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Design, synthesis, and biological evaluation of a novel series of 1,2,4-oxadiazole inhibitors of SLACK potassium channels: Identification of *in vitro* tool VU0935685

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Abstract

Malignant migrating partial seizure of infancy (MMPSI) is a devastating and pharmacoresistant form of infantile epilepsy. MMPSI has been linked to multiple gain-of-function (GOF) mutations in the *KCNT1* gene, which encodes for a potassium channel often referred to as SLACK. SLACK channels are sodium-activated potassium channels distributed throughout the central nervous system (CNS) and the periphery. The investigation described here aims to discover SLACK channel inhibitor tool compounds and profile their pharmacokinetic and pharmacodynamic properties. A SLACK channel inhibitor VU0531245 (VU245) was identified via a high-throughput screen (HTS) campaign. Structure-activity relationship (SAR) studies were conducted in five distinct regions of the hit VU245. VU245 analogs were evaluated for their ability to affect SLACK channel activity using a thallium flux assay in HEK-293 cells stably expressing wild-type (WT) human SLACK. Selected analogs were tested for metabolic stability in mouse liver microsomes and plasma-protein binding in mouse plasma. The same set of analogs was tested via thallium flux for activity versus human A934T SLACK and other structurally related potassium channels, including SLICK and Maxi-K. In addition, potencies for selected VU245 analogs were obtained using whole-cell electrophysiology (EP) assays in CHO cells stably expressing WT human SLACK through an automated patch clamp system. Results revealed that this scaffold tolerates structural changes in some regions, with some analogs demonstrating improved SLACK inhibitory activity, good selectivity against the other channels tested, and modest improvements in metabolic clearance. Analog VU0935685 represents a new, structurally distinct small-molecule inhibitor of SLACK channels that can serve as an *in vitro* tool for studying this target.

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Declaration of Competing Interest

C.D.W. is an owner of WaveFront Biosciences and ION biosciences, makers of the Panoptic plate reader and Thallos, Tl^+-sensitive fluorescent indicators used in the these studies, respectively.

Keywords

KCNT1 ; Slack; $K_{Na}1.1$; Slo2.2; MMPSI; EIMFS; 1,2,4-oxadiazoles

1. Introduction

Malignant migrating partial seizure of infancy (MMPSI) or epilepsy of infancy with migrating focal seizures (EIMFS) is a devastating and rare form of infantile epilepsy.[1] MMPSI patients usually present with pharmacoresistant seizures within the first six months of life, characterized by continuous multifocal partial seizures migrating to different brain regions. MMPSI is accompanied by deterioration of psychomotor and cognitive development, visual impairment, hypotonia, and nearly 25% of the patients die within the first year of life. The etiology of MMPSI is linked to multiple gain-of-function (GOF) *de novo* missense heterozygous mutations in the *KCNT1* gene in approximately 50% of the patients.[2–4] *KCNT1* encodes for the sodium (Na^+)-activated potassium (K^+) SLACK (Sequence Like A Calcium-Activated K^+) channel. SLACK ($K_{Na}1.1$, Slo2.2) is a member of the Slo family of K^+ channels, which also includes Slo1 (Maxi-K, BK, or $K_{Ca}1.1$), Slo2.1. (SLICK), and Slo3.[5–7] SLACK channels are critical regulators of electrical activity in the central nervous system (CNS) where they play an important role in regulating excitability, including afterhyperpolarization (AHP) after repetitive firing, which helps regulate the rate of action potentials.[3–5, 8, 9]

SLACK channels are found throughout the CNS, including the brainstem, olfactory bulb, cortical embryonic neurons, and hippocampus, as well as in the gonads, muscle tissues, cardiomyocytes, and pituitary glands.[3, 5, 6, 10] SLACK channels are a tetramer of subunits comprised of six hydrophobic transmembrane domains (S1 – S6) and a pore-forming region between S5 – S6, in addition to an extended *C*-terminal cytoplasmic domain, which contains the regulator of potassium conductance (RCK) domain, a region that confers Na^+ sensitivity to SLACK currents, as well as a nicotinamide adenine dinucleotide-binding (NAD^+) domain.[2, 7, 9, 11–14] Intracellular Na^+ level is a key regulator of SLACK channel activity;[13, 15] however, it is also regulated by the transmembrane voltage and various other factors, including direct phosphorylation of the channel by protein kinase C (PKC), or indirect modulation by protein kinase A (PKA), cytoplasmic NAD^+ and ATP levels, estradiol, phosphatidyinostitol 4,5-bisphosphate, Cl^- and others.[13, 16, 17]

Over 30 *KCNT1* GOF mutations associated with MMPSI have been reported.[3, 18–21] Previous studies have shown that these mutations are mainly present in the C-terminal region, which includes both the NAD^+ and RCK binding domains, as well as the transmembrane pore-forming region.[3, 7, 8, 14, 22, 23] Furthermore, *KCNT1* GOF mutations are linked to epilepsy phenotypes other than MMPSI, such as early-onset epileptic encephalopathy (EOEE, i.e., West syndrome, and Ohtahara syndrome),[23] autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE),[24, 25] and others.[26 27] GOF mutations in *KCNT1* lead to SLACK channels with increased neuronal excitability by several potential mechanisms. Present data are most consistent with SLACK GOF mutations decreasing the activity of inhibitory interneurons resulting in an excitatory/inhibitory

imbalance.[14, 19] Activation of SLACK channels also results in an increased AHP amplitude, which correspondingly leads to an increased neuronal firing frequency.[18, 28] It has also been noted that SLACK mutations may disrupt interactions with cell signaling pathways such as FMRP and Phactr-1.[28] In fact, the cytoplasmic domain of SLACK channels interacts with developmentally relevant proteins such as the fragile X mental retardation protein (FMRP),[29] cytoplasmic FMR1-interacting protein 1 (Cyfip1), and actin regulator 1 (Phactr1).[30]

To date, no broadly effective and safe drug therapy has been approved for the treatment of MMPSI. However, quinidine, clofilium, and bepridil inhibit SLACK currents *in vitro*, and were used as investigational treatments for MMPSI. Unfortunately, they have limited efficacy, and use of quinidine led to toxicity due to lack of selectivity and unfavorable pharmacokinetic properties.[4, 8, 14, 20, 25, 31–37] Recently, researchers have shown increased interest in the discovery of small molecule selective SLACK channel inhibitors. To identify pore blockers, a virtual screen of 100,000 compounds was performed by researchers utilizing cryo-electron microscopy-derived chicken $K_{Na}1.1$ structure. Six compounds that inhibited $K_{Na}1.1$ channels at micromolar potencies were identified (compounds **1** and **2** in Figure 1 are representative examples); however, four compounds inhibited hERG ($K_V11.1$) channels at 10 μM and showed a reduction in cell viability.[38]

A lead optimization campaign that culminated in characterization and *in vivo* studies of 1,2,4-oxadiazole tool compound **3** was recently reported.[39] Additional analogs of **3** and related scaffolds have been disclosed in the patent literature as well.[40] The activity of **3** was assessed in cell lines stably expressing human *KCNT1* GOF variants and revealed IC_{50} values ranging from 221 nM to 1768 nM versus numerous human and mouse SLACK variants. Compound **3** was evaluated in brain slices from wild-type (WT) mice and mice homozygous for SLACK variant P905L ($Kcnt1^{L/L}$), and a significant reduction in the firing was observed in neurons from $Kcnt1^{L/L}$ mice at 10 μM. However, **3** was an inhibitor of the hERG channel (IC_{50} = 11.9 μM). Likewise, it displayed 74% displacement at $GABA_A$ Cl^- channels in a binding displacement assay, raising the possibility that GABAergic activity contributed to the observed anti-seizure effect.[17, 39, 41]

Our early research on SLACK inhibitors started with a high-throughput screen (HTS) using a thallium (TI^+) flux assay in HEK-293 cells stably expressing WT human SLACK and a 110,000 member compound library. These efforts resulted in the discovery of the 2-amino-*N*-phenylacetamide VU0606170 (VU170) (**4**), which was found to be a selective SLACK channel inhibitor with low micromolar potency versus WT and select mutant SLACK channels (Figure 1). VU170 also effectively decreased the frequency of spontaneous, synchronized calcium (Ca^{2+}) oscillations in a cortical neuronal/glial co-culture. Furthermore, the discovery of VU170 provided further evidence that SLACK channels can be effectively inhibited by small molecules.[7] Finally, we recently disclosed the structure-activity relationship (SAR) studies that were executed in five distinct regions around VU170, which unfortunately revealed relatively flat SAR. Selected VU170 analogs were tested versus the A934T SLACK mutant revealing a potency on par with WT. In addition, results in whole-cell electrophysiology studies with the same compounds mirrored those obtained with the TI^+ flux assay.[17]

Higher quality tool compounds that selectively target SLACK channels are needed to further study the role of SLACK modulation on *KCNT1*-associated epilepsies. As a result, we pursued a new, distinct hit series for optimization towards an improved SLACK channel tool compound. The same cell-based HTS campaign also led to the identification of 1,2,4-oxadiazole hit compound VU0531245 (VU245, **5**), which inhibited WT SLACK channels with an IC_{50} value of 2.1 μM (Figure 1). In addition, compound **5** demonstrated high permeability in a MDCK-MDR1 bidirectional permeability assay (31.1×10^{-6} cm/sec) with no evidence of P-glycoprotein (Pgp)-mediated efflux (efflux ratio = 0.65). It likewise possessed calculated properties consistent with CNS penetrant molecules (MW = 391 g/mol, cLog P = 2.3, tPSA = 81, H-bond donors = 0, H-bond acceptors = 7).[42–44] Nonetheless, the hit compound **5** suffered from high metabolic clearance in mouse liver microsomes (<10% remaining after 10 minutes), which hinders its usefulness for in *vivo* studies in mouse models of MMPSI, which are a relevant species given the prevalence of genetic models of epilepsy in that species.[45] For example, see the successful generation of a mouse line bearing SLACK mutations by Quraishi *et al.*[18] Still, the scaffold was viewed as highly synthetically tractable, and it was thus selected as a starting point for the discovery of SLACK tool compounds. Herein, we report the discovery, synthesis, SAR studies, *in vitro* pharmacokinetic, selectivity assessment, and electrophysiology studies within this series of 1,2,4-oxadiazoles as novel SLACK inhibitors.

2. Results and Discussion

2.1. Chemistry

Our medicinal chemistry strategy targeted 5 different regions of VU245 as illustrated in Figure 2. Initially, we explored SAR within ring A utilizing the synthetic route shown in Scheme 1. First, commercially available nitrile **6** was treated with hydroxylamine hydrochloride in basic medium to afford amidoxime **7**, which was subsequently reacted with 1-(*tert*-butoxycarbonyl)azetidine-3-carboxylic acid **8** in the presence of the coupling agent HATU, followed by cyclization at high temperature to afford 1,2,4-oxadiazole **9**. Next, cleavage of the BOC-protecting group via 4M HCl in 1,4-dioxane yielded the secondary amine **10** in the form of its hydrochloride salt. Penultimate intermediate **10** was reacted with different sulfonyl chlorides under basic conditions to generate **5** and the library compounds **11** – **25**. The synthesis of related ring A analogs **26** - **30** is described in the Supplementary Material.

SAR around the linker region was evaluated by replacing the sulfonamide with alkyl, urea, and carbamate groups as illustrated in Scheme 2. The methylene (**31**) and ethylene (**32**) linkers were prepared by reductive amination of amine **10** with 2-ethoxybenzaldehyde and 2-ethoxyacetophenone, respectively. Amide linkers **33** and **34** were synthesized by coupling amine **10** with 2-ethoxybenzoic acid and 2-ethoxyphenyl acetic acid, respectively. The urea linker in **35** was generated by reacting the amine intermediate **10** with 2-ethoxyisocyanate. Finally, the carbamate linker **36** was synthesized by reacting 4-nitrophenyl chloroformate with amine **10**, followed reaction with 2-ethoxyphenol in the presence of potassium hydroxide under reflux conditions (Scheme 2).

Next, ring D was systematically examined by replacing the 3-thiophene ring with unsubstituted and substituted phenyl rings, pyridines, and other 5-membered ring heterocycles. This library was prepared as outlined in Scheme 3. The amine **37** was reacted with 2-ethoxybenzene sulfonyl chloride to afford the ester **38**, which was subsequently hydrolyzed in the presence of aqueous lithium hydroxide to yield the carboxylic acid **39**. Acid **39** was reacted with assorted amidoximes to afford oxadiazoles **40** – **68** utilizing a variety of different synthetic conditions (Scheme 3).

To further explore SAR around ring C, 5-membered heterocycles other than 1,2,4-oxadiazole were prepared. 1,3,4-Oxadiazole **81** was synthesized according to Scheme 4. First, carboxylic acid **8** was converted into hydrazide **78**, followed by coupling with 4-fluorobenzoic acid and cyclization with Burgess reagent to afford the intermediate **79**. BOC-deprotection using 4M HCl in 1,4-dioxane generated amine **80** in the form of its HCl salt, which was further reacted with 2-ethoxybenzene sulfonyl chloride to afford final compound **81**. Next, 1,3,4-triazole **86** was prepared by reacting the hydrazide intermediate **78** with 4-fluorobenzonitrile at 160 °C in the presence of potassium carbonate to yield intermediate **82**. The triazole nitrogen atom was then protected with a benzyl group to afford **83**, followed by BOC-deprotection to generate amine **84** in the form of its HCl salt. Reaction of **84** with 2-ethoxybenzene sulfonyl chloride generated intermediate **85**, which was subsequently subjected to catalytic hydrogenolysis to yield final compound **86** (Scheme 4).

Isoxazole analog **92** was prepared from the alcohol **87** (Scheme 5). Alcohol **87** was oxidized utilizing pyridine-sulfur trioxide complex into aldehyde **88**, which was converted via Bestmann-Ohira reagent into alkyne **89**. Cyclization of **89** with 4-fluorobenzaldehyde oxime via bis(trifluoroacetoxy)iodo)benzene (PIFA) generated isoxazole intermediate **90**, which was BOC-deprotected using 4M HCl in 1,4-dioxane to yield amine **91** in the form of its HCl salt. Reaction of **91** with 2-ethoxybenzene sulfonyl chloride provided isoxazole analog **92**. For the synthesis of 1,2,3-triazole analog **95**, click chemistry between alkyne **89** and 1-azido-4-fluorobenzene yielded 1,2,3-triazole **93** as outlined in Scheme 5. BOC deprotection in the presence of 4M HCl in 1,4-dioxane to generate the amine **94** in the form of its HCl salt, which was further reacted with 2-ethoxybenzene sulfonyl chloride to yield analog **95**.

For the synthesis of oxazole analog **97**, carboxylic acid intermediate **39** was reacted with 2-amino-1-(4-fluorophenyl)ethenone hydrochloride to yield intermediate **96** as illustrated in Scheme 6. Dehydration of **96** was accomplished under reflux conditions in phosphorus oxychloride ($POCl_3$) to generate oxazole analog **97**. Imidazole analogs **99** and **100** were generated by reacting carboxylic acid **39** with 2-bromo-4'-fluoroacetophenone (**98**) under basic conditions in the presence of ammonium acetate at reflux to yield imidazole **99**. *N*-methylation of **99** was accomplished with methyl iodide to afford analog **100**.

Certain analogs with different alkoxy or alkyl amine groups at position 2 of the phenyl ring were prepared according to Scheme 7. First, intermediate **104** (synthesis described in Supplementary Material) was reacted with the corresponding alcohols or amines via nucleophilic aromatic substitution to yield analogs **112** – **118**. On the other hand, analogs

109 and **110** were prepared via nucleophilic aromatic substitution of intermediate **104** with allyl alcohol to yield intermediate **105**. Removal of the allyl group by palladium catalysis generated phenol intermediate **106**, which was further reacted with the tosylated intermediates **107** and **108** to afford analogs **109** and **110**, respectively.

A library of bicyclic ring analogs in the ring A region (**119** – **122**) was prepared by following a synthetic route similar to that shown in Scheme 1 (detailed synthesis described in the Supplementary Material). However, analogs **125**, **126**, **129**, **130**, **132**, and **133** were prepared according to the synthetic route outlined in Scheme 8.[46] First, intermediate **123** (synthesis described in the Supplementary Material) was refluxed in ammonium hydroxide to yield amine **124**. Cyclization of **124** with either 2-bromo-1,1-diethoxyethane or 1-bromo-2,2-dimethoxypropane under basic conditions afforded the imidazo[1,2-*a*]pyridine analogs **125** and **126**, respectively. Synthesis of [1,2,4]triazolo[1,5-*a*]pyridine analogs **129** and **130** began with intermediate **124**, which was treated with either dimethylformamide dimethyl acetal or dimethylacetamide dimethyl acetal to afford the amidoxime intermediates **127** and **128**, respectively. Next, cyclization of the amidoxime intermediates was achieved through treatment with trifluoroacetic anhydride (TFAA) to yield the [1,2,4]triazolo[1,5-*a*]pyridine analogs **129** and **130**. Finally, synthesis of [1,2,4]triazolo[4,3-*a*]pyridine analogs was initiated from intermediate **123**. Nucleophilic aromatic substitution of **123** with hydrazine under microwave irradiation afforded intermediate **131**. Cyclization of **131** via microwave heating in either trimethyl orthoformate or trimethyl orthoacetate provided [1,2,4]triazolo[4,3-*a*]pyridines **132** and **133**, respectively.

2.2. Pharmacology

2.2.1. Structure Activity Relationship Studies—Optimization of the hit compound and subsequent SAR elucidation was achieved via our Tl^+ flux assay in HEK-293 cells stably expressing WT human SLACK to determine the potency (IC_{50}) and efficacy of all new analogs.[7] This assay uses Tl^+ as a surrogate for potassium ions along with an intracellular dye that exhibits fluorescent activity upon binding Tl^+ ions. Thus, fluorescence activity is used as the read out for this assay. Our investigation began with a SAR study of ring A by systematically scanning all positions of the phenyl ring employing common functional groups (**11** – **25**). Different electron withdrawing and electron donating groups led to mode switching from an inhibitor into an activator, or loss of activity, as most of the analogs were either weak activators of the channel or inactive (Table 1). The same observation was noted in our earlier hit optimization effort,[17] and is consistent with the possibility that the tested analogs modulate SLACK function by a mechanism other than pore blockade. Interestingly, while 2-methoxy analog **23** was a weak activator, extending the alkoxy chain at position 2 of the phenyl ring into *n*-propyl (**26**) or *i*-propyl (**27**) maintained the SLACK inhibitory activity seen with **5**, while the *n*-butyl (**28**) led to a loss of potency. On the other hand, replacing the oxygen with nitrogen (**29**) or carbon (**30**) led to a loss of activity, suggesting that the oxygen atom, in addition to a medium length alkyl chain (2–3 carbons) are necessary for optimal SLACK inhibitory activity. Next, replacing the sulfonamide linker with other linkers such as methylene (**31**), ethylene (**32**), amide (**33**, **34**), urea (**35**), and carbamate (**36**) were examined (Table 2). It was noted that alternative linkers led to mode switching, resulting in weak activators, and that the sulfonamide was

preferred for SLACK inhibitory activity. We postulate that the loss of activity upon changing the linker region is due to its unique properties, including the pyramidal structure adopted by the sulfonamide nitrogen atom, which presumably affects the orientation of the different functional groups that participate in the binding of the molecules to their binding sites.[47]

Next, we moved our attention to replacement of the 3-thiophenyl group in the ring D region of VU245, which is considered a structural alert due to its potential metabolism into reactive metabolites such as thiophene S-oxide and thiophene epoxide,[48] and potentially contributes to the high clearance observed in VU245. In order to avoid these metabolic liabilities, the bioisosteric replacement of the 3-thiophene with a phenyl ring was explored, in addition to installing different functional groups (analogs **40** – **58**, Table 3).[49] The unsubstituted phenyl ring (**40**) led to a loss of potency; however, other modifications were generally tolerated, such as the *ortho*- and *para*-substituted halogens (**41, 43, 44,** and **46**), while others led to mode switching or loss of potency. Next, we decided to further explore the effect of 2,4-di-substituted halogens on the phenyl ring (**56 – 58**), which retained good to moderate potency. Additionally, several heterocycles at the same position were prepared and tested (**59** – **68**). Interestingly, the SLACK channel activity showed a preference for the 5-membered (**59** – **64**) over the 6-membered heterocycles (**65** – **68**). Notably, 5-fluorothiophen-2-yl and 4-isothiazolyl rings demonstrated comparable SLACK inhibitory activity to the hit compound. The 4-fluorophenyl was selected as the basis for subsequent SAR elucidation, since the fluorine introduced minimal changes to the physiochemical properties while blocking a potential metabolic site. Likewise, this functional group offered a better balance of potency and lipophilicity than other similarly potent analogs. For instance, the cLogP value for **43** was 2.6 versus 3.3 and 3.0 for **49** and **58**, respectively.[44] Additionally, the 4-fluorophenyl group was expected to mitigate the potential toxicity concerns and offer improved metabolic stability compared to its 3-thiophene counterpart.

Having identified a potential replacement for the 3-thiophene ring, we turned our attention to the ring B region (Table 4). Installing a methyl on the azetidine ring (**69**) led to mode switching, while fluorine substitution (**70)** reduced inhibitor potency. Subsequently, we attempted to explore the effect of changing ring B and the sulfonamide bond together in order to eliminate any potential glutathione *S*-transferase-mediated cleavage of the sulfonamide.[50] We flipped the azetidine ring orientation (**71**), installed a cyclobutane ring both in the *cis* (**73a**) and *trans* (**73b**) configurations, and replaced the azetidine with a benzene ring (**77**). However, none of these attempts proved successful, which accords with our earlier observations about the critical configuration adopted by the sulfonamide for optimal SLACK inhibitory activity. Expanding the azetidine ring into pyrrolidine in both *R* (**74**) and *S* (**75**) configurations was detrimental for SLACK activity. Finally, installing a piperidine ring (**76**) in place of the azetidine largely retained SLACK inhibitory activity (Table 4).

Subsequently, with the aim to explore modifications of the 1,2,4-oxadiazole core (ring C), we tested several five-membered heterocycles at that position (Table 5). It was noted that even the most closely related 1,2,4-oxadiazole regioisomer **101** led to a loss of SLACK activity. Not surprisingly, other 5-membered heterocycles, including isoxazole isomers (**92** and **102**) and *N*-methyl imidazole **100** also led to a loss of activity. On the other hand,

heterocycles such as 1,3,4-oxadiazole **81**, oxazole **97**, triazole **86**, imidazole **99**, pyrazole **103**, and triazole **95** exhibited mode switching. Overall, the results demonstrated that the SAR around ring C region was narrow and that the 1,2,4-oxadiazole found in the VU245 hit was optimal for SLACK inhibitory activity. Thus, we chose to maintain that core for further SAR exploration.

Having identified some tolerated modifications around VU245, we revisited the 2-ethoxy group at ring A, which while proving ideal for SLACK inhibitory activity, could represent a metabolic liability through P450-mediated dealkylation.[51] As a result, several approaches were followed in an attempt to address this potential metabolic instability. Table 6 shows a selection of analogs (**109** – **118**) with alkoxy replacements that were introduced in this exercise. Replacing the benzene ring with a 3-pyridyl to lower lipophilicity as a means to improve metabolic stability[52] in **111** led to a loss of activity. However, the isosteric replacement of the protium atoms of the ethoxy group with deuterium in **109** retained SLACK activity and offered an opportunity to slow down P450-mediated metabolism.[53] In another attempt to increase metabolic stability, the trifluoroethyl isostere was installed at the same position;[54] however, it led to a slight loss of potency (**113**). Surprisingly, the difluoromethyl group (**112**) led to mode switching and loss of activity. Pleasingly, this position was found tolerant of different aliphatic and alicyclic groups such as methyl cyclopropyl (**114**), methyl cyclobutyl (**110**), and cyclopentyl (**116**); however, the oxetanyl substitution at this position (**115**) led to mode switching, similar to the pattern observed in the first library (Table 1). This observation is potentially explained by the effect of electronic properties on mode switching, as electron density affects molecular geometry and binding characteristics.[55] Replacing the oxygen with nitrogen in our first library (Table 1) introduced a new hydrogen bond donor and led to a loss of SLACK activity; interestingly, eliminating this hydrogen as in pyrrolidine **117** was tolerated. Nonetheless, expanding the ring to piperidine **118** led to a loss of activity. Taken together, SAR exploration within ring A in both libraries (Tables 1 and 6) implies that a hydrogen-bond acceptor at position 2 of the phenyl ring is critical for SLACK inhibitory activity, and this region is tolerant of only small alkyl chains.

Finally, a heterobicyclic ring library at the ring A region was pursued, maintaining what we postulate as critical structural features for SLACK inhibitory activity: a hydrogen bond acceptor directly attached to position 2 of the phenyl ring and a medium-length alkyl chain (Table 7). 8-Chromane **119** retained SLACK inhibitory activity, while 7-benzofuran **120** exhibited mode switching into an activator. Next, we turned our attention to nitrogen-containing heterocycles, such as 8-quinoline **121,** which retained SLACK inhibitory activity. Installation of a methyl group at the 2-position of the 8-quinoline ring (**122)** led to enhanced potency and was expected to block a potential site for certain types of metabolism while simultaneously lowering the risk for mutagenicity.[56, 57] Finally, given our objective to generate brain-penetrant analogs, we attempted to lower the lipophilicity of our compounds, which could also potentially lead to higher metabolic stability.[42] Different nitrogen-containing heterocycles were prepared and evaluated as part of this exercise; unfortunately, both unsubstituted (**125**, **129**, and **132**) and methylated (**126**, **130**, and **133**) analogs proved detrimental for SLACK inhibitory activity (Table 7).

2.2.2. Activity versus A934T SLACK—We decided to further evaluate the pharmacological profile of a selected set of analogs. In choosing this set, we of course selected some of our most potent analogs, e.g., **5**, **43**, **58**, and **122**. We also chose some more moderately potent analogs with unique structural elements. For example, we chose **27** to investigate the effect of branching on the 2-alkoxy group. We likewise chose **109** and **113** to investigate the impact of deuteration and fluorination, respectively, at the same position. We selected **60** as an example of a heteroaryl ring at the Ring D position and **76** as an example of piperidine at the Ring B position. Finally, we included **119** and **121** as additional examples of fused rings, one saturated and one unsaturated, at the Ring A position.

These 11 selected compounds were screened against A934T mutant SLACK, a GOF mutation commonly associated with MMPSI (Table 8).[2, 3] All of the tested molecules showed inhibitory activity on the SLACK variant A934T, and were equipotent or slightly more potent against A934T compared to WT. Furthermore, two of our new analogs (**60** and **122)** showed submicromolar potency versus this mutant channel. This behavior matches that observed in an earlier hit optimization effort for another structurally distinct series of SLACK inhibitors,[17] suggesting that our analogs exert their pharmacological action by the same mechanism. Moreover, it has been reported that SLACK mutants associated with epilepsy have an increased open channel probability compared to WT SLACK.[15] Hence, it could be hypothesized that our analogs may inhibit an open conformation of the channel in a similar fashion to the binding of quinidine to other SLACK mutants associated with GOF behavior.[14]

2.2.3. Selectivity Profile—We next assessed the selectivity profile for the same set of analogs against two additional members of the Slo family: SLICK (Slo2.1) and Maxi-K (Slo1 α1/β3) (Table 9). SLICK (Sequence like an intermediate conductance K^+ channel) is another sodium-activated K^+ channel, also known as Slo 2.1, encoded by the *KCNT2* gene. SLICK shares almost 74% sequence homology with SLACK; furthermore, the RCK and transmembrane domains are almost identical. SLACK and SLICK mainly differ at the distal C-terminal region.[13] Interestingly, SLICK and SLACK-B isoforms can exist as heteromeric channels with different properties than either channel expressed alone. For example, the time required to achieve 90% activation of the heteromeric channel is significantly longer than the time needed to activate either SLICK or SLACK-B homomeric currents.[58] SLICK channels are implicated in epileptic encephalopathy and somatosensory processing.[59–61] The selected analogs showed weak inhibition of SLICK (Table 10); however, analogs **43** and **121** showed an IC_{50} of 2.3 and 5.9 μM, and the efficacy was 50% and 56% of the positive control, respectively. The utility of analogs with SLICK/SLACK dual action is yet to be determined. On the other hand, Maxi-K or Slo1 (BK) channels are large-conductance Ca^{2+}-activated potassium channels encoded by the *KCNMA1* gene.[62] Maxi-K channels share almost 7% identity with SLACK. It was demonstrated that SLACK and Maxi-K channels are similar at the pore-forming domain and the following S6 domain, in addition to the RCK domain.[13] Satisfyingly, the selected analogs showed no activity versus Maxi-K at 30 μM.

Bepridil and quinidine are both known to inhibit hERG channels and are associated with increased risk for cardiac arrhythmias due to QT prolongation.[63, 64] Likewise, certain

preclinical SLACK inhibitors demonstrated a propensity for hERG inhibition as well.[38, 39] Thus, we were keen to evaluate the potential for hERG inhibition with our most potent compound. Gratifyingly, evaluation of a 10 μM concentration of analog **122** using our Tl^+ flux assay in HEK-293 stably expressing hERG[7] showed the compound to be inactive.

2.2.4. Electrophysiology—The SLACK inhibitory activity for the same set of analogs was assessed in whole-cell, voltage-clamp electrophysiology (EP) assays utilizing an automated patch clamp (APC) system (SyncroPatch 384) on Chinese Hamster Ovary (CHO) cell lines stably expressing human WT SLACK channels (Table 10). Recently, we have observed that on our Nanion SynchroPatch APC system, SLACK-expressing CHO cells demonstrated superior experimental success rates compared to SLACK-expressing HEK-293 cells. Thus, for our APC studies, SLACK-expressing CHO cells were used. The potency of VU0606170 is shown here and is similar across both cell lines.[7] In this study, each of our analogs demonstrated a slightly higher potency in EP compared to those observed in the Tl^+ flux assay. We were likewise pleased to observe that three analogs have submicromolar potency (**58**, **60**, and **122**) besides hit compound VU245 (**5**). In fact, 2-methylquinoline **122** represents a notable advance, representing the most potent analog to emerge from our SAR efforts to date and will make another useful *in vitro* probe compound for studying SLACK channels.

2.3. *In vitro* Drug Metabolism and Pharmacokinetics (DMPK)

Our SAR studies around VU245 (**5**) only marginally improved upon the potency of the hit compound. Still, we were able to identify analogs that were equipotent or near equipotent to the original hit VU245 (**5**). Next, we advanced the same set of 11 analogs for further assessment in our frontline *in vitro* DMPK assays. Specifically, metabolic stability was determined by measuring the percent remaining of compounds after incubation for 10 minutes in mouse liver microsomes (MLMs). Likewise, the extent to which the analogs bind to the mouse plasma proteins was measured by equilibrium dialysis (Table 11). Branching the ethoxy into an isopropyl (**27**) was not a successful approach to improve the metabolic stability within this scaffold. However, replacing the thiophene (**5**) with 4-fluorophenyl (**43**) and 2-chloro-4-fluorophenyl (**58**) improved metabolic stability to some extent, supporting our hypothesis that the 3-thiophene ring in **5** contributed to its metabolic instability. 5-Fluoro-2-thiophene ring analog **60** proved no more metabolically stable than the unsubstituted 3-thiophene hit **5**. Replacement of the azetidine (**43**) with a piperidine (**76**) ring led to increased metabolic clearance, possibly due to the introduction of additional sites for oxidative metabolism[65] and higher lipophilicity compared to its azetidine ring counterpart. Gratifyingly, isosteric replacement of hydrogen (**5**) with deuterium (**109**) and fluorine (**113**) improved metabolic stability. Chromane **119** demonstrated a slight improvement in metabolic stability relative to **5**; however, it is still a highly cleared analog. Interestingly, 8-quinoline **121** had notably improved metabolic stability to 74% remaining after 10 minutes. The introduction of a methyl group at position 2 of the quinoline ring (**122)** led to reduced metabolic stability, likely due to the introduction of a new metabolic soft spot to the molecule. We selected analog **121** for further assessment in mouse liver microsomes at multiple time points to enable the calculation of predicted hepatic clearance (CL_{HEP}). These studies found the CL_{HEP} to be 75 mL/min/kg, which is still considered high

(> 2/3 hepatic blood flow). Overall, the results presented thus far provide evidence that both the ethoxy group and the 3-thiophene ring at VU245 (**5**) represent potential metabolic soft spots. Several approaches proved successful for marginally improving metabolic stability; however, the scaffold still suffers in that regard. The metabolic instability may be attributed to the sulfonyl azetidine, part of the molecule that was critical for optimal SLACK inhibitory activity.

We next turned our attention to evaluation of mouse plasma-protein binding, which is expressed here (Table 11) in terms of fraction unbound (f_u). Interestingly, the 5-fluorothiophen-2-yl in **60** maintained the same level of plasma-protein binding as the hit compound VU245 (**5**). It seems possible that the thiophene ring is slightly more favorable in terms of protein binding compared to the halogenated phenyl ring counterparts (**43** and **58**); nonetheless, no significant difference in protein binding was observed across most of our analogs. All analogs showed high (f_u = 0.01 – 0.024) protein binding except analogs **27**, **76**, **119**, and **122** which demonstrated very high (f_u < 0.01) protein binding. While plasma-protein binding is an important parameter to understand the DMPK and safety properties of our compounds, high plasma-protein binding is not always a liability.[42]

3. Conclusion

The present study was designed to determine the SAR around VU245 to optimize selective SLACK inhibitors and evaluate its potential as a lead series. We were able to identify critical structural features associated with optimal SLACK inhibitory activity, and an improvement in potency was achieved. Furthermore, we selected a set of analogs for further characterization in mouse plasma-protein binding and microsomal stability assays. Our analogs demonstrated high plasma-protein binding, and we were able to improve the metabolic stability in some cases; however, this series still suffers from high metabolic clearance in mouse liver microsomes. Notably, this series demonstrated a high degree of selectivity for SLACK channels versus other K^+ channels from the same family: SLICK and Maxi-K. Furthermore, all selected analogs were active versus the A934T mutant SLACK and showed equivalent or increased potency versus the mutant compared to WT SLACK. Finally, select analogs were assessed for potency in whole-cell EP, and the results revealed submicromolar potency for multiple analogs, including an attractive new *in vitro* tool **122** (VU0935685) that also demonstrated selectivity versus hERG. Although the study has successfully characterized analogs with different structural features and improved some properties compared to the hit compound, the high metabolic clearance of this series likely precludes its use as an *in vivo* tool compound in mice. Hit evaluation and optimization continues in other distinct scaffolds identified through our HTS campaign in order to identify high-quality SLACK inhibitors tool compounds for use *in vivo*. Results from those ongoing studies will be reported in due course.

4. Experimental Section

Experimental procedures for synthesis and characterization of analogs **5**, **11**-**28**, **40**-**68**, **109**-**110**, and **112**-**122** are found below. Experimental procedures for pharmacology and *in vitro* DMPK studies as well as synthesis and characterization of all analogs, including ^{1}H

and ^{13}C NMR spectra for all analogs submitted for biological testing, may be found in the Supplementary Material.

4.1. Synthesis and Purification.

Air-sensitive reactions were carried out under a nitrogen atmosphere. Starting materials, reagents, intermediates, and final compounds were weighed on a Mettler Toledo™ New Classic ME analytical balance or a Mettler Toledo™ New Classic ME toploader balance. Thin-layer chromatography (TLC) was conducted on glass plates coated with Silica Gel 60 F_{254} from Millipore Sigma. Normal-phase flash chromatography was carried out on either a CombiFlash® EZ Prep or CombiFlash® Rf+ automated flash chromatography system, both from Teledyne ISCO. Normal-phase flash chromatography was carried out using Redi*Sep*® Rf normal-phase, disposable flash columns from Teledyne ISCO or SiliaSep normal-phase, disposable flash columns (40–63 micron) from SiliCycle, Inc. Reverse-phase preparative chromatography was carried out on the CombiFlash® EZ Prep using a reusable Redi*Sep*® Rf C18 reverse-phase column. Microwave reactions were carried out an Anton Paar Monowave 200 automated microwave synthesizer. The Monowave 200 has an output power of 850W with a maximum temperature of 260 °C and a maximum pressure of 290 psi and is suitable for use with reaction volumes ranging from 0.5 to 20 mL.

4.2. Characterization.

All NMR spectra were recorded on a 300 MHz Bruker Fourier 300HD NMR spectrometer equipped with a dual ^{1}H and ^{13}C probe with Z-Gradient and automatic tuning and matching, full computer control of all shims with TopShim™, 24-sample SampleCase™ automation system, and TopSpin™ software. All NMR samples were prepared with either chloroform-d with 0.03% TMS (99.8+ atom % D, Acros Organics Catalog No. 209561000) or d_6-dimethyl sulfoxide with 0.03% TMS (ACROS Organics Catalog No. 360000100). ^{1}H and ^{13}C chemical shifts are reported in δ values in ppm downfield. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, coupling constant (Hz). High resolution mass spectrometry was conducted on an Agilent 6230 Accurate-Mass Time-of-Flight (TOF) LC/MS with ESI source equipped with MassHunter Walkup software. MS parameters were as follows: fragmentor: 175 V, capillary voltage: 3500 V, nebulizer pressure: 35 psig, drying gas flow: 11 L/min, drying gas temperature: 325 °C. Samples were introduced via an Agilent 1260 Infinity UHPLC comprised of a G4225A HiP Degasser, G1312B binary pump, G1367E ALS, G1316A TCC, and G1315C DAD VL+ with a 5 μL semi-micro flow cell with a 6 mm path length. UV absorption was observed at 220 nm and 254 nm with a 4 nm bandwidth. Column: Agilent Zorbax SB-C18, Rapid Resolution HT, 1.8 μm, 2.1 × 50 mm. Gradient conditions: Hold at 5% CH_3CN in H_2O (0.1% formic acid) for 1.0 min, 5% to 95% CH_3CN in H_2O (0.1% formic acid) over 5 min, hold at 95% CH_3CN in H_2O (0.1% formic acid) for 1.0 min, 0.5 mL/min. All samples submitted for biological testing were confirmed ≥ 95% pure by ^{1}H NMR.

4.3. Chemical synthesis.

4.3.1. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (5).—

(Step 1) *N*-hydroxythiophene-3-carboximidamide (**7**). Hydroxylamine hydrochloride (639 mg, 9.16 mmol, 2.0 eq) and sodium bicarbonate (773 mg, 9.16, 2.0 eq) were stirred in methanol (10 mL) for 30 minutes at room temperature. 3-Thiophenecarbonitrile (500 mg, 4.58 mmol, 1.0 eq) was added to the previous suspension and heated at 50 °C for 2 hours. The reaction was concentrated *in vacuo*, water was added, and extracted with ethyl acetate (2x). The combined organics were washed with brine, dried over sodium sulfate (Na_2SO_4), filtered, and concentrated *in vacuo* to afford 651 mg (100%) of the title compound as a white solid that was used further without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (t, J = 2.1 Hz, 1H), 7.34 (m, 2H), 4.86 (bs, 2H). *(Step 2)* *tert*-Butyl 3-(3-(thiophen-3-yl)-1,2,4-oxadiazol-5-yl)azetidine-1-carboxylate (**9**). Intermediate **7** (651 mg, 4.58, 1.0 eq), 1-boc-azetidine-3-carboxylic acid (**8**) (1.00 g, 5.04 mmol, 1.1 eq), hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) (2.09 g, 5.50 mmol, 1.2 eq), and *N,N*-diisopropylethylamine (DIEA) (2.40 mL, 13.7 mmol, 3.0 eq) were dissolved in Dichloromethane (DCM) (15 mL) and allowed to react for 3 hours at room temperature. Water was added, and the reaction was extracted with ethyl acetate (2x), washed with brine, dried over sodium sulfate (Na_2SO_4), filtered, and concentrated *in vacuo*. The reaction was dissolved in *N*-Methyl-2-pyrrolidone (NMP) (1.0 mL) and heated at 150 °C for 1 hour. Water was added, and the reaction was extracted with ethyl acetate (2x). The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 773 mg (55%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (dd, J = 3.0, 1.19 Hz, 1H), 7.64 (dd, J = 5.1, 1.2 Hz, 1H), 7.44 (dd, J = 5.1, 3.0 Hz, 1H), 4.35 (m, 4H), 4.04 (m, 1H), 1.47 (s, 9H). *(Step 3)* 3-(3-(Thiophen-3-yl)-1,2,4-oxadiazol-5-yl)azetidine hydrochloride (**10**). Intermediate **9** (773 mg, 2.51 mmol, 1.0 eq) was dissolved in 4M hydrochloric acid (HCl) in 1,4-dioxane (5.0 mL) and stirred at room temperature for 30 minutes. The reaction was concentrated *in vacuo* to afford 604 mg (99%) of the title compound as a white solid that was used further without purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.21 (dd, J = 2.8, 1.4 Hz, 1H), 7.63 (m, 2H), 4.64 – 4.42 (m, 5H). *(Step 4)* 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (**5**). Intermediate **10** (25 mg, 0.10 mmol, 1.0 eq) and triethylamine (TEA) (28 µL, 0.20 mmol, 2.0 eq) were dissolved in DCM (2.0 mL). 2-Ethoxybeznene sulfonyl chloride (33 mg, 0.15 mmol, 1.5 eq) was added, and the reaction was stirred for 1 hour at room temperature. Water was added, and the reaction was extracted with DCM (2x). The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 15 mg (38%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (dd, J = 3.0, 1.2 Hz, 1H), 7.91 (dd, J = 7.9, 1.7 Hz, 1H), 7.60 (dd, J = 5.1, 1.2 Hz, 1H), 7.54 (m, 1H), 7.43 (dd, J = 3.0, 2.0 Hz, 1H), 7.06 (m, 2H), 4.46 (d, J = 2.7 Hz, 2H), 4.43 (d, J = 0.8 Hz, 2H), 4.20 (q, J = 7.0 Hz, 2H), 4.03 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.0, 165.0, 156.7, 135.0, 131.2, 128.0, 127.8, 127.2, 126.0, 125.9, 120.4, 113.5, 65.1, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.09 min; HRMS, calc'd for $C_{17}H_{18}N_3O_4S_2^+$ [M+H], 392.0733; found 392.0740.

4.3.2. 5-(1-((2-Fluorophenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (11).—The title compound was prepared in 47% yield (17 mg) from intermediate **10** and 2-fluorobenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (dd, J = 3.0, 1.2 Hz, 1H), 7.91 (m, 1H), 7.64 (m, 1H), 7.58 (dd, J = 5.1, 1.2 Hz, 1H), 7.43 (q, J = 3.0 Hz, 1H), 7.32 (m, 2H), 4.43 (t, J = 8.3 Hz, 2H), 4.35 (t, J = 6.9 Hz, 2H), 4.05 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5, 165.0, 159.3 (d, J(C,F) = 256.0 Hz), 135.7 (d, J(C,F) = 8.6 Hz), 131.2, 128.1, 127.7, 127.2, 126.0, 124.6 (d, J(C,F) = 3.78 Hz), 124.4 (d, J(C,F) = 14.7 Hz), 54.4 (d, J(C,F) = 2.1 Hz), 25.2 ppm. LCMS R_T = 5.00 min; HRMS, calc'd for $C_{15}H_{13}FN_3O_3S_2^+$ [M+H], 366.0377; found 366.0378.

4.3.3. 5-(1-((3-Fluorophenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (12).—The title compound was prepared in 79% yield (29 mg) from intermediate **10** and 3-fluorobenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (dd, J = 3.02, 1.20 Hz, 1H), 7.69 (dt, J = 7.65, 1.38 Hz, 1H), 7.64 – 7.58 (m, 2H), 7.56 (dd, J = 5.08, 1.17 Hz, 1H), 7.43 (q, J = 3.16 Hz, 1H), 7.37 (m, 1H), 4.32 (t, J = 8.45 Hz, 2H), 4.17 (t, J = 6.32 Hz, 2H), 3.96 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.2, 165.0, 162.6 (d, J(C,F) = 252.3 Hz), 136.4 (d, J(C,F) = 6.6 Hz), 131.2 (d, J(C,F) = 7.7 Hz), 128.1, 127.6, 127.3, 125.9, 124.2 (d, J(C,F) = 3.4 Hz), 120.9 (d, J(C,F) = 21.1 Hz), 115.7 (d, J(C,F) = 24.3 Hz), 54.5, 25.2 ppm. LCMS R_T = 5.04 min; HRMS, calc'd for $C_{15}H_{13}FN_3O_3S_2^+$ [M+H], 366.0377; found 366.0385.

4.3.4. 5-(1-((4-Fluorophenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (13).—The title compound was prepared in 68% (25 mg) yield from intermediate **10** and 4-fluorobenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (dd, J = 3.0, 1.2 Hz, 1H), 7.92 (m, 2H), 7.55 (dd, J = 5.0, 1.2 Hz, 1H), 7.43 (q, J = 3.0 Hz, 1H), 7.30 (m, 2H), 4.30 (t, J = 8.4 Hz, 2H), 4.13 (t, J = 6.3 Hz, 2H), 3.98 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.3, 165.8 (d, J(C,F) = 256 Hz), 165.0, 131.2 (d, J(C,F) = 9.4 Hz), 130.4 (d, J(C,F) = 3.3 Hz), 128.0, 127.6, 127.3, 125.9, 116.7 (d, J(C,F) = 22.6 Hz), 54.4, 25.2 ppm. LCMS R_T = 5.00 min; HRMS, calc'd for $C_{15}H_{13}FN_3O_3S_2^+$ [M+H], 366.0377; found 366.0379.

4.3.5. 5-(1-((2-Chlorophenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (14).—The title compound was prepared in 45% (17 mg) yield from intermediate **10** and 2-chlorobenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.1 – 8.0 (m, 2H), 7.62 (dd, J = 5.0, 1.2 Hz, 1H), 7.56 (m, 2H), 7.46 – 7.39 (m, 2H), 4.48 (m, 4H), 4.09 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6, 165.1, 136.1, 134.1, 132.8, 132.1, 131.3, 128.1, 127.8, 127.2, 127.1, 126.0, 54.5, 25.1 ppm. LCMS R_T = 5.20 min; HRMS, calc'd for $C_{15}H_{13}ClN_3O_3S_2^+$ [M+H], 382.0081; found 382.0089.

4.3.6. 5-(1-((3-Chlorophenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (15).—The title compound was prepared in 60% (23 mg)

yield from intermediate **10** and 3-chlorobenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (dd, J = 3.1, 1.2 Hz, 1H), 7.89 (t, J = 1.7 Hz, 1H), 7.77 (dt, J = 7.7, 1.3 Hz, 1H), 7.63 (m, 1H), 7.55 (m, 2H), 7.43 (q, J = 3.0 Hz, 1H), 4.32 (t, J = 8.5 Hz, 2H), 4.17 (t, J = 6.3 Hz, 2H), 3.99 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.3, 165.0, 136.2, 135.7, 133.7, 130.6, 128.3, 128.1, 127.6, 127.3, 126.4, 125.9, 54.5, 25.2 ppm. LCMS R_T = 5.26 min; HRMS, calc'd for $C_{15}H_{13}ClN_3O_3S_2^+$ [M+H], 382.0081; found 382.0083.

4.3.7. 5-(1-((4-Chlorophenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (16).—The title compound was prepared in 63% (24 mg) yield from intermediate **10** and 4-chlorobenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (dd, J = 3.0, 1.2 Hz, 1H), 7.84 (m, 2H), 7.63 – 7.52 (m, 3H), 7.43 (q, J = 3.0 Hz, 1H), 4.31 (t, J = 8.4 Hz, 2H), 4.13 (t, J = 6.3 Hz, 2H), 3.98 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.4, 164.9, 140.3, 132.8, 129.9, 129.7, 128.1, 127.6, 127.3, 125.9, 54.6, 25.2 ppm. LCMS R_T = 5.27 min; HRMS, calc'd for $C_{15}H_{13}ClN_3O_3S_2^+$ [M+H], 382.0081; found 382.0088.

4.3.8. 3-(Thiophen-3-yl)-5-(1-((2-(trifluoromethyl)phenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (17).—The title compound was prepared in 63% (26 mg) yield from intermediate **10** and 2-trifluoromethylbenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (m, 1H), 8.05 (dd, J = 3.0, 1.2 Hz, 1H), 7.92 (m, 1H), 7.74 (m, 2H), 7.61 (dd, J = 5.1, 1.2 Hz, 1H), 7.43 (q, J = 3.0 Hz, 1H), 4.42 (m, 4H), 4.07 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.5, 165.1, 137.0, 133.2, 132.5, 131.6, 128.5 (q, J(C,F) = 6.3 Hz), 128.4 (d, J(C,F) = 74.8 Hz), 128.1, 127.6, 127.2, 126.0, 122.5 (d, J(C,F) = 274 Hz), 54.5, 25.0 ppm. LCMS R_T = 5.34 min; HRMS, calc'd for $C_{16}H_{13}F_3N_3O_3S_2^+$ [M+H], 416.0345; found 416.0347.

4.3.9. 3-(Thiophen-3-yl)-5-(1-((3-(trifluoromethyl)phenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (18).—The title compound was prepared in 51% (21 mg) yield from intermediate **10** and 3-trifluoromethylbenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (m, 1H), 8.08 (m, 1H), 8.00 – 7.89 (m, 2H), 7.76 (m, 1H), 7.55 (dd, J = 5.1, 1.2 Hz, 1H), 7.43 (q, J = 3.0 Hz, 1H), 4.35 (t, J = 8.4 Hz, 2H), 4.19 (t, J = 6.3 Hz, 2H), 4.01 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.2, 165.0, 136.2, 132.1, 131.9, 131.5, 130.2 (m, J(C,F)), 128.1, 127.8, 127.2, 125.9, 125.3 (q, J(C,F) = 3.8 Hz), 123.2 (d, J(C,F) = 273.1 Hz), 54.5, 25.2 ppm. LCMS R_T = 5.38 min; HRMS, calc'd for $C_{16}H_{13}F_3N_3O_3S_2^+$ [M+H], 416.0345; found 416.0345.

4.3.10. 3-(Thiophen-3-yl)-5-(1-((4-(trifluoromethyl)phenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (19).—The title compound was prepared in 55% (23 mg) yield from intermediate **10** and 4-trifluoromethylbenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (m, 2H), 7.93 (dd, J = 3.0, 1.2 Hz, 1H), 7.89 (m, 2H), 7.52 (dd, J = 5.1, 1.2 Hz, 1H), 7.42 (q, J = 3.0 Hz, 1H), 4.35 (t, J = 8.4 Hz,

2H), 4.18 (t, J = 6.3 Hz, 2H), 4.00 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.3, 165.0, 136.9 (d, J(C,F) = 230 Hz), 135.0, 128.9, 128.0, 127.6, 127.3, 126.5 (q, J(C,F) = 3.7 Hz), 125.5, 123.2 (d, J(C,F) = 273.2 Hz), 54.6, 25.2 ppm. LCMS R_T = 5.42 min; HRMS, calc'd for $C_{16}H_{13}F_3N_3O_3S_2^+$ [M+H], 416.0345; found 416.0349.

4.3.11. 3-(Thiophen-3-yl)-5-(1-(*o*-tolylsulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (20).—The title compound was prepared in 17% (6 mg) yield from intermediate **10** and 2-methylbenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (dd, J = 3.0, 1.2 Hz, 1H), 7.99 (m, 1H), 7.62 (dd, J = 6, 1.2 Hz, 1H), 7.51 (td, J = 6.9, 1.4 Hz, 1H), 7.43 (q, J = 3.0 Hz, 1H), 7.39 – 7.31 (m, 2H), 4.35 (t, J = 7.7 Hz, 2H), 4.26 (t, J = 6.0 Hz, 2H), 4.05 (m, 1H), 2.69 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.7, 165.1, 138.8, 134.8, 133.6, 132.8, 130.1, 128.1, 127.8, 127.2, 126.2, 126.0, 53.3, 25.3, 20.7 ppm. LCMS R_T = 5.23 min; HRMS, calc'd for $C_{16}H_{16}N_3O_3S_2^+$ [M+H], 362.0628; found 362.0627.

4.3.12. 3-(Thiophen-3-yl)-5-(1-(*m*-tolylsulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (21).—The title compound was prepared in 42% (15 mg) yield from intermediate **10** and 3-methylbenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (dd, J = 3.0, 1.2 Hz, 1H), 7.72 – 7.67 (m, 2H), 7.56 (dd, J = 5.1, 1.2 Hz, 1H), 7.53 – 7.40 (m, 3H), 4.28 (t, J = 8.4 Hz, 2H), 4.16 (t, J = 6.3 Hz, 2H), 3.95 (m, 1H), 2.46 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.4, 164.9, 139.7, 134.5, 134.0, 129.2, 128.7, 128.0, 127.7, 127.2, 125.9, 125.6, 54.3, 25.3, 21.5 ppm. LCMS R_T = 5.12 min; HRMS, calc'd for $C_{16}H_{16}N_3O_3S_2^+$ [M+H], 362.0628; found 362.0634.

4.3.13. 3-(Thiophen-3-yl)-5-(1-tosylazetidin-3-yl)-1,2,4-oxadiazole (22).—The title compound was prepared in 28% yield (10 mg) from intermediate **10** and 4-methylbenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (dd, J = 3.0, 1.2 Hz, 1H), 7.78 (m, 2H), 7.55 (dd, J = 5.1, 1.2 Hz, 1H), 7.45 – 7.36 (m, 3H), 4.28 (t, J = 8.5 Hz, 2H), 4.13 (t, J = 6.3 Hz, 2H), 3.93 (m, 1H), 2.43 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.4, 164.9, 144.6, 131.0, 130.0, 128.6, 128.0, 127.7, 127.2, 125.9, 54.3, 25.3, 21.6 ppm. LCMS R_T = 5.11 min; HRMS, calc'd for $C_{16}H_{16}N_3O_3S_2^+$ [M+H], 362.0628; found 362.0627.

4.3.14. 5-(1-((2-Methoxyphenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (23).—The title compound was prepared in 61% (23 mg) yield from intermediate **10** and 2-methoxybenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (dd, J = 3.0, 1.2 Hz, 1H), 7.92 (dd, J = 7.8, 1.7 Hz, 1H), 7.62 – 7.53 (m, 2H), 7.43 (dd, J = 5.1, 3.0 Hz, 1H), 7.08 (m, 2H), 4.42 (m, 4H), 4.05 (m, 1H), 3.96 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.9, 165.0, 157.1, 135.1, 131.3, 128.0, 127.8, 127.3, 126.0, 125.5, 120.6, 112.5, 56.2, 54.5, 25.2 ppm. LCMS R_T = 4.85 min; HRMS, calc'd for $C_{16}H_{16}N_3O_4S_2^+$ [M+H], 378.0577; found 378.0578.

4.3.15. 5-(1-((3-Methoxyphenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (24).—The title compound was prepared in 61% (23 mg) yield from intermediate **10** and 3-methoxybenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (dd, J = 3.0, 1.2 Hz, 1H), 7.58 – 7.37 (m, 5H), 7.18 (m, 1H), 4.29 (t, J = 8.5 Hz, 2H), 4.17 (t, J = 6.3 Hz, 2H), 3.95 (m, 1H), 3.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.4, 164.9, 160.2, 135.1, 130.4, 128.0, 127.7, 127.2, 125.9, 120.6, 119.9, 113.2, 55.8, 54.4, 25.2 ppm. LCMS R_T = 5.01 min; HRMS, calc'd for $C_{16}H_{16}N_3O_4S_2^+$ [M+H], 378.0577; found 378.0576.

4.3.16. 5-(1-((4-Methoxyphenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (25).—The title compound was prepared in 65% (25 mg) yield from intermediate **10** and 4-methoxybenzene sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (dd, J = 3.0, 1.3 Hz, 1H), 7.84 – 7.76 (m, 3H), 7.49 (dd, J = 5.1, 1.2 Hz, 1H), 7.22 (m, 2H), 4.21–4.04 (m, 3H), 3.94 (m, 1H), 3.83 (s, 3H). LCMS R_T = 4.93 min; HRMS, calc'd for $C_{16}H_{16}N_3O_4S_2^+$ [M+H], 378.0577; found 378.0581.

4.3.17. 5-(1-((2-Propoxyphenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (26).—Sodium hydride (NaH) (20 mg, 0.82 mmol, 10.0 eq) was added to *n*-propanol (2.0 mL) at 0 °C and was allowed to stir for 15 minutes. Analog **11** (30 mg, 0.082 mmol, 1.0 eq) was added afterwards, and the reaction was warmed to room temperature, then heated at 60 °C overnight. The reaction was cooled to room temperature and concentrated *in vacuo*. Water was added, and the reaction was extracted with DCM (2x). The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 9 mg (28%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (dd, J = 3.0, 1.0 Hz, 1H), 7.91 (dd, J = 7.8, 1.7 Hz, 1H), 7.60 (dd, J = 5.1, 1.0 Hz, 1H), 7.54 (m, 1H), 7.43 (m, 1H), 7.05 (m, 2H), 4.43 (m, 4H), 4.12 – 3.96 (m, 3H), 4.03 (m, 1H), 1.89 (m, 2H), 1.08 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.9, 165.0, 156.9, 135.0, 131.3, 128.0, 127.8, 127.2, 126.0, 125.5, 120.3, 113.5, 70.9, 54.4, 25.3, 22.5, 10.4 ppm. LCMS R_T = 5.37 min; HRMS, calc'd for $C_{18}H_{20}N_3O_4S_2^+$ [M+H], 406.0890; found 406.0887.

4.3.18. 5-(1-((2-Isopropoxyphenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (27).—The title compound was prepared in 26% (9 mg) yield from compound **11** and isopropanol using a method analogous to that described for the conversion of compound **11** into **26**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (dd, J = 3.0, 1.2 Hz, 1H), 7.91 (dd, J = 7.8, 1.6 Hz, 1H), 7.60 (dd, J = 5.1, 1.2 Hz, 1H), 7.52 (m, 1H), 7.43 (m, 1H), 7.09 – 6.99 (m, 2H), 4.75 (m, 1H), 4.50 – 4.40 (m, 4H), 4.02 (m, 1H), 1.43 (d, J = 6.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.0, 165.0, 155.9, 134.8, 131.3, 128.0, 127.9, 127.2, 126.8, 126.0, 120.1, 114.7, 72.0, 54.4, 25.3, 22.0 ppm. LCMS R_T = 5.33 min; HRMS, calc'd for $C_{18}H_{20}N_3O_4S_2^+$ [M+H], 406.0890; found 406.0889.

4.3.19. 5-(1-((2-Butoxyphenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-3-yl)-1,2,4-oxadiazole (28).—The title compound was prepared in 17% (6 mg) yield

from compound **11** and *n*-butanol using a method analogous to that described for the conversion of compound **11** into **26**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 3.0, 1.2 Hz, 1H), 7.91 (m, 1H), 7.60 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.54 (m, 1H), 7.43 (m, 1H), 7.05 (m, 2H), 4.49 – 4.37 (m, 4H), 4.15 – 3.96 (m, 3H), 1.84 (m, 2H), 1.53 (m, 2H), 0.97 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.0, 165.0, 156.9, 135.0, 131.3, 128.0, 127.8, 127.2, 126.0, 125.5, 120.3, 113.5, 69.2, 54.4, 31.2, 25.3, 19.1, 13.8 ppm. LCMS R_T = 5.59 min; HRMS, calc'd for $C_{19}H_{22}N_3O_4S_2^+$ [M+H], 420.1046; found 420.1053.

4.3.20. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-phenyl-1,2,4-oxadiazole (40).—*(Step 1)* Methyl 1-((2-ethoxyphenyl)sulfonyl)azetidine-3-carboxylate (**38**). Methyl azetidine-3-carboxylate hydrochloride (**37**) (618 mg, 4.08 mmol, 1.2 eq) and *N,N*-diisopropylethylamine (1.78 mL, 10.2 mmol, 3.0 eq) were dissolved in DCM (10 mL), followed by the addition of 2-ethoxybenzene sulfonyl chloride (750 mg, 3.40 mmol, 1.0 eq). The reaction was stirred for 2 hours at room temperature. Water was added, and the reaction was extracted with DCM (2x). The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 830 mg (82%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.53 (m, 1H), 7.08 – 6.98 (m, 2H), 4.27 – 4.12 (m, 6H), 3.70 (s, 3H), 3.35 (m, 1H), 1.51 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 156.7, 134.8, 131.1, 126.1, 120.3, 113.5, 65.0, 53.1, 52.4, 31.5, 14.7 ppm. LCMS R_T = 3.94 min; HRMS, calc'd for $C_{13}H_{18}NO_5S^+$ [M+H], 300.0900; found 300.0899. *(Step 2)* 1-((2-Ethoxyphenyl)sulfonyl)azetidine-3-carboxylic acid (**39**). Intermediate **38** (830 mg, 2.77 mmol, 1.0 eq) was dissolved in THF (5.0 mL) and H_2O (5.0 mL), followed by the addition of lithium hydroxide (LiOH) monohydrate (233 mg, 5.54 mmol, 2.0 eq). The reaction was stirred at room temperature for 2 hours. The solvents were removed *in vacuo*, and the reaction was acidified with 1N HCl. The aqueous layer was extracted with ethyl acetate (2x), the combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford 720 mg (91%) as a white solid that was used further without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.53 (m, 1H), 7.08 – 6.99 (m, 2H), 4.30 – 4.12 (m, 6H), 3.38 (m, 1H), 1.51 (t, *J* = 7.0 Hz, 3H). *(Step 3)* 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-phenyl-1,2,4-oxadiazole (**40**). Intermediate **39** (50 mg, 0.18 mmol, 1.0 eq), *N,N*-diisopropylethylamine (94 μL, 0.54 mmol, 3.0 eq), and HATU (103 mg, 0.27 mmol, 1.5 eq) were dissolved in DMF (2.0 mL) and allowed to stir for 15 minutes, followed by the addition of benzamidoxime (49 mg, 0.36 mmol, 2.0 eq). The reaction was allowed to stir for 1 hour at room temperature, afterwards the reaction temperature was brought to 140 °C and stirred for 2 hours. Water was added, and the reaction was extracted with ethyl acetate (2x). The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 28 mg (40%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (m, 2H), 7.91 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.59 – 7.43 (m, 4H), 7.10 – 6.99 (m, 2H), 4.46 (m, 4H), 4.20 (q, *J* = 7.0 Hz, 2H), 4.05 (m, 1H), 1.50 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 168.6, 156.7, 135.0, 131.5, 131.2, 128.9, 127.5, 126.4, 125.9, 120.4, 113.5, 65.1, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.27 min; HRMS, calc'd for $C_{19}H_{20}N_3O_4S^+$ [M+H], 386.1169; found 386.1177.

4.3.21. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(2-fluorophenyl)-1,2,4-oxadiazole (41).—Intermediate **39** (50 mg, 0.18 mmol, 1.0 eq), DIEA (63 μL, 0.36 mmol, 2.0 eq), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (52 mg, 0.27 mmol, 1.5 eq), and Hydroxybenzotriazole (HOBt) (41 mg, 0.27 mmol, 1.5 eq) were dissolved in 1,4-dioxane (2.0 mL). The reaction was allowed to stir for 30 minutes at room temperature, followed by the addition of 2-fluorobenzamidoxime (41 mg, 0.27 mmol, 1.5 eq). The reaction was stirred at room temperature for 2 hours, afterwards the reaction temperature was brought to 90 °C and stirred overnight. Water was added, and the reaction was extracted with ethyl acetate (2x). The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 4 mg (6%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (td, J = 7.3, 1.8 Hz, 1H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H), 7.58 – 7.46 (m, 2H), 7.34 – 7.19 (m, 2H), 7.10 – 6.99 (m, 2H), 4.46 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.08 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.8, 163.9 (d, J(C,F) = 228 Hz), 159.0, 156.7, 135.0, 133.0 (d, J(C,F) = 8.6 Hz), 131.2, 130.7 (d, J(C,F) = 2.2 Hz), 125.9, 124.5 (d, J(C,F) = 3.8 Hz), 120.4, 116.8 (d, J(C,F) = 21.1 Hz), 114.7 (d, J(C,F) = 12.4 Hz), 113.5, 65.1, 54.6, 25.3, 14.7 ppm. LCMS R_T = 5.15 min; HRMS, calc'd for $C_{19}H_{19}FN_3O_4S^+$ [M+H], 404.1075; found 404.1080.

4.3.22. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(3-fluorophenyl)-1,2,4-oxadiazole (42).—The title compound was prepared in 11% yield (8 mg) from intermediate **39** and 3-fluorobenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (dd, J = 7.8, 1.7 Hz, 1H), 7.84 (dt, J = 8.0, 1.2 Hz, 1H), 7.73 (m, 1H), 7.60 – 7.41 (m, 2H), 7.22 (m, 2H), 7.11 – 7.01 (m, 2H), 4.46 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.05 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.6, 166.2 (d, J(C,F) = 244.4 Hz), 161.2, 156.7, 135.0, 131.2, 130.7 (d, J(C,F) = 8.0 Hz), 128.4 (d, J(C,F) = 8.6 Hz), 125.9, 123.2 (d, J(C,F) = 3.2 Hz), 120.4, 118.5 (d, J(C,F) = 21.3 Hz), 114.6 (d, J(C,F) = 23.8 Hz), 113.6, 65.1, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.38 min; HRMS, calc'd for $C_{19}H_{19}FN_3O_4S^+$ [M+H], 404.1075; found 404.1076.

4.3.23. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (43).—The title compound was prepared in 10% yield (7 mg) from intermediate **39** and 4-fluorobenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (m, 2H), 7.92 (dd, J = 7.9, 1.7 Hz, 1H), 7.54 (m, 1H), 7.17 (m, 2H), 7.10 – 6.99 (m, 2H), 4.46 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.04 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.3, 167.8, 164.7 (d, J(C,F) = 252 Hz), 156.7, 135.0, 131.1, 129.6 (d, J(C,F) = 8.8 Hz), 126.0, 122.6 (d, J(C,F) = 3.4 Hz), 120.4, 116.2 (d, J(C,F) = 22.1 Hz), 113.5, 65.1, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.35 min; HRMS, calc'd for $C_{19}H_{19}FN_3O_4S^+$ [M+H], 404.1075; found 404.1084.

4.3.24. 3-(2-Chlorophenyl)-5-(1-((2-ethoxyphenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (44).—The title compound was prepared in 5% yield (4 mg) from intermediate **39** and 2-chlorobenzamidoxime using a method analogous to that described for the

conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (m, 2H), 7.59 – 7.33 (m, 4H), 7.10 – 6.99 (m, 2H), 4.47 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.09 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.7, 167.4, 156.7, 135.0, 133.5, 131.9, 131.7, 131.2, 131.0, 126.9, 125.9, 125.6, 120.4, 113.6, 65.1, 54.5, 25.3, 14.8 ppm. LCMS R_T = 5.34 min; HRMS, calc'd for $C_{19}H_{19}ClN_3O_4S^+$ [M+H], 420.0779; found 420.0788.

4.3.25. 3-(3-Chlorophenyl)-5-(1-((2-ethoxyphenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (45).—The title compound was prepared in 7% yield (5 mg) from intermediate **39** and 3-chlorobenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (t, J = 1.6 Hz, 1H), 7.93 (m, 2H), 7.61 – 7.38 (m, 3H), 7.12 – 7.01 (m, 2H), 4.46 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.05 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.6, 167.6, 156.8, 155.0, 135.1, 131.5, 131.3, 130.3, 128.1, 127.6, 125.8, 125.5, 120.4, 113.6, 65.1, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.34 min; HRMS, calc'd for $C_{19}H_{19}ClN_3O_4S^+$ [M+H], 420.0779; found 420.0787.

4.3.26. 3-(4-Chlorophenyl)-5-(1-((2-ethoxyphenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (46).—The title compound was prepared in 5% yield (4 mg) from intermediate **39** and 4-chlorobenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (m, 2H), 7.91 (dd, J = 7.8, 1.8 Hz, 1H), 7.54 (m, 1H), 7.47 (m, 2H), 7.11 – 6.99 (m, 2H), 4.46 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.05 (m, 1H), 1.50 (t, J = 7. 0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.5, 167.8, 156.7, 137.7, 135.0, 131.1, 129.3, 128.8, 126.0, 124.9, 120.4, 113.5, 65.1, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.34 min; HRMS, calc'd for $C_{19}H_{19}ClN_3O_4S^+$ [M+H], 420.0779; found 420.0788.

4.3.27. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(2-(trifluoromethyl)phenyl)-1,2,4-oxadiazole (47).—The title compound was prepared in 11% yield (9 mg) from intermediate **39** and 2-trifluoromethylbenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (m, 1H), 7.87 – 7.81 (m, 1H), 7.79 – 7.73 (m, 1H), 7.71 – 7.63 (m, 2H), 7.53 (m, 1H), 7.05 (m, 2H), 4.46 (m, 4H), 4.25 – 4.02 (m, 3H), 1.49 (t, J = 7. 0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 167.8, 156.7, 135.0, 131.9, 131.8, 131.1, 131.0, 129.6, 129.2, 127.0 (q, J(C,F) = 5.3 Hz), 125.8, 125.2 (q, J(C,F) = 3.4 Hz), 120.4, 113.5, 65.1, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.38 min; HRMS, calc'd for $C_{20}H_{19}F_3N_3O_4S^+$ [M+H], 454.1043; found 454.1044.

4.3.28. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(3-(trifluoromethyl)phenyl)-1,2,4-oxadiazole (48).—The title compound was prepared in 17% yield (14 mg) from intermediate **39** and 3-trifluoromethylbenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 – 8.19 (m, 2H), 7.92 (dd, J = 7.8, 1.6 Hz, 1H), 7.79 (m, 1H), 7.63 (m, 1H), 7.55 (m, 1H), 7.06 (m, 2H), 4.47 (m, 4H), 4.21 (q, J = 7.0 Hz, 2H), 4.07 (m, 1H), 1.51 (t, J = 7. 0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.9, 167.6, 156.8, 135.1, 131.8, 131.3, 130.5, 129.6, 128.0 (q, J(C,F) = 3.7 Hz), 127.3,

125.8, 125.5, 124.5 (q, J(C,F) = 3.9 Hz), 120.4, 113.6, 65.1, 54.5, 25.2, 14.7 ppm. LCMS R_T = 5.69 min; HRMS, calc'd for $C_{20}H_{19}F_3N_3O_4S^+$ [M+H], 454.1043; found 454.1043.

4.3.29. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazole (49).—The title compound was prepared in 11% yield (9 mg) from intermediate **39** and 4-trifluoromethylbenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, J = 8.1 Hz, 2H), 7.92 (dd, J = 7.8, 1.7 Hz, 1H), 7.57 (d, J = 8.2 Hz, 2H), 7.55 (m, 1H), 7.06 (m, 2H), 4.47 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.07 (m, 1H), 1.51 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.8, 167.6, 156.6, 135.0, 133.1 (q, J(C,F) = 32.8 Hz), 131.1, 129.8, 125.8, 125.9 (q, J(C,F) = 3.5 Hz), 125.5, 121.9, 120.5, 113.6, 65.1, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.71 min; HRMS, calc'd for $C_{20}H_{19}F_3N_3O_4S^+$ [M+H], 454.1043; found 454.1042.

4.3.30. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(*o*-tolyl)-1,2,4-oxadiazole (50).—Intermediate **39** (30 mg, 0.11 mmol, 1.0 eq) and 1,1'-carbonyldiimidazole (CDI) (27 mg, 0.17 mmol, 1.5 eq) were dissolved in anhydrous DMF (2.0 mL), and the reaction was allowed to stir for 30 minutes at room temperature followed by the addition of 2-methylbenzamidoxime (25 mg, 0.17 mmol, 1.5 eq), and another 1.5 eq of CDI. The reaction was stirred overnight at 80 °C. Water was added, and the reaction was extracted with DCM (2x). The combined organics were washed with water, brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 19 mg (43%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (dd, J = 7.8, 1.7 Hz, 1H), 7.88 – 7.79 (m, 2H), 7.53 (m, 1H), 7.41 – 7.29 (m, 2H), 7.11 – 6.98 (m, 2H), 4.46 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.04 (m, 1H), 2.43 (s, 3H), 1.50 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.1, 168.7, 156.7, 138.7, 135.0, 132.2, 131.2, 128.8, 128.0, 126.2, 126.0, 124.6, 120.4, 113.6, 65.1, 54.5, 25.4, 21.3, 14.7 ppm. LCMS R_T = 5.54 min; HRMS, calc'd for $C_{20}H_{22}N_3O_4S^+$ [M+H], 400.1326; found 400.1327.

4.3.31. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(*m*-tolyl)-1,2,4-oxadiazole (51).—The title compound was prepared in 43% yield (29 mg) from intermediate **39** and 3-methylbenzamidoxime using a method analogous to that described for the conversion of compound **39** into **50**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 – 7.87 (m, 2H), 7.53 (m, 1H), 7.44 – 7.35 (m, 1H), 7.34 – 7.27 (m, 2H), 7.09 – 6.99 (m, 2H), 4.46 (m, 4H), 4.19 (q, J = 7.0 Hz, 2H), 4.06 (m, 1H), 2.60 (s, 3H), 1.49 (t, J = 7.0 Hz, 3H). LCMS R_T = 5.50 min; ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.1, 169.1, 156.7, 138.3, 135.0, 131.5, 131.2, 130.8, 130.1, 126.0, 125.9, 125.6, 120.4, 113.6, 65.1, 54.6, 25.3, 22.2, 14.7 ppm. LCMS R_T = 5.50 min; HRMS, calc'd for $C_{20}H_{22}N_3O_4S^+$ [M+H], 400.1326; found 400.1326.

4.3.32. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(*p*-tolyl)-1,2,4-oxadiazole (52).—The title compound was prepared in 39% yield (26 mg) from intermediate **39** and 4-methylbenzamidoxime using a method analogous to that described for the conversion of compound **39** into **50**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, J = 8.3 Hz, 3H), 7.53 (m, 1H), 7.28 (m,

2H), 7.09 – 6.99 (m, 2H), 4.45 (m, 4H), 4.19 (q, *J* = 7.0 Hz, 2H), 4.03 (m, 1H), 2.42 (s, 3H), 1.49 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.0, 168.6, 156.7, 141.8, 135.0, 131.2, 129.6, 127.4, 125.9, 123.5, 120.4, 113.6, 65.1, 54.5, 25.3, 21.6, 14.7 ppm. LCMS R_T = 5.53 min; HRMS, calc'd for $C_{20}H_{22}N_3O_4S^+$ [M+H], 400.1326; found 400.1327.

4.3.33. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(2-methoxyphenyl)-1,2,4-oxadiazole (53).—The title compound was prepared in 11% yield (8 mg) from intermediate **39** and 2-methoxyamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 7.92 (m, 2H), 7.58 – 7.44 (m, 2H), 7.12 – 6.98 (m, 4H), 4.45 (m, 4H), 4.19 (q, *J* = 7.0 Hz, 2H), 4.07 (m, 1H), 3.97 (s, 3H), 1.49 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.9, 167.1, 158.1, 156.7, 135.0, 132.6, 131.4, 131.1, 125.8, 120.7, 120.4, 115.3, 113.5, 111.7, 65.1, 56.0, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.00 min; HRMS, calc'd for $C_{20}H_{22}N_3O_5S^+$ [M+H], 416.1275; found 416.1284.

4.3.34. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(3-methoxyphenyl)-1,2,4-oxadiazole (54).—The title compound was prepared in 17% yield (12 mg) from intermediate **39** and 3-methoxybenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 7.92 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.63 (dt, *J* = 1.2 Hz, 1H), 7.59 – 7.49 (m, 2H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.11 – 6.99 (m, 3H), 4.46 (m, 4H), 4.20 (q, *J* = 7.0 Hz, 2H), 4.05 (m, 1H), 3.88 (s, 3H), 1.50 (t, *J* = 7. 0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 168.5, 159.9, 156.7, 135.0, 131.2, 130.1, 127.5, 125.9, 120.4, 119.9, 117.9, 113.5, 112.1, 65.1, 56.0, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.30 min; HRMS, calc'd for $C_{20}H_{22}N_3O_5S^+$ [M+H], 416.1275; found 416.1278.

4.3.35. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(4-methoxyphenyl)-1,2,4-oxadiazole (55).—The title compound was prepared in 15% yield (11 mg) from intermediate **39** and 4-methoxybenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 8.01 – 7.87 (m, 3H), 7.54 (m, 1H), 7.11 – 6.92 (m, 4H), 4.45 (m, 4H), 4.19 (q, *J* = 7.0 Hz, 2H), 4.03 (m, 1H), 3.88 (s, 3H), 1.50 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.9, 168.3, 162.1, 156.7, 135.0, 131.2, 129.1, 125.9, 120.4, 118.8, 114.3, 113.5, 65.1, 55.4, 54.5, 25.3, 14.7 ppm. LCMS R_T = 5.25 min; HRMS, calc'd for $C_{20}H_{22}N_3O_5S^+$ [M+H], 416.1275; found 416.1284.

4.3.36. 3-(2,4-Difluorophenyl)-5-(1-((2-ethoxyphenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (56).—The title compound was prepared in 18% yield (13 mg) from intermediate **39** and 2,4-difluorobenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 8.02 (m, 1H), 7.91 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.54 (m, 1H), 7.12 – 6.94 (m, 4H), 4.46 (m, 4H), 4.20 (q, *J* = 7.0 Hz, 2H), 4.07 (m, 1H), 1.50 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.0, 164.8 (d, *J*(C,F) = 260 Hz), 164.9, 164.8, 164.8 (d, *J*(C,F) = 253 Hz), 159.6, 169.5, 156.7, 135.0, 132.1 (d, *J*(C,F) = 10.5 Hz), 132.02 (d, *J*(C,F) = 10.1 Hz), 131.1, 126.0, 120.4, 113.6, 112.2 (d, *J*(C,F) = 21.6 Hz), 112.1 (d, *J*(C,F) = 21.5 Hz), 111.3 (d, *J*(C,F) = 4.4 Hz),

111.1 (d, J(C,F) = 3.8 Hz), 105.3 (d, J(C,F) = 25.2 Hz), 65.1, 54.4, 25.2, 14.7 ppm. LCMS R_T = 5.27 min; HRMS, calc'd for $C_{19}H_{18}F_2N_3O_4S^+$ [M+H], 422.0981; found 422.0985.

4.3.37. 3-(4-Chloro-2-fluorophenyl)-5-(1-((2-ethoxyphenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (57).—The title compound was prepared in 8% yield (6 mg) from intermediate **39** and 4-chloro-2-fluorobenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 – 7.86 (m, 2H), 7.54 (m, 1H), 7.34 – 7.23 (m, 2H), 7.11 – 6.99 (m, 2H), 4.46 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.07 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.1, 164.9, 163.5 (d, J(C,F) = 192.4 Hz), 158.7, 156.7, 138.4 (d, J(C,F) = 9.9 Hz), 135.0, 131.4 (d, J(C,F) = 3.1), 131.1, 126.0, 125.1 (d, J(C,F) = 3.7 Hz), 120.4, 117.6 (d, J(C,F) = 24.4 Hz), 113.5, 65.1, 54.4, 25.2, 14.7 ppm. LCMS R_T = 5.56 min; HRMS, calc'd for $C_{19}H_{18}ClFN_3O_4S^+$ [M+H], 438.0685; found 438.0689.

4.3.38. 3-(2-Chloro-4-fluorophenyl)-5-(1-((2-ethoxyphenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (58).—The title compound was prepared in 11% yield (8 mg) from intermediate **39** and 2-chloro-4-fluorobenzamidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 – 7.86 (m, 2H), 7.53 (m, 1H), 7.29 (dd, J = 8.5, 2.5 Hz, 1H), 7.18 – 6.98 (m, 3H), 4.46 (m, 4H), 4.20 (q, J = 7. 0 Hz, 2H), 4.08 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.8, 166.7, 163.7 (d, J(C,F) = 255.5 Hz), 156.7, 135.0, 134.8 (d, J(C,F) = 10.6 Hz), 133.3, 131.1, 126.0, 122.0 (d, J(C,F) = 5.5 Hz), 120.4, 118.6 (d, J(C,F) = 25.0 Hz), 114.6 (d, J(C,F) = 21.5 Hz), 113.6, 65.1, 54.5, 25.3, 14.8 ppm. LCMS R_T = 5.47 min; HRMS, calc'd for $C_{19}H_{18}ClFN_3O_4S^+$ [M+H], 438.0685; found 438.0688.

4.3.39. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(thiophen-2-yl)-1,2,4-oxadiazole (59).—The title compound was prepared in 6% yield (4 mg) from intermediate **39** and thiophene-2-amidoxime using a method analogous to that described for the conversion of compound **39** into **40**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 8.3, 1.6 Hz, 1H), 7.76 (dd, J = 3.7, 1.2 Hz, 1H), 7.59 – 7.49 (m, 2H), 7.16 (m, 1H), 7.06 (m, 2H), 4.44 (m, 4H), 4.21 (q, J = 7.0 Hz, 2H), 4.04 (m, 1H), 1.51 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.1, 164.6, 156.7, 135.1, 131.2, 129.8, 129.6, 128.1, 127.8, 125.8, 120.4, 113.5, 65.1, 54.4, 25.3, 14.8 ppm. LCMS R_T = 5.10 min; HRMS, calc'd for $C_{17}H_{18}N_3O_4S_2^+$ [M+H], 392.0733; found 392.0753.

4.3.40. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(5-fluorothiophen-2-yl)-1,2,4-oxadiazole (60).—CDI (24 mg, 0.15 mmol, 1.2 eq) was added to a solution of intermediate **39** (35 mg, 0.12 mmol, 1.1 eq) in dimethyl sulfoxide (DMSO) (0.5 mL). The reaction was stirred for 30 minutes at room temperature, followed by the addition of 5-fluorothiophene-2-amidoxime (18 mg, 0.11, 1.0 eq). The reaction was stirred at room temperature overnight, followed by the addition of sodium hydroxide (NaOH) (6 mg, 0.15 mmol, 1.2 eq), and was stirred for additional 2 hours at room temperature. The reaction was diluted with ice-cold water and extracted with DCM (2x). The combined organics were washed with water, brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 12 mg (27%)

of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, J = 8.3, 1.8 Hz, 1H), 7.55 (m, 1H), 7.42 (t, J = 4.0 Hz, 1H), 7.06 (m, 2H), 6.56 (dd, J = 4.2, 1.6 Hz, 1H), 4.43 (m, 4H), 4.21 (q, J = 7. 0 Hz, 2H), 4.01 (m, 1H), 1.51 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 168.1, 164.2, 156.7, 135.0, 131.2, 127.1 (d, J(C,F) = 4.3 Hz), 125.9, 120.4, 116.5 (d, J(C,F) = 4.7 Hz), 113.5, 108.8 (d, J(C,F) = 11.2 Hz), 65.1, 54.4, 25.2, 14.7 ppm. LCMS R_T = 5.40 min; HRMS, calc'd for $C_{17}H_{17}FN_3O_4S_2^+$ [M+H], 410.0639; found 410.0645.

4.3.41. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(thiazol-2-yl)-1,2,4-oxadiazole (61).—The title compound was prepared in 65% yield (43 mg) from intermediate **39** and thiazole-2-amidoxime using a method analogous to that described for the conversion of compound **39** into **60**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, J = 3.2 Hz, 1H), 7.90 (dd, J = 8.3, 1.8 Hz, 1H), 7.6 (d, J = 3.1 Hz, 1H), 7.5 (m, 1H), 7.1 (m, 2H), 4.5 (m, 4H), 4.21 (q, J = 7.0 Hz, 2H), 4.10 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.2, 164.2, 156.7, 153.8, 145.2, 135.0, 131.1, 126.0, 122.8, 120.4, 113.6, 65.1, 54.3, 25.4, 14.8 ppm. LCMS R_T = 4.57 min; HRMS, calc'd for $C_{16}H_{17}N_4O_4S_2^+$ [M+H], 393.0686; found 393.0689.

4.3.42. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(isothiazol-4-yl)-1,2,4-oxadiazole (62).—The title compound was prepared in 39% yield (26 mg) from intermediate **39** and isothiazole-4-amidoxime using a method analogous to that described for the conversion of compound **39** into **60**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.26 (s, 1H), 9.00 (s, 1H), 7.91 (dd, J = 7.9, 1. 6 Hz, 1H), 7.55 (m, 1H), 7.06 (m, 2H), 4.46 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.06 (m, 1H), 1.51 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.7, 163.2, 156.7, 156.1, 149.0, 135.0, 131.1, 126.0, 126.0, 120.4, 113.6, 65.1, 54.4, 25.2, 14.7 ppm. LCMS R_T = 4.76 min; HRMS, calc'd for $C_{16}H_{17}N_4O_4S_2^+$ [M+H], 393.0686; found 393.0691.

4.3.43. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(furan-2-yl)-1,2,4-oxadiazole (63).—The title compound was prepared in 28% yield (18 mg) from intermediate **39** and furan-2-amidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, J = 8.3, 1.8 Hz, 1H), 7.62 (dd, J = 1.8, 0.8 Hz, 1H), 7.54 (m, 1H), 7.11 (dd, J = 3.5, 0.8 Hz, 1H), 7.05 (m, 2H), 6.58 (dd, J = 3.5, 1.8 Hz, 1H), 4.44 (m, 4H), 4.20 (q, J = 7. 0 Hz, 2H), 4.04 (m, 1H), 1.50 (t, J = 7.0 Hz, 3H). LCMS R_T = 4.82 min; ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 161.4, 156.7, 145.5, 141.9, 135.0, 131.1, 125.9, 120.4, 114.2, 113.5, 111.9, 65.1, 54.4, 25.3, 14.7 ppm. HRMS, calc'd for $C_{17}H_{18}N_3O_5S^+$ [M+H], 376.0962; found 376.0963.

4.3.44. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(pyridin-2-yl)-1,2,4-oxadiazole (64).—The title compound was prepared in 31% yield (20 mg) from intermediate **39** and pyridine-2-amidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (dq, J = 4.8, 0.9 Hz, 1H), 8.10 (dt, J = 7.9, 1.0 Hz, 1H), 7.96 – 7.82 (m, 2H), 7.58 – 7.39 (m, 2H), 7.05 (m, 2H), 4.48 (m, 4H), 4.26 – 4.04 (m, 3H), 1.49 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.9, 168.4, 156.7, 150.5, 145.9,

137.2, 135.0, 131.0, 126.0, 125.8, 123.3, 120.4,113.5, 65.1, 54.3, 25.5, 14.7 ppm. LCMS R_T = 4.45 min; HRMS, calc'd for $C_{18}H_{19}N_4O_4S^+$ [M+H], 387.1122; found 387.1124.

4.3.45. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(pyridin-3-yl)-1,2,4-oxadiazole (65).—The title compound was prepared in 24% yield (16 mg) from intermediate **39** and pyridine-3-amidoxime using a method analogous to that described for the conversion of compound **39** into **41**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.26 (s, 1H), 8.76 (d, J = 3.8 Hz, 1H), 8.32 (dt, J = 8.0, 1.9 Hz, 1H), 7.92 (m, 1H), 7.55 (m, 1H), 7.44 (m, 1H), 7.06 (m, 2H), 4.47 (m, 4H), 4.21 (q, J = 7.0 Hz, 2H), 4.08 (m, 1H), 1.51 (t, J = 7.0 Hz, 3H). LCMS R_T = 4.36 min; HRMS, calc'd for $C_{18}H_{19}N_4O_4S^+$ [M+H], 387.1122; found 387.1128.

4.3.46. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(pyridin-4-yl)-1,2,4-oxadiazole (66).—The title compound was prepared in 40% yield (26 mg) from intermediate **39** and pyridine-4-amidoxime using a method analogous to that described for the conversion of compound **39** into **60**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (d, J = 5.9 Hz, 2H), 7.96 – 7.84 (m, 3H), 7.55 (m, 1H), 7.06 (m, 2H), 4.47 (m, 4H), 4.20 (q, J = 7.0 Hz, 2H), 4.08 (m, 1H), 1.51 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.2, 167.1, 156.7, 150.8, 135.0, 133.8, 131.1, 126.0, 121.2, 120.5, 113.6, 65.1, 54.4, 25.3, 14.7 ppm. LCMS R_T = 4.29 min; HRMS, calc'd for $C_{18}H_{19}N_4O_4S^+$ [M+H], 387.1122; found 387.1129.

4.3.47. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(5-fluoropyridin-2-yl)-1,2,4-oxadiazole (67).—The title compound was prepared in 29% yield (20 mg) from intermediate **39** and 4-fluoropyridine-2-amidoxime using a method analogous to that described for the conversion of compound **39** into **60**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (d, J = 2.8 Hz, 1H), 8.14 (m, 1H), 7.90 (m, 1H), 7.63 – 7.47 (m, 2H), 7.05 (m, 2H), 4.48 (m, 4H), 4.28 – 4.03 (m, 3H), 1.50 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.1, 167.6, 160.4 (d, J(C,F) = 261.9 Hz), 156.7, 142.1 (d, J(C,F) = 4.2 Hz), 139.2 (d, J(C,F) = 24.8 Hz), 135.0, 131.0, 126.1, 124.7 (d, J(C,F) = 5.2 Hz), 123.9 (d, J(C,F) = 18.8 Hz), 120.4, 113.5, 65.1, 54.3, 25.5, 14.7 ppm. LCMS R_T = 4.69 min; HRMS, calc'd for $C_{18}H_{18}FN_4O_4S^+$ [M+H], 405.1027; found 405.1031.

4.3.48. 5-(1-((2-Ethoxyphenyl)sulfonyl)azetidin-3-yl)-3-(pyrimidin-2-yl)-1,2,4-oxadiazole (68).—The title compound was prepared in 29% yield (19 mg) from intermediate **39** and pyrimidine-2-amidoxime using a method analogous to that described for the conversion of compound **39** into **60**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.98 (d, J = 4.9 Hz, 1H), 7.89 (dd, J = 8.1, 1.8 Hz, 1H), 7.58 – 7.43 (m, 2H), 7.09 – 6.97 (m, 2H), 4.50 (m, 4H), 4.28 – 4.08 (m, 3H), 1.49 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.6, 167.9, 158.1, 156.6, 155.8, 135.0, 131.0, 126.0, 122.4, 120.4, 113.5, 65.1, 54.2, 25.7, 14.7 ppm. LCMS R_T = 4.04 min; HRMS, calc'd for $C_{17}H_{18}N_5O_4S^+$ [M+H], 388.1074; found 388.1077.

4.3.49. 5-(1-((2-(Ethoxy-*d*$_5$)phenyl)sulfonyl)azetidin-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (109).—*(Step 1)* 3-(4-Fluorophenyl)-5-(1-((2-

fluorophenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (**104**). The title compound was prepared in 81% yield (1.10 g) from intermediate **167** and 2-fluorobenzene-sulfonyl chloride, using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (m, 2H), 7.90 (m, 1H), 7.64 (m, 1H), 7.31 (m, 2H), 7.16 (m, 2H), 4.40 (m, 4H), 4.07 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.9, 164.7 (d, *J*(C,F) = 252 Hz), 159.4 (d, *J*(C,F) = 256 Hz), 135.7 (d, *J*(C,F) = 8.4 Hz), 131.1, 129.6 (d, *J*(C,F) = 8.8 Hz), 124.6 (d, *J*(C,F) = 3.8 Hz), 123.5 (d, *J*(C,F) = 153.8 Hz), 123.4 (d, *J*(C,F) = 135.9 Hz), 117.5 (d, *J*(C,F) = 21.8 Hz), 116.1 (d, *J*(C,F) = 22.1 Hz), 54.4 (d, *J*(C,F) = 2.1 Hz), 25.2 ppm. *(Step 2)* 5-(1-((2-(Allyloxy)phenyl)sulfonyl)azetidin-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (**105**). Intermediate **104** (1.10 g, 2.91 mmol, 1.0 eq), Cs_2CO_3 (2.84 g, 8.73 mmol, 3.0 eq), and allyl alcohol (10 mL) were heated at 70 °C overnight. The reaction was cooled to room temperature, water was added, and the reaction was extracted with DCM (2x). The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 630 mg (52%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (m, 2H), 7.93 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.54 (m, 1H), 7.17 (m, 2H), 7.12 – 7.00 (m, 2H), 6.08 (m, 1H), 5.54 (m, 1H), 5.32 (m, 1H), 4.69 (dt, *J* = 5.2, 1.5 Hz, 2H), 4.44 (m, 4H), 4.03 (m, 1H). LCMS R_T = 5.44 min; HRMS, calc'd for $C_{20}H_{19}FN_3O_4S^+$ [M+H], 416.1075; found 416.1081. *(Step 3)* 2-((3-(3-(4-Fluorophenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-yl)sulfonyl)phenol (**106**). Tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) (70 mg, 0.06 mmol, 0.01 eq) was added to a solution of intermediate **105** (630 mg, 1.52 mmol, 1.0 eq) and K_2CO_3 (840 mg, 6.08 mmol, 4.0 eq) in methanol (5.0 mL), and stirred overnight at room temperature. The reaction was concentrated *in vacuo*, followed by the addition of water, and extraction with DCM (2x). The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 435 mg (77%) of the title compound as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (m, 2H), 7.67 (dd, *J* = 9. 0, 1.5 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.23 – 6.96 (m, 4H), 4.26 (m, 4H), 3.98 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.5, 167.7, 164.7 (d, *J*(C,F) = 252.2 Hz), 136.3, 129.7 (d, *J*(C,F) = 8.8 Hz), 129.6, 122.4 (d, *J*(C,F) = 3.2 Hz), 120.1, 119.5, 117.0, 116.1 (d, *J*(C,F) = 22.1 Hz), 54.3, 25.3 ppm. LCMS R_T = 4.98 min; HRMS, calc'd for $C_{17}H_{15}FN_3O_4S^+$ [M+H], 376.0762; found 376.0769. *(Step 4)* Ethyl-d_5 4-methylbenzenesulfonate (**107**). Ethanol-d_6 (534 mg, 10.5 mmol, 1.0 eq), and triethylamine (2.86 mL, 21.0 mmol, 2.0 eq) were dissolved in DCM (15 mL), followed by the addition of *p*-toluenesulfonyl chloride (1.80 g, 9.45 mmol, 0.9 eq), and DMAP (12 mg, 0.10 mmol, 0.01 eq). The reaction was stirred at room temperature overnight. Water was added and the reaction was extracted with DCM (2x). The combined organics were washed with 1N HCl, saturated $NaHCO_3$, and brine; dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford 1.60 g (83%) of the title compound that was used further without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (m, 2H), 7.35 (m, 2H), 2.45 (s, 3H). *(Step 5)* 5-(1-((2-(Ethoxy-d_5)phenyl)sulfonyl)azetidin-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (**109**). Intermediate **106** (30 mg, 0.08 mmol, 1.0 eq), intermediate **107** (33 mg, 0.16 mol, 2.0 eq), Cs_2CO_3 (78 mg, 0.24 mmol, 3.0 eq), and DMF (0.5 mL) were added to a microwave vial and heated in a microwave reactor at 120 °C for 20 minutes. Water was added, and the reaction was extracted with DCM (2x). The combined organics were washed with

brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 23 mg (70%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (m, 2H), 7.91 (dd, J = 7.8, 1.8 Hz, 1H), 7.54 (m, 1H), 7.17 (m, 2H), 7.09 – 7.00 (m, 2H), 4.45 (m, 4H), 4.05 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.4, 167.8, 164.7 (d, J(C,F) = 252.0 Hz), 156.7, 135.0, 131.1, 129.6 (d, J(C,F) = 8.7 Hz), 125.9, 122.6 (d, J(C,F) = 3.3 Hz), 122.4, 116.1 (d, J(C,F) = 22.1 Hz), 113.5, 54.5, 25.3 ppm. LCMS R_T = 5.35 min; HRMS, calc'd for $C_{19}H_{14}D_5FN_3O_4S^+$ [M+H], 409.1389; found 409.1400.

4.3.50. 5-(1-((2-(Cyclobutylmethoxy)phenyl)sulfonyl)azetidin-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (110).—The title compound was prepared in 56% yield (12 mg) from intermediate **106** and cyclobutane methanol using a method analogous to that described for the conversion of compound **106** into **109**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (m, 2H), 7.91 (m, 1H), 7.54 (m, 1H), 7.17 (m, 2H), 7.05 (m, 2H), 4.48 – 4.36 (m, 4H), 4.08 (d, J = 6.8 Hz, 2H), 4.02 (m, 1H), 2.86 (m, 1H), 2.23 – 2.09 (m, 2H), 2.04 – 1.83 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.3, 165.4 (d, J(C,F) = 356.4 Hz), 157.1, 133.2 (d, J(C,F) = 279 Hz), 130 (d, J(C,F) = 8.8 Hz), 125.5, 122.6 (d, J(C,F) = 3.1 Hz), 120.3, 116.3, 116.0, 113.7, 73.6, 54.2, 34.4, 25.3, 25.0, 18.5 ppm. LCMS R_T = 5.87 min; HRMS, calc'd for $C_{22}H_{23}FN_3O_4S^+$ [M+H], 444.1388; found 444.1392.

4.3.51. 5-(1-((2-(2,2-Difluoroethoxy)phenyl)sulfonyl)azetidin-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (112).—The title compound was prepared in 52% yield (11 mg) from intermediate **104** and difluoroethanol using a method analogous to that described for the conversion of compound **104** into **105**. The title compound was prepared in 35% yield (8 mg) from intermediate **104** and trifluoroethanol. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (m, 2H), 7.96 (dd, J = 7.9 Hz, 1H), 7.61 (m, 1H), 7.27 – 7.07 (m, 4H), 4.59 – 4.36 (m, 6H), 4.04 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.1, 167.8, 164.7 (d, J(C,F) = 251.8 Hz), 155.0, 133.2 (d, J(C,F) = 285.3 Hz), 129.7 (d, J(C,F) = 8.8 Hz), 128.0, 124.8, 123.2, 122.6 (d, J(C,F) = 3.3 Hz), 121.1, 116.1 (d, J(C,F) = 22.1 Hz), 115.8, 67.8 (q, J(C,F) = 36.2 Hz), 54.3, 25.2 ppm. LCMS R_T = 5.43 min; HRMS, calc'd for $C_{19}H_{16}F_4N_3O_4S^+$ [M+H], 458.0792; found 458.0795.

4.3.52. 3-(4-Fluorophenyl)-5-(1-((2-(2,2,2-trifluoroethoxy)phenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (113).—The title compound was prepared in 35% yield (8 mg) from intermediate **104** and trifluoroethanol using a method analogous to that described for the conversion of compound **104** into **105**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (m, 2H), 7.96 (dd, J = 7.9 Hz, 1H), 7.61 (m, 1H), 7.27 – 7.07 (m, 4H), 4.59 – 4.36 (m, 6H), 4.04 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.1, 167.8, 164.7 (d, J(C,F) = 251.8 Hz), 155.0, 133.2 (d, J(C,F) = 285.3 Hz), 129.7 (d, J(C,F) = 8.8 Hz), 128.0, 124.8, 123.2, 122.6 (d, J(C,F) = 3.3 Hz), 121.1, 116.1 (d, J(C,F) = 22.1 Hz), 115.8, 67.8 (q, J(C,F) = 36.2 Hz), 54.3, 25.2 ppm. LCMS R_T = 5.43 min; HRMS, calc'd for $C_{19}H_{16}F_4N_3O_4S^+$ [M+H], 458.0792; found 458.0795.

4.3.53. 5-(1-((2-(Cyclopropylmethoxy)phenyl)sulfonyl)azetidin-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (114).—The title compound was prepared in 28% yield (6 mg) from intermediate **104** and cyclopropane methanol using a method analogous

to that described for the conversion of compound **104** into **105**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (m, 2H), 7.92 (dd, J = 7.9, 1.7 Hz, 1H), 7.52 (m, 1H), 7.17 (m, 2H), 7.10 – 6.95 (m, 2H), 4.52 (d, J = 7.7 Hz, 4H), 4.06 (m, 1H), 3.95 (d, J = 7.0 Hz, 2H), 1.35 (m, 1H), 0.66 (m, 2H), 0.39 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.4, 167.8, 164.7 (d, J(C,F) = 251.9 Hz), 156.7, 133.0 (d, J(C,F) = 288.7 Hz), 129.6 (d, J(C,F) = 8.7 Hz), 126.3, 122.6 (d, J(C,F) = 3.3 Hz), 120.4, 116.3, 116.0, 113.7, 74.4, 54.5, 25.3, 10.1, 3.6 ppm. LCMS R_T = 5.59 min; HRMS, calc'd for $C_{21}H_{21}FN_3O_4S^+$ [M+H], 430.1231; found 430.1238.

4.3.54. 3-(4-Fluorophenyl)-5-(1-((2-(oxetan-3-yloxy)phenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (115).—The title compound was prepared in 47% yield (10 mg) from intermediate **104** and oxetan-3-ol using a method analogous to that described for the conversion of compound **104** into **105**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (m, 2H), 7.96 (dd, J = 7.9, 1.7 Hz, 1H), 7.53 (m, 1H), 7.22 – 7.09 (m, 3H), 6.60 (dd, J = 8.3, 0.7 Hz, 1H), 5.33 (m, 1H), 5.01 (m, 2H), 4.85 (m, 2H), 4.56 – 4.42 (m, 4H), 4.09 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 167.8, 164.7 (d, J(C,F) = 252.0 Hz), 154.4, 133.4 (d, J(C,F) = 253.4 Hz), 129.7 (d, J(C,F) = 8.7 Hz), 126.4, 122.5 (d, J(C,F) = 3.3 Hz), 121.6, 116.3, 116.0, 113.5, 71.7, 54.5, 25.3 ppm. LCMS R_T = 4.96 min; HRMS, calc'd for $C_{20}H_{19}FN_3O_5S^+$ [M+H], 432.1024; found 432.1027.

4.3.55. 5-(1-((2-(cyclopentyloxy)phenyl)sulfonyl)azetidin-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (116).—The title compound was prepared in 34% yield (7 mg) from intermediate **104** and cyclopentanol using a method analogous to that described for the conversion of compound **104** into **105**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (m, 2H), 7.90 (dd, J = 7.8, 1.7 Hz, 1H), 7.52 (m, 1H), 7.17 (m, 2H), 7.08 – 6.97 (m, 2H), 4.93 (m, 1H), 4.48 – 4.35 (m, 4H), 4.02 (m, 1H), 2.00 – 1.76 (m, 6H), 1.71 – 1.55 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.3, 167.7, 164.7 (d, J(C,F) = 252 Hz), 156.1, 133.1 (d, J(C,F) = 251 Hz), 129.6 (d, J(C,F) = 8.8 Hz), 125.9, 122.6 (d, J(C,F) = 3.2 Hz), 119.9, 116.3, 116.0, 114.6, 81.1, 54.3, 32.8, 25.3, 24.0 ppm. LCMS R_T = 5.81 min; HRMS, calc'd for $C_{22}H_{23}FN_3O_4S^+$ [M+H], 444.1388; found 444.1400.

4.3.56. 3-(4-Fluorophenyl)-5-(1-((2-(pyrrolidin-1-yl)phenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (117).—NaH (26 mg, 1.1 mmol, 10.0 eq) was added to pyrrolidine (0.5 mL) at 0 °C. Intermediate **104** (40 mg, 0.11 mmol, 1.0 eq) was added afterwards, and the reaction was warmed to room temperature, then heated at 80 °C overnight. The reaction was cooled to room temperature and concentrated *in vacuo*. Water was added, and the reaction was extracted with DCM (2x). The combined organics were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel afforded 7 mg (16%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (m, 2H), 7.98 (d, J = 8.07, 1.7 Hz, 1H), 7.45 (m, 1H), 7.23 – 7.12 (m, 3H), 7.01 (m, 1H), 4.36 (m, 4H), 4.03 (m, 1H), 3.38 (m, 4H), 1.94 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.5, 167.8, 164.7 (d, J(C,F) = 252.0 Hz), 150.0, 133.9, 131.5, 129.7 (d, J(C,F) = 8.8 Hz), 128.6, 122.7 (d, J(C,F) = 3.3 Hz), 120.6 (d, J(C,F) = 5.1 Hz), 116.3, 116.0, 53.9, 53.5, 25.4, 25.1 ppm. LCMS R_T = 5.84 min; HRMS, calc'd for $C_{21}H_{22}FN_4O_3S^+$ [M+H], 429.1391; found 429.1397.

4.3.57. 3-(4-Fluorophenyl)-5-(1-((2-(piperidin-1-yl)phenyl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (118).—The title compound was prepared in 18% yield (9 mg) from intermediate **104** and piperidine using a method analogous to that described for the conversion of compound **104** into **117**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 – 7.95 (m, 3H), 7.56 (m, 1H), 7.37 (dd, J = 8.1, 1.1 Hz, 1H), 7.29 – 7.12 (m, 3H), 4.38 (m, 4H), 3.97 (m, 1H), 2.98 (m, 4H), 1.72 (m, 4H), 1.57 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.3, 167.0 (d, J(C,F) = 101 Hz), 154.6, 134.3, 132.2, 132.0, 129.6 (d, J(C,F) = 8.7 Hz), 124.4, 123.4, 122.6 (d, J(C,F) = 3.3 Hz), 116.3, 116.0, 55.8, 54.6, 26.1, 25.3, 24.0 ppm. LCMS R_T = 6.08 min; HRMS, calc'd for $C_{22}H_{24}FN_4O_3S^+$ [M+H], 443.1548; found 443.1554.

4.3.58. 5-(1-(chroman-8-ylsulfonyl)azetidin-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (119).—The title compound was prepared in 72% yield (32 mg) from 3-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-hydrochloride (prepared using a method analogous to that described for **10**) and chroman-8-sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (m, 2H), 7.72 (m, 1H), 7.26 (m, 1H), 7.17 (m, 2H), 6.94 (t, J = 7.7 Hz, 1H), 4.49 – 4.39 (m, 4H), 4.33 (t, J = 5.2 Hz, 2H), 4.06 (m, 1H), 2.83 (t, J = 6.5 Hz, 2H), 2.06 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.4, 167.7, 164.7 (d, J(C,F) = 252.1 Hz), 152.8, 135.4, 129.6 (d, J(C,F) = 8.8 Hz), 129.2, 124.6, 124.3, 122.6 (d, J(C,F) = 3.3 Hz), 119.7, 116.2 (d, J(C,F) = 22.1 Hz), 67.3, 54.5, 25.3, 24.8, 21.4 ppm. LCMS R_T = 5.33 min; HRMS, calc'd for $C_{20}H_{19}FN_3O_4S^+$ [M+H], 416.1075; found 416.1076.

4.3.59. 5-(1-(Benzofuran-7-ylsulfonyl)azetidin-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (120)—The title compound was prepared in 63% yield (28 mg) from 3-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-hydrochloride (prepared using a method analogous to that described for **10**) and benzofuran-7-sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 – 7.76 (m, 4H), 7.79 (d, J = 2.2 Hz, 1H), 7.42 (t, J = 7.8 Hz, 1H), 7.15 (m, 2H), 6.89 (d, J = 2.2 Hz, 1H), 4.40 (m, 4H), 3.97 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.9, 167.6, 164.7 (d, J(C,F) = 252 Hz), 150.5, 146.6, 129.9, 129.6 (d, J(C,F) = 8.8 Hz), 127.3, 126.3, 123.0, 122.4 (d, J(C,F) = 3.3 Hz), 119.4, 116.1 (d, J(C,F) = 22.1 Hz), 106.9, 54.4, 25.2 ppm. LCMS R_T = 5.23 min; HRMS, calc'd for $C_{19}H_{15}FN_3O_4S^+$ [M+H], 400.0762; found 400.0762.

4.3.60. 3-(4-Fluorophenyl)-5-(1-(quinolin-8-ylsulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (121).—The title compound was prepared in 57% yield (26 mg) from 3-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-hydrochloride (prepared using a method analogous to that described for **10**) and 8-quinolinesulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.05 (dd, J = 4.2, 1.8 Hz, 1H), 8.49 (dd, J = 7.4, 1.4 Hz, 1H), 8.23 (dd, J = 8.5, 1.8 Hz, 1H), 8.07 (dd, J = 8.2, 1.4 Hz, 1H), 7.95 (m, 2H), 7.66 (dd, J = 8.1, 7.5 Hz, 1H), 7.51 (q, J = 4.3 Hz, 1H), 7.15 (m, 2H), 4.71 (m, 4H), 4.04 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.5, 167.6, 164.6 (d, J(C,F) = 252 Hz), 151.4, 144.2, 136.7, 136.4, 133.8, 132.4, 129.6 (d, J(C,F) = 8.8 Hz), 129.1,

125.6, 122.6 (d, J(C,F) = 3.3 Hz), 122.2, 116.1 (d, J(C,F) = 22.1 Hz), 55.0, 25.4 ppm. LCMS R_T = 5.09 min; HRMS, calc'd for $C_{20}H_{16}FN_4O_3S^+$ [M+H], 411.0922; found 411.0927.

4.3.61. 3-(4-Fluorophenyl)-5-(1-((2-methylquinolin-8-yl)sulfonyl)azetidin-3-yl)-1,2,4-oxadiazole (122).—The title compound was prepared in 81% yield (38 mg) from 3-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)azetidin-1-hydrochloride (prepared using a method analogous to that described for **10**) and 2-methylquinoline-8-sulfonyl chloride using a method analogous to that described for the conversion of compound **10** into **5**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (dd, J = 7.4, 1.43 Hz, 1H), 8.08 (d, J = 8.5, 1H), 8.04 – 7.90 (m, 3H), 7.58 (t, J = 7.8 Hz, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.15 (m, 2H), 4.74 (m, 4H), 4.05 (m, 1H), 2.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.6, 167.6, 164.7 (d, J(C,F) = 252 Hz), 160.6, 143.8, 136.4, 136.3, 133.4, 132.1, 129.6 (d, J(C,F) = 8.8 Hz), 127.4, 124.6, 123.1, 122.6 (d, J(C,F) = 3.3 Hz), 116.1 (d, J(C,F) = 22.1 Hz), 54.9, 25.5, 25.4 ppm. LCMS R_T = 5.22 min; HRMS, calc'd for $C_{21}H_{18}FN_4O_3S^+$ [M+H], 425.1078; found 425.1074.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Data Availability

Data will be made available on request.

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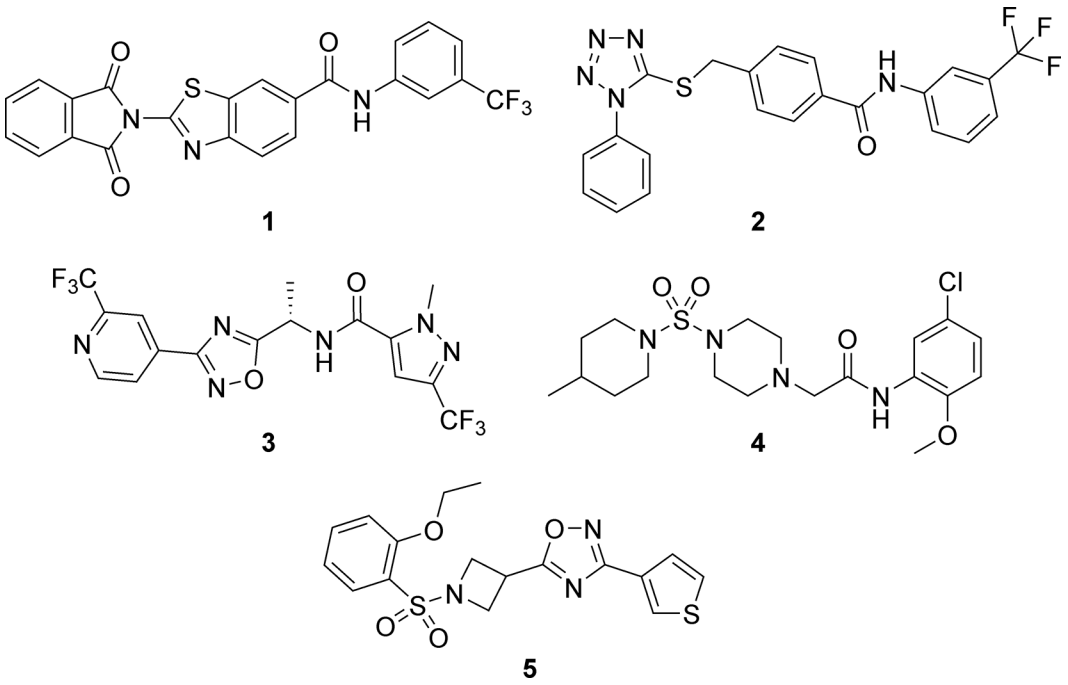


Figure 1.
Representative SLACK channel inhibitors

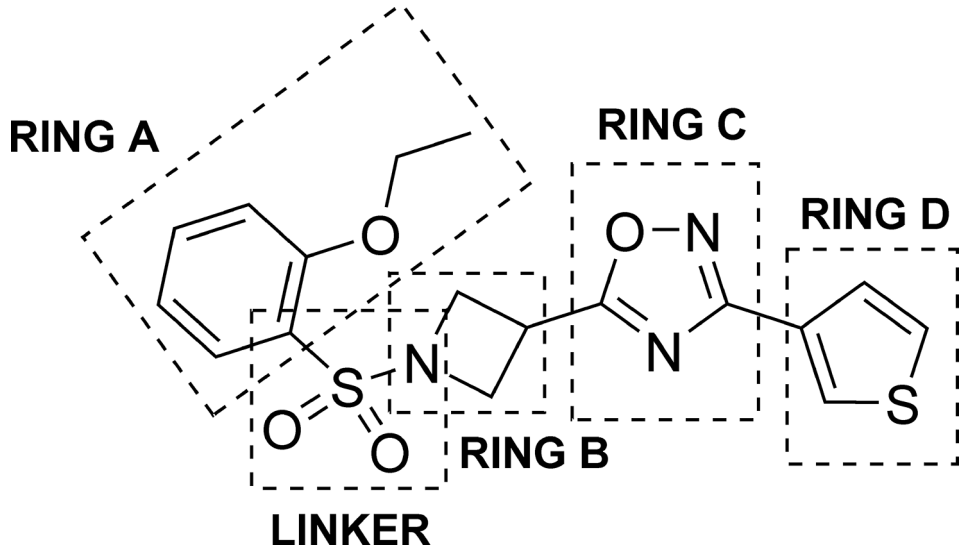
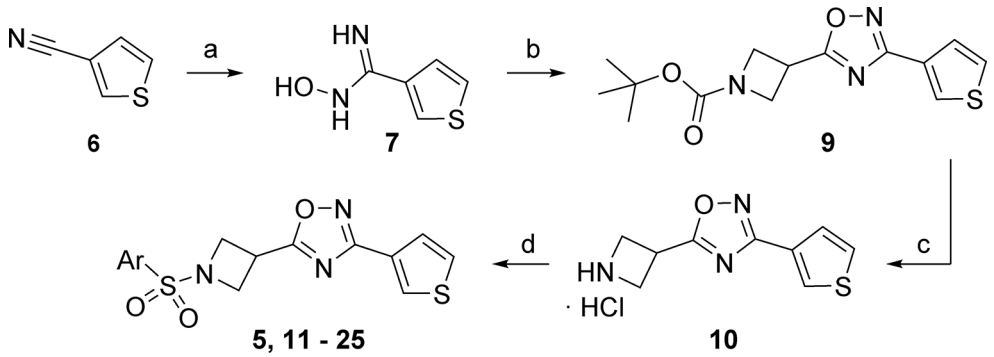
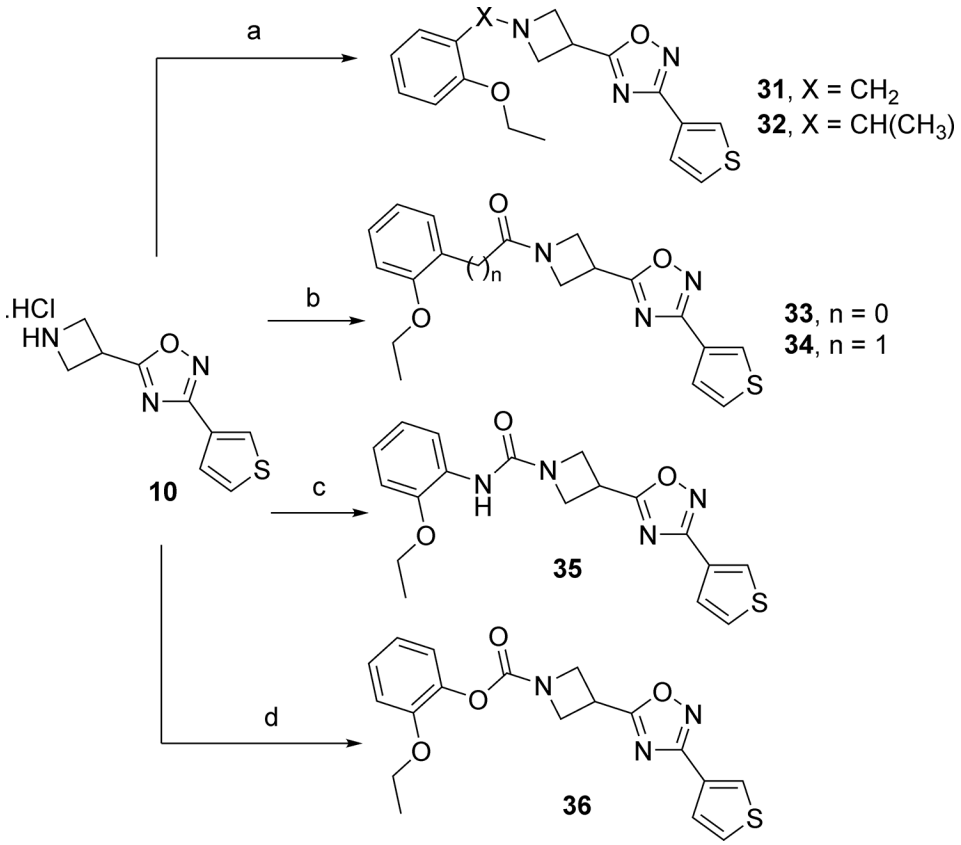


Figure 2.
Regions for SAR development in the VU0531245 scaffold.



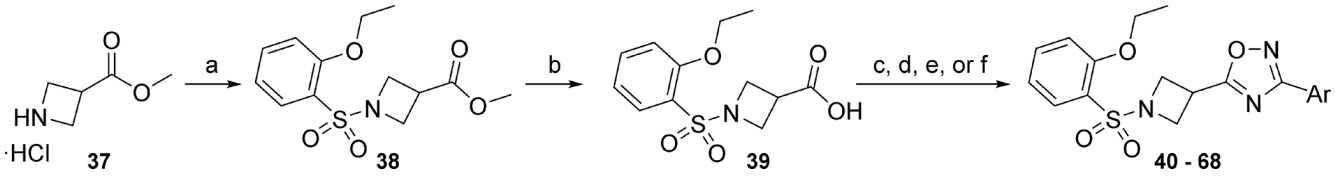
Scheme 1.

Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, $NaHCO_3$, MeOH, 60 °C, *100%*; (b) 1-(*tert*-butoxycarbonyl)azetidine-3-carboxylic acid (**8**), HATU, DIEA, DCM, NMP, 140 °C, *55%*; (c) 4M HCl in 1,4-dioxane, *99%*; (d) $ArSO_2Cl$, NEt_3, DCM, *17 – 79%*.



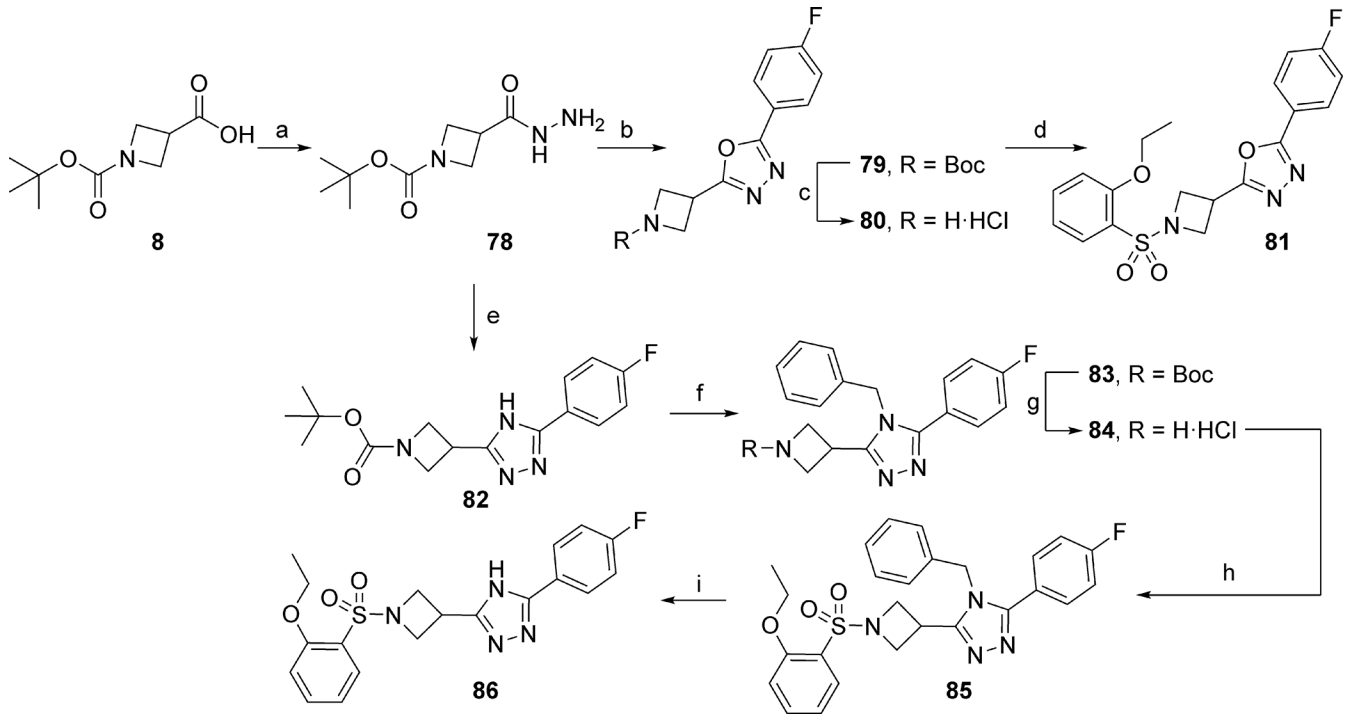
Scheme 2.

Reagents and conditions: (a) DIEA, AcOH, $NaBH(AcO)_3$, DCM, 2-ethoxybenzaldehyde (**31**, X = CH_2, *44%*), 2-ethoxyacetophenone, (**32**, X = $CHCH_3$, *52%*); (b) DIEA, HATU, DCM, 2-ethoxybenzoic acid (**33**, n = 0, *16%*), 2-ethoxyphenyl acetic acid (**34**, n = 1, *6%*); (c) 2-ethoxyphenyl isocyanate, DIEA, DCM, *13%*; (d) $ClCOOC_6H_4$-4-(NO_2), 2-ethoxyphenol, KOH, PhMe, reflux, *20%*.



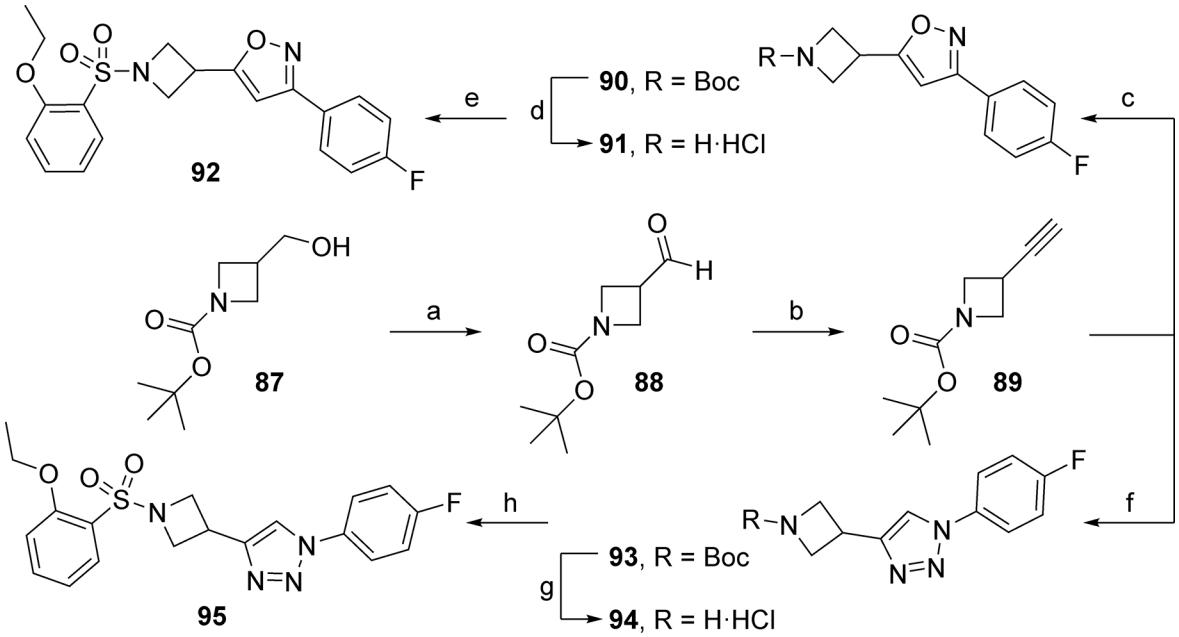
Scheme 3.

Reagents and conditions: (a) 2-ethoxybenzene sulfonyl chloride, NEt_3, DCM, *88%*; (b) LiOH, H_2O, THF, *95%*; (c) **40**, **59** ArC(NH)NHOH, HATU, DIEA, DMF, 140 °C, *6 - 40%*; (d) **41** – **49**, **53** – **58**, **63 – 65** ArC(NH)NHOH, EDC, HOBt, DIEA, 1,4-dioxane, reflux, *5 –24%,* (e) **50** – **52** ArC(NH)NHOH, CDI (2X), DMF, 80 °C, *39 – 43%* or (f) **60** – **62, 66 – 68** ArC(NH)NHOH, CDI, NaOH, DMSO, *27 – 65%*.



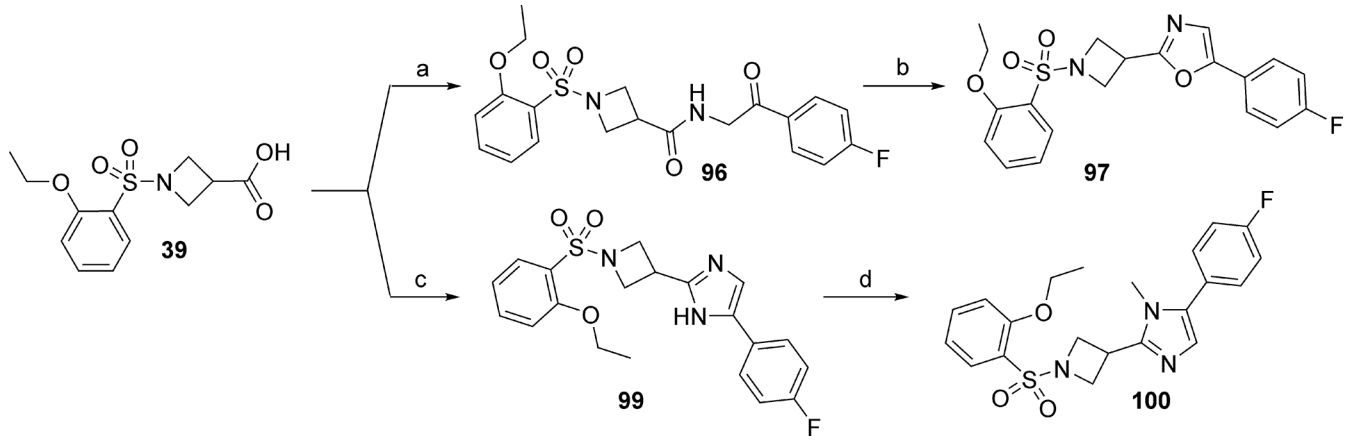
Scheme 4.

Reagents and conditions: (a) CDI, THF, $N_2H_4{\cdot}H_2O$, *87%*; (b) 4-fluorobenzoic acid, HATU, DIEA, Burgess reagent, THF, *82%*; (c) 4M HCl in 1,4-dioxane, *93%*; (d) 2-ethoxybenzene sulfonyl chloride, NEt_3, DCM, *14%*, (e) 4-fluorobenzonitrile, K_2CO_3, *n*-BuOH, 160 °C, *31%*; (f) BnBr, K_2CO_3, DMF, *64%*; (g) 4M HCl in 1,4-dioxane, *100%*; (h) 2-ethoxybenzene sulfonyl chloride, DIEA, DCM, *54%*; (i) 20% $Pd(OH)_2$/C, MeOH, H-cube®, *25%*.



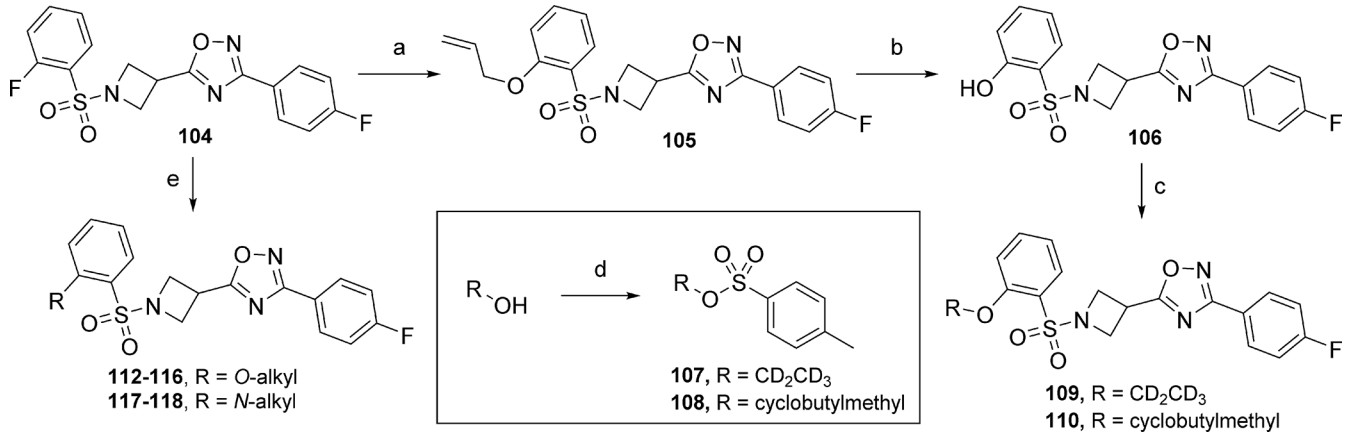
Scheme 5.

Reagents and conditions: (a) Pyridine-sulfur trioxide complex, NEt_3, DCM, DMSO, *60%*; (b) Bestmann-Ohira reagent, K_2CO_3, MeOH, *72%*; (c) 4-fluorobenzaldehyde oxime, PIFA, MeOH, H_2O, *52%*; (d) 4M HCl in 1,4-dioxane, *100%*; (e) 2-ethoxybenzene sulfonyl chloride, DIEA, DCM, *55%*; (f) 1-azido-4-fluorobenzene, sodium ascorbate, $CuSO_4$, *tert*-BuOH, H_2O, *56%*; (g) 4M HCl in 1,4-dioxane, *100%*; (h) 2-ethoxybenzene sulfonyl chloride, NEt_3, DCM, *31%*.



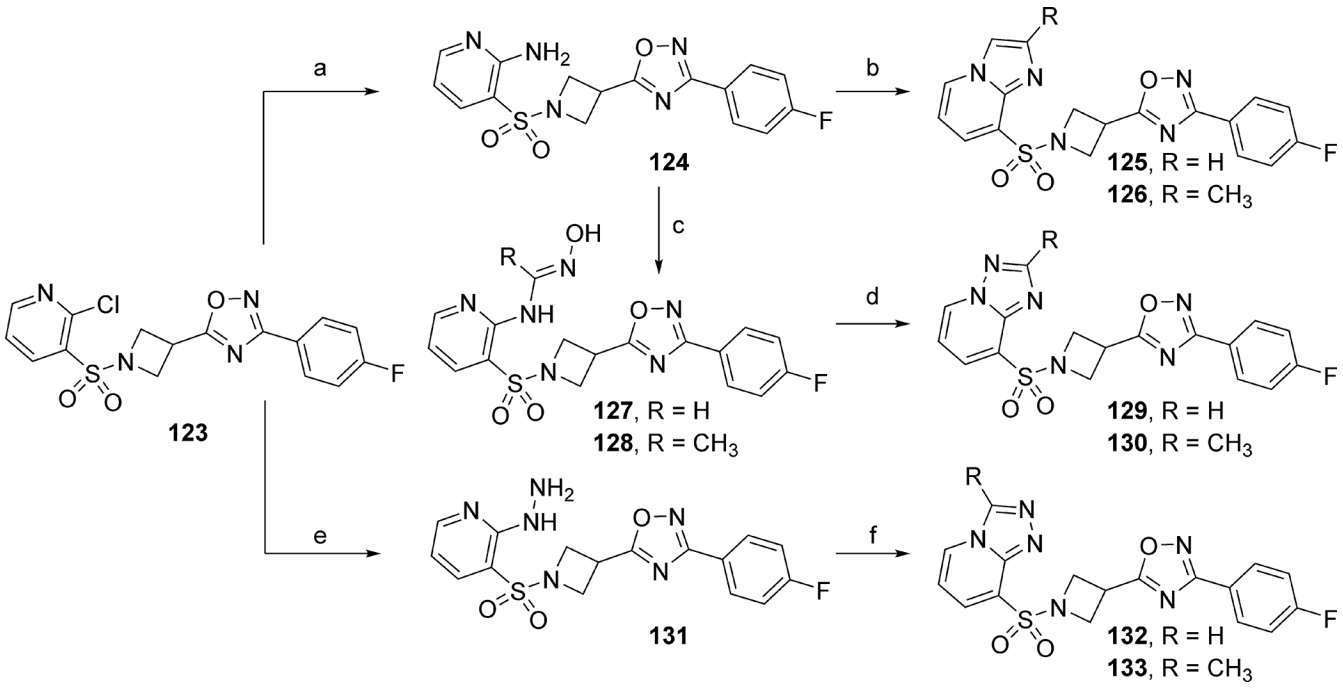
Scheme 6.

Reagents and conditions: (a) 2-amino-1-(4-fluorophenyl)ethenone hydrochloride, EDC, HOBt, DIEA, DCM, DMF, *6%*; (b) $POCl_3$, reflux, *25%*; (c) Cs_2CO_3, EtOH, 2-bromo-4'-fluoroacetophenone (**98**), DMF, NH_4OAc, PhMe, reflux, *63%*, (d) MeI, K_2CO_3, DMF, *78%*.



Scheme 7.

Reagents and conditions: (a) allyl alcohol, Cs_2CO_3, 70 °C, *47%*; (b) $Pd(PPh_3)_4$, K_2CO_3, MeOH, *77%*; (c) Cs_2CO_3, DMF, 120 °C, 20 min, μW, **107** (**109**, *70%*), **108** (**110**, *56%*); (d) TsCl, DMAP, NEt_3, DCM, (**107**, *70%*), (**108**, *75%*); (e) alkyl alcohol or alkyl amine, Cs_2CO_3, DMF, *16 – 52%*.

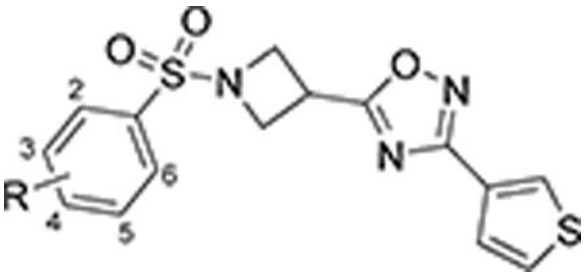


Scheme 8.

Reagents and conditions: (a) NH_4OH, 1,4-dioxane, 100 °C, *42%*; (b) R = H, $BrCH_2CH(OC_2H_5)_2$, *21%* or R = CH_3, $CH_3C(OCH_3)_2CH_2Br$, *12%*; (c) **127**: R = H, DMF.DMA, isopropanol, 80 °C, $NH_2OH{\cdot}HCl$, 50 °C; **128**: R = CH_3, DMA.DMA, isopropanol, 80 °C, $NH_2OH{\cdot}HCl$; (d) TFAA, THF; **129** (R = H), *22%*; **130** (R = CH_3), *11%*; (e) $N_2H_4.H_2O$, ethanol, μW, 120 °C, 15 min, *90%*; (f) **132**: R = H, $HC(OCH_3)_3$, μW, 180 °C, 20 min, *47%*; or **133**: R = CH_3, $CH_3C(OCH_3)_3$, μW, 180 °C, *59%*.

Table 1.

Ring A analogs



No.	R	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
5	2-OEt	Inh	2.1	102
11	2-F	Act	4.8	30
12	3-F	Act	>10	24
13	4-F		Inactive	
14	2-Cl	Act	7.5	47
15	3-Cl		Inactive	
16	4-Cl		Inactive	
17	2-CF_3	Act	6.0	36
18	3-CF_3	Act	6.2	61
19	4-CF_3		Inactive	
20	2- Me	Act	6.1	44
21	3- Me	Act	>10	<10
22	4- Me		Inactive	
23	2-OMe	Act	8.1	29
24	3-OMe		Inactive	
25	4-OMe	Act	3.2	51
26	2-O(*n*-Pr)	Inh	2.6	87
27	2-O(*i*-Pr)	Inh	3.7	103
28	2-O(*n*-Bu)		Inactive	
29	2-NHEt [d]		Inactive	
30	2-*n*-Pr [d]	Inh	>10	79

[a] Inh = inhibitor; Act = activator

[b] Concentration-response curve (CRC) from Tl^+ flux assay in HEK-293 cells expressing WT SLACK

[c] Amplitude of response in the presence of 30 μM test compound as a percentage of the maximum response for VU0606170 (inhibitors) or loxapine (activators)

[d] Synthesis described in Supplementary Material

Table 2.

Linker Library Analogs

No.	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
31	Act	>10	31
32	Act	>10	<10
33	Act	>10	18
34	Act	>10	37
35	Act	>10	<10
36	Act	>10	50

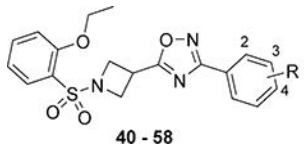
[a] Inh = inhibitor; Act = activator; NA = not active

[b] Concentration-response curve (CRC) from Tl^+ flux assay in HEK-293 cells expressing WT SLACK

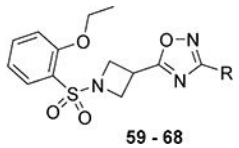
[c] Amplitude of response in the presence of 30 μM test compound as a percentage of the maximum response for VU0606170 (inhibitors) or loxapine (activators)

Table 3.

Ring D library compounds



40 - 58



59 - 68

No.	R	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
40	H	Inh	>10	51
41	2-F	Inh	7.9	88
42	3-F		Inactive	
43	4-F	Inh	3.2	105
44	2-Cl	Inh	8.0	81
45	3-Cl		Inactive	
46	4-Cl	Inh	11	94
47	2-CF_3	Act	1.9	66
48	3-CF_3	Inh	11	48
49	4-CF_3	Inh	3.8	95
50	2-Me	Act	>10	<10
51	3- Me	Inh	>10	<10
52	4- Me		Inactive	
53	2-OMe	Act	>10	18
54	3-OMe	Act	>10	<10
55	4-OMe	Act	7.1	21
56	2,4-difluoro	Inh	6.5	93
57	4-Cl,2-F	Inh	7.2	99
58	2-Cl,4-F	Inh	2.1	95
59	thiophen-2-yl	Inh	6.9	99
60	5-fluorothiophen-2-yl	Inh	4.8	105
61	2-thiazolyl		Inactive	
62	4-isothiazolyl	Inh	6.6	106
63	2-furanyl	Inh	>10	44
64	2-pyridyl		Inactive	
65	3-pyridyl		Inactive	
66	4-pyridyl		Inactive	
67	4-fluoro-2-pyridyl		Inactive	
68	2-pyrimidinyl		Inactive	

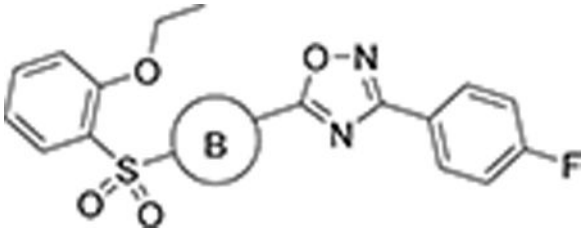
[a] Inh = inhibitor; Act = activator; NA = not active

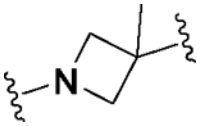
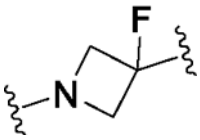
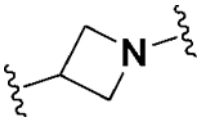
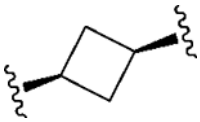
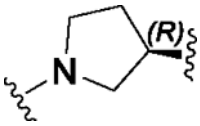
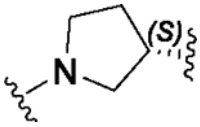
[b] Concentration-response curve (CRC) from Tl^+ flux assay in HEK-293 cells expressing WT SLACK

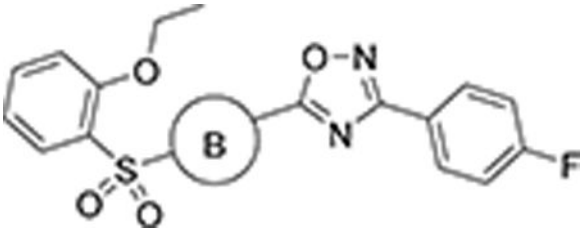
[c] Amplitude of response in the presence of 30 μM test compound as a percentage of the maximum response for VU0606170 (inhibitors) or loxapine (activators)

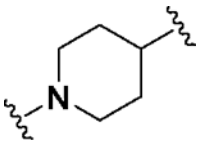
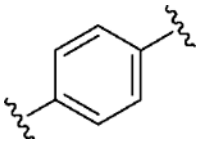
Table 4.

Ring B library compounds[d]



No.	B	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
69		Act	4.0	14
70		Inh	9.2	98
71		Inh	>10	24
73a			Inactive	
73b		Inh	>10	52
74		Inh	>10	31
75		Inh	>10	63



No.	B	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
76		Inh	4.9	105
77			Inactive	

[a] Inh = inhibitor; Act = activator; NA = not active

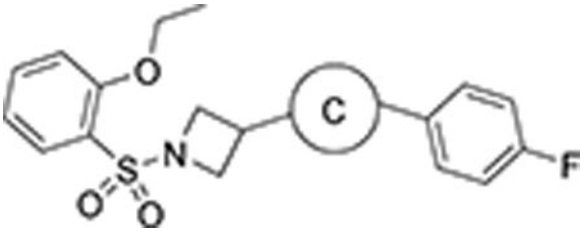
[b] Concentration-response curve (CRC) from Tl^+ flux assay in HEK-293 cells expressing WT SLACK

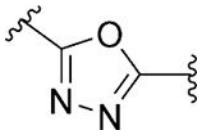
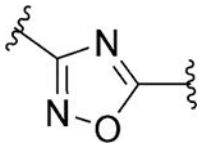
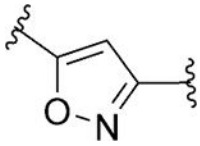
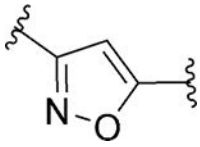
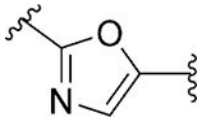
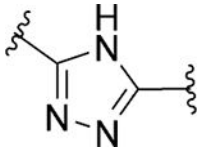
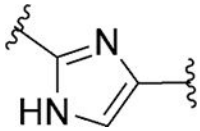
[c] Amplitude of response in the presence of 30 μM test compound as a percentage of the maximum response for VU0606170 (inhibitors) or loxapine (activators)

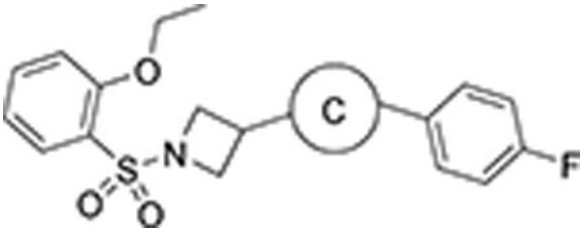
[d] Synthesis described in Supplementary Material

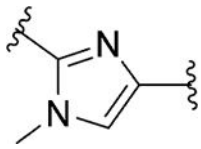
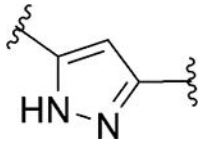
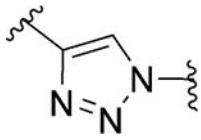
Table 5.

Ring C library compounds



No.	C	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
81		Act	>10	<10
101 [d]		Inh	>10	45
92			Inactive	
102 [d]			Inactive	
97		Act	10	34
86		Act	>10	37
99		Act	4.1	31



No.	C	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
100		Inh	>10	47
103 [d]		Act	>10	24
95		Act	>10	41

[a] Inh = inhibitor; Act = activator; NA = not active

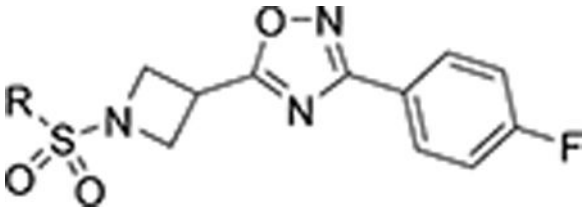
[b] Concentration-response curve (CRC) from Tl^+ flux assay in HEK-293 cells expressing WT SLACK

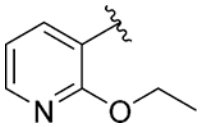
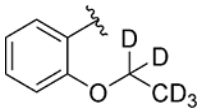
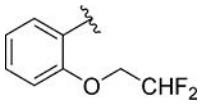
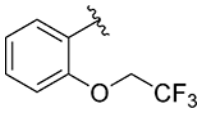
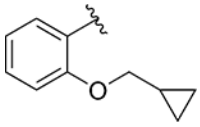
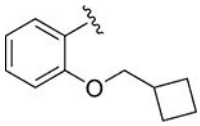
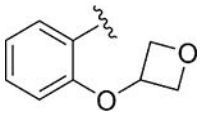
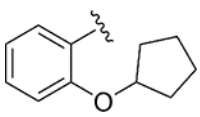
[c] Amplitude of response in the presence of 30 μM test compound as a percentage of the maximum response for VU0606170 (inhibitors) or loxapine (activators)

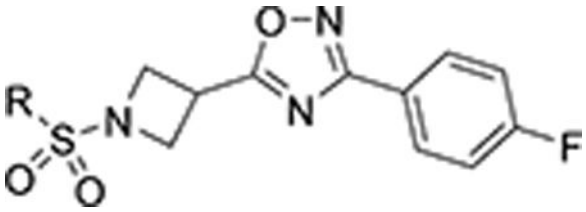
[d] Synthesis described in Supplementary Material

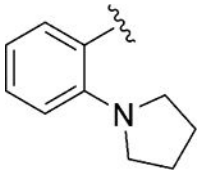
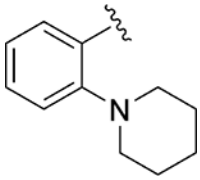
Table 6.

Ring A Further Optimization



No.	R	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
111 [d]		Inh	>10	46
109		Inh	5.4	100
112		Act		ND
113		Inh	8.8	91
114		Inh	6.0	95
110		Inh	9.3	88
115		Act	2.5	43
116		Inh	5.8	93



No.	R	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
117		Inh	8.9	92
118		Inh	>10	28

[a] Inh = inhibitor; Act = activator; NA = not active

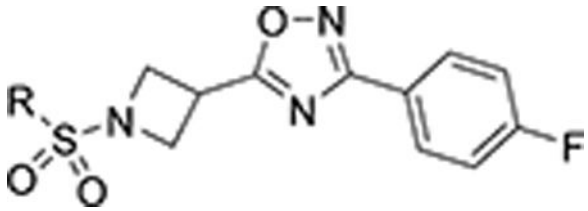
[b] Concentration-response curve (CRC) from Tl^+ flux assay in HEK-293 cells expressing WT SLACK

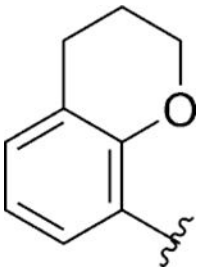
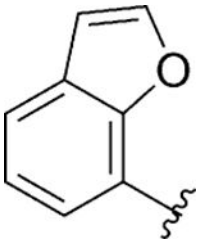
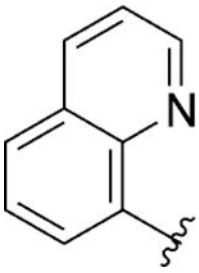
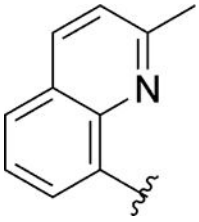
[c] Amplitude of response in the presence of 30 μM test compound as a percentage of the maximum response for VU0606170 (inhibitors) or loxapine (activators)

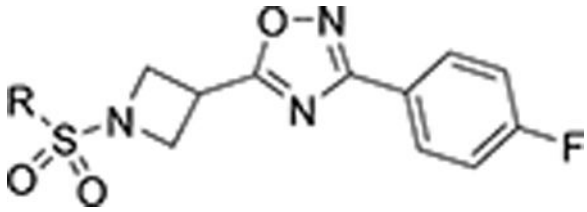
[d] Synthesis described in Supplementary Material

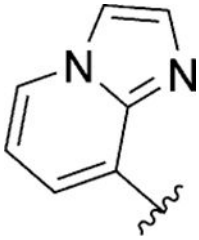
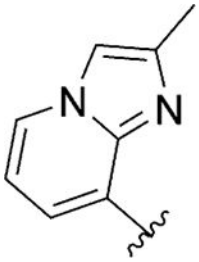
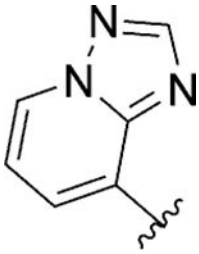
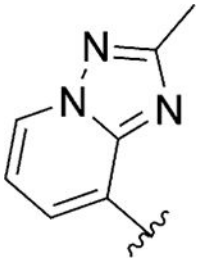
Table 7.

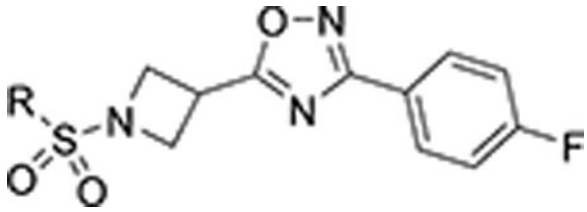
Ring A bicyclic analogs

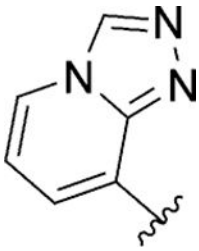
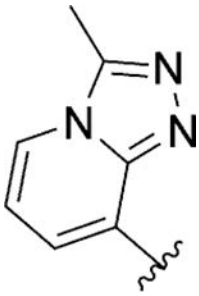


No.	R	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
119		Inh	8.7	93
120		Act	2.3	37
121		Inh	7.4	98
122		Inh	1.1	99



No.	R	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
125		Act	>10	48
126		Act	>10	19
129		Act	>10	16
130		Act	>10	16



No.	R	Mode[a]	IC_{50} / EC_{50} (μM)[b]	Efficacy (%)[b,c]
132		Act	>10	14
133			Inactive	

[a] Inh = inhibitor; Act = activator; NA = not active

[b] Concentration-response curve (CRC) from Tl^+ flux assay in HEK-293 cells expressing WT SLACK

[c] Amplitude of response in the presence of 30 μM test compound as a percentage of the maximum response for VU0606170 (inhibitors) or loxapine (activators)

Table 8.

Comparison of activity versus WT and A934T SLACK for selected inhibitors

No.	WT		A934T	
	IC_{50} (μM)[a]	Efficacy (%)[a,b]	IC_{50} (μM)[a]	Efficacy (%)[a,b]
5	2.1	102	0.82	72
27	3.7	103	1.1	109
43	3.2	105	1.0	110
58	2.1	95	2.0	95
60	4.8	105	0.97	103
76	4.9	105	4.3	129
109	5.4	100	2.3	83
113	8.8	91	2.5	72
119	8.7	93	2.6	104
121	7.4	98	1.5	112
122	1.1	99	0.67	72

[a] CRC from Tl^+ flux assay in HEK-293 cells expressing either WT or A934T SLACK

[b] Amplitude of response in the presence of 30 μM test compound as a percentage of the maximum response for VU0606170

Table 9.

Slo family selectivity for select SLACK inhibitors

No.	SLICK (Slo2.1)		Maxi-K (Slo1 α1/β3)	
	IC_{50} (μM)[a]	Efficacy (%)[a,b]	IC_{50} (μM)[a]	Efficacy (%)[a,b]
5	>10[c]	55	inactive	
27	>10[c]	47	inactive	
43	2.3	50	inactive	
58	>10[c]	44	inactive	
60	>10[c]	65	inactive	
76	>10[c]	35	inactive	
109	>10[c]	55	inactive	
113	>10[c]	27	inactive	
119	>10[c]	55	inactive	
121	5.9	56	inactive	
122	>10[c]	76	inactive	

[a] CRC from Tl^+ flux assay in HEK-293 cells expressing either SLICK (Slo2.1) or Maxi-K (Slo1 α1/β3)

[b] Amplitude of response in the presence of 30 μM test compound as a percentage of the maximum response for SKF96365 (SLICK) or paxilline (Maxi-K)

[c] CRC does not plateau

Table 10.

Whole-cell electrophysiology (EP) versus human WT SLACK for select inhibitors

No.	IC_{50} (μM)[a]	Efficacy (%)[b]
VU0606170	2.28	95
5	0.64	100
27	1.09	101
43	1.00	101
58	0.43	100
60	0.70	100
76	1.76	100
109	1.04	101
113	1.76	100
119	1.63	100
121	1.58	100
122	0.32	100

[a] CRC from whole-cell EP in CHO cells expressing WT SLACK utilizing an automated patch clamp system

[b] Amplitude of response in the presence of 30 μM test compound as a percentage of the maximum response for 60 μM bepridil.

Table 11.

In vitro DMPK data for select SLACK inhibitors

No.	cLogP[a]	% Remaining after 10 min[b]	Plasma-protein binding (f_u)[c]
5	2.32	<10	0.022
27	2.63	<10	<0.01
43	2.55	58	0.012
58	3.02	59	0.014
60	2.69	<10	0.024
76	3.52	21	<0.01
109	2.55	63	0.012
113	2.82	57	0.012
119	3.05	19	<0.01
121	2.34	71	0.014
122	2.84	26	<0.01

[a]Calculated using ChemDraw Professional. Version 16.0.

[b]Liver microsomal stability was reported as percent of parent compound remaining after 10 min incubation with NADPH-supplemented mouse liver microsomes.

[c]Determined from DMSO stock solution by equilibrium dialysis in mouse plasma.