $J = 7.7$ Hz, 2H), 7.06 (s, 1H), 4.57 – 4.49 (m, 1H), 3.58 – 3.55 (m, 1H), 3.50 – 3.46 (m, 1H), 3.30 – 3.24 (m, 2H), 2.37 (s, 3H), 2.34 – 2.22 (m, 1H), 2.19 – 1.98 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 177.71, 169.39, 164.38, 156.44, 152.69, 145.64, 138.70, 133.46, 130.22, 129.38, 126.99, 120.16, 117.99, 103.13, 57.22, 31.61, 30.06, 23.59, 21.28; HRMS (ESI+): calcd for C$_{24}$H$_{26}$N$_7$OS [M+H]$^+$: 460.1920, found: 460.1920.

**(S)-amino(2-((3-(4-((4-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20o)**. Synthesized by General Procedure 3E. 100%, off-white solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.05 – 7.97 (m, 2H), 7.90 – 7.79 (m, 2H), 7.52 – 7.41 (m, 2H), 7.40 – 7.25 (m, 5H), 7.09 – 6.98 (m, 2H), 6.83 – 6.72 (m, 2H), 6.35 – 6.25 (m, 1H), 4.71 – 4.63 (m, 1H), 3.81 – 3.73 (m, 1H), 3.73 – 3.65 (m, 1H), 3.50 – 3.42 (m, 1H), 3.38 – 3.30 (m, 1H), 3.24 – 3.16 (m, 1H), 3.14 – 3.06 (m, 1H), 3.00 – 2.92 (m, 1H), 2.90 – 2.82 (m, 1H), 2.80 – 2.72 (m, 1H), 2.70 – 2.62 (m, 1H), 2.59 – 2.51 (m, 1H), 2.50 – 2.42 (m, 1H), 2.40 – 2.32 (m, 1H), 2.30 – 2.22 (m, 1H), 2.20 – 2.12 (m, 1H), 2.10 – 2.02 (m, 1H).
4H), 6.99 – 6.93 (m, 3H), 4.57 – 4.46 (m, 1H), 3.83 (s, 3H), 3.58 – 3.50 (m, 1H), 3.50 – 3.40 (m, 1H), 3.29 (d, J = 3.7 Hz, 1H), 2.34 – 2.22 (m, 1H), 2.17 – 1.95 (m, 4H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 178.23, 168.80, 168.62, 162.30, 156.38, 144.37, 142.34, 135.53, 131.81, 130.58, 130.11, 129.43, 128.98, 125.05, 123.38, 122.10, 118.64, 115.55, 114.71, 57.17, 56.02, 31.65, 30.14, 23.66; HRMS (ESI+): calcd for C$_{24}$H$_{26}$N$_{7}$O$_{2}$S$^+$ [M+H]$^+$: 476.1869, found: 476.1858. HPLC purity: 85%.

(S)-amino(2-((3-(4-((4-ethoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20p). Synthesized by General Procedure 3E. 76%, yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.15 (d, J = 8.2 Hz, 2H), 7.81 – 7.65 (m, 4H), 7.04 – 6.96 (m, 2H), 4.58 – 4.49 (m, 1H), 4.09 (q, J = 7.0 Hz, 2H), 3.62 – 3.51 (m, 1H), 3.49 – 3.42 (m, 1H), 3.35 – 3.30 (m, 2H), 2.35 – 2.22 (m, 1H), 2.18 – 1.97 (m, 3H), 1.41 (t, J = 7.0 Hz, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 178.18, 174.32, 168.95, 161.43, 156.42, 130.58, 130.00, 129.43, 128.88, 121.55, 118.52, 115.95, 115.22, 81.41, 64.74, 57.18, 31.59, 30.06, 23.59, 15.09; HRMS (ESI+): calcd for C$_{25}$H$_{28}$N$_{7}$O$_{2}$S$^+$ [M+H]$^+$: 490.2025, found: 490.2024.

(S)-amino(2-((3-(4-((3-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20q). Synthesized by General Procedure 3E. 86%, yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.29 (s, 1H), 8.12 (d, J = 6.5 Hz, 2H), 7.78 – 7.66 (m, 2H), 7.40 – 7.27 (m, 3H), 6.97 (d, J = 7.1 Hz, 1H), 6.82 (s, 1H), 4.58 – 4.45 (m, 1H), 3.84 (s, 3H), 3.59 – 3.49 (m, 1H), 3.50 – 3.37 (m, 1H), 3.34 – 3.28 (m, 2H), 2.35 – 2.20 (m, 1H), 2.15 – 1.93 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 178.16, 168.87, 168.24, 161.59, 156.38, 145.44, 142.77, 132.82, 131.27, 130.05, 124.52, 121.75, 119.71, 116.02, 113.06, 57.21, 56.09, 31.69, 30.19, 23.69; HRMS (ESI+): calcd for C$_{24}$H$_{26}$N$_{7}$O$_{2}$S$^+$ [M+H]$^+$: 476.1869, found: 476.1847.
(S)-amino(2-(((3-((4-(4-(trifluoromethoxy)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl) methaniminium chloride (3.20r). Synthesized by General Procedure 3E. 90%, off-white solid. \(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta\) 8.06 (d, \(J = 8.3\) Hz, 4H), 7.92 (d, \(J = 8.3\) Hz, 2H), 7.35 (d, \(J = 8.3\) Hz, 2H), 7.24 (s, 1H), 4.61 – 4.50 (m, 1H), 3.63 – 3.55 (m, 1H), 3.54 – 3.45 (m, 2H), 3.33 – 3.21 (m, 1H), 2.20 – 2.14 (m, 1H), 2.14 – 2.02 (m, 3H); \(^13\)C NMR (126 MHz, CD\(_3\)OD) \(\delta\) 177.73, 169.41, 164.67, 156.47, 151.16, 149.87, 145.53, 135.35, 129.40, 128.63, 122.19, 120.33, 118.09, 104.99, 57.25, 31.62, 30.08, 23.59; \(^19\)F NMR (470 MHz, CD\(_3\)OD) \(\delta\) -59.47 (s, 3F); HRMS (ESI+): calcd for C\(_{24}\)H\(_{23}\)F\(_3\)N\(_7\)O\(_2\)S [M+H\(^+\)]: 531.1664, found: 531.1672. HPLC purity: 81%.

(S)-amino(2-(((3-((4-(3-(4-((3,4-dimethylphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)pyrrolidin-1-yl) methaniminium chloride (3.20s). Synthesized by General Procedure 3E. 100%, light yellow solid. \(^1\)H NMR (400 MHz, v) \(\delta\) 8.04 (d, \(J = 8.5\) Hz, 2H), 7.96 – 7.83 (m, 2H), 7.51 (t, \(J = 8.0\) Hz, 1H), 7.29 (s, 1H), 7.25 – 7.19 (m, 1H), 4.58 – 4.50 (m, 1H), 3.60 – 3.52 (m, 1H), 3.51 – 3.43 (m, 1H), 2.34 – 2.23 (m, 1H), 2.19 – 1.99 (m, 3H); \(^13\)C NMR (101 MHz, CD\(_3\)OD) \(\delta\) 177.75, 169.36, 164.61, 156.44, 150.98, 150.87, 145.47, 138.41, 131.34, 129.38, 125.52, 120.85, 120.37, 119.46, 118.07, 105.73, 57.23, 31.60, 30.08, 23.58; \(^19\)F NMR (376 MHz, CD\(_3\)OD) \(\delta\) -59.32 (s, 3F); HRMS (ESI+): calcd for C\(_{24}\)H\(_{23}\)F\(_3\)N\(_7\)O\(_2\)S [M+H\(^+\)]: 531.1664, found: 531.1672.

(S)-amino(2-(((3-((4-(3,4-dimethylphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl) methaniminium chloride (3.20t). Synthesized by General Procedure 3E. 89%, yellow solid. \(^1\)H NMR (400 MHz, CD\(_3\)OD) \(\delta\) 8.28 (s, 1H), 8.14 (d, \(J = 7.9\) Hz, 2H), 7.73 (d, \(J = 8.2\) Hz, 2H), 7.55 (s, 1H), 7.49 (d, \(J = 7.8\) Hz, 1H), 7.23 (d, \(J = 7.7\) Hz, 1H), 6.82 (s, 1H), 4.60 – 4.48 (m, 1H), 3.61 – 3.40 (m, 2H), 3.37 – 3.30 (m, 1H), 2.33 (s, 3H), 2.30 (s, 3H),
2.19 – 1.94 (m, 3H), 1.40 – 1.12 (m, 1H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 178.21, 168.91, 168.29, 156.42, 145.71, 142.81, 139.57, 138.58, 135.47, 131.26, 130.04, 129.15, 128.37, 124.92, 124.60, 121.76, 57.18, 31.62, 30.10, 23.62, 19.89, 19.69; HRMS (ESI+): calcd for C$_{25}$H$_{28}$N$_7$O$_3$S$^+$ [M+H]$^+$: 474.2076, found: 474.2077.

(S)-amino(2-(((3-(4-(4-(3,4-dimethoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20u). Synthesized by General Procedure 3E. 100%, orange solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.17 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.6 Hz, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.60 – 7.54 (m, 1H), 7.33 (d, J = 6.9 Hz, 2H), 7.06 (d, J = 8.5 Hz, 1H), 4.58 – 4.49 (m, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.60 – 3.51 (m, 1H), 3.49 – 3.42 (m, 1H), 3.33 (d, J = 6.0 Hz, 2H), 2.34 – 2.23 (m, 1H), 2.17 – 2.01 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 178.31, 168.99, 168.80, 156.42, 152.07, 150.90, 143.92, 142.18, 130.17, 129.46, 125.53, 123.36, 122.47, 120.66, 118.81, 113.03, 112.57, 111.17, 57.17, 56.76, 56.53, 31.60, 30.09, 23.60; HRMS (ESI+): calcd for C$_{25}$H$_{28}$N$_7$O$_3$S$^+$ [M+H]$^+$: 506.1969, found: 506.1948.

(S)-amino(2-(((3-((4-(3-chloro-4-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20v). Synthesized by General Procedure 3E. 100%, off-white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ 8.07 (d, J = 8.5 Hz, 2H), 7.90 (s, 1H), 7.83 (d, J = 8.5 Hz, 2H), 7.80 (d, J = 8.6 Hz, 1H), 7.15 (d, J = 8.6 Hz, 1H), 7.10 (s, 1H), 4.57 – 4.52 (m, 1H), 3.94 (s, 3H), 3.60 – 3.52 (m, 1H), 3.50 – 3.42 (m, 2H), 2.33 – 2.25 (m, 1H), 2.19 – 1.99 (m, 3H); $^{13}$C NMR (126 MHz, CD$_3$OD) δ 177.87, 169.26, 165.70, 156.49, 144.75, 129.59, 128.86, 128.54, 126.89, 123.76, 121.61, 119.20, 113.56, 57.24, 56.77, 31.63, 30.10, 23.60; HRMS (ESI+): calcd for C$_{24}$H$_{25}$ClN$_7$O$_2$S [M+H]$^+$: 510.1479, found: 510.1495.
(S)-amino(2-((3-(4-((4-(3,4-difluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20w). Synthesized by General Procedure 3E. 100%, yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.09 (d, $J = 8.2$ Hz, 2H), 7.91 – 7.79 (m, 3H), 7.77 – 7.70 (m, 1H), 7.35 (q, $J = 8.9$ Hz, 1H), 7.24 (s, 1H), 4.63 – 4.53 (m, 1H), 3.64 – 3.56 (m, 1H), 3.56 – 3.46 (m, 1H), 2.40 – 2.24 (m, 1H), 2.23 – 2.03 (m, 3H) $^{13}$C NMR (101 MHz, CD$_3$OD) δ 177.84, 169.26, 165.51, 156.44, 148.70, 144.82, 132.67, 129.56, 123.68 (dd, $^4$J$_{CF} = 4.0$ Hz), 123.64 (dd, $^4$J$_{CF} = 4.0$ Hz), 123.62 (dd, $^4$J$_{CF} = 4.0$ Hz), 123.58 (dd, $^4$J$_{CF} = 4.0$ Hz), 121.39, 118.99, 118.70 (d, $^2$J$_{CF} = 18.2$ Hz), 118.52 (d, $^2$J$_{CF} = 18.2$ Hz), 116.18 (d, $^2$J$_{CF} = 19.2$ Hz), 115.99 (d, $^2$J$_{CF} = 19.2$ Hz), 57.25, 31.66, 30.13, 23.63; $^{19}$F NMR (376 MHz, CD$_3$OD) δ -140.17 to -140.55 (m, 1F), -141.28 to -141.63 (m, 1F); HRMS (ESI+): calcd for C$_{23}$H$_{22}$F$_{2}$N$_{7}$OS $[M+H]^+$: 482.1575, found: 482.1571.

(S)-amino(2-((3-(4-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20x). Synthesized by General Procedure 3E. 100%, white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ 8.07 (d, $J = 8.5$ Hz, 2H), 7.90 (s, 1H), 7.83 (d, $J = 8.5$ Hz, 2H), 7.80 (d, $J = 8.6$ Hz, 1H), 7.15 (d, $J = 8.6$ Hz, 1H), 7.10 (s, 1H), 4.57 – 4.52 (m, 1H), 3.56 – 3.49 (m, 1H), 3.48 – 3.39 (m, 1H), 2.33 – 2.25 (m, 1H), 2.19 – 1.99 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 177.85, 169.26, 156.45, 146.18, 144.76, 135.36, 133.91, 133.59, 132.58, 131.14, 129.55, 128.49, 121.49, 119.08, 57.23, 54.79, 31.62, 30.08, 23.59; HRMS (ESI+): calcd for C$_{23}$H$_{22}$Cl$_{2}$N$_{7}$OS $[M+H]^+$: 514.0984, found: 514.0973.

(S)-amino(2-((3-(4-((4-(3,4-fluoro-3-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20y). Synthesized by General Procedure 3E. 100%, off-white solid. $^1$H NMR (500 MHz, CD$_3$OD) δ 8.23 – 8.10 (m, 2H), 8.04 (d, $J = 8.2$ Hz, 2H), 7.79 (d, $J = 8.2$ Hz, 2H), 7.39 (t, $J = 9.4$ Hz, 1H), 7.29 (s, 1H), 4.55 – 4.47
(m, 1H), 3.57 – 3.48 (m, 1H), 3.48 – 3.38 (m, 1H), 2.31 – 2.21 (m, 1H), 2.14 – 1.95 (m, 3H); $^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 177.94, 169.17, 166.32, 161.64 (d, $^1$J$_{CF}$ = 205.0 Hz), 159.61 (d, $^1$J$_{CF}$ = 205.0 Hz), 156.44, 147.17, 144.34, 133.33 (d, $^3$J$_{CF}$ = 7.1 Hz), 133.26 (d, $^3$J$_{CF}$ = 7.1 Hz), 131.44 (d, $^4$J$_{CF}$ = 2.0 Hz), 131.42 (d, $^4$J$_{CF}$ = 2.0 Hz), 129.64, 127.31 (q, $^1$J$_{CF}$ = 218.2 Hz), 125.97 (q, $^4$J$_{CF}$ = 4.0 Hz), 125.93 (q, $^4$J$_{CF}$ = 4.0 Hz), 125.90 (q, $^4$J$_{CF}$ = 4.0 Hz), 125.86 (q, $^4$J$_{CF}$ = 4.0 Hz), 125.14 (q, $^1$J$_{CF}$ = 218.2 Hz), 122.98 (q, $^1$J$_{CF}$ = 218.2 Hz), 122.16, 120.82 (q, $^1$J$_{CF}$ = 218.2 Hz), 119.63, 119.36 (q, $^3$J$_{CF}$ = 11.1 Hz), 119.25 (q, $^3$J$_{CF}$ = 11.1 Hz), 118.67 (d, $^3$J$_{CF}$ = 17.2 Hz), 118.50 (d, $^3$J$_{CF}$ = 17.2 Hz), 57.22, 31.61, 30.10, 23.60; $^{19}$F NMR (376 MHz, CD$_3$OD) $\delta$ -62.93 (d, $^J$ = 12.8 Hz, 3F), -117.55 to -117.83 (m, 1F); HRMS (ESI+): calcd for C$_{24}$H$_{22}$F$_4$N$_7$O$_3$[M+H]$^+$: 532.1543, found: 532.1538.

(S)-amino(2-((3-(4-((4-(3,5-difluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20z). Synthesized by General Procedure 3E. 100%, off-white solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.03 (d, $^J$ = 8.4 Hz, 2H), 7.86 (d, $^J$ = 8.4 Hz, 2H), 7.56 – 7.48 (m, 2H), 7.31 (s, 1H), 6.91 – 6.83 (m, 1H), 4.59 – 4.48 (m, 1H), 3.71 (d, $^J$ = 2.8 Hz, 1H), 3.60 – 3.51 (m, 1H), 3.49 – 3.41 (m, 1H), 3.16 – 3.10 (m, 1H), 2.34 – 2.22 (m, 1H), 2.18 – 1.97 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 177.73, 169.35, 166.08 (dd, $^1$J$_{CF}$ = 246.5 Hz), 165.95 (dd, $^1$J$_{CF}$ = 246.5 Hz), 164.57, 163.63 (dd, $^1$J$_{CF}$ = 246.5 Hz), 163.50 (dd, $^1$J$_{CF}$ = 246.5 Hz), 156.44, 150.23 (d, $^4$J$_{CF}$ = 4.0 Hz), 150.19 (d, $^4$J$_{CF}$ = 4.0 Hz), 145.35, 139.76 (dd, $^3$J$_{CF}$ = 9.1 Hz), 139.66(dd, $^3$J$_{CF}$ = 9.1 Hz), 139.56 (dd, $^3$J$_{CF}$ = 9.1 Hz), 129.40, 120.43, 118.09, 109.79 (d, $^2$J$_{CF}$ = 27.3 Hz), 109.71 (d, $^3$J$_{CF}$ = 11.1 Hz), 109.59 (d, $^3$J$_{CF}$ = 11.1 Hz), 109.52 (d, $^2$J$_{CF}$ = 27.3 Hz), 106.65, 103.70 (dd, $^2$J$_{CF}$ = 26.3 Hz), 103.44 (dd, $^3$J$_{CF}$ = 26.3 Hz), 103.18 (dd, $^2$J$_{CF}$ = 26.3 Hz), 71.34*, 67.91*, 57.20, 40.56, 31.61, 30.05, 23.59; $^{19}$F NMR (376 MHz, CD$_3$OD) $\delta$ -111.61
to -111.72 (m, 2F); HRMS (ESI+): calcd for C_{23}H_{22}F_{2}N_{7}OS [M+H]^+: 482.1575, found: 482.1534.

(S)-amino(2-((3-(4-((4-(3-fluoro-5-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20aa). Synthesized by General Procedure 3E. 100%, yellow solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 8.23 – 8.10 (m, 2H), 8.04 (d, $J = 8.2$ Hz, 2H), 7.79 (d, $J = 8.2$ Hz, 2H), 7.39 (t, $J = 9.4$ Hz, 1H), 7.29 (s, 1H), 4.55 – 4.47 (m, 1H), 3.57 – 3.48 (m, 1H), 3.48 – 3.38 (m, 1H), 2.31 – 2.21 (m, 1H), 2.14 – 1.95 (m, 3H); $^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 177.77, 169.32, 165.57, 164.91, 163.12, 156.44, 149.32, 145.21, 139.62, (d, $^3$J$_{CF}$ = 9.1 Hz) 139.53 (d, $^3$J$_{CF}$ = 9.1 Hz), 134.07 (d, $^3$J$_{CF}$ = 8.1 Hz), 133.99 (d, $^3$J$_{CF}$ = 8.1 Hz), 133.74 (d, $^3$J$_{CF}$ = 9.1 Hz), 133.65 (d, $^3$J$_{CF}$ = 9.1 Hz), 129.42, 126.24, 123.51, 120.70, 119.55 (q, $^4$J$_{CF}$ = 3.0 Hz), 119.52 (q, $^4$J$_{CF}$ = 3.0 Hz), 119.51 (q, $^4$J$_{CF}$ = 3.0 Hz), 119.48 (q, $^4$J$_{CF}$ = 3.0 Hz), 118.30, 117.37 (d, $^3$J$_{CF}$ = 17.2 Hz), 117.13 (d, $^3$J$_{CF}$ = 17.2 Hz), 112.44, 112.17, 107.28, 57.25, 31.64, 30.12, 23.61; $^{19}$F NMR (376 MHz, CD$_3$OD) $\delta$ -64.34 (s, 1F), -112.36 (t, $J = 9.1$ Hz, 1F); HRMS (ESI+): calcd for C$_{24}$H$_{22}$F$_4$N$_7$OS [M+H]^+: 532.1543, found: 532.1520.

(S)-amino(2-((3-(4-((4,5-bis(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20bb). Synthesized by General Procedure 3E. 100%, white solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.51 (s, 2H), 8.07 (d, $J = 8.4$ Hz, 2H), 7.93 – 7.86 (m, 3H), 7.60 (s, 1H), 4.62 – 4.54 (m, 1H), 3.64 – 3.55 (m, 1H), 3.55 – 3.46 (m, 1H), 2.39 – 2.27 (m, 1H), 2.21 – 2.03 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 178.12, 168.96, 167.94, 156.43, 145.08, 143.15, 133.46, 133.42, 132.82 (q, $^2$J$_{CF}$ = 32.3 Hz), 132.50 (q, $^2$J$_{CF}$ = 32.3 Hz), 132.18 (q, $^2$J$_{CF}$ = 32.3 Hz), 131.85 (q, $^2$J$_{CF}$ = 32.3 Hz), 131.02, 130.99, 129.91, 126.79, 126.64 (q, $^4$J$_{CF}$ = 3.0 Hz), 126.61 (q, $^4$J$_{CF}$ = 3.0 Hz), 126.57 (q, $^4$J$_{CF}$ = 3.0 Hz), 126.53 (q, $^4$J$_{CF}$ = 3.0 Hz), 124.09, 124.06, 124.01, 123.98, 121.24, 57.18, 31.60, 30.09, 23.59; $^{19}$F NMR
(376 MHz, CD$_3$OD) $\delta$ -64.49 (s, 3F); HRMS (ESI+): calcd for C$_{25}$H$_{22}$F$_6$N$_7$OS $[M+H]^+$: 582.1511, found: 582.1516.

(S)-amino(2-((3-(4-((2-fluoro-5-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20cc). Synthesized by General Procedure 3E. 100%, yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.57 – 8.52 (m, 1H), 8.11 – 8.03 (m, 2H), 7.90 (d, $J$ = 8.6 Hz, 2H), 7.73 – 7.66 (m, 1H), 7.50 – 7.40 (m, 2H), 4.62 – 4.54 (m, 1H), 3.65 – 3.56 (m, 1H), 3.56 – 3.46 (m, 1H), 2.40 – 2.27 (m, 1H), 2.22 – 2.04 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 183.96, 177.78, 175.04, 169.33, 164.57 (d, $^1$J$_{CF}$ = 256.5 Hz), 164.00, 162.03 (d, $^1$J$_{CF}$ = 256.5 Hz), 156.45, 145.34, 144.51, 129.37, 128.20 (q, $^1$J$_{CF}$ = 112.1 Hz), 127.09 (q, $^1$J$_{CF}$ = 112.1 Hz), 124.59 (d, $^3$J$_{CF}$ = 12.1 Hz), 124.47 (d, $^3$J$_{CF}$ = 12.1 Hz), 124.32, 123.78, 120.62, 118.54, 118.20, 117.98, 110.58, 106.88, 103.88, 57.26, 31.61, 30.11, 23.58; $^{19}$F NMR (376 MHz, CD$_3$OD) $\delta$ -63.69 (s, 3F), -110.28 to -110.99 (m, 1F); HRMS (ESI+): calcd for C$_{24}$H$_{22}$F$_4$N$_7$OS $[M+H]^+$: 532.1543, found: 532.1520. HPLC purity: 85% pure.

(S)-(2-((3-(4-((1,1'-biphenyl)-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)(amino)methaniminium chloride (3.20dd). Synthesized by General Procedure 3E. 100%, light yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.11 (d, $J$ = 8.5 Hz, 2H), 8.03 (d, $J$ = 8.0 Hz, 2H), 7.93 (d, $J$ = 8.4 Hz, 2H), 7.73 (dd, $J$ = 14.9, 7.9 Hz, 4H), 7.50 (t, $J$ = 7.6 Hz, 2H), 7.39 (t, $J$ = 7.3 Hz, 1H), 7.26 (s, 1H), 4.65 – 4.51 (m, 1H), 3.65 – 3.56 (m, 1H), 3.55 – 3.47 (m, 1H), 2.41 – 2.27 (m, 1H), 2.24 – 2.02 (m, 4H); $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 176.60, 167.69, 165.59, 155.03, 146.50, 142.48, 141.43, 140.13, 130.85, 128.55, 128.38, 127.30, 126.99, 126.46, 126.36, 121.61, 119.03, 55.78, 30.18, 28.66, 22.17; HRMS (ESI+): calcd for C$_{29}$H$_{28}$N$_7$OS $[M+H]^+$: 522.2076, found: 522.2046.
(S)-amino(2-((3-((4-(benzofuran-2-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.20ee). Synthesized by General Procedure 3E. 100%, yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.20 (d, $J = 6.5$ Hz, 1H), 7.98 (t, $J = 7.7$ Hz, 3H), 7.79 (d, $J = 7.9$ Hz, 2H), 7.49 (d, $J = 7.5$ Hz, 2H), 7.37 – 7.25 (m, 2H), 4.52 – 4.42 (m, 1H), 3.52 – 3.44 (m, 1H), 3.43 – 3.35 (m, 2H), 2.30 – 2.13 (m, 1H), 2.13 – 1.90 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 177.93, 168.95, 166.98, 156.94, 156.73, 156.32, 145.31, 143.60, 139.64, 129.74, 126.42, 126.20, 126.02, 124.57, 123.13, 121.58, 120.62, 115.61, 112.61, 57.14, 31.56, 30.08, 23.56; HRMS (ESI+): calcd for C$_{25}$H$_{24}$N$_7$O$_2$S [M+H$^+$]: 486.1712, found: 486.1683.

tert-butyl (S)-2-((3-((4-(4-fluoro-[1,1']biphenyl]-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.21a). Synthesized by General Procedure 3D. 30%, yellow amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.12 – 8.02 (m, 3H), 7.85 (dt, $J = 7.7$, 1.5 Hz, 1H), 7.77 (s, 1H), 7.69 – 7.62 (m, 2H), 7.59 – 7.43 (m, 6H), 7.40 – 7.33 (m, 1H), 6.97 (s, 1H), 4.40 – 4.22 (m, 1H), 3.52 – 3.27 (m, 3H), 3.17 – 2.98 (m, 1H), 2.14 – 2.02 (m, 1H), 1.95 – 1.78 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.20, 167.90, 162.96, 154.37, 151.61, 142.82, 141.84, 141.22, 135.00, 129.26, 128.96, 128.92, 127.54, 127.37, 127.04, 125.21, 125.15, 120.66, 117.40, 117.29, 103.13, 80.23, 79.78, 55.35, 55.22, 46.90, 46.50, 31.93, 31.15, 30.98, 30.21, 29.85, 28.63, 23.71, 22.91; HRMS (ESI+): calcd for C$_{33}$H$_{34}$N$_5$O$_3$S [M+H$^+$]: 580.2382, found: 580.2384.

tert-butyl (S)-2-((3-((4-(4-fluoro-[1,1']biphenyl]-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.21b). Synthesized by General Procedure 3D. 34%, off-white amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.07 (d, $J = 8.3$ Hz, 2H), 7.93 (d, $J = 8.0$ Hz, 2H), 7.70 – 7.65 (br s, 1H), 7.63 – 7.50 (m, 6H), 7.14 (t, $J = 8.7$ Hz, 2H), 6.95 (s, 1H), 4.39 – 4.22 (m, 1H), 3.50 – 3.27 (m, 3H), 3.15 – 2.97 (m, 1H), 2.13 – 2.01 (m, 1H), 1.95 –
1.79 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.22, 168.00, 163.88 (d, $^1J_{CF}$ = 247.5 Hz), 162.96, 161.43 (d, $^1J_{CF}$ = 247.5 Hz), 154.37, 151.25, 150.57, 142.75, 139.84, 136.93 (d, $^4J_{CF}$ = 3.0 Hz), 136.90 (d, $^4J_{CF}$ = 3.0 Hz), 133.53, 133.45, 131.92, 128.95, 128.84, 128.72 (d, $^3J_{CF}$ = 8.1 Hz), 128.64 (d, $^3J_{CF}$ = 8.1 Hz), 127.80, 127.35, 126.71, 126.07, 120.73, 117.39, 117.35, 115.92 (d, $^2J_{CF}$ = 21.2 Hz), 115.71 (d, $^2J_{CF}$ = 21.2 Hz), 103.27, 102.96, 80.23, 55.34, 46.90, 46.51, 31.93, 31.15, 31.00, 30.23, 29.83, 28.64, 23.71, 22.92; $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -114.71 to -116.15 (m, 1F); HRMS (ESI+): calcd for C$_{33}$H$_{33}$FN$_5$O$_3$S [M+H]$^+$: 598.2288, found: 598.2234.

**tert-butyl** (S)-2-((3-(4-(4′-(trifluoromethyl)-[1,1′-biphenyl]-4-y1)thiazol-2-y1)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.21c). Synthesized by General Procedure 3D. 37%, yellow amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.08 (d, $J$ = 8.2 Hz, 2H), 7.97 (d, $J$ = 8.0 Hz, 1H), 7.78 – 7.54 (m, 8H), 6.98 (s, 1H), 4.40 – 4.21 (m, 1H), 3.52 – 3.27 (m, 3H), 3.17 – 2.97 (m, 1H), 2.13 – 2.00 (m, 1H), 1.96 – 1.78 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.41, 167.99, 163.01, 154.38, 151.06, 144.29, 142.73, 139.28, 134.41, 129.69 (q, $^2J_{CF}$ = 32.3 Hz), 129.37 (q, $^2J_{CF}$ = 32.3 Hz), 128.98, 128.83 (q, $^2J_{CF}$ = 32.3 Hz), 127.67, 127.54, 127.37, 127.23, 126.84, 125.91 (q, $^4J_{CF}$ = 4.0 Hz), 125.87 (q, $^4J_{CF}$ = 4.0 Hz), 125.79 (q, $^4J_{CF}$ = 4.0 Hz), 117.39, 103.40, 80.23*, 79.81*, 55.30, 46.90*, 46.50*, 31.93*, 31.15*, 30.23*, 29.83*, 28.63, 23.71*, 22.92*; $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -62.43 (s, 3F); HRMS (ESI+): calcd for C$_{34}$H$_{32}$F$_3$N$_3$NaO$_3$S [M+H]$^+$: 670.2075, found: 670.2068.

tert-butyl (S)-2-((3-(4-(4′-(trifluoromethyl)-[1,1′-biphenyl]-4-y1)thiazol-2-y1)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.21d). Synthesized by General Procedure 3D. 35%, tan amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.12 – 8.03 (m, 2H), 7.98 (d, $J$ = 7.9 Hz, 2H), 7.88 (s, 1H), 7.81 (d, $J$ = 7.5 Hz, 1H), 7.70 – 7.51 (m, 6H), 6.98 (s, 1H),
4.39 – 4.23 (m, 1H), 3.52 – 3.27 (m, 3H), 3.15 – 2.96 (m, 1H), 2.13 – 2.01 (m, 1H), 1.95 – 1.79 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.08, 167.78, 162.84, 154.24, 150.90, 142.60, 141.42, 139.12, 134.10, 131.77, 131.65, 131.34 (q, $^2J_{CF} = 30.3$ Hz), 131.02 (q, $^2J_{CF} = 30.3$ Hz), 129.27, 128.81, 128.68, 127.64 (q, $^1J_{CF} = 155.5$ Hz), 127.41, 126.69, 126.10 (q, $^1J_{CF} = 155.5$ Hz), 125.51, 123.99 (q, $^2J_{CF} = 30.3$ Hz), 123.69 (q, $^2J_{CF} = 30.3$ Hz), 122.81, 120.63, 117.20, 103.18, 80.10, 55.15, 46.74, 46.35, 31.75, 30.98, 30.07, 28.47, 23.54, 22.76; $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -62.60 (s, 3F); HRMS (ESI+): calcd for C$_{34}$H$_{33}$F$_3$N$_5$O$_3$S $[\text{M+H}]^+$: 648.2256, found: 648.2209.

tert-butyl $(S,Z)$-((2-((3-((4-((4-(4',5'-biphenyl)-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)((tert-butoxycarbonyl)imino)methyl)carbamate (3.22a). Synthesized by General Procedure 3E. 68%, off-white oily solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 10.39 (s, 1H), 8.09 (s, 1H), 8.02 (d, $J = 8.5$ Hz, 2H), 7.86 (d, $J = 7.5$ Hz, 1H), 7.66 (d, $J = 7.4$ Hz, 2H), 7.59 – 7.53 (m, 4H), 7.52 – 7.44 (m, 3H), 7.37 (t, $J = 7.3$ Hz, 1H), 6.96 (s, 1H), 4.82 – 4.72 (m, 1H), 3.81 – 3.62 (m, 3H), 3.57 – 3.43 (m, 1H), 3.09 (dd, 1H), 2.36 – 2.26 (m, 1H), 1.95 – 1.87 (m, 1H), 1.85 – 1.73 (m, 2H), 1.48 (d, $J = 14.1$ Hz, 18H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 176.87, 167.90, 162.91, 162.64, 154.28, 151.66, 150.48, 142.80, 141.84, 141.27, 135.10, 129.26, 128.95, 127.54, 127.38, 127.00, 125.26, 125.12, 120.74, 117.20, 103.03, 82.17, 79.61, 56.62, 50.29, 30.88, 30.47, 28.38, 28.25, 24.51; HRMS (ESI+): calcd for C$_{39}$H$_{44}$N$_7$O$_5$S $[\text{M+H}]^+$: 722.3125, found: 722.3107.

tert-butyl $(S,Z)$-(((tert-butoxycarbonyl)imino)(2-((3-((4-((4'-fluoro-1,1'-biphenyl)-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.22b). Synthesized by General Procedure 3E. 50%, off-white oily solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.39 (s, 1H), 8.03 (d, $J = 8.1$ Hz, 2H), 7.94 (d, $J = 8.0$ Hz, 2H), 7.89 – 7.78 (m, 1H), 7.65 – 7.55...
(m, 5H), 7.13 (t, \(J = 8.5\) Hz, 2H), 6.94 (s, 1H), 4.84 – 4.71 (m, 1H), 3.81 – 3.61 (m, 2H), 3.58 – 3.43 (m, 1H), 3.09 (dd, \(J = 15.4, 8.7\) Hz, 1H), 2.37 – 2.24 (m, 1H), 1.96 – 1.87 (m, 1H), 1.86 – 1.72 (m, 2H), 1.54 – 1.41 (m, 18H); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 176.85, 167.88, 163.65 (d, \(^{1}J_{\text{CF}} = 100\) Hz), 162.89, 162.65 (d, \(^{1}J_{\text{CF}} = 100\) Hz), 154.31, 151.29, 150.51, 142.78, 139.81, 136.97, 133.61, 131.92, 128.97, 128.71 (d, \(^{4}J_{\text{CF}} = 7.1\) Hz), 128.64 (d, \(^{4}J_{\text{CF}} = 7.1\) Hz), 127.82, 127.35 (d, \(^{2}J_{\text{CF}} = 63.6\) Hz), 126.72 (d, \(^{2}J_{\text{CF}} = 63.6\) Hz), 120.77, 117.23, 115.91 (d, \(^{3}J_{\text{CF}} = 17.2\) Hz), 115.74 (d, \(^{3}J_{\text{CF}} = 17.2\) Hz), 103.17, 102.86, 82.17, 79.64, 56.61, 50.31, 30.92, 30.46, 29.85, 28.32, 28.17, 24.56; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)) \(\delta\) -115.56 to -115.66 (m, 1F); HRMS (ESI+): calcd for C\(_{39}\)H\(_{43}\)FN\(_{7}\)O\(_{5}\)S \([M+H]^+\): 740.3030, found: 740.2979.

tert-butyl \((S,Z)-(((\text{tert-butoxycarbonyl})\text{imino})(2-((3-(4-((4-((4-((4'-((\text{trifluoromethyl})-[1,1'-\text{biphenyl}]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxidiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.22c). Synthesized by General Procedure 3E. 75%, off-white oily solid. \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 10.32 (s, 1H), 8.09 (d, \(J = 8.3\) Hz, 2H), 7.98 (d, \(J = 7.9\) Hz, 2H), 7.81 – 7.63 (m, 6H), 7.58 (d, \(J = 8.3\) Hz, 2H), 7.46 (s, 1H), 6.98 (s, 1H), 4.79 (dt, \(J = 11.9, 5.8\) Hz, 1H), 3.79 – 3.62 (m, 2H), 3.57 – 3.44 (m, 1H), 3.15 (dd, \(J = 15.4, 8.3\) Hz, 1H), 2.33 – 2.22 (m, 1H), 1.98 – 1.91 (m, 1H), 1.89 – 1.74 (m, 2H), 1.53 – 1.44 (m, 18H); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 176.80, 167.74, 162.82, 162.54, 154.05, 150.95, 148.99, 144.17, 142.55, 139.09, 134.34, 129.52, 128.86, 127.50, 127.21, 126.69, 125.78 (q, \(^{4}J_{\text{CF}} = 2.0\) Hz), 125.75 (q, \(^{4}J_{\text{CF}} = 2.0\) Hz), 125.73 (q, \(^{4}J_{\text{CF}} = 2.0\) Hz), 125.70 (q, \(^{4}J_{\text{CF}} = 2.0\) Hz), 121.05 (q, \(^{4}J_{\text{CF}} = 2.0\) Hz), 120.79, 117.15, 103.17, 101.06, 83.41, 81.99, 56.47, 50.10, 30.69, 30.30, 29.69, 28.15, 27.98; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)) \(\delta\) -62.44 (s, 3F); HRMS (ESI+): calcd for C\(_{40}\)H\(_{43}\)F\(_{3}\)N\(_{7}\)O\(_{5}\)S \([M+H]^+\): 790.2998, found: 790.2989.
tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-((4-((4-((3'-((trifluoromethyl)-[1,1'-biphenyl]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.22d). Synthesized by General Procedure 3E. 37%, off-white oily solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.39 (s, 1H), 8.06 – 7.94 (m, 4H), 7.87 (s, 1H), 7.80 (d, $J = 7.6$ Hz, 1H), 7.68 – 7.52 (m, 6H), 6.96 (s, 1H), 4.82 – 4.73 (m, 1H), 3.82 – 3.62 (m, 2H), 3.57 – 3.45 (m, 1H), 3.09 (dd, $J = 15.5, 8.8$ Hz, 1H), 2.31 (dt, $J = 11.5, 6.7$ Hz, 1H), 1.97 – 1.88 (m, 1H), 1.84 – 1.75 (m, 1H), 1.71 – 1.62 (m, 2H), 1.55 – 1.42 (m, 18H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 176.83, 167.87, 163.01, 151.07, 150.47, 142.78, 141.61, 139.21, 134.37, 131.91, 131.75 (q, $^2$J$_{CF}$ = 25.3 Hz), 131.49 (q, $^2$J$_{CF}$ = 25.3 Hz), 131.24 (q, $^2$J$_{CF}$ = 25.3 Hz), 130.98 (q, $^2$J$_{CF}$ = 25.3 Hz), 130.36, 129.42, 128.96, 128.81 (q, $^1$J$_{CF}$ = 100.0 Hz), 127.81 (q, $^1$J$_{CF}$ = 100.0 Hz), 127.55, 126.86, 126.26, 125.41, 124.17 (q, $^4$J$_{CF}$ = 3.0 Hz), 124.14 (q, $^4$J$_{CF}$ = 3.0 Hz), 124.11 (q, $^4$J$_{CF}$ = 3.0 Hz), 124.09 (q, $^4$J$_{CF}$ = 3.0 Hz), 123.90 (q, $^4$J$_{CF}$ = 3.0 Hz), 123.87 (q, $^4$J$_{CF}$ = 3.0 Hz), 123.84 (q, $^4$J$_{CF}$ = 3.0 Hz), 123.81 (q, $^4$J$_{CF}$ = 3.0 Hz), 123.25, 120.75, 117.24, 117.16, 105.35, 103.20, 82.19, 79.67, 56.61, 50.32, 30.92, 30.45, 28.36, 28.28, 28.16; $^{19}$F NMR (376 MHz, CDCl$_3$) δ -62.63 (s, 3F); HRMS (ESI+): calcd for C$_{40}$H$_{43}$F$_3$N$_7$O$_5$S [M+H]$^+$: 790.2998, found: 790.2997.

(S)-(2-((3-(4-((3'-((trifluoromethyl)-[1,1'-biphenyl]-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)(amino)methaniminium chloride (3.23a). Synthesized by General Procedure 3E. 93%, light yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.19 – 8.14 (m, 1H), 8.02 (d, $J = 8.7$ Hz, 2H), 7.95 – 7.86 (m, 3H), 7.73 – 7.66 (m, 2H), 7.60 – 7.55 (m, 1H), 7.53 – 7.44 (m, 3H), 7.40 – 7.33 (m, 1H), 7.25 (s, 1H), 7.22 (s, 1H), 4.57 – 4.50 (m, 1H), 3.55 (ddd, $J = 9.8, 8.0, 4.2$ Hz, 1H), 3.51 – 3.41 (m, 1H), 2.36 – 2.19 (m, 1H), 2.19 – 1.97 (m, 3H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 177.73, 169.37, 164.53, 156.44, 152.44, 145.59, 142.93, 142.47, 136.66, 130.19, 129.92, 129.40, 128.48, 128.08, 127.47, 126.06, 125.64, 120.26, 118.04, 104.46, 57.23,
31.60, 30.07, 23.58; HRMS (ESI+): calcd for C_{29}H_{28}N_{7}O_{5} [M+H]^+: 522.2076, found: 522.2072.

HPLC purity: 88%.

(S)-amino(2-((3-(4-(4′-fluoro-[1,1′-biphenyl]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.23b). Synthesized by General Procedure 3E. 90%, off-white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 8.09 (d, $J = 8.5$ Hz, 2H), 7.94 (d, $J = 7.8$ Hz, 2H), 7.84 (d, $J = 8.5$ Hz, 3H), 7.72 – 7.63 (m, 3H), 7.23 (s, 1H), 7.22 – 7.15 (m, 2H), 4.57 – 4.49 (m, 1H), 3.58 – 3.50 (m, 1H), 3.49 – 3.42 (m, 1H), 3.33 – 3.31 (m, 2H), 2.35 – 2.24 (m, 1H), 2.18 – 1.97 (m, 3H); $^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 177.96, 169.20, 167.04, 166.33 (d, $^1J_{CF} = 128.3$ Hz), 165.06 (d, $^1J_{CF} = 128.3$ Hz), 156.46, 149.01, 144.38, 141.46, 138.03, 132.86, 129.76, 129.70, 129.54, 128.90, 128.26 (d, $^2J_{CF} = 49.5$ Hz), 127.77 (d, $^2J_{CF} = 49.5$ Hz), 119.81, 118.95, 116.73 (d, $^3J_{CF} = 13.7$ Hz), 116.56 (d, $^3J_{CF} = 13.7$ Hz), 57.22, 31.62, 30.08, 28.13, 23.59; $^{19}$F NMR (376 MHz, CD$_3$OD) $\delta$ -117.18 to -117.54 (m, 1F); HRMS (ESI+): calcd for C$_{29}$H$_{27}$FN$_{7}$O$_{5}$ [M+H]$^+$: 540.1982, found: 540.1978.

(S)-amino(2-((3-(4-((4′-(trifluoromethyl)-[1,1′-biphenyl]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.23c). Synthesized by General Procedure 3E. 100%, off-white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 8.11 – 7.99 (m, 3H), 7.96 – 7.83 (m, 4H), 7.80 – 7.73 (m, 2H), 7.58 (d, $J = 8.4$ Hz, 1H), 7.28 – 7.18 (m, 2H), 4.58 – 4.51 (m, 1H), 3.59 – 3.51 (m, 1H), 3.51 – 3.45 (m, 1H), 2.36 – 2.25 (m, 1H), 2.19 – 1.99 (m, 3H); $^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 177.72, 169.41, 156.48, 152.04, 151.44, 145.62, 145.54, 139.94, 136.20, 135.32, 132.74, 131.64, 129.40, 128.80, 128.45, 128.38, 127.72, 126.84 (q, $^4J_{CF} = 3$ Hz), 126.81 (q, $^4J_{CF} = 3$ Hz), 126.78 (q, $^4J_{CF} = 3$ Hz), 126.75 (q, $^4J_{CF} = 3$ Hz), 122.39, 120.29, 118.08, 104.78, 57.26, 31.63, 30.09, 23.60; $^{19}$F NMR (376 MHz, CD$_3$OD) $\delta$ -63.98 (s, 3F): calcd for C$_{30}$H$_{27}$F$_3$N$_7$O$_5$ [M+H]$^+$: 590.1950, found: 590.1954.
(S)-amino(2-((3-((4-(4′-((3′-trifluoromethyl)-[1,1′-biphenyl]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.23d). Synthesized by General Procedure 3E. 100%, off-white solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 8.16 – 8.03 (m, 2H), 7.94 (d, $J$ = 4.1 Hz, 2H), 7.88 – 7.82 (m, 2H), 7.79 – 7.75 (m, 2H), 7.68 – 7.65 (m, 2H), 4.57 – 4.50 (m, 1H), 3.60 – 3.50 (m, 1H), 3.50 – 3.41 (m, 1H), 3.34 – 3.31 (m, 1H), 2.34 – 2.22 (m, 1H), 2.17 – 2.00 (m, 4H); $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 177.96, 169.17, 166.36, 156.43, 148.71, 144.30, 142.73, 140.75, 133.87, 132.47, 131.64, 130.89, 129.70, 128.53, 127.96, 127.34, 127.06, 125.26 (q, $^4$J$_{CF}$ = 4.0 Hz), 125.22 (q, $J_{CF}$ = 4.0 Hz), 125.18 (q, $J_{CF}$ = 4.0 Hz), 125.14 (q, $J_{CF}$ = 4.0 Hz), 124.41, 124.37, 122.32, 119.83, 57.20, 31.60, 30.07, 23.59; $^{19}$F NMR (376 MHz, CD$_3$OD) $\delta$ -64.14 (s, 3F); HRMS (ESI+): calcd for C$_{30}$H$_{27}$F$_3$N$_7$OS $[M+H]^+$: 590.1950, found: 590.1949.

$N$-(4-cyanophenyl)-2,2,2-trifluoroacetamide (3.25). 4-Fluorobenzonitrile (0.5 g, 4.13 mmol) was dissolved in THF (20.6 mL) and 1 M potassium tert-butoxide in THF (10.3 mL, 10.32 mmol) was then added. The reaction mixture was refluxed for 4 h, after which the organic solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (5% EtOAc/ hexanes) to yield 2 (207 mg, 29%) as a clear liquid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.52 (d, $J$ = 8.7 Hz, 2H), 7.01 (d, $J$ = 8.7 Hz, 2H), 1.38 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 159.90, 133.36, 122.94, 119.11, 105.58, 80.17, 28.80; HRMS (ESI+): calcd for C$_{11}$H$_{14}$NO $[M+H]^+$: 176.1075, found: 176.1083.

(Z)-4-((tert-butoxy)-$N$'-hydroxybenzimidamide. Synthesized by General Procedure 3A. 87% as white solid. $^1$H NMR (400 MHz, acetone-$d_6$) $\delta$ 9.11 (s, 1H), 7.64 (d, $J$ = 8.6 Hz, 2H), 6.99 (d, $J$ = 8.6 Hz, 1H), 5.48 (s, 2H), 1.34 (s, 9H); $^{13}$C NMR (101 MHz, acetone-$d_6$) $\delta$ 157.36, 151.95,
tert-butyl (S)-2-((3-(4-tert-butoxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.26). Synthesized by General Procedure 3B. 52%, yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.98 – 7.93 (m, 2H), 7.05 (d, \(J = 8.3\) Hz, 2H), 4.35 – 4.18 (m, 1H), 3.46 – 3.23 (m, 3H), 3.04 (dd, \(J = 37.3, 14.5, 8.7\) Hz, 1H), 2.04 (q, \(J = 6.4, 4.4\) Hz, 1H), 1.91 – 1.74 (m, 3H), 1.44 (s, 9H), 1.37 (s, 9H); \(^1\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 177.30, 168.09, 158.39, 154.24, 128.43, 123.86, 121.52, 80.05, 79.37, 55.26, 46.82, 46.41, 31.83, 31.03, 30.86, 30.10, 28.97, 28.54, 23.63, 22.84; HRMS (ESI+): calcd for C\(_{22}\)H\(_{32}\)N\(_3\)O\(_4\)[M+H]\(^+\): 402.2393, found: 402.2407.

tert-butyl (S)-2-((3-(4-hydroxyphenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.27). 3.26 (212 mg, 0.529 mmol) was dissolved in DCM (2 mL) and then 1 N TFA (2 mL) was added. The reaction mixture was stirred for 4 h. At this time, TLC showed complete conversion of starting material. Organic solvent was removed under reduced pressure. The resulting product was then dissolved in dioxane (1 mL), and a mixture of di-tert-butyl dicarbonate (0.146 mL, 0.635 mmol) and TEA (0.192 mL, 1.38 mmol) was added dropwise to the solution. The reaction mixture was stirred for 1 h, after which the organic solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (30% EtOAc/hexane) to provide 27(73 mg, 40%) as a white solid. \(^1\)H NMR (400 MHz, acetone-\(d_6\)) \(\delta\) 7.92 (d, \(J = 8.5\) Hz, 2H), 6.98 (d, \(J = 8.5\) Hz, 2H), 4.29 – 4.20 (m, 1H), 3.41 – 3.25 (m, 2H), 3.13 (dd, \(J = 14.6, 8.2\) Hz, 1H), 2.95 (s, 1H), 2.15 – 2.06 (m, 1H), 1.95 – 1.80 (m, 3H), 1.47 – 1.39 (m, 9H); \(^1\)C NMR (101 MHz, CD\(_3\)OD) \(\delta\) 178.74, 169.35, 161.71, 156.09, 130.01, 118.99, 116.75, 81.52, 81.01, 56.89\(^\ast\), 56.53\(^\ast\), 47.81\(^\ast\), 47.31\(^\ast\), 32.38\(^\ast\), 32.10\(^\ast\), 31.53\(^\ast\), 31.16\(^\ast\), 28.73\(^\ast\), 28.59\(^\ast\), 24.33\(^\ast\), 23.51\(^\ast\); HRMS (ESI+): calcd for C\(_{18}\)H\(_{22}\)N\(_3\)O\(_4\)[M-H]\(^-\): 344.1610, found: 344.1620.
**tert-butyl (S)-2-((3-(4-((4-bromothiazol-2-yl)oxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.28).** 2, 4-dibromothiazole (0.025 g, 0.101 mmol) was added to a mixture of 27 (0.035 g, 0.101 mmol) and K$_2$CO$_3$ (0.017 g, 0.122 mmol) in DMF (1 mL). The reaction mixture was refluxed for 17 h, after which the solution was partitioned between EtOAc and LiBr aqueous solution. The aqueous solution was washed with EtOAc three times, and the combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated via vacuum. The resulting residue was purified by silica gel column chromatography (30% EtOAc/ hexane) to yield 28 (20 mg, 39%) as an off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.17 – 8.10 (m, 2H), 7.40 (d, $J$ = 8.4 Hz, 2H), 6.78 (s, 1H), 4.38 – 4.21 (m, 1H), 3.49 – 3.28 (m, 3H), 3.27 – 2.98 (m, 1H), 2.13 – 2.01 (m, 1H), 1.93 – 1.77 (m, 3H), 1.47 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.64, 172.10, 167.55, 156.88, 154.30, 129.48, 129.95, 124.79, 120.39, 120.10, 119.76, 110.97, 80.18*, 79.77*, 55.27*, 55.20*, 46.88*, 46.49*, 31.95*, 31.16*, 31.01*, 30.24*, 28.62, 23.71*, 22.92*; HRMS (ESI+): calcd for C$_{21}$H$_{23}$BrN$_4$NaO$_4$S [M+Na]$^+$: 529.0466, found: 529.0493.

tert-butyl (S)-2-((3-(4-((4-(trifluoromethyl)phenyl)thiazol-2-yl)oxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.29). Synthesized by General Procedure 3D. 45%, off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.16 (d, $J$ = 8.6 Hz, 2H), 7.91 (d, $J$ = 8.1 Hz, 2H), 7.64 (d, $J$ = 8.1 Hz, 2H), 7.48 (d, $J$ = 8.3 Hz, 2H), 7.17 (s, 1H), 4.39 – 4.21 (m, 1H), 3.51 – 3.30 (m, 3H), 3.17 – 3.00 (m, 1H), 2.14 – 2.03 (m, 1H), 1.94 – 1.80 (m, 3H), 1.48 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.78, 172.08, 167.71, 157.42, 148.58, 137.36, 129.50, 129.41, 126.27, 125.83, 124.50, 120.36, 110.97, 108.87, 80.20*, 79.80*, 55.29, 46.89*, 46.52*, 31.96*, 31.17*, 30.27*, 28.64, 23.73*, 22.94*; $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -62.61 (s, 3F); HRMS (ESI+): calcd for C$_{21}$H$_{23}$BrN$_4$NaO$_4$S [M+Na]$^+$: 529.0466, found: 529.0493.
tert-butyl (S,Z)-((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(trifluoromethyl)phenyl)thiazol-2-yl)oxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (3.30).

Synthesized by General Procedure 3E. 83%, white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 10.33 (s, 1H), 8.21 – 8.13 (m, 2H), 7.91 (d, \(J = 8.2\) Hz, 2H), 7.64 (d, \(J = 8.2\) Hz, 2H), 7.50 – 7.45 (m, 2H), 7.42 – 7.37 (m, 1H), 7.17 (s, 1H), 4.85 – 4.75 (m, 1H), 3.74 – 3.61 (m, 2H), 3.56 – 3.43 (m, 1H), 3.20 (dd, \(J = 15.2, 8.2\) Hz, 1H), 2.32 – 2.23 (m, 1H), 1.97 – 1.89 (m, 1H), 1.88 – 1.76 (m, 2H), 1.47 (d, \(J = 7.6\) Hz, 18H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 177.49, 172.13, 167.61, 162.67, 157.36, 156.85, 154.23, 150.45, 148.55, 137.35, 130.41, 130.24, 129.92, 129.58, 129.49, 126.26, 125.89 (q, \(4J_{CF} = 3.5\) Hz), 125.85 (q, \(4J_{CF} = 3.5\) Hz), 125.81 (q, \(4J_{CF} = 3.5\) Hz), 125.77 (q, \(4J_{CF} = 3.5\) Hz), 124.69, 121.91, 120.41, 120.35, 119.78, 110.93, 108.84, 82.12, 79.45, 56.61, 50.28, 30.76, 30.45, 28.36, 28.23, 24.54; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)) \(\delta\) 62.62 (s, 3F); HRMS (ESI+) calcd for C\(_{34}\)H\(_{38}\)F\(_3\)N\(_6\)O\(_6\)S [M+H]\(^+\): 715.2526, found: 715.2499.

\((S)\)-amino(2-((3-(4-((4-(trifluoromethyl)phenyl)thiazol-2-yl)oxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (3.31). Synthesized by General Procedure 3E. 100%, yellow solid. \(^1\)H NMR (400 MHz, CD\(_3\)OD) \(\delta\) 8.24 – 8.15 (m, 2H), 8.02 (d, \(J = 8.1\) Hz, 2H), 7.70 (d, \(J = 8.2\) Hz, 2H), 7.63 (s, 1H), 7.61 – 7.53 (m, 2H), 4.59 – 4.51 (m, 1H), 3.61 – 3.52 (m, 1H), 3.53 – 3.43 (m, 1H), 3.38 – 3.34 (m, 2H), 2.38 – 2.25 (m, 1H), 2.21 – 2.01 (m, 3H); \(^{13}\)C NMR (101 MHz, CD\(_3\)OD) \(\delta\) 178.31, 173.59, 168.87, 159.02, 156.45, 149.34, 138.97, 130.41, 130.32, 127.36, 126.75 (q, \(4J_{CF} = 4.0\) Hz), 126.71 (q, \(4J_{CF} = 4.0\) Hz), 126.67 (q, \(4J_{CF} = 4.0\) Hz), 126.64 (q, \(4J_{CF} = 4.0\) Hz), 125.48, 121.77, 121.60, 113.12, 111.34, 57.17, 31.60, 30.05, 23.58; \(^{19}\)F NMR (376 MHz, CD\(_3\)OD) \(\delta\) -64.16 (s, 3F); HRMS (ESI+): calcd for C\(_{24}\)H\(_{22}\)F\(_3\)N\(_6\)O\(_2\)S [M+H]\(^+\): 515.1477, found: 515.1445.
tert-butyl (S)-2-((3-(4-((1,1'-biphenyl)-4-yl)thiazol-2-yl)(methyl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (3.32). To a solution of tert-butyl (S)-2-((3-(4-
3.18dd (0.025 g, 0.043 mmol) in acetone (1 mL) was added K$_2$CO$_3$ (0.024 g, 0.172 mmol) and methyl iodide (0.061 g, 0.431 mmol). The reaction mixture was refluxed for 17 h. The organic solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (30% EtOAc/ hexane) to yield 3.32 (15 mg, 73%) as an off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.13 (d, $J = 8.3$ Hz, 2H), 7.94 (d, $J = 7.9$ Hz, 2H), 7.63 (t, $J = 7.2$ Hz, 5H), 7.45 (t, $J = 7.5$ Hz, 2H), 7.35 (t, $J = 7.4$ Hz, 1H), 6.85 (s, 1H), 4.40–4.22 (m, 1H), 3.68 (s, 3H), 3.52–3.28, 3H), 3.20–2.99 (m, 1H), 2.16–2.02 (m, 1H), 1.97–1.78 (m, 3H), 1.49 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.62, 168.41, 154.64, 151.48, 148.51, 140.96, 140.54, 134.00, 128.93, 128.79, 127.43, 127.40, 127.33, 127.12, 126.59, 123.52, 102.76, 80.17, 55.29, 46.92*, 46.53*, 40.39, 31.98*, 31.13*, 30.25*, 29.85*, 28.65, 23.74*, 22.94*; HRMS (ESI+): calcd for C$_{34}$H$_{36}$N$_5$O$_3$S [M+H]$^+$: 594.2538, found: 594.2553

tert-butyl (S,Z)-((2-((3-(4-((1,1'-biphenyl)-4-yl)thiazol-2-yl)(methyl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)((tert-butoxycarbonylimino)methyl)carbamate (3.33).

Synthesized by General Procedure 3E. 73%, off-white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.33 (s, 1H), 8.15 (d, $J = 8.2$ Hz, 2H), 7.94 (d, $J = 8.0$ Hz, 2H), 7.62 (dd, $J = 10.9$, 8.2 Hz, 6H), 7.45 (t, $J = 7.6$ Hz, 2H), 7.35 (t, $J = 7.4$ Hz, 1H), 6.84 (s, 1H), 4.84–4.76 (m, 1H), 3.73–3.61 (m, 5H), 3.55–3.48 (m, 1H), 3.19 (dd, $J = 15.2$, 8.2 Hz, 1H), 2.32–2.24 (m, 1H), 1.98–1.90 (m, 1H), 1.89–1.76 (m, 2H), 1.48 (s, 18H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.30, 168.44, 167.82, 158.56, 155.56, 154.18, 151.41, 148.46, 140.94, 140.50, 133.98, 128.91, 128.85, 128.71, 127.39, 127.11, 126.57, 123.65, 123.55, 102.70, 81.99, 79.53, 56.63, 50.23, 40.37, 30.76, 30.48, 28.30, 28.12, 24.54; HRMS (ESI+): calcd for C$_{40}$H$_{46}$N$_7$O$_5$S [M+H]$^+$: 736.3281, found: 736.3274.
(S)-(2-((3-(4-(4-(1,1'-biphenyl)-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)(amino)methaniminium chloride (3.34). Synthesized by General Procedure 3F. 100% yield, off-white solid. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 8.13 (d, $J = 8.6$ Hz, 2H), 7.93 (d, $J = 8.1$ Hz, 2H), 7.71 (d, $J = 8.6$ Hz, 2H), 7.66 – 7.59 (m, 5H), 7.43 (t, $J = 7.6$ Hz, 2H), 7.33 (t, $J = 7.3$ Hz, 1H), 7.10 (s, 1H), 4.58 – 4.50 (m, 1H), 3.66 (s, 3H), 3.55 (td, $J = 9.2$, 8.5, 3.9 Hz, 1H), 3.49 – 3.42 (m, 1H), 3.33 – 3.31 (m, 2H), 2.34 – 2.22 (m, 1H), 2.17 – 2.12 (m, 1H), 2.11 – 1.97 (m, 2H); $^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 178.12, 169.97, 169.08, 156.47, 152.27, 150.03, 141.96, 141.78, 135.08, 129.89, 129.60, 128.41, 128.07, 127.81, 127.58, 124.81, 124.41, 57.20, 40.80, 31.61, 30.09, 23.59; HRMS (ESI+): calcd for C$_{30}$H$_{30}$N$_7$O$_5$ [M$^+$]: 536.2233, found: 536.2230.

6.3 Structure–Activity Relationship Studies of Uncouplers of Oxidative Phosphorylation

6.3.1 Oxygen Consumption Rate Seahorse Assay

The oxygen consumption rates of the synthesized compounds was determined using a previously published method$^{20}$ with a Seahorse XF-24 Flux Analyzer (Seahorse Biosciences, North Billerica, MA). L6 myoblast cells were seeded in a Seahorse 24-well tissue culture plate at a density of 3.5 x 10$^4$ cells/well. The cells were then allowed to adhere for 24 h. Prior to the assay, the media was changed to unbuffered DMEM containing pyruvate and glutamine (Gibco #12800-017, pH =7.4 at 37 ºC) and the cells were equilibrated for 30 mins at 37 ºC. Compounds were injected during the assay and OCR was measured using 2 min measurement periods. The mitochondrial uncoupling agents 5.5 and 5.13 were used as a positive controls for increasing oxygen consumption.
6.3.2 $pK_a$ Determination

Approximately 1.0 mg of BAM-15 derivatives was dissolved in 10 mL of an aqueous DMSO solution (DMSO: H$_2$O = 9:1). Monitored by a calibrated pH meter (Fisher Scientific accumet AB15 Basic pH/mV/°C Meter), 50 mM of an aqueous NaOH solution was titrated into the sample. The reported pH values were recorded and plotted to obtain the sigmoid-like curve. The equivalence point was identified by the first derivative curve, and the pKa values were determined by pH value corresponding to the half volume to reach the equivalent point.

5.16d (1.0 mg, in 90% DMSO in H$_2$O) titrated with 10 mM NaOH in H$_2$O (not repeated)

Titration Curve

First derivative
**5.16g** (1.0 mg, in 90% DMSO in H$_2$O) titrated with 50 mM NaOH in H$_2$O

Titration Curve

![Titration Curve](image)

First derivative

![First derivative](image)

**5.16h** (1.0 mg, in 90% DMSO in H$_2$O) titrated with 50 mM NaOH in H$_2$O

Titration Curve

![Titration Curve](image)
5.16n (1.0 mg, in 90% DMSO in H₂O) titrated with 50 mM NaOH in H₂O Titration Curve
**5.16r** (1.0 mg, in 90% DMSO in H$_2$O) titrated with 50 mM NaOH in H$_2$O

**Titration Curve**

![Titration Curve](image)

**First derivative**

![First derivative](image)

**5.16t** (0.5 mg, in 90% DMSO in H$_2$O) titrated with 10 mM NaOH in H$_2$O

**Titration Curve**

![Titration Curve](image)
First derivative

$\Delta p\text{H} \text{ vs } \Delta \text{Vol}$

Avg. NaOH (µL)

5.17h (1.5 mg, in 90% DMSO in H$_2$O) titrated with 50 mM NaOH in H$_2$O

Titration Curve

First derivative

$\Delta p\text{H} \text{ vs } \Delta \text{Vol}$

Avg. Vol. NaOH (µL)
5.17i (1.0 mg, in 90% DMSO in H2O) titrated with 50 mM NaOH in H2O

Titration Curve

First derivative

5.17s (1.0 mg, in 90% DMSO in H2O) titrated with 50 mM NaOH in H2O

Titration Curve
First derivative

5.17u (1.0 mg, in 90% DMSO in H₂O) titrated with 50 mM NaOH in H₂O

Titration Curve

First derivative
5.19b (0.5 mg, in 90% DMSO in H₂O) titrated with 10 mM NaOH in H₂O (not repeated)

Titration Curve

First derivative

6.3.3  General Material and Synthetic Procedures

All solvents used were dried with a PureSolv solvent purification system prior to use. All chemical reagents were purchased from commercial sources and used without further purification. Thin layer chromatography (TLC) was performed on aluminum-backed silica gel, 200 µm, F254. Column chromatography was performed on flash grade silica gel, 40-63 µm, with a Combiflash Rf purification system. ¹H NMR spectra were recorded at 500 or 400 MHz; the corresponding ¹³C NMR resonant frequencies were 126 and 101 MHz, respectively; the corresponding ¹⁹FNMR resonant frequencies were 471 and 376 MHz, respectively. ¹HNMR chemical shifts are reported in ppm with the solvent resonance as an internal standard (Methanol-
$d_4$: 4.87 ppm; acetone-$d_6$: 2.05 ppm; acetonitrile-$d_3$: 1.94 ppm. $^{13}$C NMR, chemical shifts are reported in ppm with the solvent resonance as the internal standard (Methanol-$d_4$: 49.00 ppm; acetone-$d_6$: 206.26 ppm; acetonitrile-$d_3$: 118.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), and integration. Rotomers and tautomers are denoted by an asterisk (*).

High resolution mass spectroscopy (HRMS) was performed on an LC/MS time-of-flight mass spectrometer using electrospray ionization (ESI). HPLC analyses were performed with a Thermo Electron TSQ triple quadrupole mass spectrometer equipped with an ESI source. All compounds tested in biological assays are >95% pure by $^1$H NMR and HPLC analyses unless noted otherwise.

**General Procedure 5A: Symmetric derivative synthesis**

5,6-dichloro-[1,2,5]oxadiazolo[3,4-b]pyrazine (1 equiv) was dissolved in THF (0.6 M solution). The desired substituted arylamines or alkylamines (4 equiv) was then added. The reaction mixture was allowed reflux for 13 h. After which, the solution was concentrated via vacuum, and the residue was purified by silica gel column to yield the title compound.

**General Procedure 5B: Unsymmetric derivative synthesis**

5,6-dichloro-[1,2,5]oxadiazolo[3,4-b]pyrazine (1 equiv) was dissolved in THF (0.52 M solution) and cooled to 0 °C. The desired substituted arylamines (0.9 equiv) was then added dropwise and allowed to stir for 10 min. Triethylamine (1 equiv) was then added dropwise and allowed to stir at 0 °C for 1 h. The second desired substituted arylamine was then added dropwise and allowed to stir for 10 min. Triethylamine (1 equiv) was then added dropwise, and the reaction was then allowed to warm to rt and allowed to stir for 19 h. After which, the solution was concentrated via vacuum, and the residue was purified by silica gel column to yield the title compound.
General Procedure 5C: Amide Derivative Synthesis

[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (1 equiv 20 mg, 0.131 mmol) was dissolved in DCM (0.44 M solution) with TEA (2 equiv) and cooled to 0 °C. The select sulfonyl chloride or anhydride (3 equiv) was then added dropwise. The reaction mixture was allowed to warm up to rt and stirred for 17 h. The resulting reaction mixture was then concentrated via vacuum, and the residue was purified by silica gel column chromatography to yield the title compound.

5,6-dichloro-[1,2,5]oxadiazolo[3,4-b]pyrazine (5.15). 1,2,5-oxadiazole-3,4-diamine (1 equiv) was dissolved in 10% HCl (6 M solution). Oxalic acid (1.5 equiv) was added to the solution, and the mixture was heated to reflux for 3 h. After which, the mixture was cooled to rt, filtered, and dried. The resulting dihydroxy white solid was then carried forward crude (1 equiv) by adding phosphorous pentachloride (3 equiv) and phosphorous oxychloride (2 M solution). The resulting mixture was heated to 95 ºC for 2 h, and excess phosphorous oxychloride was removed via vacuum distillation. The mixture was cooled to 0 ºC, and cold water was added (15–20 mL), causing the title compound to precipitate out of solution. The precipitate was purified via recrystallization using an acetone/water mixture, producing the title compound as a white solid, 23%. Analytical data matches with the literature.205

N⁵,N⁶-di-tert-butyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16a). Synthesized by General Procedure 5A. 79%, white solid. ¹H NMR (400 MHz, Acetone-d₆) δ 7.96 (s, 2H), 2.68 (s, 24H); ¹³C NMR (101 MHz, Acetone-d₆) δ 150.35, 149.17, 54.65, 28.47; HRMS (ESI+): calcd for C₁₂H₂₁N₆O [M+H]+: 265.1777, found: 265.1776.

N⁵,N⁶-dicyclopenty-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16b). Synthesized by General Procedure 5A. 33%, white solid. ¹H NMR (400 MHz, Acetone-d₆) δ 7.32 (s, 2H), 3.01 (tt, J = 7.3, 3.9 Hz, 2H), 0.89 – 0.82 (m, 4H), 0.64 – 0.57 (m, 4H); ¹³C NMR (101 MHz,

$N^5,N^6$-dicyclopentyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.15c). Synthesized by General Procedure 5A. 40%, off-white solid. $^1$H NMR (400 MHz, Acetone-d$_6$) $\delta$ 7.15 (d, $J$ = 6.6 Hz, 2H), 4.44 (s, 2H), 2.14 – 1.96 (m, 4H), 1.74 – 1.42 (m, 12H); $^{13}$C NMR (101 MHz, Acetone-d$_6$) $\delta$ 151.44, 149.33, 54.54, 32.86, 24.62; HRMS (ESI+): calcd for C$_{14}$H$_{21}$N$_6$O [M+H]$^+$: 289.1777, found: 289.1774.

$N^5,N^6$-dicyclohexyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16d). Synthesized by General Procedure 5A. 72%, clear solid. $^1$H NMR (400 MHz, Acetone-d$_6$) $\delta$ 8.26 (d, 2H), 5.38 – 5.23 (m, 2H), 3.34 – 3.19 (m, 4H), 2.95 (dt, $J$ = 13.5, 3.6 Hz, 4H), 2.84 (dt, $J$ = 12.8, 3.6 Hz, 2H), 2.59 (qt, $J$ = 12.9, 3.4 Hz, 4H), 2.51 – 2.28 (m, 6H); $^{13}$C NMR (101 MHz, Acetone-d$_6$) $\delta$ 151.38, 148.70, 51.79, 32.78, 26.33, 25.85; HRMS (ESI+): calcd for C$_{16}$H$_{25}$N$_6$O [M+H]$^+$: 317.2090, found: 317.2089.

5,6-dimorpholino-[1,2,5]oxadiazolo[3,4-b]pyrazine (5.16e). Synthesized by General Procedure 5A. 95%, off-white solid. $^1$H NMR (400 MHz, Acetone-d$_6$) $\delta$ 3.81 (s, 1H); $^{13}$C NMR (101 MHz, Acetone-d$_6$) $\delta$ 154.04, 151.75, 66.80, 49.45; HRMS (ESI+): calcd for C$_{12}$H$_{17}$N$_6$O$_3$ [M+H]$^+$: 293.1362, found: 293.1361. HPLC Purity: 86% pure.

$N^5,N^6$-di-o-tolyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16f). Synthesized by General Procedure 5A. 35%, yellow solid. $^1$H NMR (400 MHz, Acetone-d$_6$) $\delta$ 9.36 (s, 2H), 7.55 (dd, $J$ = 6.9, 2.3 Hz, 2H), 7.40 – 7.25 (m, 6H), 2.34 (s, 6H); $^{13}$C NMR (101 MHz, Acetone-d$_6$) $\delta$ 152.83*, 152.45*, 151.25*, 151.05*, 136.42, 135.48, 131.69, 128.54, 127.69, 127.45, 18.18; HRMS (ESI+): calcd for C$_{18}$H$_{17}$N$_6$O [M+H]$^+$: 333.1464, found: 333.1470.
$N^5,N^6$-di-m-tolyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16g). Synthesized by General Procedure 5A. 60%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 8.82 (s, 2H), 7.61 – 7.27 (m, 6H), 7.06 (d, $J$ = 7.5 Hz, 2H), 2.38 (s, 6H); $^{13}$C NMR (101 MHz, Acetonitrile-$d_3$) δ 140.16, 130.01, 126.88, 123.25, 119.96, 116.00, 112.50, 21.52; HRMS (ESI+): calcd for C$_{18}$H$_{17}$N$_6$O [M+H]$^+$: 333.1464, found: 333.1464.

$N^5,N^6$-di-p-tolyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16h). Synthesized by General Procedure 5A. 69%, off-white solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.56 (s, 2H), 7.62 (s, 4H), 7.25 (d, $J$ = 8.1 Hz, 4H), 2.34 (s, 6H); $^{13}$C NMR (101 MHz, Acetonitrile-$d_3$) δ 151.17, 148.92, 136.01, 130.41, 123.54, 115.47, 20.97; HRMS (ESI+): calcd for C$_{18}$H$_{17}$N$_6$O [M+H]$^+$: 333.1464, found: 333.1441.

$N^5,N^6$-bis(2-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16i). Synthesized by General Procedure 5A. 16%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.03 – 8.64 (m, 2H), 7.28 – 6.97 (m, 8H), 3.92 (s, 6H); $^{13}$C NMR (126 MHz, Acetone-$d_6$) δ 151.28, 150.77, 128.30, 126.82, 121.81, 115.25, 112.88, 111.67, 56.40; HRMS (ESI+): calcd for C$_{18}$H$_{17}$N$_6$O$_3$ [M+H]$^+$: 365.1362, found: 365.1345.

$N^5,N^6$-bis(3-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16j). Synthesized by General Procedure 5A. 32%, off-white solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.64 (s, 2H), 7.70 (s, 4H), 7.04 – 6.95 (m, 4H), 3.83 (s, 6H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 161.40, 150.72, 140.11, 130.83, 114.61, 111.20, 108.21, 55.85, 55.72, 55.60; HRMS (ESI+): calcd for C$_{18}$H$_{17}$N$_6$O$_3$ [M+H]$^+$: 365.1362, found: 365.1370.

$N^5,N^6$-bis(4-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16k). Synthesized by General Procedure 5A. 47%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.26 (d, $J$ = 70.1 Hz, 2H), 7.70 (s, 4H), 7.04 – 6.95 (m, 4H), 3.83 (s, 6H); $^{13}$C NMR (101 MHz, Acetone-$d_6$)
δ 158.27, 151.21, 125.17, 123.20, 115.85, 114.89, 49.87; HRMS (ESI+): calcd for C_{18}H_{17}N_{6}O_{3}\,[M+H]^+: 365.1362, found: 365.1367.

$N^5,N^6$-bis(2-ethoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16l). Synthesized by General Procedure 5A. 54%, light yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 10.75 – 10.04 (m, 2H), 9.04 – 8.05 (m, 2H), 7.11 (dq, $J = 28.7, 9.3, 8.3$ Hz, 6H), 4.37 – 3.97 (m, 4H), 1.56 – 1.18 (m, 6H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 150.31, 126.29, 121.87, 113.51, 65.18, 15.27; HRMS (ESI+): calcd for C_{20}H_{21}N_{6}O_{3} [M+H]^+: 393.1675, found: 393.1674.

$N^5,N^6$-bis(3-ethoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16m). Synthesized by General Procedure 5A. 27%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.61 (s, 2H), 7.79 – 7.37 (m, 2H), 7.32 (t, $J = 8.1$ Hz, 4H), 6.81 – 6.65 (m, 2H), 4.08 (q, $J = 7.0$ Hz, 4H), 1.38 (t, $J = 7.0$ Hz, 6H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 160.74, 130.83, 111.78, 111.01, 108.80, 64.22, 15.22; HRMS (ESI+): calcd for C_{20}H_{21}N_{6}O_{3} [M+H]^+: 393.1675, found: 393.1656.

$N^5,N^6$-bis(4-ethoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16n). Synthesized by General Procedure 5A. 73%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.15 (s, 2H), 7.74 (s, 4H), 7.04 – 6.94 (m, 4H), 4.07 (q, $J = 7.0$ Hz, 4H), 1.38 (t, $J = 6.9$ Hz, 6H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 157.38, 151.05, 148.33, 131.76, 124.81, 115.46, 64.24, 15.17; HRMS (ESI+): calcd for C_{20}H_{21}N_{6}O_{3} [M+H]^+: 393.1675, found: 393.1673.

3,3'-([1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diylbis(azanediyl))diphenol (5.16o). Synthesized by General Procedure 5A. 43%, light yellow solid. H NMR (500 MHz, Acetone-$d_6$) δ 7.91 – 7.65 (m, 0H), 7.48 (d, $J = 91.0$ Hz, 2H), 7.09 – 6.87 (m, 1H), 8.71 (s, 2H), 7.23 (t, $J = 7.9$ Hz, 4H), 6.74 – 6.57 (m, 2H); $^{13}$C NMR (126 MHz, Acetone-$d_6$) δ 158.94, 150.93, 150.64, 140.40, 130.72, 113.53, 112.80, 109.46; HRMS (ESI+): calcd for C_{16}H_{13}N_{6}O_{3} [M+H]^+: 337.1049, found: 337.1044.
N,N’-([1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diylbis(azanediyl))bis(4,1-phenylene))bis(2,2,2-trifluoroacetamide) (5.16p). Synthesized by General Procedure 5A. 22%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 10.38 (s, 2H), 9.61 (s, 2H), 8.00 – 7.94 (m, 4H), 7.86 – 7.81 (m, 4H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 156.27 (q, $^2$$J_{CF}$ = 37.4 Hz), 155.90 (q, $^2$$J_{CF}$ = 37.4 Hz), 155.53 (q, $^2$$J_{CF}$ = 37.4 Hz), 155.16 (q, $^2$$J_{CF}$ = 37.4 Hz), 152.34, 150.84, 150.01, 135.59*, 135.15*, 124.51, 122.03, 121.28 (q, $^1$$J_{CF}$ = 288.9 Hz), 118.42 (q, $^1$$J_{CF}$ = 288.9 Hz), 115.56 (q, $^1$$J_{CF}$ = 288.9 Hz), 112.69 (q, $^1$$J_{CF}$ = 288.9 Hz); $^{19}$F NMR (376 MHz, acetone) δ -76.58 (s, 6F); HRMS (ESI+): calcd for C$_{20}$H$_{13}$F$_6$N$_8$O$_3$ [M+H]$^+$: 527.1015, found: 527.1009.

N$_5^5$,N$_6^6$-bis(2-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16q). Synthesized by General Procedure 5A. 20%, off-white solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 8.02 (s, 2H), 7.54 – 7.46 (m, 6H), 7.38 – 7.29 (m, 2H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 147.75, 145.73, 141.01, 135.57, 129.33, 126.71, 125.52 (q, $^1$$J_{CF}$ = 258.5 Hz), 123.87, 123.07, 122.95, 120.40 (q, $^1$$J_{CF}$ = 258.5 Hz), 117.84 (q, $^1$$J_{CF}$ = 258.5 Hz); $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -58.69 (s, 6F); HRMS (ESI-): calcd for C$_{18}$H$_9$F$_6$N$_6$O$_3$ [M-H]: 471.0646, found: 471.0657.

N$_5^5$,N$_6^6$-bis(3-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16r). Synthesized by General Procedure 5A. 69%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 10.00 (s, 2H), 8.01 – 7.64 (m, 4H), 7.58 (t, $J$ = 8.1 Hz, 2H), 7.17 (d, 2H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 150.42, 131.64, 125.33 (q, $^1$$J_{CF}$ = 256.5 Hz), 122.78 (q, $^1$$J_{CF}$ = 258.5 Hz), 121.11, 120.24 (q, $^1$$J_{CF}$ = 258.5 Hz), 117.74, 117.69 (q, $^1$$J_{CF}$ = 258.5 Hz), 115.00; $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -58.47 (s, 6F); HRMS (ESI+): calcd for C$_{18}$H$_{11}$F$_6$N$_6$O$_3$ [M+H]$^+$: 473.0797, found: 473.0773.

N$_5^5$,N$_6^6$-bis(4-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16s). Synthesized by General Procedure 5A. 40%, off-white solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ
9.39 – 8.65 (m, 2H), 7.80 (s, 4H), 7.49 (d, J = 8.5 Hz, 4H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 149.92, 146.42, 138.74, 125.30 (q, $^1J_{CF} = 256.5$ Hz), 123.98, 122.76, 120.22 (q, $^1J_{CF} = 256.5$ Hz), 117.68 (q, $^1J_{CF} = 256.5$ Hz); $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -58.78 (s, 6F); HRMS (ESI+): calcd for C$_{18}$H$_{11}$F$_6$N$_6$O$_3$ [M+H]$^+$: 473.0797, found: 473.0792.

$N^5,N^6$-bis(2-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16t).

Synthesized by General Procedure 5A. 52%, light yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.40 (s, 2H), 8.03 – 7.76 (m, 6H), 7.64 (dd, J = 7.7 Hz, 2H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 152.64*, 152.08*, 152.05*, 151.32*, 135.93, 134.66, 134.66, 134.64, 130.82, 130.68, 129.37, 129.06 (q, $^1J_{CF} = 274.7$ Hz), 128.12 (q, $^4J_{CF} = 5.1$ Hz), 128.07 (q, $^4J_{CF} = 5.1$ Hz), 127.97 (q, $^4J_{CF} = 5.1$ Hz), 127.31 (q, $^2J_{CF} = 30.3$ Hz), 127.01 (q, $^2J_{CF} = 30.3$ Hz), 126.34 (q, $^1J_{CF} = 274.7$ Hz), 123.63 (q, $^1J_{CF} = 274.7$ Hz), 120.92 (q, $^1J_{CF} = 274.7$ Hz); $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -61.18 (s, 6F); HRMS (ESI+): calcd for C$_{18}$H$_{11}$F$_6$N$_6$O$_3$ [M+H]$^+$: 441.0899, found: 441.0892.

$N^5,N^6$-bis(3-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16u).

Synthesized by General Procedure 5A. 50%, off-white solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 10.11 (s, 2H), 8.07 (s, 4H), 7.70 (t, J = 7.9 Hz, 2H), 7.55 (d, J = 7.8 Hz, 2H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 149.32, 141.84, 132.29 (q, $^2J_{CF} = 32.3$ Hz), 131.97 (q, $^2J_{CF} = 32.3$ Hz), 131.65 (q, $^2J_{CF} = 32.3$ Hz), 131.19, 129.27 (q, $^1J_{CF} = 272.7$ Hz), 126.56 (q, $^1J_{CF} = 272.7$ Hz), 126.08, 123.86 (q, $^1J_{CF} = 272.7$ Hz), 122.09, 121.16 (q, $^1J_{CF} = 272.7$ Hz), 119.00; $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -63.20 (s, 6F); HRMS (ESI+): calcd for C$_{18}$H$_{11}$F$_6$N$_6$O$_3$ [M+H]$^+$: 441.0899, found: 441.0887.

$N^5,N^6$-bis(4-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.16v).

Synthesized by General Procedure 5A. 63%, light yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$)
δ 10.17 (s, 2H), 7.93 (s, 4H), 7.80 (d, J = 8.3 Hz, 4H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 148.83, 145.22, 129.53 (q, $^1J_{\text{CF}} = 273.7$ Hz), 127.38 (q, $^4J_{\text{CF}} = 4.0$ Hz), 127.34 (q, $^4J_{\text{CF}} = 4.0$ Hz), 127.30 (q, $^4J_{\text{CF}} = 4.0$ Hz), 127.27 (q, $^4J_{\text{CF}} = 4.0$ Hz), 127.03 (q, $^2J_{\text{CF}} = 26.3$ Hz), 126.90 (q, $^1J_{\text{CF}} = 273.7$ Hz), 126.84 (q, $^2J_{\text{CF}} = 26.3$ Hz), 126.58 (q, $^2J_{\text{CF}} = 26.3$ Hz), 126.25 (q, $^2J_{\text{CF}} = 26.3$ Hz), 124.15 (q, $^1J_{\text{CF}} = 273.7$ Hz), 122.60, 121.45 (q, $^1J_{\text{CF}} = 273.7$ Hz); $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -62.53 (s, 6F); HRMS (ESI+): calcd for C$_{18}$H$_{11}$F$_6$N$_6$O [M+H]+: 441.0899, found: 441.0875.

$^{4,4'}$-([1,2,5]oxadiazo[3,4-b]pyrazine-5,6-diylbis(azanediyl))dibazonitrile (5.16w).

Synthesized by General Procedure 5A. 19%, off-white solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.78 (s, 2H), 8.25 – 8.17 (m, 4H), 7.92 – 7.85 (m, 4H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 152.28, 152.07, 150.92, 150.02, 142.45, 134.08, 133.99, 123.89, 123.78, 119.25, 109.63; HRMS (ESI+): calcd for C$_{18}$H$_{11}$N$_8$O [M+H]$^+$: 355.1056, found: 355.1040.

$N^5$-(2-fluorophenyl)-$N^6$-phenyl-[1,2,5]oxadiazo[3,4-b]pyrazine-5,6-diamine (5.17a).

Synthesized by General Procedure 5B. 44%, yellow solid. $^1$H NMR (400 MHz, Acetonitrile-$d_3$) δ 9.63 (s, 2H), 8.14 – 7.58 (m, 3H), 7.45 (t, J = 7.8 Hz, 3H), 7.35 – 7.14 (m, 6H); $^{13}$C NMR (101 MHz, Acetonitrile-$d_3$) δ 156.74, 156.54, 154.96, 154.29, 149.81, 148.02, 140.27 (d, $^1J_{\text{CF}} = 204.2$ Hz), 138.25 (d, $^1J_{\text{CF}} = 204.2$ Hz), 130.15, 127.83, 127.75, 127.65, 126.16 (d, $^2J_{\text{CF}} = 18.2$ Hz), 125.98 (d, $^2J_{\text{CF}} = 18.2$ Hz), 125.54, 122.65, 118.26, 117.22 (d, $^2J_{\text{CF}} = 19.2$ Hz), 117.03 (d, $^2J_{\text{CF}} = 19.2$ Hz); $^{19}$F NMR (376 MHz, Acetonitrile-$d_3$) δ -122.05 to -130.05 (m, 1F); HRMS (ESI+): calcd for C$_{16}$H$_{12}$FN$_6$O [M+H]$^+$: 323.1057, found: 323.1051. HPLC Purity: 90% pure.

$N^5$-(2-fluorophenyl)-$N^6$-(pyridin-2-yl)-[1,2,5]oxadiazo[3,4-b]pyrazine-5,6-diamine (5.17b).

Synthesized by General Procedure 5B. 12%, off-white solid. $^1$H NMR (400 MHz, Acetonitrile-$d_3$) δ 10.16 (s, 1H), 8.65 (dd, J = 8.0 Hz, 1H), 8.22 – 8.14 (m, 1H), 8.02 (ddd, J = 8.9, 7.1, 1.9 Hz, 1H), 7.44 (d, J = 8.7 Hz, 1H), 7.37 – 7.11 (m, 5H); $^{13}$C NMR (126 MHz, Acetone-$d_6$) δ
156.95, 155.85, 153.89, 152.67 (d, $^1J_{CF} = 157.6$ Hz), 152.22, 151.11 (d, $^1J_{CF} = 157.6$ Hz), 150.35, 143.49, 137.61, 127.27 (d, $^3J_{CF} = 9.1$ Hz), 127.18 (d, $^3J_{CF} = 9.1$ Hz), 126.24 (d, $^3J_{CF} = 6.1$ Hz), 126.18 (d, $^3J_{CF} = 6.1f$ Hz), 125.63 (d, $^4J_{CF} = 3.0$ Hz), 125.60 (d, $^4J_{CF} = 3.0$ Hz), 125.09, 123.50, 117.87, 117.40, 116.11 (d, $^2J_{CF} = 15.2$ Hz); $^{19}$F NMR (376 MHz, Acetonitrile-$d_3$) $\delta$ -129.95 to -130.15 (m, 1F); HRMS (ESI+): calcd for C$_{15}$H$_{11}$FN$_{7}$O [M+H]$^+$: 324.1009, found: 324.0997.

$^N$5-(2-fluorophenyl)- $^N$6-(3-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17c).

Synthesized by General Procedure 5B. 42%, off-white solid. $^1$H NMR (400 MHz, Acetone-$d_6$) $\delta$ 11.15 (s, 1H), 9.82 (s, 1H), 7.60 (s, 5H), 7.48 (ddd, $J = 8.2, 6.6$ Hz, 1H), 7.35 – 7.21 (m, 4H), 6.98 (td, $J = 8.8, 2.5$ Hz, 1H); $^{13}$C NMR (101 MHz, a Acetone-$d_6$) $\delta$ 165.21, 164.63, 162.75, 162.41, 131.64, 127.35, 125.92, 118.20, 116.93, 112.31 (d, $^2J_{CF} = 20.2$ Hz), 112.10 (d, $^2J_{CF} = 20.2$ Hz), 109.50 (d, $^2J_{CF} = 28.3$ Hz), 109.22 (d, $^2J_{CF} = 28.3$ Hz); $^{19}$F NMR (376 MHz, Acetone-$d_6$) $\delta$ -113.18 to -113.24 (m, 1F), -113.24 to -113.29 (m, 1F); HRMS (ESI+): calcd for C$_{16}$H$_{11}$F$_2$N$_6$O [M+H]$^+$: 341.0962, found: 341.0956.

$^N$5-(2-fluorophenyl)- $^N$6-(4-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17d).

Synthesized by General Procedure 5B. 28%, off-white solid. $^1$H NMR (500 MHz, Acetone-$d_6$) $\delta$ 10.82 (s, 1H), 9.72 (s, 1H), 8.28 – 7.56 (m, 4H), 7.36 – 7.17 (m, 4H); $^{13}$C NMR (126 MHz, Acetone-$d_6$) $\delta$ 160.70, 158.78, 148.45, 146.74, 126.32, 126.15, 124.87, 123.46, 116.17, 116.01, 115.85, 115.69 (d, $^2J_{CF} = 18.2$ Hz), 115.51 (d, $^2J_{CF} = 18.2$ Hz); $^{19}$F NMR (376 MHz, Acetone-$d_6$) $\delta$ -118.35 to -120.14 (m, 1F), -129.69 (dt, $J = 8.8, 4.3$ Hz, 1F); HRMS (ESI+): calcd for C$_{16}$H$_{11}$F$_2$N$_6$O [M+H]$^+$: 341.0962, found: 341.0967.

$^N$5-(2,3-difluorophenyl)-$^N$6-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17e).

Synthesized by General Procedure 5B. 32%, light yellow solid. $^1$H NMR (400 MHz,
Acetone-$d_6$ δ 10.91 (s, 1H), 9.75 (s, 1H), 8.64 – 8.03 (m, 1H), 7.86 – 7.42 (m, 1H), 7.35 – 7.22 (m, 4H), 7.22 – 7.13 (m, 1H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 152.14, 152.08, 149.71, 149.62, 144.02 (d, $^1J_{CF}$ = 246.4 Hz), 143.89 (d, $^1J_{CF}$ = 247.5 Hz), 141.58 (d, $^1J_{CF}$ = 246.4 Hz), 141.44 (d, $^1J_{CF}$ = 247.5 Hz), 124.79 (dd, $^3J_{CF}$ = 6.1 Hz), 124.72 (dd, $^3J_{CF}$ = 6.1 Hz), 124.66 (dd, $^3J_{CF}$ = 6.1 Hz), 119.03, 113.39 (d, $^2J_{CF}$ = 18.2 Hz), 113.21 (d, $^2J_{CF}$ = 18.2 Hz), $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -139.18 to -139.86 (m, 1F), -148.96 to -154.99 (m, 2F); HRMS (ESI+): calcd for C$_{16}$H$_{10}$F$_3$N$_6$O [M+H]$^+$: 359.0868, found: 359.0858.

$N^5$-(2,4-difluorophenyl)$-N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17f). Synthesized by General Procedure 5B. 16%, light yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 10.90 (s, 1H), 9.76 (s, 1H), 8.29 – 7.55 (m, 2H), 7.34 – 7.25 (m, 2H), 7.24 – 7.16 (m, 2H), 7.17 – 7.08 (m, 2H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 161.14, 161.02, 158.70, 158.59, 155.67 (d, $^1J_{CF}$ = 247.5 Hz), 155.38 (d, $^1J_{CF}$ = 249.5 Hz), 153.22 (d, $^1J_{CF}$ = 247.5 Hz), 152.91 (d, $^1J_{CF}$ = 249.5 Hz), 147.46, 126.17, 125.16, 124.86, 124.82, 123.68, 115.81 (d, $^2J_{CF}$ = 21.2 Hz), 115.60 (d, $^2J_{CF}$ = 21.2 Hz), 111.70 (d, $^2J_{CF}$ = 22.2 Hz), 111.48 (d, $^2J_{CF}$ = 22.2 Hz), 104.60 (dd, $^2J_{CF}$ = 25.3 Hz), 104.35 (dd, $^2J_{CF}$ = 25.3 Hz), 104.09; $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -114.32 to -116.24 (m, 2F), -119.15 to -121.61 (m, 1F); HRMS (ESI+): calcd for C$_{16}$H$_{10}$F$_3$N$_6$O [M+H]$^+$: 359.0868, found: 359.0862.

$N^5$-(2-fluorophenyl)$-N^6$-(o-tolyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17g).

Synthesized by General Procedure 5B. 17%, off-white solid. $^1$H NMR (500 MHz, Acetone-$d_6$) δ 9.60 (s, 1H), 8.63 (s, 1H), 7.47 – 7.41 (m, 1H), 7.40 – 7.36 (m, 2H), 7.35 – 7.25 (m, 5H), 2.28 (s, 3H); $^{13}$C NMR (126 MHz, Acetone-$d_6$) δ 152.69, 152.61 (d, $^1J_{CF}$ = 131.3 Hz), 151.31 (d, $^1J_{CF}$ = 131.3 Hz), 150.93, 135.83 (d, $^3J_{CF}$ = 7.1 Hz), 135.76 (d, $^3J_{CF}$ = 7.1 Hz), 131.78, 129.76, 128.88, 127.72 (d, $^3J_{CF}$ = 10.1 Hz), 127.62 (d, $^3J_{CF}$ = 10.1 Hz), 125.72, 125.61, 124.36, 17.98; $^{19}$F NMR
(376 MHz, Acetonitrile-$d_3$) δ -122.81 to -123.46 (m, 1F); HRMS (ESI+): calcd for C$_{17}$H$_{14}$FN$_6$O [M+H]$^+$: 337.1213, found: 337.1200

$N^5$-(2-fluorophenyl)-$N^6$-(m-tolyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17h).

Synthesized by General Procedure 5B. 43%, yellow solid. $^1$H NMR (400 MHz, Acetonitrile-$d_3$) δ 9.10 (s, 1H), 7.62 (s, 1H), 7.47 (s, 2H), 7.39 – 7.21 (m, 5H), 7.07 (d, $J = 7.5$ Hz, 1H), 2.39 (s, 3H); $^{13}$C NMR (101 MHz, Acetonitrile-$d_3$) δ 156.74, 154.29, 140.21, 130.05, 127.79 (d, $^3J_{CF} = 7.1$ Hz), 127.72 (d, $^3J_{CF} = 7.1$ Hz), 126.92, 126.01, 125.97, 125.50, 123.07, 119.75, 117.20 (d, $^2J_{CF} = 19.2$ Hz), 117.01 (d, $^2J_{CF} = 19.2$ Hz), 21.52; $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -118.84 to -127.22 (m, 1F); HRMS (ESI+): calcd for C$_{17}$H$_{14}$FN$_6$O [M+H]$^+$: 337.1213, found: 337.1203.

$N^5$-(2-fluorophenyl)-$N^6$-(p-tolyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17i).

Synthesized by General Procedure 5B. 40%, light yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.68 (s, 2H), 8.01 – 7.52 (m, 3H), 7.37 – 7.18 (m, 5H), 2.35 (s, 3H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 156.52, 154.28, 140.77, 135.42, 130.49, 127.36, 125.88, 124.79, 122.46, 117.08 (d, $^3J_{CF} = 19.6$ Hz), 116.90 (d, $^3J_{CF} = 19.6$ Hz), 21.52; $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -117.35 to -129.39 (m, 1F); HRMS (ESI+): calcd for C$_{17}$H$_{14}$FN$_6$O [M+H]$^+$: 337.1213, found: 337.1201.

$N^5$-(2-fluorophenyl)-$N^6$-(2-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17j).

Synthesized by General Procedure 5B. 55%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 10.05 (s, 1H), 8.78 (s, 1H), 7.35 – 7.03 (m, 8H), 3.97 (s, 3H); $^{13}$C NMR (126 MHz, Acetone-$d_6$) δ 157.15, 155.87, 149.72, 128.48, 126.31, 126.19, 126.09, 125.57, 125.04, 124.85, 123.88 (d, $^2J_{CF} = 34.3$ Hz), 123.54 (d, $^2J_{CF} = 34.3$ Hz), 120.72, 116.43 (d, $^3J_{CF} = 6.1$ Hz), 116.27 (d, $^3J_{CF} = 6.1$ Hz), 110.69, 55.60; $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -123.87 to -126.02 (m, 1F); HRMS (ESI-): calcd for C$_{17}$H$_{12}$FN$_6$O$_2$ [M-H]$^-$: 351.1011, found: 351.1015.
**N^5-(2-fluorophenyl)-N^6-(3-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine**

(5.17k). Synthesized by General Procedure 5B. 50%, yellow solid. \(^1\)H NMR (400 MHz, Acetone-\(d_6\)) \(\delta\) 10.05 (s, 1H), 8.78 (s, 1H), 7.35 – 7.03 (m, 8H), 3.97 (s, 3H); \(^13\)C NMR (126 MHz, Acetone-\(d_6\)) \(\delta\) 161.39, 156.28, 154.31, 130.84, 127.27, 125.85, 125.82, 116.97 (d, \(^2J_{CF} = 19.1\) Hz), 116.81 (d, \(^2J_{CF} = 19.1\) Hz), 114.50, 111.29, 108.09, 55.71; \(^19\)F NMR (376 MHz, Acetone-\(d_6\)) \(\delta\) -124.26 to -126.08 (m, 1F); HRMS (ESI-): calcd for C\(_{17}\)H\(_{12}\)FN\(_6\)O\(_2\) [M-H]: 351.1011, found: 351.1003.

**N^5-(2-fluorophenyl)-N^6-(4-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine**

(5.17l). Synthesized by General Procedure 5B. 38%, yellow solid. \(^1\)H NMR (400 MHz, Acetone-\(d_6\)) \(\delta\) 10.65 (s, 1H), 9.65 (s, 1H), 8.20 – 7.46 (m, 3H), 7.27 (dt, \(J = 5.4, 2.7\) Hz, 3H), 7.16 – 6.90 (m, 2H), 3.83 (s, 3H); \(^13\)C NMR (101 MHz, Acetone-\(d_6\)) \(\delta\) 166.60, 165.69, 158.31, 154.18, 127.55, 126.05, 124.30, 117.27 (d, \(^2J_{CF} = 19.2\) Hz), 117.08 (d, \(^2J_{CF} = 19.2\) Hz), 115.31, 56.05; \(^19\)F NMR (376 MHz, Acetone-\(d_6\)) \(\delta\) -124.26 to -126.08 (m, 1F); HRMS (ESI+): calcd for C\(_{17}\)H\(_{14}\)FN\(_6\)O\(_2\) [M+H]: 353.1162, found: 353.1177.

**N^5-(2-ethoxyphenyl)-N^6-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine**

(5.17m). Synthesized by General Procedure 5B. 39%, yellow solid. \(^1\)H NMR (400 MHz, Acetone-\(d_6\)) \(\delta\) 10.81 (s, 1H), 10.20 (s, 1H), 8.76 (s, 1H), 8.20 – 7.46 (m, 3H), 7.36 (s, 1H), 7.31 – 7.20 (m, 3H), 4.22 (q, \(J = 7.0\) Hz, 2H), 1.42 (t, \(J = 6.9\) Hz, 3H); \(^13\)C NMR (126 MHz, Acetone-\(d_6\)) \(\delta\) 156.44, 154.37, 151.11, 150.02, 127.34 (d, \(^4J_{CF} = 3.0\) Hz), 127.31 (d, \(^4J_{CF} = 3.0\) Hz), 126.21, 126.20, 126.04, 124.99, 121.93, 121.59, 117.53 (d, \(^3J_{CF} = 14.1\) Hz), 117.39 (d, \(^3J_{CF} = 14.1\) Hz), 113.04; \(^19\)F NMR (376 MHz, Acetone-\(d_6\)) \(\delta\) -124.26 to -126.08 (m, 1F); HRMS (ESI+): calcd for C\(_{18}\)H\(_{16}\)FN\(_6\)O\(_2\) [M+H]: 367.1319, found: 367.1322. HPLC Purity: 85% pure.
$N^6$-(3-ethoxyphenyl)-$N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17n).

Synthesized by General Procedure 5B. 22%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 10.76 (s, 1H), 9.77 (s, 1H), 7.95 – 7.41 (m, 2H), 7.39 – 7.20 (m, 5H), 6.82 – 6.71 (m, 1H), 4.09 (q, $J = 7.0$ Hz, 2H), 1.39 (t, $J = 7.0$ Hz, 3H); $^{13}$C NMR (126 MHz, Acetonitrile-$d_3$) δ 160.63, 156.32, 154.39, 131.03, 130.76, 127.66, 125.98, 125.10, 123.94, 118.48, 118.26, 118.03, 117.03 (d, $^3J_{CF} = 16.2$ Hz), 116.87 (d, $^3J_{CF} = 16.2$ Hz), 114.53, 112.01, 108.78, 108.01, 104.20 (d, $^1J_{CF} = 275.7$ Hz), 101.47 (d, $^1J_{CF} = 275.7$ Hz), 64.44, 15.02; $^{19}$F NMR (376 MHz, Acetonitrile-$d_3$) δ -121.14 to -130.75 (m, 1F); HRMS (ESI+): calcd for C$_{18}$H$_{16}$FNO$_2$ [M+H]$^+$: 367.1319, found: 367.1324.

$N^5$-(4-ethoxyphenyl)-$N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17o).

Synthesized by General Procedure 5B. 55%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.64 (s, 2H), 7.80 (s, 3H), 7.27 (dd, $J = 7.4$, 4.0 Hz, 3H), 7.03 – 6.96 (m, 2H), 4.08 (q, $J = 7.0$ Hz, 2H), 1.38 (t, $J = 7.0$ Hz, 3H); $^{13}$C NMR (101 MHz, acetone) δ 157.24, 156.79 (d, $^1J_{CF} = 247.5$ Hz), 154.34 (d, $^1J_{CF} = 247.5$ Hz), 150.29, 148.35 (d, $^3J_{CF} = 11.1$ Hz), 148.24 (d, $^3J_{CF} = 11.1$ Hz), 145.51, 133.19, 130.96 (d, $^3J_{CF} = 12.2$ Hz), 130.84 (d, $^3J_{CF} = 12.2$ Hz), 127.05 (d, $^3J_{CF} = 8.1$ Hz), 126.97 (d, $^3J_{CF} = 8.1$ Hz), 125.66, 125.63, 123.95, 116.92 (d, $^2J_{CF} = 19.2$ Hz), 116.73 (d, $^2J_{CF} = 19.2$ Hz), 115.52, 64.26, 15.20; $^{19}$F NMR (376 MHz, Acetonitrile-$d_6$) δ -125.05 to -127.41 (m); HRMS (ESI+): calcd for C$_{18}$H$_{16}$FN$_6$O$_2$ [M+H]$^+$: 367.1319, found: 367.1313.

3-((6-((2-fluorophenyl)amino)-[1,2,5]oxadiazolo[3,4-b]pyrazin-5-yl)amino)phenol (5.17p).

Synthesized by General Procedure 5B. 33%, yellow solid. $^1$H NMR (400 MHz, Acetone-$d_6$) δ 9.42 (s, 1H), 8.27 – 8.17 (m, 1H), 7.42 – 7.27 (m, 3H), 7.19 (t, $J = 8.1$ Hz, 1H), 6.73 (t, $J = 2.2$ Hz, 1H), 6.67 (ddd, $J = 8.1$, 2.2, 0.9 Hz, 1H), 6.63 (ddd, $J = 8.1$, 2.3, 0.9 Hz, 1H), 5.00 (s, 2H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) δ 157.61 (d, $^1J_{CF} = 248.5$ Hz), 156.02 (d, $^1J_{CF} = 243.4$ Hz),
155.15 (d, $^1J_{CF} = 248.5$ Hz), 153.61 (d, $^1J_{CF} = 243.4$ Hz), 152.07, 151.25, 150.74, 148.59, 130.93, 128.35 (d, $^3J_{CF} = 7.1$ Hz), 128.28 (d, $^3J_{CF} = 7.1$ Hz), 126.68, 126.18 (d, $^3J_{CF} = 11.1$ Hz), 126.07 (d, $^3J_{CF} = 11.1$ Hz), 125.56, 125.52, 116.73 (d, $^2J_{CF} = 19.2$ Hz), 116.54 (d, $^2J_{CF} = 19.2$ Hz), 113.58, 110.08, 108.03; $^{19}$F NMR (376 MHz, Acetone-$d_6$) $\delta$ -124.79 to -124.95 (m, 1F); HRMS (ESI+): calcd for C$_{16}$H$_{12}$FN$_6$O$_2$ [M+H]$^+$: 339.1006, found: 339.1004.

2,2,2-trifluoro-N-(4-((2-fluorophenyl)amino)-[1,2,5]oxadiazolo[3,4-b]pyrazin-5-yl)amino)phenyl)acetamide (5.17q). Synthesized by General Procedure 5B. 18%, yellow solid.

$^1$H NMR (400 MHz, Acetonitrile-$d_3$) $\delta$ 9.23 (s, 2H), 7.87 – 7.46 (m, 6H), 7.36 – 7.21 (m, 3H); $^{13}$C NMR (126 MHz, CD$_3$CN) $\delta$ 156.39, 156.24 (q, $^2J_{CF} = 29.3$ Hz), 155.95 (q, $^2J_{CF} = 29.3$ Hz), 155.65 (q, $^2J_{CF} = 29.3$ Hz), 155.35 (q, $^2J_{CF} = 29.3$ Hz), 154.59, 149.83, 133.91, 127.79, 125.99, 125.49, 123.19, 122.75, 120.35 (q, $^1J_{CF} = 230.3$ Hz), 117.21 (d, $^3J_{CF} = 16.2$ Hz), 117.05 (d, $^3J_{CF} = 16.2$ Hz), 115.78 (q, $^1J_{CF} = 230.3$ Hz), 113.49 (q, $^1J_{CF} = 230.3$ Hz); $^{19}$F NMR (376 MHz, Acetonitrile-$d_3$) $\delta$ -76.46 to -76.75 (m, 3F), -125.89 (s, 1F); HRMS (ESI+): calcd for C$_{18}$H$_{12}$F$_4$N$_7$O$_2$ [M+H]$^+$: 434.0989, found: 434.0988.

$^{N^5}$-(2-fluorophenyl)-$^{N^6}$-(2-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17r). Synthesized by General Procedure 5B. 14%, off-white solid. $^1$H NMR (400 MHz, Acetone-$d_6$) $\delta$ 11.10 (s, 1H), 9.88 (s, 1H), 7.56 – 7.40 (m, 2H), 7.39 – 7.23 (m, 6H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) $\delta$ 155.32, 155.11, 152.93, 152.69, 140.24, 128.33, 126.13, 125.96, 125.86, 124.88, 124.50 (q, $^1J_{CF} = 258.6$ Hz), 123.69, 123.15, 122.12, 121.95 (q, $^1J_{CF} = 258.6$ Hz), 119.39 (q, $^1J_{CF} = 258.6$ Hz), 116.83, 115.88, 115.74, 115.57; $^{19}$F NMR (376 MHz, Acetone-$d_6$) $\delta$ -58.48 (s, 3F), -123.69 to -131.84 (m, 1F); HRMS (ESI+): calcd for C$_{17}$H$_{11}$F$_4$N$_6$O$_2$ [M+H]$^+$: 407.0880, found: 407.0897.
$N^5$-(2-fluorophenyl)-$N^6$-(3-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17s). Synthesized by General Procedure 5B. 59%, yellow solid. $^1$H NMR (400 MHz, Acetone- $d_6$) $\delta$ 10.83 – 9.59 (m, 2H), 7.87 (d, $J = 49.5$ Hz, 3H), 7.58 (t, $J = 8.2$ Hz, 1H), 7.33 – 7.22 (m, 3H), 7.20 – 7.14 (m, 1H); $^{13}$C NMR (101 MHz, Acetone- $d_6$) $\delta$ 156.45, 154.01, 150.43, 131.64, 127.36, 125.91 (d, $^4J_{CF} = 3.0$ Hz), 125.88 (d, $^4J_{CF} = 3.0$ Hz), 125.38, 125.10, 122.84 (q, $^1J_{CF} = 257.6$ Hz), 121.09, 120.29 (q, $^1J_{CF} = 257.6$ Hz), 117.76, 117.19 (q, $^3J_{CF} = 15.2$ Hz), 117.04 (q, $^3J_{CF} = 15.2$ Hz), 116.86 (q, $^3J_{CF} = 15.2$ Hz), 116.71 (q, $^3J_{CF} = 15.2$ Hz), 114.97; $^{19}$F NMR (376 MHz, Acetone- $d_6$) $\delta$ -58.45 (s, 3F), -120.56 to -130.21 (m, 1F); HRMS (ESI+): calcd for C$_{17}$H$_{11}$F$_4$N$_6$O$_2$ [M+H]$^+$: 407.0880, found: 407.0887.

$N^5$-(2-fluorophenyl)-$N^6$-(4-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17t). Synthesized by General Procedure 5B. 28%, light yellow solid. $^1$H NMR (400 MHz, Acetone- $d_6$) $\delta$ 10.96 (s, 1H), 9.80 (s, 1H), 8.36 – 7.53 (m, 3H), 7.47 – 7.37 (m, 2H), 7.34 – 7.11 (m, 3H); $^{13}$C NMR (101 MHz, Acetone) $\delta$ 150.62, 131.83, 127.47, 126.06, 126.02, 125.54 (q, $^1J_{CF} = 257.6$ Hz), 122.99 (q, $^1J_{CF} = 257.6$ Hz), 121.27, 120.45 (q, $^1J_{CF} = 257.6$ Hz), 117.93, 117.19 (d, $^2J_{CF} = 17.2$ Hz), 117.02 (d, $^2J_{CF} = 17.2$ Hz), 115.15; $^{13}$C NMR (126 MHz, Acetone- $d_6$) $\delta$ 155.35, 153.39, 145.48, 126.36, 124.89 (d, $^4J_{CF} = 3.0$ Hz), 124.86 (d, $^4J_{CF} = 3.0$ Hz), 123.64 (q, $^1J_{CF} = 205.0$ Hz), 123.00, 121.87, 121.61 (q, $^1J_{CF} = 205.0$ Hz), 119.58 (q, $^1J_{CF} = 205.0$ Hz), 117.55 (q, $^1J_{CF} = 205.0$ Hz), 116.01 (d, $^3J_{CF} = 15.2$ Hz), 115.86 (d, $^3J_{CF} = 15.2$ Hz); $^{19}$F NMR (376 MHz, Acetone- $d_6$) $\delta$ -59.45 (s, 3F), -122.88 to -126.12 (m, 1F); HRMS (ESI+): calcd for C$_{17}$H$_{11}$F$_4$N$_6$O$_2$ [M+H]$^+$: 407.0880, found: 407.0872.

$N^5$-(2-fluorophenyl)-$N^6$-(2-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17u). Synthesized by General Procedure 5B. 27%, off-white solid. $^1$H NMR (400 MHz, Acetone- $d_6$) $\delta$ 11.13 (s, 1H), 9.82 (s, 1H), 8.67 (s, 1H), 7.85 – 7.67 (m, 2H), 7.51 – 7.19...
(m, 5H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) $\delta$ 155.85, 153.41, 151.47, 150.72, 144.88, 139.26, 134.76, 129.15 (q, $^1J_{CF} = 273.7$ Hz), 127.85 (q, $^4J_{CF} = 4.0$ Hz), 127.81 (q, $^4J_{CF} = 4.0$ Hz), 127.76 (q, $^4J_{CF} = 4.0$ Hz), 127.72 (q, $^4J_{CF} = 4.0$ Hz), 126.63 (d, $^4J_{CF} = 7.1$ Hz), 126.56 (d, $^4J_{CF} = 7.1$ Hz), 126.44 (q, $^1J_{CF} = 273.7$ Hz), 125.96, 125.83 (d, $^4J_{CF} = 3.0$ Hz), 125.80 (d, $^4J_{CF} = 3.0$ Hz), 123.73 (q, $^3J_{CF} = 18.2$ Hz); $^{19}$F NMR (376 MHz, Acetone-$d_6$) $\delta$ -62.71 (s, 3F), -131.01 to -131.82 (m, 1F); HRMS (ESI+): calcd for C$_{17}$H$_{11}$F$_4$N$_6$O [M+H]$^+$: 391.0930, found: 391.0932.

$N^5$-(2-fluorophenyl)-$N^6$-(3-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17v). Synthesized by General Procedure 5B. 49%, off-white solid. $^1$H NMR (400 MHz, Acetone-$d_6$) $\delta$ 10.82 (s, 1H), 9.90 (s, 1H), 8.49 – 7.92 (m, 3H), 7.70 (dd, $J = 8.0$ Hz, 1H), 7.55 (d, $J = 7.8$ Hz, 1H), 7.36 – 7.19 (m, 3H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) $\delta$ 157.30, 154.11, 132.04 (d, $^2J_{CF} = 37.4$ Hz), 131.67 (d, $^2J_{CF} = 37.4$ Hz), 131.20, 129.34 (q, $^1J_{CF} = 272.7$ Hz), 127.35, 127.19, 126.64 (q, $^1J_{CF} = 272.7$ Hz), 126.11, 125.93 (d, $^4J_{CF} = 3.0$ Hz), 125.90 (d, $^4J_{CF} = 3.0$ Hz), 125.25, 123.94 (q, $^1J_{CF} = 272.7$ Hz), 122.12, 119.03, 117.23 (q, $^2J_{CF} = 20.2$ Hz), 117.03 23 (q, $^2J_{CF} = 20.2$ Hz), 116.84 23 (q, $^2J_{CF} = 20.2$ Hz), 111.02; $^{19}$F NMR (376 MHz, Acetone-$d_6$) $\delta$ -63.20 , -123.46 to -132.24 (m); HRMS (ESI+): calcd for C$_{17}$H$_{11}$F$_4$N$_6$O [M+H]$^+$: 391.0930, found: 391.0917. HPLC Purity: 92% pure.

$N^5$-(2-fluorophenyl)-$N^6$-(4-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.17w). Synthesized by General Procedure 5B. 44%, off-white solid. $^1$H NMR (400 MHz, Acetone-$d_6$) $\delta$ 10.97 (s, 1H), 9.87 (s, 1H), 8.60 – 7.85 (m, 3H), 7.80 (d, $J = 8.4$ Hz, 2H), 7.28 (dt, $J = 10.7$, 4.1 Hz, 3H); $^{13}$C NMR (101 MHz, Acetone-$d_6$) $\delta$ 161.37, 158.62, 152.49, 151.70, 127.16, 126.70, 126.40, 125.88, 125.75 (d, $^4J_{CF} = 1.0$ Hz), 125.74 (d, $^4J_{CF} = 1.0$ Hz), 124.84, 124.01, 122.38, 121.78, 117.05 (q, $^3J_{CF} = 15.2$ Hz), 116.90 (q, $^3J_{CF} = 15.2$ Hz), 116.71
(q, $^3J_{CF} = 15.2$ Hz), 116.57 (q, $^3J_{CF} = 15.2$ Hz); $^{19}$F NMR (376 MHz, Acetone-$d_6$) δ -62.51 (s, 3F), -124.05 to -131.33 (m, 1F); HRMS (ESI+): calcd for C$_{17}$H$_{10}$F$_4$N$_6$NaO $[M+Na]^+$: 413.0750, found: 413.0740. HPLC Purity: 86% pure.

$[1,2,5]$oxadiazolo[3,4-b]pyrazine-5,6-diamine (5.18). 5.15 (1 equiv.) was dissolved in ACN (6M solution), and 30% aqueous ammonium (2M solution) was added to the solution at 0 °C. The reaction mixture was allowed to warm to rt and stirred for 3 h, resulting in precipitate formation. The precipitated was filtered, washed with water, and dried, giving the title compound as a yellow solid in quantitative yield that was carried forward as is. Analytical data matches with the literature.$^{205}$

$N,N'-([1,2,5]$oxadiazolo[3,4-b]pyrazine-5,6-diyl)bis(2,2,2-trifluoroacetamide) (5.19a).

Synthesized by General Procedure 5C. 40%, clear solid. $^{13}$C NMR (126 MHz, CD$_3$OD) δ 152.18, 151.10, 124.52 (q, $^1J_{CF} = 229.3$ Hz), 122.25 (q, $^1J_{CF} = 229.3$ Hz), 119.98 (q, $^1J_{CF} = 229.3$ Hz), 117.71 (q, $^1J_{CF} = 229.27$ Hz; $^{19}$F NMR (376 MHz, CD$_3$OD) δ -86.90 (s, 6F); GCMS (EI): calcd for C$_8$H$_2$F$_6$N$_6$O$_3$ $[M^*-2F]$: 306.0, found: 306.1.

$N,N'-([1,2,5]$oxadiazolo[3,4-b]pyrazine-5,6-diyl)bis(4-methylbenzenesulfonamide) (5.19b).

Synthesized by General Procedure 5C. 13%, off-white solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 7.60 (d, J = 7.9 Hz, 4H), 7.05 (d, J = 7.9 Hz, 4H), 2.34 (s, 6H); $^{13}$C NMR (126 MHz, CD$_3$OD) δ 152.40*, 152.04*, 142.81*, 142.10*, 129.68*, 128.78*, 127.74*, 115.52*, 115.04*, 21.35; HRMS (ESI-): calcd for C$_{18}$H$_{20}$N$_7$O$_5$S$_2$ $[M+NH^+]$: 478.0962, found: 478.0997.

$N,N'-([1,2,5]$oxadiazolo[3,4-b]pyrazine-5,6-diyl)bis(4-fluorobenzenesulfonamide) (5.19c).

Synthesized by General Procedure 5C. 13%, yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 7.77 – 7.70 (m, 4H), 7.09 – 7.02 (m, 4H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 166.77 (d, $^1J_{CF} = 251.5$ Hz), 164.28 (d, $^1J_{CF} = 251.5$ Hz), 152.41, 152.03, 141.82, 130.52 (d, $^3J_{CF} = 8.1$ Hz), 130.43 (d, $^3J_{CF} =
8.1 Hz), 116.13 (d, $^{2}J_{CF} = 23.2$ Hz), 115.90 (d, $^{2}J_{CF} = 23.2$ Hz); $^{19}$F NMR (376 MHz, CD$_3$OD) δ -111.07 to -111.22 (m, 2F); HRMS (ESI-): calcd for C$_{16}$H$_{13}$F$_{2}$N$_{7}$O$_{5}$S$_{2}$ [M+NH$_{4}^-$]: 485.0388, found: 485.0124.

$N,N'$-([1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diyl)bis(3-(trifluoromethyl)benzenesulfonamide) (5.19d). Synthesized by General Procedure 5C. 13%, golden yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 7.95 (dt, $J = 7.9, 1.4$ Hz, 2H), 7.90 (t, $J = 1.9$ Hz, 2H), 7.70 (dt, $J = 7.9, 1.9, 1.0$ Hz, 2H), 7.57 – 7.51 (m, 2H); $^{13}$C NMR (126 MHz, Methanol-d$_4$) δ 152.41, 152.04, 146.39, 131.97 (q, $^{2}J_{CF} = 26.3$ Hz), 131.71 (q, $^{2}J_{CF} = 26.3$ Hz), 131.45 (q, $^{2}J_{CF} = 26.3$ Hz), 131.39, 131.19 (q, $^{2}J_{CF} = 26.3$ Hz), 130.47, 128.84, 128.81, 128.78, 128.23 (q, $^{1}J_{CF} = 218.2$ Hz), 126.07 (q, $^{1}J_{CF} = 218.2$ Hz), 124.55, 124.52, 124.49, 124.47, 123.91 (q, $^{1}J_{CF} = 218.2$ Hz), 121.75 (q, $^{1}J_{CF} = 218.2$ Hz); $^{19}$F NMR (376 MHz, Methanol-d$_4$) δ -64.27 (s, 3F); HRMS (ESI-): calcd for C$_{18}$H$_{14}$F$_{6}$N$_{7}$O$_{5}$S$_{2}$ [M+NH$_{4}^-$]: 586.0402, found: 586.0390.

$N,N'$-([1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diyl)bis(2-nitrobenzenesulfonamide) (5.19e). Synthesized by General Procedure 5C. 29%, yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 8.07 (s, 1H), 8.05 (s, 1H), 7.65 – 7.54 (m, 8H); $^{13}$C NMR (101 MHz, CD$_3$OD) δ 152.40, 152.04, 149.47, 137.53, 133.40, 132.11, 131.52, 124.49; HRMS (ESI+): calcd for C$_{16}$H$_{14}$N$_{9}$O$_{9}$S$_{2}$ [M+NH$_{4}^+$]: 540.0356, found: 540.0319.
6.3.4 Oxygen Consumption Rate Data

Lines are provided as an eye guide. They are not a trendline.
6.3.5  NMR Spectra

5,6-dichloro-[1,2,5]oxadiazolo[3,4-b]pyrazine $^{13}$CNMR (5.13)

$N^5,N^6$-di-tert-butyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16a)
$N^5,N^6$-di-tert-butyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16a)

$N^5,N^6$-dicyclopropyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16b)
$N^5,N^6$-dicyclopentyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16b)

$N^5,N^6$-dicyclopentyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR(5.16c)

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$N^5,N^6$-dicyclopentyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16c)

$N^5,N^6$-dicyclohexyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16d)
$N^5,N^6$-dicyclohexyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16d)

5,6-dimorpholino-[1,2,5]oxadiazolo[3,4-b]pyrazine $^1$HNMR (5.16e)
5,6-dimorpholino-[1,2,5]oxadiazolo[3,4-b]pyrazine $^{13}$CNMR(5.16e)

$^{1}H$NMR(5.16f)
$N^5,N^6$-di-o-tolyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$C NMR (5.16f)

$N^5,N^6$-di-m-tolyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$H NMR (5.16g)
$N^5,N^6$-di-$m$-tolyl-$[1,2,5]$oxadiazolo$[3,4-b]$pyrazine-$5,6$-diamine $^{13}$CNMR (5.16g)

$N^5,N^6$-di-$p$-tolyl-$[1,2,5]$oxadiazolo$[3,4-b]$pyrazine-$5,6$-diamine $^1$HNMR(5.16h)
$N^5,N^6$-di-$p$-tolyl-$[1,2,5]$oxadiazolo[3,4-$b$]pyrazine-5,6-diamine $\text{CNMR}(5.16h)$

$N^5,N^6$-bis(2-methoxyphenyl)-$[1,2,5]$oxadiazolo[3,4-$b$]pyrazine-5,6-diamine $\text{HNMR}(5.16i)$
$N^5,N^6$-bis(2-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16i)

$N^5,N^6$-bis(3-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16j)
$N^5,N^6$-bis(3-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16j)

$N^5,N^6$-bis(4-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16k)
$N^5,N^6$-bis(4-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16k)

$N^5,N^6$-bis(2-ethoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16l)
$N^5,N^6$-bis(2-ethoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16l)

$N^5,N^6$-bis(3-ethoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16m)
$N^5,N^6$-bis(3-ethoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16m)

$N^5,N^6$-bis(4-ethoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{1}$HNMR (5.16n)
N^5,N^6-bis(4-ethoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$CNMR (5.16n)

3,3'-([1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diylbis(azanediyl))diphenol $^1$HNMR (5.16o)
$3,3'-(1,2,5)$ oxadiazolo[3,4-$b$]pyrazine-5,6-diylbis(azanediyl))diphenol $^{13}$CNMR (5.16o)

$N,N'-(1,2,5)$ oxadiazolo[3,4-$b$]pyrazine-5,6-diylbis(azanediyl)bis(4,1-phenylene)bis(2,2,2-trifluoroacetamide) $^1$HNMR (5.16p)
N,N'-((1,2,5)oxadiazolo[3,4-b]pyrazine-5,6-diylbis(azanediyl))bis(4,1-phenylene)bis(2,2,2-trifluoroacetamide) $^{19}$F-NMR (5.16p)

N,N'-((1,2,5)oxadiazolo[3,4-b]pyrazine-5,6-diylbis(azanediyl))bis(4,1-phenylene)bis(2,2,2-trifluoroacetamide) $^{13}$C-NMR (5.16p)
$N^5,N^6$-bis(2-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16q)

$N^5,N^6$-bis(2-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.16q)
$N^5,N^6$-bis(2-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

**$^{13}$CNMR (5.16q)**

$N^5,N^6$-bis(3-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

**$^1$HNMR (5.16r)**

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$N^5,N^6$-bis(3-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{10}\text{FNMR}$ (5.16r)

$N^5,N^6$-bis(3-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}\text{CNMR}$ (5.16r)
$N^6,N^6$-bis(4-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16s)

$N^5,N^6$-bis(4-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.16s)
$N^5,N^6$-bis(4-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$FNMR (5.16s)

$N^5,N^6$-bis(2-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16t)
$N^5,N^6$-bis(2-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.16t)

$N^5,N^6$-bis(2-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16t)
$N^5, N^6$-bis(3-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16u)

$N^5, N^6$-bis(3-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$FNMR (5.16u)
$N^5, N^6$-bis(3-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.16u)

$N^5, N^6$-bis(4-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.16v)
$N^5, N^6$-bis(4-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$F-NMR (5.16v)

$N^5, N^6$-bis(4-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$C-NMR (5.16v)
$4,4'-([1,2,5]\text{oxadiazolo}[3,4-b]\text{pyrazine}-5,6 \text{-diylbis(azanediyl)})\text{dibzonitrile}$ $^1\text{HNMR}$ (5.16w)

$4,4'-([1,2,5]\text{oxadiazolo}[3,4-b]\text{pyrazine}-5,6 \text{-diylbis(azanediyl)})\text{dibzonitrile}$ $^{13}\text{CNMR}$ (5.16w)
$N^5$-(2-fluorophenyl)- $N^6$-phenyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.17a)

$N^5$-(2-fluorophenyl)- $N^6$-phenyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.17a)
$N^5$-(2-fluorophenyl)- $N^6$-phenyl-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.17a)

$N^5$-(2-fluorophenyl)- $N^6$-(pyridin-2-yl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.17b)
\( N^5-(2\text{-fluorophenyl})-N^6-(\text{pyridin-2-yl})-[1,2,5]\text{oxadiazolo}[3,4-b]\text{pyrazine-5,6-diamine}^{10}\text{FNMR} \)

(5.17b)

\( N^5-(2\text{-fluorophenyl})-N^6-(\text{pyridin-2-yl})-[1,2,5]\text{oxadiazolo}[3,4-b]\text{pyrazine-5,6-diamine}^{13}\text{CNMR} \)

(5.17b)
$N^5$-(2-fluorophenyl)- $N^6$-(3-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine
$^1$HNMR (5.17c)

$N^5$-(2-fluorophenyl)- $N^6$-(3-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine
$^1$FPNMR (5.17c)
$N^5$-(2-fluorophenyl)-$N^6$-(3-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine  
$^{13}$CNMR (5.17c)

$N^5$-(2-fluorophenyl)-$N^6$-(4-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine  
$^1$HNMR (5.17d)
$N^5$-$(2$-fluorophenyl)- $N^6$-$(4$-fluorophenyl)$-[1,2,5]$oxadiazolo[3,4-$b$]pyrazine-5,6-diamine

$^{19}$FNMR (5.17d)

$N^5$-$(2$-fluorophenyl)- $N^6$-$(4$-fluorophenyl)$-[1,2,5]$oxadiazolo[3,4-$b$]pyrazine-5,6-diamine

$^{13}$CNMR (5.17d)
$N^5$-(2,3-difluorophenyl)- $N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^1$HNMR (5.17e)

$N^5$-(2,3-difluorophenyl)- $N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{19}$FNMR (5.17e)
$N^5$-(2,3-difluorophenyl) - $N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{13}$C NMR (5.17e)

$N^5$-(2,4-difluorophenyl) - $N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^1$H NMR (5.17f)
$N^5$-(2,4-difluorophenyl)-$N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{19}$FNMR (5.17f)

$N^5$-(2,4-difluorophenyl)-$N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{13}$CNMR (5.17f)
$N^\delta$-(2-fluorophenyl)-$N^\delta$-(o-tolyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.17g)

$N^\delta$-(2-fluorophenyl)-$N^\delta$-(o-tolyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.17g)
$N^5-(2$-fluorophenyl)-$ $N^6-(o$-tolyl)$-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6$-diamine $^{13}$CNMR (5.17g)

$N^5-(2$-fluorophenyl)-$ $N^6-(m$-tolyl)$-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6$-diamine $^1$HNMR (5.17h)
$N^8$-(2-fluorophenyl)- $N^4$-(m-tolyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{19}$FNMR

(5.17h)

$N^8$-(2-fluorophenyl)- $N^4$-(m-tolyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{13}$CNMR

(5.17h)
$N^5$-(2-fluorophenyl)- $N^6$-(p-tolyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.17i)

$N^5$-(2-fluorophenyl)- $N^6$-(p-tolyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.17i)
$N^5$-(2-fluorophenyl)-$N^6$-(p-tolyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$C NMR (5.17i)

$N^5$-(2-fluorophenyl)-$N^6$-(2-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$H NMR (5.17j)
$N^5$-(2-fluorophenyl)-$N^6$-(2-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^1$H NMR (5.17)
$N^5$-(2-fluorophenyl)-$N^6$-(3-methoxyphenyl)-[1,2,5]oxadiazo[3,4-b]pyrazine-5,6-diamine

$^1$HNMR (5.17k)

$N^5$-(2-fluorophenyl)-$N^6$-(3-methoxyphenyl)-[1,2,5]oxadiazo[3,4-b]pyrazine-5,6-diamine

$^{19}$FNMR (5.17k)
$N^5$-(2-fluorophenyl)-$N^6$-(3-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{13}$CNMR (5.17k)

$N^5$-(2-fluorophenyl)-$N^6$-(4-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^1$HNMR (5.17l)
$N^5$-(2-fluorophenyl)-$N^6$-(4-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{19}$F NMR (5.17 ppm)

$N^5$-(2-fluorophenyl)-$N^6$-(4-methoxyphenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{13}$C NMR (5.17 ppm)
$N^5$-(2-ethoxyphenyl)- $N^5$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^1$HNMR (5.17m)

$N^5$-(2-ethoxyphenyl)- $N^5$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{19}$FNMR (5.17m)
$N^\delta$-(2-ethoxyphenyl)- $N^\delta$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

**$^{13}$CNMR (5.17m)**

$N^\delta$-(3-ethoxyphenyl)- $N^\delta$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

**$^1$HNMR (5.17n)**
$N^5$-(3-ethoxyphenyl)- $N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{13}$FNMR (5.17n)

$N^5$-(3-ethoxyphenyl)- $N^6$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{13}$CNMR (5.17n)
$N^5$-(4-ethoxyphenyl)- $N^5$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^1$HNMR (5.17o)

$N^5$-(4-ethoxyphenyl)- $N^5$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^{19}$FNMR (5.17o)
$N^5$-(4-ethoxyphenyl)-$N^5$-(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine

$^1$H NMR (5.17o)

3-((6-((2-fluorophenyl)amino)-[1,2,5]oxadiazolo[3,4-b]pyrazin-5-yl)amino)phenol

$^1$HNMR (5.17p)
3-((6-((2-fluorophenyl)amino)-[1,2,5]oxadiazolo[3,4-b]pyrazin-5-yl)amino)phenol $^{19}$F-NMR (5.17p)

3-((6-((2-fluorophenyl)amino)-[1,2,5]oxadiazolo[3,4-b]pyrazin-5-yl)amino)phenol $^{19}$C-NMR (5.17p)
2,2,2-trifluoro-N-(4-((6-(2-fluorophenyl)amino)-[1,2,5]oxadiazolo[3,4-b]pyrazin-5-yl)amino)phenyl)acetamide $^1$HNMR (5.17q)

2,2,2-trifluoro-N-(4-((6-(2-fluorophenyl)amino)-[1,2,5]oxadiazolo[3,4-b]pyrazin-5-yl)amino)phenyl)acetamide $^{19}$FNMR (5.17q)
2,2,2-trifluoro-N-(4-((6-(2-fluorophenyl)amino)-[1,2,5]oxadiazolo[3,4-b]pyrazin-5-yl)amino)phenyl)acetamide $^{13}$CNMR (5.17q)

$N^5$-(2-fluorophenyl)-$N^6$-(2-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.17r)
$N^5$-(2-fluorophenyl)- $N^6$-(2-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.17r)

$N^5$-(2-fluorophenyl)- $N^6$-(2-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.17r)

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$N^5$-(2-fluorophenyl)-$N^6$-(3-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.17s)

$N^5$-(2-fluorophenyl)-$N^6$-(3-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.17s)
$N^5$-(2-fluorophenyl)- $N^6$-(3-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.17s)

$N^5$-(2-fluorophenyl)- $N^6$-(4-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.17t)

314
$N^5$-(2-fluorophenyl)- $N^4$-(4-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.17t)

$N^5$-(2-fluorophenyl)- $N^4$-(4-(trifluoromethoxy)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.17t)

315
$N^5$-(2-fluorophenyl)-$N^6$-(2-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.17u)

$N^5$-(2-fluorophenyl)-$N^6$-(2-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.17u)
$N^5$-(2-fluorophenyl)- $N^6$-(2-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.17u)

$N^5$-(2-fluorophenyl)- $N^6$-(3-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.17v)
$N^5$-(2-fluorophenyl)-$N^6$-(3-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.17v)

$N^5$-(2-fluorophenyl)-$N^6$-(3-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.17v)
$N^5$-(2-fluorophenyl)-$N^6$-(4-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^1$HNMR (5.17w)

$N^5$-(2-fluorophenyl)-$N^6$-(4-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{19}$FNMR (5.17w)

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$N^6$-(2-fluorophenyl) - $N^6$-(4-(trifluoromethyl)phenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (5.17w)

$[1,2,5]$oxadiazolo[3,4-b]pyrazine-5,6-diamine $^{13}$CNMR (18)
$N,N'-([1,2,5]\text{oxadiazolo}[3,4-b]pyrazine-5,6-diyl)bis(2,2,2$-trifluoroacetamide) $^{13}$CNMR (5.19a)

$N,N'-([1,2,5]\text{oxadiazolo}[3,4-b]pyrazine-5,6-diyl)bis(2,2,2$-trifluoroacetamide) $^{19}$FNMR (5.19a)
$N,N'-(\text{[1,2,5]oxadiazolo}[3,4-b]pyrazine-5,6-diyl)\text{bis(4-methylbenzenesulfonamide})$ $^{1}\text{HNMR}$

(5.19b)

$N,N'-(\text{[1,2,5]oxadiazolo}[3,4-b]pyrazine-5,6-diyl)\text{bis(4-methylbenzenesulfonamide})$ $^{13}\text{CNMR}$

(5.19b)
$N,N'-(\text{[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diyl]} \text{bis(4-fluorobenzensulfonamide}) \ ^1\text{HNMR (5.19c)}$

$N,N'-(\text{[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diyl]} \text{bis(4-fluorobenzensulfonamide}) \ ^{19}\text{FNMR (5.19c)}$
$N,N'-([1,2,5] \text{oxadiazolo}[3,4-b] \text{pyrazine}-5,6$-diyl)bis(4-fluorobenzenesulfonamide) $^{13}$CNMR (5.19c)

$N,N'-([1,2,5] \text{oxadiazolo}[3,4-b] \text{pyrazine}-5,6$-diyl)bis(3-(trifluoromethyl)benzenesulfonamide) $^{1}$HNMR (5.19d)
$\text{N,N'-(\{1,2,5\}oxidazolo[3,4-b]pyrazine-5,6-diyl)bis(3-(trifluoromethyl)benzenesulfonamide)}$

$\text{CNMR (5.19d)}$

$\text{FNMR (5.19d)}$
$N,N'-(1,2,5)$oxadiazolo$[3,4-b]$pyrazine-$5,6$-diyl$\text{bis}(2$-nitrobenzenesulfonamide)$^1$HNMR ($5.19e$)

$N,N'-(1,2,5)$oxadiazolo$[3,4-b]$pyrazine-$5,6$-diyl$\text{bis}(2$-nitrobenzenesulfonamide)$^{13}$CNMR ($5.19e$)

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6.4 References


