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Probing the Substitution Pattern of Indole-Based Scaffold Reveals Potent and Selective Sphingosine Kinase 2 Inhibitors

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Abstract

Elevated levels of sphingosine 1-phosphate (S1P) and increased expression of sphingosine kinase isoforms (SphK1 and SphK2) have been implicated in a variety of disease states including cancer, inflammation, autoimmunity, among others. Consequently, the S1P signaling axis has become an attractive target for drug discovery. Selective inhibition of either SphK1 or SphK2 has been demonstrated to be effective in modulating S1P levels in animal models. While SphK1 inhibitors have received much attention, the development of potent and selective SphK2 inhibitors are emerging. Previously, our group reported a SphK2 naphthalene-based selective inhibitor, **SLC5081308**, which displays approximately 7-fold selectivity for hSphK2 over hSphK1 and has a SphK2 K_i value of 1.0 μ M. To improve SphK2 potency and selectivity, we designed, synthesized, and evaluated a series of indole-based compounds derived from **SLC5081308**. After investigating substitution patterns around the indole ring, we discovered that 1,5-disubstitution promoted optimal binding in the SphK2 substrate binding site and subsequent inhibition of enzymatic activity. Our studies led to the identification of **SLC5101465 (6r)**, SphK2 K_i = 90 nM, >110 fold selective for SphK2 over SphK1). Molecular modeling studies revealed key nonpolar interactions with Val308, Phe548, His556, and Cys533 and hydrogen bonds with both Asp211 and Asp308 as responsible for the high SphK2 inhibition and selectivity.

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Author Contributions

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Supporting Information.

The following files are available free of charge.

HPLC purity analysis, ¹H NMR, and ¹³C NMR spectra of novel compounds (PDF)

The authors declare the following competing financial interest(s): W.L.S. and K.R.L. are among the co-founders of Flux Therapeutics Inc, which was created to commercialize S1P-related discoveries, including SphK inhibitors, discovered and characterized in their laboratories.

Keywords

Sphingosine Kinase; SphK2; Sphingosine; Sphingosine 1-Phosphate; SAR; Molecular Docking

INTRODUCTION

Sphingosine 1-phosphate (S1P) is a pleiotropic signaling lipid involved in a variety of physiological processes such as cellular proliferation, chemotaxis, and vascular integrity. S1P has been implicated in disease states including cancer,^[1] sickle cell,^[2] renal fibrosis,^[3] osteoporosis,^[4] inflammation,^[5] and autoimmunity.^[6] Given its importance in multiple disease states, S1P signaling is an attractive target for drug discovery. S1P exerts its effects through a variety of intra and extracellular targets with the most well-defined being five G-protein coupled receptors (S1P1–5).^[7] S1P is synthesized intracellularly from sphingosine and ATP by one of two sphingosine kinase isoforms, SphK1 and SphK2. While genetic knockout of either isoform infers some degree of redundancy,^[8] SphK1 and SphK2 possess distinctions in structure and cellular localization. SphK1 is located in the cytosol and generates S1P that can interact with intracellular targets or be exported and engage in autocrine and paracrine signaling through S1P1–5.^[7] On the other hand, SphK2 is distributed into subcellular compartments such as the nucleus and mitochondria in addition to the cytosol. S1P generated in the mitochondria can promote apoptotic pathways,^[9] whereas nuclear S1P is purported to be involved in transcriptional regulation and cell survival.^[10]

S1P signaling is a well-established and a clinically validated target for drug discovery. In 2010, fingolimod was approved by the FDA for treatment of relapsing and remitting multiple sclerosis (Figure 1). Serving as a prodrug that following phosphorylation (primarily by SphK2), fingolimod acts as a nonselective S1P receptor agonist and validated the S1P pathway as a therapeutic target.^[11] Consequently, significant efforts have been made to develop selective S1P1,5 modulators.^[12] Two important examples, siponimod^[12] and onazimod,^[13] are orally bioavailable S1P1,5 agonists that were recently approved for the treatment of multiple sclerosis. In contrast to fingolimod, siponimod demonstrates faster clearance and allows for more rapid recovery from immune system suppression following treatment cessation. However, cardiac side effects previously associated with fingolimod's S1P receptor activity (initially ascribed to S1P3 activity) were retained by siponimod, despite being S1P3-sparing.^[14] In addition to onazimod's indications for multiple sclerosis, a phase III trial for Crohn's disease is underway.^[15] Given the clinical relevance of S1P and the success of S1P receptor modulators, there is considerable interest in identifying therapeutic targets in other S1P pathways including biosynthesis.

SphK1 and SphK2 are targets to modulate S1P signaling. In addition to the differences mentioned (*vide supra*), selective inhibition of SphK1/2 results in distinct physiological responses. In contrast to SphK1, inhibition of SphK2 results in the seemingly counterintuitive increase in circulating S1P.^[16] Kharel and co-workers postulated that this is due to SphK2's role in the clearance of blood S1P by the liver.^[17] While SphK1 has been the more prominent focus of academia and the pharmaceutical industry, the physiological

relevance of SphK2 inhibition is under investigation.^[18] The X-ray crystal structure of SphK1 indicates the presence of a bent or “J-shaped” lipophilic binding pocket.^[19] Unfortunately, the crystal structure of SphK2 has yet to be reported. However, homology modeling (SphKs are 50% identical in regions of overlap) suggests structural differences between the two isoforms in the sphingosine binding pocket.^[16a, 20] Continued development of highly selective SphK2 inhibitors is necessary to elaborate the role of SphK2 in biological systems and validate SphK2 as a drug target.

A variety of compounds have been identified as either SphK1/2 selective or dual inhibitors (Figure 2). PF-543, Amgen 23, and SLP7111228 are the three most potent human SphK1-selective inhibitors to date, which have been used as tools for the study of S1P signaling.^[19b, 21] Significant efforts have also been made to develop SphK dual inhibitors. For example, Amgen 82^[19b] is a potent dual inhibitor. Indeed, oral administration of Amgen 82 to mice demonstrated remarkable reduction in blood and plasma S1P although it was unsuccessful at altering tumor volume in xenograft models.^[22] Schnute and co-workers reported **27a** that displayed nanomolar activity for both human SphK1/2 isoforms. Interestingly, molecular docking studies with **27a** suggest that π -hydrophobic and π - π interactions with Phe259 predominate.^[23] Only a limited number of compounds have been reported as SphK2-selective inhibitors (Figure 2). The first SphK2-selective inhibitor was ABC294640 ($K_i = 10 \mu\text{M}$), although the extent of selectivity for SphK2 is not known.^[24] While ABC294640 is reported to have off-target estrogen receptor activity,^[25] it is the only SphK2 inhibitor to advance to clinical trials and is currently in phase II clinical trials for advanced bile duct cancer.^[26]

To further elucidate SphK2's role *in vivo*, our group focuses on the development of potent and selective SphK2 inhibitors.^[16a, 21a, 27] SLR080811 (SphK2 $K_i = 1.3 \mu\text{M}$) was the first SphK2 selective inhibitor to reveal an increase in blood S1P levels upon SphK2 inhibition in mice.^[16] Initial investigations established a dependence of SphK2 inhibition and selectivity on alkyl tail length and the internal phenyl ring.^[16a, 28] Furthermore, the importance of a positively charged head group, such as a quaternary amine or guanidine moiety, as well as stereochemistry of the proline ring has been established.^[21a] An SAR study led to the incorporation of a 2,6-disubstituted naphthyl ring in place of the phenyl ring and an ether linkage led to the development of SLC5081308 ($K_i = 1.0 \mu\text{M}$) and SLC5091592 ($K_i = 1.0 \mu\text{M}$), 7-fold and >20-fold selective for SphK2, respectively.^[29] The increase in selectivity of SLC5091592 is postulated to be enhanced by π -stacking interactions between the naphthyl moiety and Phe548 and nonpolar interactions with Cys533, Tyr566 and His556 in the tail region of the binding pocket.^[29] Inspired by these initial SAR results, we hypothesized that the space occupied by the naphthyl ring in the SphK2 binding cavity could be exploited to increase SphK2 inhibitor activity and selectivity. Herein, we describe the synthesis and biological evaluation of a series of indole-based SphK2-selective inhibitors. By varying the substitution pattern on the indole ring, we explored the available space in the SphK2 binding pocket and, through molecular modeling, identified key Van der Waals and hydrogen bonding interactions in the substrate binding cavity that are proposed to facilitate inhibitor potency and selectivity. Target engagement, inhibition, and selectivity was validated in both mammalian U937 cells and in yeast *Saccharomyces cerevisiae*.

RESULTS AND DISCUSSION

Inhibitor Design.

The success of the 2,6-disubstituted naphthyl moiety in SLC5081308 and SLC5091592 necessitated exploration of different substitution patterns to optimize π -stacking with Phe548 in SphK2 and additional interactions in the SphK2 binding pocket. Because an indole heterocycle is a privileged scaffold and its electronic and steric properties can be modulated, we installed the indole in place of the naphthyl ring. Not only did this substitution provide a moiety with more favorable biological properties, it allowed for rapid synthetic access to all relevant substitution patterns *via* readily prepared common intermediates. Alkyl tails ranging from heptyl through decyl were prepared for each substitution pattern. Previous investigations into the tail region of SLR080811, SLC5081308, and earlier lead compounds concluded that the ideal compound length is 18–22 atoms in length from the charged guanidine to the omega carbon.^[16a, 28–29] Thus, undecyl or dodecyl tails were synthesized on the 1,3-disubstituted indole scaffold to account for the shorter distance through the indole ring while maintaining the ideal atom length.

Amidoximes **2a-d** were readily prepared in excellent yields *via* reaction of the corresponding commercially available cyanoindole (**1a-d**) with hydroxylamine hydrochloride and triethylamine in refluxing ethanol (Scheme 1). Subsequent HCTU-mediated coupling of the amidoxime products (**2a-d**) with *N*-Boc-*L*-proline under heated conditions afforded the 1,2,4-oxadiazole cyclization products (**3a-d**) in moderate to good yield.^[21a] Compounds **3a-d** were utilized as common intermediates for each substitution scaffold. Treatment of **3a-d** with sodium hydride and subsequent substitution with alkyl bromides afforded the corresponding *N*-substituted indole products (**4e-w**). Boc-deprotection with methanolic hydrogen chloride followed by reaction with DIEA and *N,N'*-Di-Boc-1*H*-pyrazole-1-carboxamidinium afforded the di-Boc-protected guanidine intermediates **5e-w**.^[21a] Final products **6e-w** were then readily prepared in good yield as hydrogen chloride salts *via* a second methanolic hydrogen chloride deprotection. Given that only one homologated compound (**11**) was synthesized, the alkyl chain was added first to simplify purification of intermediates and a homologated *N*-Boc-*L*-Proline was used to for the 1,2,4-oxadiazole ring (Scheme 2).

Structure-Activity Relationship Studies and Biologic Evaluation of Inhibitors.

With analogues **6e-w** and **11** in hand, the inhibitory effects on human SphK1 and SphK2 were determined using a previously established protocol.^[30] In summary, cell lysate containing either recombinant hSphK1 or hSphK2, sphingosine, and [γ -³²P]ATP was incubated for 20 minutes with or without inhibitor. Afterwards the reaction mixture was extracted, separated by thin layer chromatography, and quantified by scintillation counting. The level of hSphK activity was determined by the amount of [γ -³²P]-S1P produced as a function of inhibitor concentration. Compounds were screened at 1 μ M inhibitor concentrations in hSphK1 and 0.3 μ M concentrations with hSphK2. The results of the inhibition assay are displayed in Table 1. With all scaffolds, inhibition of SphK2 increased with increasing tail length. Both the decyl and dodecyl tails (**6h** and **6j**) of the 1,3-disubstituted scaffold inhibited hSphK2 by over 50%; however, **6h** with a decyl tail

possessed greater SphK2-selectivity and inhibition (53%) with minimal (4%) inhibition of hSphK1 activity. The top performing inhibitors for the 1,4- and 1,6-disubstituted scaffolds, **6n** and **6w**, also contained decyl tails, and were able to reduce hSphK2 activity by 36% and 32%, respectively. Likewise, the decyl tail also exhibited excellent inhibition (70%) and selectivity for hSphK2 in the 1,5-disubstituted scaffold (**6s**); however, the nonyl tail (**6r**) was slightly more potent and exhibited no effect on hSphK1 activity. The optimal length in all cases was 21 atoms, as counted from the tail ω -carbon to the guanidine nitrogen, except for the 1,3-disubstituted scaffold; in this case, the most potent inhibitor was 20 atoms long (**6h**). Furthermore, the 1,5-disubstituted scaffold (**6o-s**) was optimal, and **6r** was identified as the most potent and SphK2-selective inhibitor of this series.

Determination of K_i Values for Select Compounds.

As the preliminary screen revealed several compounds with good potency and selectivity, we validated our initial observations by determining the K_i values for a subset of analogues that inhibited SphK2 activity by at least 50% (Table 2). The 1,3-disubstituted indoles **6h** (SLC5101463) and **6j** (SLC5121467) had K_i values of 300 nM for hSphK2 and >8000 nM for hSphK1. Compared to SLC5081308, both compounds displayed improved selectivity (>28) towards SphK2 over SphK1. To our delight, the 1,5-disubstituted indoles **6r** (SLC5101465) and **6s** (SLC5101464) were both more potent and selective towards SphK2. While both compounds had K_i values of >10 μ M for hSphK1, **6r** and **6s** had a K_i values of 90 nM and 120 nM for hSphK2, respectively. This is a substantial improvement when compared to the naphthalene leads (SLC5081308 and SLC5091592) in terms of potency and selectivity. Taken together, these studies identified **6r** as the most potent and selective compound in the series.

The results of our studies in Tables 1 and 2 identified SLC5101465 (**6r**) for further investigations; therefore, we validated its ability to inhibit SphK2 *in vitro* in whole cell assays by monitoring S1P levels via mass spectrometry (Figure 3). S1P levels were assessed in histiocytic lymphoma U937 cells, which express both SphK1 and SphK2. As shown in Figure 3A, vehicle control demonstrated maximal S1P levels in cells. Upon treatment with SLC5101465 (**6r**) from 0.1 to 1 μ M, a dose-dependent decrease in S1P levels was observed suggesting SphK inhibition. To confirm that SphK2 inhibition is occurring, we treated U937 cells with the SphK2-specific substrate FTY720^[31] and monitored the formation of phosphorylated-FTY720 (FTY720-P). In this assay, FTY720-P is the read-out for SphK2-specific activity (Figure 3B, Vehicle only), which should decrease upon administration of SphK2 inhibitor. As shown in Figure 3B, increasing SLC5101465 (**6r**) concentration from 0.1 μ M to 1 μ M resulted in concomitant decrease in FTY720-P in a dose dependent manner. These results indicate direct action of SLC5101465 (**6r**) on SphK2 inhibition. To further validate this activity, we subjected SLC5101465 (**6r**) in a previously described *S. cerevisiae* SphK2 inhibition assay.^[32] In this assay, forced expression of SphK2 in yeast results in intracellular accumulation of phosphorylated long-chain bases that reaches toxic concentrations. In the absence of SphK2 inhibitor, the yeast is non-viable while with SphK2 inhibitor, the yeast survives and proliferates resulting in increased optical density. As shown in Figure 3C, treatment of *S. cerevisiae* with SLC5101465 (**6r**) demonstrated a dose-

dependent growth ($EC_{50} = 170 \pm 15$ nM) (Figure 3C). Taken together, these studies validate inhibition of SphK2 with SLC5101465 (**6r**).

Molecular Modeling.

To understand the molecular basis for the increased potency and selectivity as well as the structure-activity relationship profile of the indole-based inhibitors, docking studies were performed using a previously reported homology model of hSphK2.^[29] This homology model was generated based on the published crystal structure of hSphK1 complexed with ADP and magnesium (PDB ID: 3VZB).^[19a] For the purpose of this study, ADP was converted to ATP in the ATP binding site and energy minimizing the resulting structure. The SphK2 energy minimized homology model used in this work was further validated with metrics that analyze peptide backbone favorability, side chain positioning, and energetic metrics.^[20b] Additionally, we previously have shown the ability to replicate ligand poses of known crystal structure ligands, as well as sphingosine, with reasonable accuracy (root-mean-square deviation < 0.2 nm), using the molecular docking protocol previously published and discussed for multiple isoforms and orthologues of SphK. Residue-inhibitor interactions influential for SphK isoform selectivity are utilized in this work as well. The most potent and selective structure SLC5101465 (**6r**) as well as the corresponding compounds possessing the nonyl tail of the other scaffolds [**6g** (88% and 59%), **6m** (90% and 68%), and **6v** (107% and 79%) SphK1 and SphK2 activity, respectively] were subsequently docked into the substrate-binding cavity of hSphK2 (Figure 4A–D). Flexible ligand, receptor rigid docking of the 1,3-disubstitution pattern in **6g** places the indole ring out of line with the alkyl tail and pushes the compound into the back of the active site towards His556 (Figure 4A). In this case, **6g** is positioned deeper towards the tail portion of the active site, and the indole ring is forced further into the pocket to avoid steric clash with Phe548 (Figure 4A), a residue previously identified as being influential in ligand orientation in the SphK binding cavity. This change in position within the active site eliminates hydrogen bonding interactions with Asp308 and Asp211 and explains the observed decrease in hSphK2-selectivity. However, increased non-polar interactions in the tail region of the active site with Cys533, His556, and Tyr566 provide an explanation for why **6g** retains SphK2 inhibitory activity. In contrast, the 1,4-, 1,5-, and 1,6-disubstituted scaffolds appear to situate the indole moiety in a more horizontal or linear alignment with the alkyl tail in the binding cavity (Figure 4B–D). This linear alignment allows for a central positioning within the binding cavity and key nonpolar interactions with Phe548 and Val304, and more similarly mimics sphingosine positioning in the binding pocket. Furthermore, this centralized location of the scaffolds, in what we have previously called the hydrophobic core,^[20b] allows for optimal positioning of ring groups with nonpolar residues to then promote and stabilize electrostatic interactions of the guanidine moiety with Asp211 and/or Asp308, interactions previously reported to afford SphK2-selectivity.^[29] The distance of the guanidine moiety from ATP is beyond the threshold for interaction, highlighting the positioning further into the pocket as to best fill the space and not be dependent on ATP presence for inhibition (Figure 4C). In addition to electrostatic and hydrogen bonding with Asp308 and ATP, the 1,4-disubstituted scaffold (**6m**) engages in Van der Waals interactions with both Val304 and Phe548 (Figure 4B). In comparison, the 1,5-disubstituted scaffold (SLC5101465, **6r**) engages in hydrogen bonding with both Asp308 and Asp211 as well as

Van der Waals interactions with Val304 and Phe548 (Figure 4C). In accordance with the experimental observations, SLC5101465 (**6r**) is predicted to be the most potent and selective inhibitor given the nonpolar interactions with Val308, Phe548, His556, and Cys533 and strong electrostatic interactions and hydrogen bonding with both Asp211 and Asp308. In the case of the 1,6-disubstituted scaffold (**6v**), interactions with both Asp211 and Asp308 are maintained near the ATP binding site (Figure 4D); however, **6v**'s hydrogen bonding and electrostatic interactions with Asp211 and Asp308 are weakened due to longer bond distances in comparison with SLC5101465 [**6r** (4.0 Å vs 3.8 Å and 4.3 Å vs. 3.8 Å, respectively)]. Moreover, nonpolar interactions with SphK2 specific Cys533 are also diminished in **6v**. Between the 1,4- and 1,6-disubstituted scaffolds, **6m** is closer to Asp308 than **6v** (3.0 Å vs. 4.0 Å) and consequently has greater SphK2 inhibition; however, SphK2-selectivity begins to diminish in **6m** due to lack of interactions with Asp211. These results suggest that the volume between Val304 and Phe548 can be a limiting factor on functional groups linked to the 1,2,4-oxadiazole ring and influence interactions with Asp211 and Asp308.^[20b]

To further understand the unique binding mode of the 1,3-disubstituted indole scaffold, **11**, which contains a methylene spacer between the oxadiazole and pyrrolidine rings, was also docked in the homology model. Based on previous work, incorporation of the homopyrrolidine head group increased compound potency for hSphK1;^[21a] however, in this study, it was hypothesized that the addition of a methylene linker would position the inhibitor forward towards the ATP binding site while maintaining interactions with Cys533, His556, and Tyr566. Surprisingly, this compound was inactive in both SphK isoforms. Docking results revealed that the indole moiety of **11** is predicted to be sterically clashed with Phe548 and Val304, resulting in loss of nonpolar interactions deep in the pocket (Figure 4E). This change in position of **11** was caused by the adjustment of the guanidine group, being driven towards Asp308 as a result of the extra methylene. It is possible that extending the methylene linker may alleviate steric clash and allow for favorable interactions with Asp308 and the nonpolar tail. Additionally, these results highlight that the positioning of scaffolds impact the flexibility and position of aromatic moieties of ligands within the binding cavity and their position relevant to Val304 and Phe548, which impact SphK selectivity.

CONCLUSIONS

SphK inhibition represents a promising strategy for the treatment of variety of SIP mediated disease states including inflammation, renal fibrosis, autoimmunity, cancer, among others. While SphK1 inhibition has been extensively studied, much less is known regarding the therapeutic relevance of SphK2 inhibition due to a scarcity of SphK2-selective inhibitors. In this study, we performed a structure–activity relationship profiling of SphK2 inhibitors based on an indole scaffold. By varying substitution patterns around the indole ring, we identified that decorations at the 1,5 position is ideal for SphK2 selectivity. Moreover, homologation studies of the alkyl tail revealed an optimal tail length of either 10 or 9 methylene units depending on the indole scaffold. Molecular docking studies indicate that the linear alignment of the 1,5-disubstituted scaffold allows for optimal nonpolar interactions deep into the substrate binding pocket and electrostatic interactions with the

guanidine head group. Additionally, the position of the indole ring in the nonpolar channel is important. In the binding cavity, alternative substitution patterns sacrifice either nonpolar tail interactions or electrostatic interactions with Asp308 and Asp211 to minimize steric clash between the indole and Phe548/Val304 residues resulting in decreased biological activity. Identified as a result of this study is SLC5101465 (**6r**) which, to the best of our knowledge, is the most selective SphK2-inhibitor (>110-fold) with an equivalent K_i to the most potent SphK2 inhibitor described to date. The remarkable potency and selectivity displayed *in vitro* render SLC5101465 (**6r**) a valuable lead for further optimization and development. We expect these results to aid in the design of future SphK2 selective inhibitors for use as molecular tools and therapeutics for the treatment of disease. Further development of lead compounds and *in vivo* results will be reported in due course.

EXPERIMENTAL

Sphingosine Kinase Inhibition Assays.

The SphK1/2 inhibitory activity of all compounds was determined using a previously described method.^[18c, 21a] Recombinant human SphK1 or SphK2 isolated from cell lysate was incubated with (0.3 μ M, SphK2; 1 μ M SphK1) or without compound, sphingosine (5 μ M SphK1; 10 μ M SphK2), and 250 μ M γ -[32P]ATP. Radiolabeled S1P was isolated by extraction and thin-layer chromatography and quantified by scintillation counting.

The growth of recombinant yeast (*S. cerevisiae*) cells expressing hSphK2 was performed according to our previously described protocol.^[32] Briefly, yeast strain KYA1 harboring a plasmid encoding hSphK2 was selected, maintained, and grown in SC-URA media with 2% glucose overnight at 30°C. The growth media was diluted 1:100 into SC-URA media supplemented with 2% galactose and various concentrations of the test inhibitor. After a 24–48 h incubation at 30°C, cellular growth was quantified by measuring absorbance at 600 nm.

U937 cells were cultured as previously described.^[16b] Protocols for sample preparation and determination of S1P and FTY720-P concentrations *via* LC-MS/MS were performed as previously described.^[16b, 27b]

Molecular Docking.

Molecular docking was performed using compounds experimentally shown to be inhibitors to elucidate protein-ligand interactions that contribute to activity. The generated SphK2 receptor model and the SphK1 template structure model (PDB ID: 3VZB) were used as the receptors for docking.^[29] Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions (Marvin 6.2.2, 2014, ChemAxon; <http://www.chemaxon.com>). AutoDock Tools^[33] was used to prepare the protein and ligand files, while AutoDock Vina^[33b] was used to perform the docking. The grid box was set to 20 $\times$ 20 $\times$ 28 Å, with a 1.000 Å grid spacing was used. The center of the box (50.0, 62.5, 7.3) was placed at the approximate center of the ligand-binding cavity, with a portion of the ATP binding cavity included. Nine docked poses were predicted for each compound. The lowest energy (kcal/mol) pose for each docked ligand, with the guanidine group oriented towards the head of the binding cavity, was considered as the best pose for the given compound. Free

energy of binding scores were cataloged for each docked compound and used as one level of comparison between compounds. The second, more informative aspect of analysis was distance measurements between protein and ligand heavy atoms that would allow for prediction of interactions. Distances were assessed to predict hydrogen bonding (less than 3.5 Å), nonpolar interactions (3.4–3.9 Å), and distal, weaker interactions (greater than 4.0 Å) that would translate to ligand binding and stability in the binding cavity.

General Materials and Synthetic Procedures.

All reagents were purchased from commercial sources and used without further purification unless noted otherwise. Solvents were dried with 4.0 Å molecular sieves. Microwave reactions were conducted in a CEM microwave. Aluminum-backed silica gel, 200 µm, F254 plates were utilized for all thin-layer chromatography. Flash chromatography was performed on silica gel (Zeoprep 60 ECO, 43–60 µm) using either a Combiflash Rf or Biotage Isolera purification system and TLC on silica gel 200µm, F254. ¹H and ¹³C NMR spectra were obtained with either a Bruker Avance-II 500 MHz, Agilent 400-MR 400 MHz, or an Agilent U4-DD2–400 MHz spectrometer. Chemical shifts are reported in ppm and referenced to the residual solvent signal (CDCl₃ or CD₃OD). Low resolution mass spectra and HPLC traces were obtained with a Thermo Electron TSQ triple quadrupole mass spectrometer equipped with and ESI source. HPLC and high-resolution mass spectroscopy (HRMS) was performed on Thermo Electron TSQ triple quadrupole mass spectrometer equipped with an ESI source. HPLC condition: Phenomenex LUNA column (150 mm × 2.0 mm, 5 µm, C18) using a solvent system of 1% formic acid in H₂O (mobile phase A) and 1% formic acid in acetonitrile (mobile phase B) on an Agilent 1100 binary pump with a gradient of 50–95% mobile phase A→B at a flow rate of 0.2 mL/min. Unless otherwise noted, HPLC condition 1 was used. All compounds tested in biological assays are >95% pure by HPLC analyses unless noted otherwise.

General Procedure A: Amidoxime Formation.

Cyanoindole (1 equiv) and HONH₂•HCl (3 equiv), and TEA (3 equiv) were added to a round bottom flask containing EtOH (10 mL EtOH/1mmol cyanoindole). The reaction mixture was heated to 80 °C for 12 hours or until the reaction appeared to have gone to completion via monitoring by TLC. The solution was cooled to room temperature and the solvent was removed under reduced pressure. The resulting solid was loaded onto Celite and purified on a silica gel column with 0–10% MeOH in EtOAc to afford the corresponding amidoxime product.

General Procedure B: 1,2,4-Oxadiazole Formation.

Amidoxime derivative (1 equiv), *N*-Boc-*L*-Proline (1.4 equiv), and DIEA (1.4 equiv) were added to a round bottom flask containing DMF (10 mL DMF/1 mmol amidoxime-indole). After adding HCTU (1.8 equiv) to the reaction, the solution was heated to 120 °C for 12–16 hours. Once the reaction cooled to room temperature, the solution was extracted with EtOAc and saturated LiBr solution. The combined organic layers were washed with brine and dried over Na₂SO₄. After filtration to remove the Na₂SO₄ and concentration via reduced pressure, the resulting brown oil was purified on a silica column with hexanes and EtOAc to afford the pure 1,2,4-oxadiazole product.

General Procedure C: N-Alkylation of Indoles.

Indole intermediate (1 equiv) was added to a round bottom flask which was subsequently purged with N₂ and sealed with a rubber septum. The flask was placed in an ice bath and DMF (1 mL) was added. NaH (1.5 equiv) was added to the flask in one addition and the septum was replaced on the vessel. The reaction mixture was stirred for 30 minutes at 0 °C and the ice bath was replenished. Alkyl halide (3 equiv) was added dropwise to the cooled solution. Liquid alkyl halides were added neat while solid alkyl halides were dissolved in 0.5 mL of DMF. The reaction solution was stirred for an additional 2 hours while the solution warmed to room temperature. The reaction was quenched with the slow addition of deionized water. The product was extracted with EtOAc and saturated LiBr. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentration *via* reduced pressure. The resulting oil was purified on a silica column with hexanes and EtOAc to afford the corresponding N-alkylated indole product.

General Procedure D: Boc Deprotection.

Boc-protected intermediate was dissolved in MeOH (5 mL). HCl gas was bubbled into the solution for 1 minute. The solution was stirred until TLC indicated that the starting material had been consumed. The solvent was removed under reduced pressure. The resulting white to light yellow solid was washed with Et₂O to afford Boc deprotected product as the hydrochloride salt.

General Procedure E: Guanylation.

To the corresponding secondary amine hydrochloride salt in a round bottom flask was added CH₃CN (1 mL) and DIEA (3 equiv). The solution was allowed to stir for 10 minutes before being transferred to a microwave vial containing *N,N'*-di-Boc-1*H*-pyrazole-1-carboximidine, (1.05 equiv). The vessel was capped and placed in a CEM microwave where it was heated to 50 °C for 2 hours. The solvent was removed under reduced pressure and the resulting yellow oil was purified on silica gel with 10–40% EtOAc in hexanes to afford the desired di-Boc protected guanylation product.

***N'*-hydroxy-1*H*-indole-3-carboximidamide (2a).**

Prepared from **1a** according to general procedure A. Purification on a silica gel column with 0–10% MeOH in EtOAc produced **2a** as a mixture of diastereomers as tan solid (0.61 g, 99%). ¹H NMR (400 MHz, CD₃OD) δ 7.94 (ddd, *J* = 8.0, 1.3, 0.8 Hz, 1H), 7.59 (s, 1H), 7.39–7.36 (m, 1H), 7.14 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 7.07 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 153.6, 138.2, 126.3, 125.8, 123.2, 121.9, 121.1, 112.5, 109.2. HRMS (ESI+): Calcd for C₉H₁₀N₃O⁺ [M+H]⁺: 176.0818, Found: 176.0813.

***N'*-hydroxy-1*H*-indole-4-carboximidamide (2b).**

Prepared from **1b** according to general procedure A. Purification on a silica gel column with 70–100% EtOAc in hexanes produced **2b** as a mixture of diastereomers as tan solid (1.12 g, 91%). ¹H NMR (500 MHz, CD₃OD) δ 7.45 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.28 (dd, *J* = 3.4, 1.8 Hz, 1H), 7.24 (dd, *J* = 7.2, 1.6 Hz, 1H), 7.13 (td, *J* = 7.8, 1.8 Hz, 1H), 6.78 (dd, *J* = 3.0, 1.8

Hz, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 156.6, 138.1, 127.1, 126.3, 125.7, 121.8, 119.3, 113.8, 102.5. HRMS (ESI⁺): Calcd for $\text{C}_9\text{H}_{10}\text{N}_3\text{O}^+$ $[\text{M}+\text{H}]^+$: 176.0818, Found: 176.0825.

***N'*-hydroxy-1*H*-indole-5-carboximidamide (2c).**

Prepared from **1c** according to general procedure A. Purification on a silica gel column with 70–100% EtOAc in hexanes produced **2c** as a mixture of diastereomers as tan solid (1.78 g, 96%). ^1H NMR (400 MHz, CD_3OD) δ 7.88 (dd, J = 1.6, 0.8 Hz, 1H), 7.43–7.37 (m, 2H), 7.25 (d, J = 3.2 Hz, 1H), 6.49 (dd, J = 3.2, 0.8 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 157.6, 148.2, 138.5, 129.1, 128.8, 127.3, 126.9, 126.8, 124.3, 121.9, 121.8, 120.7, 119.7, 112.21, 112.16, 111.9, 103.6, 103.06, 103.01. HRMS (ESI⁺): Calcd for $\text{C}_9\text{H}_{10}\text{N}_3\text{O}^+$ $[\text{M}+\text{H}]^+$: 176.0818, Found: 176.0812.

***N'*-hydroxy-1*H*-indole-6-carboximidamide (2d).**

Prepared from **1d** according to general procedure A. Purification on a silica gel column with 70–100% EtOAc in hexanes produced **2d** as a mixture of diastereomers as tan solid (1.12 g, 91%). ^1H NMR (400 MHz, CD_3OD) δ 7.72 (dt, J = 1.7, 0.8 Hz, 1H), 7.61–7.54 (m, 1H), 7.34 (dd, J = 8.3, 1.6 Hz, 1H), 7.27 (d, J = 3.2 Hz, 1H), 6.46 (dd, J = 3.1, 1.0 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 157.2, 137.0, 130.7, 128.9, 127.6, 127.4, 126.6, 121.1, 120.9, 119.2, 118.4, 112.6, 110.5, 102.6, 102.4. HRMS (ESI⁺): Calcd for $\text{C}_9\text{H}_{10}\text{N}_3\text{O}^+$ $[\text{M}+\text{H}]^+$: 176.0818, Found: 176.0812.

***tert*-Butyl (S)-2-(3-(1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (3a).**

Prepared from **2a** according to general procedure B. Purification on a silica gel column with 10–30% EtOAc produced **3a** as a yellow solid (100 mg, 5%). ^1H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 500 MHz, CDCl_3) δ 9.62* (s, 1H), 9.10 (s, 1H), 8.25 (dd, J = 6.2, 3.2 Hz, 1H), 8.19–8.16* (m, 1H), 8.13* (d, J = 2.8 Hz, 1H), 7.92 (d, J = 2.8 Hz, 1H), 7.45 (dd, J = 6.3, 3.2 Hz, 1H), 7.42–7.37* (m, 1H), 7.32–7.26 (m, 2H), 7.26–7.21* (m, 1H), 5.29–5.24* (m, 1H), 5.08 (dd, J = 8.2, 3.8 Hz, 1H), 3.78–3.69 (m, 1H), 3.62–3.48 (m, 1H), 2.43–2.32 (m, 1H), 2.26–2.10 (m, 2H), 2.05–1.93 (m, 1H), 1.51* (s, 3H), 1.31 (s, 6H). ^{13}C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 126 MHz, CDCl_3) δ 179.3, 178.4*, 165.4, 154.7*, 154.0, 136.7, 128.4*, 127.4, 124.9, 123.4, 123.1*, 121.9, 121.7*, 121.6, 121.4*, 111.7, 104.3, 80.7, 60.6, 54.0, 47.0*, 46.5, 32.5, 31.6*, 28.6*, 28.3, 24.5*, 23.8. HRMS (ESI⁺): Calcd for $\text{C}_{19}\text{H}_{22}\text{N}_4\text{O}_3$ $[\text{M}+\text{Na}]$: 377.1584, Found: 377.1582.

***tert*-Butyl (S)-2-(3-(1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (3b).**

Prepared from **2b** according to general procedure B. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **3b** as tan solid (0.65 g, 32%). ^1H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl_3) δ 8.71 (s, 1H), 7.96 (d, J = 7.4 Hz, 1H), 7.92* (d, J = 7.4 Hz, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.52* (d, J = 8.1 Hz, 1H), 7.36 (t, J = 2.6 Hz, 1H), 7.31* (s, 1H), 7.30–7.26 (m, 1H), 7.25–7.20* (m, 1H), 5.33–5.23* (m, 1H), 5.13 (dd, J = 8.2, 3.7 Hz, 1H), 3.81–3.67 (m, 1H), 3.62–3.48 (m, 1H), 2.49–2.35 (m, 1H), 2.24–2.12 (m, 2H), 2.06–1.97 (m, 1H), 1.49* (s, 3H), 1.31 (s, 6H). ^{13}C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl_3) δ 179.7, 169.0, 153.9, 136.5, 126.0, 125.8, 125.7, 125.6, 121.8, 121.7, 121.3, 121.2, 118.3, 114.42, 114.37, 114.3,

104.0, 103.9, 80.7, 80.5, 54.0, 46.8*, 46.5, 32.6, 31.7*, 28.6*, 28.3, 24.4*, 23.8. HRMS (ESI⁺): Calcd for C₁₉H₂₂N₄O₃ [M+Na]: 377.1584, Found: 377.1587.

***tert*-Butyl (S)-2-(3-(1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (3c).**

Prepared from **2c** according to general procedure B. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **3c** as tan solid (3.59 g, 45%). ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 9.52 (s, 1H), 9.40* (s, 1H), 8.43 (d, *J* = 1.6 Hz, 1H), 8.31* (s, 1H), 7.93 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.86* (dd, *J* = 6.3, 3.1 Hz, 1H), 7.81* (d, *J* = 8.6 Hz, 1H), 7.48 (d, *J* = 8.6 Hz, 1H), 7.40–7.35 (m, 1H), 7.27–7.23 (m, 1H), 7.22–7.19* (m, 1H), 6.60 (s, 1H), 6.55* (s, 1H), 5.26–5.21* (m, 1H), 5.09 (dd, *J* = 8.2, 3.9 Hz, 1H), 3.80–3.65 (m, 1H), 3.60–3.45 (m, 1H), 2.42–2.27 (m, 1H), 2.19–2.07 (m, 2H), 2.01–1.90 (m, 1H), 1.48* (s, 3H), 1.32 (s, 6H). ¹³C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl₃) δ 180.0, 179.5*, 171.4*, 169.5, 154.5*, 154.0, 137.6, 137.5*, 128.0, 127.8*, 125.9, 125.8, 125.7*, 120.8, 120.7*, 117.8, 114.9, 111.8, 111.6*, 103.0, 80.8, 60.5, 53.9, 46.7*, 46.4, 32.3, 31.5*, 28.4*, 28.2, 24.3*, 23.7, 21.0, 14.1. HRMS (ESI⁺): Calcd for C₁₉H₂₂N₄O₃ [M+Na]: 377.1584, Found: 377.1589.

***tert*-Butyl (S)-2-(3-(1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (3d).**

Prepared from **2d** according to general procedure B. Purification on a silica gel column with 15–40% EtOAc in hexanes produced **3d** as tan solid (0.57 g, 28%). ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 500 MHz, CDCl₃) δ 8.85 (s, 1H), 8.18 (s, 1H), 8.08* (s, 1H), 7.85 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.81–7.77* (m, 1H), 7.72 (d, *J* = 8.3 Hz, 1H), 7.66* (d, *J* = 8.2 Hz, 1H), 7.34–7.31 (m, 1H), 7.31–7.28* (m, 1H), 6.59 (t, *J* = 2.5 Hz, 1H), 6.56* (s, 1H), 5.24* (dd, *J* = 8.4, 2.6 Hz, 1H), 5.09 (dd, *J* = 8.2, 3.8 Hz, 1H), 3.80–3.72 (m, 1H), 3.71–3.66* (d, *J* = 7.4 Hz, 1H), 3.63–3.55 (m, 1H), 3.54–3.47* (m, 1H), 2.48–2.31 (m, 1H), 2.22–2.10 (m, 2H), 2.07–1.93 (m, 1H), 1.48* (s, 3H), 1.31 (s, 6H). ¹³C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 126 MHz, CDCl₃) δ 180.2, 169.3, 153.9, 135.6, 130.3, 126.7, 121.1, 120.1, 118.8, 111.0, 102.9, 80.7, 53.9, 46.8*, 46.4, 32.4, 31.5*, 28.4*, 28.2, 24.4*, 23.7. HRMS (ESI⁺): Calcd for C₁₉H₂₂N₄O₃ [M+H]⁺: 377.3927, Found: 377.1600.

***tert*-Butyl (S)-2-(3-(1-heptyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4e).**

Prepared from **3a** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4e** (34 mg, 89%) as yellow oil. ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 8.30–8.21 (m, 1H), 7.83 (s, 1H), 7.42–7.36 (m, 1H), 7.35–7.26 (m, 2H), 5.23* (d, *J* = 7.2 Hz, 1H), 5.07 (dd, *J* = 8.2, 3.7 Hz, 1H), 4.16 (t, *J* = 7.0 Hz, 2H), 3.82–3.66 (m, 1H), 3.62–3.44 (m, 1H), 2.44–2.30 (m, 1H), 2.23–2.11 (m, 2H), 2.04–1.93 (m, 1H), 1.88 (p, *J* = 7.0 Hz, 2H), 1.47* (s, 3H), 1.39–1.16 (m, 13H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 179.2, 165.3, 153.8, 136.9, 130.6, 125.6, 122.9, 122.7, 122.1, 121.3, 121.2, 110.0, 102.9, 80.5, 53.9, 47.0, 46.8, 46.5, 32.5, 31.8, 31.6, 30.1, 29.0, 28.6, 28.3, 27.0, 24.5, 23.8, 22.7, 14.2. HRMS (ESI⁺): Calcd for C₂₆H₃₆N₄O₃ [M+K]⁺: 491.2419, Found: 491.2428.

***tert*-Butyl (S)-2-(3-(1-octyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4f).**

Prepared from **3a** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4f** (15 mg, 9%) as yellow oil. ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 8.24 (dd, *J* = 7.0, 2.0 Hz, 1H), 7.83 (s, 1H), 7.42–7.36 (m, 1H), 7.34–7.27 (m, 2H), 5.22* (d, *J* = 8.2 Hz, 1H), 5.07 (dd, *J* = 8.1, 3.6 Hz, 1H), 4.17 (t, *J* = 7.1 Hz, 2H), 3.80–3.67 (m, 1H), 3.61–3.49 (m, 1H), 2.47–2.30 (m, 1H), 2.21–2.12 (m, 2H), 2.07–1.97 (m, 1H), 1.94–1.82 (m, 2H), 1.47* (s, 3H), 1.37–1.20 (m, 18H), 0.86 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 179.2, 165.3, 153.8, 136.9, 130.6, 125.7, 122.9, 122.2, 121.4, 110.0, 102.9, 80.5, 53.9, 47.1, 46.5, 32.6, 31.9, 30.1, 29.3, 29.3, 28.6, 28.3, 27.1, 23.9, 22.8, 14.2. HRMS (ESI+): Calcd for C₂₇H₃₈N₄O₃ [M+K]: 505.2575, Found: 505.2567.

***tert*-Butyl (S)-2-(3-(1-nonyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4g).**

Prepared from **3a** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4g** (40 mg, 74%) as yellow oil. ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 8.31–8.20 (m, 1H), 7.83 (s, 1H), 7.44–7.35 (m, 1H), 7.35–7.21 (m, 2H), 5.29–5.20* (m, 1H), 5.07 (dd, *J* = 8.2, 3.6 Hz, 1H), 4.16 (t, *J* = 7.1 Hz, 2H), 3.81–3.63 (m, 1H), 3.61–3.50 (m, 1H), 2.45–2.31 (m, 1H), 2.26–2.09 (m, 2H), 2.05–1.95 (m, 1H), 1.89 (p, *J* = 7.0 Hz, 2H), 1.47* (s, 3H), 1.38–1.19 (m, 16H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 179.2, 165.3, 153.8, 136.9, 130.6, 125.6, 122.9, 122.7, 122.1, 121.3, 110.0, 102.9, 80.5, 77.5, 77.2, 76.8, 53.9, 47.0, 46.5, 32.5, 31.9, 31.6, 30.1, 29.5, 29.3, 28.5, 28.3, 27.0, 24.5, 23.8, 22.8, 22.7, 14.2. HRMS (ESI+): Calcd for C₂₈H₄₀N₄O₃ [M+K]⁺: 519.2732, Found: 519.2736.

***tert*-Butyl (S)-2-(3-(1-decyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4h).**

Prepared from **3a** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4h** (42 mg, 100%) as yellow oil. ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 8.26–8.20 (m, 1H), 7.84 (s, 1H), 7.44–7.35 (m, 1H), 7.35–7.24 (m, 2H), 5.27–5.19* (m, 1H), 5.07 (dd, *J* = 8.2, 3.7 Hz, 1H), 4.16 (t, *J* = 7.0 Hz, 2H), 3.80–3.66 (m, 1H), 3.61–3.52 (m, 1H), 2.46–2.31 (m, 1H), 2.26–2.12 (m, 2H), 2.07–1.94 (m, 1H), 1.93–1.83 (m, 2H), 1.47* (s, 3H), 1.39–1.20 (m, 19H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 179.2, 165.3, 153.8, 136.9, 130.6, 125.6, 122.9, 122.7, 122.1, 121.3, 121.2, 110.0, 102.9, 80.5, 53.9, 47.0, 46.8, 46.5, 32.5, 32.0, 31.6, 30.1, 29.61, 29.56, 29.4, 29.3, 28.5, 28.3, 27.0, 24.5, 23.8, 22.8, 14.2. HRMS (ESI+): Calcd for C₂₉H₄₂N₄O₃ [M+K]⁺: 533.2888, Found: 533.2902.

***tert*-Butyl (S)-2-(3-(1-undecyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4i).**

Prepared from **3a** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4i** (22 mg, 77%) as yellow oil. ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 8.24 (dd, *J* = 6.9, 2.0 Hz, 1H), 7.83 (s, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.28 (d, *J* = 14.4 Hz, 2H), 5.27–5.17* (m, 1H), 5.07 (dd, *J* = 8.3, 3.6 Hz, 1H), 4.16 (t, *J* = 7.1 Hz, 2H), 3.80–3.70 (m, 1H), 3.62–3.51 (m, 1H), 2.45–2.29 (m, 1H), 2.24–2.10 (m, 2H), 2.06–1.98 (m, 1H), 1.89 (p, *J* = 6.6 Hz, 2H),

1.47* (s, 3H), 1.38–1.14 (m, 21H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 179.2, 165.3, 153.8, 137.0, 130.6, 125.7, 122.9, 122.2, 121.3, 110.0, 103.0, 80.5, 54.0, 47.1, 46.5, 32.6, 32.0, 30.2, 29.7, 29.6, 29.43, 29.36, 28.6, 28.3, 27.1, 23.9, 22.8, 14.3. HRMS (ESI+): Calcd for $\text{C}_{30}\text{H}_{44}\text{N}_4\text{O}_3$ $[\text{M}+\text{Na}]^+$: 531.3311, Found: 531.3354.

***tert*-Butyl (S)-2-(3-(1-dodecyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4j).**

Prepared from **3a** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4j** (26 mg, 59%) as yellow oil. ^1H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl_3) δ 8.24 (d, J = 6.8, 1H), 7.83 (s, 1H), 7.40 (d, J = 7.7 Hz, 1H), 7.3–7.25 (m, 2H), 5.22* (d, J = 8.3 Hz, 1H), 5.07 (dd, J = 8.2, 3.6 Hz, 1H), 4.16 (t, J = 7.1 Hz, 2H), 3.80–3.65 (m, 1H), 3.61–3.50 (m, 1H), 2.46–2.31 (m, 1H), 2.22–2.12 (m, 2H), 2.04–1.95 (m, 1H), 1.88 (p, J = 6.9 Hz, 2H), 1.47* (s, 3H), 1.39–1.14 (m, 30H), 0.89 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 179.2, 165.3, 153.8, 136.9, 130.6, 125.6, 122.9, 122.2, 121.3, 110.0, 102.9, 80.5, 54.0, 47.1, 46.5, 32.6, 32.1, 30.2, 29.74, 29.69, 29.6, 29.5, 29.4, 28.6, 28.3, 27.1, 23.9, 22.8, 14.3. HRMS (ESI+): Calcd for $\text{C}_{31}\text{H}_{46}\text{N}_4\text{O}_3$ $[\text{M}+\text{Na}]^+$: 545.3468, Found: 545.3447.

***tert*-Butyl (S)-2-(3-(1-heptyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4k).**

Prepared from **3b** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4k** as yellow oil (22 mg, 57%). ^1H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 500 MHz, CDCl_3) δ 7.97–7.91 (m, 1H), 7.50 (d, J = 8.2 Hz, 1H), 7.30 (t, J = 7.8 Hz, 1H), 7.26–7.22 (m, 1H), 7.20 (d, J = 3.1 Hz, 1H), 5.30–5.20* (m, 1H), 5.12 (dd, J = 8.2, 3.6 Hz, 1H), 4.17 (t, J = 7.0 Hz, 2H), 3.77–3.61 (m, 1H), 3.61–3.48 (m, 1H), 2.44–2.35 (m, 1H), 2.24–2.13 (m, 2H), 2.05–1.95 (m, 1H), 1.85 (p, J = 8.0, 7.4 Hz, 2H), 1.47* (s, 3H), 1.37–1.20 (m, 16H), 0.86 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 179.7, 169.0, 153.8, 136.7, 129.5, 126.3, 121.2, 120.8, 118.5, 112.6, 102.5, 80.6, 54.0, 46.8, 46.5, 32.6, 31.8, 30.5, 29.0, 28.6, 28.3, 27.1, 23.9, 22.7, 14.2. HRMS (ESI+): Calcd for $\text{C}_{26}\text{H}_{36}\text{N}_4\text{O}_3$ $[\text{M}+\text{Na}]^+$: 475.2685, Found: 475.2673.

***tert*-Butyl (S)-2-(3-(1-octyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4l).**

Prepared from **3b** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4l** as yellow oil (30 mg, 76%). ^1H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 500 MHz, CDCl_3) δ 7.93 (d, J = 7.3 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.30 (t, J = 7.8 Hz, 1H), 7.25 (d, J = 3.2 Hz, 1H), 7.20 (d, J = 3.2 Hz, 1H), 5.36–5.20* (m, 1H), 5.12 (dd, J = 8.2, 3.6 Hz, 1H), 4.17 (t, J = 7.0 Hz, 2H), 3.80–3.66 (m, 1H), 3.54–3.45 (m, 1H), 2.40–2.30 (m, 1H), 2.24–2.14 (m, 2H), 2.07–1.95 (m, 1H), 1.85 (p, J = 7.0 Hz, 2H), 1.47* (s, 3H), 1.34–1.21 (m, 18H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 179.7, 169.0, 153.8, 136.6, 129.5, 126.3, 121.2, 120.8, 118.5, 112.6, 102.5, 80.6, 54.0, 46.8, 46.5, 32.6, 31.9, 30.5, 29.33, 29.28, 28.6, 28.3, 27.1, 23.9, 22.7, 14.3, 14.2. HRMS (ESI+): Calcd for $\text{C}_{27}\text{H}_{38}\text{N}_4\text{O}_3$ $[\text{M}+\text{Na}]^+$: 489.2842, Found: 489.2820.

***tert*-Butyl (S)-2-(3-(1-nonyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4m).**

Prepared from **3b** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4m** as yellow oil (29 mg, 71%). ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 7.93 (dd, *J* = 7.4, 0.9 Hz, 1H), 7.50 (d, *J* = 8.3 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.26–7.23 (m, 1H), 7.20 (d, *J* = 3.0 Hz, 1H), 5.37–5.20* (m, 1H), 5.12 (dd, *J* = 8.2, 3.6 Hz, 1H), 4.16 (t, *J* = 7.1 Hz, 2H), 3.81–3.68 (m, 1H), 3.62–3.49 (m, 1H), 2.47–2.34 (m, 1H), 2.26–2.13 (m, 2H), 2.06–1.96 (m, 1H), 1.86 (p, *J* = 7.2 Hz, 2H), 1.47* (m, 3H), 1.35–1.19 (m, 19H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 179.7, 169.0, 153.8, 136.6, 129.5, 126.3, 121.1, 120.7, 118.4, 112.6, 102.4, 80.5, 54.0, 46.7, 46.5, 32.6, 31.9, 30.5, 29.5, 29.33, 29.31, 28.5, 28.3, 27.1, 23.8, 22.7, 14.2. HRMS (ESI⁺): Calcd for C₂₈H₄₀N₄O₃ [M+Na]⁺: 503.2998, Found: 503.2987.

***tert*-Butyl (S)-2-(3-(1-decyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4n).**

Prepared from **3b** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4n** as yellow oil (30 mg, 72%). ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 7.93 (dd, *J* = 7.4, 0.9 Hz, 1H), 7.50 (d, *J* = 8.3 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.22 (dd, *J* = 19.7, 3.1 Hz, 2H), 5.37–5.22* (m, 1H), 5.12 (dd, *J* = 8.2, 3.6 Hz, 1H), 4.16 (t, *J* = 7.1 Hz, 2H), 3.78–3.62 (m, 1H), 3.60–3.52 (m, 1H), 2.44–2.31 (m, 1H), 2.24–2.11 (m, 2H), 2.05–1.94 (m, 1H), 1.85 (p, *J* = 7.0 Hz, 2H), 1.47* (s, 3H), 1.31–1.20 (m, 21H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 179.7, 169.0, 153.8, 136.6, 129.5, 126.3, 121.2, 121.1, 120.8, 118.4, 112.6, 102.4, 80.5, 54.0, 46.7, 46.5, 32.6, 32.0, 30.5, 29.62, 29.60, 29.38, 29.35, 28.6, 28.3, 27.1, 23.8, 22.8, 14.2. HRMS (ESI⁺): Calcd for C₂₉H₄₂N₄O₃ [M+Na]⁺: 517.3155, Found: 517.3177.

***tert*-Butyl (S)-2-(3-(1-hexyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4o).**

Prepared from **3c** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4o** as yellow oil (72 mg, 97%). ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 8.38 (d, *J* = 1.6 Hz, 1H), 7.92 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 7.14 (dt, *J* = 3.6 Hz, 1H), 6.57 (d, *J* = 3.8 Hz, 1H), 5.23* (d, *J* = 6.7 Hz, 1H), 5.07 (dd, *J* = 8.1, 3.8 Hz, 1H), 4.13 (t, *J* = 7.1 Hz, 2H), 3.78–3.65 (m, 1H), 3.60–3.45 (m, 1H), 2.48–2.34 (m, 1H), 2.22–2.08 (m, 2H), 2.06–1.94 (m, 1H), 1.84 (p, *J* = 7.2 Hz, 2H), 1.47* (s, 3H), 1.30 (s, 14H), 0.87 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl₃) δ 180.2, 169.5, 153.8, 137.6, 129.1, 129.0*, 128.6, 121.3, 120.8*, 120.6, 117.8, 109.9, 109.8*, 102.2, 80.5, 54.0, 46.7, 46.5*, 32.5*, 31.5, 30.4, 28.5*, 28.3, 26.7, 24.5*, 23.8, 22.6, 14.1. HRMS (ESI⁺): Calcd for C₂₅H₃₄N₄O₃ [M+Na]⁺: 461.2529, Found: 461.2525.

***tert*-Butyl (S)-2-(3-(1-heptyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4p).**

Prepared from **3c** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4p** as yellow oil (52 mg, 82%). ¹H NMR (2:1 rotamer

ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 8.38 (d, J = 1.6 Hz, 1H), 7.92 (dd, J = 8.6, 1.7 Hz, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.14 (d, J = 3.5 Hz, 1H), 6.57 (d, J = 3.2 Hz, 1H), 5.22* (d, J = 8.1 Hz, 1H), 5.07 (dd, J = 8.1, 3.7 Hz, 1H), 4.13 (t, J = 7.1, 2H), 3.78–3.65 (m, 1H), 3.61–3.51 (m, 1H), 2.46–2.32 (m, 1H), 2.25–2.13 (m, 2H), 2.06–1.95 (m, 2H), 1.84 (p, J = 7.0 Hz, 2H), 1.47* (s, 3H), 1.36–1.23 (m, 14H), 0.86 (t, J = 6.9 Hz, 3H). ¹³C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl₃) δ 180.2, 169.5, 153.8, 137.6, 129.1, 129.0*, 128.6, 121.3, 120.8*, 120.6, 118.1*, 117.8, 109.9, 109.8*, 102.2, 80.5, 54.0, 46.7, 46.5*, 32.5, 31.8, 31.7*, 30.4, 29.0, 28.5*, 28.3, 27.0, 24.5*, 23.8, 22.7, 14.2. HRMS (ESI+): Calcd for C₂₆H₃₆N₄O₃ [M+Na]⁺: 475.2685, Found: 475.2681.

***tert*-Butyl (S)-2-(3-(1-octyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4q).**

Prepared from **3c** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4q** as yellow oil (76 mg, 97%). ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 8.38 (d, J = 1.6 Hz, 1H), 7.92 (dd, J = 8.6, 1.6 Hz, 1H), 7.41 (d, J = 8.7 Hz, 1H), 7.15 (d, J = 3.2 Hz, 1H), 6.58 (d, J = 3.2 Hz, 1H), 5.31–5.18* (m, 1H), 5.07 (dd, J = 8.2, 3.7 Hz, 1H), 4.13 (t, J = 7.1 Hz, 2H), 3.78–3.64 (m, 1H), 3.62–3.48 (m, 1H), 2.42–2.32 (m, 1H), 2.24–2.12 (m, 2H), 2.07–1.94 (m, 1H), 1.83 (p, J = 6.8 Hz, 2H), 1.47* (s, 3H), 1.36–1.21 (m, 18H), 0.86 (t, J = 6.5 Hz, 3H). ¹³C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl₃) δ 180.2, 169.5, 153.8, 137.6, 129.1, 129.0*, 128.6, 121.3, 120.8*, 120.6, 117.8, 110.1, 109.9, 109.8*, 102.2, 80.5, 77.5, 77.4, 77.2, 76.8, 54.0, 46.7, 46.5*, 32.5, 31.9, 31.7*, 30.4, 29.3, 29.3, 28.5*, 28.3, 27.1, 24.5*, 23.9, 22.7, 14.2. HRMS (ESI+): Calcd for C₂₇H₃₈N₄O₃ [M+Na]⁺: 489.6054, Found: 489.2839.

***tert*-Butyl (S)-2-(3-(1-nonyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4r).**

Prepared from **3c** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4r** as yellow oil (28 mg, 52%). ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 8.38 (d, J = 1.6 Hz, 1H), 7.92 (dd, J = 8.6, 1.6 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.15 (d, J = 3.1 Hz, 1H), 6.57 (d, J = 3.2 Hz, 1H), 5.22* (d, J = 8.2 Hz, 1H), 5.07 (dd, J = 8.1, 3.7 Hz, 1H), 4.13 (t, J = 7.2 Hz, 3H), 3.79–3.66 (m, 1H), 3.63–3.51 (m, 1H), 2.45–2.33 (m, 1H), 2.22–2.10 (m, 2H), 2.06–1.96 (m, 1H), 1.85 (p, J = 7.4 Hz, 2H), 1.47* (s, 3H), 1.37–1.21 (m, 21H), 0.87 (t, J = 7.1 Hz, 3H). ¹³C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl₃) δ 169.5, 153.8, 137.6, 129.1, 129.0*, 128.7, 121.3, 120.8*, 120.7, 117.9, 109.9, 109.8*, 102.3, 80.5, 54.0, 46.7, 46.5*, 32.6*, 31.9, 30.4, 29.6, 29.4, 29.3, 28.6*, 28.3, 27.1, 23.9*, 22.8, 14.2. HRMS (ESI+): Calcd for C₂₈H₄₀N₄O₃ [M+Na]⁺: 503.2993, Found: 503.2966.

***tert*-Butyl (S)-2-(3-(1-decyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4s).**

Prepared from **3c** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4s** as yellow oil (56 mg, 80%). ¹H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl₃) δ 8.39 (d, J = 1.6 Hz, 1H), 7.92 (dd, J = 8.6, 1.6 Hz, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.14 (d, J = 3.3 Hz, 1H), 6.57 (d, J = 3.2 Hz, 1H), 5.22* (d, J = 8.1 Hz, 1H), 5.07 (dd, J = 8.0, 3.5 Hz, 1H), 4.13 (t, J = 7.1 Hz, 2H),

3.78–3.67 (m, 1H), 3.62–3.49 (m, 1H), 2.45–2.33 (m, 1H), 2.23–2.11 (m, 2H), 2.09–1.97 (m, 1H), 1.84 (p, $J = 7.5$ Hz, 2H), 1.47* (s, 3H), 1.3–1.19 (m, 20H), 0.87 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl_3) δ 180.2, 169.5, 153.8, 137.6, 129.1, 128.9*, 128.7, 121.3, 120.8*, 120.7, 118.1*, 117.9, 109.9*, 109.8, 102.2, 80.5, 54.0, 46.7, 46.5*, 32.5*, 32.0, 30.4, 29.60, 29.58, 29.5, 29.4, 29.3, 28.5*, 28.3, 27.1, 23.8*, 22.8, 14.2. HRMS (ESI+): Calcd for $\text{C}_{29}\text{H}_{42}\text{N}_4\text{O}_3$ $[\text{M}+\text{Na}]^+$: 517.3149, Found: 517.3111.

***tert*-Butyl (S)-2-(3-(1-heptyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4t).**

Prepared from **3d** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4t** as yellow oil (55 mg, 86%). ^1H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl_3) δ 8.09 (d, $J = 1.3$ Hz, 1H), 7.83 (dd, $J = 8.2, 1.5$ Hz, 1H), 7.69 (d, $J = 8.4$ Hz, 1H), 7.20 (t, $J = 3.6$ Hz, 1H), 6.53 (d, $J = 3.2$ Hz, 1H), 5.31–5.19* (m, 1H), 5.09 (dd, $J = 8.2, 3.9$ Hz, 1H), 4.18 (t, $J = 7.0$ Hz, 2H), 3.80–3.67 (m, 1H), 3.62–3.42 (m, 1H), 2.48–2.33 (m, 1H), 2.26–2.09 (m, 2H), 2.06–1.96 (m, 1H), 1.86 (p, $J = 7.1$ Hz, 2H), 1.47* (s, 3H), 1.37–1.20 (m, 14H), 0.86 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl_3) δ 180.3, 169.5, 153.8, 135.9, 131.0, 130.2, 130.0*, 121.4, 121.3*, 119.7, 118.6*, 118.5, 109.4*, 109.2, 101.5, 80.5, 54.0, 46.8*, 46.7, 46.5*, 32.5, 31.8, 31.7*, 30.5, 29.0, 28.5*, 28.3, 27.0, 24.5*, 23.8, 22.7, 14.2. HRMS (ESI+): Calcd for $\text{C}_{26}\text{H}_{36}\text{N}_4\text{O}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$: 475.2680, Found 475.2677.

***tert*-Butyl (S)-2-(3-(1-octyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4u).**

Prepared from **3d** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4u** as yellow oil (51 mg, 97%). ^1H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl_3) δ 8.09 (s, 1H), 7.83 (d, $J = 8.1$ Hz, 1H), 7.69 (d, $J = 8.4$ Hz, 1H), 7.21 (d, $J = 3.2$ Hz, 1H), 6.53 (d, $J = 3.2$ Hz, 1H), 5.28–5.18* (m, 1H), 5.09 (dd, $J = 8.1, 3.9$ Hz, 1H), 4.18 (t, $J = 7.1$ Hz, 2H), 3.81–3.69 (m, 1H), 3.64–3.46 (m, 1H), 2.48–2.32 (m, 1H), 2.28–2.13 (m, 2H), 2.07–1.96 (m, 1H), 1.86 (p, $J = 7.2$ Hz, 2H), 1.47* (s, 3H), 1.37–1.19 (m, 15H), 0.86 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl_3) δ 180.3, 169.6, 153.8, 135.9, 131.0, 130.2, 130.0*, 121.4, 121.3*, 119.7, 118.6*, 118.5, 109.4*, 109.2, 101.5, 80.6, 54.0, 46.7, 46.5, 32.6, 31.9, 31.7*, 30.5, 29.33, 29.28, 28.5*, 28.3, 27.1, 24.5*, 23.9, 22.7, 14.2. HRMS (ESI+): Calcd for $\text{C}_{27}\text{H}_{38}\text{N}_4\text{O}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$: 489.2836, Found: 489.2836.

***tert*-Butyl (S)-2-(3-(1-nonyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4v).**

Prepared from **3d** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4v** as yellow oil (62 mg, 65%). ^1H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl_3) δ 8.09 (p, $J = 0.9$ Hz, 1H), 7.83 (d, $J = 8.2$ Hz, 1H), 7.69 (d, $J = 8.4$ Hz, 1H), 7.21 (d, $J = 3.1$ Hz, 1H), 6.53 (d, $J = 3.1$ Hz, 1H), 5.37–5.16* (m, 1H), 5.09 (dd, $J = 8.2, 3.9$ Hz, 1H), 4.18 (t, $J = 7.0$ Hz, 2H), 3.80–3.69 (m, 1H), 3.63–3.49 (m, 1H), 2.48–2.34 (m, 1H), 2.26–2.10 (m, 2H), 2.05–1.98 (m, 1H), 1.86

(p, $J = 7.2$ Hz, 2H), 1.47* (s, 3H), 1.36–1.19 (m, 17H), 0.86 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl_3) δ 180.3, 169.5, 153.8, 135.9, 131.0, 130.2, 130.0*, 121.4, 121.3*, 119.7, 118.6*, 118.5, 109.4*, 109.2, 101.5, 80.6, 54.0, 46.7*, 46.5, 32.6, 31.9, 31.7*, 30.5, 29.6, 29.4, 29.3, 28.5*, 28.3, 27.1, 24.5*, 23.9, 22.8, 14.2. HRMS (ESI+): Calcd for $\text{C}_{28}\text{H}_{40}\text{N}_4\text{O}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$: 503.2993, Found: 503.2975.

***tert*-Butyl (S)-2-(3-(1-decyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidine-1-carboxylate (4w).**

Prepared from **3d** according to general procedure C. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **4w** as yellow (92 mg, 94%). ^1H NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 400 MHz, CDCl_3) δ 8.09 (s, 1H), 7.83 (d, $J = 8.1$ Hz, 1H), 7.69 (d, $J = 8.4$ Hz, 1H), 7.21 (d, $J = 3.1$ Hz, 1H), 6.53 (d, $J = 3.1$ Hz, 1H), 5.23* (t, $J = 4.3$ Hz, 1H), 5.09 (dd, $J = 8.2, 3.9$ Hz, 1H), 4.18 (t, $J = 7.1$ Hz, 2H), 3.82–3.68 (m, 1H), 3.63–3.50 (m, 1H), 2.48–2.32 (m, 1H), 2.25–2.13 (m, 2H), 2.07–1.97 (m, 1H), 1.85 (p, $J = 7.1$ Hz, 2H), 1.47* (s, 3H), 1.37–1.19 (m, 19H), 0.87 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (2:1 rotamer ratio, asterisk denote minor rotamer peak, 101 MHz, CDCl_3) δ 180.3, 169.5, 153.8, 135.8, 131.0, 130.2, 130.1*, 121.4, 121.3*, 119.7, 118.6*, 118.5, 109.4*, 109.2, 101.5, 101.4*, 80.5, 54.0, 46.8*, 46.7, 46.5*, 32.5, 32.0, 31.7*, 30.5, 29.61, 29.60, 29.55, 29.37, 29.36, 28.5*, 28.3, 27.1, 24.5*, 23.9, 22.8, 14.2. HRMS (ESI+): Calcd for $\text{C}_{29}\text{H}_{42}\text{N}_4\text{O}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$: 517.3149, Found: 517.3124.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-heptyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5e).**

Prepared from **4e** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5e** (21 mg, 53%) as clear oil. ^1H NMR (500 MHz, CDCl_3) δ 8.27–8.12 (m, 1H), 7.84 (s, 1H), 7.41–7.36 (m, 1H), 7.32–7.24 (m, 3H), 5.59 (s, 1H), 4.16 (t, $J = 7.2$ Hz, 2H), 3.93–3.86 (m, 1H), 3.84–3.76 (m, 1H), 2.49–2.40 (m, 1H), 2.34–2.24 (m, 1H), 2.22–2.12 (m, 1H), 2.08–1.99 (m, 1H), 1.88 (p, $J = 7.3$ Hz, 2H), 1.50–1.45 (m, 19H), 1.35–1.31 (m, 4H), 1.28–1.22 (m, 4H), 0.86 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 177.5, 165.3, 161.9, 153.5, 151.1, 136.9, 130.8, 125.6, 122.9, 122.1, 121.4, 110.0, 102.8, 81.8, 79.8, 55.4, 49.5, 47.1, 36.8, 31.8, 31.5, 30.2, 29.0, 28.3, 28.1, 27.0, 22.7, 14.2. HRMS (ESI+): Calcd for $\text{C}_{32}\text{H}_{47}\text{N}_6\text{O}_5^+$ $[\text{M}+\text{H}]^+$: 595.3602, Found: 595.3608.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-octcyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5f).**

Prepared from **4f** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5f** (15 mg, 76%) as clear oil. ^1H NMR (500 MHz, CDCl_3) δ 8.24–8.18 (m, 1H), 7.83 (s, 1H), 7.42–7.36 (m, 1H), 7.32–7.25 (m, 2H), 5.60 (s, 1H), 4.16 (t, $J = 7.2$ Hz, 2H), 3.93–3.88 (m, 1H), 3.85–3.77 (m, 1H), 2.51–2.41 (m, 1H), 2.33–2.23 (m, 1H), 2.21–2.13 (m, 1H), 2.09–2.01 (m, 1H), 1.89 (p, $J = 7.1$ Hz, 2H), 1.53–1.41 (m, 23H), 1.38–1.31 (m, 4H), 1.31–1.21 (M, 6H), 0.86 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 177.5, 165.4, 162.0, 153.5, 150.7, 137.0, 130.8, 125.7, 122.9,

122.2, 121.4, 110.0, 102.9, 81.7, 79.8, 55.5, 49.6, 47.1, 31.9, 31.5, 30.2, 29.3, 29.2, 28.3, 28.1, 27.7, 27.1, 24.0, 22.7, 14.2. HRMS (ESI⁺): Calcd for C₃₃H₄₈N₆O₅ [M+H]⁺: 609.3759, Found: 609.3371.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-nonyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5g).**

Prepared from **4g** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5g** (25 mg, 64%) as clear oil. ¹H NMR (500 MHz, CDCl₃) δ 8.31–8.19 (m, 1H), 7.86 (s, 1H), 7.46–7.41 (m, 1H), 7.37–7.28 (m, 2H), 5.61 (s, 1H), 4.18 (t, *J* = 7.2 Hz, 2H), 3.94–3.88 (m, 1H), 3.85–3.79 (m, 1H), 2.51–2.45 (m, 1H), 2.36–2.26 (m, 1H), 2.24–2.15 (m, 1H), 2.08–1.98 (m, 1H), 1.90 (p, *J* = 7.4 Hz, 3H), 1.64–1.43 (m, 18H), 1.40–1.32 (m, 4H), 1.32–1.21 (m, 8H), 0.89 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 177.5, 165.3, 162.1, 153.6, 150.6, 136.9, 130.8, 125.6, 122.8, 122.1, 121.4, 110.0, 102.8, 82.2, 79.7, 55.4, 49.5, 47.1, 31.9, 31.5, 31.4, 30.2, 29.5, 29.4, 28.3, 28.1, 27.1, 22.8, 14.2. HRMS (ESI⁺): Calcd for C₃₄H₅₁N₆O₅⁺ [M+H]⁺: 623.3915, Found: 623.3915.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-decyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5h).**

Prepared from **4h** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5h** (23 mg, 52%) as clear oil. ¹H NMR (500 MHz, CDCl₃) δ 8.17 – 8.12 (m, 1H), 7.77 (s, 1H), 7.34–7.28 (m, 1H), 7.26–7.20 (m, 2H), 5.52 (s, 1H), 4.09 (t, *J* = 7.2 Hz, 2H), 3.86–3.79 (m, 1H), 3.76–3.68 (m, 1H), 2.39–2.34 (m, 1H), 2.29–2.17 (m, 1H), 2.16–2.06 (m, 1H), 2.01–1.92 (m, 1H), 1.81 (p, *J* = 7.4 Hz, 2H), 1.48–1.32 (m, 22H), 1.27–1.23 (m, 4H), 1.23–1.08 (m, 10H), 0.80 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 177.46, 165.30, 162.12, 153.53, 150.94, 136.94, 130.79, 125.64, 122.84, 122.13, 121.35, 109.98, 102.78, 82.17, 79.82, 55.42, 49.53, 47.07, 31.97, 31.53, 31.37, 30.21, 29.64, 29.57, 29.37, 28.27, 28.12, 27.98, 27.07, 22.79, 14.23. HRMS (ESI⁺): Calcd for C₃₅H₅₃N₆O₅⁺ [M+H]⁺: 637.4072, Found: 637.4073.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-undecyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5i).**

Prepared from **4i** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5i** (21 mg, 100%), a clear oil. ¹H NMR (500 MHz, CDCl₃) δ 8.26–8.19 (m, 1H), 7.83 (s, 1H), 7.44–7.36 (m, 1H), 7.33–7.26 (m, 2H), 5.59 (s, 1H), 4.16 (t, *J* = 7.2 Hz, 2H), 3.97–3.88 (m, 1H), 3.85–3.77 (m, 1H), 2.51–2.42 (m, 1H), 2.35–2.24 (m, 1H), 2.22–2.14 (m, 1H), 2.09–1.99 (m, 1H), 1.89 (t, *J* = 7.2 Hz, 2H), 1.58–1.41 (m, 18H), 1.37–1.30 (m, 4H), 1.29–1.21 (m, 12H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 177.4, 165.3, 161.8, 153.3, 150.3, 137.0, 130.8, 125.7, 122.9, 122.2, 121.4, 110.0, 102.8, 82.2, 79.7, 55.5, 49.6, 47.1, 32.0, 31.5, 30.2, 29.7, 29.6, 29.4, 28.3, 27.1, 24.1, 22.8, 14.3. HRMS (ESI⁺): Calcd for C₃₆H₅₅N₆O₅⁺ [M+H]⁺: 651.4228, Found: 651.4249.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-dodecyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5j).**

Prepared from **4j** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5j** (21 mg, 99%) as clear oil. ¹H NMR (500 MHz, CDCl₃) δ 8.26–1.18 (m, 1H), 7.83 (s, 1H), 7.45–7.35 (m, 1H), 7.32–7.26 (m, 2H), 5.58 (s, 1H), 4.16 (t, *J* = 7.2 Hz, 2H), 3.93–3.86 (m, 1H), 3.84–3.76 (m, 1H), 2.50–2.38 (m, 1H), 2.22–2.18 (m, 1H), 2.09–1.99 (m, 1H), 1.88 (p, *J* = 7.5 Hz, 2H), 1.54–1.34 (m, 18H), 1.36–1.30 (m, 4H), 1.28–1.20 (m, 14H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 177.5, 165.3, 162.1, 152.9, 150.6, 148.7, 136.9, 130.8, 125.6, 122.8, 122.1, 121.4, 110.0, 102.8, 82.2, 79.7, 55.4, 49.5, 47.1, 32.0, 31.5, 30.2, 29.8, 29.73, 29.70, 29.62, 29.58, 29.5, 29.4, 28.3, 28.1, 27.1, 22.8, 14.3. HRMS (ESI⁺): Calcd for C₃₇H₅₇N₆O₅⁺ [M+H]⁺: 665.4385, Found: 665.4401.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)amino)(2-(3-(1-heptyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methylene)carbamate (5k).**

Prepared from **4k** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5k** (13 mg, 43%) as clear oil. ¹H NMR (400 MHz, CDCl₃) δ 7.93 (dd, *J* = 7.4, 0.9 Hz, 1H), 7.54–7.47 (m, 1H), 7.32–7.27 (m, 1H), 7.23 (t, *J* = 1.6 Hz, 1H), 7.18 (dd, *J* = 3.1, 0.8 Hz, 1H), 5.64 (t, *J* = 6.2 Hz, 1H), 4.17 (t, *J* = 7.1 Hz, 2H), 3.95–3.87 (m, 1H), 3.86–3.76 (m, 1H), 2.52–2.41 (m, 1H), 2.37–2.26 (m, 1H), 2.25–2.13 (m, 2H), 2.10–1.99 (m, 1H), 1.86 (p, *J* = 7.1 Hz, 2H), 1.53–1.39 (m, 17H), 1.35–1.29 (m, 4H), 1.29–1.20 (m, 6H), 0.86 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 178.0, 169.0, 162.2, 153.8, 150.7, 136.6, 129.5, 129.4, 126.3, 121.1, 121.0, 118.3, 112.7, 102.5, 81.9, 79.8, 55.5, 49.6, 46.8, 31.8, 31.6, 30.5, 29.8, 29.0, 28.3, 27.1, 24.1, 22.7, 14.2. HRMS (ESI⁺): Calcd for C₃₂H₄₆N₆O₅Na⁺ [M+Na]⁺: 617.3422, Found: 617.3426.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)amino)(2-(3-(1-octyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methylene)carbamate (5l).**

Prepared from **4l** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5l** (7 mg, 15%) as clear oil. ¹H NMR (500 MHz, CDCl₃) δ 7.93 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.28 (dd, *J* = 8.2, 7.4 Hz, 1H), 7.24–7.22 (m, 1H), 7.18 (dd, *J* = 3.1, 0.9 Hz, 1H), 5.65 (s, 1H), 4.17 (t, *J* = 7.1 Hz, 2H), 3.94–3.87 (m, 1H), 3.87–3.77 (m, 1H), 2.50–2.42 (m, 1H), 2.35–2.26 (m, 1H), 2.25–2.3 (m, 1H), 2.10–2.01 (m, 1H), 1.85 (p, *J* = 7.5 Hz, 2H), 1.51–1.38 (m, 20H), 1.33–1.29 (m, 4H), 1.28–1.20 (m, 8H), 0.86 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 178.0, 169.0, 162.1, 153.6, 150.6, 136.7, 129.5, 126.3, 121.1, 121.0, 118.4, 112.7, 102.5, 82.2, 79.8, 55.5, 49.6, 46.8, 31.9, 31.5, 30.5, 29.8, 29.32, 28.28, 27.1, 24.0, 22.8, 14.2. HRMS (ESI⁺): Calcd for C₃₃H₄₉N₆O₅⁺ [M+H]⁺: 609.3759, Found: 609.3785.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)amino)(2-(3-(1-nonyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methylene)carbamate (5m).**

Prepared from **4m** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5m** (20 mg, 45%) as clear oil. ¹H NMR (500 MHz, CDCl₃) δ 7.93 (d, *J* = 7.3 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.33–7.26 (m, 2H),

7.24–7.22 (m, 1H), 7.18 (d, $J = 3.1$ Hz, 1H), 5.65 (s, 1H), 4.16 (t, $J = 7.1$ Hz, 2H), 3.95–3.90 (m, 1H), 3.84–3.78 (m, 1H), 2.52–2.46 (m, 1H), 2.22–2.18 (m, 1H), 2.11–2.03 (m, 1H), 1.85 (p, $J = 7.2$ Hz, 2H), 1.56–1.34 (m, 21H), 1.33–1.28 (m, 4H), 1.27–1.18 (m, 10H), 0.86 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 178.0, 169.0, 162.1, 153.6, 150.6, 136.6, 129.5, 126.3, 121.1, 121.0, 118.3, 112.7, 102.5, 82.3, 79.8, 55.5, 49.5, 46.8, 32.0, 31.6, 30.5, 29.9, 29.6, 29.38, 29.35, 28.3, 28.1, 27.1, 24.1, 22.8, 14.2. HRMS (ESI $^{+}$): Calcd for $\text{C}_{34}\text{H}_{51}\text{N}_6\text{O}_5^{+}$ $[\text{M}+\text{H}]^{+}$: 623.3915, Found: 623.3915.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)amino)(2-(3-(1-decyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methylene)carbamate (5n).**

Prepared from **4n** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5n** (10 mg, 34%) as clear oil. ^1H NMR (400 MHz, CDCl_3) δ 7.93 (dd, $J = 7.4$, 0.9 Hz, 1H), 7.49 (d, $J = 8.2$ Hz, 1H), 7.28 (dd, $J = 8.2$, 7.4 Hz, 1H), 7.24–7.22 (m, 1H), 7.18 (dd, $J = 3.1$, 0.8 Hz, 1H), 5.64 (s, 1H), 4.16 (t, $J = 7.1$ Hz, 2H), 3.96–3.87 (m, 1H), 3.86–3.76 (m, 1H), 2.51–2.41 (m, 1H), 2.30 (s, 1H), 2.24–2.13 (m, 1H), 2.10–2.00 (m, 1H), 1.85 (p, $J = 7.0$ Hz, 2H), 1.53–1.38 (m, 21H), 1.35–1.28 (m, 6H), 1.28–1.19 (m, 12H), 0.86 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 178.0, 169.0, 162.1, 153.5, 150.6, 136.6, 129.5, 129.4, 126.3, 121.1, 121.0, 118.3, 112.7, 102.5, 82.3, 79.7, 55.5, 49.5, 46.8, 32.0, 31.6, 30.5, 29.9, 29.6, 29.4, 28.3, 27.1, 24.0, 22.8, 14.2. HRMS (ESI $^{+}$): Calcd for $\text{C}_{35}\text{H}_{53}\text{N}_6\text{O}_5^{+}$ $[\text{M}+\text{H}]^{+}$: 637.4072, Found: 637.4083.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-hexyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5o).**

Prepared from **4o** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5o** (30 mg, 48%) as clear oil. ^1H NMR (500 MHz, CDCl_3) δ 8.37 (d, $J = 1.6$ Hz, 1H), 7.90 (d, $J = 1.6$ Hz, 1H), 7.39 (d, $J = 8.6$ Hz, 1H), 7.14 (d, $J = 3.2$ Hz, 1H), 6.56 (dd, $J = 3.1$, 0.8 Hz, 1H), 5.59 (s, 1H), 4.13 (t, $J = 7.2$ Hz, 2H), 3.92–3.86 (m, 1H), 3.84–3.75 (m, 1H), 2.49–2.39 (m, 1H), 2.34–2.24 (m, 1H), 2.22–2.15 (m, 1H), 2.09–1.99 (m, 1H), 1.85 (p, $J = 7.2$ Hz, 2H), 1.54–1.35 (m, 22H), 1.34–1.22 (m, 9H), 0.87 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 178.4, 169.5, 162.1, 153.5, 150.6, 137.7, 129.1, 128.7, 121.5, 120.8, 117.7, 109.9, 102.3, 82.3, 79.7, 55.5, 49.6, 46.7, 31.5, 30.4, 28.3, 28.1, 26.8, 24.1, 22.7, 14.1. HRMS (ESI $^{+}$): Calcd for $\text{C}_{31}\text{H}_{45}\text{N}_6\text{O}_5^{+}$ $[\text{M}+\text{H}]^{+}$: 581.3446, Found: 581.3475.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-heptyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5p).**

Prepared from **4p** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5p** (25 mg, 52%) as clear oil. ^1H NMR (500 MHz, CDCl_3) δ 8.37 (d, $J = 1.7$ Hz, 1H), 7.91 (dd, $J = 8.7$, 1.7 Hz, 1H), 7.39 (d, $J = 8.7$ Hz, 1H), 7.14 (d, $J = 3.2$ Hz, 1H), 6.56 (dd, $J = 3.1$, 0.8 Hz, 1H), 5.59 (s, 1H), 4.13 (t, $J = 7.2$ Hz, 2H), 3.93–3.85 (m, 1H), 3.83–3.77 (m, 1H), 2.49–2.40 (m, 1H), 2.34–2.25 (m, 1H), 2.22–2.14 (m, 1H), 2.09–2.02 (m, 1H), 1.85 (p, $J = 7.0$ Hz, 2H), 1.52–1.39 (m, 17H), 1.34–1.21 (m, 9H), 0.86 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 178.4, 169.5, 162.0, 153.7, 150.7, 137.7, 129.1, 128.7, 121.5, 120.8, 117.7, 109.9, 102.3, 82.3, 79.7, 55.5, 49.6,

46.8, 31.8, 31.5, 30.4, 29.0, 28.5, 28.3, 28.1, 27.1, 24.0, 22.7, 14.4, 14.2. HRMS (ESI+): Calcd for $C_{32}H_{47}N_6O_5^+$ $[M+H]^+$: 595.3602, Found: 595.3620.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-octyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5q).**

Prepared from **4q** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5q** (32 mg, 50%) as clear oil. 1H NMR (400 MHz, $CDCl_3$) δ 8.37 (dd, $J = 1.6, 0.7$ Hz, 1H), 7.91 (dd, $J = 8.6, 1.6$ Hz, 1H), 7.39 (dt, $J = 8.7, 0.8$ Hz, 1H), 7.14 (d, $J = 3.1$ Hz, 1H), 6.56 (dd, $J = 3.2, 0.8$ Hz, 1H), 5.59 (s, 1H), 4.13 (t, $J = 7.2$ Hz, 2H), 3.93–3.87 (m, 1H), 3.85–3.76 (m, 1H), 2.48–2.40 (m, 1H), 2.35–2.26 (m, 1H), 2.23–2.15 (m, 1H), 2.05 (p, $J = 6.4$ Hz, 1H), 1.85 (p, $J = 7.2$ Hz, 2H), 1.51–1.37 (m, 19H), 1.34–1.21 (m, 11H), 0.86 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.4, 169.5, 162.0, 153.5, 150.6, 137.6, 129.1, 128.6, 121.5, 120.7, 117.7, 109.9, 102.3, 82.3, 79.7, 55.4, 49.6, 46.7, 31.9, 31.5, 30.4, 29.3, 28.3, 28.1, 27.1, 22.7, 14.2. HRMS (ESI+): Calcd for $C_{33}H_{49}N_6O_5^+$ $[M+H]^+$: 609.3759, Found: 609.3793.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-nonyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5r).**

Prepared from **4r** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5r** (11 mg, 41%) as clear oil. 1H NMR (500 MHz, $CDCl_3$) δ 8.37 (d, $J = 1.5$ Hz, 1H), 7.91 (dd, $J = 8.6, 1.6$ Hz, 1H), 7.39 (d, $J = 8.7$ Hz, 1H), 7.14 (t, $J = 1.6$ Hz, 1H), 6.56 (dd, $J = 3.1, 0.8$ Hz, 1H), 5.59 (dd, $J = 7.9, 4.6$ Hz, 1H), 4.13 (t, $J = 7.2$ Hz, 2H), 3.94–3.89 (m, 1H), 3.84–3.78 (m, 1H), 2.50–2.42 (m, 1H), 2.34–2.26 (m, 1H), 2.23–2.15 (m, 1H), 2.09–2.01 (m, 1H), 1.86–1.79 (m, 2H), 1.55–1.38 (m, 20H), 1.37–1.17 (m, 14H), 0.86 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.4, 169.5, 153.5, 137.7, 129.1, 128.6, 121.5, 120.8, 117.7, 109.9, 102.3, 81.1, 55.5, 49.6, 46.8, 32.0, 31.5, 30.4, 29.9, 29.6, 29.4, 28.3, 28.0, 27.1, 24.1, 22.8, 14.2. HRMS (ESI+): Calcd for $C_{34}H_{50}N_6O_5Na^+$ $[M+Na]^+$: 645.3735, Found: 645.3714.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-decyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5s).**

Prepared from **4s** according to general procedures D and E. Purification on a silica gel column with 10–30% EtOAc in hexanes produced **5s** as clear oil (36 mg, 61%). 1H NMR (500 MHz, $CDCl_3$) δ 8.37 (d, $J = 1.5$ Hz, 1H), 7.91 (dd, $J = 8.7, 1.6$ Hz, 1H), 7.39 (d, $J = 8.7$ Hz, 1H), 7.14 (d, $J = 1.7$ Hz, 1H), 6.56 (d, $J = 3.1$ Hz, 1H), 5.59 (t, $J = 6.0$ Hz, 1H), 4.13 (t, $J = 7.2$ Hz, 2H), 3.94–3.86 (m, 1H), 3.85–3.77 (m, 1H), 2.47–2.41 (m, 1H), 2.35–2.25 (m, 1H), 2.26–2.16 (m, 1H), 2.11–1.98 (m, 1H), 1.84 (p, $J = 7.9, 7.0$ Hz, 2H), 1.54–1.41 (m, 18H), 1.35–1.20 (m, 16H), 0.87 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.4, 169.5, 161.8, 153.4, 150.6, 137.6, 129.1, 129.0, 128.6, 128.5, 121.5, 120.7, 117.7, 109.9, 102.3, 82.1, 79.8, 55.5, 49.6, 46.7, 32.0, 31.5, 30.4, 29.8, 29.6, 29.4, 28.6, 28.3, 28.1, 27.6, 27.1, 24.8, 24.0, 22.8, 14.2. HRMS (ESI+): Calcd for $C_{35}H_{52}N_6O_5Na^+$ $[M+Na]^+$: 659.3891, Found: 659.3884.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-heptyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5t).**

Prepared from **4t** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5t** as clear oil (31 mg, 41%). ¹H NMR (500 MHz, CDCl₃) δ 8.08 (s, 1H), 7.81 (d, *J* = 8.2 Hz, 1H), 7.68 (d, *J* = 8.3 Hz, 1H), 7.21 (d, *J* = 3.0 Hz, 1H), 6.52 (d, *J* = 3.0 Hz, 1H), 5.62 (dd, *J* = 7.8, 4.8 Hz, 1H), 4.17 (t, *J* = 7.2 Hz, 2H), 3.96–3.88 (m, 1H), 3.85–3.78 (m, 1H), 2.52–2.42 (m, 1H), 2.34–2.16 (m, 2H), 2.09–2.00 (m, 1H), 1.86 (p, *J* = 7.3 Hz, 2H), 1.53–1.37 (m, 20H), 1.32 (p, *J* = 3.2 Hz, 4H) 1.29–1.21 (m, 6H), 0.85 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 178.6, 169.6, 162.1, 153.7, 150.9, 135.9, 131.0, 130.2, 121.3, 119.7, 118.7, 109.3, 101.5, 82.0, 79.9, 55.5, 49.6, 46.7, 31.8, 30.5, 29.8, 29.1, 28.3, 28.1, 27.1, 27.0, 24.1, 22.7, 14.2. HRMS (ESI+): Calcd for C₃₂H₄₇N₆O₅⁺ [M+H]⁺: 595.3602, Found: 595.3616.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-octyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5u).**

Prepared from **4u** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5u** as clear oil (33 mg, 55%). ¹H NMR (500 MHz, CDCl₃) δ 8.08 (s, 1H), 7.81 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.68 (d, *J* = 8.2 Hz, 1H), 7.20 (d, *J* = 3.1 Hz, 1H), 6.52 (d, *J* = 3.0 Hz, 1H), 5.62 (dd, *J* = 7.8, 4.7 Hz, 1H), 4.17 (t, *J* = 7.2 Hz, 2H), 3.95–3.89 (m, 1H), 3.86–3.77 (m, 1H), 2.51–2.41 (m, 1H), 2.34–2.12 (m, 2H), 2.09–1.99 (m, 1H), 1.85 (p, *J* = 7.0 Hz, 2H), 1.56–1.37 (m, 18H), 1.35–1.29 (m, 4H), 1.29–1.18 (m, 6H), 0.86 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 178.6, 169.6, 162.1, 153.7, 150.5, 135.9, 131.0, 130.2, 130.1, 121.3, 121.3, 119.6, 118.7, 109.3, 101.5, 82.3, 79.7, 49.6, 46.7, 31.9, 30.5, 29.33, 29.28, 28.3, 28.1, 27.1, 22.7, 14.2. HRMS (ESI+): Calcd for C₃₃H₄₉N₆O₅⁺ [M+H]⁺: 609.7794, Found: 609.3791.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-nonyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5v).**

Prepared from **4v** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5v** as clear oil (30 mg, 67%). ¹H NMR (500 MHz, CDCl₃) δ 8.08 (s, 1H), 7.81 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.68 (d, *J* = 8.3 Hz, 1H), 7.21 (d, *J* = 3.1 Hz, 1H), 6.52 (d, *J* = 3.0 Hz, 1H), 5.62 (dd, *J* = 7.9, 4.7 Hz, 1H), 4.17 (t, *J* = 7.2 Hz, 2H), 3.96–3.89 (m, 1H), 3.87–3.76 (m, 1H), 2.52–2.42 (m, 1H), 2.32–2.15 (m, 2H), 2.08–2.01 (m, 1H), 1.85 (p, *J* = 7.1 Hz, 2H), 1.54–1.37 (m, 20H), 1.34–1.18 (m, 15H), 0.86 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 178.6, 169.6, 162.1, 153.7, 150.4, 135.9, 131.0, 130.8, 121.3, 119.7, 118.7, 109.3, 101.5, 82.2, 79.7, 55.5, 49.6, 46.7, 36.8, 31.9, 30.5, 29.6, 29.4, 29.3, 28.6, 27.1, 22.8, 14.2. HRMS (ESI+): Calcd for C₃₄H₅₁N₆O₅ [M+H]: 623.3915, Found: 623.3909.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)imino)(2-(3-(1-decyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methyl)carbamate (5w).**

Prepared from **4w** according to general procedures D and E. Purification on a silica gel column with 10–40% EtOAc in hexanes produced **5w** as clear oil (19 mg, 43%). ¹H NMR (500 MHz, CDCl₃) δ 8.08 (s, 1H), 7.81 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.68 (d, *J* = 8.3 Hz, 1H),

7.21 (d, $J = 3.1$ Hz, 1H), 6.52 (d, $J = 3.1$ Hz, 1H), 5.63 (dd, $J = 7.9, 4.7$ Hz, 1H), 4.17 (t, $J = 7.2$ Hz, 2H), 3.96–3.88 (m, 1H), 3.86–3.78 (m, 1H), 2.51–2.42 (m, 1H), 2.32–2.16 (m, 2H), 2.09–1.99 (m, 1H), 1.85 (p, $J = 7.2$ Hz, 2H), 1.46 (s, 19H), 1.34–1.20 (m, 18H), 0.87 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 178.6, 169.6, 162.2, 153.8, 150.5, 135.9, 131.1, 130.2, 121.3, 119.7, 118.7, 109.3, 101.5, 82.1, 80.0, 55.5, 49.6, 46.7, 36.8, 32.0, 30.5, 29.9, 29.7, 29.4, 28.6, 28.3, 28.1, 27.2, 24.8, 24.1, 22.8, 14.2. HRMS (ESI+): Calcd for $\text{C}_{35}\text{H}_{53}\text{N}_6\text{O}_5\text{Na}^+ [\text{M}+\text{Na}]^+$: 659.3891, Found: 659.3884.

(S)-Amino(2-(3-(1-heptyl-1H-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6e).

Prepared from **5e** according to general procedure D. Compound **6e** was isolated as yellow solid (11 mg, 100%). ^1H NMR (400 MHz, CD_3OD) δ 8.10 (ddd, $J = 7.9, 1.3, 0.8$ Hz, 1H), 7.98 (s, 1H), 7.51 (dt, $J = 8.3, 0.9$ Hz, 1H), 7.29 (ddd, $J = 8.3, 7.1, 1.3$ Hz, 1H), 7.22 (ddd, $J = 8.0, 7.1, 1.0$ Hz, 1H), 5.44 (dd, $J = 7.8, 2.0$ Hz, 1H), 4.26 (t, $J = 7.0$ Hz, 2H), 3.82–3.74 (m, 1H), 3.67–3.56 (m, 1H), 2.61–2.45 (m, 2H), 2.31–2.21 (m, 1H), 2.17–2.06 (m, 1H), 1.88 (p, $J = 7.1$ Hz, 2H), 1.40–1.18 (m, 9H), 0.87 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 177.3, 166.7, 157.1, 138.4, 132.3, 126.7, 123.9, 122.5, 122.2, 111.4, 103.1, 56.4, 47.7, 32.8, 32.7, 31.2, 30.0, 27.8, 24.4, 23.6, 14.3. HRMS (ESI+): Calcd for $\text{C}_{22}\text{H}_{30}\text{N}_6\text{O}^+ [\text{M}+\text{H}]^+$: 395.2554, Found: 395.2543.

(S)-Amino(2-(3-(1-octyl-1H-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6f).

Prepared from **5f** according to general procedure D. Compound **6f** was isolated as yellow solid (10 mg, 91%). ^1H NMR (500 MHz, CD_3OD) δ 8.12 (dt, $J = 8.0, 1.0$ Hz, 1H), 8.00 (s, 1H), 7.53 (dt, $J = 8.3, 0.9$ Hz, 1H), 7.31 (ddd, $J = 8.3, 7.1, 1.2$ Hz, 1H), 7.26–7.21 (m, 1H), 5.45 (dd, $J = 7.9, 1.9$ Hz, 1H), 4.29 (t, $J = 7.0$ Hz, 2H), 3.84–3.77 (m, 1H), 3.67–3.58 (m, 1H), 2.63–2.48 (m, 2H), 2.29–2.22 (m, 1H), 2.18–2.07 (m, 1H), 1.92–1.85 (m, 2H), 1.38–1.19 (m, 12H), 0.88 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (126 MHz, $\text{CD}_3\text{C}_9\text{H}_{19}\text{OD}$) δ 177.3, 166.7, 157.1, 138.4, 132.3, 126.7, 123.9, 122.5, 122.2, 111.4, 103.1, 56.5, 47.7, 32.9, 32.7, 31.2, 30.3, 30.2, 27.8, 24.4, 23.6, 14.4. HRMS (ESI+): Calcd for $\text{C}_{23}\text{H}_{33}\text{N}_6\text{O}^+ [\text{M}+\text{H}]^+$: 409.2710, Found: 409.2728.

(S)-Amino(2-(3-(1-nonyl-1H-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6g).

Prepared from **5g** according to general procedure D. Compound **6g** was isolated as yellow solid (15 mg, 100%). ^1H NMR (400 MHz, CD_3OD) δ 8.10 (ddd, $J = 7.9, 1.3, 0.7$ Hz, 1H), 7.98 (s, 1H), 7.51 (dt, $J = 8.3, 0.9$ Hz, 1H), 7.32–7.26 (m, 1H), 7.22 (ddd, $J = 8.0, 7.1, 1.0$ Hz, 1H), 5.45–5.41 (m, 1H), 4.27 (t, $J = 7.0$ Hz, 2H), 3.83–3.76 (m, 1H), 3.66–3.59 (m, 1H), 2.60–2.48 (m, 2H), 2.30–2.20 (m, 1H), 2.17–2.08 (m, 1H), 1.92–1.83 (p, $J = 7.1$ Hz, 2H), 1.35–1.21 (m, 14H), 0.86 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 177.3, 166.7, 157.1, 138.4, 132.3, 126.7, 123.9, 122.5, 122.2, 111.4, 103.1, 56.4, 47.7, 32.9, 32.7, 31.2, 30.5, 30.3, 27.8, 24.4, 23.7, 14.4. HRMS (ESI+): Calcd for $\text{C}_{24}\text{H}_{35}\text{N}_6\text{O}^+ [\text{M}+\text{H}]^+$: 423.2867, Found: 423.2890.

(S)-Amino(2-(3-(1-decyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6h).

Prepared from **5h** according to general procedure D. Compound **6h** was isolated as yellow solid (13 mg, 100%). ¹H NMR (500 MHz, CD₃OD) δ 8.14–8.09 (m, 1H), 7.98 (s, 1H), 7.55–7.50 (m, 1H), 7.29 (ddd, *J* = 8.3, 7.0, 1.2 Hz, 1H), 7.22 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 5.43 (dd, *J* = 7.9, 1.9 Hz, 1H), 4.27 (t, *J* = 7.0 Hz, 2H), 3.81–3.76 (m, 1H), 3.65–3.57 (m, 1H), 2.60–2.47 (m, 2H), 2.28–2.21 (m, 1H), 1.89 (p, *J* = 6.9 Hz, 1H), 1.40–1.24 (m, 14H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 177.3, 166.7, 157.1, 138.4, 132.3, 126.7, 123.9, 122.5, 122.2, 111.4, 103.1, 56.4, 47.7, 33.0, 32.7, 31.2, 30.6, 30.4, 30.3, 27.8, 24.4, 23.7, 14.4. HRMS (ESI+): Calcd for C₂₅H₃₆N₆O⁺ [M+H]⁺: 437.3023, Found: 437.3010.

(S)-Amino(2-(3-(1-undecyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6i).

Prepared from **5i** according to general procedure D. Compound **6i** was isolated as yellow solid (6 mg, 80%). ¹H NMR (500 MHz, CD₃OD) δ 8.10 (d, *J* = 7.8 Hz, 1H), 7.98 (s, 1H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.29 (ddd, *J* = 8.3, 7.0, 1.2 Hz, 1H), 7.22 (t, *J* = 7.5 Hz, 1H), 5.43 (dd, *J* = 7.9, 1.8 Hz, 1H), 4.27 (t, *J* = 7.0 Hz, 2H), 3.82–3.75 (m, 1H), 3.66–3.59 (m, 1H), 2.60–2.52 (m, 1H), 2.52–2.46 (m, 1H), 2.28–2.19 (m, 1H), 2.17–2.08 (m, 1H), 1.88 (p, *J* = 7.1 Hz, 2H), 1.39–1.20 (m, 18H), 0.88 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 177.3, 166.7, 157.1, 138.4, 132.3, 126.7, 123.9, 122.5, 122.2, 111.4, 103.1, 56.5, 47.7, 33.0, 32.7, 31.2, 30.7, 30.60, 30.56, 30.4, 30.3, 27.8, 24.4, 23.7, 14.4. HRMS (ESI+): Calcd for C₂₆H₃₉N₆O⁺ [M+H]⁺: 451.3180, Found: 451.3192.

(S)-Amino(2-(3-(1-dodecyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6j).

Prepared from **5j** according to general procedure D. Compound **6j** was isolated as yellow solid (10 mg, 88%). ¹H NMR (400 MHz, CD₃OD) δ 8.10 (ddd, *J* = 7.9, 1.3, 0.8 Hz, 1H), 7.98 (s, 1H), 7.51 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.29 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.22 (ddd, *J* = 8.0, 7.1, 1.0 Hz, 1H), 5.44 (dd, *J* = 7.8, 2.0 Hz, 1H), 4.27 (t, *J* = 7.0 Hz, 2H), 3.85–3.75 (m, 1H), 3.66–3.58 (m, 1H), 2.63–2.46 (m, 2H), 2.31–2.20 (m, 1H), 2.21–2.07 (m, 1H), 1.88 (p, *J* = 7.1 Hz, 2H), 1.25 (s, 18H), 0.89 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CD₃OD) δ 177.3, 166.7, 157.1, 138.4, 132.3, 126.7, 123.9, 122.5, 122.2, 111.4, 103.1, 56.4, 47.7, 33.0, 32.7, 31.2, 30.70, 30.69, 30.58, 30.55, 30.4, 30.3, 27.8, 24.4, 23.7, 14.4. HRMS (ESI+): Calcd for C₂₇H₄₁N₆O⁺ [M+H]⁺: 465.3336, Found: 465.3352.

(S)-Amino(2-(3-(1-heptyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6k).

Prepared from **5k** according to general procedure D. Compound **6k** was isolated as yellow solid (9 mg, 100%). ¹H NMR (500 MHz, CD₃OD) δ 7.86 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.64 (dd, *J* = 8.3, 0.9 Hz, 1H), 7.45–7.38 (m, 2H), 7.29 (dd, *J* = 8.3, 7.4 Hz, 1H), 7.09 (dd, *J* = 3.2, 0.8 Hz, 1H), 5.47 (dd, *J* = 7.7, 2.1 Hz, 1H), 4.25 (t, *J* = 7.0 Hz, 1H), 3.84–3.78 (m, 1H), 3.67–3.61 (m, 1H), 2.64–2.50 (m, 2H), 2.31–2.22 (m, 1H), 2.18–2.09 (m, 1H), 1.86 (p, *J* = 7.1 Hz, 2H), 1.32–1.24 (m, 10H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 177.8,

170.3, 157.1, 138.1, 131.0, 130.9, 127.4, 121.8, 121.6, 118.6, 114.3, 56.5, 47.3, 32.9, 32.7, 31.5, 30.0, 28.1, 27.9, 24.4, 23.6, 14.3. HRMS (ESI+): Calcd for $C_{22}H_{31}N_6O^+$ [M+H]⁺: 395.2554, Found: 395.2577.

(S)-Amino(2-(3-(1-octyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6l).

Prepared from **5l** according to general procedure D. Compound **6l** was isolated as yellow solid (6 mg, 100%). ¹H NMR (500 MHz, CD₃OD) δ 7.86 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.64 (dd, *J* = 8.3, 0.9 Hz, 1H), 7.39 (s, 1H), 7.29 (dd, *J* = 8.2, 7.4 Hz, 1H), 5.48 (dd, *J* = 7.8, 2.0 Hz, 1H), 4.25 (t, *J* = 7.0 Hz, 2H), 3.83–3.78 (m, 1H), 3.66–3.61 (m, 1H), 2.62–2.50 (m, 2H), 2.29–2.22 (m, 1H), 2.18–2.07 (m, 1H), 1.85 (p, *J* = 6.9 Hz, 2H), 1.36–1.22 (m, 14H), 0.87 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 177.8, 170.3, 157.1, 138.1, 131.0, 130.9, 127.4, 121.8, 121.6, 118.6, 114.3, 103.0, 61.5, 56.5, 47.3, 32.9, 32.7, 31.5, 30.28, 30.26, 27.9, 24.4, 23.6, 20.9, 14.5, 14.4. HRMS (ESI+): Calcd for $C_{23}H_{33}N_6O^+$ [M+H]⁺: 409.2710, Found: 409.2731.

(S)-Amino(2-(3-(1-nonyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6m).

Prepared from **5m** according to general procedure D. Compound **6m** was isolated as yellow solid (7 mg, 95%). ¹H NMR (500 MHz, CD₃OD) δ 7.72 (dd, *J* = 7.3, 2.0 Hz, 1H), 7.45 (s, 1H), 7.42–7.38 (m, 1H), 7.36–7.33 (m, 1H), 7.11 (d, *J* = 3.1 Hz, 1H), 5.56–5.46 (m, 1H), 4.26 (t, *J* = 6.4 Hz, 2H), 3.86–3.79 (m, 1H), 3.70–3.58 (m, 1H), 2.65–2.55 (m, 1H), 2.54–2.47 (m, 1H), 2.29–2.21 (m, 1H), 2.18–2.09 (m, 1H), 1.87 (p, *J* = 7.0 Hz, 2H), 1.42–1.20 (m, 14H), 0.87 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 178.0, 157.1, 137.3, 128.7, 123.7, 122.8, 121.8, 118.9, 114.3, 104.2, 103.0, 56.5, 56.3, 47.5, 47.3, 33.0, 32.8, 32.7, 31.5, 31.3, 30.6, 30.3, 27.9, 27.8, 24.3, 23.7, 14.4. HRMS (ESI+): Calcd for $C_{24}H_{34}N_6O$ [M+H]⁺: 423.2867, Found: 424.2938.

(S)-Amino(2-(3-(1-decyl-1*H*-indol-4-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6n).

Prepared from **5n** according to general procedure D. Compound **6n** was isolated as yellow solid (6 mg, 81%). ¹H NMR (500 MHz, CD₃OD) δ 7.88 (d, *J* = 7.3 Hz, 1H), 7.66 (dd, *J* = 8.3, 5.0 Hz, 1H), 7.40 (d, *J* = 3.1 Hz, 1H), 7.35–7.29 (m, 1H), 7.10 (dd, *J* = 6.0, 3.1 Hz, 1H), 5.50 (dd, *J* = 7.8, 2.0 Hz, 1H), 4.27 (t, *J* = 6.7 Hz, 2H), 3.85–3.79 (m, 1H), 3.70–3.63 (m, 1H), 2.65–2.52 (m, 2H), 2.30–2.20 (m, 1H), 2.19–2.07 (m, 1H), 1.86 (q, *J* = 6.9 Hz, 2H), 1.35–1.22 (m, 20H), 0.89 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 177.8, 170.3, 157.1, 138.1, 131.0, 130.9, 128.7, 127.5, 123.8, 122.9, 121.8, 121.6, 114.3, 103.0, 56.5, 56.3, 47.3, 33.03, 33.01, 32.7, 31.4, 31.3, 30.7, 30.6, 30.5, 30.39, 30.36, 30.3, 28.1, 27.9, 24.4, 24.3, 23.72, 23.68, 14.4. HRMS (ESI+): Calcd for $C_{25}H_{36}N_6O$ [M+H]⁺: 437.6009, Found: 437.3033.

(S)-Amino(2-(3-(1-hexyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6o).

Prepared from **5o** according to general procedure D. Compound **6o** was isolated as yellow solid (9 mg, 82%). ¹H NMR (500 MHz, CD₃OD) δ 8.34–8.00 (m, 1H), 7.79 (ddd, *J* = 67.4, 8.7, 1.6 Hz, 1H), 7.49 (dd, *J* = 46.9, 8.8 Hz, 1H), 7.36–7.27 (m, 1H), 7.10–6.99 (m, 1H), 6.73–6.60 (m, 1H), 5.43 (ddd, *J* = 31.8, 7.9, 1.9 Hz, 1H), 4.23 (q, *J* = 7.5 Hz, 2H), 3.88–3.75 (m, 1H), 3.68–3.57 (m, 1H), 2.58–2.40 (m, 2H), 2.32–2.20 (m, 1H), 2.17–2.03 (m, 1H), 1.92–1.82 (m, 2H), 1.43–1.21 (m, 8H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 178.2, 170.9, 157.1, 156.0, 143.5, 140.4, 130.9, 130.1, 128.7, 128.1, 127.3, 126.2, 122.0, 121.9, 121.24, 121.19, 117.0, 115.5, 111.1, 111.0, 102.9, 56.4, 47.3, 47.1, 42.5, 35.9, 32.7, 32.6, 32.5, 31.7, 31.4, 31.0, 27.6, 27.5, 24.4, 23.59, 23.55, 14.34, 14.27. HRMS (ESI⁺): Calcd for C₂₁H₂₉N₆O [M+H]⁺: 381.2397, Found: 381.2419.

(S)-Amino(2-(3-(1-heptyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6p).

Prepared from **5p** according to general procedure D. Compound **6p** was isolated as yellow solid (8 mg, 80%). ¹H NMR (500 MHz, CD₃OD) δ 8.29 (d, *J* = 1.5 Hz, 1H), 7.84 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.32 (d, *J* = 3.0 Hz, 1H), 6.83 (dd, *J* = 180.1, 8.7 Hz, 1H), 6.56 (d, *J* = 3.1 Hz, 1H), 5.44 (d, *J* = 7.5 Hz, 1H), 4.22 (t, *J* = 7.0 Hz, 2H), 3.83–3.71 (m, 1H), 3.66–3.60 (m, 1H), 2.61–2.44 (m, 2H), 2.28–2.18 (m, 1H), 2.17–2.03 (m, 1H), 1.84 (p, *J* = 7.0 Hz, 2H), 1.35–1.16 (m, 10H), 0.87 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 178.3, 170.8, 157.1, 139.1, 130.8, 130.0, 128.7, 121.9, 121.2, 118.1, 115.5, 111.1, 56.5, 47.3, 32.9, 32.7, 31.4, 30.0, 27.9, 24.4, 23.6, 14.3. HRMS (ESI⁺): Calcd for C₂₂H₃₁N₆O⁺ [M+H]⁺: 395.2554, Found: 395.2573.

(S)-Amino(2-(3-(1-octyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6q).

Prepared from **5q** according to general procedure D. Compound **6q** was isolated as yellow solid (13 mg, 87%). ¹H NMR (1:1 rotamer ratio, 500 MHz, CD₃OD) δ 8.29 (dd, *J* = 1.7, 0.6 Hz, 1H), 7.84 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.52* (dd, *J* = 8.7, 0.8 Hz, 1H), 7.32 (d, *J* = 3.2 Hz, 1H), 7.02* (d, *J* = 8.7 Hz, 1H), 6.66 (s, 1H), 6.56 (dd, *J* = 3.2, 0.8 Hz, 1H), 5.44 (dd, *J* = 7.9, 2.0 Hz, 1H), 4.22 (t, *J* = 7.0 Hz, 2H), 3.85–3.76 (m, 1H), 3.62 (t, *J* = 7.2 Hz, 1H), 2.60–2.54 (m, 1H), 2.53–2.47 (m, 1H), 2.27–2.22 (m, 1H), 2.17–2.08 (m, 1H), 1.84 (p, *J* = 7.0 Hz, 2H), 1.32–1.19 (m, 14H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 178.3, 170.8, 157.1, 156.0, 143.5, 139.2, 130.9, 130.8, 130.1, 130.0, 128.7, 121.97, 121.95, 121.2, 118.2, 115.5, 111.1, 102.9, 56.5, 47.3, 42.5, 32.9, 32.7, 31.7, 31.4, 30.3, 30.3, 27.9, 24.4, 23.6, 14.4. HRMS (ESI⁺): Calcd for C₂₃H₃₃N₆O⁺ [M+H]⁺: 409.2710, Found: 409.2717.

(S)-Amino(2-(3-(1-nonyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6r).

Prepared from **5r** according to general procedure D. Compound **6r** was isolated as yellow solid (7 mg, 95%). ¹H NMR (500 MHz, CD₃OD) δ 8.29 (d, *J* = 1.6 Hz, 1H), 7.84 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.52 (d, *J* = 8.7 Hz, 1H), 7.32 (d, *J* = 3.2 Hz, 1H), 6.56 (dd, *J* = 3.2, 0.8 Hz, 1H), 5.44 (dd, *J* = 7.9, 1.9 Hz, 1H), 4.22 (t, *J* = 7.0 Hz, 2H), 3.87–3.72 (m, 1H), 3.67–3.58

(m, 1H), 2.63–2.51 (m, 1H), 2.25–2.19 (m, 1H), 2.16–2.07 (m, 1H), 1.84 (p, $J = 7.0$ Hz, 2H), 1.34–1.07 (m, 14H), 0.86 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 178.4, 170.8, 157.1, 139.2, 130.9, 130.1, 122.3, 122.0, 121.2, 118.2, 112.0, 111.2, 102.9, 56.5, 47.3, 33.0, 32.7, 31.4, 30.6, 30.30, 30.29, 27.9, 24.4, 23.7, 14.4. HRMS (ESI⁺): Calcd for $\text{C}_{24}\text{H}_{35}\text{N}_6\text{O}^+$ $[\text{M}+\text{H}]^+$: 423.2867, Found: 423.2858.

(S)-Amino(2-(3-(1-decyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6s).

Prepared from **5s** according to general procedure D. Compound **6s** was isolated as yellow solid (17 mg, 92%). ^1H NMR (1:1 rotamer ratio, 500 MHz, CD_3OD) δ 8.29 (dd, $J = 1.7, 0.6$ Hz, 1H), 8.04* (d, $J = 1.7$ Hz, 1H), 7.84 (dd, $J = 8.7, 1.6$ Hz, 1H), 7.70* (s, 1H), 7.52 (dd, $J = 8.7, 0.8$ Hz, 1H), 7.43* (d, $J = 8.9$ Hz, 1H), 7.34–7.31 (m, 1H), 7.27* (s, 1H), 5.41 (ddd, $J = 29.5, 8.0, 1.9$ Hz, 1H), 4.21 (t, $J = 7.2$ Hz, 2H), 3.81–3.71 (m, 1H), 3.67–3.56 (m, 1H), 2.59–2.39 (m, 2H), 2.28–2.18 (m, 1H), 2.16–2.05 (m, 1H), 1.85 (t, $J = 7.1$ Hz, 2H), 1.32–1.19 (m, 14H), 0.87 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 178.3, 170.9, 170.8, 157.1, 139.2, 130.9, 130.1, 122.0, 121.2, 118.2, 111.2, 102.9, 56.5, 56.4, 47.3, 33.0, 32.72, 32.69, 31.4, 31.3, 30.6, 30.54, 30.50, 30.49, 30.38, 30.37, 30.3, 30.1, 27.9, 27.8, 24.4, 23.7, 14.44, 14.41. HRMS (ESI⁺): Calcd for $\text{C}_{25}\text{H}_{37}\text{N}_6\text{O}^+$ $[\text{M}+\text{H}]^+$: 437.3023, Found: 437.3018.

(S)-Amino(2-(3-(1-heptyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6t).

Prepared from **5t** according to general procedure D. Compound **6t** was isolated as yellow solid (6 mg, 83%). ^1H NMR (500 MHz, CD_3OD) δ 8.12 (dd, $J = 21.5, 1.2$ Hz, 1H), 7.73 (dd, $J = 8.3, 1.4$ Hz, 1H), 7.66 (d, $J = 8.2$ Hz, 1H), 7.39 (t, $J = 1.5$ Hz, 1H), 6.54–6.49 (m, 1H), 5.45 (dd, $J = 7.9, 2.0$ Hz, 1H), 4.25 (t, $J = 7.1$ Hz, 2H), 3.81 (td, $J = 9.1, 2.6$ Hz, 1H), 3.66–3.60 (m, 1H), 2.63–2.55 (m, 1H), 2.54–2.47 (m, 1H), 2.30–2.29 (m, 1H), 2.17–2.06 (m, 1H), 1.90–1.82 (m, 2H), 1.42–1.18 (m, 14H), 0.87 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 177.1, 169.5, 155.7, 135.7, 131.3, 130.5, 120.8, 118.6, 117.6, 108.7, 100.9, 55.1, 45.8, 31.5, 31.42, 31.39, 30.03, 29.99, 28.62, 28.57, 28.53, 26.46, 26.4, 22.3, 22.20, 22.16, 13.03, 12.98, 12.9. HRMS (ESI⁺): Calcd for $\text{C}_{22}\text{H}_{31}\text{N}_6\text{O}^+$ $[\text{M}+\text{H}]^+$: 395.2554, Found: 395.2573.

(S)-Amino(2-(3-(1-octyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6u).

Prepared from **5u** according to general procedure D. Compound **6u** was isolated as yellow solid (6 mg, 82%). ^1H NMR (500 MHz, CD_3OD) δ 8.15 (dd, $J = 1.3, 0.7$ Hz, 1H), 7.75 (dd, $J = 8.3, 1.4$ Hz, 1H), 7.68 (dd, $J = 7.1, 0.7$ Hz, 1H), 7.44–7.38 (m, 1H), 6.54 (dd, $J = 3.1, 0.9$ Hz, 1H), 5.50–5.46 (m, 1H), 4.27 (t, $J = 7.0$, 2H), 3.82 (td, $J = 8.2, 7.2, 5.3$ Hz, 1H), 3.67–3.62 (m, 1H), 2.65–2.58 (m, 1H), 2.56–2.51 (m, 1H), 2.33–2.27 (m, 1H), 2.17–2.08 (m, 1H), 1.92–1.83 (m, 2H), 1.38–1.20 (m, 16H), 0.89 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 178.5, 170.9, 157.1, 137.2, 132.0, 128.8, 122.3, 120.0, 119.9, 119.6, 119.1, 110.7, 110.1, 102.4, 56.5, 47.3, 32.9, 32.8, 31.43, 31.38, 30.29, 30.26, 30.2, 28.0, 27.8, 24.4, 23.6, 14.4. HRMS (ESI⁺): Calcd for $\text{C}_{23}\text{H}_{33}\text{N}_6\text{O}^+$ $[\text{M}+\text{H}]^+$: 409.2710, Found: 409.2732.

(S)-Amino(2-(3-(1-nonyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6v).

Prepared from **5v** according to general procedure D. Compound **6v** was isolated as yellow solid (7 mg, 95%). ¹H NMR (500 MHz, CD₃OD) δ 8.11–8.08 (m, 1H), 7.73 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.66 (d, *J* = 8.3 Hz, 1H), 7.38 (d, *J* = 3.1 Hz, 1H), 6.52 (d, *J* = 3.0 Hz, 1H), 5.46 (dd, *J* = 7.8, 1.9 Hz, 1H), 4.24 (t, *J* = 6.9 Hz, 2H), 3.81 (td, *J* = 9.1, 2.5 Hz, 1H), 3.66–3.62 (m, 1H), 2.59–2.55 (m, 1H), 2.53–2.47 (m, 1H), 2.28–2.22 (m, 1H), 2.16–2.09 (m, 1H), 1.86 (p, *J* = 7.2 Hz, 2H), 1.33–1.19 (m, 16H), 0.86 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 178.5, 170.9, 157.1, 137.1, 132.8, 132.7, 132.1, 132.0, 122.3, 122.2, 120.0, 119.1, 110.1, 102.4, 56.5, 47.3, 32.99, 32.95, 32.8, 31.4, 30.58, 30.55, 30.28, 30.26, 28.1, 27.9, 24.4, 23.69, 23.67, 14.5, 14.4. HRMS (ESI⁺): Calcd for C₂₄H₃₅N₆O⁺ [M+H]⁺: 423.2867, Found: 423.2868.

(S)-Amino(2-(3-(1-decyl-1*H*-indol-6-yl)-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl)methaniminium chloride (6w).

Prepared from **5w** according to general procedure D. Compound **6w** was isolated as yellow solid (7 mg, 94%). ¹H NMR (500 MHz, CD₃OD) δ 8.11 (d, *J* = 1.2 Hz, 1H), 7.73 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.39 (d, *J* = 3.1 Hz, 1H), 6.52 (dd, *J* = 3.1, 0.9 Hz, 1H), 5.46 (dd, *J* = 7.9, 2.0 Hz, 1H), 4.25 (t, *J* = 7.0 Hz, 2H), 3.84–3.77 (m, 1H), 3.67–3.60 (m, 1H), 2.62–2.54 (m, 1H), 2.53–2.48 (m, 1H), 2.28–2.21 (m, 1H), 2.17–2.08 (m, 1H), 1.85 (p, *J* = 7.0 Hz, 2H), 1.34–1.17 (m, 18H), 0.88 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ 178.5, 170.9, 157.14, 137.2, 132.8, 132.1, 122.3, 120.0, 119.1, 110.1, 102.4, 56.5, 47.3, 33.0, 32.8, 31.4, 30.6, 30.5, 30.4, 30.3, 27.9, 24.4, 23.7, 14.4. HRMS (ESI⁺): Calcd for C₂₅H₃₇N₆O⁺ [M+H]⁺: 437.3023, Found: 437.3017.

1-Nonyl-1*H*-indole-3-carbonitrile (7).

Prepared from **1a** according to general procedure C. Purification on a silica gel column with 0–10% EtOAc in hexanes produced **7** as yellow oil (360 mg, 95%). ¹H NMR (400 MHz, CDCl₃) δ 7.76–7.68 (m, 1H), 7.53 (s, 1H), 7.40–7.37 (m, 1H), 7.31 (ddd, *J* = 8.3, 7.0, 1.3 Hz, 1H), 7.25 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 4.08 (t, *J* = 7.2 Hz, 2H), 1.80 (p, *J* = 7.2 Hz, 2H), 1.37–1.15 (m, 12H), 0.89 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 135.1, 134.2, 127.7, 123.4, 121.7, 119.4, 115.9, 110.5, 85.0, 47.0, 31.6, 29.6, 29.2, 29.0, 28.9, 26.5, 22.5, 13.9. HRMS (ESI⁺): Calcd for C₁₈H₂₅N₂⁺ [M+H]⁺: 269.2012, Found: 269.2025.

***N*'-hydroxy-1-nonyl-1*H*-indole-3-carboximidamide (8).**

Prepared from **7** according to general procedure A. Purification on a silica gel column with 20–40% EtOAc in hexanes produced **8** as white solid (240 mg, 97%). ¹H NMR (400 MHz, CDCl₃) δ 8.05–8.01 (m, 1H), 7.43 (s, 1H), 7.36–7.31 (m, 1H), 7.25 (ddd, *J* = 8.2, 6.9, 1.3 Hz, 1H), 7.19 (ddd, *J* = 8.0, 6.9, 1.2 Hz, 1H), 4.95 (s, 2H), 4.07 (t, *J* = 7.2 Hz, 2H), 1.86–1.78 (m, 2H), 1.32–1.21 (m, 16H), 0.88 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 150.1, 136.6, 127.2, 125.6, 122.3, 121.2, 120.6, 109.8, 107.8, 46.7, 34.2, 31.9, 30.2, 29.5, 29.3, 27.0, 22.7, 14.2. HRMS (ESI⁺): Calcd for C₁₈H₂₈N₃O⁺ [M+H]⁺: 302.2227, Found: 302.2235.

***tert*-Butyl (S)-2-((3-(1-nonyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidine-1-carboxylate (**9**).**

Prepared from **8** according to general procedure B; however, homo-*N*-Boc-*L*-Proline (1.4 equiv.) was used instead of *N*-Boc-*L*-Proline. Purification on a silica gel column with 0–20% EtOAc in hexanes produced **9** as clear oil (65 mg, 40%). ¹H NMR (400 MHz, CDCl₃) δ 8.24 (dd, *J* = 6.8, 1.9 Hz, 1H), 7.82 (s, 1H), 7.41–7.35 (m, 1H), 7.33–7.24 (m, 2H), 4.38–4.26 (m, 1H), 4.15 (t, *J* = 7.1 Hz, 2H), 3.50–3.40 (m, 1H), 3.39–3.31 (m, 1H), 3.16–3.08 (m, 1H), 3.04–2.96 (m, 1H), 2.12–2.03 (m, 2H), 1.87 (p, *J* = 7.0 Hz, 5H), 1.48 (s, 8H), 1.36–1.18 (m, 12H), 0.87 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 175.8, 165.2, 154.3, 136.9, 130.4, 125.6, 122.8, 122.1, 121.3, 110.0, 103.0, 80.1, 79.6, 55.3, 47.0, 46.4, 34.2, 31.9, 31.1, 30.8, 30.1, 29.6, 29.5, 29.3, 28.6, 27.0, 23.7, 22.9, 22.7, 14.19. HRMS (ESI⁺): Calcd for C₂₉H₄₃N₄O₃⁺ [M+H]⁺: 495.3330, Found: 495.3340.

***tert*-Butyl (S,Z)-(((*tert*-butoxycarbonyl)amino)(2-((3-(1-nonyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methylene)carbamate (**10**).**

Prepared from **9** according to general procedures D and E. Purification on a silica gel column with 0–10% EtOAc in hexanes produced **10** as clear oil (30 mg, 64%). ¹H NMR (400 MHz, CDCl₃) δ 8.26–8.22 (m, 1H), 7.86 (s, 1H), 7.40–7.37 (m, 1H), 7.32–7.24 (m, 2H), 4.83–4.75 (m, 1H), 4.16 (t, *J* = 7.2 Hz, 2H), 3.73–3.63 (m, 2H), 3.56–3.46 (m, 1H), 3.12 (dd, *J* = 15.1, 8.7 Hz, 1H), 2.29–2.22 (m, 1H), 1.94–1.79 (m, 5H), 1.48 (s, 18H), 1.37–1.19 (m, 14H), 0.87 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 175.7, 165.2, 162.6, 154.0, 150.5, 136.9, 130.6, 125.7, 122.8, 122.2, 122.1, 121.3, 110.0, 109.9, 103.0, 81.7, 79.6, 56.7, 47.0, 31.9, 30.6, 30.3, 30.2, 29.5, 29.3, 28.3, 27.1, 22.8, 14.2. HRMS (ESI⁺): Calcd for C₃₅H₅₃N₆O₅⁺ [M+H]⁺: 637.4072, Found: 637.4090.

(S)-Amino(2-((3-(1-nonyl-1*H*-indol-3-yl)-1,2,4-oxadiazol-5-yl)methyl)pyrrolidin-1-yl)methaniminium chloride (11**).**

Prepared from **10** according to general procedure D. Compound **11** was isolated as white solid (10 mg, 100%). ¹H NMR (400 MHz, CD₃OD) δ 8.10 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.95 (s, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.29 (t, *J* = 7.0 Hz, 1H), 7.21 (t, *J* = 7.4 Hz, 1H), 4.55 (d, *J* = 6.3 Hz, 1H), 4.26 (t, *J* = 6.8 Hz, 2H), 3.58–3.53 (m, 1H), 3.50–3.41 (m, 1H), 2.33–2.24 (m, 1H), 2.18–2.10 (m, 1H), 2.08–2.01 (m, 2H), 1.87 (t, *J* = 6.7 Hz, 2H), 1.37–1.15 (m, 14H), 0.86 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (101 MHz, CD₃OD) δ 176.4, 166.5, 156.4, 138.4, 132.1, 126.6, 123.9, 122.5, 122.1, 111.4, 103.3, 57.3, 47.6, 33.0, 31.6, 31.2, 30.5, 30.3, 30.0, 27.8, 23.68, 23.67, 23.6, 14.4. HRMS (ESI⁺): Calcd for C₂₅H₃₇N₆O⁺ [M+H]⁺: 437.3023, Found: 437.3046.

Supplementary Material

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Abbreviations:

S1P	sphingosine-1-phosphate
Sph	sphingosine
SphK	sphingosine kinase
DIEA	diisopropylethanamine
HCTU	2-(6-Chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylammonium hexafluorophosphate
SAR	structure-activity-relationship

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Figure 1.
FDA approved therapies targeting S1P signaling

SphK1-selective inhibitors *SphK dual inhibitors* *SphK2-selective inhibitors*

Figure 2.
Select sphingosine kinase inhibitors

Figure 3.

SphK inhibitory activity of SLC5101465 (**6r**) in whole cell assays. (A) U937 cells were treated with or without SLC5101465 (**6r**) as indicated for 2 hours. (B) Cells were treated with 1 μ M FTY720 with or without SLC5101465 (**6r**) as indicated for 2 hours. Cells were harvested by centrifugation and processed followed by determination of S1P or FTY720-P levels. S1P and FTY720-P levels were measured using LC-MS/MS. Experiments performed in duplicate. (C) Rescue of yeast strain KYA1 growth expressing SphK2 in the presence of SLC5101465 (**6r**) over 24 h. The yeast proliferation was determined by measuring the optical density (OD) of the sample at 600 nm. The experiment was performed in triplicate.

Figure 4.

Lowest energy poses of SphK2 selective inhibitors in the hSphK2 homology model. (A) **6g**-blue; (B) **6m**-purple; (C) SLC5101465 (**6r**)-green; (D) **6v**-gold; (E) **11**-pink. hSphK2 is shown as a light grey cartoon with key amino acids being shown as sticks and labeled. ATP is shown as green sticks and colored by element (oxygen in red and phosphorus in orange). Magnesium is not shown for clarity. Red dashed lines with associated numbers indicate distances (Å) and potential hydrogen bonds as appropriate.

Scheme 1.

Synthesis of analogues **6e-w**

(a) $\text{NH}_2\text{OH}\cdot\text{HCl}$ (3 equiv), TEA (3 equiv), EtOH, reflux, 6 h, (91–99%); (b) *N*-Boc-L-proline (1.4 equiv), DIEA (1.4 equiv), HCTU (1.8 equiv), DMF, 110 °C, 18 h, (5–28%); (c) R-Br (3 equiv), NaH (1.5 equiv), DMF, 0 °C - RT, 30 min, (9–97%); (d) HCl/MeOH, (80–100%); (e) DIEA (3 equiv.), *N,N'*-DiBoc-1*H*-pyrazole-1carboxamidine (1.05 equiv), CH_3CN , 80 °C, microwave, 2 h, (41–99%).

Scheme 2.**Synthesis of analogue **11****

(a) $n\text{-C}_9\text{H}_{18}\text{-Br}$ (3 equiv), NaH (1.5 equiv), DMF, 0 °C - RT, 30 min, (95%); (b) $\text{NH}_2\text{OH}\cdot\text{HCl}$, (3 equiv), TEA (3 equiv), EtOH, 80 °C, 6 h, (97%); (c) Homologated-*N*-Boc-L-proline (1.4 equiv), DIEA (1.4 equiv), HCTU (1.8 equiv), DMF, 110 °C, 18 h, (40%); (d) HCl/MeOH , (quant.); (e) DIEA (3 equiv.), *N,N'*-DiBoc-1*H*-pyrazole-1-carboxamide (1.05 equiv), CH_3CN , 80 °C, microwave, 2 h, (64%).

Table 1.

SphK1 and SphK2 activity upon treatment with inhibitors^a

Cmpd	Indole	R	hSphK1	hSphK2	Cmpd	Indole	R	hSphK1	hSphK2
SLC 5081308	---	---	90 ± 7	95 ± 5	6o		C ₆ H ₁₃	106 ± 1	72 ± 10
6e		C ₇ H ₁₅	110 ± 4	96 ± 5	6p		C ₇ H ₁₅	96 ± 2	93 ± 7
6f		C ₈ H ₁₇	93 ± 2	73 ± 3	6q		C ₈ H ₁₇	101 ± 5	87 ± 9

Cmpd	Indole	R	hSphK1	hSphK2	Cmpd	Indole	R	hSphK1	hSphK2
6g		C ₉ H ₁₉	88 ± 1	59 ± 7	6r		C ₉ H ₁₉	103 ± 1	25 ± 3
6h		C ₁₀ H ₂₁	96 ± 6	47 ± 1	6s		C ₁₀ H ₂₁	99 ± 6	31 ± 1
6i		C ₁₁ H ₂₃	107 ± 4	79 ± 12	6t		C ₇ H ₁₅	98 ± 6	103 ± 1
6j		C ₁₂ H ₂₅	76 ± 3	46 ± 7	6u		C ₈ H ₁₇	94 ± 1	93 ± 6

Cmpd	Indole	R	hSphK1	hSphK2	Cmpd	Indole	R	hSphK1	hSphK2
6k		C ₇ H ₁₅	105 ± 6	89 ± 4	6v		C ₉ H ₁₉	107 ± 2	79 ± 5
6l		C ₈ H ₁₇	98 ± 7	84 ± 2	6w		C ₁₀ H ₂₁	98 ± 2	68 ± 2
6m		C ₉ H ₁₉	90 ± 1	68 ± 4	11 ^b		C ₉ H ₁₉	98 ± 1	98 ± 3

Cmpd	Indole	R	hSphK1	hSphK2	Cmpd	Indole	R	hSphK1	hSphK2
6n		C ₁₀ H ₂₁	87 ± 2	64 ± 3					

^aSphK activity is presented as % control (no inhibitor added). Recombinant hSphK1 or hSphK2 were 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 in triplicate at 0.3 μM (SphK2) or 1 μM (SphK1).

^bSee scheme 2 for structure.

Cmpd	Structure	hSphK1 K_i (μM)	hSphK2 K_i (μM)	SphK2 selectivity
SLC5081308		7.2 ± 0.9	0.98 ± 0.2	4
SLC5091592		>20	1.02 ± 0.2	>10
6h (SLC5101463)		>10	0.30 ± 0.05	>33
6j (SLC5121467)		8 ± 1.7	0.29 ± 0.08	28
6r (SLC5101465)		>10	0.09 ± 0.01	>111
6s (SLC5101464)		>10	0.12 ± 0.03	>83

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