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

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Abstract

Sphingosine 1-phosphate (S1P) is a pleiotropic signaling molecule that interacts with its five G-protein coupled receptors S1P₁₋₅ 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 report, 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 **20i** (SLC4011540) (hSphK1 K_i = 120 nM, hSphK2 K_i = 90 nM) and SphK2 inhibitor **20dd** (SLC4101431) (K_i = 90 nM, 100-fold SphK2 selectivity). These compounds effectively decrease S1P levels *in vitro*. *In vivo* administration of **20dd** validated that inhibition of SphK2 increases blood S1P levels.

Graphical abstract

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Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI:

¹H, ¹³C and ¹⁹F NMR spectra of intermediates and final products.

Molecular formula strings

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Keywords

Sphingosine; sphingosine 1-phosphate; sphingosine kinase; aminothiazole; structure-activity relationships

INTRODUCTION

Sphingosine 1-phosphate (S1P) is a ubiquitous cellular signaling molecule that has been implicated in a variety of diseases including cancer,^{1–4} fibrosis,^{5–7} Alzheimer's,^{8, 9} sickle cell disease,^{10, 11} and viral infections such as Chikungunya virus.¹² 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, S1P_{1–5}.^{13–16} Although its function as an intracellular ligand is less well defined, S1P is reported to control epigenetic modifications through regulation of HDAC 1/2 activity via sphingosine kinase isotype 2 (SphK2) and alter erythrocyte glycolysis to increase O₂ release under hypoxic conditions.^{17–19} 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 identity²⁰ 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,^{21, 22} whereas some SphK2 is found in the nucleus^{17, 18} but can relocate to the cytosol on phosphorylation.^{23, 24} Depending on this localization, SphK2 can promote either apoptosis^{12, 13} or cell proliferation.^{17, 18, 25, 26} Although isotype-specific SphK null mice are viable, fertile, and phenotypically unremarkable,²⁷ SphK1-null mice have about a two-fold reduction in blood S1P levels while SphK2-null mice have 2–4 fold increased blood S1P levels.^{28–32} 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.^{27, 32}

The therapeutic potential of drugging the S1P pathway was realized by the approval of Fingolimod by the Food and Drug Administration for the treatment of multiple sclerosis.^{33, 34} Fingolimod is a pro-drug that is phosphorylated by SphK2 to act as a functional antagonist of S1P receptors, S1P_{1/3}. Other approaches have also been employed to manipulate S1P activity including targeting SphKs to control S1P synthesis.^{3, 35–37} There are currently multiple reports of potent SphK1-selective inhibitors and SphK1/SphK2-dual inhibitors (Figure 1). Inhibitors such as the SphK1-selective inhibitor **1** (PF-543)^{38, 39} and the SphK1/SphK2-dual inhibitor **5** (SKI-II)^{40–42} have been co-crystallized with SphK1 and have been useful in developing next generation inhibitors.^{43, 44} Indeed, medicinal chemists at Amgen improved **5** to realize a selective SphK1 inhibitor, **2** (Amgen 23),⁴⁵ while preserving the aminothiazole region. While **5** is recently reported to have off-target activity against dihydroceramide desaturase,⁴⁶ **1** has shown promise for disease states such as sickle cell disease, where inflammation, cell sickling, and hemolysis were reduced in treated mouse models.¹¹

In contrast with SphK1 inhibitors, highly potent SphK2-selective inhibitors are lacking. Early SphK2 inhibitors (**8** (SLR080811),^{29, 47} **9** (ABC294640),⁴⁸ and **10** (K145)^{49, 50}),

which have low micromolar potency and are modestly selective vs. SphK1, are active in cultured cells as measured by lowering of cellular S1P levels. Among these, **9** has been deployed in a variety of animal disease models, including ulcerative colitis,⁵¹ Crohns disease,⁵² ischaemia/reperfusion injury,⁵³ osteoarthritis,⁵⁴ and colon cancer.⁵⁵ However, **9** has recently been reported to have an off-target effect of acting as a tamoxifen-like molecule with the estrogen receptor.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 **8**.^{57, 58} In addition, an oncogenic role for SphK2 is emerging where high-level overexpression is associated with reduced cell survival and proliferation.⁵⁹

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: the SphK1/SphK2 dual-inhibitor **7** (SLC5111312)^{28, 60} and the SphK2-selective inhibitor **11** (SLM6031434)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 *in vivo* activity as selective SphK2 inhibitors.

RESULTS AND DISCUSSION

Inhibitor Design and Development

Our group reported the synthesis of **3** (SLP7111228),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.^{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 (**8**) into a >100-fold SphK1-selective inhibitor. In this work, we sought to improve **3**'s potency and selectivity further by replacing the octyl chain with an aminothiazole group, as present in SphK1 inhibitors such as **2** (Figure 2). Docking of **2** into the binding pocket of a homology model of SphK2 and superimposition with **2**'s "aminothiazole tail" linked to an oxadiazole phenyl ring suggested binding interactions mimicking **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 the aminothiazole-based inhibitors **2** and **5**.^{44, 45} Gustin *et al.* recently developed potent aminothiazole-based SphK1/SphK2-dual inhibitors derived from **5**, including **2**.⁴⁵ Although most compounds reported were dual-inhibitors, some compounds in their series show a bias towards SphK1 inhibition

with attractive pharmacokinetic properties. Vogt and co-workers subsequently reported the synthesis of aminothiazole-based derivatives of **5** that are SphK1/SphK2-dual inhibitors with several inhibitors showing a slight bias for SphK2 selectivity.⁶¹ However, these molecules show only micromolar potency, and extensive biological studies have yet to be reported. Aurelio *et al.* also recently reported the synthesis of derivatives of **5** that show limited success as SphK1/SphK2 dual inhibitors and SphK1- and SphK2-selective inhibitors.⁶² Due to the prominence of aminothiazole moieties in SphK inhibitor scaffolds, we incorporated aminothiazole moieties into our guanidine-based scaffold, specifically SphK1-selective inhibitors.

Inhibitor Synthesis

In prior studies,^{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 1. 4-Trifluoromethylacetamide benzonitrile **13** was synthesized by the acetylation of 4-aminobenzonitrile **12** with trifluoroacetic anhydride. Benzonitrile **13** was then reacted with hydroxylamine hydrochloride and triethylamine in ethanol using a microwave reactor to yield amidoxime **14**, which was reacted with homoproline using HCTU and Hunig's base at 100 °C to afford the 1,2,4-oxadiazole **15**. The trifluoroacetate group was removed using lithium hydroxide to afford amine **16**. Treatment of **16** with thiocarbodiimidazole followed by ammonia provided the key intermediate thiourea **17**. Aminothiazoles **18a-ee** were produced by reacting the required α -bromoketone with thiourea **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-1*H*-pyrazole-1-carboxamidine in a microwave reactor to afford the *bis*-Boc-protected guanidines **19a-ee**. Bubbling HCl gas subsequently provided the desired guanidine derivatives **20a-ee** (see Tables 1 and 2 for structures).

Biphenyl derivatives were synthesized as outlined in Scheme 2. Using either compound **18f** or **18i**, Suzuki-Miyaura cross-coupling with various aryl boronic acids yielded the biaryl aminothiazoles **21a-d**. The standard synthetic sequence of deprotection, guanylation, and deprotection was then followed to yield the desired biaryl aminothiazole derivatives **23 a-d**.

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 (**20k**, Table 1) and its analogous oxathiazole was done. The corresponding oxathiazole derivative was synthesized as presented in Scheme 3. 4-*Tert*-butoxy benzonitrile **25** was synthesized via nucleophilic aromatic substitution of 4-fluorobenzonitrile **24** with 1 M potassium *tert*-butoxide. The benzonitrile **25** was then reacted with hydroxylamine hydrochloride and triethylamine in ethanol using a microwave reactor to yield its amidoxime that was then reacted with homoproline using HCTU and Hunig's base at 100 °C to afford the 1,2,4-oxadiazole **26**. The phenol derivative **27** was achieved via treatment of 1,2,4-oxadiazole **26** with trifluoroacetic acid followed by reprotection with Boc-anhydride. Nucleophilic aromatic substitution with the phenol derivative **27** on 2,4-dibromothiazole afforded the

oxathiazole **28**. Suzuki-Miyaura cross-coupling with 4-trifluoromethylphenylboronic acid yielded the desired oxathiazole **29**. The standard synthetic sequence of deprotection, guanylation, and deprotection was followed for compound **29** to yield the desired oxathiazole derivative **31**.

To further probe the significance of the hydrogen bond effect of the amino moiety in **20dd**, its methylated derivative was synthesized as illustrated in Scheme 4. The biaryl aminothiazole **18dd** was methylated with methyl iodine in refluxing acetone to yield the **32**. The standard synthetic sequence of deprotection, guanylation, and deprotection was then followed to yield the desired methylated aminothiazole derivative **34**.

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.^{47, 64} In particular, synthesized inhibitors were tested at 0.3 μ M 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 1, substituting the thiazole ring with an unsubstituted phenyl ring (**20a**) resulted in poor inhibition of either SphK isotype in our assay. Replacement of the phenyl ring with either a 4' (**20b**) or 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 head group⁴⁷ generates an SphK1 inhibitor when the 'tail' is an unsubstituted octyl group. Furthermore, aminothiazoles in the Amgen series are also selective for SphK1,⁴⁵ although their scaffold differed in other aspects also. The identity of the halogen atom – fluorine, chlorine, or bromine – did not greatly affect the compounds' (**20d-f**) SphK2 potency, reducing the enzyme activity to ~ 50%. Moving the halogen group to the *meta* or *ortho* position (**20g-j**) resulted in a retention of SphK2 potency, but with a loss in SphK2 selectivity, producing only moderately SphK2 selective inhibitors.

Because of the SphK2 selectivity observed with halogen moieties at the *para* position, a bulkier electron-withdrawing trifluoromethyl group at the *para* position (**20k** (SLC4071411)) was synthesized. **20k** was more potent but less selective for SphK2. Interestingly, placement of the trifluoromethyl group at the *meta* position (**20l**) produced a more potent SphK inhibitor (28% inhibition). However, this molecule showed no selectivity for SphK1 vs. SphK2. The *ortho* trifluoromethyl analogue (**20m**) did not improve potency nor selectivity. These results suggest that the key binding interactions were lost with the *ortho*-substituted derivative likely due to a loss in CF₃ interactions with the residues Cys533, His556 and Tyr566 that are found at the end of the hydrophobic tunnel of the SphK2 binding pocket, which was shown⁶⁰ by our group to be important for strong inhibitor binding.

Replacement of the electron-withdrawing groups with electron-donating groups— methyl (**20n**), methyl ether (**20o**), and ethyl ether (**20p**) – at the *para* position markedly diminished SphK2 inhibition (<30%) (Table 1). Likewise, positioning the methyl ether to the *meta* position (**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^{65, 66} to the aryl ring. The resulting molecule (**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 only diminished by ~10%. However, shifting the trifluoromethyl ether moiety from the *para* position to the *meta* position (**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 di-substitution on the aryl ring of the aminothiazole were also explored (Table 2). Although poor inhibition activities were observed with mono-substituted aryl rings containing traditional electron-donating groups, we were curious about the effects of having di-substituted aryl rings. Compounds with either two methyl (**20t**) or two methyl ether moieties (**20u**) showed minimal activity. Placement of a methyl ether at the *para* position and the chlorine moiety at the *meta* position (**20v**) led to modest SphK2 activity (~70%) and no SphK1 inhibition. In contrast, di-substitution with electron-withdrawing groups reestablished SphK2 potency, particularly fluorines (**20w**, **20z**), chlorine (**20x**), or a combination of fluorine with a trifluoromethyl group (**20y** (SLC4091423), **20aa** (SLC4091424), **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 (**20bb**) led to a loss in compound potency, but this loss in potency was met with a gain in selectivity, as it was inactive towards SphK1 at concentrations up to 1 μ M. Together, these results reinforce the preference for electron deficient aryl ring.

Our previous studies^{63, 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 2). We discovered that the *para*-substituted biphenyl derivative (**20dd**) was not only potent towards SphK2 but also selective for SphK2. The *meta*-substituted biphenyl derivative (**23a**) essentially lost activity. However, a fluorine on the 4-position (**23b**) maintained the potency and selectivity observed with **20dd**. Attempts to substitute **20dd** with a trifluoromethyl moiety at either the 4- (**23c**) or 3-position (**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 (**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 2).

The co-crystal structure of SphK1 with **5** reported by Wang *et al.* notes a key hydrogen bonding interaction between the aminothiazole NH and SphK1 threonine-196.⁴⁴ 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 (**31**) or methylating (**34**) the amino group. The N-Me derivative **34** was found to be completely inactive in both SphKs up to 1 μ M while the ether derivative **31** showed moderate activity towards both SphKs, decreasing SphK1 and SphK2 activity by $\sim$ 45% at 0.3 μ M. These results suggest that the amino moiety plays a key role in enzyme binding likely through hydrogen bonding (compare **34** with **20dd**) although we cannot rule out the loss in SphK2 potency may also be due to steric effects created from the methylation. Comparison of **31** with the amino moiety **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 towards SphK1 and SphK2, we determined the inhibitory constant (K_i) of the most potent compounds in the library (Table 3). Consistent with the results of the initial screen (Tables 1 & 2), fluoro and trifluoromethyl positional isomers of disubstituted aryls **20y** and **20aa** had good activity with both enzymes and partial selectivity towards SphK2 ($\sim$ 5 fold) (entries 1-2). Fortunately, monosubstitution with a *meta*-trifluoromethyl group (**20l**) by removal of the fluorine atom from **20y** or **20aa** resulted in improved binding inhibition (entry 3). **20l** is a dual inhibitor with K_i of 120 nM and 90 nM with 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 trifluoromethylether on the *para* position (**20r**) improved the K_i towards SphK2 (250 nM) while decreasing inhibitory activity against SphK1 (K_i 5 μ M), affording 20-fold selectivity towards SphK2 (entry 5). The introduction of a more lipophilic and larger substituent such as a phenyl ring on the *para* position (**20dd**) resulted in a potent and selective SphK2 inhibitor (K_i 90 nM). To the best of our knowledge, **20dd** is the most potent SphK2 reported to date; this compound is 100-fold selective towards SphK2 vs SphK1. In comparing compounds **20k**, **20r**, and **20dd**, it is interesting to note that as the steric bulk and lipophilicity of the molecules (cLogP 5.18, 5.42 and 6.18, respectively) increased, the inhibition constant and selectivity improved. These results are consistent with the observation that the hydrophobic binding pocket of SphK2 is larger than SphK1.

We selected the most potent, SphK2 selective (**20dd**) and SphK1/2 dual inhibitors (**20l**) towards 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 4A/C) while Sph levels remained constant (Figure 4B/D), indicating that the compounds are cell permeable and capable of inhibiting SphK activity in whole cells. Further, the selectivity of **20dd** towards SphK2 *in vitro* is observed as its effect on S1P level is lower than **20l**—S1P is still generated by functional SphK1. To assess the *in vivo* properties of **20dd**, C57BL/6 mice were treated with a single 10 mg/kg intraperitoneal dose. S1P concentration in blood was monitored over a course of 24 hours via LC-MS-MS. As shown in Figure 5, blood S1P

levels increased on treatment with the SphK2 selective inhibitor **20dd** at two and a six-hour time points and returned to basal levels after 24 hours. Such an observation is in accordance with three SphK2 selective inhibitors^{28, 29, 47} and supports the notion that SphK2 selective inhibitors drive elevated S1P levels in whole blood.²⁸ To date, **20dd** is the most potent and selective SphK2 inhibitor reported with *in vivo* activity.

CONCLUSIONS

Herein, we disclose the discovery and development of the most potent and selective SphK2 inhibitor reported to date. The scaffold contains a 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** and aminothiazole from **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 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 significant loss in activity.

Biological analysis of the aminothiazole inhibitors **20l** and **20dd** effectively lower S1P levels in U937 cells. *In vivo* study of the SphK2 selective **20dd** caused elevated blood S1P levels in wildtype mice, recapitulating our previous findings^{28, 29, 47, 60} with SphK2 selective inhibitors. Collectively, our work provides a novel chemical biology approach towards selective SphK2 and dual SphK inhibition. We expect that these studies will aid in elucidating the *in vivo* function of SphK2 as well as the development of improved SphK inhibitors.

EXPERIMENTAL SECTION

Sphingosine Kinase assays

The inhibitory activity of the synthesized compounds on human SphK1 and SphK2 was determined using a previously published method.^{28, 47} Recombinant human SphK1 or SphK2 isolated from a cell lysate was briefly incubated with (0.3 μ M) 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.

Sample Preparation and LC-MS-MS Analysis

Sample preparation protocols were from our previous publication²⁹ with minor modifications. Cell pellets ($2-3 \times 10^6$ cells), whole blood (10 μ l) or plasma (10 μ l) was mixed with 2 ml of a methanol:chloroform solution (3:1) and transferred to a capped glass vial. Suspensions were supplemented with 10 μ l of internal standard solution containing 10 pmoles 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 μ l 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 μ l of glacial acetic acid and transferred to 2 ml microcentrifuge tubes. Samples were then centrifuged at 12,000 $\times$ 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 $\times$ g for 12 min at 4 °C. Fifty μ L 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 $\times$ 2.1 mm, 5 μ m 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 $\rightarrow$ 264.4); deuterated (D7)C₁₈S1P (387.4 $\rightarrow$ 271.3); sphingosine (300.5 $\rightarrow$ 264.4); deuterated (D7) sphingosine (307.5 $\rightarrow$ 271.3).

Pharmacokinetic Analysis

Mouse studies were conducted using a previously reported method.⁴⁷ **20dd** (10 mg/kg) and **20l** (3 mg/kg) were 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.

Molecular Docking

Molecular docking was performed using compounds (**2**, **20x**) to assess potential difference in position in the binding pocket of SphK2. The SphK2 model, with ATP and Mg²⁺ bound, was generated with Molecular Operating Environment (MOE) and energy minimized as previously described.⁶⁰ 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 Tools⁶⁸ was used to prepare the protein and ligand files, while AutoDock Vina⁶⁹ was used to perform the docking for pose prediction. The grid box was set to 20 $\times$ 20 $\times$ 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 performed⁶⁰ 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.

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 μ m, F254, and column chromatography was performed on flash grade silica gel, 40-63 μ m, using a Combiflash Rf purification system. ^1H 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. ^1H NMR chemical shifts are reported in ppm with the solvent resonance as an internal standard (CDCl_3 : 7.26 ppm; CD_3OD : 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_3OD : 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 ^1H NMR and HPLC analyses unless noted otherwise.

General Procedure A: 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 $^\circ\text{C}$. 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 B: 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 $^\circ\text{C}$ 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_2SO_4 , filtered, and concentrated *via* vacuum. The resulting residue was purified by silica gel column chromatography.

General Procedure C: Coupling of 6 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 **6** (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 D: Suzuki Coupling of aryl bromides with phenyl boronic acids—Cs₂CO₃ (2 equiv) was added to a solution 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 E: 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 F: 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.

N-(4-cyanophenyl)-2,2,2-trifluoroacetamide (13): 4-aminobenzonitrile **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 **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, ²*J*_{CF} = 38.4 Hz), 156.76 (q, ²*J*_{CF} = 38.4 Hz), 142.17, 122.19, 121.26 (q, ¹*J*_{CF} = 281.3 Hz), 119.35, 118.57 (q, ¹*J*_{CF} = 281.3 Hz), 115.70 (q, ¹*J*_{CF} = 281.3 Hz), 112.85 (q, ¹*J*_{CF} = 281.3 Hz), 109.84; ¹⁹F NMR (376 MHz, CD₃OD) δ -75.64 (s, 3F); HRMS (ESI-): Calcd for C₉H₄F₃N₂O [M-H]⁻: 213.0275, Found: 213.0273.

(Z)-2,2,2-trifluoro-N-(4-(N'-hydroxycarbamimidoyl)phenyl)acetamide

(14): Synthesized by General Procedure A. 330 mg, 57% as white solid. ¹H NMR (400 MHz, CD₃OD) δ 7.39 (d, *J* = 8.9 Hz, 2H), 6.70 (d, *J* = 8.9 Hz, 2H); ¹³C NMR (101 MHz, acetone-*d*₆) δ 156.22 (q, ²*J*_{CF} = 38.4 Hz), 155.85 (q, ²*J*_{CF} = 38.4 Hz), 155.48 (q, ²*J*_{CF} = 38.4 Hz), 155.11 (q, ²*J*_{CF} = 38.4 Hz), 152.17, 138.14, 131.54, 127.11, 121.23, 120.91 (q, ¹*J*_{CF} = 280.2 Hz), 118.29 (q, ¹*J*_{CF} = 280.2 Hz), 115.42 (q, ¹*J*_{CF} = 280.2 Hz), 112.56 (q, ¹*J*_{CF} = 280.2 Hz); ¹⁹F NMR (471 MHz, acetone-*d*₆) δ -76.14 (s, 3F); HRMS (ESI⁺): Calcd for C₉H₈F₃N₃O₂ [M+H]⁺: 248.0659, Found: 248.1822.

tert-butyl (S)-2-((3-(4-(2,2,2-trifluoroacetamido)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (15):

Synthesized by General Procedure B. 454 mg, 67%, yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, cdcl₃) δ 177.39, 167.67, 155.56, 155.19 (q, ²*J*_{CF} = 53.5 Hz), 154.79 (q, ²*J*_{CF} = 53.5 Hz), 154.47 (q, ²*J*_{CF} = 53.5 Hz), 153.94 (q, ²*J*_{CF} = 53.5 Hz), 138.54, 128.45, 124.30, 120.89, 120.11 (q, ¹*J*_{CF} = 289.9 Hz), 117.24 (q, ¹*J*_{CF} = 289.9 Hz), 114.37 (q, ¹*J*_{CF} = 289.9 Hz), 111.51 (q, ¹*J*_{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*; ¹⁹F NMR (470 MHz, CDCl₃) δ -74.47 (s, 3F); HRMS (ESI⁺): Calcd for C₂₀H₂₃F₃N₄NaO₄ [M+Na]⁺: 463.1569, Found: 463.1546.

tert-butyl (S)-2-((3-(4-aminophenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-

carboxylate (16): (*S*)-*tert*-butyl-2-(3-(4-iodophenyl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate **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 **16** (45 mg, 82%) as a clear solid without further purification. ¹H 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 C₁₈H₂₄N₄NaO₃ [M+Na]⁺: 367.1746, Found: 367.1743.

tert-butyl (S)-2-((3-(4-thioureidophenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-

carboxylate (17): *tert*-butyl (*S*)-2-((3-(4-aminophenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate **16** (100 mg, 0.290 mmol) was dissolved in THF (4 mL). Di(1*H*-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 **17** (101 mg, 86%) as an off-white solid. ¹H 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); ^{13}C NMR (101 MHz, CDCl_3) 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 $\text{C}_{19}\text{H}_{25}\text{N}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 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 (18a): Synthesized by General Procedure C. 42 mg, 56%, yellow amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 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*, 29.85*, 28.64, 23.72*, 22.93*; HRMS (ESI+): Calcd for $\text{C}_{27}\text{H}_{29}\text{N}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 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 (18b): Synthesized by General Procedure C. 25 mg, 39%, off-white solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 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 $\text{C}_{26}\text{H}_{29}\text{N}_6\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 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 (18c): Synthesized by General Procedure C. 28 mg, 75%, off-white solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 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 $\text{C}_{26}\text{H}_{29}\text{N}_6\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 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 (18d): Synthesized by General Procedure C. 21 mg, 66%, light yellow solid. ^1H 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); ^{13}C NMR (101 MHz, CDCl_3) δ 177.23, 167.97, 164.00 (d, $f^1 J_{\text{CF}} = 248.5$ Hz), 163.13, 161.54 (d, $f^1 J_{\text{CF}} = 248.5$ Hz), 158.61, 154.41, 151.31, 150.70, 142.80, 130.86 (d, $f^4 J_{\text{CF}} = 3.0$), 130.83 (d, $f^4 J_{\text{CF}} = 3.0$ Hz), 129.23, 128.95, 128.03 (d, $f^3 J_{\text{CF}} = 8.1$ Hz),

127.95 (d, $^3J_{\text{CF}} = 8.1$ Hz), 125.54, 120.95, 117.37, 115.83 (d, $^2J_{\text{CF}} = 22.2$), 115.6 (d, $^2J_{\text{CF}} = 22.2$ 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 – -116.29 (m, 1F); HRMS (ESI+): Calcd for $\text{C}_{27}\text{H}_{28}\text{FN}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 522.1975, Found: 522.1966.

tert-butyl (S)-2-((3-(4-((4-(4-chlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18e): Synthesized by General Procedure C. 30 mg, 77%, yellow solid. ^1H 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*, 46.36*, 31.75*, 31.00*, 30.10*, 29.68*, 28.48, 23.54*, 22.76*; HRMS (ESI+): Calcd for $\text{C}_{27}\text{H}_{28}\text{ClN}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{Na}]^+$: 560.1499, Found 560.1502.

tert-butyl (S)-2-((3-(4-((4-(4-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18f): Synthesized by General Procedure C. 73 mg, 84%, off-white amorphous solid. ^1H 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), 3.50–3.27 (m, 3H), 3.15 – 2.99 (m, 1H), 2.13 – 2.03 (m, 1H), 1.95–1.78 (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 $\text{C}_{27}\text{H}_{28}\text{BrN}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{Na}]^+$: 604.0993, Found: 604.0967.

tert-butyl (S)-2-((3-(4-((4-(3-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18g): Synthesized by General Procedure C. 58 mg, 90%, off-white solid. ^1H 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, $J = 8.5, 1\text{H}$), 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, $^1J_{\text{CF}} = 246.4$ Hz), 163.06, 162.08 (d, $^1J_{\text{CF}} = 246.4$ Hz), 154.42, 150.44, 142.71, 136.72 (d, $^3J_{\text{CF}} = 8.1$ Hz), 136.64 (d, $^3J_{\text{CF}} = 8.1$ Hz), 130.35 (d, $^3J_{\text{CF}} = 9.1$ Hz), 130.26 (d, $^3J_{\text{CF}} = 9.1$ Hz), 128.97, 121.80 (d, $^4J_{\text{CF}} = 2.0$ Hz), 121.78 (d, $^4J_{\text{CF}} = 2.0$ Hz), 121.11, 120.88 117.44, 115.05 (d, $^2J_{\text{CF}} = 21.2$ Hz), 114.84 (d, $^2J_{\text{CF}} = 21.2$ Hz), 113.36 (d, $^2J_{\text{CF}} = 23.2$ Hz), 113.13 (d, $^2J_{\text{CF}} = 21.2$ 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*, 22.93*; ^{19}F NMR (376 MHz, CDCl_3) δ -112.92 – -113.24 (m, 1F); HRMS (ESI+): Calcd for $\text{C}_{27}\text{H}_{28}\text{FN}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{Na}]^+$: 544.1795, Found: 544.1784.

tert-butyl (S)-2-((3-(4-((4-(3-chlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18h): Synthesized by General Procedure C. 43 mg, 63%, white solid. ^1H NMR (400 MHz, CDCl_3) 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 (ddd, J = 22.4, 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); ^{13}C NMR (101 MHz, CDCl_3) 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 $\text{C}_{27}\text{H}_{28}\text{ClN}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 538.1680, Found: 538.1679.

tert-butyl (S)-2-((3-(4-((4-(3-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18i): Synthesized by General Procedure C. 90 mg, 78%, yellow amorphous solid. ^1H NMR (400 MHz, CDCl_3) 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); ^{13}C NMR (101 MHz, CDCl_3) 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*, 28.64*, 23.71*, 22.92*; HRMS (ESI+): Calcd for $\text{C}_{27}\text{H}_{28}\text{BrN}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{Na}]^+$: 604.0993, Found: 604.0988.

tert-butyl (S)-2-((3-(4-((4-(2-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18j): Synthesized by General Procedure C. 25 mg, 56%, off-white amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 8.16 (td, J = 7.7, 2.0 Hz, 1H), 8.06 (d, J = 7.2 Hz, 2H), 7.75 (s, 1H), 7.58 (d, J = 8.5 Hz, 2H), 7.32 – 7.18 (m, 3H), 7.13 (ddd, J = 12.0, 7.9, 1.5 Hz, 1H), 4.40 – 4.22 (m, 1H), 3.54 – 3.24 (m, 3H), 3.16 – 2.99 (m, 1H), 2.14 – 2.01 (m, 1H), 1.96 – 1.77 (m, 3H), 1.48 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ 191.63, 177.19, 168.00, 161.95, 161.71 (d, $^1J_{\text{CF}}$ = 251.5 Hz), 159.22 (d, $^1J_{\text{CF}}$ = 251.5 Hz), 154.41, 145.30, 142.90, 130.07 (d, $^4J_{\text{CF}}$ = 3.0 Hz), 130.04 (d, $^4J_{\text{CF}}$ = 3.0 Hz), 129.17 (d, $^3J_{\text{CF}}$ = 9.1 Hz), 129.08 (d, $^3J_{\text{CF}}$ = 9.1 Hz), 128.92, 124.54, 124.50, 122.39 (d, $^3J_{\text{CF}}$ = 11.1 Hz), 122.28 (d, $^3J_{\text{CF}}$ = 11.1 Hz), 120.69, 117.34, 117.24, 116.15 (d, $^2J_{\text{CF}}$ = 22.2 Hz), 115.93 (d, $^2J_{\text{CF}}$ = 22.2 Hz), 108.05 (d, $^3J_{\text{CF}}$ = 16.2 Hz), 107.89 (d, $^3J_{\text{CF}}$ = 16.2 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) δ -114.11 (s, 1F); HRMS (ESI+): Calcd for $\text{C}_{27}\text{H}_{28}\text{FN}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{Na}]^+$: 544.1795, Found: 544.1782.

tert-butyl (S)-2-((3-(4-((4-(4-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18k): Synthesized by General Procedure C. 83 mg, 70%, off-white solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 177.19, 167.99, 163.35, 154.44, 150.17, 142.83, 137.74, 130.26 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 129.94 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 129.62 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 129.30 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 128.89, 126.38, 125.82 (q, $^3J_{\text{CF}}$ = 4.0 Hz), 125.78 (q, $^3J_{\text{CF}}$ = 4.0 Hz), 125.74 (q, $^3J_{\text{CF}}$ = 4.0 Hz), 125.70 (q, $^3J_{\text{CF}}$ = 4.0 Hz), 122.97 (q, $^1J_{\text{CF}}$ = 224.2 Hz), 120.90, 120.75 (q, $^1J_{\text{CF}}$ = 224.2 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) δ -62.49 (s, 3F); HRMS (ESI+): Calcd for $\text{C}_{28}\text{H}_{29}\text{F}_3\text{N}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 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 (18l): Synthesized by General Procedure C. 45 mg, 79%, yellow amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 177.12, 168.02, 163.39, 154.49, 150.03, 142.94, 135.24, 131.58 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 131.25 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 130.93 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 130.61 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 129.32, 129.22, 128.82, 125.58 (q, $^1J_{\text{CF}}$ = 165.2 Hz), 124.56 (q, $^3J_{\text{CF}}$ = 4.0 Hz), 124.52 (q, $^3J_{\text{CF}}$ = 4.0 Hz), 124.49 (q, $^3J_{\text{CF}}$ = 4.0 Hz; q, $^1J_{\text{CF}}$ = 165.2 Hz), 124.45 (q, $^3J_{\text{CF}}$ = 4.0 Hz), 122.95, 122.91 (q, $^1J_{\text{CF}}$ = 165.2 Hz), 122.87, 120.65 (q, $^1J_{\text{CF}}$ = 165.2 Hz), 120.50, 117.37, 103.95, 80.40*, 79.90*, 55.35*, 55.20*, 46.86*, 46.51⁸, 31.81*, 31.10*, 30.99*, 30.25*, 28.59, 23.62*, 22.86*; ^{19}F NMR (376 MHz, CDCl_3) δ -62.71 (s, 3F). HRMS (ESI+): Calcd for $\text{C}_{28}\text{H}_{28}\text{F}_3\text{N}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{Na}]^+$: 594.1763, Found: 594.1767.

tert-butyl (S)-2-((3-(4-((4-(2-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18m): Synthesized by General Procedure C. 45 mg, 79%, yellow amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 191.63, 177.20, 167.97, 162.95, 154.38, 148.62, 142.83, 134.47, 132.09, 131.72, 129.01 (q, $^2J_{\text{CF}}$ = 30.3 Hz), 128.86, 128.71 (q, $^2J_{\text{CF}}$ = 30.3 Hz), 128.40, 128.38, 128.32 (q, $^2J_{\text{CF}}$ = 30.3 Hz), 128.10 (q, $^1J_{\text{CF}}$ = 275.7 Hz), 126.66 (q, $^3J_{\text{CF}}$ = 6.1 Hz), 126.60 (q, $^3J_{\text{CF}}$ = 6.1 Hz), 126.55 (q, $^3J_{\text{CF}}$ = 6.1 Hz), 126.49 (q, $^3J_{\text{CF}}$ = 6.1 Hz), 125.59 (q, $^1J_{\text{CF}}$ = 275.7 Hz), 122.87 (q, $^1J_{\text{CF}}$ = 275.7 Hz), 120.93, 120.73, 120.14 (q, $^1J_{\text{CF}}$ = 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 $\text{C}_{28}\text{H}_{28}\text{F}_3\text{N}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{H}]^+$: 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 (18n): Synthesized by General Procedure C. 30 mg, 47%, off-white amorphous solid. ^1H 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 $\text{C}_{28}\text{H}_{32}\text{N}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 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 (18o): Synthesized by General Procedure C. 30 mg, 75%, clear amorphous solid. ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (101 MHz, CDCl₃) 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 C₂₈H₃₂N₅O₄S [M + H]⁺: 534.2175, Found: 534.2160.

tert-butyl (S)-2-((3-(4-((4-(4-ethoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18p): Synthesized by General Procedure C. 81 mg, 92%, light yellow amorphous solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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 C₂₉H₃₄N₅O₄S [M + H]⁺: 548.2332, Found: 548.2308.

tert-butyl (S)-2-((3-(4-((4-(3-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18q): Synthesized by General Procedure C. 28 mg, 70%, yellow amorphous solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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 C₂₈H₃₂N₅O₄S [M + H]⁺: 534.2175, Found: 534.2182.

tert-butyl (S)-2-((3-(4-((4-(4-(trifluoromethoxy)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18r): Synthesized by General Procedure C. 27 mg, 30%, yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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 Hz), 121.09, 119.16 (q, ¹*J*_{CF} = 258.6 Hz), 117.22, 103.12, 80.09*, 79.64*, 55.18*, 55.06*, 46.73*, 46.34*, 31.73*, 30.98*, 30.83*, 30.06*, 29.68, 28.46*, 23.53*, 22.74*; ¹⁹F NMR (376 MHz, CDCl₃) δ –57.82 (s, 3F); HRMS (ESI+): Calcd for C₂₈H₂₈F₃N₅O₄S [M + H]⁺: 588.1892, Found: 588.1916.

tert-butyl (S)-2-((3-(4-((4-(4-(trifluoromethoxy)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate

(18s): Synthesized by General Procedure C. 27 mg, 30%, yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.08 (d, *J* = 8.3 Hz, 2H), 7.78 (dt, *J* = 7.9, 1.3 Hz, 1H), 7.73 (dt, *J* = 2.9, 1.4 Hz, 1H), 7.64 (s, 1H), 7.57 (d, *J* = 7.7 Hz, 2H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.17 (ddt, *J* = 8.1, 2.3, 1.1 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); ¹³C NMR (101 MHz, CDCl₃) δ 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 Hz), 120.96, 120.32 (q, ¹*J*_{CF} = 156.6 Hz), 119.41, 118.87 (q, ¹*J*_{CF} = 156.6 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*; ¹⁹F NMR (376 MHz, CDCl₃) δ –57.82 (s, 3F); HRMS (ESI+): Calcd for C₂₈H₂₈F₃N₅NaO₄S [M+Na]⁺: 610.1712, Found: 610.1721.

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

(18t): Synthesized by General Procedure C. 30 mg, 75%, yellow amorphous solid. ¹H NMR (400 MHz, CDCl₃) δ 8.30 – 8.08 (m, 1H), 8.03 (d, *J* = 8.1 Hz, 2H), 7.63 (s, 1H), 7.57 (d, *J* = 7.7, 2.1 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.15 (d, *J* = 7.9 Hz, 1H), 6.82 (s, 1H), 4.30 (d, *J* = 29.1 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); ¹³C NMR (101 MHz, CDCl₃) 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 C₂₉H₃₄N₅O₃S [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

(18u): Synthesized by General Procedure C. 25 mg, 63%, yellow amorphous solid. ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (101 MHz, cdcl₃) 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 C₂₉H₃₄N₅O₅S [M+H]⁺: 564.2281, Found: 564.2271.

tert-butyl (S)-2-((3-(4-((4-(3-chloro-4-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate

(18v): Synthesized by General Procedure C. 83 mg, 91%, light yellow amorphous solid. ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (101 MHz, CDCl₃) 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 C₂₈H₃₁ClN₅O₄S [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 (18w): Synthesized by General Procedure C. 21 mg, 58%, off-white solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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), 151.57 (dd, ²*J*_{CF} = 36.4 Hz, ³*J*_{CF} = 13.1 Hz), 151.45 (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*; ¹⁹F NMR (376 MHz, CDCl₃) δ -137.57, -138.63; HRMS (ESI+): Calcd for C₂₇H₂₇F₂N₅NaO₃S [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 (18x): Synthesized by General Procedure C. 42 mg, 59%, yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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 C₂₇H₂₈Cl₂N₅O₃S [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 (18y): Synthesized by General Procedure C. 21 mg, 24%, clear yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 177.27, 167.92, 163.40, 160.64 (d, ¹*J*_{CF} = 257.6 Hz), 158.09 (d, ¹*J*_{CF} = 257.6 Hz), 154.42, 149.25, 142.64, 131.46 (d, ³*J*_{CF} = 8.1 Hz), 131.38 (d, ³*J*_{CF} = 8.1 Hz), 131.14 (d, ⁴*J*_{CF} = 4.0 Hz), 131.10 (d, ⁴*J*_{CF} = 4.0 Hz), 128.96, 126.76 (q, ¹*J*_{CF} = 272.7 Hz), 125.04 (qd, ³*J*_{CF} = 5.1 Hz, ⁴*J*_{CF} = 1.0 Hz), 125.03 (qd, ³*J*_{CF} = 5.1 Hz, ⁴*J*_{CF} = 1.0 Hz), 125.00 (qd, ³*J*_{CF} = 5.1 Hz, ⁴*J*_{CF} = 1.0 Hz), 124.98 (qd, ³*J*_{CF} = 5.1 Hz, ⁴*J*_{CF} = 1.0 Hz), 124.95, 124.94, 124.05 (q, ¹*J*_{CF} = 272.7 Hz), 121.35 (q, ¹*J*_{CF} = 272.7 Hz), 120.99, 118.96 (d, ³*J*_{CF} = 13.1 Hz), 118.83 (d, ³*J*_{CF} = 13.1 Hz), 118.63 (q, ¹*J*_{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) δ -61.47 (d, J = 12.6 Hz 3F), -115.74 (br. s, 1F); HRMS (ESI+): Calcd for $\text{C}_{28}\text{H}_{27}\text{F}_4\text{N}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{Na}]^+$: 612.1668, Found: 612.1663.

tert-butyl (S)-2-((3-(4-((4-(3,5-difluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18z):

Synthesized by General Procedure C. 37 mg, 76%, yellow amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 177.21, 168.01, 164.70 (dd, $^1J_{\text{CF}}$ = 248.5 Hz, $^3J_{\text{CF}}$ = 13.1 Hz), 164.57 (dd, $^1J_{\text{CF}}$ = 248.5 Hz, $^3J_{\text{CF}}$ = 13.1 Hz), 163.17, 162.24 (dd, $^1J_{\text{CF}}$ = 248.5 Hz, $^3J_{\text{CF}}$ = 13.1 Hz), 162.11 (dd, $^1J_{\text{CF}}$ = 248.5 Hz, $^3J_{\text{CF}}$ = 13.1 Hz), 154.47, 149.46, 149.42, 142.70, 137.75 (d, $^2J_{\text{CF}}$ = 20.2 Hz), 137.65, 137.55 (d, $^2J_{\text{CF}}$ = 20.2 Hz), 128.93, 120.87, 117.48, 109.16 (d, $^2J_{\text{CF}}$ = 27.3 Hz), 109.08 (d, $^3J_{\text{CF}}$ = 11.1 Hz), 108.97 (d, $^3J_{\text{CF}}$ = 11.1 Hz), 108.89 (d, $^2J_{\text{CF}}$ = 27.3 Hz), 104.66, 103.48 (d, $^2J_{\text{CF}}$ = 51.5 Hz), 103.23, 102.97 (d, $^2J_{\text{CF}}$ = 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 $\text{C}_{27}\text{H}_{28}\text{F}_2\text{N}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 540.1881, Found: 540.1845.

tert-butyl (S)-2-((3-(4-((4-(3-fluoro-5-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate

(18aa): Synthesized by General Procedure C. 39 mg, 44%, yellow amorphous solid. ^1H 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, 0H), 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, $^1J_{\text{CF}}$ = 248.5 Hz), 163.39, 161.70 (d, $^1J_{\text{CF}}$ = 248.5 Hz), 154.47, 149.04, 142.61, 137.75 (d, $^3J_{\text{CF}}$ = 8.1 Hz), 137.67 (d, $^3J_{\text{CF}}$ = 8.1 Hz), 133.44 (qd, $^2J_{\text{CF}}$ = 33.3 Hz, $^3J_{\text{CF}}$ = 8.1 Hz), 133.36 (qd, $^2J_{\text{CF}}$ = 33.3 Hz, $^3J_{\text{CF}}$ = 8.1 Hz), 133.11(qd, $^2J_{\text{CF}}$ = 33.3 Hz, $^3J_{\text{CF}}$ = 8.1 Hz), 133.03, 132.78 (qd, $^2J_{\text{CF}}$ = 33.3 Hz, $^3J_{\text{CF}}$ = 8.1 Hz), 132.70 (qd, $^2J_{\text{CF}}$ = 33.3 Hz, $^3J_{\text{CF}}$ = 8.1 Hz), 132.45 (qd, $^2J_{\text{CF}}$ = 33.3 Hz, $^3J_{\text{CF}}$ = 8.1 Hz), 132.37 (qd, $^2J_{\text{CF}}$ = 33.3 Hz, $^3J_{\text{CF}}$ = 8.1 Hz), 128.96, 127.50 (q, $^1J_{\text{CF}}$ = 272.7 Hz), 124.81 (q, $^1J_{\text{CF}}$ = 272.7 Hz), 122.10 (q, $^1J_{\text{CF}}$ = 272.7 Hz), 121.00, 119.36 (q, $^1J_{\text{CF}}$ = 272.7 Hz), 118.70, 118.66, 118.63, 117.53, 116.59 (d, $^2J_{\text{CF}}$ = 23.2 Hz), 116.36 (d, $^2J_{\text{CF}}$ = 23.2 Hz), 112.11 (dq, $^2J_{\text{CF}}$ = 23.2 Hz, $^3J_{\text{CF}}$ = 4.0 Hz), 112.08 (dq, $^2J_{\text{CF}}$ = 23.2 Hz, $^3J_{\text{CF}}$ = 4.0 Hz), 111.87 (dq, $^2J_{\text{CF}}$ = 23.2 Hz, $^3J_{\text{CF}}$ = 4.0 Hz), 111.83 (dq, $^2J_{\text{CF}}$ = 23.2 Hz, $^3J_{\text{CF}}$ = 4.0 Hz), 105.06, 80.35*, 79.91*, 55.33, 46.90*, 46.54*, 31.89*, 31.16*, 30.31, 28.63, 23.69*, 22.93*; ^{19}F NMR (376 MHz, cdcl_3) δ ^{19}F NMR (376 MHz, CDCl_3) δ -62.87 (s, 3F), -110.68 (q, J = 8.2, 7.6 Hz, 1F); HRMS (ESI+): Calcd for $\text{C}_{28}\text{H}_{28}\text{F}_4\text{N}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 590.1849, Found: 590.1843.

tert-butyl (S)-2-((3-(4-((4-(3,5-bis(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate

(18bb): Synthesized by General Procedure C. 33 mg, 49%, yellow amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 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_3) δ 177.28, 167.93, 163.64, 154.46, 148.68, 142.53, 136.44, 132.65 (d, $^2J_{\text{CF}}$ = 33.3 Hz), 132.32 (q, $^2J_{\text{CF}}$ = 33.3 Hz), 131.99 (q, $^2J_{\text{CF}}$ = 33.3 Hz), 131.66 (d, $^2J_{\text{CF}}$ = 33.3 Hz), 129.00, 127.56 (q, $^1J_{\text{CF}}$ = 273.7 Hz), 126.12, 124.85 (q, $^1J_{\text{CF}}$ = 273.7 Hz), 122.14 (q, $^1J_{\text{CF}}$ = 273.7 Hz), 121.35, 119.42 (q, $^1J_{\text{CF}}$ = 273.7 Hz), 117.59, 105.45, 80.36*, 79.91*, 55.31, 46.90*, 46.55*, 31.91*, 31.18*, 30.32, 28.64, 23.69*, 22.94*; ^{19}F NMR (376 MHz, CDCl_3) δ –62.96 (s, 6F); HRMS (ESI+): Calcd for $\text{C}_{29}\text{H}_{27}\text{F}_6\text{N}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{Na}]^+$: 662.1636, Found: 662.1627.

tert-butyl (S)-2-((3-(4-((4-(2-fluoro-5-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate

(18cc): Synthesized by General Procedure C. 57 mg, 78%, yellow amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 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_3) δ 177.24, 167.94, 163.19 (d, $^1J_{\text{CF}}$ = 256.5 Hz), 162.29, 160.65 (d, $^1J_{\text{CF}}$ = 256.6 Hz), 154.46, 143.80, 142.71, 128.95, 128.00 (q, $^1J_{\text{CF}}$ = 272.7 Hz), 127.72, 127.67, 127.61, 127.38 (q, $^2J_{\text{CF}}$ = 33.3 Hz), 127.05 (q, $^2J_{\text{CF}}$ = 33.3 Hz), 126.72 (q, $^2J_{\text{CF}}$ = 33.3 Hz), 126.00, 125.30 (q, $^1J_{\text{CF}}$ = 272.7 Hz), 123.11 (d, $^3J_{\text{CF}}$ = 12.1 Hz), 122.99 (d, $^3J_{\text{CF}}$ = 12.1 Hz), 122.59 (q, $^1J_{\text{CF}}$ = 272.7 Hz), 120.89, 119.89 (q, $^1J_{\text{CF}}$ = 272.7 Hz), 117.43, 117.08, 116.81 (d, $^2J_{\text{CF}}$ = 24.2 Hz), 116.57 (d, $^2J_{\text{CF}}$ = 24.2 Hz), 109.27 (d, $^2J_{\text{CF}}$ = 16.2 Hz), 109.11 (d, $^2J_{\text{CF}}$ = 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 $\text{C}_{28}\text{H}_{27}\text{F}_4\text{N}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{Na}]^+$: 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 (18dd):

Synthesized by General Procedure C. 13 mg, 19%, light yellow amorphous solid. ^1H 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 $\text{C}_{33}\text{H}_{34}\text{N}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 580.2382, Found: 580.2377.

tert-butyl (S)-2-((3-(4-((4-(benzofuran-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (18ee):

Synthesized by General Procedure C. 24 mg, 71%, tan amorphous solid. ^1H 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 C₂₉H₂₉N₅NaO₄S [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 (19a): Synthesized by General Procedure E. 21 mg, 82%, off-white oily solid. ¹H NMR (500 MHz, CDCl₃) δ 8.07 (d, *J* = 8.3 Hz, 2H), 7.87 (d, *J* = 7.6 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 C₃₃H₄₀N₇O₅S [M+H]⁺: 646.2812, Found: 646.2805.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(pyridin-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19b): Synthesized by General Procedure E. 3 mg, 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 C₃₂H₃₉N₈O₅S [M+H]⁺: 647.2764, Found: 647.2772.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(pyridin-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19c): Synthesized by General Procedure E. 18 mg, 78%, yellow oily solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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 C₃₂H₃₈N₈NaO₅S [M+Na]⁺: 669.2584, Found: 669.2589.

tert-butyl (S,E)-(((tert-butoxycarbonyl)amino)(2-((3-(4-((4-(3-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (19d): Synthesized by General Procedure E. 10 mg, 43%, light yellow oily solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 176.79, 167.82, 163.97 (d, ¹*J*_{CF} = 247.5 Hz), 163.03, 161.52 (d, ¹*J*_{CF} = 247.5 Hz), 154.28, 150.72, 150.53,

142.67, 130.91, 130.76, 128.93, 128.00 (d, $^3J_{\text{CF}} = 8.1$ Hz), 127.92 (d, $^3J_{\text{CF}} = 8.1$ Hz), 120.72, 117.21, 115.83 (d, $^2J_{\text{CF}} = 21.2$ Hz), 115.62 (d, $^2J_{\text{CF}} = 21.2$ Hz), 102.16, 81.89, 79.74, 56.61, 50.34, 30.94, 30.44, 28.30; ^{19}F NMR (376 MHz, CDCl_3) δ -113.86 (p, $J = 6.7$ Hz, 1F); HRMS (ESI⁺): Calcd for $\text{C}_{33}\text{H}_{39}\text{FN}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 664.2717, Found: 664.2710.

tert-butyl (S,E)-(((tert-butoxycarbonyl)amino)(2-((3-(4-((4-(4-chlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (19e): Synthesized by General Procedure E. 16 mg, 57%, light yellow oily solid. ^1H 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 $\text{C}_{33}\text{H}_{38}\text{ClN}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 680.2422, Found: 680.2425.

tert-butyl (S,Z)-((2-((3-(4-((4-(4-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)((tert-butoxycarbonyl)imino)methyl)carbamate (19f): Synthesized by General Procedure E. 10 mg, 83%, off-white oily solid. ^1H 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 $\text{C}_{33}\text{H}_{39}\text{BrN}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 724.1917, Found: 724.1912.

tert-butyl (S,E)-(((tert-butoxycarbonyl)amino)(2-((3-(4-((4-(3-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (19g): Synthesized by General Procedure E. 10 mg, 57%, yellow oily solid. ^1H 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}C NMR (101 MHz, CDCl_3) δ 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}F NMR (376 MHz, CDCl_3) δ -113.07 – -113.17 (m, 1F); HRMS (ESI⁺): Calcd for $\text{C}_{33}\text{H}_{39}\text{FN}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 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 (19h): Synthesized by General Procedure E. 37 mg, 77%, light yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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 C₃₃H₃₈ClN₇O₅S [M+H]⁺: 680.2422, Found: 680.2435.

tert-butyl (S,Z)-((2-((3-(4-((4-(3-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)((tert-butoxycarbonyl)imino)methyl)carbamate (19i): Synthesized by General Procedure E. 21 mg, 57%, off-white oily solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) 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 C₃₃H₃₉BrN₇O₅S [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 (19j): Synthesized by General Procedure E. 25 mg, 86%, light yellow oily solid. ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (101 MHz, CDCl₃) 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} = 11.1 Hz), 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; ¹⁹F NMR (376 MHz, CDCl₃) δ –114.04 – –114.17 (m, 1F); HRMS (ESI⁺): Calcd for C₃₃H₃₈FN₇NaO₅S [M+Na]⁺: 686.2537, Found: 686.2550.

tert-butyl (S,E)-(((tert-butoxycarbonyl)amino)(2-((3-(4-((4-(4-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (19k): Synthesized by General Procedure E. 52 mg, 45%, off-white solid. ¹H NMR (400 MHz, CDCl₃) 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); ^{13}C NMR (101 MHz, CDCl_3) δ 176.49, 167.75, 163.18, 154.41, 150.11, 149.03, 142.73, 137.81, 130.19 (q, $^2J_{\text{CF}}$ = 32.8 Hz), 129.86 (q, $^2J_{\text{CF}}$ = 32.8 Hz), 129.54 (q, $^2J_{\text{CF}}$ = 32.8 Hz), 129.22 (q, $^2J_{\text{CF}}$ = 32.8 Hz), 128.77, 128.39, 126.38, 125.80 (q, $^4J_{\text{CF}}$ = 4.0 Hz), 125.75 (q, $^4J_{\text{CF}}$ = 4.0 Hz), 125.71 (q, $^4J_{\text{CF}}$ = 4.0 Hz), 125.68 (q, $^4J_{\text{CF}}$ = 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; ^{19}F NMR (376 MHz, CDCl_3) δ –62.53 (s, 3F); HRMS (ESI⁺): Calcd for $\text{C}_{34}\text{H}_{39}\text{F}_3\text{N}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 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 (19l): Synthesized by General Procedure E. 5 mg, 26%, off-white oily solid. ^1H NMR (400 MHz, CDCl_3) 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); ^{13}C NMR (101 MHz, CDCl_3) δ 176.38, 167.74, 163.12, 162.49, 154.55, 150.41, 150.18, 142.79, 135.41, 131.61 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 131.29 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 130.97 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 130.65 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 129.45, 129.24, 128.88, 128.73, 125.66, 124.53 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 124.48 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 124.45 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 124.41 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 122.96 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 122.92 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 122.89 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 122.85 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 120.34, 117.04, 103.79, 82.32, 79.98, 56.50, 50.48, 31.17, 30.47, 28.35, 28.22, 28.09; ^{19}F NMR (376 MHz, CDCl_3) δ –62.66; HRMS (ESI⁺): Calcd for $\text{C}_{34}\text{H}_{39}\text{F}_3\text{N}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 714.2685, Found: 714.2682.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((2-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19m): Synthesized by General Procedure E. 10 mg, 37%, yellow oily solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 191.61, 176.91, 167.90, 162.66 48 (q, $^3J_{\text{CF}}$ = 19.2 Hz), 162.47 48 (q, $^2J_{\text{CF}}$ = 319.2 Hz), 154.20, 150.48, 148.79, 142.69, 134.55 48 (q, $^1J_{\text{CF}}$ = 285.8 Hz), 132.10, 131.72 (q, $^1J_{\text{CF}}$ = 285.8 Hz), 128.97 48 (q, $^1J_{\text{CF}}$ = 285.8 Hz), 128.65, 128.32, 126.58 48 (q, $^1J_{\text{CF}}$ = 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; ^{19}F NMR (376 MHz, CDCl_3) δ –57.78; HRMS (ESI⁺): Calcd for $\text{C}_{34}\text{H}_{38}\text{F}_3\text{N}_7\text{NaO}_5\text{S}$ $[\text{M}+\text{Na}]^+$: 736.2505, Found: 714.2500.

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

(19n).Synthesized by General Procedure E: 21 mg, 82%, off-white oily solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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, 30.93, 30.44, 29.81, 28.30, 24.53, 21.44; HRMS (ESI⁺): Calcd for C₃₄H₄₂N₇O₅S [M+H]⁺: 660.2968, Found: 660.2991.

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

(19o): Synthesized by General Procedure E. 23 mg, 61%, clear oily solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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, 30.93, 30.44, 29.81, 28.30, 24.53, 21.44; HRMS (ESI⁺): Calcd for C₃₄H₄₂N₇O₆S [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

(19p): Synthesized by General Procedure E. 67 mg, 68%, light yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 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, 21H); ¹³C NMR (126 MHz, CDCl₃) δ 176.96, 167.94, 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 C₃₅H₄₄N₇O₆S [M+H]⁺: 690.3074, Found: 690.3084.

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

(19q): Synthesized by General Procedure E. 18 mg, 51%, clear oily solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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, 30.96, 30.43, 29.83, 28.29, 28.10, 24.50; HRMS (ESI⁺): Calcd for C₃₄H₄₂N₇O₆S [M+H]⁺: 676.2917, Found: 676.2900.

tert-butyl (S,E)-(((tert-butoxycarbonyl)amino)(2-((3-(4-((4-(4-(trifluoromethoxy)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (19r): Synthesized by General Procedure E. 27 mg, 70%) as a yellow oily solid. ¹H NMR (500 MHz, CDCl₃) δ 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); ¹³C NMR (126 MHz, CDCl₃) δ 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; ¹⁹F NMR (470 MHz, CDCl₃) δ –57.82 (s, 3F); HRMS (ESI+): Calcd for C₃₄H₃₈F₃N₇O₆S [M+H]⁺: 730.2634, Found: 730.2672.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(3-(trifluoromethoxy)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19s): Synthesized by General Procedure E. 20 mg, 83%, yellow oily solid. ¹H NMR (400 MHz, CDCl₃) δ 10.38 (s, 1H), 8.04 – 8.00 (m, 2H), 7.80 (ddd, *J* = 7.8, 1.6, 1.0 Hz, 1H), 7.73 (dt, *J* = 2.5, 1.3 Hz, 1H), 7.60 – 7.54 (m, 2H), 7.43 (t, *J* = 8.0 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 Hz, 1H), 2.32 (d, *J* = 11.6 Hz, 1H), 1.97 – 1.88 (m, 1H), 1.86 – 1.75 (m, 1H), 1.67 – 1.56 (m, 1H), 1.50 (s rot., 9H), 1.46 (s rot., 9H); ¹³C NMR (101 MHz, CDCl₃) δ 176.81, 163.00, 150.47 (q, ³*J*_{CF} = 32.8 Hz), 150.20 (q, ³*J*_{CF} = 32.8 Hz), 149.82 (q, ³*J*_{CF} = 32.8 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*, 28.24*, 28.14; ¹⁹F NMR (376 MHz, CDCl₃) δ –57.66; HRMS (ESI+): Calcd for C₃₄H₃₈F₃N₇O₆S [M+H]⁺: 730.2634, Found: 730.2635.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(3,4-dimethylphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19t): Synthesized by General Procedure E. 35 mg, 92%, clear oily solid. ¹H NMR (400 MHz, CDCl₃) δ 10.38 (s, 1H), 7.99 (d, *J* = 8.3 Hz, 2H), 7.67 – 7.51 (m, 6H), 7.17 (d, *J* = 7.8 Hz, 1H), 6.81 (s, 1H), 6.36 (d, *J* = 2.4 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 Hz, 1H), 2.30 (d, *J* = 11.3 Hz, 6H), 1.98 – 1.85 (m, 1H), 1.85 – 1.72 (m, 2H), 1.47 (s, 18H); ¹³C NMR (101 MHz, CDCl₃) δ 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; HRMS (ESI+): Calcd for C₃₅H₄₄N₇O₅S [M+H]⁺: 674.3125, Found: 674.3134.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(3,4-dimethoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19u): Synthesized by General Procedure E. 25 mg, 78%, clear oily solid. ¹H NMR (400 MHz, CDCl₃) 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 $\text{C}_{35}\text{H}_{44}\text{N}_7\text{O}_7\text{S}$ $[\text{M}+\text{H}]^+$: 706.3023, Found: 706.3028.

tert-butyl (S,Z)-((((tert-butoxycarbonyl)imino)(2-((3-(4-((3-chloro-4-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19v): Synthesized by General Procedure E. 17 mg, 34%, light yellow oil. ^1H 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 $\text{C}_{34}\text{H}_{41}\text{ClN}_7\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$: 710.2528, Found: 710.2542.

tert-butyl (S,Z)-((((tert-butoxycarbonyl)imino)(2-((3-(4-((3,4-difluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19w): Synthesized by General Procedure E. 10 mg, 43%, off-white solid. ^1H 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, $^1J_{\text{CF}}$ = 228.3 Hz, $^3J_{\text{CF}}$ = 17.2 Hz), 117.49 (dd, $^1J_{\text{CF}}$ = 228.3 Hz, $^3J_{\text{CF}}$ = 17.2 Hz), 117.19, 115.40 (dd, $^1J_{\text{CF}}$ = 228.3 Hz, $^3J_{\text{CF}}$ = 17.2 Hz), 115.22 (dd, $^1J_{\text{CF}}$ = 228.3 Hz, $^3J_{\text{CF}}$ = 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 – -137.74 (m, 1F), -138.64 – -138.84 (m, 1F); HRMS (ESI⁺): Calcd for $\text{C}_{33}\text{H}_{38}\text{F}_2\text{N}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 682.2623, Found: 682.2590.

tert-butyl (S,Z)-((((tert-butoxycarbonyl)imino)(2-((3-(4-((3,5-dichlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19x): Synthesized by General Procedure E. 20 mg, 45, clear yellow solid. ^1H 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 $\text{C}_{33}\text{H}_{38}\text{Cl}_2\text{N}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 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 (19y): Synthesized by General Procedure E. 12 mg, 48%, off-white solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 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, $^3J_{\text{CF}}$ = 9.1 Hz), 131.43 (d, $^3J_{\text{CF}}$ = 9.1 Hz), 131.19, 128.94, 126.81 (q, $^1J_{\text{CF}}$ = 273.7 Hz), 124.94, 124.09 (q, $^1J_{\text{CF}}$ = 273.7 Hz), 121.38 (q, $^1J_{\text{CF}}$ = 273.7 Hz), 120.93, 119.32, 118.64 (q, $^1J_{\text{CF}}$ = 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) δ –61.44 (d, J = 12.7 Hz, 3F), –115.54 – –116.19 (m, 1F); HRMS (ESI+): Calcd for $\text{C}_{34}\text{H}_{38}\text{F}_4\text{N}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 732.2591, Found: 732.2542.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(3,5-difluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19z): Synthesized by General Procedure E. 26 mg, 79%, yellow oil. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 176.41, 167.72, 164.69 (dd, $^1J_{\text{CF}}$ = 248.5 Hz, $^3J_{\text{CF}}$ = 13.1 Hz), 164.56 (dd, $^1J_{\text{CF}}$ = 248.5 Hz, $^3J_{\text{CF}}$ = 13.1 Hz), 162.95, 162.49, 162.23 (dd, $^1J_{\text{CF}}$ = 248.5 Hz, $^3J_{\text{CF}}$ = 13.1 Hz), 162.10 (dd, $^1J_{\text{CF}}$ = 248.5 Hz, $^3J_{\text{CF}}$ = 13.1 Hz), 154.55, 150.38, 149.46, 142.68, 137.86 (dd, $^3J_{\text{CF}}$ = 10.1 Hz), 137.76 (dd, $^3J_{\text{CF}}$ = 10.1 Hz), 137.66 (dd, $^3J_{\text{CF}}$ = 10.1 Hz), 128.77, 120.40, 117.07, 109.14 (dd, $^3J_{\text{CF}}$ = 19.2 Hz, $^4J_{\text{CF}}$ = 7.1 Hz), 109.07 (dd, $^3J_{\text{CF}}$ = 19.2 Hz, $^4J_{\text{CF}}$ = 7.1 Hz), 108.95 (dd, $^3J_{\text{CF}}$ = 19.2 Hz, $^4J_{\text{CF}}$ = 7.1 Hz), 108.88 (dd, $^3J_{\text{CF}}$ = 19.2 Hz, $^4J_{\text{CF}}$ = 7.1 Hz), 104.44, 103.40 (dd, $^2J_{\text{CF}}$ = 25.8 Hz), 103.14 (dd, $^2J_{\text{CF}}$ = 25.8 Hz), 102.89 (dd, $^2J_{\text{CF}}$ = 25.8 Hz), 82.33, 79.97, 56.53, 50.56, 31.17, 30.44, 28.34*, 28.24*, 24.68; ^{19}F NMR (376 MHz, CDCl_3) δ –109.87 (t, J = 8.2 Hz, 2F); HRMS (ESI+): Calcd for $\text{C}_{33}\text{H}_{38}\text{F}_2\text{N}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 682.2623, Found: 682.2615.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(3-fluoro-5-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (19aa): Synthesized by General Procedure E. 22 mg, 51%, clear yellow solid. ^1H NMR (400 MHz, CDCl_3) δ 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, 1.9 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, 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); ^{13}C NMR (101 MHz, CDCl_3) δ 176.54, 167.74, 164.19 (d, $^1J_{\text{CF}}$ = 248.5 Hz), 163.20, 162.52, 161.73 (d, $^1J_{\text{CF}}$ = 248.5 Hz), 154.49, 150.43, 149.10, 142.57, 137.89 (q, $^3J_{\text{CF}}$ = 9.1 Hz), 137.80 (d, $^3J_{\text{CF}}$ = 9.1 Hz), 133.40 (qd, $^2J_{\text{CF}}$ = 34.3 Hz, $^3J_{\text{CF}}$ = 9.1 Hz), 133.32 (qd, $^2J_{\text{CF}}$ = 34.3 Hz, $^3J_{\text{CF}}$ = 9.1 Hz), 133.08 (qd, $^2J_{\text{CF}}$ = 34.3 Hz, $^3J_{\text{CF}}$ = 9.1 Hz), 132.99 (qd, $^2J_{\text{CF}}$ = 34.3 Hz, $^3J_{\text{CF}}$ = 9.1 Hz), 132.74 (qd, $^2J_{\text{CF}}$ = 34.3 Hz, $^3J_{\text{CF}}$ = 9.1 Hz), 132.66 (qd, $^2J_{\text{CF}}$ = 34.3 Hz, $^3J_{\text{CF}}$ = 9.1 Hz), 132.41 (qd, $^2J_{\text{CF}}$ = 34.3 Hz, $^3J_{\text{CF}}$ = 9.1 Hz), 132.33 (qd, $^2J_{\text{CF}}$ = 34.3 Hz, $^3J_{\text{CF}}$ = 9.1 Hz), 128.84, 127.56 (q, $^1J_{\text{CF}}$ = 274.7 Hz),

124.85 (q, $^1J_{\text{CF}} = 274.7$ Hz), 122.13 (q, $^1J_{\text{CF}} = 274.7$ Hz), 120.72, 119.41 (q, $^1J_{\text{CF}} = 274.7$ Hz), 118.61, 117.20, 116.68 (d, $^2J_{\text{CF}} = 23.2$ Hz), 116.45 (d, $^2J_{\text{CF}} = 23.2$ Hz), 112.02 (d, $^2J_{\text{CF}} = 25.3$ Hz), 111.77 (d, $^2J_{\text{CF}} = 25.3$ Hz), 104.90, 82.32, 79.92, 56.54, 50.46, 31.13, 30.49, 28.37*, 28.24*, 24.62; ^{19}F NMR (376 MHz, CDCl_3) δ -62.84 (s, 3F), -110.73 (t, $J = 9.0$ Hz, 1F); HRMS (ESI+): Calcd for $\text{C}_{34}\text{H}_{38}\text{F}_4\text{N}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 732.2591, Found: 732.2602.

tert-butyl (S,Z)-((2-((3-(4-((4-(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 (19bb):

Synthesized by General Procedure E. 31 mg, 84%, off-white solid. ^1H NMR (400 MHz, CDCl_3) 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); ^{13}C NMR (101 MHz, CDCl_3) δ 176.75, 167.74, 163.51, 162.55, 150.45, 148.74, 142.42, 136.53, 132.64 (q, $^2J_{\text{CF}} = 33.3$ Hz), 132.31 (q, $^2J_{\text{CF}} = 33.3$ Hz), 131.98 (q, $^2J_{\text{CF}} = 33.3$ Hz), 131.66 (q, $^2J_{\text{CF}} = 33.3$ Hz), 128.94, 127.60 (q, $^1J_{\text{CF}} = 274.7$ Hz), 126.15, 124.88 (q, $^1J_{\text{CF}} = 274.7$ Hz), 122.16 (q, $^1J_{\text{CF}} = 274.7$ Hz), 121.35, 121.05, 119.45 (q, $^1J_{\text{CF}} = 274.7$ Hz), 117.35, 105.34, 82.27, 79.83, 56.56, 50.40, 31.04, 30.53, 28.37*, 28.24*; ^{19}F NMR (376 MHz, CDCl_3) δ -62.94 (s, 6F); HRMS (ESI+): Calcd for $\text{C}_{35}\text{H}_{38}\text{F}_6\text{N}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 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 (19cc):

Synthesized by General Procedure E. 27 mg, 51%, clear yellow solid. ^1H NMR (400 MHz, CDCl_3) 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); ^{13}C NMR (101 MHz, CDCl_3) δ 176.91, 167.83, 163.22 (d, $^1J_{\text{CF}} = 256.5$ Hz), 162.25, 160.68 (d, $^1J_{\text{CF}} = 256.5$ Hz), 158.33, 154.26, 148.80, 143.85, 142.54, 129.01, 127.72, 126.04, 123.36, 123.26 (q, $^3J_{\text{CF}} = 12.1$ Hz), 123.15 (q, $^3J_{\text{CF}} = 12.1$ Hz), 123.03 (q, $^3J_{\text{CF}} = 12.1$ Hz), 122.90 (q, $^3J_{\text{CF}} = 12.1$ Hz), 122.61, 121.09, 117.33, 116.82, 116.58, 109.22 (d, $^3J_{\text{CF}} = 16.2$ Hz), 109.06 (d, $^3J_{\text{CF}} = 16.2$ 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 – -109.28 (m, 1F); HRMS (ESI+): Calcd for $\text{C}_{34}\text{H}_{38}\text{F}_4\text{N}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 732.2591, Found: 732.2605.

tert-butyl (S,Z)-((2-((3-(4-((4-([1,1'-biphenyl]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)((tert-butoxycarbonyl)imino)methyl)carbamate (19dd):

Synthesized by General Procedure E. 7 mg, 83%, light yellow solid. ^1H 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 C₃₉H₄₄N₇O₅S [M+H]⁺: 722.3125, Found: 722.3107.

tert-butyl (S,Z)-((2-((3-(4-((4-(benzofuran-2-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)((tert-butoxycarbonyl)imino)methyl)carbamate (19ee): Synthesized by General Procedure E. 18 mg, 69%, off-white solid. ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (101 MHz, CDCl₃) 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 C₃₅H₄₀N₇O₆S [M+H]⁺: 686.2761, Found: 686.2760.

(S)-amino(2-((3-(4-((4-phenylthiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20a): Synthesized by General Procedure E. 8 mg, 85%, yellow solid. ¹H NMR (500 MHz, CD₃OD) 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 (ddd, *J* = 12.1, 10.3, 5.8 Hz, 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, CD₃OD) 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 C₂₃H₂₄N₇OS [M+H]⁺: 446.1763, Found: 446.1755.

(S)-amino(2-((3-(4-((4-(pyridin-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium trifluoroacetate (20b): Synthesized by General Procedure E. 3 mg, 100%, yellow solid. ¹H NMR (400 MHz, CD₃OD) 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, CD₃OD) δ 176.41, 167.86, 163.98, 155.01, 148.91, 146.25, 143.48, 142.84, 128.02, 122.18, 119.62, 117.04, 114.31, 55.77, 30.14, 28.63, 22.15; HRMS (ESI+): Calcd for C₂₂H₂₃N₈OS [M+H]⁺: 447.1716, Found: 447.1704. HPLC purity: 87%.

(S)-amino(2-((3-(4-((4-(pyridin-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20c): Synthesized by General Procedure E. 8 mg, 85%, yellow solid. ¹H NMR (400 MHz, CD₃OD) 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); ¹³C NMR (101 MHz, CD₃OD) 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 C₂₂H₂₃N₈OS [M+H]⁺: 447.1716, Found: 447.1698.

(S)-amino(2-((3-(4-((4-(4-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20d): Synthesized by General Procedure E. 5 mg, 100%, yellow solid. ^1H NMR (500 MHz, CD_3OD) δ 8.09 (d, J = 8.3 Hz, 2H), 7.89 (dd, J = 8.4, 5.3 Hz, 2H), 7.82 (d, J = 8.4 Hz, 2H), 7.19 (t, J = 8.6 Hz, 2H), 7.15 (s, 1H), 4.57 – 4.51 (m, 1H), 3.60 – 3.51 (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_3OD) δ 176.54, 167.77, 165.04, 163.86 (d, $^1J_{\text{CF}}$ = 199.0 Hz), 161.89 (d, $^1J_{\text{CF}}$ = 199.0 Hz), 155.04, 146.83, 142.85, 129.13, 128.29, 127.95 (d, $^4J_{\text{CF}}$ = 6.1 Hz), 127.89 (d, $^4J_{\text{CF}}$ = 6.1 Hz), 120.97, 118.47, 115.30 (d, $^3J_{\text{CF}}$ = 18.2 Hz), 115.12 (d, $^3J_{\text{CF}}$ = 18.2 Hz), 55.80, 36.84, 30.19, 28.67, 22.18; ^{19}F NMR (376 MHz, CD_3OD) δ –116.45 – –116.59 (m, 1F); HRMS (ESI $^+$): Calcd for $\text{C}_{23}\text{H}_{23}\text{FN}_7\text{OS}$ $[\text{M}+\text{H}]^+$: 464.1669, Found: 464.1681.

(S)-amino(2-((3-(4-((4-(4-chlorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20e): Synthesized by General Procedure E. 5 mg, 100%, off-white solid. ^1H NMR (500 MHz, CD_3OD) 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_3OD) 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 $\text{C}_{23}\text{H}_{23}\text{ClN}_7\text{OS}$ $[\text{M}+\text{H}]^+$: 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 (20f): Synthesized by General Procedure E. 5 mg, 100% light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 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_3OD) δ 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 $\text{C}_{23}\text{H}_{23}\text{BrN}_7\text{OS}$ $[\text{M}+\text{H}]^+$: 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 (9g): Synthesized by General Procedure E. 5 mg, 100%, yellow solid. ^1H NMR (500 MHz, CD_3OD) δ 8.08 (d, J = 8.7 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H), 7.67 (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_3OD) δ 177.96, 169.19, 166.35, 165.52 (d, $^1J_{\text{CF}}$ = 195.9 Hz), 163.58 (d, $^1J_{\text{CF}}$ = 195.9 Hz), 156.46, 148.11, 144.28, 136.47 (d, $^4J_{\text{CF}}$ = 6.1 Hz), 136.41 (d, $^4J_{\text{CF}}$ = 6.1 Hz), 131.74 (d, $^4J_{\text{CF}}$ = 7.1 Hz), 131.67 (d, $^4J_{\text{CF}}$ = 7.1 Hz), 129.71, 123.04, 123.02, 122.35, 119.82, 116.20 (d, $^3J_{\text{CF}}$ = 17.2 Hz), 116.03 (d, $^3J_{\text{CF}}$ = 17.2 Hz), 114.06 (d, $^3J_{\text{CF}}$ = 18.2 Hz), 113.88 (d, $^3J_{\text{CF}}$ = 18.2 Hz), 105.55, 57.22, 31.62, 30.08, 23.60; ^{19}F NMR (376 MHz, CD_3OD) δ –77.20 (s, 1F); HRMS (ESI $^+$): Calcd for $\text{C}_{23}\text{H}_{23}\text{FN}_7\text{OS}$ $[\text{M}+\text{H}]^+$: 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 (20h): Synthesized by General Procedure E. 26 mg, 100%, light yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 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); ¹³C NMR (101 MHz, CD₃OD) 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 C₂₃H₂₃ClN₇OS [M+H]⁺: 480.1373, Found: 480.1370.

(S)-amino(2-((3-(4-((4-(3-bromophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20i): Synthesized by General Procedure E. 3 mg, 100%, off-white solid. ¹H NMR (400 MHz, CD₃OD) 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); ¹³C NMR (101 MHz, CD₃OD) 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 C₂₃H₂₃BrN₇OS [M+H]⁺: 524.0868, Found: 524.0869.

(S)-amino(2-((3-(4-((4-(2-fluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20j): Synthesized by General Procedure E. 14 mg, 100%, light yellow solid. ¹H NMR (400 MHz, CD₃OD) 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); ¹³C NMR (101 MHz, CD₃OD) 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; ¹⁹F NMR (471 MHz, CD₃OD) δ –116.05 – –116.22 (m, 1F); HRMS (ESI⁺): Calcd for C₂₃H₂₃FN₇OS [M+H]⁺: 464.1669, Found: 464.1658.

(S)-amino(2-((3-(4-((4-(4-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20k): Synthesized by General Procedure E. 24 mg, 100%, yellow solid. ¹H NMR (500 MHz, CD₃OD) δ 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); ¹³C NMR (126 MHz, CD₃OD) δ 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; ¹⁹F NMR (471 MHz, CD₃OD) δ –64.02 (s, 3F); HRMS (ESI⁺): Calcd for C₂₄H₂₃F₃N₇OS [M+H]⁺: 515.1715, Found: 515.1718.

(S)-amino(2-((3-(4-((4-(3-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20l): Synthesized by

General Procedure E. 3 mg, 100%, light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 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_3OD) δ 177.72, 169.34, 164.73, 156.46, 150.89, 145.42, 137.08, 134.58, 132.49 (q, $^2J_{\text{CF}} = 31.3$ Hz), 132.17 (q, $^2J_{\text{CF}} = 31.3$ Hz), 131.86 (q, $^2J_{\text{CF}} = 31.3$ Hz), 131.54 (q, $^2J_{\text{CF}} = 31.3$ Hz), 130.49, 130.43, 129.95, 129.80 (q, $^1J_{\text{CF}} = 272.7$ Hz), 129.37, 127.10, 126.52 (q, $^1J_{\text{CF}} = 272.7$ Hz), 125.14 (q, $^4J_{\text{CF}} = 4.0$ Hz), 125.11 (q, $^4J_{\text{CF}} = 4.0$ Hz), 125.06 (q, $^4J_{\text{CF}} = 4.0$ Hz), 125.03 (q, $^4J_{\text{CF}} = 4.0$ Hz), 124.40 (q, $^1J_{\text{CF}} = 272.7$ Hz), 123.98, 123.59 (q, $^4J_{\text{CF}} = 4.0$ Hz), 123.56 (q, $^4J_{\text{CF}} = 4.0$ Hz), 123.51 (q, $^4J_{\text{CF}} = 4.0$ Hz), 123.48 (q, $^4J_{\text{CF}} = 4.0$ Hz), 121.70 (q, $^1J_{\text{CF}} = 272.7$ Hz), 120.38, 118.08, 105.77, 57.22; ^{19}F NMR (376 MHz, CD_3OD) δ -64.27 (s, 3F); HRMS (ESI⁺): Calcd for $\text{C}_{24}\text{H}_{23}\text{F}_3\text{N}_7\text{OS}$ [M+H]⁺: 514.1637, Found: 514.1625.

(S)-amino(2-((3-(4-((2-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20m): 5 mg, 93%, off-white solid. ^1H NMR (400 MHz, CD_3OD) 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_3OD) δ 178.06, 169.06, 166.70, 156.45, 148.60, 144.53, 143.47, 133.49, 133.34, 132.47, 130.78, 129.82, 129.34 (q, $^2J_{\text{CF}} = 29.3$ Hz), 129.05 (q, $^2J_{\text{CF}} = 29.3$ Hz), 127.68 (q, $^3J_{\text{CF}} = 5.1$ Hz), 127.63 (q, $^4J_{\text{CF}} = 5.1$ Hz), 127.57 (q, $^4J_{\text{CF}} = 5.1$ Hz), 127.52 (q, $^4J_{\text{CF}} = 5.1$ Hz), 123.47, 120.86, 108.89, 57.20, 31.60, 30.08, 23.59; ^{19}F NMR (376 MHz, CD_3OD) δ -59.48 (s, 3F); HRMS (ESI⁺): Calcd for $\text{C}_{24}\text{H}_{23}\text{F}_3\text{N}_7\text{OS}$ [M+H]⁺: 515.1715, Found: 515.1663.

(S)-amino(2-((3-(4-((4-(p-tolyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20n): Synthesized by General Procedure E. 6 mg, 85%, light yellow solid. ^1H NMR (400 MHz, CD_3OD) 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_3OD) 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 $\text{C}_{24}\text{H}_{26}\text{N}_7\text{OS}$ [M+H]⁺: 460.1920, Found: 460.1920.

(S)-amino(2-((3-(4-((4-(4-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20o): Synthesized by General Procedure E. 15 mg, 100%, off-white solid. ^1H NMR (400 MHz, CD_3OD) δ 8.05 – 7.97 (m, 2H), 7.90 – 7.79 (m, 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_3OD) 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 $\text{C}_{24}\text{H}_{26}\text{N}_7\text{O}_2\text{S}^+$ [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 (20p): Synthesized by General Procedure E. 26 mg, 76%, yellow solid. ¹H NMR (400 MHz, CD₃OD) 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); ¹³C NMR (101 MHz, CD₃OD) 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 C₂₅H₂₈N₇O₂S [M + H]⁺: 490.2025, Found: 490.2024.

(S)-amino(2-((3-(4-((4-(3-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20q): Synthesized by General Procedure E. 12 mg, 86%, yellow solid. ¹H NMR (400 MHz, CD₃OD) 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); ¹³C NMR (101 MHz, CD₃OD) 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 C₂₄H₂₆N₇O₂S⁺ [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 (20r): Synthesized by General Procedure E. 9 mg, 90%, off-white solid. ¹H NMR (500 MHz, CD₃OD) δ 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); ¹³C NMR (126 MHz, CD₃OD) δ 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; ¹⁹F NMR (470 MHz, CD₃OD) δ –59.47 (s, 3F); HRMS (ESI⁺): Calcd for C₂₄H₂₃F₃N₇O₂S [M + H]⁺: 531.1664, Found: 531.1672. HPLC purity: 81%.

(S)-amino(2-((3-(4-((4-(3-(trifluoromethoxy)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20s): Synthesized by General Procedure E. 9 mg, 100%, light yellow solid. ¹H NMR (400 MHz, v) δ 8.04 (d, *J* = 8.5 Hz, 2H), 7.96 – 7.83 (m, 4H), 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); ¹³C NMR (101 MHz, CD₃OD) δ 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, 30.07, 23.58; ¹⁹F NMR (376 MHz, CD₃OD) δ –59.32 (s, 3F); HRMS (ESI⁺): Calcd for C₂₄H₂₃F₃N₇O₂S [M + H]⁺: 531.1664, Found: 531.1712.

(S)-amino(2-((3-(4-((4-(3,4-dimethylphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20t): Synthesized by General Procedure E. 25 mg, 89%, yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 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_3OD) 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 $\text{C}_{25}\text{H}_{28}\text{N}_7\text{O}_3\text{S}^+$ $[\text{M}+\text{H}]^+$: 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 (20u): Synthesized by General Procedure E. 19 mg, 100%, orange solid. ^1H NMR (400 MHz, CD_3OD) 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_3OD) 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 $\text{C}_{25}\text{H}_{28}\text{N}_7\text{OS}^+$ $[\text{M}+\text{H}]^+$: 506.1969, Found: 506.1948.

(S)-amino(2-((3-(4-((4-(3-chloro-4-methoxyphenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20v): Synthesized by General Procedure E. 11 mg, 100%, off-white solid. ^1H NMR (500 MHz, CD_3OD) 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_3OD) 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 $\text{C}_{24}\text{H}_{25}\text{ClN}_7\text{O}_2\text{S}^+$ $[\text{M}+\text{H}]^+$: 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 (20w): Synthesized by General Procedure E. 6 mg, 100%, yellow solid. ^1H NMR (400 MHz, CD_3OD) 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_3OD) δ 177.84, 169.26, 165.51, 156.44, 148.70, 144.82, 132.67, 129.56, 123.68 (dd, $^4J_{\text{CF}}$ = 4.0 Hz), 123.64 (dd, $^4J_{\text{CF}}$ = 4.0 Hz), 123.62 (dd, $^4J_{\text{CF}}$ = 4.0 Hz), 123.58 (dd, $^4J_{\text{CF}}$ = 4.0 Hz), 121.39, 118.99, 118.70 (d, $^2J_{\text{CF}}$ = 18.2 Hz), 118.52 (d, $^2J_{\text{CF}}$ = 18.2 Hz), 116.18 (d, $^2J_{\text{CF}}$ = 19.2 Hz), 115.99 (d, $^2J_{\text{CF}}$ = 19.2 Hz), 57.25, 31.66, 30.13, 23.63; ^{19}F NMR (376 MHz, CD_3OD) δ -140.17 – -140.55 (m, 1F), -141.28 – -141.63 (m, 1F); HRMS (ESI⁺): Calcd for $\text{C}_{23}\text{H}_{22}\text{F}_2\text{N}_7\text{OS}^+$ $[\text{M}+\text{H}]^+$: 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 (20x): Synthesized by General Procedure E. 15 mg, 100%, white solid. ^1H NMR (500 MHz, CD_3OD) 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_3OD) 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 C₂₃H₂₂Cl₂N₇OS [M+H]⁺: 514.0984, Found: 514.0973.

(S)-amino(2-((3-(4-((4-(4-fluoro-3-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20y): Synthesized by General Procedure E. 9 mg, 100%, off-white solid. ¹H NMR (500 MHz, CD₃OD) 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); ¹³C NMR (126 MHz, CD₃OD) δ 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; ¹⁹F NMR (376 MHz, CD₃OD) δ –62.93 (d, *J* = 12.8 Hz, 3F), –117.55 – –117.83 (m, 1F); HRMS (ESI⁺): Calcd for C₂₄H₂₂F₄N₇OS [M+H]⁺: 532.1543, Found: 532.1538.

(S)-amino(2-((3-(4-((3,5-difluorophenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20z): Synthesized by General Procedure E. 8 mg, 100%, off-white solid. ¹H NMR (400 MHz, CD₃OD) δ 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); ¹³C NMR (101 MHz, CD₃OD) δ 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; ¹⁹F NMR (376 MHz, CD₃OD) δ –111.61 – –111.72 (m, 2F); HRMS (ESI⁺): Calcd for C₂₃H₂₂F₂N₇OS [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 (20aa): Synthesized by General Procedure E. 6 mg, 100%, yellow solid. ¹H NMR (500 MHz, CD₃OD) 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); ¹³C NMR (126 MHz, CD₃OD) δ 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, $^4J_{\text{CF}} = 3.0$ Hz), 119.51 (q, $^4J_{\text{CF}} = 3.0$ Hz), 119.48 (q, $^4J_{\text{CF}} = 3.0$ Hz), 118.30, 117.37 (d, $^3J_{\text{CF}} = 17.2$ Hz), 117.13 (d, $^3J_{\text{CF}} = 17.2$ Hz), 112.44, 112.17, 107.28, 57.25, 31.64, 30.12, 23.61; ^{19}F NMR (376 MHz, CD_3OD) δ -64.34 (s, 1F), -112.36 (t, $J = 9.1$ Hz, 1F); HRMS (ESI+): Calcd for $\text{C}_{24}\text{H}_{22}\text{F}_4\text{N}_7\text{OS}$ $[\text{M}+\text{H}]^+$: 532.1543, Found: 532.1520.

(S)-amino(2-((3-(4-((4-(3,5-bis(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20bb): Synthesized by General Procedure E. 22 mg, 100%, white solid. ^1H NMR (400 MHz, CD_3OD) δ 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_3OD) δ 178.12, 168.96, 167.94, 156.43, 145.08, 143.15, 133.46, 133.42, 132.82 (q, $^2J_{\text{CF}} = 32.3$ Hz), 132.50 (q, $^2J_{\text{CF}} = 32.3$ Hz), 132.18 (q, $^2J_{\text{CF}} = 32.3$ Hz), 131.85 (q, $^2J_{\text{CF}} = 32.3$ Hz), 131.02, 130.99, 129.91, 126.79, 126.64 (q, $^4J_{\text{CF}} = 3.0$ Hz), 126.61 (q, $^4J_{\text{CF}} = 3.0$ Hz), 126.57 (q, $^4J_{\text{CF}} = 3.0$ Hz), 126.53 (q, $^4J_{\text{CF}} = 3.0$ 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_3OD) δ -64.49; HRMS (ESI+): Calcd for $\text{C}_{25}\text{H}_{22}\text{F}_6\text{N}_7\text{OS}$ $[\text{M}+\text{H}]^+$: 582.1511, Found: 582.1516.

(S)-amino(2-((3-(4-((4-(2-fluoro-5-(trifluoromethyl)phenyl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20cc): Synthesized by General Procedure E. 6 mg, 100%, yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 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_3OD) δ 183.96, 177.78, 175.04, 169.33, 164.57 (d, $^1J_{\text{CF}} = 256.5$ Hz), 164.00, 162.03 (d, $^1J_{\text{CF}} = 256.5$ Hz), 156.45, 145.34, 144.51, 129.37, 128.20 (q, $^1J_{\text{CF}} = 112.1$ Hz), 127.09 (q, $^1J_{\text{CF}} = 112.1$ Hz), 124.59 (d, $^3J_{\text{CF}} = 12.1$ Hz), 124.47 (d, $^3J_{\text{CF}} = 12.1$ 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_3OD) δ -63.69 (s, 3F), -110.28 – -110.99 (m, 1F); HRMS (ESI+): Calcd for $\text{C}_{24}\text{H}_{22}\text{F}_4\text{N}_7\text{OS}$ $[\text{M}+\text{H}]^+$: 532.1543, Found: 532.1520. HPLC purity: 85% pure.

(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 (20dd): Synthesized by General Procedure E. 5 mg, 100%, light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 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_3OD) δ 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 $\text{C}_{29}\text{H}_{28}\text{N}_7\text{OS}$ $[\text{M}+\text{H}]^+$: 522.2076, Found: 522.2046.

(S)-amino(2-((3-(4-((4-(benzofuran-2-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (20ee): Synthesized by General Procedure E. 13 mg, 100%, yellow solid. ^1H NMR (400 MHz, CD_3OD) 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_3OD) 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 $\text{C}_{25}\text{H}_{24}\text{N}_7\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$: 486.1712, Found: 486.1683.

tert-butyl (S)-2-((3-(4-((4-([1,1'-biphenyl]-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-

oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (21a): Synthesized by General Procedure D. 12 mg, 30%, yellow amorphous solid. ^1H 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 $\text{C}_{33}\text{H}_{34}\text{N}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 580.2382, Found: 580.2384.

tert-butyl (S)-2-((3-(4-((4-(4'-fluoro-[1,1'-biphenyl]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate

(21b): Synthesized by General Procedure D. 14 mg, 34%, off-white amorphous solid. ^1H 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) δ 177.22, 168.00, 163.88 (d, $^1J_{\text{CF}}$ = 247.5 Hz), 162.96, 161.43 (d, $^1J_{\text{CF}}$ = 247.5 Hz), 154.37, 151.25, 150.57, 142.75, 139.84, 136.93 (d, $^4J_{\text{CF}}$ = 3.0 Hz), 136.90 (d, $^4J_{\text{CF}}$ = 3.0 Hz), 133.53, 133.45, 131.92, 128.95, 128.84, 128.72 (d, $^3J_{\text{CF}}$ = 8.1 Hz), 128.64 (d, $^3J_{\text{CF}}$ = 8.1 Hz), 127.80, 127.35, 126.71, 122.07, 120.73, 117.39, 117.35, 115.92 (d, $^2J_{\text{CF}}$ = 21.2 Hz), 115.71 (d, $^2J_{\text{CF}}$ = 21.2 Hz), 103.27, 102.96, 80.23, 55.34, 46.90*, 46.51*, 31.93*, 31.15*, 31.00*, 30.23*, 29.85*, 28.64*, 23.71*, 22.92*; ^{19}F NMR (376 MHz, CDCl_3) δ -114.71 – -116.15 (m, 1F); HRMS (ESI⁺): Calcd for $\text{C}_{33}\text{H}_{33}\text{FN}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 598.2288, Found: 598.2234.

tert-butyl (S)-2-((3-(4-((4-(4-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate

(21c): Synthesized by General Procedure D. 11 mg, 37%, yellow amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 177.41, 167.99, 163.01, 154.38, 151.06, 144.29, 142.73, 139.28, 134.41, 129.69 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 129.37 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 128.98, 128.83 (q, $^2J_{\text{CF}}$ = 32.3 Hz), 127.67, 127.54, 127.37, 127.23, 126.84, 125.91 (q, $^4J_{\text{CF}}$ = 4.0 Hz), 125.87 (q, $^4J_{\text{CF}}$ = 4.0 Hz), 125.79 (q, $^4J_{\text{CF}}$ = 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) δ -62.43 (s, 3F); HRMS (ESI+): Calcd for $\text{C}_{34}\text{H}_{32}\text{F}_3\text{N}_5\text{NaO}_3\text{S}$ $[\text{M}+\text{H}]^+$: 670.2075, Found: 670.2068.

tert-butyl (S)-2-((3-(4-((4-(3'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate

(21d): Synthesized by General Procedure D. 12 mg, 35%, tan amorphous solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 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, $^2J_{\text{CF}}$ = 30.3 Hz), 131.02 (q, $^2J_{\text{CF}}$ = 30.3 Hz), 130.22, 129.27, 128.81, 128.68, 127.64 (q, $^1J_{\text{CF}}$ = 155.5 Hz), 127.41, 126.69, 126.10 (q, $^1J_{\text{CF}}$ = 155.5 Hz), 125.51, 123.99 (q, $^2J_{\text{CF}}$ = 30.3 Hz), 123.69 (q, $^2J_{\text{CF}}$ = 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) δ -62.60 (s, 3F); HRMS (ESI+): Calcd for $\text{C}_{34}\text{H}_{33}\text{F}_3\text{N}_5\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 648.2256, Found: 648.2209.

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

(22a): Synthesized by General Procedure E. 13mg, 68%, off-white oily solid. ^1H NMR (500 MHz, CDCl_3) δ 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) δ 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 $\text{C}_{39}\text{H}_{44}\text{N}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 722.3125, Found: 722.3107.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((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

(22b): Synthesized by General Procedure E. 25 mg, 50%, off-white oily solid. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 176.85, 167.88, 163.65 (d, $^1J_{\text{CF}}$ = 100 Hz), 162.89, 162.65 (d, $^1J_{\text{CF}}$ = 100 Hz), 154.31, 151.29, 150.51, 142.78, 139.81, 136.97, 133.61, 131.92, 128.97, 128.71 (d, $^4J_{\text{CF}}$ = 7.1 Hz), 128.64 (d, $^4J_{\text{CF}}$ = 7.1 Hz), 127.82, 127.35 (d, $^2J_{\text{CF}}$ = 63.6 Hz), 126.72 (d, $^2J_{\text{CF}}$ = 63.6 Hz), 120.77, 117.23, 115.91 (d, $^3J_{\text{CF}}$ = 17.2 Hz), 115.74 (d, $^3J_{\text{CF}}$ = 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) δ -115.56 – -115.66 (m, 1F); HRMS (ESI+): Calcd for $\text{C}_{39}\text{H}_{43}\text{FN}_7\text{O}_5\text{S}$ $[\text{M}+\text{H}]^+$: 740.3030, Found: 740.2979.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(4'-trifluoromethyl)-[1,1'-biphenyl]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (22c): Synthesized by General Procedure E. 10 mg, 75%, off-white oily solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (126 MHz, CDCl₃) δ 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*_{CF} = 2.0 Hz), 125.75 (q, ⁴*J*_{CF} = 2.0 Hz), 125.73 (q, ⁴*J*_{CF} = 2.0 Hz), 125.70 (q, ⁴*J*_{CF} = 2.0 Hz), 121.05 (q, ⁴*J*_{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; ¹⁹F NMR (376 MHz, CDCl₃) δ –62.44 (s, 3F); HRMS (ESI⁺): Calcd for C₄₀H₄₃F₃N₇O₅S [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 (22d): Synthesized by General Procedure E. 7 mg, 37%, off-white oily solid. ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (126 MHz, CDCl₃) 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; ¹⁹F NMR (376 MHz, CDCl₃) δ –62.63; HRMS (ESI⁺): Calcd for C₄₀H₄₃F₃N₇O₅S [M+H]⁺: 790.2998, Found: 790.2997.

(S)-(2-((3-(4-((4-([1,1'-biphenyl]-3-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)(amino)methaniminium chloride (23a): Synthesized by General Procedure E. 5 mg, 93%, light yellow solid. ¹H NMR (400 MHz, CD₃OD) 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); ¹³C NMR (101 MHz, CD₃OD) 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 C₂₉H₂₈N₇OS [M+H]⁺: 522.2076, Found: 522.2072. HPLC purity: 88%.

(S)-amino(2-((3-(4-((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 (23b): Synthesized by General Procedure E. 8 mg, 90%, off-white solid. ¹H NMR (500 MHz, CD₃OD) δ 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 – 2.22 (m, 1H), 2.18 – 1.97 (m, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 177.96, 169.20, 167.04, 166.33 (d, $^1J_{\text{CF}}$ = 128.3 Hz), 165.06 (d, $^1J_{\text{CF}}$ = 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, $^2J_{\text{CF}}$ = 49.5 Hz), 127.77 (d, $^2J_{\text{CF}}$ = 49.5 Hz), 119.81, 118.95, 116.73 (d, $^3J_{\text{CF}}$ = 17.3 Hz), 116.56 (d, $^3J_{\text{CF}}$ = 17.3 Hz), 57.22, 31.62, 30.08, 28.13, 23.59; ^{19}F NMR (376 MHz, CD_3OD) δ –117.18 – –117.54 (m, 1F); HRMS (ESI+): Calcd for $\text{C}_{29}\text{H}_{27}\text{FN}_7\text{OS}$ [$\text{M} + \text{H}$] $^+$: 540.1982, Found: 540.1978.

(S)-amino(2-((3-(4-((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 (23c): Synthesized by General Procedure E. 2 mg, 100%, off-white solid. ^1H NMR (500 MHz, CD_3OD) δ 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_3OD) δ 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, $^4J_{\text{CF}}$ = 3.0 Hz), 126.81 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 126.78 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 126.75 (q, $^4J_{\text{CF}}$ = 3.0 Hz), 122.39, 120.29, 118.08, 104.78, 57.26, 31.63, 30.09, 23.60; ^{19}F NMR (376 MHz, CD_3OD) δ –63.98 (s, 3F); Calcd for $\text{C}_{30}\text{H}_{27}\text{F}_3\text{N}_7\text{OS}$ [$\text{M} + \text{H}$] $^+$: 590.1950, Found: 590.1954.

(S)-amino(2-((3-(4-((3-(4'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)thiazol-2-yl)amino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (23d): Synthesized by General Procedure E. 5 mg, 100%, off-white solid. ^1H NMR (400 MHz, CD_3OD) δ 8.16 – 8.03 (m, 2H), 8.02 – 7.97 (m, 1H), 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_3OD) δ 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, $^4J_{\text{CF}}$ = 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_3OD) δ –64.14 (s, 3F); HRMS (ESI+): Calcd for $\text{C}_{30}\text{H}_{27}\text{F}_3\text{N}_7\text{OS}$ [$\text{M} + \text{H}$] $^+$: 590.1950, Found: 590.1949.

N-(4-cyanophenyl)-2,2,2-trifluoroacetamide (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. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 159.90, 133.36, 122.94, 119.11, 105.58, 80.17, 28.80; HRMS (ESI+): Calcd for $\text{C}_{11}\text{H}_{14}\text{NO}$ [$\text{M} + \text{H}$] $^+$: 176.1075, Found: 176.1083.

(Z)-4-(tert-butoxy)-N'-hydroxybenzimidamide: Synthesized by General Procedure A. 215 mg, 87% as white solid. ^1H NMR (400 MHz, acetone- d_6) δ 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) δ 157.36, 151.95, 128.89, 126.94, 123.92, 78.87, 28.95; HRMS (ESI $^{+}$): Calcd for $\text{C}_{11}\text{H}_{17}\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^{+}$: 209.1290, Found: 209.1290.

tert-butyl (S)-2-((3-(4-(tert-butoxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (26): Synthesized by General Procedure B. 214 mg, 52%, yellow oil. ^1H NMR (400 MHz, CDCl_3) δ 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 (ddd, 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); ^{13}C NMR (101 MHz, CDCl_3) δ 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 $\text{C}_{22}\text{H}_{32}\text{N}_3\text{O}_4$ $[\text{M}+\text{H}]^{+}$: 402.2393, Found: 402.2407.

tert-butyl (S)-2-((3-(4-hydroxyphenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (27): **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. ^1H NMR (400 MHz, acetone- d_6) δ 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); ^{13}C NMR (101 MHz, CD_3OD) δ 178.74, 169.35, 161.71, 156.09, 130.01, 118.99, 116.75, 81.52, 81.01, 56.89*, 56.53*, 47.81*, 47.31*, 32.38*, 32.10*, 31.53*, 31.16*, 28.73*, 28.59*, 24.33*, 23.51*; HRMS (ESI $^{+}$): Calcd for $\text{C}_{18}\text{H}_{22}\text{N}_3\text{O}_4$ $[\text{M}-\text{H}]^{-}$: 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 (28): **27** (0.035 g, 0.101 mmol) and K_2CO_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_2SO_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. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 177.64, 172.10, 167.55, 156.88, 154.30, 129.48, 129.19, 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 $C_{21}H_{23}BrN_4NaO_4S$ $[M+Na]^+$: 529.0466, Found: 529.0493.

tert-butyl (S)-2-((3-(4-((4-(4-(trifluoromethyl)phenyl)thiazol-2-yl)oxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (29): Synthesized by General Procedure D. 30 mg, 45%, off-white solid. 1H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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, $CDCl_3$) δ –62.61 (s, 3F); HRMS (ESI+): Calcd for $C_{21}H_{23}BrN_4NaO_4S$ $[M+Na]^+$: 529.0466, Found: 529.0493.

tert-butyl (S,Z)-(((tert-butoxycarbonyl)imino)(2-((3-(4-((4-(4-(trifluoromethyl)phenyl)thiazol-2-yl)oxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methyl)carbamate (30): Synthesized by General Procedure E. 10 mg, 83%, white solid. 1H NMR (400 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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, $CDCl_3$) δ –62.62 (s, 3F); HRMS (ESI+): Calcd for $C_{34}H_{38}F_3N_6O_6S$ $[M+H]^+$: 715.2526, Found: 715.2499.

(S)-amino(2-((3-(4-((4-(4-(trifluoromethyl)phenyl)thiazol-2-yl)oxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (31): Synthesized by General Procedure E. 6 mg, 100%, yellow solid. 1H NMR (400 MHz, CD_3OD) δ 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_3od) δ 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_3OD) δ –64.16 (s, 3F); HRMS (ESI+): Calcd for $C_{24}H_{22}F_3N_6O_2S$ $[M+H]^+$: 515.1477, Found: 515.1445.

tert-butyl (S)-2-((3-(4-((4-([1,1'-biphenyl]-4-yl)thiazol-2-yl)(methylamino)phenyl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (32): To a solution of *tert*-butyl (S)-2-((3-(4-**18dd** (0.025 g, 0.043 mmol) in acetone (1 mL) was added K_2CO_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 **32** (15 mg, 73%) as an off-white solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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 C₃₄H₃₆N₅O₃S [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-butoxycarbonyl)imino)methyl)carbamate (33): Synthesized by General Procedure E. 15 mg, 73%, off-white solid. ¹H NMR (400 MHz, CDCl₃) δ 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); ¹³C NMR (101 MHz, CDCl₃) δ 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 C₄₀H₄₆N₇O₅S [M+H]⁺: 736.3281, Found: 736.3274.

(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 (34): Synthesized by General Procedure F. 5mg, 100% yield, off-white solid. ¹H NMR (500 MHz, CD₃OD) δ 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); ¹³C NMR (126 MHz, CD₃OD) δ 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 C₃₀H₃₀N₇OS [M*]⁺: 536.2233, Found: 536.2230.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

Sph sphingosine

S1P	sphingosine-1-phosphate
SphK	sphingosine kinase
HCTU	O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

References

1. Heffernan-Stroud LA, Obeid LM. Sphingosine kinase 1 in cancer. *Adv Cancer Res.* 2013; 117:201–235. [PubMed: 23290781]
2. Pyne NJ, Tonelli F, Lim KG, Long JS, Edwards J, Pyne S. Sphingosine 1-phosphate signalling in cancer. *Biochem Soc Trans.* 2012; 40:94–100. [PubMed: 22260672]
3. Plano D, Amin S, Sharma AK. Importance of sphingosine kinase (sphk) as a target in developing cancer therapeutics and recent developments in the synthesis of novel sphk inhibitors. *J Med Chem.* 2014; 57:5509–5524. [PubMed: 24471412]
4. Pyne NJ, Pyne S. Sphingosine 1-phosphate and cancer. *Nat Rev Cancer.* 2010; 10:489–503. [PubMed: 20555359]
5. Schwalm S, Pfeilschifter J, Huwiler A. Sphingosine-1-phosphate: A janus-faced mediator of fibrotic diseases. *Biochim Biophys Acta.* 2013; 1831:239–250. [PubMed: 22889995]
6. Yang L, Yue S, Yang L, Liu X, Han Z, Zhang Y, Li L. Sphingosine kinase/sphingosine 1-phosphate (S1P)/S1P receptor axis is involved in liver fibrosis-associated angiogenesis. *J Hepatol.* 2013; 59:114–123. [PubMed: 23466305]
7. Gellings Lowe N, Swaney JS, Moreno KM, Sabbadini RA. Sphingosine-1-phosphate and sphingosine kinase are critical for transforming growth factor-beta-stimulated collagen production by cardiac fibroblasts. *Cardiovasc Res.* 2009; 82:303–312. [PubMed: 19228708]
8. Takasugi N, Sasaki T, Suzuki K, Osawa S, Isshiki H, Hori Y, Shimada N, Higo T, Yokoshima S, Fukuyama T, Lee VM, Trojanowski JQ, Tomita T, Iwatsubo T. Bace1 activity is modulated by cell-associated sphingosine-1-phosphate. *J Neurosci.* 2011; 31:6850–6857. [PubMed: 21543615]
9. Prager B, Spampinato SF, Ransohoff RM. Sphingosine 1-phosphate signaling at the blood-brain barrier. *Trends Mol Med.* 2015; 21:354–363. [PubMed: 25939882]
10. Proia RL, Hla T. Emerging biology of sphingosine-1-phosphate: Its role in pathogenesis and therapy. *J Clin Invest.* 2015; 125:1379–1387. [PubMed: 25831442]
11. Zhang Y, Berka V, Song A, Sun K, Wang W, Zhang W, Ning C, Li C, Zhang Q, Bogdanov M, Alexander DC, Milburn MV, Ahmed MH, Lin H, Idowu M, Zhang J, Kato GJ, Abdulmalik OY, Zhang W, Dowhan W, Kellems RE, Zhang P, Jin J, Safo M, Tsai AL, Juneja HS, Xia Y. Elevated sphingosine-1-phosphate promotes sickling and sickle cell disease progression. *J Clin Invest.* 2014; 124:2750–2761. [PubMed: 24837436]
12. Reid SP, Tritsch SR, Kota K, Chiang CY, Dong L, Kenny T, Brueggemann EE, Ward MD, Cazares LH, Bavari S. Sphingosine kinase 2 is a chikungunya virus host factor co-localized with the viral replication complex. *Emerg Microbes Infect.* 2015; 4:e61. [PubMed: 26576339]
13. Rosen H, Gonzalez-Cabrera PJ, Sanna MG, Brown S. Sphingosine 1-phosphate receptor signaling. *Annu Rev Biochem.* 2009; 78:743–768. [PubMed: 19231986]
14. Mendelson K, Evans T, Hla T. Sphingosine 1-phosphate signalling. *Development.* 2014; 141:5–9. [PubMed: 24346695]
15. Blaho VA, Hla T. An update on the biology of sphingosine 1-phosphate receptors. *J Lipid Res.* 2014; 55:1596–1608. [PubMed: 24459205]
16. Dyckman AJ. Modulators of Sphingosine-1-Phosphate Pathway Biology: Recent Advances of Sphingosine-1-Phosphate Receptor 1 (S1P1) Agonists and Future Perspectives. *J Med Chem.* [Online early access]. Published Online: March 14, 2017.
17. Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, Luo C, Marmorstein R, Kordula T, Milstien S, Spiegel S. Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. *Science.* 2009; 325:1254–1257. [PubMed: 19729656]

18. Igarashi N, Okada T, Hayashi S, Fujita T, Jahangeer S, Nakamura S. Sphingosine kinase 2 is a nuclear protein and inhibits DNA synthesis. *J Biol Chem*. 2003; 278:46832–46839. [PubMed: 12954646]
19. Sun K, Zhang Y, D'Alessandro A, Nemkov T, Song A, Wu H, Liu H, Adebisi M, Huang A, Wen YE, Bogdanov MV, Vila A, O'Brien J, Kellems RE, Dowhan W, Subudhi AW, Jameson-Van Houten S, Julian CG, Lovering AT, Safo M, Hansen KC, Roach RC, Xia Y. Sphingosine-1-phosphate promotes erythrocyte glycolysis and oxygen release for adaptation to high-altitude hypoxia. *Nat Commun*. 2016; 7:12086. [PubMed: 27417539]
20. Liu H, Sugiura M, Nava VE, Edsall LC, Kono K, Poulton S, Milstien S, Kohama T, Spiegel S. Molecular cloning and functional characterization of a novel mammalian sphingosine kinase type 2 isoform. *J Biol Chem*. 2000; 275:19513–19520. [PubMed: 10751414]
21. Kunkel GT, Maceyka M, Milstien S, Spiegel S. Targeting the sphingosine-1-phosphate axis in cancer, inflammation and beyond. *Nat Rev Drug Discov*. 2013; 12:688–702. [PubMed: 23954895]
22. Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. *Trends Cell Biol*. 2012; 22:50–60. [PubMed: 22001186]
23. Ding G, Sonoda H, Yu H, Kajimoto T, Goparaju SK, Jahangeer S, Okada T, Nakamura S. Protein kinase D-mediated phosphorylation and nuclear export of sphingosine kinase 2. *J Biol Chem*. 2007; 282:27493–27502. [PubMed: 17635916]
24. Okada T, Ding G, Sonoda H, Kajimoto T, Haga Y, Khosrowbeygi A, Gao S, Miwa N, Jahangeer S, Nakamura S. Involvement of N-terminal-extended form of sphingosine kinase 2 in serum-dependent regulation of cell proliferation and apoptosis. *J Biol Chem*. 2005; 280:36318–36325. [PubMed: 16103110]
25. Neubauer HA, Pitson SM. Roles, regulation and inhibitors of sphingosine kinase 2. *FEBS J*. 2013; 1–20.
26. Sankala HM, Hait NC, Paugh SW, Shida D, Lepine S, Elmore LW, Dent P, Milstien S, Spiegel S. Involvement of sphingosine kinase 2 in p53-independent induction of p21 by the chemotherapeutic drug doxorubicin. *Cancer Res*. 2007; 67:10466–10474. [PubMed: 17974990]
27. Hait NC, Oskeritzian CA, Paugh SW, Milstien S, Spiegel S. Sphingosine kinases, sphingosine 1-phosphate, apoptosis and diseases. *Biochim Biophys Acta*. 2006; 1758:2016–2026. [PubMed: 16996023]
28. Kharel Y, Morris EA, Congdon MD, Thorpe SB, Tomsig JL, Santos WL, Lynch KR. Sphingosine kinase 2 inhibition and blood sphingosine 1-phosphate levels. *J Pharmacol Exp Ther*. 2015; 355:23–31. [PubMed: 26243740]
29. Kharel Y, Raje M, Gao M, Gellett AM, Tomsig JL, Lynch KR, Santos WL. Sphingosine kinase type 2 inhibition elevates circulating sphingosine 1-phosphate. *Biochem J*. 2012; 447:149–157. [PubMed: 22747486]
30. Maceyka M, Sankala H, Hait NC, Le Stunff H, Liu H, Toman R, Collier C, Zhang M, Satin LS, Merrill AH Jr, Milstien S, Spiegel S. Sphk1 and Sphk2, sphingosine kinase isoenzymes with opposing functions in sphingolipid metabolism. *J Biol Chem*. 2005; 280:37118–37129. [PubMed: 16118219]
31. Allende ML, Sasaki T, Kawai H, Olivera A, Mi Y, van Echten-Deckert G, Hajdu R, Rosenbach M, Keohane CA, Mandala S, Spiegel S, Proia RL. Mice deficient in sphingosine kinase 1 are rendered lymphopenic by FTY720. *J Biol Chem*. 2004; 279:52487–52492. [PubMed: 15459201]
32. Mizugishi K, Yamashita T, Olivera A, Miller GF, Spiegel S, Proia RL. Essential role for sphingosine kinases in neural and vascular development. *Mol Cell Biol*. 2005; 25:11113–11121. [PubMed: 16314531]
33. Cohen JA, Barkhof F, Comi G, Hartung HP, Khatami BO, Montalban X, Pelletier J, Capra R, Gallo P, Izquierdo G, Tiel-Wilck K, de Vera A, Jin J, Stites T, Wu S, Aradhye S, Kappos L. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med*. 2010; 362:402–415. [PubMed: 20089954]
34. Kappos L, Radue EW, O'Connor P, Polman C, Hohlfeld R, Calabresi P, Selmaj K, Agoropoulou C, Leyk M, Zhang-Auberson L, Burtin P. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N Engl J Med*. 2010; 362:387–401. [PubMed: 20089952]

35. Pitman MR, Costabile M, Pitson SM. Recent advances in the development of sphingosine kinase inhibitors. *Cell Signalling*. 2016; 28:1349–1363. [PubMed: 27297359]
36. Lynch KR, Thorpe SB, Santos WL. Sphingosine kinase inhibitors: A review of patent literature (2006–2015). *Expert Opin Ther Pat*. 2016; 26:1409–1416. [PubMed: 27539678]
37. Vogt D, Stark H. Therapeutic strategies and pharmacological tools influencing S1P signaling and metabolism. *Med Res Rev*. 2017; 37:3–51. [PubMed: 27480072]
38. Schnute ME, McReynolds MD, Kasten T, Yates M, Jerome G, Rains JW, Hall T, Chrencik J, Kraus M, Cronin CN, Saabye M, Highkin MK, Broadus R, Ogawa S, Cukyne K, Zawadzke LE, Peterkin V, Iyanar K, Scholten JA, Wendling J, Fujiwara H, Nemirovskiy O, Wittwer AJ, Nagiec MM. Modulation of cellular S1P levels with a novel, potent and specific inhibitor of sphingosine kinase-1. *Biochem J*. 2012; 444:79–88. [PubMed: 22397330]
39. Schnute ME, McReynolds MD, Carroll J, Chrencik J, Highkin MK, Iyanar K, Jerome G, Rains JW, Saabye M, Scholten JA, Yates M, Nagiec MM. Discovery of a potent and selective sphingosine kinase 1 inhibitor through the molecular combination of chemotype-distinct screening hits. *J Med Chem*. 2017; 60:2562–2572. [PubMed: 28231433]
40. French KJ, Schrecengost RS, Lee BD, Zhuang Y, Smith SN, Eberly JL, Yun JK, Smith CD. Discovery and evaluation of inhibitors of human sphingosine kinase. *Cancer Res*. 2003; 63:5962–5969. [PubMed: 14522923]
41. Gao G, Peterson UK, Smith RA, Smith CD. Characterization of isoenzyme-selective inhibitors of human sphingosine kinases. *PloS one*. 2012; 7:1–11.
42. Orr Gandy KA, Obeid LM. Targeting the sphingosine kinase/sphingosine 1-phosphate pathway in disease: Review of sphingosine kinase inhibitors. *Biochim Biophys Acta*. 2013; 1831:157–166. [PubMed: 22801037]
43. Wang J, Knapp S, Pyne NJ, Pyne S, Elkins JM. Crystal structure of sphingosine kinase 1 with PF-543. *ACS Med Chem Lett*. 2014; 5:1329–1333. [PubMed: 25516793]
44. Wang Z, Min X, Xiao SH, Johnstone S, Romanow W, Meininger D, Xu H, Liu J, Dai J, An S, Thibault S, Walker N. Molecular basis of sphingosine kinase 1 substrate recognition and catalysis. *Structure*. 2013; 21:798–809. [PubMed: 23602659]
45. Gustin DJ, Li Y, Brown ML, Min X, Schmitt MJ, Wanska M, Wang X, Connors R, Johnstone S, Cardozo M, Cheng AC, Jeffries S, Franks B, Li S, Shen S, Wong M, Wesche H, Xu G, Carlson TJ, Plant M, Morgenstern K, Rex K, Schmitt J, Coxon A, Walker N, Kayser F, Wang Z. Structure guided design of a series of sphingosine kinase (SphK) inhibitors. *Bioorg Med Chem Lett*. 2013; 23:4608–4616. [PubMed: 23845219]
46. Cingolani F, Casasampere M, Sanllehi P, Casas J, Bujons J, Fabrias G. Inhibition of dihydroceramide desaturase activity by the sphingosine kinase inhibitor SKI-II. *J Lipid Res*. 2014; 55:1711–1720. [PubMed: 24875537]
47. Patwardhan NN, Morris EA, Kharel Y, Raje MR, Gao M, Tomsig JL, Lynch KR, Santos WL. Structure-activity relationship studies and in vivo activity of guanidine-based sphingosine kinase inhibitors: Discovery of SphK 1- and SphK 2-selective inhibitors. *J Med Chem*. 2015; 58:1879–1899. [PubMed: 25643074]
48. French KJ, Zhuang Y, Maines LW, Gao P, Wang W, Beljanski V, Upson JJ, Green CL, Keller SN, Smith CD. Pharmacology and antitumor activity of ABC294640, a selective inhibitor of sphingosine kinase-2. *J Pharmacol Exp Ther*. 2010; 333:129–139. [PubMed: 20061445]
49. Liu K, Guo TL, Hait NC, Allegood J, Parikh H, Xu W, Kellogg GE, Grant S, Spiegel S, Zhang S. Biological characterization of 3-(2-amino-ethyl)-5-[3-(4-butoxyl-phenyl)-propylidene]-thiazolidine-2,4-dione (K145) as a selective sphingosine kinase-2 inhibitor and anticancer agent. *PloS one*. 2013; 8:1–13.
50. Santos WL, Lynch KR. Drugging sphingosine kinases. *ACS Chem Biol*. 2015; 10:225–233. [PubMed: 25384187]
51. Maines LW, Fitzpatrick LR, French KJ, Zhuang Y, Xia Z, Keller SN, Upson JJ, Smith CD. Suppression of ulcerative colitis in mice by orally available inhibitors of sphingosine kinase. *Dig Dis Sci*. 2008; 53:997–1012. [PubMed: 18058233]

52. Maines LW, Fitzpatrick LR, Green CL, Zhuang Y, Smith CD. Efficacy of a novel sphingosine kinase inhibitor in experimental Crohn's disease. *Inflammopharmacology*. 2010; 18:73–85. [PubMed: 20151210]
53. Shi Y, Rehman H, Ramshesh VK, Schwartz J, Liu Q, Krishnasamy Y, Zhang X, Lemasters JJ, Smith CD, Zhong Z. Sphingosine kinase-2 inhibition improves mitochondrial function and survival after hepatic ischemia-reperfusion. *J Hepatol*. 2012; 56:137–145. [PubMed: 21756852]
54. Maines L, Fitzpatrick L, French K, Zhuang Y, Xia Z, Keller S, Upson J, Smith C. Suppression of ulcerative colitis in mice by orally available inhibitors of sphingosine kinase. *Dig Dis Sci*. 2008; 53:997–1012. [PubMed: 18058233]
55. Chumanevich AA, Poudyal D, Cui X, Davis T, Wood PA, Smith CD, Hofseth LJ. Suppression of colitis-driven colon cancer in mice by a novel small molecule inhibitor of sphingosine kinase. *Carcinogenesis*. 2010; 31:1787–1793. [PubMed: 20688834]
56. Antoon JW, White MD, Meacham WD, Slaughter EM, Muir SE, Elliott S, Rhodes LV, Ashe HB, Wiese TE, Smith CD, Burow ME, Beckman BS. Antiestrogenic effects of the novel sphingosine kinase-2 inhibitor ABC294640. *Endocrinology*. 2010; 151:5124–5135. [PubMed: 20861237]
57. Dimasi DP, Pitson SM, Bonder CS. Examining the role of sphingosine kinase-2 in the regulation of endothelial cell barrier integrity. *Microcirculation*. 2016; 23:248–265. [PubMed: 26822263]
58. Bajwa A, Huang L, Kurmaeva E, Ye H, Dondeti KR, Chroscicki P, Foley LS, Balogun ZA, Alexander KJ, Park H, Lynch KR, Rosin DL, Okusa MD. Sphingosine kinase 2 deficiency attenuates kidney fibrosis via IFN- γ . *J Am Soc Nephrol*. 2017; 28:1145–1161. [PubMed: 27799486]
59. Neubauer HA, Pham DH, Zebol JR, Moretti PAB, Peterson AL, Leclercq TM, Chan H, Powell JA, Pitman MR, Samuel MS, Bonder CS, Creek DJ, Gliddon BL, Pitson SM. An oncogenic role for sphingosine kinase 2. *Oncotarget*. 2016; 7:64886–64899. [PubMed: 27588496]
60. Congdon MD, Kharel Y, Brown AM, Lewis SN, Bevan DR, Lynch KR, Santos WL. Structure–activity relationship studies and molecular modeling of naphthalene-based sphingosine kinase 2 inhibitors. *ACS Med Chem Lett*. 2016; 7:229–234. [PubMed: 26985306]
61. Vogt D, Weber J, Ihlefeld K, Bruggerhoff A, Proschak E, Stark H. Design, synthesis and evaluation of 2-aminothiazole derivatives as sphingosine kinase inhibitors. *Bioorg Med Chem*. 2014; 22:5354–5367. [PubMed: 25150091]
62. Aurelio L, Scullino CV, Pitman MR, Sexton A, Oliver V, Davies L, Rebello RJ, Furic L, Creek DJ, Pitson SM, Flynn BL. From sphingosine kinase to dihydroceramide desaturase: A structure–activity relationship (SAR) study of the enzyme inhibitory and anticancer activity of 4-((4-(4-chlorophenyl)thiazol-2-yl)amino)phenol (SKI-II). *J Med Chem*. 2016; 59:965–984. [PubMed: 26780304]
63. Congdon MD, Childress ES, Patwardhan NN, Gumkowski J, Morris EA, Kharel Y, Lynch KR, Santos WL. Structure-activity relationship studies of the lipophilic tail region of sphingosine kinase 2 inhibitors. *Bioorg Med Chem Lett*. 2015; 25:4956–4960. [PubMed: 25862200]
64. Kharel Y, Mathews TP, Kennedy AJ, Houck JD, Macdonald TL, Lynch KR. A rapid assay for assessment of sphingosine kinase inhibitors and substrates. *Anal Biochem*. 2011; 411:230–235. [PubMed: 21216217]
65. Leroux FR, Manteau B, Vors JP, Pazenok S. Trifluoromethyl ethers-synthesis and properties of an unusual substituent. *Beilstein J Org Chem*. 2008; 4:13. [PubMed: 18941485]
66. Sheppard WA. The effect of fluorine substitution on the electronic properties of alkoxy, alkylthio and alkylsulfonyl groups. *J Am Chem Soc*. 1963; 85:1314–1318.
67. Knott K, Kharel Y, Raje MR, Lynch KR, Santos WL. Effect of alkyl chain length on sphingosine kinase 2 selectivity. *Bioorg Med Chem Lett*. 2012; 22:6817–6820. [PubMed: 22321213]
68. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. Autodock4 and autodocktools4: Automated docking with selective receptor flexibility. *J Comput Chem*. 2009; 30:2785–2791. [PubMed: 19399780]
69. Trott O, Olson AJ. Autodock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010; 31:455–461. [PubMed: 19499576]

70. Baek DJ, MacRitchie N, Anthony NG, Mackay SP, Pyne S, Pyne NJ, Bittman R. Structure-Activity Relationships and Molecular Modeling of Sphingosine Kinase Inhibitors. *J Med Chem.* 2013; 56:9310–9327. [PubMed: 24164513]

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Sphingosine Kinase 1 Inhibitors

1 (PF-543)³⁸
SphK1 K_i = 3.6 nM

2 (Amgen 23)⁴⁵
hSphK1 IC_{50} = 0.02 μ M
hSphK2 IC_{50} = 1.6 μ M

3 (SLP7111228)⁴⁷
SphK1 K_i = 48 nM $\pm$ 0.01
SphK2 K_i > 10 μ M

4 (CB5468139)⁴¹
SphK1 K_i = 0.3 μ M

Sphingosine Kinase Dual Inhibitors

5 (SKI-II)⁴⁰
SphK1 K_i = 16 $\pm$ 1.3 μ M
SphK2 K_i = 7.9 $\pm$ 0.6 μ M

6 (RB-042)⁷⁰
SphK1 IC_{50} = 5.3 $\pm$ 0.5 μ M
SphK2 IC_{50} = 5.0 $\pm$ 1.3 μ M

7 (SLC5111312)²⁸
hSphK1 K_i = 0.73 $\pm$ 0.2 μ M
hSphK2 K_i = 0.9 $\pm$ 0.2 μ M

Sphingosine Kinase 2 Inhibitors

8 (SLR080811)²⁹
hSphK1 K_i = 12 $\pm$ 1.2 μ M
mSphK2 K_i = 1.3 $\pm$ 0.4 μ M

9 (ABC294640)⁴⁸
SphK2 K_i = 9.8 $\pm$ 1.4 μ M

10 (K145)⁴⁹
SphK2 K_i = 6.4 $\pm$ 0.7 μ M

11 (SLM6031434)²⁸
mSphK1 K_i = 0.4 μ M
mSphK2 K_i = > 20 μ M

Figure 1.
Select SphK Inhibitors

Figure 2.
Tail group modifications towards the development of new “j-shaped” SphK1 inhibitors.

Figure 3. Superimposition of lowest energy docked poses of **2** (purple sticks, colored by atom) and aminothiazole analogue of **3** (teal stick, color by atom) in homology model of SphK2. Key residues in the binding cavity are shown as grey sticks, ATP is shown green colored by atom, and the overall protein structure is shown as grey cartoon for perspective.

Figure 4.

Effect of **20l** and **20dd** on sphingolipids in U937 cells. Following 2 h of incubations U937 cells were harvested by centrifugation, lysed, and 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 pmoles 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).

Figure 5.

Detected S1P blood levels in mice following injection with **20dd**. Wild-type mice were treated with a single 10 mg/kg ip dose of **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.

Scheme 1.

Synthesis of aryl-substituted aminothiazole derivatives **20a-ee**.^a

^a Reagents and conditions: (a) trifluoroacetic anhydride, DCM, 0 °C – rt, 19 h, 88%; (b) $\text{NH}_2\text{OH}\cdot\text{HCl}$, TEA, ACN, 150 °C microwave, 6 min, 57%; (c) Boc-L-homoproline, HCTU, DIEA, DMF, rt – 100 °C, 4 h, 67%; (d) 1:1 1M LiOH:MeOH, 100 °C, 3 h, 82%; (e) Thio-CDI, THF, rt, 4 h; (f) NH_3 (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-carboxamide, DIEA, ACN, 50 °C, microwave, 2 h, 26-87%; (j) HCl (g), MeOH, 90-100%.

Scheme 2.

Synthesis of biphenyl aminothiazole derivatives **23 a-d**.^a

^aReagents and conditions: (a) aryl boronic acid, $\text{PdCl}_2(\text{dppf})$, Cs_2CO_3 , DMF, 150 °C microwave, 90 min; (b) 1:1 TFA:DCM; (c) *N,N'*-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, ACN, 50 °C, microwave, 2 h; (d) HCl (*g*), MeOH.

Scheme 3.

Synthesis of the oxathiazole analogue of inhibitor **20k**.^a

^aReagents and conditions: (a) 1M KO^tBu; THF, reflux, 15 h; (b) NH₂OH·HCl, TEA, EtOH, 150 °C microwave, 6 min; (c) Boc-L-homoproline, HCTU, DIEA, DMF, rt – 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-1*H*-pyrazole-1-carboxamide, DIEA, ACN, 50 °C, microwave, 2 h; (i) HCl (*g*), MeOH.

^dReagents and conditions: (a) MeI, K₂CO₃, acetone, reflux, 17 h; (b) 1:1 TFA:DCM; (c) *N*, *N'*-di-Boc-1*H*-pyrazole-1-carboxamidine, DIEA, ACN, 50 °C, microwave, 2 h; (d) HCl (*g*), MeOH.

Table 1

SphK1 and SphK2 Inhibitory Activity for Mono-Substituted Aminothiazole Derivatives.^a

Compound	R	X	hSphK1	hSphK2	Compound	R	X	hSphK1	hSphK2
20a		NH	100 ± 6	83 ± 4	20k		NH	57 ± 0.4	36 ± 1
20b		NH	100 ± 5	94 ± 4	20l		NH	31 ± 12	28 ± 0.7
20c		NH	95 ± 10	94 ± 7	20m		NH	69 ± 4	72 ± 6
20d		NH	100 ± 13	55 ± 1	20n		NH	89 ± 0.7	44 ± 12
20e		NH	70 ± 3	51 ± 0	20o		NH	58 ± 4	82 ± 6
20f		NH	88 ± 4	46 ± 6	20p		NH	75 ± 0.2	80 ± 7

Compound	R	X	hSphK1	hSphK2	Compound	R	X	hSphK1	hSphK2
20g		NH	60 ± 1	45 ± 2	20q		NH	91 ± 2	100 ± 2
20h		NH	64 ± 1	45 ± 10	20r		NH	100 ± 9	84 ± 2
20i		NH	79 ± 12	44 ± 8	20s		NH	90 ± 1	100 ± 2
20j		NH	50 ± 4	44 ± 9					

^a 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.

Table 2

SphK1 and SphK2 Inhibitory Activity for Di-Substituted and Bulky Aminothiazole Derivatives.^a

Compound	R	X	hSphK1	hSphK2	Compound	R	X	hSphK1	hSphK2
20t		NH	91 ± 4	67 ± 12	20cc		NH	65 ± 2	48 ± 1
20u		NH	100 ± 7	100 ± 7	20dd		NH	100 ± 2	27 ± 12
20v		NH	100 ± 0.2	71 ± 11	23a		NH	92 ± 8	73 ± 7
20w		NH	51 ± 5	51 ± 5	23b		NH	82 ± 9	31 ± 7
20x		NH	51 ± 4	46 ± 11	23c		NH	100 ± 2	96 ± 1
20y		NH	58 ± 5	33 ± 5	23d		NH	99 ± 6	85 ± 3

Compound	R	X	hSphK1	hSphK2	Compound	R	X	hSphK1	hSphK2
20z		NH	68 ± 7	43 ± 8	20ee		NH	68 ± 7	67 ± 5
20aa		NH	51 ± 4	30 ± 15	31		O	62 ± 4	55 ± 2
20bb		NH	100 ± 4	63 ± 4	34		NCH ₃	100 ± 5	100 ± 5

^aSphK 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 μ M (SphK1) or 10 μ M (SphK2) sphingosine and 250 μ M γ -[³²P]ATP. Compounds were assayed at 0.3 μ M in triplicate.

Table 3

K_i and cLogP^a values of Selected Inhibitors of SphK1 and SphK2.

Entry	Compound	Structure	hSphK1 $K_i(\mu\text{M})$	hSphK2 $K_i(\mu\text{M})$	hSphK2 Selectivity	cLogP
1	20y		0.7 ± 0.05	0.17 ± 0.15	4	5.33
2	20aa		0.8 ± 0.07	0.17 ± 0.17	5	5.33
3	20l		0.12 ± 0.04	0.09 ± 0.01	1	5.18
4	20k		0.82 ± 0.25	0.39 ± 0.09	2	5.18
5	20r		5 ± 0.22	0.25 ± 0.10	20	5.42
6	20dd		9 ± 2	0.09 ± 0.02	100	6.18

^a cLogP was calculated for the protonated inhibitor with Chemdraw Professional 13.0.