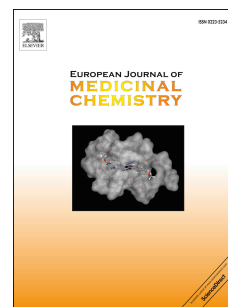


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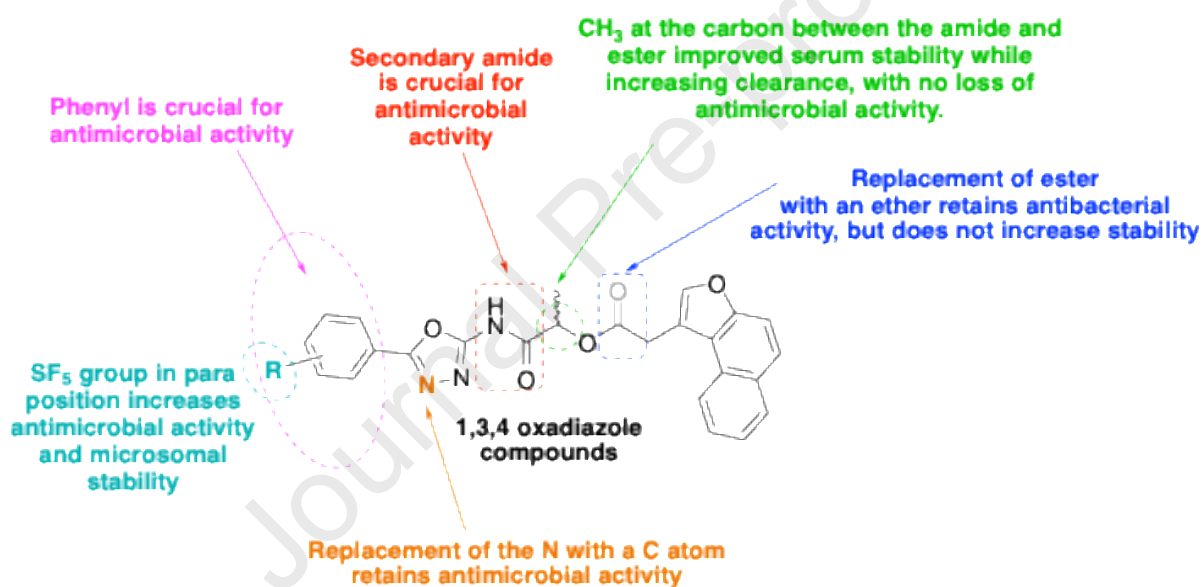
Structural optimization of novel 1,3,4 oxadiazole containing antibacterial compounds toward improved stability

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Abstract

A 1,3,4-oxadiazole-based compound named **1771** and its derivatives have been reported to exhibit antibacterial activity against Gram-positive bacteria, with activity extending to Gram-negative pathogens upon disruption of the outer membrane permeability barrier and/or efflux pump systems. However, the precise mode of action of these compounds remains unknown, and their utility is limited by low metabolic stability. Here, we describe the design and evaluation of analogues aimed at improving metabolic stability while defining the structural features required for antibacterial activity. Structure activity relationship analysis identify the secondary amide and the phenyl group as essential for antibacterial activity. Introduction of a *para*-pentafluorosulfanyl (SF₅) substituent yielded compound **9b**, which showed improved antibacterial potency and microsomal stability when compared to **1771**. Although **9b** remained suboptimal in microsomal stability, it provided the best overall balance of antibacterial activity and metabolic profile. Overall these results define key structural requirements for activity and provide a foundation for further optimization of this oxadiazole-based antibacterial scaffold, while providing key insights that may aid in identifying the compound's molecular target

1. Introduction

The WHO has identified antimicrobial resistance (AMR) as one of the gravest threats to global public health due to the significant mortality, morbidity and economic costs associated with multi-drug resistant (MDR) infections.¹ The global rise of AMR is a result of multiple, complex factors such as poor antimicrobial stewardship combined with constraints on the development of new antimicrobials and

alternative therapies, which are significant drivers in healthcare environments. As the antibiotic discovery pipeline has shrunk sharply in recent decades, while the multidrug resistance in ESKAPE pathogens has reached critical levels, there is an urgent need to develop new antibacterial agents.² To avoid cross-resistance in the fight against AMR, scientists are actively seeking new biological targets and novel antimicrobial agents.³ In 2013 Richter *et al.*⁴ conducted a high-throughput screen to identify antibacterial small molecules, identifying a 1,3,4-oxadiazole-based small molecule, named **1771** (Figure 1). This molecule was shown to inhibit *in vitro* growth of methicillin-resistant *Staphylococcus aureus* (MRSA), a bacterial opportunistic pathogen recognized as the second of the top six pathogens for deaths associated and attributed to AMR, and deemed by the WHO as a high-priority bacterium for the discovery of new antibiotics.^{5, 6} This compound has also been shown to have activity against vancomycin-resistant *Enterococcus faecium* (VRE) strains.⁷ Interestingly, in a *S. aureus* sepsis mouse model, compound **1771**, despite its significant instability in blood, demonstrated some *in vivo* efficacy, prolonging mice survival.⁴ Compound **1771** blocks the synthesis of lipoteichoic acid (LTA), essential membrane glycopolymers integral to the survival of exclusively Gram-positive bacteria (Figure 1).⁸ The authors suggested that compound **1771** binds and inhibits lipoteichoic acid synthase (LtaS), a key conditionally essential enzyme that catalyses the formation of the poly-glycerol phosphate backbone of LTA.⁴ This binding was supported by our and others' molecular modelling studies.^{9, 10}

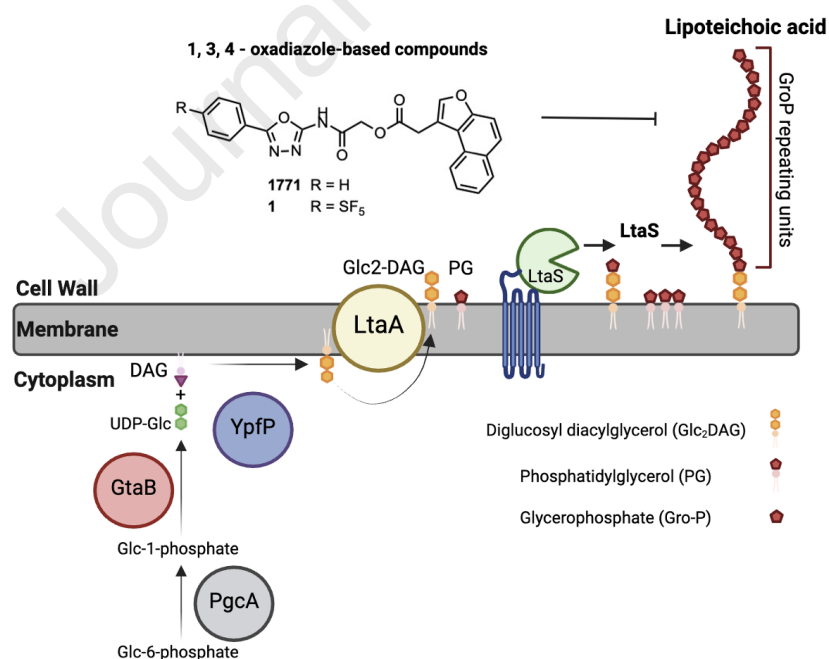


Figure 1. LTA biosynthesis in *S. aureus* begins with the synthesis of the diglucosyl-diacylglycerol (Glc₂-DAG) anchor. PgcA and GtaB are responsible for generating nucleotide-activated sugar UDP-glucose. The diacylglycerol β -glucosyltransferase YpfP then combines DAG with UDP-glucose to form the membrane anchor. The glycolipid permease LtaA subsequently transfers the Glc₂-DAG anchor from the inner to the outer leaflet of the membrane. The LtaS enzyme then hydrolyzes the glycerolphosphate

headgroup from the membrane lipid phosphatidyl glycerol (PG) and transfers between 15 and 50 of these moieties to the Glc₂-DAG anchor. LTA inhibiting compounds **1771** and **1** are included.

In search for more potent LtaS inhibitors, we have previously reported compound **1** (Figure 1), the pentafluorosulfanyl derivative of **1771**, identified among a small family of **1771** derivatives.¹¹ Compound **1** shows a 16-32-fold increase in antibacterial activity compared to **1771** when tested against a cohort of MDR *S. aureus* strains, together with low toxicity and resistance potential.¹¹ However, we found that neither deletion nor overexpression of LtaS altered susceptibility to **1771** or **1**, suggesting that LtaS is not the target of these molecules.¹¹ In addition, although the study by Ritcher *et al.*⁴ indicated that **1771** activity was due to LtaS inhibition, in a subsequent study, Vickery *et al.* developed an *in vitro* model of LTA polymerization employing artificial liposomes with reconstituted LtaS, and found that **1771** did not show LtaS inhibitory activity.¹² At the same time, other oxadiazole-based compounds were reported to reduce LTA in *S. aureus*, via direct binding to other enzymes involved in the synthesis of Glc₂-DAG and phosphatidylglycerol phosphate (PG-P).¹³ Further supporting the hypothesis that LtaS is not the target of **1771**, we found that the activity of both compounds (**1771** and **1**) is extended to Gram-negative species, if the outer membrane is first permeabilized, challenging their classification as strict LtaS inhibitors.¹¹

To date, many antibacterial oxadiazole derivatives have been reported, yet their primary targets often remain unknown. As a versatile scaffold, the oxadiazole core can support multiple substitution-dependent mechanisms of action that vary with bacterial species. Given that the whole-cell antibacterial activity of **1771** directly reflects its therapeutic potential and may point to a novel class of antibiotics with a distinct mode of action, we selected it as a lead compound for structural optimisation to improve stability and define the key structural requirements for antibacterial activity. The presence of an ester and amide moieties, both liable to esterase-mediated hydrolysis in serum, as shown in a previous study,⁹ makes neither **1771** nor **1** an appropriate drug candidate. In this study, new 2-amino-1,3,4-oxadiazole-based molecules were designed, synthesized, and assessed for their metabolic stability and for their antibacterial activity against a panel of MDR *S. aureus* strains.

1.1 Rational design

To obtain a candidate compound with improved metabolic stability while retaining or ideally enhancing its antibacterial activity, we synthesized derivatives in which the reference template **1771** was sequentially modified at the ester and amide moieties. First, we investigated the effect of modifying the ester with an ether functionality, then we examined the effect of replacing the amide

moiety with an amine, and finally, we determined the impact of both structural modifications (Figure 2).

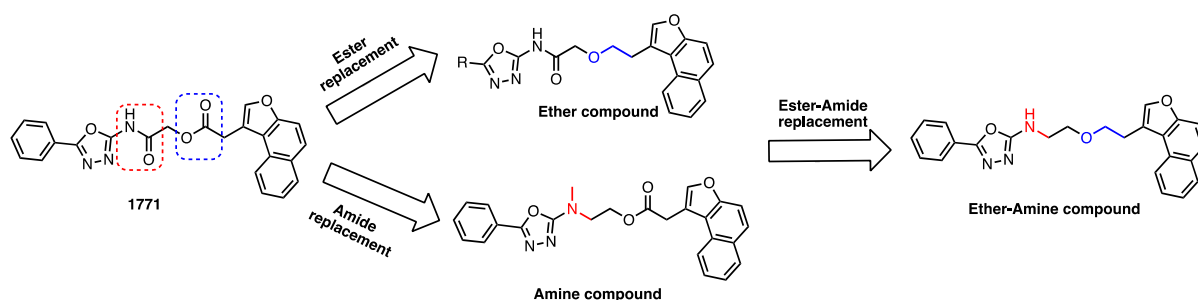


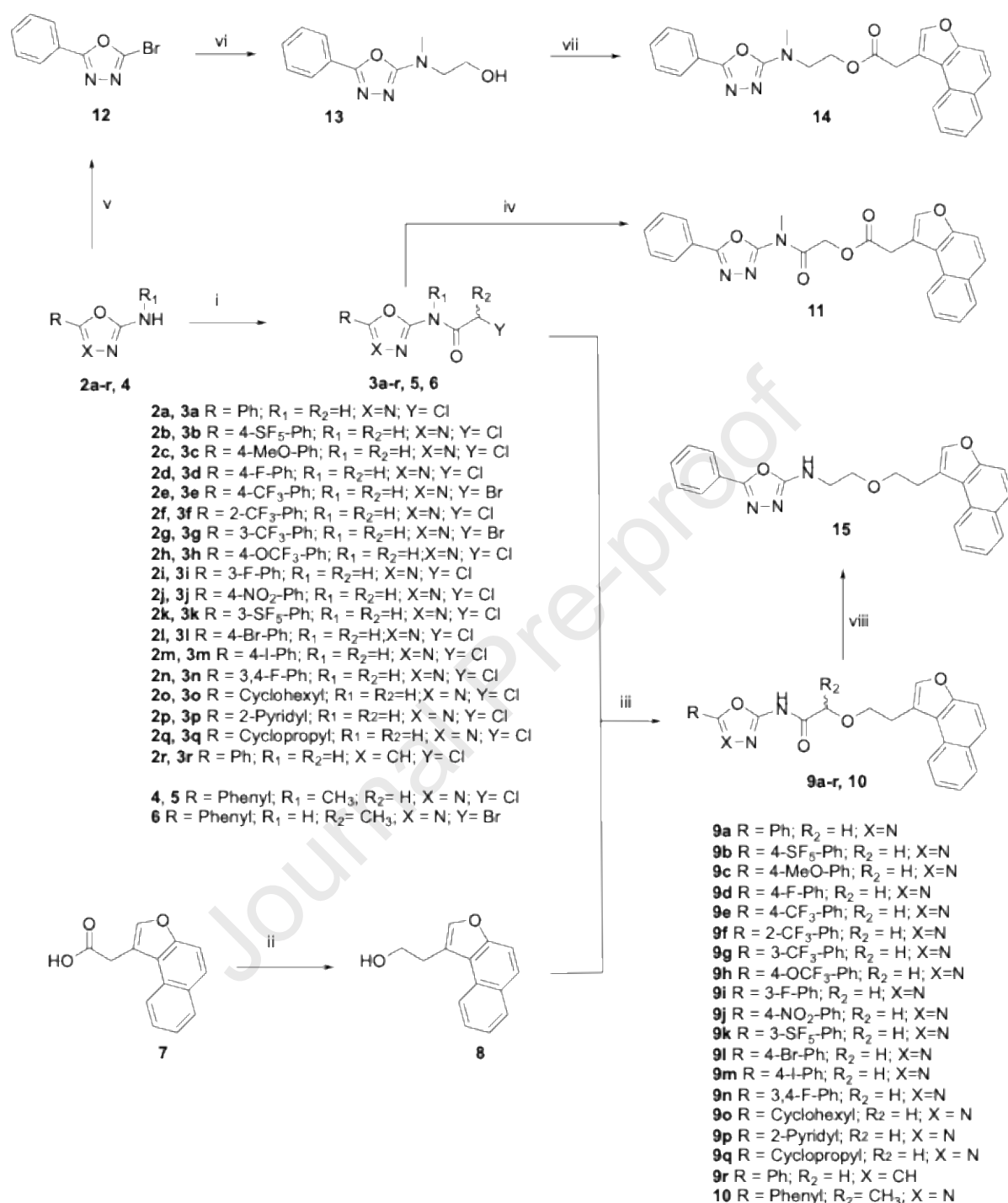
Figure 2. Rational drug design of the newly synthesized compounds.

2. Results

2.1 Chemistry

The ether compounds (**9a-r**, **10**) were synthesised as reported in scheme 1. Reaction of amino oxadiazole **2a-r**, either commercially available or prepared according to literature procedures¹⁴ (see scheme 1 SI), with either chloroacetyl chloride or bromoacetyl bromide afforded acetamides **3a-r**.¹⁵ 2-Bromopropionamide **6** was synthesized in 76% yield in a similar way, by reaction of the amino oxadiazole **2a** with 2-bromopropionyl chloride. Naphthofuran acid **7**, prepared as previously reported,⁹ was reduced to the corresponding naphthofuran alcohol **8** using borane-dimethyl sulphide complex. The final coupling reactions of chloroacetamides **3a-r** and **6** with naphthofuran alcohol **8** were achieved by modified Williamson etherification, using an excess of NaH and 1 equivalent of 15-crown-5 in anhydrous THF to afford compounds **9a-r** and **10**.⁹ To improve the stability of the amide moiety toward metabolic degradation, the *N*-methyl amide derivative **11** was synthesized. The required *N*-methyl-1,3,4-oxadiazole intermediate **4** was prepared following a reported protocol¹⁶ (see scheme 2 SI) and then reacted with 2-chloroacetyl chloride in toluene, affording compounds **5** in 87% yield.¹⁵ Coupling of **5** with **7** in the presence of triethylamine and a catalytic amount of sodium iodide in anhydrous dimethylformamide afforded the desired compound **11** in 79% yield.¹⁷

Replacing the amide moiety of compound **1771** with an amine functionality proved more difficult. Oxadiazole **2a** was converted to bromide **12** via a Sandmeyer reaction using *tert*-butyl nitrite and copper (II) bromide.¹⁸ Reaction of **12** with *N*-methyl aminoethanol afforded intermediate **13**, which was then esterified with naphthofuran acid **7** using HATU coupling reagent, affording the *N*-methyl amide derivative **14** in 57% yield. Compound **15**, bearing both structural modifications at the amide and ester functionality, was synthesised in 47% yield by lithium aluminium hydride reduction of **9a**.



Scheme 1. Reagents and Conditions: i) chloroacetyl chloride or bromoacetyl chloride (for **3a-r, 5**) or 2-bromopropionyl chloride (for **6**), toluene, 80 °C, 3-18 h (50-99%); ii) BH₃•Me₂S, THF, 0 °C – rt, 3 h (63%); iii) NaH, 15-crown-5, THF, rt, 18 h (10-69%); iv) **7**, NaI, Et₃N, DMF, 80 °C, 20 h (79%); v) ^tBuONO, CuBr₂, MeCN, reflux, 30 min. (91%); vi) *N*-methylaminoethanol, *N*(*i*Pr)₂Et, DMF, 80 °C, 20 min. (54%); vii) **4**, HATU, *N*(*i*Pr)₂Et, DMF, rt, 3 h (57%); viii) LiAlH₄, THF, rt 2h (47%).

2.2 Chemical and Metabolic Stability in Rat Serum and Rat and Human Liver Microsomes

All the synthesized final compounds **9a-r**, **10**, **11**, **14** and **15**, along with **1771** were tested for chemical and metabolic stability, respectively, in buffer and in rat serum, and their estimated half-lives were calculated. All compounds showed to be chemically robust in buffer at pH 7.4 at 37 °C without any significant degradation up to 10 hours.

Compound **1771** in rat serum showed comparable metabolic stability to that observed in mice serum in previous studies⁹ (Table 1, Entry 1). The metabolic profile was also consistent with previous finding,⁹ with three main metabolites observed in LC-MS due to the breakdown of the ester and amide moieties. To our surprise, the ether derivative **9a** showed low stability with only 14% remaining after 77 min when compared to 50% for **1771** (Table 1, Entry 2 vs 1; Figure 3). When looking at the substituent on the phenyl ring for the ether series of compounds, we found that it influences, to a certain extent the rate of degradation. In particular, SF₅, CF₃, NO₂ and F groups in the para position, as in **9b**, **9d**, **9e** and **9j**, appear to stabilise the molecule (Table 1, Entries 3,5,6 and 11), while OCH₃, OCF₃, Br and I groups in the same position as in **9c**, **9h**, **9l** and **9m**, have the opposite effect (Table 1, Entries 4, 9 and 13). Groups such as F, CF₃ and SF₅ in the meta position of the phenyl ring do not affect the stability, with compounds **9g**, **9i** and **9k** showing similar half-lives to **9a** (Table 1, Entries 8,10 and 12). If a CF₃ group in the ortho position of the phenyl reduced the stability (**9f** having half live <20 min), the 3,4-difluoro substitution as in compound **9n** provided instead a modest improvement (Table 1, Entries 7 vs 15). Replacement of the phenyl ring with a cyclohexane or cyclopropyl ring significantly decreased stability (Table 1, Entries 16 and 18), whereas substitution with a 2-pyridyl ring enhanced stability (Table 1, Entry 17). Compound **9r**, where the 1,3,4-oxadiazole ring is replaced by an oxazole ring, exhibits a significant decrease in stability (Table 1, Entry 19 vs 2).

A substantial increase in rat serum metabolic stability in the ether series was achieved with compound **10**, wherein a methyl group was added on the methylene carbon alpha to the carbonyl of the amide bond. Compound **10** showed a half-life nearly nine times longer than that of the unmethylated ether analogue **9a** (Table 1, Entry 20 vs 2, Figure 3), and 3 times longer when compared to **1771** (Table 1, Entry 20 vs 1, Figure 3).

Neither methylation nor replacement of the amide in **1771** with an *N*-methyl amine was tolerated, as the concentrations of compounds **11** and **14** decreased from 100% to 0% within 20 minutes (half-life < 20 min; Table 1, Entries 21 and 22).

When the metabolic profiles of **9a** and **14** were analysed by LC-MS, two metabolites were observed, formed by the breakdown of the amide moiety in **9a** and the ester moiety in **14** (Figures S1 and S2). The concurrent replacement of both amide and ester bonds of **1771** with an amine and an ether group instead produced the highly stable analogue **15** (Table 1, Entry 23, Figure 3).

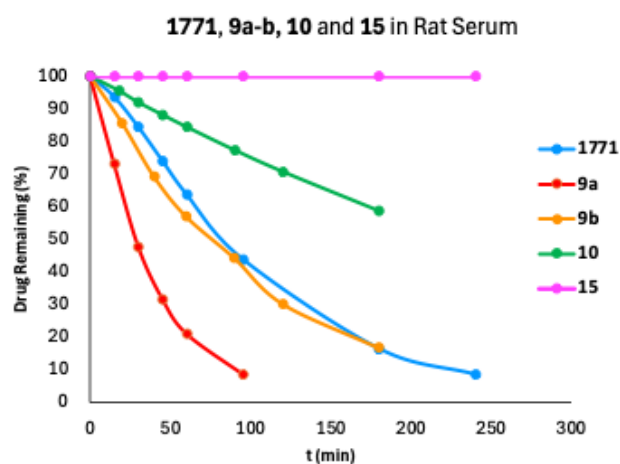
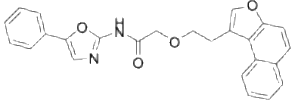
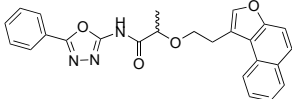
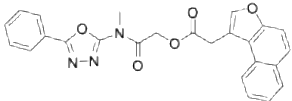
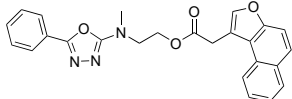
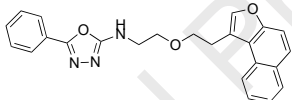


Figure 3. Metabolic stability of compound **1771**, and its derivatives **9a-b**, **10** and **15** in rat serum. Data are expressed as percent remaining relative to 0 min.

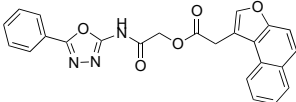
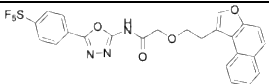
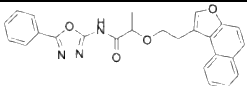
Table 1. Half-lives for **1771** and compounds **9a-r**, **10,11**, **14** and **15** in in rat serum (pH 7.3) at 37°C.

Entry	Cpd	Structure	R	Half-lives, $t_{1/2}$ (min)
1	1771		-	77
2	9a		Ph	27
3	9b		4-SF ₅ -Ph	69
4	9c		4-CH ₃ O-Ph	<20
5	9d		4-F-Ph	96
6	9e		4-CF ₃ Ph	52
7	9f		2-CF ₃ -Ph	<20
8	9g		3-CF ₃ -Ph	31
9	9h		4-CF ₃ O-Ph	24
10	9i		3-F	20
11	9j		4-NO ₂ -Ph	101
12	9k		3-SF ₅ -Ph	31
13	9l		4-Br-Ph	25
14	9m		4-I-Ph	17

15	9n		3,4-F-Ph	60
16	9o		cyclohexyl	<20
17	9p		2 pyridinyl	85
18	9q		cyclopropyl	16
19	9r		-	<10
20	10		-	236
21	11		-	<20
22	14		-	<20
23	15		-	>1440

The in vitro metabolism of **1771** and compounds **9b** and **10** was further evaluated by monitoring their stability over 2 h in rat and human liver microsomes (Table 2).¹⁹ All three compounds exhibited low metabolic stability in this assay. However, compound **9b** appeared to be slightly more stable than the others, with half-lives of 12.6 min and 15.7 min in human and rat liver microsomes, respectively. In comparison, compound **1771** showed half-lives of 4.32 min and 1.73 min, while compound **10** displayed the lowest microsomal stability, with half-lives of 1.70 min and 0.95 min in human and rat liver microsomes, respectively. Consistent with these short half-lives, all compounds exhibited relatively high intrinsic clearance, with **9b** showing the lowest clearance of the series and compounds **1771** and **10** being cleared more rapidly.

Table 2. Half-lives and intrinsic clearances for **1771** and compounds **9b** and **10** in rat and human microsomes.

Cpd	Structure	Rat Microsome		Human Microsome	
		$t_{1/2}^a$ (min)	CL_{int}^b ($\mu\text{L}/\text{min}/\text{mg}$ protein)	$t_{1/2}^a$ (min)	CL_{int}^b ($\mu\text{L}/\text{min}/\text{mg}$ protein)
1771		1.73	801	4.32	321
9b		15.7	88.4	12.6	110
10		0.95	1450	1.79	775

^a $t_{1/2}$: half-life ^b CL_{int} : intrinsic clearance.

2.3 Biology

2.3.1. *In vitro* antibacterial activity

In vitro antibacterial activities of the prepared derivatives against a panel of MDR *S. aureus* strains were depicted as minimal inhibitory concentration (MIC) in Table 3. The results show that the replacement of the ester with an ether bond has a positive impact on the antibacterial activities, with compounds **9a** and **9b** showing a 4-fold increase in potency when compared to **1771** and **1**, respectively. Substitutions at the phenyl ring are, in general, well tolerated with only compounds **9h** and **9m**, bearing an iodo and trifluoromethoxy group in the para position, respectively, being inactive within the ether series. Given the good tolerance for a range of substituents, we decided to test if a phenyl ring is required for activity, replacing it with a pyridin-2-yl (**9p**), a cyclohexyl (**9o**) or a cyclopropyl (**9q**) ring. However, compounds **9o-q** showed a significant, if not full, loss of antibacterial activity across all strains, suggesting that the phenyl ring is essential for activity. When testing the bioisosteric replacement of an oxadiazole ring with an oxazole ring (**9r**), only a minor decrease in the antibacterial activity was observed. Introduction of an extra methyl group on the methylene carbon adjacent to the carbonyl group of the amide bond, as in compound **10**, resulted in slightly lower antibacterial activity when compared to compound **9a**. A complete loss of antibacterial activity across all strains was observed when the amide bond was either methylated or replaced with an amine, as in compounds **11**, **14** and **15**. Despite the excellent *in vitro* stability of compound **15** in serum, its lack of antibacterial activity was striking.

Table 3. MIC values ($\mu\text{g/mL}$) for **1771** and compounds **1**, **9a-r**, **10**, **11**, **14** and **15** against Gram-positive bacteria.

[illegible]

[illegible]

Compound **9b** combined with either 0 or 100 $\mu\text{g/mL}$ of a non-lethal efflux pump inhibitor, phenyl-alanine-arginine β -naphthylamide (PA β N), was tested against *E. coli* and monitored for growth over 18 h (Figure 4). As seen before for **1771** and its ester derivative **1**, permeabilization of the outer membrane renders *E. coli* susceptible to **9b**.¹¹ The bacterial growth (OD600nm) was plotted against time following 18 h of growth at 37 °C. In the absence of Pa β N, **9b** displayed no growth inhibitory activity at concentrations up to 32 $\mu\text{g/mL}$. With the addition of 100 $\mu\text{g/mL}$ of PA β N, growth was completely inhibited at a concentration of 1 $\mu\text{g/mL}$. Similar to **9b**, compound **10** exhibited antibacterial activity against *E. coli* only in the presence of 100 $\mu\text{g/mL}$ PA β N, with an MIC of 4 $\mu\text{g/mL}$ whereas no activity was observed in the absence of the efflux pump inhibitor. In contrast, when compounds **9l** and **15** were tested against *E. coli* in the presence or absence of 100 $\mu\text{g/mL}$ PA β N, no antibacterial activity was observed under either condition.

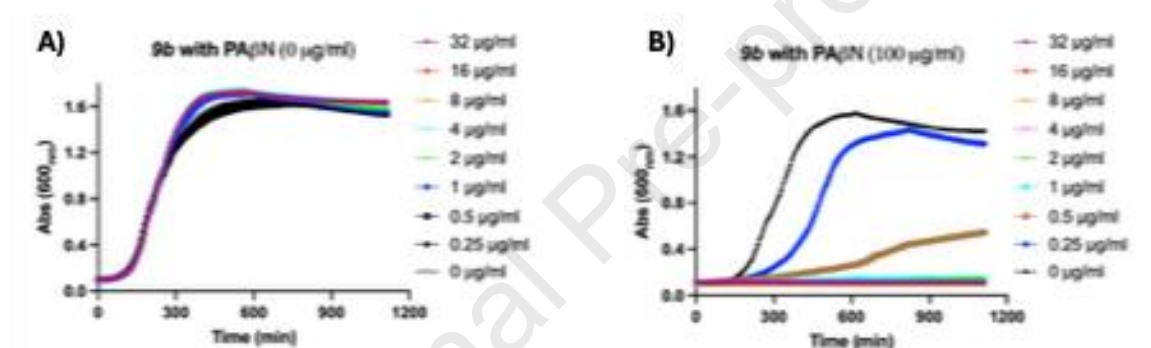


Figure 4. *E. coli* bacterial growth curves of **9b** (0–32 $\mu\text{g/mL}$) without (A) and in combination with 100 $\mu\text{g/mL}$ of Pa β N (B).

2.3.2 Compound **9b** is an inhibitor of LTA Synthesis

As a structurally similar phenylamino-oxadiazole compound such as **KKL-35** (Figure S4A) does not inhibit LTA biosynthesis (Figure S4B), a Western blot experiment was performed in triplicate with **9b** to confirm that the ether analogues reduce LTA production to the same extent of **1771** as we have previously shown.¹¹ When *S. aureus* strain LAC was incubated with 0.5 $\times$, 1 $\times$ and 4 $\times$ the MIC of **9b** a significant reduction in LTA abundance was observed (Figure 5 and Figure S4C-D).

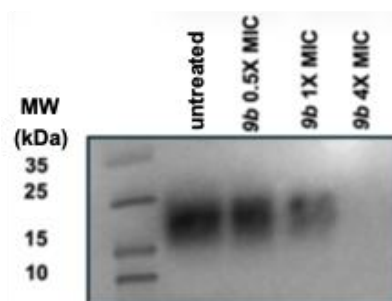


Figure 5. Western blot analysis of LTA level in *S. aureus* LAC strain after treatment for 2 h at 37 °C with **9b** at 0.5×, 1× and 4× MIC, alongside an untreated control.

2.3.3 Bacterial resistance tendency

A limiting therapeutic value of many existing and pre-clinical antimicrobials is the propensity of bacteria to acquire resistance. To evaluate whether *S. aureus* could develop resistance to these 1,3,4-oxadiazole-based molecules, we conducted an *in vitro* serial passage experiment in the presence of increasing concentrations of **9b** and **10**. Using two MRSA strains, we found that after 30 passages in the presence of **9b**, *S. aureus* was unable to develop spontaneous resistance (Figure 6). An identical outcome was observed with compound **10** (Figure S5). In contrast, *S. aureus* gains stable resistance to multiple clinically relevant antibiotics after 10-20 passages; for example, daptomycin (**DAP**, Figure. 6), and ceftaroline, ceftobiprole, and levofloxacin.²⁰⁻²²

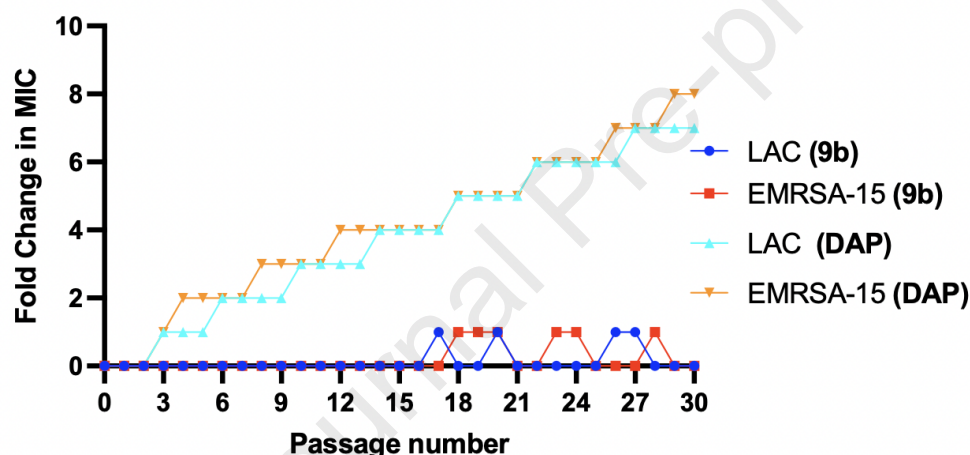


Figure 6. Serial passage of two MRSA *S. aureus* strains (LAC and EMRSA-15) in increasing concentrations of **9b** or daptomycin, displaying the fold change in MIC over 30 passages.

2.3.4 Cytotoxicity assay

As we did previously with the ester derivatives of **1771**,¹¹ we tested selected ether derivatives for acute toxicity using the AsedaSciences SYSTEMETRIC Cell Health Screen in HL-60 cell lines.^{23,24} This system measures eight parameters using a fluorescent readout of multiple dyes that increase or decrease the fluorescence on a per-cell basis, depending on how a compound affects that specific biological parameter. The parameters measured included cell morphology (Morph), cytoplasmic, membrane integrity (CMI), mitochondrial reactive oxygen species (ROS), glutathione levels (GSH), nuclear membrane integrity (two dyes are used, NMI 1–2), cell cycle activity (CC), and mitochondrial membrane potential (MMP). Tested compounds **9a** and **9b** were within the lower toxicity risks, where the cell health index (CHI), a final probability score that quantifies the similarity of a test compound's

cell stress phenotype to the high-risk class in the training set, was below 0.5. Both compounds scored high on one of the NMI tests, indicating potential cellular damage (Table 4).

Table 4. Toxicity profile of **1771**, **1** and compounds **9a-b**.

Cpd	Morph	CMI	ROS	GSH	NMI1	CC	NMI2	MMP	CHI
1771	0.92	0.64	0.03	0.07	0.92	0.05	0.05	0.09	0.37
1	0.04	0.49	0.11	0.02	0.64	0.06	0.34	0.55	0.34
9a	0.00	0.21	0.02	0.02	0.68	0.03	0.54	0.20	0.21
9b	0.36	0.64	0.26	0.04	0.62	0.14	0.51	0.36	0.37

2.3.5 Membrane permeability

To exclude non-specific membrane damage, we incubated **9b** with fluorescently encapsulated phospholipid vesicles. In contrast to daptomycin (Figure 7A), a well-known membrane-active antibiotic that disrupts bacterial phospholipid membranes, **9b** did not induce any increase in fluorescence (Figure 7B), indicating that the compound does not permeabilize or disrupt phospholipid membranes. Membrane permeability in *S. aureus* was further assessed using a propidium iodide (PI) uptake assay in the presence of daptomycin (Figure 7C), **9b** (Figure 7D), or **10** (Figure S6). Consistent with the vesicle leakage assay, neither compound caused an increase in PI fluorescence, demonstrating that they do not compromise bacterial membrane integrity.

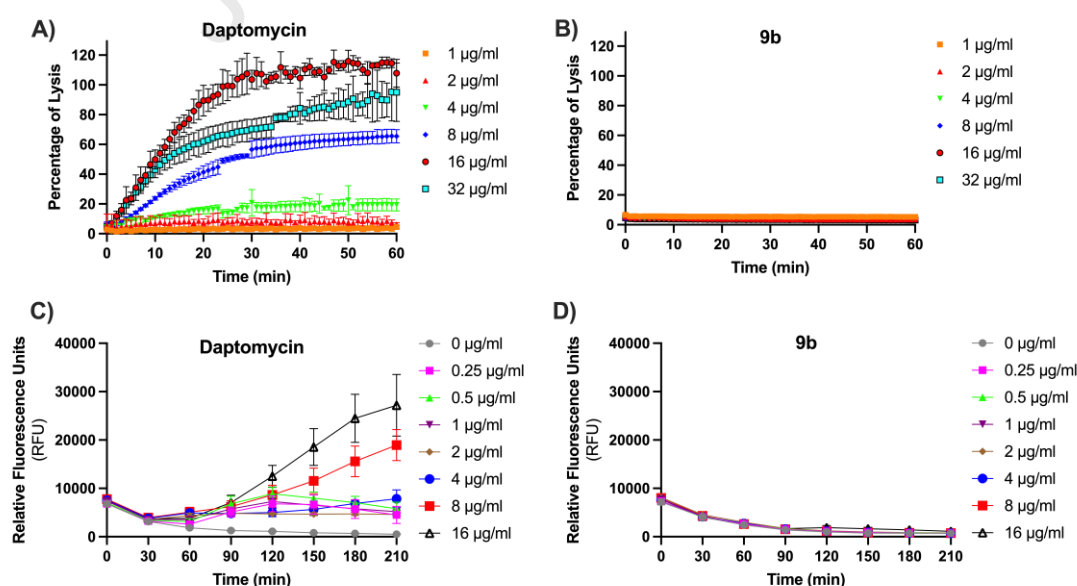


Figure 7: Carboxylfluorescein filled - phospholipid vesicles based on the Gram-positive cell membrane (60% [wt/wt] phosphatidylglycerol, 40% cardiolipin) were incubated with increasing concentrations of daptomycin (**A**) or **9b**, using triton-x100 and HEPES buffer as positive and negative controls (**B**). *S. aureus* was grown in the presence of increasing concentrations of daptomycin (**C**) or **9b** (**D**) and in the presence of membrane impermeable dye, propidium iodide (PI). Experiments were performed in biological triplicate and error bars represent the mean + SD.

4. Discussion and Conclusion

In response to the growing threat of antimicrobial resistance, particularly among Gram-positive pathogens such as *S. aureus*, this study explored a series of 1,3,4-oxadiazole-based analogues of **1771** designed to improve metabolic stability while retaining antibacterial activity. The results demonstrate that individual replacement of the ester and amide functionalities with ether and amine groups, respectively, generates chemically robust analogues and prevents the hydrolysis in rat serum previously observed for the corresponding ester and amide bonds in **1771**. However, neither modification stabilises the molecule as a whole, as each appears to increase the susceptibility of the remaining labile bond to esterase-mediated cleavage in rat serum. Importantly, while replacement of the ester with an ether preserves antibacterial activity, *N*-methylation of the amide nitrogen or replacement of the amide with an amine results in complete loss of activity. Consistent with this observation, compound **15**, the analogue containing both modifications, exhibited the highest serum stability in the series but was completely devoid of antibacterial activity. These findings highlight the critical role of the secondary amide functionality in maintaining biological activity. Specifically, the secondary amide provides both a hydrogen-bond donor (NH) and a hydrogen-bond acceptor (carbonyl oxygen) in a defined geometric arrangement. *N*-methylation removes the donor capability of the amide NH, while replacement with an amine removes the carbonyl acceptor and alters the conformational characteristics of the molecule. Together, these modifications likely disrupt key interactions with the biological target, resulting in loss of antibacterial activity.

Additional SAR studies revealed that removal of the nitrogen atom at the 3-position of the 1,3,4-oxadiazole ring has little effect on activity against Gram-positive bacteria, whereas the nature of the aromatic substituent is a major determinant of potency. In particular, the para-pentafluorosulfanyl-substituted analogue **9b** displayed the highest antibacterial activity in the series, suggesting the presence of a predominantly hydrophobic region within the target binding site. Unfortunately, despite its improved antibacterial potency, compound **9b** exhibited stability in rat serum comparable to that of **1771**. Improved serum stability without compromising antibacterial activity was achieved through steric hindrance of the amide bond, as demonstrated by the α -methyl-substituted analogue **10**. The

additional methyl group likely reduces susceptibility to esterase-mediated hydrolysis by limiting access to the amide bond. Since compound **10** demonstrated that improved serum stability could be achieved without compromising antibacterial activity, and compound **9b** exhibited the highest antibacterial potency in the series, both compounds were prioritised for further investigation, including assessment of their activity against Gram-negative pathogens, propensity for resistance development, and ability to permeabilise or disrupt phospholipid membranes.

As observed for their ester-containing counterparts,³ compounds **9b** and **10** exhibited activity against Gram-negative bacteria when combined with a well-characterised efflux pump inhibitor and outer membrane permeabiliser. In contrast, derivatives lacking antibacterial activity against *S. aureus* remained inactive against Gram-negative bacteria under the same conditions. These findings are consistent with our previous observations and further support the potential of this oxadiazole scaffold for the development of broad-spectrum antibacterial agents. Moreover, the correlation between activity against *S. aureus* and Gram-negative bacteria suggests the presence of a common molecular target across these species. Importantly, no resistance development was observed for either **9b** or **10** during the course of these studies, and both compounds exert their antibacterial activity through a mechanism that does not involve membrane disruption.

However, despite the improved stability in serum, in subsequent studies **10** exhibited very low microsomal stability, undergoing rapid clearance in both rat and human liver microsomes. This finding suggests that introduction of the α -methyl group created a metabolic soft spot that is readily oxidized by hepatic enzymes, resulting in accelerated metabolic turnover. In contrast, incorporation of the pentafluorosulfanyl group not only enhanced the antibacterial potency of **9b**, but also improved its microsomal stability compared to **1771**. However, despite its improved microsomal stability, compound **9b** still exhibited high clearance indicating that its metabolic stability remains suboptimal. Similar to the ester-containing analogues, compound **9b** inhibited LTA biosynthesis and displayed minimal toxicity in mammalian cells. Combined with its potent activity against Gram-positive bacteria, these properties identify compound **9b** as the most promising analogue in the series. Nevertheless further optimisation will be required to improve its metabolic stability and achieve intrinsic activity against Gram-negative pathogens without the need for efflux pump inhibitors or outer membrane permeabilisers.

While the precise molecular target remains unknown both in Gram-positive and Gram-negative bacteria, our results clearly demonstrate that our 1,3,4-oxadiazole-based compounds do not function via nonspecific bacterial membrane disruption, a mechanism widely regarded as undesirable in drug discovery, due to poor selectivity, elevated toxicity to host cells, and difficulties in achieving effective delivery. While our results should guide the development of promising **1771** analogues that have

potent activities against MDR bacteria as well as reduced toxicities and optimal metabolic profile, the identification of the molecular target remains a crucial step for advancing this class of compounds. Ongoing studies in our laboratories are focused on elucidating the molecular target of this class of compounds, determining whether their intracellular accumulation is limited by restricted influx, active efflux, or a combination of both, and optimizing their pharmacokinetic properties to support their further development as antibacterial agents.

5. Experimental Part

5.1 Chemistry

5.1.1 Material and method

All commercially available reagents were supplied by Sigma-Aldrich, Fisher, Apollo, Acros Organics or Fluorochem and used without further purification. Compound **1771** was purchased from Enamine. Solvents were supplied by Fisher or Acros Organics, anhydrous solvents were supplied by Acros Organics stored over molecular sieves and under nitrogen. For analytical thin-layer chromatography (TLC), precoated aluminium-backed plates (60 F-254, 0.2 mm thickness; supplied by E. Merck AG, Darmstadt, Germany) were used and developed by an ascending elution method. After solvent evaporation, compounds were detected by quenching of fluorescence at 254 nm upon irradiation with a UV lamp and basic KMnO₄ dip then heating until yellow spots appear. Column chromatography purifications were performed by automatic Biotage Isolera One or manually using 40-60 µm silica. Fractions containing the product were identified by TLC, pooled and the solvent removed *in vacuo*. ¹H, ¹³C and ¹⁹F NMR spectra were recorded on either a Bruker Ascend 500, Bruker Ultrashield 400 or Bruker Fourier 300 spectrometer at 500 MHz, 400 MHz and 300 MHz respectively for ¹H NMR; at 126 MHz, 101 MHz, 75 MHz for ¹³C NMR and at 471 MHz and 376 MHz respectively for ¹⁹F NMR. Spectra were calibrated to the deuterated residual solvent peak in ¹H NMR and ¹³C NMR. All ¹³C NMR spectra were proton-decoupled. Chemical shifts were given in parts per million (ppm) and coupling constants (*J*) measured in Hertz (Hz). The following abbreviations were used in the assignment of NMR signals: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dt (doublet of triplets), td (triplet of doublets), m (multiplet), and br (broad). Analytical High-Performance Liquid Chromatography (HPLC) analysis was performed using an Agilent 1260 Infinity HPLC system on an Agilent Pursuit C18 3 x 100 mm, 5 µm column. The purity of all test compounds was assessed by HPLC and area % purity was measured at 220 or 270 nm. Low and high-resolution mass spectrometry were performed on a Thermo Scientific Exactive GC orbitrap (chemical ionisation) or a Waters Xevo G2XS (electrospray ionisation).

5.1.2 General procedure A for the synthesis of 5-phenyl 2-amino oxadiazoles **2a-r**

Step 1: To a solution of semicarbazide hydrochloride (1 eq.) and sodium acetate (1 eq.) in water was added a solution of an appropriate benzaldehyde (1 eq.) in CH₃OH. After stirring for 20 minutes at room temperature, the precipitate was filtered off and the solid washed with diethyl ether then dried under vacuum pump to afford the desired semicarbazone.

Step 2: To a solution of crude semicarbazone (1 eq) in anhydrous 1,4-dioxane, at rt and under N₂ was added anhydrous K₂CO₃ (3 – 4.5 eq.) and iodine (1.2 eq.). The reaction was stirred for 16-24 hours at 80-95°C. After cooling to rt, the solution is treated with 5% Na₂S₂O₃ (w/v) and extracted with a mixture of CH₂Cl₂ and CH₃OH 9:1. The combined organic layers were dried over anhydrous Mg₂SO₄ and concentrated. Unless differently indicated the crude material was then washed with diethyl ether, and water to afford the desired products **2a-r** in 25-99% yield.

5.1.3 5-phenyl-1,3,4-oxadiazol-2-amine (**2a**) (commercially available)

5.1.4 5-(4-(pentafluoro-*l*-sulfaneyl)phenyl)-1,3,4-oxadiazol-2-amine (**2b**). Prepared according to general procedure **A** using for **Step 1** 4-(pentafluoro-*l*-sulfaneyl)benzaldehyde (504.2 mg, 2.17 mmol), semicarbazide hydrochloride (252.4 mg, 2.26 mmol) sodium acetate (184.1 mg, 2.25 mmol), CH₃OH (5 mL) and water (5 mL) to afford crude semicarbazone (550.93 mg, 99%); for **Step 2** crude semicarbazone (456.9 mg, 1.80 mmol), anhydrous K₂CO₃ (657.0 mg, 4.8 mmol), iodine (456.9 mg, 1.80 mmol) and dry 1,4-dioxane (5 mL) at 95 °C, 18 h. Crude product was taken up with diethyl ether and water, the organic phase dried over anhydrous MgSO₄, filtered and concentrated under vacuum to afford pure product **2b** as an orange solid (235 mg, 54%). ¹H-NMR (300 MHz, DMSO-*d*₆): δ_H 8.13 – 8.03 (2 H, m), 7.97 (2 H, d, *J* 9.0), 7.49 (2 H, s). ¹³C-NMR (126 MHz, DMSO-*d*₆): δ_C 164.52 (s), 155.99 (s), 153.41 (p, *J*_{CF} 16.6), 127.85 (s), 127.04 (p, *J*_{CF} 3.9), 125.80 (s). ¹⁹F-NMR (471 MHz, DMSO-*d*₆): δ_F 86.30 (1F, p, *J*_{FF} = 151.2 Hz, 1F), 63.74 (4F, d, *J*_{FF} = 151.2 Hz, 4F). HRMS (ESI⁺): *m/z* calc. for C₈H₇F₅N₃OS 288.0230 [M+H]⁺, found 288.0233 [M+H]⁺.

5.1.5 5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (**2c**). Prepared according to general procedure **A** using for **Step 1** 4-methoxybenzaldehyde (1210 mg, 10 mmol), semicarbazide hydrochloride (1130 mg, 10.31 mmol) sodium acetate (973 mg, 11.8 mmol), CH₃OH (3 mL) and water (5 mL) to afford crude semicarbazone (1670 mg, 87%); for **Step 2** crude semicarbazone (1498 mg, 7.76 mmol), anhydrous K₂CO₃ (3177 mg, 23.0 mmol), iodine (2396 mg, 9.44 mmol) and dry 1,4-dioxane (20 mL) at 80 °C, 18 h. Crude product was washed with petroleum ether, CH₂Cl₂ and acetone to afford pure product **2c** as an off-white amorphous powder (977 mg, 66%). ¹H-NMR (300 MHz, DMSO-*d*₆): δ_H 7.72 (2 H, d, *J* = 9.0 Hz),

7.15 (2 H, s, $J = 13.1$ Hz), 7.07 (2 H, d, $J = 9.0$ Hz), 3.81 (3 H, s). ^{13}C -NMR (75 MHz, DMSO- d_6): δ_{C} 163.6, 160.9, 157.3, 126.8, 117.0, 114.7, 55.4. HRMS (ESI $^{+}$): m/z calc. for $\text{C}_9\text{H}_{10}\text{N}_3\text{O}_2$ 192.0773, $[\text{M}+\text{H}]^{+}$, found 192.0764 $[\text{M}+\text{H}]^{+}$.

5.1.6 5-(4-(fluoro)phenyl)-1,3,4-oxadiazol-2-amine (2d). Prepared according to general procedure **A** using for the **Step 1** 4-fluorobenzaldehyde (215 μL , 249 mg, 2.00 mmol), semicarbazide hydrochloride (249 mg, 2.23 mmol) sodium acetate (168 mg, 2.05 mmol), CH_3OH (2 mL) and water (2 mL) to afford crude semicarbazone (304 mg, 84 %) ; for **Step 2** crude semicarbazone (253.9 mg, 1.40 mmol), anhydrous K_2CO_3 (574.4 mg, 4.16 mmol), iodine (456.9 mg, 1.80 mmol) and dry 1,4-dioxane (4 mL) at 80 $^{\circ}\text{C}$, 18 h. Crude product was washed with petroleum ether, CH_2Cl_2 and acetone to afford pure product **2d** as an off-white amorphous powder (977 mg, 66%). ^1H -NMR (300 MHz, DMSO- d_6): δ_{H} 7.83 (1H, dd, $J = 8.8, 5.4$ Hz), 7.37 (1H, t, $J = 8.9$ Hz), 7.27 (1H, s). ^{13}C -NMR (126 MHz, DMSO- d_6): δ_{C} 163.9, 163.1 (d, $J = 248.2$ Hz), 156.6, 127.5 (d, $J = 8.8$ Hz), 121.1 (d, $J = 3.1$ Hz), 116.4 (d, $J = 22.4$ Hz). ^{19}F -NMR (376 MHz, DMSO- d_6): δ_{F} -109.86 (1F, s). HRMS (ESI $^{+}$): m/z calc. for $\text{C}_8\text{H}_7\text{FN}_3\text{O}$ 180.0573, $[\text{M}+\text{H}]^{+}$, found 180.0868 $[\text{M}+\text{H}]^{+}$.

5.1.7 5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-amine (2e). Prepared according to general procedure **A** using for the **Step 1** 4-(trifluoromethyl)benzaldehyde (1045 mg, 6.00 mmol) semicarbazide hydrochloride (669 mg, 6.00 mmol) sodium acetate (492 mg, 6.00 mmol) CH_3OH (10 mL) and water (10 mL) to afford crude semicarbazone (1280 mg, 92 %); for **Step 2** crude semicarbazone (506 mg, 2.19 mmol), anhydrous K_2CO_3 (1042 mg, 6.6 mmol), iodine (661 mg, 2.6 mmol) and dry 1,4-dioxane (15 mL) at 80 $^{\circ}\text{C}$, 18 h. Pure product **2e** was afforded as an orange solid (125 mg, 25%). ^1H -NMR (300 MHz, DMSO- d_6): δ_{H} 7.99 (2 H, d, $J = 8.2$ Hz), 7.89 (2 H, d, $J = 8.4$ Hz), 7.45 (2 H, s). ^{13}C -NMR (101 MHz, DMSO- d_6): δ_{C} 164.34, 156.33, 129.99 (q, $J_{\text{CF}} = 32.1$ Hz), 128.02, 126.24 (q, $J_{\text{CF}} = 3.7$ Hz), 125.66, 123.90 (app. d, $J_{\text{CF}} = 272.2$ Hz). ^{18}F -NMR (376 MHz, DMSO- d_6): δ_{F} -61.46 (3F, s).

5.1.8 5-(2-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-amine (2f). Prepared according to general procedure **A** using for the **Step 1** 2-(trifluoromethyl)benzaldehyde (1045 mg, 6.00 mmol) semicarbazide hydrochloride (669 mg, 6.00 mmol) sodium acetate (492 mg, 6.00 mmol) CH_3OH (15 mL) and water (15 mL) to afford crude semicarbazone (1385 mg, 99%); for **Step 2** crude semicarbazone (464.2 mg, 2.01 mmol), anhydrous K_2CO_3 (1169 mg, 7.4 mmol, iodine (629.5 mg, 2.5 mmol) and dry 1,4-dioxane (10 mL) at 80 $^{\circ}\text{C}$, 18 h. Pure product **2f** was afforded as an orange amorphous solid (328.1 mg, 71 %). ^1H -NMR (300 MHz, DMSO- d_6): δ_{H} 8.00 – 7.89 (2 H, m), 7.88 – 7.80 (1 H, m), 7.80 – 7.73 (1 H, m), 7.36 (2 H, s). ^{13}C -NMR (101 MHz, Acetone- d_6): δ_{C} 165.6, 157.0, 133.5 (q $J = 1.0$ Hz), 132.1, 131.8,

128.4 (q, $J = 31.8$ Hz), 127.8 (q, $J = 5.6$ Hz), 124.5 (q, $J = 272.7$ Hz), 124.3 (q, $J = 1.5$ Hz). ^{19}F -NMR (376 MHz, Acetone- d_6): δ_{F} -60.43 (3F, s).

5.1.9 5-(3-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-amine (2g). Prepared according to general procedure A using for the first step 3-(trifluoromethyl)benzaldehyde (1045 mg, 6.00 mmol) semicarbazide hydrochloride (669 mg, 6.00 mmol) sodium acetate (492 mg, 6.00 mmol) CH_3OH (10 mL) and water (10 mL) to afford crude semicarbazone (1160 mg, 84 %); for **Step 2** crude semicarbazone (502 mg, 2.17 mmol), anhydrous K_2CO_3 (898 mg, 4.1 mmol), iodine (666 mg, 2.6 mmol) and dry 1,4-dioxane (15 mL) at 80 °C, 18 h. Pure product **2g** as an orange solid (172 mg, 35%). ^1H -NMR (300 MHz, DMSO- d_6): δ_{H} 8.08 (1 H, d, $J = 7.8$ Hz), 8.01 (1 H, s), 7.89 (1 H, d, $J = 7.9$ Hz), 7.78 (1 H, t, $J = 7.8$ Hz), 7.41 (2 H, s). ^{19}F -NMR (376 MHz, DMSO- d_6): δ_{F} -61.67 (3 F, s). ^{13}C -NMR (101 MHz, DMSO- d_6): δ_{C} 164.2, 156.2, 130.7 (q, $J = 32.3$ Hz), 129.9, 127.18 (d, $J = 3.0$ Hz), 121.62 (q, $J = 3.8$ Hz), 124.2 (q, $J = 272.4$ Hz).

5.1.10 5-(4-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-2-amine (2h). Prepared according to general procedure A using for the first step 4-(trifluoromethoxy)benzaldehyde (1.05 g, 6.00 mmol) semicarbazide hydrochloride (669 mg, 6.00 mmol) sodium acetate (492 mg, 6.00 mmol) CH_3OH (10 mL) and water (10 mL) to afford crude semicarbazone (506.5 mg, 2.05 mmol); for **Step 2** crude semicarbazone (506.5 mg, 2.05 mmol), anhydrous K_2CO_3 (829.9 mg, 6.01 mmol), iodine (610.1 mg, 2.40 mmol) and dry 1,4-dioxane (10 mL) at 80 °C, 18 h. Pure product **2h** was afforded as an off-white amorphous solid (438 mg, 87 %). ^1H -NMR (400 MHz, DMSO- d_6): δ_{H} 7.90 (1 H, d, $J = 8.7$ Hz), 7.52 (1 H, d, $J = 8.6$ Hz), 7.35 (1 H, s). ^{13}C -NMR (101 MHz, DMSO- d_6): δ_{C} 164.1, 156.4, 131.4 (q, $J = 33.0$ Hz), 129.4 (q, $J = 1.8$ Hz), 127.7 (q, $J = 4.0$ Hz), 127.2, 125.34 (q, $J = 270.2$ Hz). ^{19}F -NMR (376 MHz, DMSO- d_6): δ_{F} -56.81 (3F, s). HRMS (ESI $^+$): m/z calc. for $\text{C}_9\text{H}_7\text{F}_3\text{N}_3\text{O}_2$ 246.0490, $[\text{M}+\text{H}]^+$, found 246.0488 $[\text{M}+\text{H}]^+$.

5.1.11 5-(3-fluorophenyl)-1,3,4-oxadiazol-2-amine (2i). Prepared according to general procedure A using for **Step 1** 3-(trifluoromethyl)benzaldehyde 3-fluorobenzaldehyde (210 μL , 2.0 mmol) semicarbazide hydrochloride (237 mg, 1.0 mmol) sodium acetate (165 mg, 1.0 mmol) CH_3OH (2 mL) and water (2 mL) to afford crude semicarbazone (291 mg, 80%); for **Step 2** crude semicarbazone (250 mg, 1.4 mmol), anhydrous K_2CO_3 (579 mg, 3.6 mmol), iodine (428 mg, 1.7 mmol) and dry 1,4-dioxane (4 mL) at 80 °C, 18 h. Pure product **2i** was afforded as an orange amorphous solid (115 mg, 47 %). ^1H -NMR (500 MHz, DMSO- d_6): δ_{H} 7.63 (1 H, dt, $J = 7.8, 1.3$ Hz), 7.58 (1 H, td, $J = 7.9, 5.7$ Hz), 7.53 (1 H, ddd, $J = 9.7, 2.4, 1.5$ Hz), 7.39 – 7.33 (3 H, m). ^{13}C -NMR (126 MHz, DMSO- d_6): δ_{C} 164.09, 162.26 (d, $J = 244.6$ Hz), 156.37 (d, $J = 3.5$ Hz), 131.65 (d, $J = 8.5$ Hz), 126.42 (d, $J = 8.8$ Hz), 121.22 (d, $J = 2.9$ Hz), 117.26 (d, $J = 21.1$ Hz), 111.66 (d, $J = 22.7$ Hz). ^{19}F -NMR (471 MHz, DMSO- d_6): δ_{F} -111.69 (1F, s).

5.1.12 5-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine (2j). Prepared according to general procedure **A** using for **Step 1** 3-(trifluoromethyl)benzaldehyde 4-nitrobenzaldehyde (454 mg, 3.2 mmol) semicarbazide hydrochloride (758 mg, 3.2 mmol) sodium acetate (528 mg, 3.2 mmol) CH₃OH (5 mL) and water (5 mL) to afford crude semicarbazone (585 mg, 88%); for **Step 2** crude semicarbazone (557 mg, 2.68 mmol), anhydrous K₂CO₃ (1110 mg, 7. mol), iodine (812 mg, 3.2 mmol) and dry 1,4-dioxane (10 mL) at 80 °C, 5h. Pure product **2j** was afforded as a yellow microcrystalline solid (381 mg, 69%) ¹H-NMR (500 MHz, DMSO-*d*₆): δ_H 8.36 (2 H, d, *J* = 9.1 Hz), 8.02 (2 H, d, *J* = 9.1 Hz), 7.54 (2 H, s). ¹³C-NMR (126 MHz, DMSO-*d*₆): δ_C 164.6, 156.1, 148.0, 129.9, 126.0, 124.7. (Consistent with *J. Org. Chem.* 2015, 80, 2, 1018–1024)

5.1.13 5-(3-(pentafluoro-*l*-6-sulfaneyl)phenyl)-1,3,4-oxadiazol-2-amine (2k). Prepared according to general procedure **A** using for **Step 1** 3-(pentafluorosulfaneyl)benzaldehyde (228.0 mg, 0.983 mmol) semicarbazide hydrochloride (111.5 mg, 1.00 mmol) sodium acetate (82 mg, 1.00 mmol) CH₃OH (1 mL) and water (2 mL) to afford crude semicarbazone (226.9 mg, 80 %); for **Step 2** crude semicarbazone (220 mg, 0.760 mol), anhydrous K₂CO₃ (318 mg, 2.30 mmol), iodine (235 mg, 0.925 mmol) and dry 1,4-dioxane (5 mL) at 80 °C, 18h. Pure product **2k** was afforded as an orange amorphous solid (221.4 mg, 99%). ¹H-NMR (500 MHz, DMSO-*d*₆): δ_H 8.14 – 8.11 (1 H, m), 8.08 – 8.03 (2 H, m), 7.80 (1 H, q, *J* = 7.7 Hz), 7.44 (2 H, s). ¹³C-NMR (126 MHz, DMSO-*d*₆): δ_C 164.3, 155.8, 153.2, 131.0, 128.8, 127.5 – 127.2 (m), 125.6, 121.9 – 121.6 (m). ¹⁹F-NMR (471 MHz, DMSO-*d*₆): δ_F 85.96 (1F, p, *J*_{FF} = 150.8 Hz), 63.56 (4F, d, *J*_{FF} = 150.8 Hz).

5.1.14 5-(4-bromophenyl)-1,3,4-oxadiazol-2-amine (2l). Prepared according to general procedure **A** using for **Step 1** 4-bromobenzaldehyde (699.8 mg, 4.0 mmol) semicarbazide hydrochloride (448 mg, 4.0 mmol) sodium acetate (328 mg, 4 mmol) CH₃OH (5 mL) and water (5 mL) to afford crude semicarbazone (844 mg, 87%); for **Step 2** crude semicarbazone (807 mg, 3.51 mmol), anhydrous K₂CO₃ (1457 mg, 9.2 mmol), iodine (1072 mg, 4.2 mmol) and dry 1,4-dioxane (10 mL) at 80 °C, 24h. Pure product **2l** was to afforded as a crème amorphous powder (679.6 mg, 99%). ¹H-NMR (300 MHz, DMSO-*d*₆): δ_H 7.69 (2 H, d, *J* = 8.5 Hz), 7.56 (2 H, d, *J* = 8.5 Hz), 6.54 (2 H, s). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ_C 156.7, 137.9, 134.2, 131.5, 128.5, 122.1. HRMS (ESI⁺): *m/z* calc. for C₈H₇BrN₃O 241.9929 [M+H]⁺, found 241.9933.

5.1.15 5-(4-iodophenyl)-1,3,4-oxadiazol-2-amine (2m). Prepared according to general procedure **A** using for the **Step 1** 4-iodobenzaldehyde (463.5 mg, 1.997 mol) semicarbazide hydrochloride (224.6

mg, 2.01 mmol) sodium acetate (166 mg, 2.03 mmol) CH₃OH (5 mL) and water (5 mL) to afford crude semicarbazone (480 mg, 83 %); for **Step 2** crude semicarbazone (404.4 mg, 1.34 mmol), anhydrous K₂CO₃ (612 mg, 3.9 mmol), iodine (408 mg, 1.6 mmol, 1.2 eq) and dry 1,4-dioxane (15 mL) at 80 °C, 21h. Pure product **2m** was afforded as a fine crème powder (305 mg, 76 %). ¹H-NMR (300 MHz, DMSO-*d*₆): δ_H 7.90 (1 H, d, *J* = 8.6 Hz), 7.56 (1 H, d, *J* = 8.6 Hz), 7.31 (1 H, br s). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ_C 164.0, 156.8, 138.1, 126.8, 123.8, 97.2. HRMS (ESI⁺): *m/z* calc. for C₈H₆IN₃O 287.9634 [M+H]⁺, found 287.9648 [M+H]⁺.

5.1.16 5-(3,4-difluorophenyl)-1,3,4-oxadiazol-2-amine (2n). Prepared according to general procedure **A** using for **Step 1** 3,4-difluorobenzaldehyde (1.3 mL, 1670 mg, 7 mmol) semicarbazide hydrochloride (790 mg, 7 mmol) sodium acetate (579 mg, 7 mmol) CH₃OH (5 mL) and water (5 mL) to afford crude semicarbazone (1.2 g, 88 %); for **Step 2** crude semicarbazone (1 g, 5 mmol), anhydrous K₂CO₃ (2100 mg, 15 mmol), iodine (1500 mg, 6 mmol) and dry 1,4-dioxane (20 mL) at 80 °C, 18h. Pure product **2n** was afforded as an orange solid (566 mg, 57 %). ¹H-NMR (300 MHz, DMSO-*d*₆): δ_H 7.82 – 7.71 (m, 1H), 7.67 – 7.54 (m, 2H), 7.34 (s, 2H); ¹³C-NMR (75 MHz, DMSO-*d*₆): δ_C 164.2, 155.8, 153.4 (dd, *J* = 250.0, 12.5 Hz), 150.2 (dd, *J* = 247.0, 13.92 Hz), 122.8 (dd, *J* = 7.2, 3.7 Hz), 122.3 (dd, *J* = 7.4, 3.9 Hz), 118.8 (d, *J* = 18.1 Hz), 114.3 (d, *J* = 19.7 Hz). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ_F -135.36 (1F, d, *J*_{FF} = 22.3 Hz), -136.78 (1F, d, *J*_{FF} = 22.2 Hz). HRMS (ESI⁺): *m/z* calc. for C₈H₆F₂N₃O 198.0479 [M+H]⁺, found 198.0486 [M+H]⁺.

5.1.17 5-cyclohexyl-1,3,4-oxadiazol-2-amine (2o) (commercially available).

5.1.18 5-(pyridin-2-yl)-1,3,4-oxadiazol-2-amine (2p) (commercially available).

5.1.19 5-cyclopropyl-1,3,4-oxadiazol-2-amine (2q) (commercially available).

5.1.20 5-phenyloxazol-2-amine (2r) (commercially available).

5.1.21 General procedure B for the synthesis of chloroacetamides 3a-r. To a suspension of compound **2a-r** (1 eq.) in dry toluene chloroacetyl chloride (1.1 eq) was added under N₂. The suspension was then heated at 80 °C for 18 h under an anhydrous calcium chloride guard tube. The resulting mixture is allowed to reach rt then the toluene and residual chloroacetyl chloride are evaporated under vacuum to afford the desired product **3a-r** in 50-99% yield.

5.1.22 2-chloro-N-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (3a). Prepared according to general procedure **B** using commercial **2a** (1610 mg, 10 mmol) anhydrous toluene (20 mL) chloroacetyl chloride (800 μ L, 1134 mg, 10 mmol). Pure product **3a** was obtained as an off-white amorphous solid (1726 mg, 73%). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ_{H} 12.27 (1 H, s), 7.98 – 7.87 (2 H, m), 7.67 – 7.54 (3 H, m), 4.46 (2 H, s). $^{13}\text{C-NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ_{C} 164.6, 160.6, 157.0, 131.8, 129.5, 126.0, 123.3, 43.2. LRMS (ESI⁺): m/z calc. for $\text{C}_{10}\text{H}_9\text{ClN}_3\text{O}_2$ 238.04 $[\text{M}+\text{H}]^+$, found 238.04 $[\text{M}+\text{H}]^+$.

5.1.23 2-chloro-N-(5-(4-(pentafluoro-*l*-sulfaneyl)phenyl)-1,3,4-oxadiazol-2-yl)acetamide (3b)
Prepared according to general procedure **B** using **2b** (450 mg, 1.57 mmol) anhydrous toluene (7 mL) chloroacetyl chloride (260 μ L, 369 mg, 3.30 mmol). Pure product **3b** was obtained as a yellow amorphous solid (550 mg, 96%). $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ_{H} 12.44 (1 H, s), 8.17 (2 H, d, $J = 9.2$ Hz), 8.13 (2 H, d, $J = 9.0$ Hz), 4.48 (2 H, s). $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ_{C} 164.44, 159.07, 157.65, 127.20, 127.14, 127.03, 126.90, 43.16. $^{19}\text{F-NMR}$ (376 MHz, $\text{DMSO-}d_6$): δ_{F} 86.33 (1F, q $J_{\text{FF}} = 150.4$ Hz), 63.73 (4F, d $J_{\text{FF}} = 150.4$ Hz).

5.1.24 2-chloro-N-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)acetamide (3c). Prepared according to general procedure **B** using **2c** (579.1 mg, 3.03 mmol) anhydrous toluene (5 mL) chloroacetyl chloride (262 μ L, 358 mg, 3.2 mmol). Pure product **3c** was obtained as an off-white powder (808 mg, 99%). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ_{H} 7.91 – 7.80 (2 H, m), 7.28 – 7.08 (2 H, m), 4.46 (2 H, s), 3.84 (3 H, s). $^{13}\text{C-NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ_{C} 164.8, 161.9, 160.7, 156.5, 127.9, 115.6, 115.0, 55.6, 43.1.

5.1.24 2-chloro-N-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)acetamide (3d). Prepared according to general procedure **B** using **2d** (81.4 mg, 0.455 mmol) anhydrous toluene (1 mL) chloroacetyl chloride (40 μ L, 57 mg, 0.50 mmol). Pure product **3d** was obtained as an off-white amorphous solid (103 mg, 88%). $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$): δ_{H} 12.25 (1 H, s), 8.01 – 7.94 (2 H, m), 7.50 – 7.41 (2 H, m), 4.45 (2 H, s). $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$): δ_{C} 168.6, 163.9 (d, $J = 250.0$ Hz), 157.0, 128.7 (d, $J = 9.1$ Hz), 120.0 (d, $J = 3.1$ Hz), 116.7 (d, $J = 22.5$ Hz), 43.1. $^{19}\text{F-NMR}$ (471 MHz, $\text{DMSO-}d_6$): δ_{F} -107.72 (1F, s).

5.1.25 2-bromo-N-(5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)acetamide (3e). Prepared according to general procedure **B** using **2e** (111.2 mg, 0.485 mmol) anhydrous toluene (1 mL), 2-bromoacetyl bromide (45 μ L, 104 mg, 1.1 eq). Pure product **3e** was obtained as an off-white amorphous solid (147 mg, 99%). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ_{H} 8.13 (2 H, d, $J = 8.1$ Hz), 7.98 (2 H, d, $J = 8.3$ Hz), 5.90 (1 H, br s), 4.21 (2 H, s)

5.1.26 2-chloro-N-(5-(2-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)acetamide (3f). Prepared according to general procedure **B** using **2f** (60.8 mg, 0.264 mmol) anhydrous toluene (3 mL) 2-chloroacetyl chloride (23 μ L, 33 mg, 0.29 mmol). Pure product **3f** was obtained as an off-white amorphous solid (92 mg, 99%). $^1\text{H-NMR}$ (500 MHz, CD_3OD): δ_{H} 8.04 – 7.98 (1 H, m), 7.98 – 7.92 (1 H, m), 7.87 – 7.79 (2 H, m), 4.85 (2 H, s).

5.1.27 2-bromo-N-(5-(3-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)acetamide (3g)

Prepared according to general procedure B using 2g (117 mg, 0.511 mmol) anhydrous toluene (1 mL), 2-bromoacetyl bromide (45 μ L, 104 mg, 1.1 eq). Pure product was obtained as an orange amorphous solid (174 mg, 99%) $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ_{H} 8.22 (2 H, d, $J = 7.8$ Hz), 8.14 (1 H, s), 8.01 (2 H, d, $J = 6.8$ Hz), 7.90 – 7.83 (1H, m), 4.21 (2 H, s).

5.1.28 2-chloro-N-(5-(4-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-2-yl)acetamide (3h). Prepared according to general procedure **B** using **2h** (198.2 mg, 0.809 mmol) anhydrous toluene (4 mL) 2-chloroacetyl chloride (71 μ L, 99 mg, 0.89 mmol). Pure product **3h** was obtained as an off-white amorphous solid (215 mg, 83%). $^1\text{H-NMR}$ (500 MHz, DMSO-d_6): δ_{H} 8.08 – 8.02 (1 H, m), 7.60 (1 H, d, $J = 8.1$ Hz), 4.47 (1 H, s). $^{13}\text{C-NMR}$ (126 MHz, DMSO-d_6): δ_{C} 169.0, 164.0, 159.0, 156.66, 150.37 (q, $J = 1.7$ Hz), 130.94, 128.37, 127.36, 122.50, 121.90, 119.97 (q, $J_{\text{CF}} 257.6$ Hz), 43.11. $^{19}\text{F-NMR}$ (471 MHz, DMSO-d_6): δ_{F} -56.72 (3F, s). HRMS (ESI $^+$): m/z calc. for $\text{C}_{11}\text{H}_8\text{ClF}_3\text{N}_3\text{O}_3$ 322.0206 $[\text{M}+\text{H}]^+$, found 322.0201 $[\text{M}+\text{H}]^+$.

5.1.29 2-chloro-N-(5-(3-fluorophenyl)-1,3,4-oxadiazol-2-yl)acetamide (3i). Prepared according to general procedure **B** using **2i** (102.4 mg, 0.572 mmol) anhydrous toluene (3 mL) 2-chloroacetyl chloride (50 μ L, 71 mg, 0.629 mmol). Pure product **3i** was obtained as an orange solid (122.2 mg, 83%). $^1\text{H-NMR}$ (500 MHz, DMSO-d_6): δ_{H} 12.43 (1 H, s), 7.76 (1 H, ddd, $J = 7.7, 1.5, 1.0$ Hz), 7.70 – 7.63 (2 H, m), 7.52 – 7.45 (1 H, m), 4.48 (2 H, s). $^{13}\text{C-NMR}$ (126 MHz, DMSO-d_6): δ_{C} 164.5, 162.2 (d, $J = 245.3$ Hz), 159.6, 157.3, 132.0 (d, $J = 8.4$ Hz), 125.3 (d, $J = 8.9$ Hz), 122.3 (d, $J = 2.9$ Hz), 118.8 (d, $J = 21.1$ Hz), 112.7 (d, $J = 24.3$ Hz), 43.1. $^{19}\text{F-NMR}$ (471 MHz, DMSO-d_6): δ_{F} -111.28 (1F, s). HRMS (ESI $^+$): m/z calc. for $\text{C}_{10}\text{H}_8\text{ClFN}_3\text{O}_2$ 256.0289 $[\text{M}+\text{H}]^+$, found 256.0292 $[\text{M}+\text{H}]^+$.

5.1.30 2-chloro-N-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)acetamide (3j). Prepared according to general procedure **B** using **2j** (299.1 mg, 1.45) anhydrous toluene (7 mL) 2-chloroacetyl chloride (130 μ L, 177 mg, 1.6 mmol). Pure product **3j** was obtained as an off-white amorphous solid (399 mg, 97%). $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): δ_{H} 12.47 (1 H, s), 8.43 (2 H, d, $J = 9.0$ Hz), 8.17 (2 H, d, $J = 9.0$ Hz), 4.48 (2 H, s). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ_{C} 157.8, 148.9, 128.9, 127.3, 124.8, 43.2.

5.1.31 2-chloro-N-(5-(3-(pentafluoro-1,6-sulfaneyl)phenyl)-1,3,4-oxadiazol-2-yl)acetamide (3k).

Prepared according to general procedure **B** using **2k** (183mg, 0.637 mmol) anhydrous toluene (5 mL) 2-chloroacetyl chloride (56 μ L, 78.4 mg, 0.7 mmol). Pure product **3k** was obtained as a tan amorphous solid (116 mg, 50%). $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$): δ_{H} 12.48 (1 H, s), 8.25 (1 H, s), 8.22 – 8.16 (2 H, m), 7.88 (1 H, t, $J = 8.1$ Hz), 4.48 (2 H, s). $^{19}\text{F-NMR}$ (471 MHz, $\text{DMSO-}d_6$): δ_{F} 85.63 (1F, p, $J_{\text{FF}} = 154.3$ Hz), 63.63 (4F, d, $J_{\text{FF}} = 151.5$ Hz).

5.1.32 2-chloro-N-(5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)acetamide (3l). Prepared according to general procedure **B** using **2l** (599 mg, 2.49 mmol) anhydrous toluene (15 mL) 2-chloroacetyl chloride (220 μ L, 307 mg 2.74 mmol). Pure product **3l** was obtained as an off-white amorphous solid (748 mg, 95%). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ_{H} 7.83 (4 H, app. d, $J = 4.1$ Hz), 4.47 (2 H, s). $^{13}\text{C-NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ_{C} 164.6 (s), 160.0, 157.2, 132.6, 127.9, 125.3, 122.5, 43.1. HRMS (ESI^+): m/z calc. for $\text{C}_{10}\text{H}_8\text{BrClN}_3\text{O}_2$ 315.9488 $[\text{M}+\text{H}]^+$, found 315.9489 $[\text{M}+\text{H}]^+$.

5.1.33 2-chloro-N-(5-(4-iodophenyl)-1,3,4-oxadiazol-2-yl)acetamide (3m). Prepared according to general procedure **B** using **2m** (249.1 mg, 0.868 mmol) anhydrous toluene (5 mL) 2-chloroacetyl chloride (76 μ L, 107 mg, 0.95 mmol). Pure product **3m** was obtained as an off-white amorphous solid **3m** as a crème amorphous powder (289.7 mg, 92%). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ_{H} 12.29 (1 H, s), 7.98 (2 H, d, $J = 8.5$ Hz), 7.68 (2 H, d, $J = 8.5$ Hz), 4.45 (2 H, s). $^{13}\text{C-NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ_{C} 164.4, 160.2, 157.1, 138.4, 127.7, 122.7, 99.3, 43.2. HRMS (ESI^+): m/z calc. for $\text{C}_{10}\text{H}_8\text{ClIN}_3\text{O}_2$ 363.9350 $[\text{M}+\text{H}]^+$, found 363.9358 $[\text{M}+\text{H}]^+$.

5.1.34 2-chloro-N-(5-(3,4-difluorophenyl)-1,3,4-oxadiazol-2-yl)acetamide (3n). Prepared according to general procedure **B** using **2n** (517 mg, 2.2 mmol) anhydrous toluene (15 mL) 2-chloroacetyl chloride (192 μ L, 271 mg, 2.4 mmol). Pure product **3n** was obtained as a yellow amorphous powder (368 mg, 66%). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ_{H} 12.37 (s, 1H), 7.99 – 7.85 (m, 1H), 7.81 – 7.60 (m, 2H), 4.46 (s, 2H). $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$): δ_{C} 164.9, 159.1, 157.2, 152.0 (dd $J = 251.8, 13.6$ Hz), 150.3 (dd $J = 247.5, 13.7$ Hz) 124.1 (dd, $J = 7.6, 3.7$ Hz), 120.8 (dd, $J = 6.9, 4.2$ Hz), 119.1 (d, $J = 17.9$ Hz), 115.5 (d, $J = 19.8$ Hz), 43.1. $^{19}\text{F-NMR}$ (376 MHz, $\text{DMSO-}d_6$): δ_{F} -133.16 (1F, d, $J_{\text{FF}} = 22.3$ Hz), -136.25 (1F, d, $J_{\text{FF}} = 22.3$ Hz). HRMS (ESI^+): m/z calc. for $\text{C}_{10}\text{H}_7\text{ClF}_2\text{N}_3\text{O}_2$ 274.0195 $[\text{M}+\text{H}]^+$, found 274.0188 $[\text{M}+\text{H}]^+$.

5.1.35 2-chloro-N-(5-cyclohexyl-1,3,4-oxadiazol-2-yl)acetamide (3o). Prepared according to general procedure **B** using commercial 5-(cyclohexyl)-2-amino-1,3,4-oxadiazole (**2o**) (334.4 mg, 2 mmol),

anhydrous toluene (5 mL), 2-chloroacetyl chloride (180 μ L, 247 mg, 2.2 mmol). Pure product **3o** was obtained as an off-white amorphous solid (506 mg, 99%). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ_{H} 4.40 (2 H, s), 2.90 (1 H, tt, $J = 10.8, 3.6$ Hz), 2.03 – 1.89 (2 H, m), 1.79 – 1.67 (2 H, m), 1.67 – 1.57 (1 H, m), 1.57 – 1.16 (5 H, m). $^{13}\text{C-NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ_{C} 166.2, 164.8, 156.6, 43.0, 34.1, 29.4, 25.2, 24.61. HRMS (ESI^+): m/z calc. for $\text{C}_{10}\text{H}_{15}\text{ClN}_3\text{O}_2$ 244.0853 $[\text{M}+\text{H}]^+$, found 244.0852 $[\text{M}+\text{H}]^+$

5.1.36 2-chloro-N-(5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl)acetamide (3p). Prepared according to general procedure **B** using commercial 5-(pyridin-2-yl)-1,3,4-oxadiazol-2-amine (**3p**) (0.617 mmol, 100 mg), dry toluene (5 mL), 2-chloroacetyl chloride (0.679 mmol, 54 μ L, 1.1 eq) Pure product **3p** was obtained as a yellowish white solid. (146 mg, 99%) $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ_{H} 8.76 (1 H, d, $J = 4.27$), 8.13-8.02 (2 H, m), 7.64-7.60 (1 H, m), 4.51 (2H, s). LRMS (ESI^+): m/z calc. for $\text{C}_9\text{H}_8\text{ClN}_4\text{O}_2$ $[\text{M}+\text{H}]^+$ requires 239.03, found 239.03 $[\text{M}+\text{H}]^+$.

5.1.37 2-chloro-N-(5-cyclopropyl-1,3,4-oxadiazol-2-yl)acetamide (3q). Prepared according to general procedure **B** using commercial 5-cyclopropyl-1,3,4-oxadiazol-2-amine (**2q**) (100.4 mg, 0.80 mmol), anhydrous toluene (2 mL), 2-chloroacetyl chloride (70 μ L, 99.4 mg, 0.88 mmol). Pure product **3q** was obtained an off-white amorphous powder (154.2 mg, 95 %). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ_{H} 4.40 (2 H, s), 2.23 – 2.08 (1 H, m), 1.16 – 1.03 (2 H, m), 0.99 – 0.88 (2 H, m). $^{13}\text{C-NMR}$ (126 MHz, $\text{DMSO-}d_6$): δ_{C} 164.8, 156.0, 42.9, 7.5, 5.8. HRMS (ESI^+): m/z calc. for $\text{C}_7\text{H}_9\text{ClN}_3\text{O}_2$ 202.0383 $[\text{M}+\text{H}]^+$, found 202.0383 $[\text{M}+\text{H}]^+$.

5.1.38 2-chloro-N-(5-phenyloxazol-2-yl)acetamide (3r). Prepared according to general procedure **B** using commercial 5-phenyloxazol-2-amine (**2r**) (101.5 mg, 0.634 mmol) anhydrous toluene (1 mL), 2-chloroacetyl chloride (55.4 μ L, 78 mg, 0.7 mmol). Pure product **3r** was obtained as an amorphous white powder (148 mg, 99%). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ_{H} 7.64 (1 H, t, $J = 1.6$ Hz), 7.63 – 7.61 (1 H, m), 7.61 (1 H, s), 7.50 – 7.42 (2 H, m), 7.37 – 7.28 (1 H, m), 4.43 (2 H, s). $^{13}\text{C-NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ_{C} 168.6, 164.7, 157.6, 146.9, 129.2, 128.2, 123.4, 121.8, 43.2. HRMS (ESI^+): m/z calc. for $\text{C}_{11}\text{H}_9\text{ClN}_2\text{O}_2$ 237.0431 $[\text{M}+\text{H}]^+$, found 237.0431 $[\text{M}+\text{H}]^+$.

5.1.39 N-methyl-5-phenyl-1,3,4-oxadiazol-2-amine (4). Benzoic acid (220mg, 1.8 mmol), 4-methyl-3-thiosemicabazide (190 mg, 1.8 mmol), EDC (1g, 5.3 mmol) were dissolved in dichloromethane (15 mL) and left to stir at room temperature for 17 hours. After that the reaction mixture was washed with water (2 x 20 mL) and organic layer was dried over anhydrous MgSO_4 . and concentrated under reduced pressure. The product was then purified by flash chromatography to the expected compound

4 as a white powder. (196 mg, 24 %). Rf : 0.28 (Ethyl acetate: Hexane 75:25). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.95 – 7.86 (m, 2H), 7.53 – 7.41 (m, 3H), 4.74 (s, 1H), 3.12 (d, J = 5.2 Hz, 3H). $^{13}\text{C-NMR}$ (126 MHz, CDCl_3) δ 164.29, 159.16, 131.44, 130.61, 128.75, 125.89, 124.61, 30.05.

5.1.402-chloro-N-methyl-N-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (5). Prepared according to general procedure **B** using **4** (100 mg, 0.571 mmol), anhydrous toluene (3 mL), 2-chloroacetyl chloride (77 mg, 0.054 mL, 0.6852 mmol). Pure product **5** was obtained as a yellow powder. (125 mg, 87 %). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.04 – 8.00 (m, 2H), 7.58 – 7.54 (m, 3H), 4.72 (s, 2H), 3.54 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 166.51, 162.89, 159.82, 132.23, 129.35, 126.69, 123.25, 43.62, 34.19. HRMS (EI⁺): m/z calc. for $\text{C}_{11}\text{H}_{10}\text{N}_3\text{O}_2\text{Cl}$ 251.0462 [M], found m/z 252.0545 [M+H].

5.1.41 2-(RS)-bromo-N-(5-phenyl-1,3,4-oxadiazol-2-yl)propenamide (6). To a solution of 2-(RS)-bromopropionyl chloride (515 mg, 3 mmol, 1.5 eq) in dry toluene (1 mL), 2-amino-5-phenyl-1,3,4-oxadiazole **2a** (329 mg, 2 mmol, 1 eq) was added and the mixture heated at 60 °C for 2 hours. The reaction mixture was then diluted with petroleum ether to precipitate the product which was filtered. The solids were washed with water and petroleum ether and then dried under high vacuum to afford **6** as an off-white amorphous powder (449 mg, 76%) $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) (mixture of two rotamers ratio 2:1): δ_{H} 12.33 (1 H, s), 8.05 – 7.84 (2 H, m), 7.73 – 7.47 (3 H, m), 4.77 (1 H, q, J = 6.2 Hz), 1.77 (1.5 H, d, J = 6.7 Hz, rotamer 1), 1.64 (1.5 H, d, J = 6.7 Hz rotamer 2). $^{13}\text{C-NMR}$ (75 MHz, DMSO-d_6): δ_{C} 167.26, 160.64, 157.09, 131.79, 129.51, 126.07, 123.30, 42.41, 20.82 (CH_3 , rotamer 1), 20.58 (CH_3 , rotamer 2).

5.1.42 2-(naphtho[2,1-b]furan-1-yl)ethanol (8). To a solution of 2-(naphtho[2,1-b]furan-1-yl)acetic acid (**7**) (100.8 mg, 0.446 mmol) in anhydrous THF (2.5 mL) at 0 °C under N_2 , a 1 M solution of $\text{BH}_3 \cdot \text{DMS}$ in 2-methyltetrahydrofuran (0.67 mL, 0.67 mmol) was added dropwise. The solution was allowed to reach room temperature and then stirred for 4 hours. After that, CH_3OH (~1 mL) was added and the mixture was partitioned between water (10 mL), EtOAc (10 mL) and sat. aq. solution NaHCO_3 (1 mL). The organic phase was washed additional times with sat. aq. solution NaHCO_3 (2 x 5 mL) and then dried over anhydrous MgSO_4 , filtered off and the solvent evaporated to afford crude product which was purified by manual column chromatography eluting with 50 % EtOAc in hexane to afford **5** as a yellow amorphous solid (59.7 mg, 63%). $^1\text{H-NMR}$ (500 MHz, DMSO-d_6): δ_{H} 8.36 (1 H, ddt, J = 8.4, 1.2, 0.7 Hz), 8.08 – 8.03 (1 H, m), 7.93 (1 H, t, J = 1.1 Hz), 7.82 (1 H, d, J = 8.9 Hz), 7.77 (1 H, d, J = 8.9 Hz), 7.65 (1 H, ddd, J = 8.3, 6.9, 1.3 Hz), 7.53 (1 H, ddd, J = 8.1, 6.9, 1.2 Hz), 4.91 (1 H, t, J = 5.4 Hz), 3.85 (2 H, td, J = 7.1, 5.4 Hz), 3.22 (2 H, td, J = 7.1, 1.1 Hz). $^{13}\text{C-NMR}$ (126 MHz, DMSO-d_6): δ_{C} 152.6, 142.3,

130.3, 129.0, 128.1, 126.6, 125.4, 124.3, 122.9, 121.0, 119.0, 112.7, 60.4, 29.0. HRMS (EI⁻): m/z calc. for C₁₄H₁₂O₂ 212.0832 [M⁻], found 212.0825 [M⁻].

5.1.43 General procedure C for compound **9a-r**

To a solution of compound **8** (1 eq.), **3a-r** (1 eq.) and 15-crown-5 (1 eq.) in dry THF at 0°C, was added 60% NaH dispersion in mineral oil (3 - 8 eq.) and the mixture was stirred for 18 hours at rt. The mixture was then quenched with HCl, partitioned between EtOAc and water and the organic layer washed with water, dried over anhydrous MgSO₄ and then evaporated to obtain crude product. The crude mixture was purified by automated column chromatography on prepacked Biotage Sfär C18 cartridge (60 g) chromatography using a linear gradient CH₃CN in H₂O (10-100%) over 10 column volumes to afford the desired compound **9a-r**.

5.1.44 2-oxo-2-((5-phenyl-1,3,4-oxadiazol-2-yl)amino)ethyl 2-(naphtho[2,1-*b*]furan-1-yl)acetate (**9a**).

Prepared according to general procedure **C** using **8** (42.5 mg, 0.200 mmol), 15-crown-5 (40 µL, 44 mg, 0.2 mmol), 60 % NaH (8.0 mg, 0.2 mmol), dry THF (2 mL) and **3a** (47 mg, 0.200 mmol). Purification afforded **9a** as a pale-yellow solid. (9.1 mg, 19 %). ¹H-NMR (400 MHz, CDCl₃): δ_H 9.32 (1 H, s), 8.30 (1 H, d, J = 8.3 Hz), 8.01 (2 H, dd, J = 7.9, 1.4 Hz), 7.95 (1 H, d, J = 8.1 Hz), 7.73 (1 H, d, J = 9.0 Hz), 7.65 (1 H, s), 7.64 (2 H, d, J = 10.7 Hz), 7.62 – 7.58 (1 H, m), 7.55 – 7.45 (4 H, m), 4.25 (2 H, s), 4.08 (2 H, t, J = 6.8 Hz), 3.48 (2 H, t, J = 6.7 Hz). ¹³C-NMR (101 MHz, CDCl₃): δ_C 153, 141, 131, 130, 129, 129, 128, 126, 126, 125, 124, 123, 122, 120, 118, 112, 71, 70, 26 (s). HRMS (ESI⁺): m/z calc. for C₂₄H₁₉N₃O₄ 414.1454 [M+H]⁺, found 414.1452 [M+H]⁺. Reverse-phase HPLC, eluting with 0.1 % TFA H₂O/ 0.1% TFA CH₃CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, λ = 220 nm, t_R = 17.55 min (99%).

5.1.45 2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)-*N*-(5-(4-(pentafluoro-*l*6-sulfaneyl)phenyl)-1,3,4-

oxadiazol-2-yl)acetamide (**9b**). Prepared according to general procedure **C** using **8** (99.6 mg, 0.470 mmol), 15-crown-5 (82 µL, 88 mg, 0.412 mmol), 60 % NaH (73.5 mg, 1.84 mmol), dry THF (5 mL) and **3b** (151.7 mg, 0.417 mmol) purification afforded **9b** as a white amorphous solid (80 mg, 36%) ¹H-NMR (500 MHz, CDCl₃): δ_H 9.41 (1 H, s), 8.28 (1 H, d, J = 8.4 Hz), 8.07 (2 H, d, J = 8.7 Hz), 7.94 (1 H, d, J = 8.1 Hz), 7.89 – 7.82 (2 H, m), 7.73 (1 H, d, J = 8.9 Hz), 7.67 – 7.63 (2 H, m), 7.60 (1 H, ddd, J = 8.3, 6.9, 1.3 Hz), 7.47 (1 H, ddd, J = 8.1, 7.0, 1.1 Hz), 4.26 (2 H, s), 4.11 (2 H, t, J = 6.7 Hz), 3.48 (2 H, td, J = 6.6, 0.7 Hz). ¹³C-NMR (126 MHz, CDCl₃): δ_C 166.4, 160.4, 157.0, 155.9 (t, J 18.3), 153.7, 141.7, 130.9, 129.5, 128.6, 123.0 (d, J 2.5), 127.0, 126.8, 126.5, 126.2, 124.5, 122.8, 121.0, 118.4, 113.0, 71.5, 70.2, 26.4. ¹⁹F-NMR (471 MHz, CDCl₃): δ_F 82.86 (p, J_{FF} = 151.9 Hz), 62.45 (d, J_{FF} = 150.7 Hz). HRMS (ESI⁺): m/z calc.

for $C_{24}H_{18}F_5N_3O_4S$ 540.1016 $[M+H]^+$, found 540.1016 $[M+H]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 10/100 in 30 min, Flow = 1 mL/min, λ = 270 nm, t_R = 20.72 min (94%).

5.1.46 *N*-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)acetamide (**9c**). Prepared according to general procedure **C** using **8** (185.5 mg, 0.874 mmol), 15-crown-5 (110 μ L, 120 mg, 0.56 mmol), 60 % NaH (151.5 mg, 3.79 mmol), dry THF (6 mL) and **3c** (149.9 mg, 0.560 mmol, 1 eq). Purification afforded **9c** as a white amorphous solid (29.2 mg, 12%). 1H -NMR (500 MHz, $CDCl_3$): δ_H 9.72 (1 H, s), 8.28 (1 H, d, J = 8.2 Hz), 7.93 (1 H, d, J = 7.8 Hz), 7.91 (2 H, d, J = 8.9 Hz), 7.71 (1 H, d, J = 8.9 Hz), 7.64 (1 H, s), 7.63 (1 H, d, J = 8.9 Hz), 7.58 (1 H, ddd, J = 8.3, 7.0, 1.2 Hz), 7.46 (1 H, ddd, J = 8.1, 7.0, 1.1 Hz), 6.94 (2 H, d, J = 8.9 Hz), 4.25 (2 H, s), 4.07 (2 H, t, J = 6.8 Hz), 3.85 (3 H, s), 3.46 (2 H, t, J = 6.8 Hz). ^{13}C -NMR (126 MHz, $CDCl_3$): δ_C 162.4, 153.6, 141.6, 130.8, 129.4, 128.6, 128.5, 126.7, 126.0, 124.4, 122.8, 121.0, 118.4, 116.0, 114.5, 112.9, 71.3, 70.3, 55.5, 26.3. HRMS (ESI $^+$): m/z calc. for $C_{24}H_{22}N_3O_5$ 444.1559 $[M+H]^+$, found 444.1560 $[M+H]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, λ = 220 nm, t_R = 17.67 min (91%).

5.1.47 *N*-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)acetamide (**9d**). Prepared according to general procedure **C** using **8** (128 mg, 0.60 mmol), 15-crown-5 (79 μ L, 88 mg, 0.4 mmol), 60 % NaH (64 mg, 1.6 mmol), dry THF (5 mL) and **3d** (102.5 mg, 0.40 mmol). Purification afforded **9d** as a white amorphous solid (17.2 mg, 10 %). 1H -NMR (500 MHz, $DMSO-d_6$): δ_H 11.76 (1 H, s), 8.36 (1 H, d, J = 8.3 Hz), 8.05 (2 H, t, J = 3.8 Hz), 8.02 – 7.94 (2 H, m), 7.83 (1 H, d, J = 9.0 Hz), 7.77 (1 H, d, J = 8.9 Hz), 7.64 (1 H, ddd, J = 8.3, 7.0, 1.3 Hz), 7.52 (1 H, ddd, J = 8.0, 7.0, 1.0 Hz), 7.49 – 7.40 (2 H, m), 4.34 (2 H, s), 3.98 (2 H, t, J = 6.8 Hz), 3.38 (2 H, t, J = 6.5 Hz). ^{13}C -NMR (126 MHz, $DMSO-d_6$): δ_C 168.6, 163.9 (d, J_{CF} = 250.0 Hz), 160.1, 157.1, 152.6, 142.4, 130.3, 129.0, 128.7 (d, J_{CF} = 9.1 Hz), 128.0, 126.6, 125.5, 124.3, 123.0, 120.8, 120.0 (d, J_{CF} = 3.2 Hz), 118.6, 116.7 (d, J_{CF} = 22.5 Hz), 112.7, 69.9, 69.5, 25.8. ^{19}F -NMR (471 MHz, $DMSO-d_6$): δ_F -107.80 (1F, s). HRMS (ESI $^+$): m/z calc. for $C_{24}H_{19}FN_3O_4$ 432.1360 $[M+H]^+$, found 432.1358 $[M+H]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, λ = 220 nm, t_R = 17.93 min (94%).

5.1.48 2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)-*N*-(5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)acetamide (**9e**). Prepared according to general procedure **C** using **8** (104.4 mg, 0.492 mmol), 15-crown-5 (85 μ L, 95 mg, 0.434 mmol), 60 %NaH (66 mg, 1.65 mmol), dry THF (5 mL) and **3e** (151.9 mg,

0.434 mmol). Purification afforded **9e** as a white amorphous solid (9.5 mg, 9%) ^1H -NMR (500 MHz, CDCl_3): δ_{H} .32 (1 H, s), 8.29 (1 H, d, J = 8.4 Hz), 8.12 (2 H, app. d, J = 8.1 Hz), 7.94 (1 H, d, J = 8.2 Hz), 7.76 (1 H, s), 7.73 (2 H, d, J = 9.5 Hz), 7.66 (1 H, s), 7.64 (1 H, d, J = 8.9 Hz), 7.61 (1 H, ddd, J = 8.3, 6.9, 1.3 Hz), 7.47 (1 H, ddd, J = 8.1, 5.5, 1.1 Hz), 4.26 (2 H, s), 4.10 (2 H, t, J = 6.7 Hz), 3.48 (2 H, td, J = 6.7, 0.8 Hz). ^{13}C -NMR (126 MHz, CDCl_3): δ_{C} 166.4, 161.0, 156.8, 153.7, 141.7, 133.4 (q, J_{CF} = 32.8 Hz), 130.9, 129.5, 128.6, 127.1, 126.8, 126.0 (q, J = 3.7 Hz), 125.9.2, 124.5, 123.5 (q, J_{CF} = 272.5 Hz), 122.8, 121.0, 118.4, 113.0, 71.5, 70.2, 26.4. ^{19}F -NMR (471 MHz, CDCl_3): δ_{F} -63.10 (3F, s). HRMS (ESI $^+$): m/z calc. for $\text{C}_{25}\text{H}_{19}\text{F}_3\text{N}_3\text{O}_4$ 482.1328 $[\text{M}+\text{H}]^+$, found 482.1325 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, λ = 220 nm, t_{R} = 19.92 min (96%).

5.1.49 2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)-*N*-(5-(2-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)acetamide (9f). Prepared according to general procedure **C** using **8** (56.8 mg, 0.173 mmol), 15-crown-5 (34 μL , 37 mg, 0.17 mmol), 60 % NaH (64 mg, 1.6 mmol), dry THF (2 mL) and **3f** (53 mg, 0.268 mmol). Purification afforded **9f** as a white amorphous solid (40.1 mg, 48%) ^1H -NMR (500 MHz, CDCl_3): δ_{H} 9.63 (1 H, s), 8.27 (1 H, d, J = 8.3 Hz), 7.97 – 7.90 (2 H, m), 7.86 – 7.81 (1 H, m), 7.72 – 7.61 (5 H, m), 7.59 (1 H, ddd, J = 8.3, 7.0, 1.3 Hz), 7.46 (1 H, ddd, J = 8.1, 6.9, 1.1 Hz), 4.25 (2 H, s), 4.07 (2 H, t, J = 6.9 Hz), 3.46 (2 H, td, J = 6.8, 0.8 Hz). ^{13}C -NMR (126 MHz, CDCl_3): δ_{C} 166.6, 160.0, 157.4, 153.6, 141.6, 132.2 (q, J_{CF} = 1.0 Hz), 131.9, 131.7, 130.9, 129.4, 129.1 (q, J_{CF} = 32.5 Hz), 128.6, 127.2 (q, J_{CF} = 5.4 Hz), 126.7, 126.0, 124.4, 122.8 (q, J_{CF} = 263.4 Hz), 122.8, 122.0 (q, J_{CF} = 2.0 Hz), 121.0, 118.3, 112.9, 71.3, 70.2, 26.3. ^{19}F -NMR (471 MHz, CDCl_3): δ_{F} -59.9 (1F, s). HRMS (ESI $^+$): m/z calc. for $\text{C}_{25}\text{H}_{19}\text{F}_3\text{N}_3\text{O}_4$ 482.1328 $[\text{M}+\text{H}]^+$, found 482.1326 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, λ = 220 nm, t_{R} = 18.94 min (97%).

5.1.50 2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)-*N*-(5-(3-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)acetamide(9g). Prepared according to general procedure **C** using **8** (88.1 mg, 0.416 mmol), 15-crown-5 (55 μL , 62 mg, 0.28 mmol), 60 % NaH (45.4 mg, 2.73 mmol), dry THF (2.8 mL) and **3g** (97.5 mg, 0.279 mmol). Purification afforded **9g** as a white amorphous solid (53.5 mg, 40%). ^1H -NMR (500 MHz, CDCl_3): δ_{H} 9.21 (1 H, s), 8.30 (1 H, d, J = 8.3 Hz), 8.25 (1 H, s), 8.20 (1 H, d, J = 7.9 Hz), 7.94 (1 H, d, J = 8.1 Hz), 7.79 (1 H, d, J = 7.9 Hz), 7.73 (1 H, d, J = 8.9 Hz), 7.68 – 7.58 (4 H, m), 7.47 (1 H, ddd, J = 8.1, 6.9, 1.1 Hz), 4.25 (2 H, s), 4.10 (2 H, t, J = 6.7 Hz), 3.48 (2 H, td, J = 6.7, 0.8 Hz). ^{13}C -NMR (75 MHz, CDCl_3): δ_{C} 166.6, 160.8, 156.7, 153.7, 151.5, 141.7, 130.9, 129.8, 129.5, 128.5, 128.3 (q, J_{CF} = 3.7 Hz), 126.7, 126.1, 124.5, 124.3, 123.6 (q, J_{CF} = 3.9 Hz), 123.5 (q J = 270.0 Hz), 122.64 121.0, 118.4, 112.9,

71.5, 70.2, 26.3. ^{19}F -NMR (471 MHz, CDCl_3): δ_{F} -62.91 (3F, s). HRMS (ESI $^-$): m/z calc. for $\text{C}_{25}\text{H}_{17}\text{F}_3\text{N}_3\text{O}_4$ 480.1171 $[\text{M}-\text{H}]^-$, found 480.1174 $[\text{M}-\text{H}]^-$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, λ = 220 nm, t_{R} = 19.79 min (98%).

5.1.51 *2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)-N-(5-(4-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-2-yl)acetamide (9h)*. Prepared according to general procedure **C** using **8** (94.0 mg, 0.443 mmol), 15-crown-5 (62 μL , 48 mg, 0.220 mmol), 60 %NaH (54 mg, 1.3 mmol), dry THF (3 mL) and **3h** (105.7 mg, 0.329 mmol). Purification afforded **9h** as a white amorphous solid (44.1 mg, 20%). ^1H -NMR (500 MHz, CDCl_3): δ_{H} 9.34 (1 H, s), 8.29 (1 H, d, J = 8.2 Hz), 8.04 (2 H, d, J = 8.8 Hz), 7.94 (1 H, d, J = 8.0 Hz), 7.73 (1 H, d, J = 9.0 Hz), 7.70 – 7.56 (3 H, m), 7.56 – 7.41 (1 H, m), 7.32 (2 H, d, J = 8.0 Hz), 7.29 – 7.15 (1 H, m), 4.25 (2 H, s), 4.09 (2 H, t, J = 6.6 Hz), 3.48 (2 H, d, J = 6.5 Hz). ^{13}C -NMR (126 MHz, CDCl_3): δ_{C} 166.5, 161.1, 156.6, 153.7, 151.7, 141.7, 130.9, 129.5, 128.5, 126.7, 126.1, 124.4, 122.7, 122.0, 121.3, 120.9, 120.4 (q, J = 260.6 Hz), 118.3, 112.9, 71.4, 70.2, 26.4. ^{19}F -NMR (471 MHz, CDCl_3): δ_{F} -57.67 (3F, s). HRMS (ESI $^+$): m/z calc. for $\text{C}_{25}\text{H}_{19}\text{F}_3\text{N}_3\text{O}_5$ 498.1277 $[\text{M}+\text{H}]^+$, found 498.1277 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, λ = 220 nm, t_{R} = 20.66 min (96%).

5.1.52 *N-(5-(3-fluorophenyl)-1,3,4-oxadiazol-2-yl)-2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)acetamide (9i)*. Prepared according to general procedure **C** using **8** (75.5 mg, 0.356 mmol), 15-crown-5 (59 μL , 65 mg, 0.295 mmol), 60 % NaH (52 mg, 1.3 mmol), dry THF (5 mL) and **3i** (75.9 mg, 0.296 mmol). Purification afforded **9i** as a white amorphous solid (30.7 mg, 24%). ^1H -NMR (500 MHz, CDCl_3): δ_{H} 9.61 (1 H, s), 8.28 (1 H, d, J = 8.2 Hz), 7.93 (1 H, d, J = 8.0 Hz), 7.80 – 7.74 (1 H, m), 7.71 (1 H, d, J = 8.9 Hz), 7.68 – 7.65 (2 H, m), 7.63 (1 H, d, J = 8.9 Hz), 7.58 (1 H, ddd, J = 8.3, 7.0, 1.3 Hz), 7.48 – 7.40 (2 H, m), 7.21 (1 H, tdd, J = 8.4, 2.6, 0.7 Hz), 4.26 (2 H, s), 4.09 (2 H, t, J = 6.7 Hz), 3.47 (2 H, td, J = 6.6, 0.5 Hz). ^{13}C -NMR (101 MHz, CDCl_3): δ_{C} 162.9 (d, J_{CF} = 247.7 Hz), 156.7, 153.6, 141.7, 131.0 (d, J = 8.1 Hz), 130.9, 129.45, 128.5, 126.7, 126.1, 125.3 (d, J = 8.8 Hz), 124.4, 122.8, 122.5 (d, J = 3.2 Hz), 121.0, 118.8 (d, J = 21.2 Hz), 118.4, 113.8 (d, J = 24.4 Hz), 112.9, 71.4, 70.2, 26.3. ^{19}F -NMR (376 MHz, CDCl_3): δ_{F} -111.12 (1F, s). HRMS (ESI $^+$): m/z calc. for $\text{C}_{24}\text{H}_{19}\text{FN}_3\text{O}_4$ 432.1360 $[\text{M}+\text{H}]^+$, found 432.1360 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, λ = 220 nm, t_{R} = 18.43 min (95%).

5.1.53 *2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)-N-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)acetamide (9j)*. Prepared according to general procedure **C** using **8** (126.1 mg, 0.595 mmol), 15-crown-5 (100

μL , 110 mg, 0.5 mmol), 60 % NaH (114 mg, 2.84 mmol), dry THF (5 mL) and **3j** (140.5 mg, 0.498 mmol) purification afforded **9j** as a yellow crystalline solid (34.5 mg, 15%). $^1\text{H-NMR}$ (500 MHz, DMSO-d_6): δ_{H} 11.98 (1 H, s), 8.46 – 8.39 (2 H, m), 8.35 (1 H, d, $J = 8.4$ Hz), 8.20 – 8.11 (2 H, m), 8.05 (2 H, d, $J = 7.9$ Hz), 7.83 (1 H, d, $J = 9.0$ Hz), 7.77 (1 H, d, $J = 8.9$ Hz), 7.64 (1 H, ddd, $J = 8.3, 6.9, 1.3$ Hz), 7.52 (1 H, ddd, $J = 8.1, 6.9, 1.1$ Hz), 4.36 (2 H, s), 3.99 (2 H, t, $J = 6.8$ Hz), 3.38 (2 H, td, $J = 6.8, 0.9$ Hz). $^{13}\text{C-NMR}$ (126 MHz, DMSO-d_6): δ_{C} 168.4, 159.2, 158.0, 152.5, 148.9, 142.4, 130.3, 129.0, 128.9, 128.0, 127.2, 126.6, 125.5, 124.7, 124.3, 123.0, 120.8, 118.6, 112.7, 69.9, 69.6, 25.8. HRMS (ESI $^+$): m/z calc. for $\text{C}_{24}\text{H}_{19}\text{N}_4\text{O}_6$ 459.1305 $[\text{M}+\text{H}]^+$, found 459.1306 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN 90/10; to 0/100 in 30 min, Flow = 1 mL/min, $\lambda = 270$ nm, $t_{\text{R}} = 18.09$ min (99%)

5.1.54 2-(2-(naphtho[2,1-b]furan-1-yl)ethoxy)-N-(5-(3-(pentafluoro-*l*6-sulfaneyl)phenyl)-1,3,4-oxadiazol-2-yl)acetamide (9k). Prepared according to general procedure **C** using **8** (56.2 mg, 0.265 mmol), 15-crown-5 (44 μL , 48 mg, 0.220 mmol), 60 %NaH (52 mg, 1.3 mmol), dry THF (5 mL) and **3k** (80.9 mg, 0.222 mmol). Purification afforded **9k** as a yellow amorphous solid (21.9 mg, 18%). $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ_{H} 9.32 (1 H, s), 8.41 – 8.36 (1 H, m), 8.29 (1 H, d, $J = 8.3$ Hz), 8.13 (1 H, d, $J = 7.9$ Hz), 7.96 – 7.86 (2 H, m), 7.72 (1 H, d, $J = 8.9$ Hz), 7.66 (1 H, s), 7.64 (2 H, d, $J = 9.0$ Hz), 7.61 – 7.57 (1 H, m), 7.46 (1 H, ddd, $J = 8.1, 6.9, 1.2$ Hz), 4.25 (2 H, s), 4.09 (2 H, t, $J = 6.7$ Hz), 3.48 (2 H, td, $J = 6.6, 0.8$ Hz). $^{13}\text{C-NMR}$ (126 MHz, CDCl_3): δ_{C} 166.6, 160.4, 156.8, 154.5 (app. t, $J = 18.7$ Hz), 153.7, 141.7, 130.9, 129.8, 129.6, 129.5, 128.8 (p, $J = 4.5$ Hz), 128.5, 126.7, 126.1, 124.5, 124.5, 124.2 (p, $J = 4.5$ Hz), 122.8, 121.0, 118.4, 113.0, 71.5, 70.2, 26.3. $^{19}\text{F-NMR}$ (471 MHz, CDCl_3): δ_{F} 82.72 (1F, p, $J_{\text{FF}} = 150.8$ Hz), 62.66 (4F, d, $J_{\text{FF}} = 150.5$ Hz). HRMS (ESI $^+$): m/z calc. for $\text{C}_{24}\text{H}_{19}\text{F}_5\text{N}_3\text{O}_4\text{S}$ 540.1016 $[\text{M}+\text{H}]^+$, found 540.1016 $[\text{M}+\text{H}]^+$.

5.1.55 N-(5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)-2-(2-(naphtho[2,1-b]furan-1-yl)ethoxy)acetamide (9l). Prepared according to general procedure **C** using **8** (184 mg, 0.87 mmol), 15-crown-5 (155 μL , 171 mg, 0.78 mmol), 60 % NaH (150 mg, 3.75 mmol), dry THF (8 mL) and **3l** (230 mg, 0.73 mmol). Purification afforded **9l** as a white amorphous solid (249 mg, 69%). $^1\text{H-NMR}$ (500 MHz, DMSO-d_6): δ_{H} 11.82 (1 H, s), 8.35 (1 H, d, $J = 8.1$ Hz), 8.08 – 8.02 (2 H, m), 7.85 (2 H, d, $J = 8.8$ Hz), 7.83 – 7.79 (3 H, m), 7.77 (1 H, d, $J = 8.9$ Hz), 7.64 (1 H, ddd, $J = 8.3, 6.9, 1.3$ Hz), 7.52 (1 H, ddd, $J = 8.1, 6.9, 1.1$ Hz), 4.34 (2 H, s), 3.98 (2 H, t, $J = 6.8$ Hz), 3.38 (2 H, td, $J = 6.6, 0.8$ Hz). $^{13}\text{C-NMR}$ (126 MHz, DMSO-d_6): δ_{C} 168.53, 160.09, 157.32, 152.56, 142.45, 132.59, 130.32, 129.05, 128.03, 127.91, 126.65, 125.52, 125.24, 124.33, 123.02, 122.57, 120.84, 118.64, 112.71, 69.91, 69.54, 25.77. HRMS (ESI $^+$): m/z calc. for $\text{C}_{24}\text{H}_{19}^{81}\text{BrN}_3\text{O}_4$ 494.0533 $[\text{M}+\text{H}]^+$, found 494.0547 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, $\lambda = 220$ nm, $t_{\text{R}} = 19.55$ min (95%).

5.1.56 *N*-(5-(4-iodophenyl)-1,3,4-oxadiazol-2-yl)-2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)acetamide (**9m**). Prepared according to general procedure **C** using **6** (105.5 mg, 0.498 mmol), 15-crown-5 (100 μ L, 110 mg, 0.50 mmol), 60 % NaH (110 mg, 2.75 mmol), dry THF (5 mL) and **3m** (182.7 mg, 0.502 mmol). Purification afforded **9m** as a white amorphous solid (184 mg, 68%). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ_{H} 11.83 (1 H, s), 8.35 (1 H, d, $J = 8.3$ Hz), 8.05 (2 H, d, $J = 6.6$ Hz), 7.98 (2 H, d, $J = 8.5$ Hz), 7.83 (1 H, d, $J = 9.0$ Hz), 7.77 (1 H, d, $J = 8.9$ Hz), 7.72 – 7.60 (3 H, m), 7.52 (1 H, ddd, $J = 7.9, 7.0, 0.8$ Hz), 4.34 (2 H, s), 3.97 (2 H, t, $J = 6.7$ Hz), 3.38 (2 H, t, $J = 6.7$ Hz). $^{13}\text{C-NMR}$ (101 MHz, $\text{DMSO-}d_6$): δ_{C} 168.5, 160.3, 157.3, 152.6, 142.4, 138.4, 130.3, 129.0, 128.0, 127.6, 126.6, 125.5, 124.3, 123.0, 122.8, 120.8, 118.6, 112.7, 99.2, 69.9, 69.5, 25.8. HRMS (ESI $^+$): m/z calc. for $\text{C}_{24}\text{H}_{19}\text{IN}_3\text{O}_4$ 540.0420 $[\text{M}+\text{H}]^+$, found 540.0421 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, $\lambda = 220$ nm, $t_{\text{R}} = 20.07$ min (92%).

5.1.57 *N*-(5-(3,4-difluorophenyl)-1,3,4-oxadiazol-2-yl)-2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)acetamide (**9n**). Prepared according to general procedure **C** using **8** (42.2 mg, 0.199 mmol), 15-crown-5 (20 μ L, 22 mg, 0.1 mmol), 60 % NaH (47 mg, 1.18 mmol), dry THF (3 mL) and **3n** (61.1 mg, 0.223 mmol). Purification afforded **9n** as a white amorphous solid (29.2 mg, 33 %). $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ_{H} 9.66 (1 H, s), 8.26 (1 H, d, $J = 8.3$ Hz), 7.93 (1 H, dd, $J = 8.1, 0.5$ Hz), 7.76 (1 H, ddd, $J = 10.2, 7.4, 2.1$ Hz), 7.71 (2 H, d, $J = 8.9$ Hz), 7.65 (1 H, s), 7.63 (1 H, d, $J = 8.9$ Hz), 7.58 (1 H, ddd, $J = 8.3, 6.9, 1.3$ Hz), 7.46 (1 H, ddd, $J = 8.1, 6.9, 1.1$ Hz), 7.29 – 7.20 (1 H, m), 4.26 (2 H, s), 4.09 (2 H, t, $J = 6.7$ Hz), 3.47 (2 H, td, $J = 6.6, 0.7$ Hz). $^{13}\text{C-NMR}$ (126 MHz, CDCl_3): δ_{C} 166.6, 160.2, 156.7, 153.5, 152.47 (dd, $J_{\text{CF}} = 250.7, 12.4$ Hz), 150.48 (dd, $J_{\text{CF}} = 246.0, 13.2$ Hz), 141.6, 130.8, 129.4, 128.5, 126.7, 126.1, 124.4, 123.5 (dd, $J_{\text{CF}} = 7.0, 3.9$ Hz), 122.8, 121.0, 120.4 (dd, $J_{\text{CF}} = 6.9, 3.8$ Hz), 118.3, 118.2 (d, $J_{\text{CF}} = 18.4$ Hz), 116.1 (d, $J_{\text{CF}} = 19.7$ Hz), 112.9, 71.3, 70.1, 26.3. $^{19}\text{F-NMR}$ (376 MHz, none): δ_{F} -132.87 (1F, d, $J_{\text{FF}} = 20.7$ Hz), -136.66 (d, $J_{\text{FF}} = 20.6$ Hz). HRMS (ESI $^+$): m/z calc. for $\text{C}_{24}\text{H}_{18}\text{F}_2\text{N}_3\text{O}_4$ 450.1265 $[\text{M}+\text{H}]^+$, found 450.1259 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, $\lambda = 220$ nm, $t_{\text{R}} = 18.55$ min (98%).

5.1.58 2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)-*N*-(5-phenyloxazol-2-yl)acetamide (**9o**). Prepared according to general procedure **C** using **8** (174.6 mg, 0.823 mmol), 15-crown-5 (163 μ L, 181 mg, 0.824 mmol), 60 % NaH (140 mg, 3.5 mmol), dry THF (6 mL) and **3o** (131.0 mg, 0.823 mmol). Purification afforded **9o** as a white amorphous solid (81.9 mg, 36%) $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ_{H} 9.16 (1 H, s), 8.28 (1 H, d, $J = 8.3$ Hz), 7.94 (1 H, d, $J = 8.1$ Hz), 7.74 – 7.68 (1 H, m), 7.66 – 7.52 (5 H, m), 7.47

(1 H, t, $J = 7.5$ Hz), 7.40 (2 H, t, $J = 7.6$ Hz), 7.31 (1 H, t, $J = 7.4$ Hz), 7.19 (1 H, s), 4.20 (2 H, s), 4.03 (2 H, t, $J = 6.5$ Hz), 3.44 (2 H, t, $J = 6.5$ Hz). ^{13}C -NMR (126 MHz, CDCl_3): δ_{C} 153.6, 151.5, 147.9, 141.6, 130.9, 129.4, 128.9, 128.6, 128.4, 127.5, 126.7, 126.0, 124.4, 123.9, 122.8, 121.0, 118.4, 112.9, 71.2, 70.3, 26.4. HRMS (ESI⁺): m/z calc. for $\text{C}_{25}\text{H}_{20}\text{N}_2\text{O}_4$ 413.1501 $[\text{M}+\text{H}]^+$, found 413.1496 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, $\lambda = 270$ nm, $t_{\text{R}} = 18.84$ min (93.5 %).

5.1.59 *N*-(5-cyclohexyl-1,3,4-oxadiazol-2-yl)-2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)acetamide (**9p**). Prepared according to general procedure **C** using **8** (127.4 mg, 0.601 mmol), 15-crown-5 (100 μL , 110 mg, 0.5 mmol), 60 % NaH (117 mg, 3 mmol), dry THF (5 mL) and **3p** (121.0 mg, 0.496 mmol). Purification afforded **9p** as a white amorphous solid (32.8 mg, 16%). ^1H -NMR (400 MHz, CDCl_3): δ_{H} 9.49 (1 H, s), 8.28 (1 H, d, $J = 8.3$ Hz), 7.96 (1 H, d, $J = 8.1$ Hz), 7.73 (1 H, d, $J = 9.0$ Hz), 7.66 – 7.63 (2 H, m), 7.60 (1 H, ddd, $J = 8.3, 7.0, 1.3$ Hz), 7.49 (1 H, ddd, $J = 8.1, 7.0, 1.1$ Hz), 4.21 (2 H, s), 4.06 (2 H, dt, $J = 13.7, 6.7$ Hz), 3.44 (2 H, td, $J = 6.8, 0.7$ Hz), 2.83 (1 H, tt, $J = 11.3, 3.6$ Hz), 2.11 – 1.98 (2 H, m), 1.88 – 1.75 (2 H, m), 1.75 – 1.64 (1 H, m), 1.64 – 1.49 (2 H, m), 1.43 – 1.18 (3 H, m). ^{13}C -NMR (101 MHz, CDCl_3): δ_{C} 153.6, 141.6, 130.9, 129.4, 128.6, 126.7, 126.0, 124.4, 122.8, 121.0, 118.3, 112.9, 71.3, 70.2, 35.3, 30.0, 26.3, 25.6, 25.4. HRMS (ESI⁺): m/z calc. for $\text{C}_{24}\text{H}_{26}\text{N}_3\text{O}_4$ $[\text{M}+\text{H}]^+$, found 420.1923 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA CH_3CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, $\lambda = 220$ nm, $t_{\text{R}} = 18.66$ min (96%).

5.1.60 2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)-*N*-(5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl)acetamide (**9q**). Prepared according to general procedure **C** using **8** (93 mg, 0.44 mmol), 15-crown-5 (64 μL , 0.209 mmol), 60% NaH (47 mg, 1.17 mmol), dry tetrahydrofuran (2 mL) and **3q** (70 mg, 0.29 mmol). Purification by Biotage C18 (60g) chromatography [30% Acetonitrile in water over 6 column volumes] to afford the desired compound **9q** as a white amorphous powder (13 mg, 23 %). ^1H -NMR (500 MHz, Acetonitrile- d_3): δ_{H} 8.73 (1 H, d, $J = 4.1$ Hz), 8.41 (1 H, d, $J = 8.3$ Hz), 8.12 (1 H, d, $J = 7.9$ Hz), 8.00 (1 H, d, $J = 8.1$ Hz), 7.94 (1 H, td, $J = 7.8, 1.4$ Hz), 7.82 (1 H, s), 7.79 (1 H, d, $J = 9.0$ Hz), 7.68 (1 H, d, $J = 8.9$ Hz), 7.65 (1 H, ddd, $J = 8.2, 6.9, 1.0$ Hz), 7.56 – 7.45 (2 H, m), 4.26 (2 H, s), 4.05 (2 H, t, $J = 6.6$ Hz), 3.46 (2 H, t, $J = 6.6$ Hz). ^{13}C -NMR (126 MHz, Acetonitrile- d_3): δ_{C} 168.77, 162.00, 158.32, 154.13, 151.13, 144.30, 143.15, 138.42, 131.70, 129.98, 129.42, 127.55, 126.83, 126.58, 125.32, 124.17, 123.38, 122.03, 119.94, 113.56, 71.52, 70.77, 26.72. HRMS (ESI⁺): m/z calc. for $\text{C}_{23}\text{H}_{19}\text{N}_4\text{O}_4$ requires 415.1401 $[\text{M}+\text{H}]^+$ found 415.1408 $[\text{M}+\text{H}]^+$. Reverse-phase HPLC, eluting with 0.1 % TFA H_2O / 0.1% TFA

CH₃CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, λ = 220 nm, t_R = 22.51 min (98%).

5.1.61 *N*-(5-cyclopropyl-1,3,4-oxadiazol-2-yl)-2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)acetamide (**9r**). Prepared according to general procedure **C** using **8** (75.9 mg, 0.36 mmol), 15-crown-5 (74 μ L, 82 mg, 0.37 mmol), 60 % NaH (85.9 mg, 2.16 mmol), dry THF (3.7 mL) and **3r** (74.6 mg, 0.37 mmol). Purification afforded **9r** as a white amorphous solid (48 mg, 35%). ¹H-NMR (400 MHz, Acetone-*d*₆): δ_H 10.19 (1 H, s), 8.40 (1 H, d, *J* = 8.3 Hz), 8.03 (1 H, d, *J* = 8.2 Hz), 7.93 (1 H, s), 7.81 (1 H, d, *J* = 9.0 Hz), 7.69 (1 H, d, *J* = 8.9 Hz), 7.62 (1 H, ddd, *J* = 8.3, 7.0, 1.3 Hz), 7.50 (1 H, ddd, *J* = 8.1, 7.0, 1.1 Hz), 4.32 (2 H, s), 4.09 (2 H, t, *J* = 6.8 Hz), 3.46 (2 H, td, *J* = 6.7, 0.8 Hz), 2.18 – 2.08 (1 H, m), 1.18 – 1.05 (2 H, m), 1.05 – 0.93 (2 H, m). ¹³C-NMR (101 MHz, Acetone-*d*₆): δ_C 154.1, 143.1, 131.7, 129.9, 129.5, 127.4, 126.5, 126.5, 125.1, 124.0, 122.0, 119.8, 113.4, 71.4, 70.7, 26.8, 8.0, 6.6. HRMS (ESI⁺): *m/z* calc. for C₂₁H₁₉N₃O₄ 378.1454 [M+H]⁺, found 378.1452 [M+H]⁺.

5.1.62 2-(*RS*)-((1-(naphtho[2,1-*b*]furan-1-yl)propan-2-yl)oxy)-*N*-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (**10**). Prepared according to general procedure **C** using **6** (133.1 mg, 0.628 mmol) 15-crown-5 (100 μ L, 113 mg, 0.513 mmol), 60% NaH (100 mg, 2.5 mmol) dry THF (5 mL) and **7** (147.7 mg, 0.490 mmol). Purification by Biotage Sfär C18 cartridge (60 g) chromatography using a linear gradient of CH₃CN in H₂O (50-100%) over 6 column volumes afforded the desired compound **10** as a white amorphous solid (57.1 mg, 27%). ¹H-NMR (500 MHz, CDCl₃) δ_H 9.52 (1 H, s), 8.28 (1 H, d, *J* = 8.3 Hz), 8.00 (2 H, d, *J* = 6.9 Hz), 7.92 (1 H, d, *J* = 8.1 Hz), 7.70 (1 H, d, *J* = 8.9 Hz), 7.66 (1 H, s), 7.62 (1 H, d, *J* = 8.9 Hz), 7.61 – 7.56 (1 H, m), 7.55 – 7.44 (4 H, m), 4.21 – 4.09 (2 H, m), 4.09 – 3.89 (1 H, m), 3.46 (2 H, t, *J* = 6.4 Hz), 1.53 (3 H, d, *J* = 6.8 Hz). ¹³C-NMR (126 MHz, CDCl₃): δ_C 170.1, 162.0, 156.6, 153.7, 141.7, 131.7, 130.8, 129.4, 129.1, 128.5, 126.8, 126.7, 126.1, 124.4, 123.6, 122.9, 120.9, 118.5, 112.9, 69.5, 26.6, 18.0. HRMS (ESI⁺): *m/z* calc. for C₂₅H₂₂N₃O₄ 428.1610 [M+H]⁺, found 428.1613 [M+H]⁺. Reverse-phase HPLC, eluting with H₂O/CH₃CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min, λ = 220 nm, t_R = 18.23 min (96%).

5.1.63 2-(methyl(5-phenyl-1,3,4-oxadiazol-2-yl)amino)ethyl 2-(naphtho[2,1-*b*]furan-1-yl)acetate (**11**). Compounds **6** (100 mg, 0.397 mmol), **7** (106 mg, 0.397 mmol) and sodium iodide (6 mg, 0.0397 mmol) were dissolved in triethylamine (50 μ L, 0.4367 mmol) and DMF (1.5 mL). The solution was stirred at 80°C for 20 hr. The reaction mixture was washed with water (2x10 mL) and brine (1x10 mL). The organic layer was dried over MgSO₄ and concentrated under reduced pressure to obtain the expected compound **11** as a yellow solid. (138 mg, 79%). ¹H-NMR (500 MHz, CDCl₃) δ 8.26 (d, *J* = 5.0

Hz, 1H), 7.96 (t, $J = 7.7$ Hz, 3H), 7.84 (s, 1H), 7.73 (d, $J = 9.0$ Hz, 1H), 7.67–7.57 (m, 2H), 7.57–7.44 (m, 4H), 5.32 (s, 2H), 4.26 (s, 2H), 3.52 (s, 3H). ^{13}C -NMR (126 MHz, CDCl_3) δ 170.38, 166.80, 162.31, 159.53, 153.41, 142.93, 132.00, 130.77, 129.20, 129.06, 128.36, 126.56, 126.50, 125.92, 124.33, 123.11, 121.00, 114.23, 112.67, 64.15, 33.11, 31.39. HRMS (ESI⁺): m/z calc. for $\text{C}_{25}\text{H}_{19}\text{N}_3\text{O}_5$ m/z 441.1325 [M], found m/z 442.1407 [M+H]⁺.

5.1.64 2-bromo-5-phenyl-1,3,4-oxadiazole (12). To a suspension of **2a** (333.5 mg, 2.07 mmol) and copper (II) bromide (1334 mg, 8 mmol) in dry CH_3CN (30 mL) at rt, was added *tert*-butyl nitrite (0.95 mL, 618 mg, 6 mmol) and the mixture refluxed for 30 mins. The mixture was then allowed to reach rt, the solvent and excess of nitrite evaporated under vacuum, and the residue redissolved in 5% CH_3OH in CH_2Cl_2 (25 mL). The resulting solution was washed with water (2 x 20 mL), 2M HCl (2 x 20 mL), sat. aq. NaHCO_3 (2 x 20 mL), dried over MgSO_4 , filtered off to afford compound **12** as a pale-yellow solid (425 mg, 91%). ^1H -NMR (300 MHz, $\text{DMSO}-d_6$): δ_{H} 8.06–7.93 (2 H, m), 7.74–7.54 (3 H, m). ^{13}C -NMR δ_{C} (75 MHz, $\text{DMSO}-d_6$) 167.0, 140.5, 132.5, 129.5, 126.6, 122.6. HRMS (ESI⁺): m/z calc. for $\text{C}_8\text{H}_6^{81}\text{BrN}_2\text{O}$ calc. 226.9643 [M+H]⁺, found 226.9646 [M+H]⁺.

5.1.65 2-(methyl(5-phenyl-1,3,4-oxadiazol-2-yl)amino)ethan-1-ol (13). Compound **12** (103.1 mg, 0.458 mmol), *N*-methylethanolamine (106 μL , 99.6 mg, 1.33 mmol) and DIPEA (232 μL , 171.7 mg, 1.33 mmol) were dissolved in dry DMF (2.2 mL) and the reaction heated at 80 °C for 20 mins. The reaction mixture was partitioned between EtOAc (10 mL) and water (10 mL) and the aqueous phase extracted with EtOAc (4 x 10 mL). The combined organic extracts were then evaporated to obtain the desired compound **13** as a white amorphous solid (55 mg, 54%). ^1H -NMR (500 MHz, CDCl_3): δ_{H} 7.92–7.81 (2 H, m), 7.48–7.34 (3 H, m), 3.93 (2 H, t, $J = 4.8$ Hz), 3.64 (2 H, t, $J = 5.2$ Hz), 3.45 (1 H, s), 3.21 (3 H, s). ^{13}C -NMR (126 MHz, CDCl_3): δ_{C} 164.9, 159.1, 130.5, 129.0, 125.8, 124.6, 60.4, 53.8, 37.2. HRMS (ESI⁺): m/z calc. for $\text{C}_{11}\text{H}_{11}\text{N}_3\text{O}_2$ 220.1086 [M+H]⁺, found 220.1086 [M+H]⁺.

5.1.66 2-(methyl(5-phenyl-1,3,4-oxadiazol-2-yl)amino)ethyl 2-(naphtho[2,1-*b*]furan-1-yl)acetate (14). Compound **13** (55.3 mg, 0.244 mmol), HATU (92.0 mg, 0.242 mmol) and DIPEA (77 μL , 57 mg, 0.44 mmol) were dissolved in dry DMF (0.5 mL) and the mixture was stirred for 10 minutes at rt under N_2 . This solution was added to a solution of **7** (48.4 mg, 0.219 mmol) in dry DMF (0.5 mL) and stirred at ambient temperature for 3 hours. The mixture was then partitioned between EtOAc (5 mL) and water (5 mL) and the aqueous phase extracted with EtOAc (3 x 1 mL) and the combined organic extracts washed with brine (2 x 5 mL), dried over anhydrous MgSO_4 , filtered off and evaporated. The crude product was purified first by automated column chromatography on a prepacked Biotage S ϕ ar cartridge

(10 g) using a linear gradient of CH₃OH in CH₂Cl₂ (0-5%) over 6 column volumes and then on a prepacked Biotage Sfär C18 cartridge (30 g) chromatography using as eluent a gradient of CH₃CN in H₂O (10% CH₃CN in H₂O for 1 CV, 10-100% CH₃CN over 6 CV) to afford the desired compound **14** as an amorphous white powder (53.5 mg, 57%). ¹H-NMR (400 MHz, CDCl₃): δ_H 8.13 (1 H, d, *J* = 8.3 Hz), 7.91 (1 H, d, *J* = 8.1 Hz), 7.88 – 7.82 (2 H, m), 7.69 (1 H, d, *J* = 9.0 Hz), 7.68 – 7.65 (1 H, m), 7.58 (1 H, d, *J* = 8.9 Hz), 7.54 (1 H, ddd, *J* = 8.3, 7.0, 1.4 Hz), 7.47 – 7.39 (4 H, m), 4.41 (2 H, t, *J* = 5.3 Hz), 4.05 (2 H, d, *J* = 0.8 Hz), 3.64 (2 H, t, *J* = 5.3 Hz), 2.87 (3 H, s). ¹³C-NMR (101 MHz, CDCl₃): δ_C 170.76, 164.1, 159.2, 153.5, 142.9, 130.8, 130.5, 129.3, 128.9, 128.3, 126.6, 126.1, 125.8, 124.6, 124.5, 122.8, 121.0, 114.6, 112.8, 62.2, 49.7, 36.5, 31.8. HRMS (ESI⁺): *m/z* calc. for C₂₅H₂₂N₃O₄ 428.1610 [M+H]⁺, found 428.1611 [M+H]⁺. Reverse-phase HPLC, eluting with 0.1 % TFA H₂O/ 0.1% TFA CH₃CN 90/10; to 0/100 in 30 min, Flow = 1 mL/min, λ = 270 nm, *t_R* = 18.23 min (99%).

5.1.67 *N*-(2-(2-(naphtho[2,1-*b*]furan-1-yl)ethoxy)ethyl)-5-phenyl-1,3,4-oxadiazol-2-amine (**15**).

To a solution of compound **9a** (71.9 mg, 0.174 mmol) in dry THF (6.8 mL) at 0°C, was added dropwise 2.4 M LiAlH₄ in THF (0.40 mL, 0.96 mmol) and the mixture was stirred at rt for 2h. The reaction was then quenched with acetone (2 mL), treated with sat. aq. solution of sodium potassium tartrate (3 mL) then with 5 M NaOH (5 mL) before extracting with EtOAc (3 x 5 mL) and the combined organic extracts were evaporated under vacuum. Crude was purified first on a strong cation exchange resin (Biotage ISOLUTE® SCX-2 g/3 mL, 0.6 meq/g; 1:9 v/v conc. aq. ammonia:methanol solution (10 mL) and then by automated chromatography on a prepacked Biotage Sfär C18 cartridge (30 g) using a linear gradient of 0.1% NH₃ CH₃CN (10-100%) in H₂O over 10 CV to afford **15** as a white amorphous solid (32 mg, 47 %). ¹H-NMR (500 MHz, Acetone-*d*₆): δ_H 8.39 (1 H, d, *J* = 8.3 Hz), 8.01 (1 H, dd, *J* = 8.2, 1.1 Hz), 7.89 – 7.83 (2 H, m), 7.82 (1 H, app. s), 7.80 (1 H, d, *J* = 8.9 Hz), 7.66 (1 H, d, *J* = 8.9 Hz), 7.62 (1 H, ddd, *J* = 8.3, 6.9, 1.3 Hz), 7.53 – 7.46 (4 H, m), 6.82 (1 H, s), 3.96 (2 H, t, *J* = 6.8 Hz), 3.80 (2 H, t, *J* = 5.5 Hz), 3.61 (2 H, q, *J* = 5.0 Hz), 3.37 (2 H, td, *J* = 6.8, 1.1 Hz). ¹³C-NMR (126 MHz, Acetone-*d*₆): δ_C 164.8, 159.2, 154.0, 143.0, 131.7, 131.1, 129.9, 129.9, 129.6, 127.3, 126.4, 126.2, 125.9, 125.1, 124.0, 122.2, 120.2, 113.4, 70.6, 69.7, 44.0, 27.1. HRMS (ESI⁺): *m/z* calc. for C₂₄H₂₂N₃O₃ 400.1661 [M+H]⁺, found 400.1662 [M+H]⁺. Reverse-phase HPLC, eluting with 0.1 % TFA H₂O/ 0.1% TFA CH₃CN isocratic 90/10 for 2 min and then gradient to 0/100 in 30 min, Flow = 1 mL/min λ = 220 nm, *t_R* = 17.44 min (97%).

5.2 Stability Studies

5.2.1 Buffer stability assay

Compound **1771**, **9a-r**, **10**, **11**, **14**, and **15** were incubated in 100 mM phosphate buffer at pH 7.4 and acetonitrile at 37 °C in a water bath. The incubation mixture was prepared by dissolving 20 µL of a 1 mg/mL stock solution of tested compound in DMSO in preheated buffer acetonitrile 1:1 solution (360 µL). Aliquots of 25 µL were withdrawn at appropriate intervals and analysed by HPLC eluting with isocratic 0.1 % TFA H₂O/ 0.1% TFA CH₃CN 90/10 for 1 min and then gradient to 0/100 in 10 min, Flow = 1 mL/min λ = 254 nm.

5.2.2 Rat Serum stability assay

Compound **1771**, **9a-r**, **10**, **11**, **14**, and **15** were incubated in rat serum and 100 mM phosphate buffer at pH 7.4 at 37 °C in a water bath. The incubation mixture was prepared by dissolving 40 µL of a 1 mg/mL stock solution of tested compound in DMSO in preheated buffer (260 µL) and then adding a thawed aliquot of rat serum (120 µL). Aliquots (25 µL) were drawn at appropriate intervals, mixed with methanol (25 µL), centrifuged for at least 5 minutes (6000 rpm, RCF 2000 g) then the supernatant (25 µL) analysed by HPLC eluting with isocratic 0.1 % TFA H₂O/ 0.1% TFA CH₃CN 90/10 for 1 min and then gradient to 0/100 in 10 min, Flow = 1 mL/min λ = 254 nm.

5.2.3 Description of Analytical LC-MS Conditions

LC-MS analysis of compounds **9a** and **14** was performed at 60 and 20 min incubation in rat serum
LC-MS analysis of compounds **9a** and **14** was performed at 60 and 20 min incubation in rat serum on a ACE 3 C18, , particle size 3 µm, pore size 300 Å HPLC column (3 µm, 2.1 x 100 mm) with a 5-95% CH₃CN gradient in 0.1 % formic acid, at a flow rate of 0.3 mL/min. The quadrupole mass spectrometer equipped with an ES-API source was operated in the positive and negative-ion mode. Full scan mass spectra were recorded over a range of m/z 150-1000. The UV detector was operated at 210, 254 & 280 nm.

5.2.3 Rat and human and liver microsome stability assay¹⁹

Compound **1771**, **9b** and **10** (final substrate concentration 1 µM; final DMSO concentration 0.25 %) were preincubated at 37 °C in rat or human liver microsomes (final protein concentration 0.5 mg/mL), in 0.1 M phosphate buffer pH 7.4 prior to the addition of NADPH (final concentration 1 mM) to initiate the reaction. Two species-specific positive controls are also incubated alongside the compounds for each species. All incubations are performed singularly for each compound. Each compound is incubated for 0, 5, 15, 30 and 45 min. The reactions are stopped by transferring incubate into acetonitrile at the appropriate time points, in a 1:3 ratio. The termination plates are centrifuged at 3,000 rpm for 10 min at 4 °C to precipitate the protein. Following protein precipitation, the sample supernatants are combined in cassettes of up to four compounds, internal standard is added and

samples analysed using Cypotex generic LC-MS conditions on a high-resolution accurate mass instrumentation (TOF-MS) in positive ion mode. From a plot of $\ln$ peak area ratio (peak area/internal standard peak area) against time, the gradient of the line is determined. Subsequently, half-life and intrinsic clearance are calculated using the equations below:

Elimination rate constant (k) = (- gradient)

Half life ($t_{1/2}$)(min) $0.693/k$

Intrinsic clearance (CL_{int})($\mu\text{L}/\text{min}/\text{mg protein}$) = $V \times 0.693/t_{1/2}$

where V = Incubation volume (μL)/Microsomal protein (mg)

5.3 Biological evaluation

5.3.1 Bacterial Strains and Culture Conditions

Bacterial strains are listed in Table S1. *S. aureus* strains were routinely grown on tryptic soy agar (TSA) for 18 h at 37 °C. Single colonies of *S. aureus* were subsequently transferred into 2 mL of tryptic soy broth (TSB) and grown for a further 18 h at 37 °C while being shaken at 180 rpm. *E. coli* was grown in LB broth at 37 °C. Phenylalanine-arginine β -naphthylamide (PA β N) (Sigma) was dissolved in sterile water.

5.3.2 Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MIC) of **1771** and its derivatives **1**, **9a-q**, **11**, **14** and **15** were performed using the micro broth dilution method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. *S. aureus* strains were grown in Mueller-Hinton broth (MHB) for 18 h at 37°C. The overnight cultures were subsequently sub-cultured 1:200 and grown to exponential phase ($OD_{600\text{nm}} = 0.5-0.6$) in fresh broth. **1771** and its derivatives were re-constituted in DMSO and further diluted by performing a 1:2 serial dilution ranging between 256-0.125 $\mu\text{g}/\text{mL}$ in a 96-well micro-titre plate. One hundred microlitres of 0.5 McFarland standardized inoculum (approximately equal to 5×10^5 CFU) were added into each well. The plate was incubated for 24 h at 37°C and the MIC was determined as the lowest concentration that completely inhibited bacterial growth.

5.3.3 LTA Quantification by Western Blot

Overnight cultures of *S. aureus* strains were diluted 1:200 in fresh tryptic soy broth (TSB) and grown to mid-log phase ($OD_{600} \approx 0.3$). Cultures were then treated with compound **9b** or **KKL-35** at either 0.25x, 0.5x, 1x or 4x their respective MICs and incubated for a further 2 h at 37 °C. Following treatment, cultures were adjusted to a total volume of 10 mL at $OD_{600} \approx 0.6$, harvested by centrifugation at 4000 rpm, and resuspended in 300 μ L phosphate-buffered saline (PBS) containing 200 μ g/mL lysostaphin. Samples were incubated at 37 °C for 45 min to facilitate cell wall digestion. Subsequently, 100 μ L of 4x SDS sample buffer was added, and samples were heated at 95 °C for 20 min. Insoluble debris was removed by centrifugation at 16,000 rpm for 10 min. The resulting supernatant, containing lipoteichoic acid (LTA), was separated by SDS-PAGE using a 4–20% Tris–glycine gel (Bio-Rad) and transferred to a PVDF membrane with a Trans-Blot Turbo transfer system (Bio-Rad). Membranes were blocked overnight in TBST supplemented with 5% semi-skimmed milk, then incubated with a mouse anti-LTA primary antibody (Hycult, HM2048; 1:2000 dilution), followed by a goat anti-mouse HRP-conjugated secondary antibody (Proteintech, SA00001; 1:2000 dilution). Detection was performed using an enhanced chemiluminescence kit (Amersham ECL, Cytiva).

5.3.4 AsedaSciences SYSTEMETRIC Cell Health Screen

Compounds **1771** and **1**, **9a**, and **9b** were evaluated across a concentration range of 100 μ M to 5 nM using the AsedaSciences SYSTEMETRIC cell health screening platform. This assay is a multiparametric acute cell stress screen conducted in HL-60 cells, integrating automated flow cytometry, a panel of fluorescent physiological reporter dyes, and machine learning, as previously described.^{23, 24} In brief, the machine learning classifier was trained on a dataset of 300 compounds comprising marketed drugs, withdrawn drugs, and research compounds, encompassing the full spectrum of phenotypic responses observed in the cell health assay, from no detectable effect to acute toxicity. For test compounds, the classifier integrates all measured cell stress parameters simultaneously to generate a cell health index (CHI), a probabilistic score ranging from 0 to 1 that reflects the likelihood of the compound being classified within the high-toxicity risk category

5.4.5 Membrane permeability

S. aureus strain LAC was grown O/N in 2 ml of MHB. Bacterial culture was normalised to an $OD_{600nm} = 0.1$ in 10 ml and subsequently pelleted by centrifugation at 4000 rpm for 10 min. Bacterial pellet was then resuspended in fresh 10 mL MHB containing 5 μ M propidium iodide (PI) (Sigma). Using a 96-well, black-walled, clear-bottom microplate (ThermoFisher) antibiotic concentrations were serially diluted in MHB at 2X the desired concentration in 100 μ L, following which 100 μ L of the prepared bacterial

suspension containing PI was added. Fluorescence was measured every 30 min for 4 h at 37 °C with shaking between readings, using an excitation and emission wavelength of 530-615 nm respectively (BMG Labtech, ClarioStar).

5.4.6 Phospholipid vesicle synthesis and lysis assay.

Gram-positive cytoplasmic membranes were synthesised using carboxyfluorescein-filled vesicles with a phospholipid content mirroring that of *S. aureus*, specifically 60% [wt/wt] phosphatidylglycerol, 40% cardiolipin.²⁵ Phospholipids were purchased from Avanti polar lipids and vesicles generated as previously described.^{26, 27} 50 µL of serially diluted antibiotic was incubated with 50 µL of vesicles and fluorescence was measured every 60 s using an excitation and emission wavelength of 485-520 nm respectively (BMG Labtech, ClarioStar). Normalised fluorescence was calculated using the equation $(F_t - F_0)/(F_m - F_0) * 100$ where F_t is the average fluorescence value at a specific time point, F_0 is the minimum and F_m is the maximum fluorescence value in determined using the negative and positive controls of HEPES buffer and 0.1 % Triton X-100, respectively.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

The following is the Supplementary data to this article:

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Brandon Marshall: Data curation, Formal analysis, Investigation, Validation, Writing – review & editing.

Edward J.A. Douglas: Formal analysis, Investigation, Writing – review and editing

Kyle Perrins: Formal analysis, Investigation

Ebba Qviberg: Formal analysis, Investigation, Writing – review and editing

Maisem Laabei: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

Michaela Serpi: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Highlights

- This study investigates the structural modifications of 2-amino-5-phenyl-1,3,4-oxadiazole-based compound **1771**, which serves as LTA inhibitors and antibacterial agents, with the aim of improving its stability in serum.
- Structural requirements for antibacterial activity were defined
- A compound with increased stability and good antibacterial activity was identified.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: