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Article

Development of 1,2,4-Oxadiazole Antimicrobial Agents to Treat Enteric Pathogens within the Gastrointestinal Tract

Noel P. Pitcher, Jitendra R. Harjani, Yichao Zhao, Jianwen Jin, Daniel R. Knight, Lucy Li, Papanin Putsathit, Thomas V. Riley, Glen P. Carter,*,○ and Jonathan B. Baell*,○

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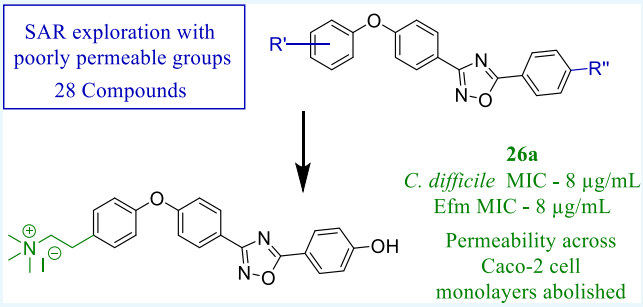
ABSTRACT: Colonization of the gastrointestinal (GI) tract with pathogenic bacteria is an important risk factor for the development of certain potentially severe and life-threatening healthcare-associated infections, yet efforts to develop effective decolonization agents have been largely unsuccessful thus far. Herein, we report modification of the 1,2,4-oxadiazole class of antimicrobial compounds with poorly permeable functional groups in order to target bacterial pathogens within the GI tract. We have identified that the quaternary ammonium functionality of analogue **26a** results in complete impermeability in Caco-2 cell monolayers while retaining activity against GI pathogens *Clostridioides difficile* and multidrug-resistant (MDR) *Enterococcus faecium*. Low compound recovery levels after oral administration in rats were observed, which suggests that the analogues may be susceptible to degradation or metabolism within the gut, highlighting a key area for optimization in future efforts. This study demonstrates that modified analogues of the 1,2,4-oxadiazole class may be potential leads for further development of colon-targeted antimicrobial agents.



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■ INTRODUCTION

The gastrointestinal (GI) tract serves as an important site of colonization for many pathogenic bacteria prior to the initiation of infection. The "healthy" microbiota that resides in the GI tract is normally considered to offer "colonization resistance" against invading pathogens but this can be compromised by exposure to antimicrobials or in certain illnesses.[1−3] Such disruption can lead to the overgrowth of GI pathogens, which is a major risk factor in the development of several different healthcare-associated infections.[4−8] Among the most significant nosocomial pathogens that colonize the GI tract is *Clostridioides difficile*, which causes a range of colonic infections collectively referred to as *C. difficile* infections (CDI). CDI may lead to mild to severe diarrhoea and can lead to potentially fatal complications such as pseudomembranous colitis and toxic mega-colon.[9,10] Indeed, *C. difficile* has been classified as an urgent threat to public health by the United States Centers for Disease Control and Prevention (CDC).[11] Traditional first-line treatments including metronidazole and vancomycin are effective for most CDI cases but approximately 15−30% of patients will suffer a recurrence of infection after cessation of treatment.[12,13] More recently, fidaxomicin, a narrow spectrum macrocyclic antimicrobial from the tiacumicin family, has become a recommended treatment for CDI. This antimicrobial is associated with lower rates of recurrence than vancomycin or metronidazole.[14,15] Also, metronidazole is no longer recommended as a first line therapy of CDI due to treatment failures in the US.[14]

Another important nosocomial pathogen is *Enterococcus faecium*.[16] Colonization of the GI tract by *E. faecium* is recognized as a major risk factor for the onset of potentially life-threatening diseases such as bacteraemia and infective endocarditis.[8,17] *E. faecium* has a high propensity for the development of antimicrobial resistance due to its malleable genome which has resulted in the emergence of multidrug-resistant (MDR) strains,[18] most notably vancomycin-resistant *E. faecium* (VREfm). The CDC has declared VREfm a serious threat to human health[11] and it has been included in the 2017 World Health Organization priority pathogen list for the development of new antimicrobials,[19] which highlights the limited therapeutic options available for treating this pathogen. High rates of VREfm infection have rendered the traditional first-line treatment of vancomycin increasingly ineffective against *E. faecium* infections, with clinicians now increasingly using linezolid and daptomycin.[17] Reports documenting the emergence of linezolid and daptomycin resistance in VREfm

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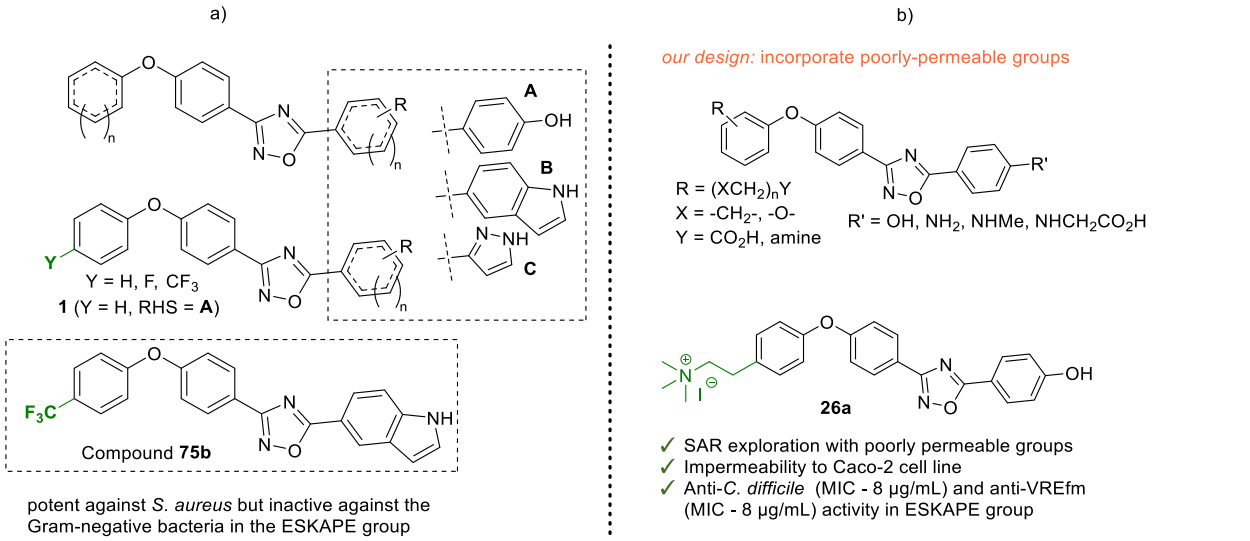

Figure 1. (a) Reported antimicrobials of a new oxadiazole class, with leading compounds **1** and **75b**; (b) modifications reported here, leading to **26a** that exhibits potent anti-*C. difficile* and anti-VREfm activity. (a) Previous work by Chang and Mobashery et al.[26,28−30] (b) This work.

are therefore of great clinical concern.[20,21] Furthermore, the development of decolonization agents that can be used to eradicate *E. faecium* from the gut has been an aim for several years without success.[22−25]

Despite exhibiting different clinical manifestations, the disease states caused by both of these pathogens arise following initial colonization of the GI tract. There is therefore an urgent need for novel antimicrobials that selectively target pathogens within the GI tract while maintaining the integrity of gut microbiota. Such agents would undoubtedly improve treatment outcomes for difficult cases of *C. difficile* and *E. faecium* infections.

The 1,2,4-oxadiazole antimicrobial compound class was reported in 2014 by Chang and Mobashery et al. and displayed potent activity against important Gram-positive pathogens in the ESKAPE panel.[26,27] Analogue **1** emerged as a lead compound in the study with activity against *Staphylococcus aureus*, *Enterococcus faecalis*, and *E. faecium*, and this activity was maintained across multiple strains, including those exhibiting vancomycin resistance. Analogue **75b** was another leading compound. Significantly, there was no activity observed against the Gram-negative bacteria in the ESKAPE group, potentially demonstrating a selectivity profile that may be sparing of important members of the GI tract microbiota. Chang and Mobashery et al. have advanced several analogues in the class with a focus on developing effective treatments for methicillin-resistant *S. aureus* (MRSA) (Figure 1a).[28−30]

Here, we explore the utility of the 1,2,4-oxadiazole compound class for the treatment of GI pathogens by attaching substituents that are predicted to reduce permeability in the GI tract. The idea is that such compounds may therefore reach the colon in relatively higher concentrations (Figure 1b). In an important proof-of-concept, several analogues modified to be less Caco-2 permeable maintained antimicrobial activity. Quaternary ammonium derivative **26a** is of particular note with complete impermeability to Caco-2 cells and notable anti-*C. difficile* and anti-VREfm activity.

■ RESULTS AND DISCUSSION

Minimum Inhibitory Concentration and Time-Kill Assays of Lead Compound 1. The lead 1,2,4-oxadiazole compound **1** was assessed by minimum inhibitory concentration (MIC) assay and exhibited a modest potency of 6 μg/mL against *C. difficile*, comparable to that of vancomycin (2 μg/mL). Compound **1** also showed concentration-dependent killing kinetics in time-kill assays and rapid bactericidal activity when using ≥2 × MIC (12−48 μg/mL), which was similar to the activity observed for the positive control compound benzalkonium chloride (a quaternary ammonium compound with known membrane lysing activity) and superior to the bacteriostatic activity of vancomycin (Figure 2).

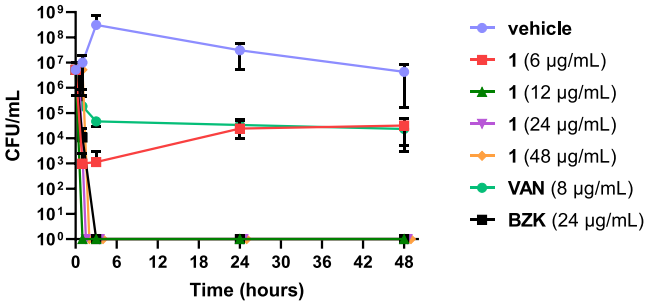

Figure 2. Time-kill analysis of **1** against *C. difficile* strain EDN0008. Blue circles are DMSO-containing untreated cultures (vehicle), red squares are cultures treated with 6 μg/mL **1**, green triangles are cultures treated with 12 μg/mL **1**, purple triangles are cultures treated with 24 μg/mL **1**, orange diamonds are cultures treated with 48 μg/mL **1**, turquoise circles are cultures treated with 8 μg/mL vancomycin, and black squares are cultures treated with 24 μg/mL benzalkonium chloride. Compounds were tested using three independent biological replicates with the data shown representing the mean ± SEM. VAN—vancomycin, BZK—benzalkonium chloride, and CFU—colony-forming units.

Our previous findings demonstrated that compound **1** also displayed potent activity against a range of clinically relevant *E. faecium* strains, including both VREfm and daptomycin-resistant strains.[31] Compound **1** was not toxic against the human cell line HepG2 at the *E. faecium* MIC_{90} (2 μg/mL); however, reduced viability was observed at higher concentrations. This was deemed to represent a potentially narrow therapeutic window, but analogues of compound **1** have been

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reported by Spink et al. in 2015 with activity against *S. aureus* and reduced toxicity against HepG2 cells.[28] Thus, we hypothesized that optimization of the therapeutic index for this compound class would be highly feasible.

General Strategy. To address the high bioavailability reported for the 1,2,4-oxadiazole compound class,[26] we proposed that their physicochemical properties could be modified to reduce "drug-likeness", thereby decreasing systemic exposure and leading to accumulation in the colon. Amine and carboxylic acid functional groups that are charged at physiological pH were linked to the oxadiazole structure to induce ionization after oral administration and reduce compound permeability across the small intestine.[32,33] Indeed, similar approaches have shown success previously by utilizing the poorly permeable tetramic acid moiety to localize metronidazole in the GI tract.[34]

Suitable positions for linking to the oxadiazole structure needed to be carefully selected as to not decrease antimicrobial activity. It was noted in similar oxadiazole analogues reported for the treatment of MRSA[28] that trifluoromethyl groups at the 4 position of ring A had minimal effect on *in vitro* activity but improved oral bioavailability (Figure 3). Therefore, the

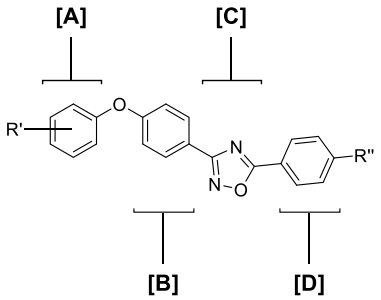

Figure 3. General structure of the oxadiazole class, consisting of four main aromatic rings (A−D). R′ and R″ represent the positions explored to link poorly permeable groups.

trifluoromethyl group was excluded from our oxadiazole analogues and ring A was instead used to introduce the poorly permeable groups. Our structure−activity relationship (SAR) investigation probed all aromatic positions of ring A while exploring different linkers to attach the ionizable functional groups. The hydroxyl in compound **1** was maintained consistent for the analogues in the series as it was previously recognized that a hydrogen bond donor at the position was essential for activity against MRSA.[28]

Additionally, we explored replacement of the phenol with an aniline to probe modification of ring D while maintaining the critical hydrogen bond donor. Primary and secondary aniline groups were synthesized to investigate if substitution from ring D was tolerated and if it could offer an alternative site to link the poorly permeable groups.

Synthetic Chemistry. Modified oxadiazole analogues were synthesized as outlined in Scheme 1. Reagents **2a−h** were used to prepare *t*-Bu protected carboxylic acid substrates **3a−h**, followed by installation of a benzonitrile moiety using a known Ullmann-type reaction procedure.[26] Hydroxylamine addition to the nitrile yielded amidoximes **6a−h** which then underwent coupling, cyclization, and dehydration with benzoic acid **7** in a "one-pot" process to form the 1,2,4-oxadiazole ring C.[35] An acetyl group was used as a transient protecting group to inhibit the ring D phenol from reacting during coupling and was subsequently cleaved in situ at high temperature to reveal the free phenol in compounds **8a−h**. Protecting group removal was achieved under acidic conditions to afford final analogues **9a−h**.

A more concise scheme was also devised to introduce the derivatized phenols at the penultimate step, as outlined in Scheme 2. Hydroxylamine addition to 4-iodobenzonitrile **4** and then reaction with acyl chloride **11** yielded the key intermediate **12**.[26] Optimized Ullmann-type reaction conditions were used to couple the derivatized phenols, during which the *tert*-butyldimethylsilyl (TBS) provided a transient protecting group that was cleaved in situ. The derivatized phenol reagents **13a−i** were individually prepared from commercially available material as described in the Supporting Information. Deprotection of the boc groups under acidic conditions yielded final analogues **15a−i**.

Aniline series analogues were synthesized as outlined in Scheme 3. Amine- and nitro-substituted aryl carboxylate substrates **17a−d** were subjected to coupling, cyclization, and dehydration with amidoxime intermediate **18** to form the 1,2,4-oxadiazole ring C and afford analogues **19c** and **19d**.[35] Treatment with 4 M HCl in 1,4-dioxane then afforded compounds **20a** and **20b**. Compound **22** was synthesized *via* sodium hydride-mediated alkylation of intermediate **19a** using 2-bromoethyl methyl ether, followed by protecting group removal. Alkylation of **20a** using *tert*-butyl bromoacetate and subsequent acidic removal of the protecting group was undertaken to synthesize **24**.

Dimethylamine and quaternary ammonium substituted analogues **25a**, **25b**, **26a**, and **26b** were synthesized from their corresponding primary amine analogues, as outlined in Scheme 4. Boc-deprotection reactions of **14e** and **14h** were undertaken using standard conditions followed by careful neutralization to remove the trifluoroacetic acid (TFA) salts of the amines while also avoiding removal of the acidic phenolic protons. Treatment with formaldehyde and $NaBH(OAc)_3$ was undertaken to convert the primary amine intermediates to the dimethylamine groups,[36] and subsequent reaction with iodomethane selectively yielded the quaternary ammonium analogues without phenol methylation.[37]

MIC and Time-Kill Assays of Oxadiazole Analogues. The antimicrobial activity of the modified oxadiazole analogues against *C. difficile* and *E. faecium* was evaluated using standardized broth microdilution MIC assays. The initial SAR exploration sought to identify the best position for substitution from ring A and which poorly permeable groups were suitable for maintaining potency. The attachment of certain amine and carboxylic acid groups was tolerated in terms of *C. difficile* activity but was dependent on linker length and aromatic position (Table 1). Carboxylic acid substituents with ethylene linkers **9b** (4 μg/mL) and **9d** (8 μg/mL) were favored from the 2 and 3 positions, displaying similar activities to analogue **1** (6 μg/mL). However, a 4-fold loss in potency was observed for the same substituent in the 4 position (**9g**, 25 μg/mL). Decreasing the linker length from ethyl (**9b**, **9d**, and **9g**) to methyl (**9a**, **9c**, and **9f**) was not favored with at least a 2-fold loss in potency against *C. difficile* for the carboxylic acid groups at all aromatic positions on ring A. The methyl ether linkers (**9e** and **9h**) also caused a loss of activity compared with their corresponding ethyl linkers (**9d** and **9g**), thereby demonstrating that additional hydrogen bond accepting or hydrophilic interactions were not favored from the benzylic site. The trend of a 2-fold increase in potency for the ethyl

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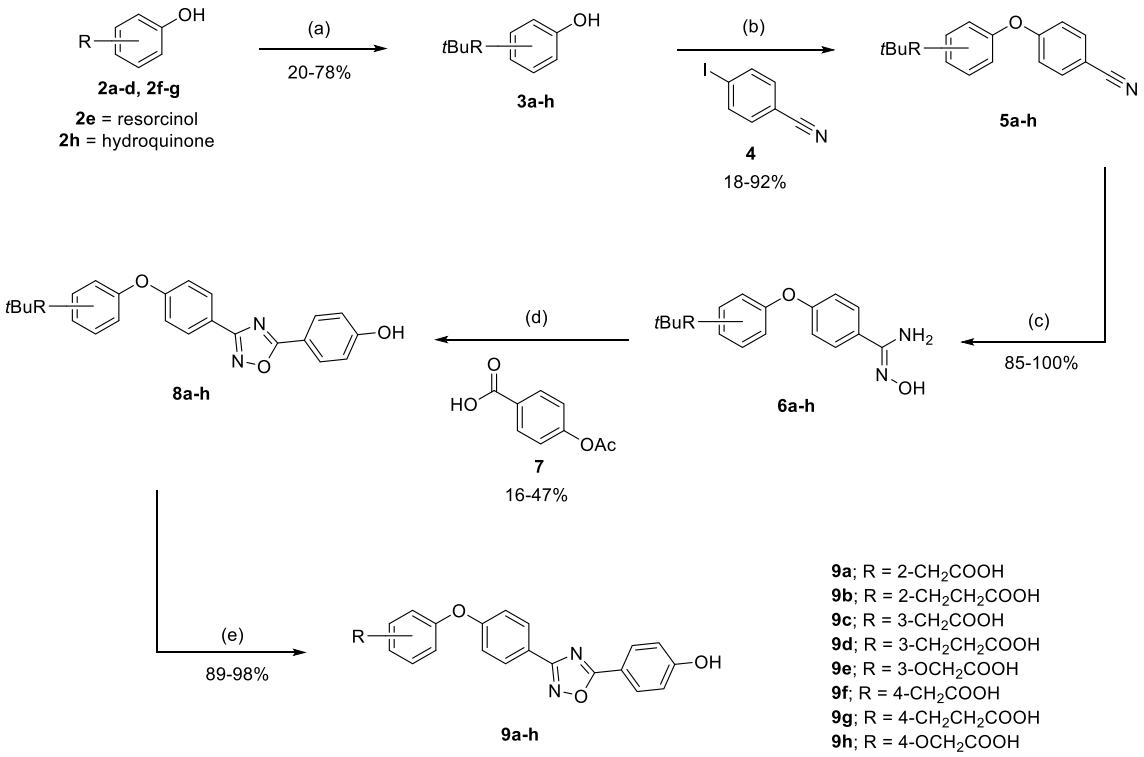
Scheme 1. Synthesis for Modified Oxadiazole Analogues 9a–h[a]



[a]Reagents and conditions: (a) (i) 2-*tert*-butyl-1,3-diisopropylisourea, DMAP, DCM, rt, 96 h; (ii) 1,1′-carbonyldiimidazole, 1,8-diazabicyclo[5.4.0]-undec-7-ene, *tert*-butanol, DMF, 65 °C, 72 h; (iii) *tert*-butyl bromoacetate, K_2CO_3, DMF, rt, 16 h; (b) **4**, CuI, Cs_2CO_3, *N*,*N*-dimethylglycine, 1,4-dioxane, 100 °C, 48 h; (c) $NH_2OH{\cdot}HCl$, Et_3N, MeOH, 60 °C, 6 h; (d) **7**, HOBt, EDC·HCl, DMF, rt for 16 h to 100 °C for 16 h; (e) TFA, DCM, rt, 8 h.

linkers (**15b** and **15e**) compared with methyl (**15a** and **15d**) was also observed for the amine substituents at both the 2 position and 4 position. Ethylamine substitution was favored at the 3 and 4 positions with analogues **15c** (4 μg/mL) and **15e** (3 μg/mL) giving slight increases in potency against *C. difficile* as compared to analogue **1** (6 μg/mL).

The replacement of the phenol ring D with an unsubstituted aniline (**20a**, >50 μg/mL) was detrimental to *C. difficile* activity. Interestingly, the activity was able to be somewhat restored upon functionalization of the aniline with methyl (**20b**, 8 μg/mL), methoxyethyl (**22**, 8 μg/mL), acetic acid (**24**, 13 μg/mL), or conversion to nitro groups (**19d**, 8 μg/mL). The analogue bearing a nitro substituent (**19d**) had similar potency to the unsubstituted phenol analogue (**1**), and it was apparent that a hydrogen bond donor on ring D was not essential for activity against *C. difficile*.

Modification of the oxadiazole structure was less tolerated in terms of *E. faecium* activity, with analogue **1** remaining the most potent at 2 μg/mL. Carboxylic acid substitution led to a complete loss of potency, regardless of the aromatic position or linker variant. The decrease in activity was less pronounced when attaching the amine substituents, with only a 2-fold loss in activity at the 4 position (**15d**, **15e**, 4 μg/mL) and a 4-fold loss at the 2 position (**15a**, **15b**, 8 μg/mL). Linking with methyl or ethyl groups had no effect on the activity for these analogues. There was a complete loss of potency for all analogues from the aniline series which suggested that the hydroxyl on ring D was important for activity against *E. faecium*.

The initial SAR exploration suggested that modification with amino groups could be made while maintaining antimicrobial activity. Specifically, analogue **15e** with ethylamine substitution from the 4-position of ring A appeared to be suitable, exhibiting modest activity against both *C. difficile* (3 μg/mL) and *E. faecium* (4 μg/mL). Further, analogue **15e** displayed rapid killing when assessed in time-kill assays, with a 100% kill of *C. difficile* at 4× MIC (12 μg/mL) within 3 h and VREfm at 2× MIC (8 μg/mL) within 1 h (Figure 4). This rapid rate of killing was comparable to the positive control benzalkonium chloride. When benchmarked against **1**, we observed superior killing activity of **15e** against *C. difficile* at 6 μg/mL and indistinguishable kill kinetics at 12 μg/mL. Against VREfm, **15e** displayed comparable killing kinetics to **1** at 4 μg/mL despite the 2-fold loss of activity identified above, with both compounds killing 100% of VREfm within 24 h. Of note, while growth of VREfm remained undetectable after exposure to 4 μg/mL of **15e** for the duration of the assays, rebounding growth of VREfm was observed after 48 h in cultures exposed to **1**. At a concentration of 8 μg/mL, however, the killing kinetics of **1** and **15e** were indistinguishable, with 100% of VREfm being killed within 1 h of exposure and no rebounding growth observed for either compound.

1 has previously been shown to possess membrane-lytic activity, with concentrations in excess of 64 μg/mL leading to haemolysis in human erythrocytes.[31] To determine whether **15e** displayed a similar membrane-lytic activity as **1**, the compound was tested against human erythrocytes as before (Figure 4c). Limited haemolysis was observed following incubation of human erythrocytes with 4 μg/mL **15e**

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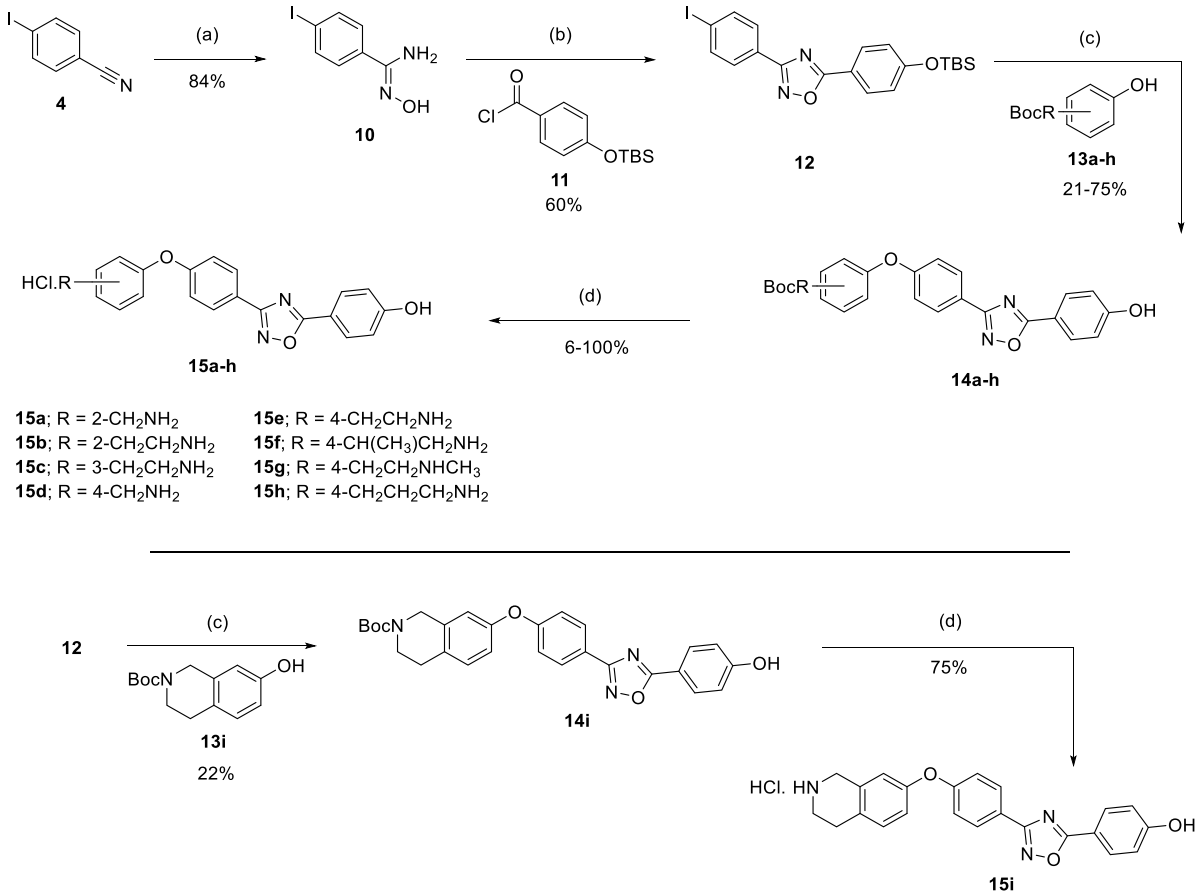
Scheme 2. Synthesis for Modified Oxadiazole Analogues 15a−i[a]



[a]Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, Et_3N, MeOH, 60 °C, 6 h; (b) 11 (synthesis previously described[26]), toluene, 120 °C, 27 h; (c) **13a−i**, 1,3-diphenyl-1,3-propanedione, Cs_2CO_3, copper(II) acetylacetonate, DMSO, 90 °C, 24 h; (d) 4 M HCl/1,4-dioxane, rt, 7 h

(approximate MIC of *C. difficile* and *E. faecium*), with only 2.5% (range: 0−7%) of erythrocytes undergoing lysis. As previously observed for **1**, increasing concentrations of **15e** resulted in increased levels of hemolysis in a dose-dependent manner, with a concentration of ≥64 μg/mL leading to substantial levels of haemolysis. These data therefore provide evidence to show that **15e** most likely operates through a membrane-lysing mechanism.

Further SAR studies of analogue **15e** were undertaken to identify the optimal linker for use and explore functionalization of the amine (Table 2). Against *C. difficile*, the introduction of a branched methyl group at the benzylic position of the linker (**15f**, 16 μg/mL) saw a 5-fold loss in potency compared with the ethyl linker (**15e**, 3 μg/mL), which suggested that flexibility of the linkers may be important for activity. Conversely, activity was retained when further restricting the linker flexibility by adding an N-methylene bridge to the 3 position of ring A to form a tetrahydroisoquinoline moiety (**15i**, 4 μg/mL). Methylation of the ethylamine group to form both secondary (**15g**) and tertiary amines (**25a**) caused slight decreases in activity to 4 μg/mL. The observation that the dimethylamine group retained activity suggested that favorable interactions were made *via* the amine basicity or a hydrogen bond acceptor effect, rather than through hydrogen bond donating. The conversion of the amine group into a quaternary ammonium (**26a**, 8 μg/mL) was also tolerated but with another slight drop in *C. difficile* activity observed. Nonetheless, the analogue was of particular interest because it represented an active compound that also possessed a permanently cationic group which would greatly reduce permeability across the small intestine.[32,33] A further look at modifying the linker identified that a slight increase in activity against *C. difficile* was obtained when the length was increased to a propyl (**15h**, 2 μg/mL). Unfortunately, this increase in potency was not maintained when forming the tertiary amine **25b** (16 μg/mL) or quaternary ammonium **26b** (8 μg/mL).

The SAR for *E. faecium* was generally similar to the trends observed for changes in activity against *C. difficile*. Restriction of the linkers in analogues **15f** and **15i** had no effect on *E. faecium* activity. Methylation of the ethylamine to form secondary and tertiary amines **15g** and **25a** also saw no change in potency, and quaternization (**26a**, 8 μg/mL) caused only a 2-fold decrease in activity compared with the primary amine **15e** (4 μg/mL). Extension of the linker to a propylamine (**15h**, 2 μg/mL) gave a 2-fold increase in activity; however, again this increased activity was not translated when forming the quaternary ammonium (**26b**, 16 μg/mL).

SAR Summary. Modification of the oxadiazole analogues with amine substituents was generally more favored than the carboxylic acid groups. Exploring changes to the linkers indicated that while different variations were tolerated, they did not lead to significant increases in activity. With the discovery of analogue **26a**, we identified that a poorly permeable quaternary ammonium group could be linked to

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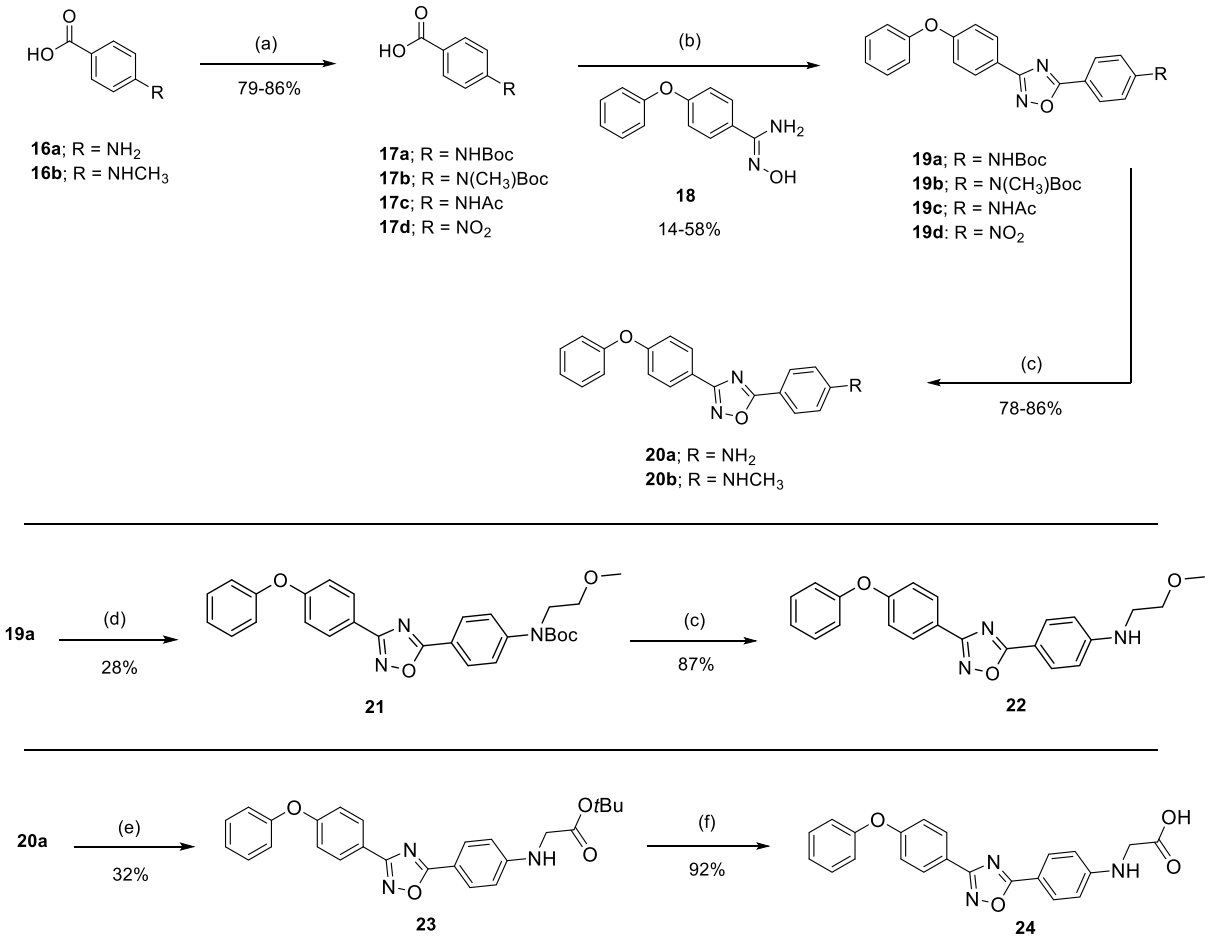
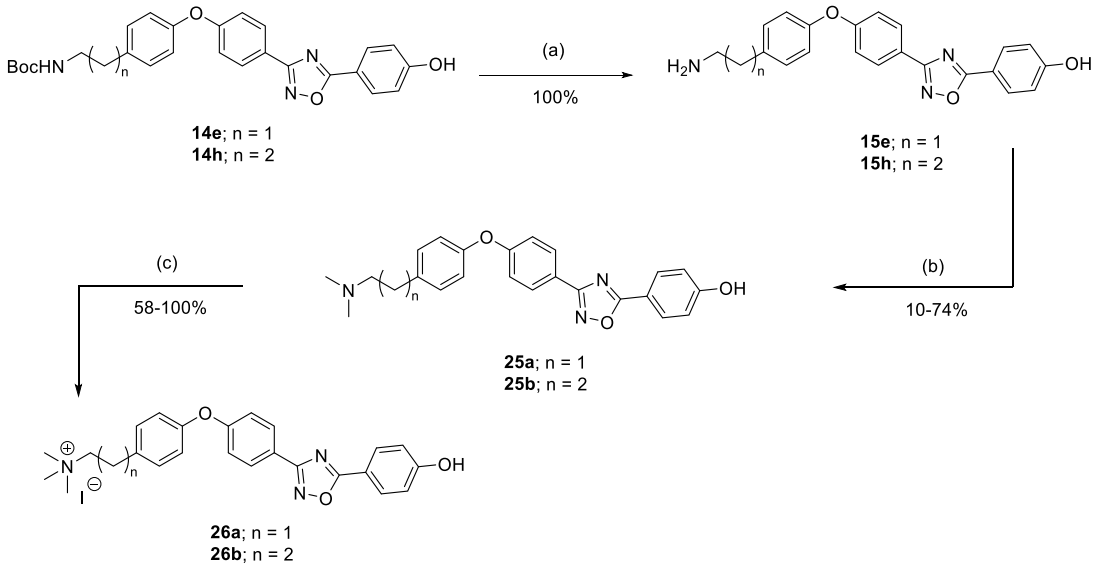
Scheme 3. Synthesis of Aniline Series Analogues[a]



[a]Reagents and conditions: (a) Boc_2O, $NaHCO_3$, $MeOH/H_2O$, rt, 24 h; (b) **18** (synthesis previously described[26]), HOBt, EDC·HCl, DMF, rt for 16 h to 100 °C for 16 h; (c) 4 M HCl/1,4-dioxane, rt, 6 h; (d) NaH (60% in mineral oil), DMF, 0 °C to rt, 1 h, 1-bromo-2-methoxyethane, 0 °C to rt, 2 h; (e) *tert*-butyl bromoacetate, DIPEA, acetonitrile, 80 °C, 48 h; (f) TFA, DCM, rt, 8 h

Scheme 4. Synthesis of Methylated-Amine Substituted Analogues[a]



[a]Reagents and conditions: (a) TFA, DCM, rt, 7 h; (b) 37% aq formaldehyde, $NaBH(OAc)_3$, acetonitrile/H_2O, rt, 50 min; (c) MeI, DCM/MeOH, rt, 24 h.

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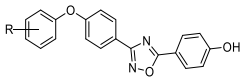
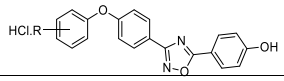
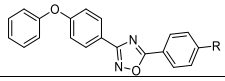
Table 1. Structure and Activity of Oxadiazole Analogues

Entry	R	*C. difficile* MIC (μg/mL)[a]	VREfm MIC (μg/mL)[a]
			
1	H	6	2
9a	2-CH_2COOH	>50	>32
9b	2-CH_2CH_2COOH	4	>32
9c	3-CH_2COOH	50	>32
9d	3-CH_2CH_2COOH	8	>32
9e	3-OCH_2COOH	50	>32
9f	4-CH_2COOH	50	>32
9g	4-CH_2CH_2COOH	25	>32
9h	4-OCH_2COOH	50	>32
			
15a	2-CH_2NH_2	16	8
15b	2-$CH_2CH_2NH_2$	8	8
15c	3-$CH_2CH_2NH_2$	4	n.d.
15d	4-CH_2NH_2	6	4
15e	4-$CH_2CH_2NH_2$	3	4
			
19c	-NHAc	>50	>32
19d	-NO_2	8	>32
20a	-NH_2	>50	>32
20b	-$NHCH_3$	8	n.d.
22	-$NHCH_2CH_2OCH_3$	8	>32
24	-$NHCH_2COOH$	13	n.d.
Vancomycin		0.5 - 2	n.d.
Daptomycin		n.d.	2

[a]n.d.—not determined.

the oxadiazole structure while retaining moderate MICs of 8 μg/mL for both *C. difficile* and *E. faecium*.

***In Vitro* Permeability Studies.** We sought to measure the effect that each modification had on compound permeability by using differentiated Caco-2 cell monolayers. Four analogues were assessed to represent the different types of ionizable groups and linkers (Table 3). Analogues **9e** and **9g** bearing carboxylic acid groups displayed moderate permeability with P_{app} (10^{-6} cm/s) values of 5.3 ± 0.20 and 18 ± 1.0. Measurable concentrations of the primary amine-substituted analogue **15e** could not be detected in the acceptor chamber, prohibiting the determination of a P_{app} value. However, the low mass balance indicated that the analogue was likely being retained in the Caco-2 cell monolayer rather than having intrinsically low permeability.[38] Quaternary ammonium-substituted analogue **26a** could also not be detected in the acceptor chamber but was fully recovered in the donor chamber and therefore displayed no detectable permeability across the Caco-2 cell monolayer. This finding strongly suggested that the quaternary ammonium-linked analogue would be ideally suited to exhibit minimal absorption across the small intestine.

***In Vivo* Pharmacokinetic Studies.** To investigate compound exposure to the colon, the *in vivo* pharmacokinetic properties of analogues **15e** and **26a** were assessed after oral administration in healthy male Sprague Dawley rats (Table 4). Importantly, there were no adverse effects observed in the rats as a result of oxadiazole compound administration. Plasma concentrations of the oxadiazole analogues were measurable but low after oral administration, with $AUC_{0\text{-inf}}$ values of only 0.90 and 0.28 h*μM reached by analogues **15e** and **26a**, respectively. However, the cumulative recoveries of unchanged compounds in the faeces were also very low for both compounds. Of the oral doses, only 0.42% of **15e** and 1.5% of **26a** were recovered in the faeces over a 48 h period and even less detected in the urine. Based on the poor recovery levels, it was thought that the compounds may have been degraded or metabolized in the GI lumen by chemical or enzymatic processes.

■ CONCLUSIONS

The synthesis and evaluation of colon-targeted antimicrobial agents were accomplished *via* the attachment of poorly permeable functional groups to the 1,2,4-oxadiazole class of compounds. Modified oxadiazole analogues were produced which displayed antimicrobial activity against two important Gram-positive pathogens. Rapid bactericidal activity was a hallmark feature of these analogues. Our study identified that modification with the quaternary ammonium moiety at ring A of the oxadiazole scaffold led to a suitable physicochemical profile for colon-targeted delivery, as these compounds were completely impermeant toward Caco-2 cell monolayers yet maintained good antimicrobial potency. The recovery of analogues **15e** and **26a** after oral administration in rats was low and this was identified as a key target for future optimization in order to progress the analogues toward exhibiting *in vivo* efficacy. Analysis of the resulting metabolites may be a reasonable approach toward identifying the structural

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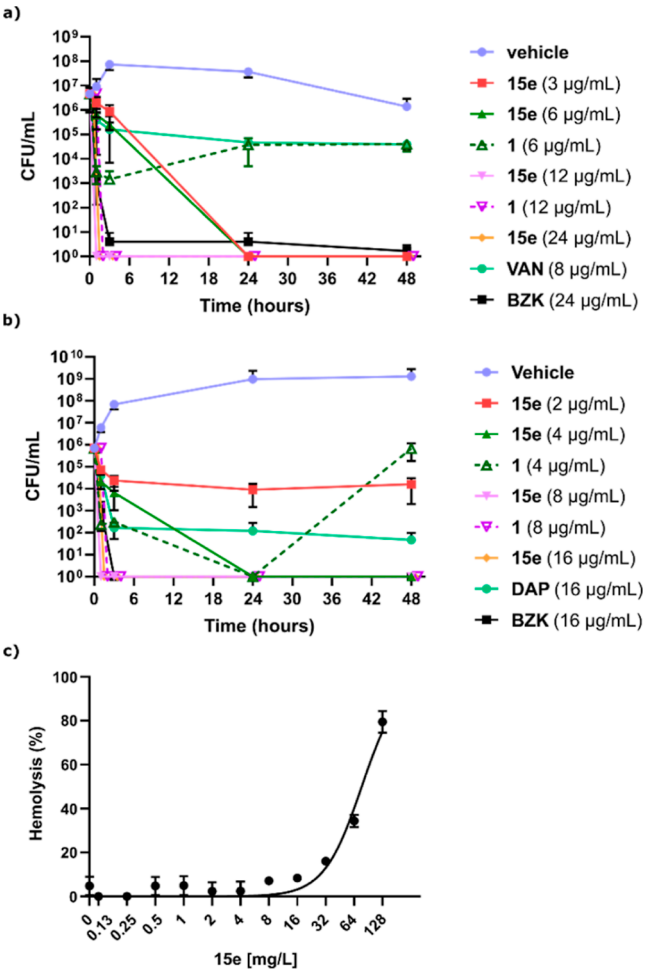

Figure 4. Time-kill analyses of **15e** against GI pathogens: (a) *C. difficile* strain EDN0008, blue circles are DMSO-containing untreated cultures (vehicle), red squares are cultures treated with 3 μg/mL **15e**, closed green triangles are cultures treated with 6 μg/mL **15e**, open green triangles are cultures treated with 6 μg/mL 1, closed purple triangles are cultures treated with 12 μg/mL **15e**, open purple triangles are culture treated with 12 μg/mL **1**, orange diamonds are cultures treated with 24 μg/mL **15e**, turquoise circles are cultures treated with 8 μg/mL vancomycin, and black squares are cultures treated with 24 μg/mL benzalkonium chloride; (b) *E. faecium* strain Aus0085, blue circles are DMSO-containing untreated cultures (vehicle), red squares are cultures treated with 2 μg/mL **15e**, closed green triangles are cultures treated with 4 μg/mL **15e**, open green triangles are cultures treated with 4 μg/mL 1, closed purple triangles are cultures treated with 8 μg/mL **15e**, open purple triangles are cultures treated with 8 μg/mL **1**, orange diamonds are cultures treated with 16 μg/mL **15e**, turquoise circles are cultures treated with 16 μg/mL daptomycin, and black squares are cultures treated with 16 μg/mL benzalkonium chloride; (c) haemolysis assays of **15e** against human erythrocytes. All assays were performed using three independent biological replicates with the data shown representing the mean ± SEM. VAN—vancomycin, DAP—daptomycin, BZK—benzalkonium chloride, and CFU—colony-forming units.

changes needed to avoid such metabolism or degradation. In summary, we believe our results contribute important data toward the future development of decolonization agents with utility against difficult-to-treat infections caused by *C. difficile* and *E. faecium*.

■ EXPERIMENTAL SECTION

General Chemistry. Reactions were monitored by analytical thin-layer chromatography (TLC) using silica gel 60/F254 pre-coated aluminium sheets (0.25 mm, Merck). Flash column chromatography was performed with silica gel 60, 0.63−0.20 mm (70−230 mesh, Merck). The solvents; ethyl acetate (EtOAc); dichloromethane (DCM); dimethyl formamide (DMF); 1,4-dioxane; dimethyl sulfoxide (DMSO); methanol (MeOH); tetrahydrofuran; and diethyl ether (Et_2O) were of analytical grade or distilled laboratory grade. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 Hz, respectively, using an Avance III Nanobay 400 MHz Bruker spectrometer coupled to the BACS 60 automatic sample changer. Chemical shifts (δ, ppm) are reported relative to the solvent peak [$CDCl_3$: 7.26 (^{1}H NMR), 77.16 (^{13}C NMR), DMSO-d_6: 2.50 (^{1}H NMR), 39.52 (^{13}C NMR), methanol-d_4: 3.31 (^{1}H NMR), 49.00 (^{13}C NMR)]. Each proton resonance was assigned with the following annotations; chemical shift (δ, ppm), singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad signal (br), coupling constant (J, Hz), and number of protons. Analytical HPLC was performed on an Agilent 1260 Infinity analytical HPLC coupled with a G1322A degasser, G1312B binary pump, G1367E high-performance autosampler, and G4212B diode array detector. The conditions were Zorbax Eclipse Plus C18 Rapid Resolution column (4.6 × 100 mm) with UV detection at 254 and 214 nm, 30 °C; the sample was eluted using a gradient system. Solvent A: water 0.1% TFA. Solvent B: acetonitrile 0.1% TFA. PP-gradient method—gradient: 5-95% B over 9 min and 100% B over 1 min. Detection: 254 or 214 nm. Hydrophobic PP method—gradient: 5−80% B over 0.1 min, 80−100% B from 0.6 to 9 min, and 100% B for 1 min. The PP-gradient method was used for all characterization unless otherwise specified. LCMS was obtained on an Agilent 6100 Series Single Quad LC/MS coupled with an Agilent 1200 Series HPLC, 1200 Series G1311A quaternary pump, 1200 series G1329A thermostatted autosampler and 1200 series G1314B variable wavelength detector. The liquid chromatography conditions were reverse-phase HPLC analysis fitted with a Phenomenex Luna C8(2) 5 μm (50 × 4.6 mm) 100 Å column; column temperature: 30 °C; injection volume: 5 μL; solvent: 99.9% acetonitrile, 0.1% formic acid; gradient: 5−100% of solvent over 10 min; detection: 254 nm. The mass spectrometry conditions were quadrupole ion source; ion mode: multimode-ES; drying gas temp: 300 °C; vaporizer temperature: 200 °C; capillary voltage: 2000 V (positive), 4000 (negative); scan range: 100−1000 m/z; step size: 0.1 s Acquisition time: 10 min. HRMS were obtained on an Agilent 6224 TOF LC/MS mass spectrometer coupled to an Agilent 1290 Infinity (Agilent, Palo Alto, CA). All data were acquired, and reference mass was corrected via a dual-spray electrospray ionization (ESI) source. Each scan or data point on the total ion chromatogram (TIC) is an average of 13,700 transients, producing a spectrum every second. Mass spectra were created by averaging the scans across each peak and background subtracted against the first 10 s of the TIC. Acquisition was performed using the Agilent Mass Hunter Data Acquisition software version B.05.00 Build 5.0.5042.2 and analysis was performed using MassHunter Qualitative Analysis version B.05.00 Build 5.0.519.13. Mass spectrometer conditions: ionization mode: ESI. Drying gas flow: 11 L/min; nebulizer: 45 psi; drying gas temperature: 325 °C; capillary voltage

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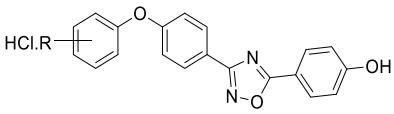
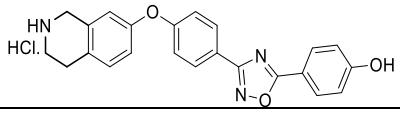
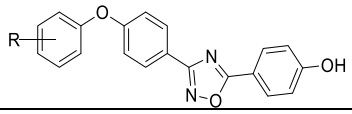
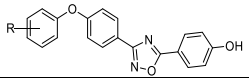
Table 2. Structure and Activity of Oxadiazole Analogues

Entry	Structure or R	*C. difficile* MIC (μg/mL)[a]	VREfm MIC (μg/mL)[a]
			
15f	4-$CH(CH_3)CH_2NH_2$	16	4
15g	4-$CH_2CH_2NHCH_3$	4	4
15h	4-$CH_2CH_2CH_2NH_2$	2	2
15i		4	4
			
25a	4-$CH_2CH_2N(CH_3)_2$	4	4
25b	4-$CH_2CH_2CH_2N(CH_3)_2$	16	n.d.
26a	4-$CH_2CH_2N^+(CH_3)_3$	8	8
26b	4-$CH_2CH_2CH_2N^+(CH_3)_3$	8	16
Vancomycin		0.5 - 2	n.d.
Daptomycin		n.d.	2

[a]n.d.—not determined.

Table 3. Caco-2 Cell Permeability Assay of Key Oxadiazole Analogues

Entry	R	A-B P_{app} (10^{-6} cm/s)[a]	Mass balance (%)[a]
			
9e	3-OCH_2COOH	5.3 ± 0.20	96 ± 5.3
9g	4-CH_2CH_2COOH	18 ± 1.0	55 ± 1.7
15e	4-$CH_2CH_2NH_2$	c.n.d.	13 ± 2.1
26a	4-$CH_2CH_2N^+(CH_3)_3$	c.n.d.	105 ± 7.6

[a]Values are presented as the mean ± SD of n = 3 transwells. C.n.d.—could not be determined.

Table 4. Plasma Exposure Parameters and Urinary and Faecal Recovery from Rats of Unchanged Analogues after Oral Administration

entry	$t_{1/2}$ (h)[a]	C_{max} (μM)[a]	T_{max} (h)[a]	$AUC_{0\text{-inf}}$ (h*μM)[a]	% dose in urine (0–48 h)[a]	% dose in faeces (0–48 h)[a]
15e	2.0 ± 0.30	0.16 ± 0.038	2.5 ± 0.0	0.90 ± 0.22	0.08 ± 0.03	0.42 ± 0.47
26a	5.3 ± 2.1	0.039 ± 0.046	2.8 ± 2.0	0.28 ± 0.27	0.06 ± 0.03	1.5 ± 0.98

[a]Values are presented as the mean ± SD of n = 3. Measured dose—30 mg/kg.

(V_{cap}): 4000 V; fragmentor: 160 V; skimmer: 65 V; OCT RFV: 750 V; and scan range acquired: 100–1500 m/z. Internal reference ions: positive ion mode = m/z = 121.050873 & 922.009798. Chromatographic separation was performed using an Agilent Zorbax SB-C18 Rapid Resolution HT 2.1 × 50 mm, 1.8 μm column (Agilent Technologies, Palo Alto, CA) using an acetonitrile gradient (5–100%) over 3.5 min at 0.5 mL/min. Solvent A = aqueous 0.1% formic acid. Solvent B = acetonitrile/0.1% formic acid. Compounds **6a–h** were obtained as unpurified intermediates and were used directly in subsequent reactions. Therefore, these intermediates were not fully characterized. Characterization and syntheses of the derivatized phenol reagents **13a–i** in Scheme 2 are described in the Supporting Information. The purity of all compounds submitted for biological testing was ≥95% as determined using HPLC analysis.

General Procedure A. A solution of phenol (1.2 equiv) in DCM (0.27 M) was treated with 2-*tert*-butyl-1,3-diisopropylisourea (1 equiv) and a catalytic amount of DMAP and then stirred at rt for 96 h. The reaction mixture was concentrated in vacuo, diluted with a 10% solution of aq Na_2CO_3, and then, Et_2O was added to extract the product. The separated organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified using flash chromatography

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eluting with 15% EtOAc in petroleum ether to yield the product.

General Procedure B. Phenol (1 equiv), 4-iodobenzonitrile (1 equiv), Cs_2CO_3 (2 equiv), and *N*,*N*-dimethylglycine (0.3 equiv) were dissolved in 1,4-dioxane (0.59 M) in a sealed tube and purged with N_2 for 15 min. CuI (0.2 equiv) was then added, the reaction vessel was sealed, and the solution was stirred at 100 °C for 48 h. The reaction mixture was filtered, washed with Et_2O, and the collected filtrate was evaporated in vacuo. The residue was purified using flash chromatography eluting with 2−15% EtOAc in petroleum ether to yield the product.

General Procedure C. $NH_2OH{\cdot}HCl$ (2 equiv) and Et_3N (2 equiv) were added to a solution of benzonitrile (1 equiv) in MeOH (0.2 M). The solution was heated to 60 °C and allowed to stir for 6 h. The solvent was removed in vacuo, and the residue was diluted with water and then extracted with DCM. The organic layers were combined, dried over $MgSO_4$, and the solvent removed in vacuo to yield the product that was used in the subsequent step without further purification.

General Procedure D. A solution of amidoxime (1 equiv) in DMF (0.22 M) was treated with 4-acetoxybenzoic acid **7** (1.5 equiv), HOBt (2 equiv), and EDC·HCl (2 equiv) then the solution was stirred at rt for 16 h. The temperature was increased to 100 °C, and the stirring was continued for 16 h. The reaction mixture was concentrated in vacuo, diluted with a 10% solution of aq Na_2CO_3, and then, DCM was added to extract the product. The separated organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified using flash chromatography eluting with 0−15% EtOAc and 1% acetic acid in petroleum ether to yield the product.

General Procedure E. A solution of the *tert*-butyl ester substrate in DCM (1 mL per 100 mg substrate) was treated with TFA (0.1 mL per 100 mg substrate) and stirred at rt for 8 h. The reaction mixture was concentrated in vacuo, and the remaining residue suspended in water and sonicated. The solid was filtered, washed with water, and air-dried to yield the pure product.

*4-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)phenol (**1**).* The title compound was synthesized as previously reported to yield the product as a yellow solid (287 mg, 84%).[26] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13−8.07 (m, 4H), 7.41−7.35 (m, 2H), 7.17 (t, J = 7.4 Hz, 1H), 7.12−7.06 (m, 4H), 7.01−6.96 (m, 2H); spectroscopic data consistent with the literature.[26]

*tert-Butyl 2-(2-Hydroxyphenyl)acetate (**3a**).* The title compound was synthesized from 2-(2-hydroxyphenyl)acetic acid **2a** (9.12 mmol, 1.39 g) according to general procedure A and obtained as a yellow solid (800 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (br, 1H), 7.19 (td, J = 7.9, 1.7 Hz, 1H), 7.07 (dd, J = 7.5, 1.4 Hz, 1H), 6.96 (dd, J = 8.1, 1.0 Hz, 1H), 6.87 (td, J = 7.4, 1.2 Hz, 1H), 3.60 (s, 2H), 1.47 (s, 9H); spectroscopic data consistent with the literature.[39]

*tert-Butyl 3-(2-Hydroxyphenyl)propanoate (**3b**).* 3-(2-Hydroxyphenyl) propionic acid **2b** (1 equiv, 18.1 mmol, 3.00 g) was dissolved in DMF (16 mL), and 1,1′-carbonyldiimidazole (1 equiv, 18.1 mmol, 2.93 g) was added carefully. The solution was stirred for 2 h at 40 °C. 1,8-Diazabicyclo[5.4.0]undec-7-ene (2 equiv, 36.1 mmol, 5.40 mL) and anhydrous *tert*-butanol (2.5 equiv, 45.2 mmol, 4.30 mL) were added, and the solution was stirred at 65 °C for 3 days. Solvents were removed in vacuo, and the residue was diluted with water and aq 2 M HCl solution. The aqueous layer was washed with EtOAc, then the organic layers were combined, dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was purified using flash chromatography (10% EtOAc in petroleum ether) to yield the product as light yellow oil (804 mg, 20%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (br, 1H), 7.14−7.04 (m, 2H), 6.93−6.83 (m, 2H), 2.90−2.81 (m, 2H), 2.66−2.61 (m, 2H), 1.42 (s, 9H); spectroscopic data consistent with the literature.[40]

*tert-Butyl 2-(3-Hydroxyphenyl)acetate (**3c**).* The title compound was synthesized from 2-(3-hydroxyphenyl)acetic acid **2c** (15.9 mmol, 2.42 g) according to general procedure A and obtained as clear oil (0.796 g, 29%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.16 (t, J = 7.8 Hz, 1H), 6.81 (d, J = 7.4 Hz, 1H), 6.74 (s, 1H), 6.72 (d, J = 8.1 Hz, 1H), 5.47 (br, 1H), 3.47 (s, 2H), 1.44 (s, 9H); spectroscopic data consistent with the literature.[41]

*tert-Butyl 3-(3-Hydroxyphenyl)propanoate (**3d**).* 3-(3-Hydroxyphenyl) propionic acid **2d** (1 equiv, 18.1 mmol, 3.00 g) was dissolved in DMF (16 mL) and 1,1′-carbonyldiimidazole (1 equiv, 18.1 mmol, 2.93 g) was added carefully. The solution was stirred for 2 h at 40 °C. 1,8-Diazabicyclo[5.4.0]undec-7-ene (2 equiv, 36.1 mmol, 5.40 mL) and anhydrous *tert*-butanol (2.5 equiv, 45.2 mmol, 4.30 mL) were added, and the solution was stirred at 65 °C for 3 days. Solvents were removed in vacuo, and the residue was diluted with water and aq 2 M HCl solution. The aqueous layer was washed with EtOAc, then the organic layers were combined, dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was purified using flash chromatography (10% EtOAc in petroleum ether) to yield the product as clear oil (1.95 g, 49%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.26 (br, 1H), 7.04 (t, J = 7.7 Hz, 1H), 6.64−6.53 (m, 3H), 2.70 (t, J = 7.5 Hz, 2H), 2.45 (t, J = 7.5 Hz, 2H), 1.36 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.5, 155.8, 142.8, 129.7, 120.9, 115.4, 113.2, 80.6, 37.0, 31.1, 28.2. HPLC: t_R = 6.6 min, 99% at 254 nm. HRMS m/z: calcd for $2\times(C_{13}H_{18}O_3)-H^+$ [2M − H$^+$], 443.2439; found, 443.2440.

*tert-Butyl 2-(3-Hydroxyphenoxy)acetate (**3e**).* A solution of resorcinol **2e** (50 mmol, 5.50 g, 5 equiv) in DMF (50 mL) was treated with anhydrous K_2CO_3 (50 mmol, 6.91 g, 5 equiv) and *tert*-butyl bromoacetate (10 mmol, 1.48 mL, 1 equiv); then, the solution was stirred at rt for 16 h. The reaction mixture was concentrated in vacuo, and a 6 M solution of aq HCl was added while stirring until the solution became until acidic. DCM was added to extract the product, and the separated organic layer was dried over anhydrous $MgSO_4$ and then concentrated in vacuo. The residue was purified using flash chromatography (25% EtOAc in petroleum ether) to yield the product as orange oil (1.18 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10 (t, J = 8.1 Hz, 1H), 6.50−6.35 (m, 3H), 5.76 (br, 1H), 4.48 (s, 2H), 1.49 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 168.6, 159.2, 157.2, 130.2, 109.1, 106.4, 102.8, 82.9, 65.8, 28.2. HPLC: t_R = 4.8 min, 99% at 254 nm. HRMS m/z: calcd for $C_{12}H_{16}O_4$ + H$^+$ [M + H$^+$], 225.1121; found, 225.1119.

*tert-Butyl 2-(4-Hydroxyphenyl)acetate (**3f**).* The title compound was synthesized from 2-(4-hydroxyphenyl)acetic acid **2f** (24 mmol, 3.65 g) according to general procedure A and obtained as a white solid (1.15 g, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12−7.08 (m, 2H), 6.75−6.70 (m, 2H), 5.33 (br, 1H), 3.45 (s, 2H), 1.44 (s, 9H); spectroscopic data consistent with the literature.[39]

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*tert-Butyl 3-(4-Hydroxyphenyl)propanoate (**3g**).* The title compound was synthesized from 3-(4-hydroxyphenyl)-propanoic acid **2g** (24.0 mmol, 3.98 g) according to general procedure A and obtained as yellow oil (1.17 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05 (d, J = 8.4 Hz, 2H), 6.76−6.70 (m, 2H), 5.31 (br, 1H), 2.83 (t, J = 7.7 Hz, 2H), 2.50 (t, J = 7.7 Hz, 2H), 1.42 (s, 9H); spectroscopic data consistent with the literature.[40]

*tert-Butyl 2-(4-Hydroxyphenoxy)acetate (**3h**).* A solution of hydroquinone **2h** (50 mmol, 5.50 g, 5 equiv) in DMF (50 mL) was treated with anhydrous K_2CO_3 (50 mmol, 6.91 g, 5 equiv) and *tert*-butyl bromoacetate (10 mmol, 1.48 mL, 1 equiv); then, the solution was stirred at rt for 16 h. The reaction mixture was concentrated in vacuo and a 6 M solution of aq HCl was added while stirring until the solution became until acidic. DCM was added to extract the product, and the separated organic layer was dried over anhydrous $MgSO_4$ and then concentrated in vacuo. The residue was purified using flash chromatography eluting with 25% EtOAc in petroleum ether to yield the product as an off-white amorphous solid (1.75 g, 78%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.96 (br, 1H), 6.75−6.69 (m, 2H), 6.68−6.63 (m, 2H), 4.49 (s, 2H), 1.41 (s, 9H); spectroscopic data consistent with the literature.[42]

*tert-Butyl 2-(2-(4-Cyanophenoxy)phenyl)acetate (**5a**).* The title compound was synthesized from *tert*-butyl 2-(2-hydroxyphenyl)acetate **3a** (2.89 mmol, 600 mg) according to general procedure B and purified using flash chromatography (15% EtOAc in petroleum ether) to yield the product as clear oil (454 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60−7.56 (m, 2H), 7.36 (dd, J = 7.5, 1.5 Hz, 1H), 7.31 (td, J = 7.8, 1.8 Hz, 1H), 7.21 (td, J = 7.5, 1.3 Hz, 1H), 7.00−6.95 (m, 3H), 3.52 (s, 2H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.3, 161.6, 153.0, 134.2, 132.2, 129.1, 127.7, 125.6, 120.7, 119.0, 117.8, 105.9, 81.2, 37.1, 28.1. HPLC: t_R = 6.3 min, 99% at 254 nm. HRMS m/z: calcd for $C_{19}H_{19}NO_3$ + H^+ [M + H^+], 310.1438; found, 310.1433.

*tert-Butyl 3-(2-(4-Cyanophenoxy)phenyl)propanoate (**5b**).* The title compound was synthesized from *tert*-butyl 3-(2-hydroxyphenyl)propanoate **3b** (3.54 mmol, 785 mg) according to general procedure B and purified using flash chromatography (2% EtOAc in petroleum ether) to yield the product as light yellow oil (208 mg, 18%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62−7.56 (m, 2H), 7.32 (dd, J = 7.5, 1.7 Hz, 1H), 7.25 (td, J = 7.7, 1.8 Hz, 1H), 7.17 (td, J = 7.5, 1.3 Hz, 1H), 6.98−6.92 (m, 3H), 2.83 (t, J = 7.7 Hz, 2H), 2.49 (dd, J = 9.0, 6.5 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.1, 161.8, 152.7, 134.3, 133.1, 131.2, 128.3, 125.7, 121.0, 119.0, 117.4, 105.8, 80.6, 35.6, 28.2, 25.8. HPLC: t_R = 7.6 min, 99% at 254 nm. HRMS m/z: calcd for $C_{20}H_{21}NO_3$ + H^+ [M + H^+], 324.1594; found, 324.1597.

*tert-Butyl 2-(3-(4-Cyanophenoxy)phenyl)acetate (**5c**).* The title compound was synthesized from *tert*-butyl 2-(3-hydroxyphenyl)acetate **3c** (3.10 mmol, 645 mg) according to general procedure B and purified using flash chromatography (15% EtOAc in petroleum ether) to yield the product as clear oil (786 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62−7.57 (m, 2H), 7.35 (t, J = 7.9 Hz, 1H), 7.13 (d, J = 8.1 Hz, 1H), 7.02−6.99 (m, 3H), 6.96 (dd, J = 8.1, 1.7 Hz, 1H), 3.53 (s, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.4, 161.7, 155.0, 137.3, 134.3, 130.3, 126.2, 121.4, 119.0, 118.9, 118.1, 106.0, 81.3, 42.5, 28.2. HPLC: t_R = 6.4 min, 96% at 254 nm. HRMS m/z: calcd for $C_{19}H_{19}NO_3$ + H^+ [M + H^+], 310.1438; found, 310.1428.

*tert-Butyl 3-(3-(4-Cyanophenoxy)phenyl)propanoate (**5d**).* The title compound was synthesized from *tert*-butyl 3-(3-hydroxyphenyl)propanoate **3d** (3.94 mmol, 875 mg) according to general procedure B and purified using flash chromatography (2% EtOAc in petroleum ether) to yield the product as light yellow oil (600 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61−7.57 (m, 2H), 7.31 (dd, J = 9.1, 6.5 Hz, 1H), 7.07 (dd, J = 7.6, 0.5 Hz, 1H), 7.01−6.96 (m, 2H), 6.90 (ddd, J = 8.1, 2.9, 1.7 Hz, 2H), 2.91 (t, J = 7.7 Hz, 2H), 2.54 (t, J = 7.7 Hz, 2H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.0, 161.8, 155.0, 143.7, 134.7, 130.3, 125.4, 120.5, 119.0, 118.3, 118.0, 105.9, 80.7, 36.9, 31.0, 28.2. HPLC: t_R = 8.4 min, 99% at 254 nm. HRMS m/z: calcd for $C_{20}H_{21}NO_3$ + H^+ [M + H^+], 324.1594; found, 324.1594.

*tert-Butyl 2-(3-(4-Cyanophenoxy)phenoxy)acetate (**5e**).* The title compound was synthesized from *tert*-butyl 2-(3-hydroxyphenoxy)acetate **3e** (4.69 mmol, 1.05 g) according to general procedure B and purified using flash chromatography (15% EtOAc in petroleum ether) to yield the product as clear oil (1.08 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62−7.57 (m, 2H), 7.30 (t, J = 8.2 Hz, 1H), 7.04−6.99 (m, 2H), 6.75 (ddd, J = 8.4, 2.5, 0.8 Hz, 1H), 6.68 (ddd, J = 8.1, 2.2, 0.8 Hz, 1H), 6.60 (t, J = 2.3 Hz, 1H), 4.50 (s, 2H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.7, 161.4, 159.6, 156.1, 134.3, 130.9, 118.9, 118.3, 113.3, 111.3, 107.3, 106.2, 82.8, 65.8, 28.2. HPLC: t_R = 6.3 min, 99% at 254 nm. HRMS m/z: calcd for $C_{19}H_{19}NO_4$ + H^+ [M + H^+], 326.1387; found, 326.1375.

*tert-Butyl 2-(4-(4-Cyanophenoxy)phenyl)acetate (**5f**).* The title compound was synthesized from *tert*-butyl 2-(4-hydroxyphenyl)acetate **3f** (4.66 mmol, 970 mg) according to general procedure B and purified using flash chromatography (5% EtOAc in petroleum ether) to yield the product as white crystals (1.20 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62−7.56 (m, 2H), 7.31 (d, J = 8.5 Hz, 2H), 7.05−6.96 (m, 4H), 3.54 (s, 2H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.9, 161.8, 153.8, 134.3, 131.8, 131.2, 120.5, 119.0, 118.0, 105.9, 81.3, 41.9, 28.2. HPLC: t_R = 7.2 min, 99% at 254 nm. HRMS m/z: calcd for $C_{19}H_{19}NO_3$ + H^+ [M + H^+], 310.1438; found, 310.1435.

*tert-Butyl 3-(4-(4-Cyanophenoxy)phenyl)propanoate (**5g**).* The title compound was synthesized from *tert*-butyl 3-(4-hydroxyphenyl)propanoate **3g** (4.90 mmol, 1.09 g) according to general procedure B and purified using flash chromatography (15% EtOAc in petroleum ether) to yield the product as a white solid (1.39 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60−7.55 (m, 2H), 7.26−7.21 (m, 2H), 7.00−6.94 (m, 4H), 2.92 (t, J = 7.7 Hz, 2H), 2.55 (t, J = 7.7 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.2, 162.0, 153.1, 138.0, 134.2, 130.2, 120.6, 119.0, 117.8, 105.7, 80.7, 37.1, 30.6, 28.2. HPLC: t_R = 6.7 min, 99% at 254 nm. HRMS m/z: calcd for $C_{20}H_{21}NO_3$ + H^+ [M + H^+], 324.1594; found, 324.1585.

*tert-Butyl 2-(4-(4-Cyanophenoxy)phenoxy)acetate (**5h**).* The title compound was synthesized from *tert*-butyl 2-(4-hydroxyphenoxy)acetate **3h** (4.69 mmol, 1.05 g) according to general procedure B and purified using flash chromatography (15% EtOAc in petroleum ether) to yield the product as white crystals (1.40 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60−7.54 (m, 2H), 7.02−6.89 (m, 6H), 4.52 (s, 2H), 1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 168.0, 162.4, 155.4, 148.7, 134.2, 121.9, 119.1, 117.4, 116.2, 105.6, 82.7, 66.2, 28.2.

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HPLC: t_R = 6.3 min, 99% at 254 nm. HRMS *m/z*: calcd for $C_{19}H_{19}NO_4$ + H^+ [M + H^+], 326.1387; found, 326.1373.

*tert-Butyl (Z)-2-(2-(4-(N′-Hydroxycarbamimidoyl)-phenoxy)phenyl)acetate (**6a**).* The title compound was synthesized from *tert*-butyl 2-(2-(4-cyanophenoxy)phenyl)-acetate **5a** (1.25 mmol, 385 mg) according to general procedure C and obtained as yellow oil (426 mg, 100%). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59−7.54 (m, 2H), 7.32 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.24 (dt, *J* = 8.0, 2.2 Hz, 1H), 7.13 (td, *J* = 7.5, 1.2 Hz, 1H), 6.98−6.93 (m, 2H), 6.91 (dd, *J* = 8.1, 1.1 Hz, 1H), 4.86 (br, 2H), 3.56 (s, 2H), 1.36 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 159.1, 154.2, 131.6, 129.3, 128.6, 127.3, 127.0, 126.9, 124.3, 119.6, 117.8, 117.2, 80.8, 67.1, 37.0, 27.9, 27.9. LCMS (ESI$^+$) *m/z*: 343.8 [M + H]$^+$, t_R = 4.9 min.

*tert-Butyl (Z)-3-(2-(4-(N′-Hydroxycarbamimidoyl)-phenoxy)phenyl)propanoate (**6b**).* The title compound was synthesized from *tert*-butyl 3-(2-(4-cyanophenoxy)phenyl)-propanoate **5b** (0.529 mmol, 171 mg) according to general procedure C and obtained as yellow oil (155 mg, 85%). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.55 (br, 1H), 7.69−7.61 (m, 2H), 7.34 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.25 (td, *J* = 7.8, 1.7 Hz, 1H), 7.13 (td, *J* = 7.5, 1.2 Hz, 1H), 6.92−6.87 (m, 3H), 5.76 (br, 2H), 2.78 (t, *J* = 7.6 Hz, 2H), 2.50−2.45 (m, 2H), 1.34 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.9, 157.9, 156.6, 150.8, 143.5, 130.3, 128.8, 127.6, 124.3, 119.4, 118.2, 117.1, 55.4, 36.5, 30.7, 28.2. LCMS (ESI$^+$) *m/z*: 301.2 [M − (C_4H_{10}) + H]$^+$, t_R = 0.5 min.

*tert-Butyl (Z)-2-(3-(4-(N′-Hydroxycarbamimidoyl)-phenoxy)phenyl)acetate (**6c**).* The title compound was synthesized from *tert*-butyl 2-(3-(4-cyanophenoxy)phenyl)-acetate **5c** (2.30 mmol, 712 mg) according to general procedure C and obtained as clear oil (787 mg, 100%). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62−7.56 (m, 2H), 7.30 (t, *J* = 7.8 Hz, 1H), 7.05 (d, *J* = 7.6 Hz, 1H), 7.00 (dd, *J* = 8.6, 1.1 Hz, 2H), 6.95 (s, 1H), 6.93 (d, *J* = 8.1 Hz, 1H), 4.87 (br, 2H), 3.51 (s, 2H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 158.9, 156.5, 152.3, 136.8, 129.8, 127.4, 127.2, 124.8, 120.2, 118.5, 117.8, 42.5, 28.0. LCMS (ESI$^+$) *m/z*: 343.2 [M + H]$^+$, t_R = 5.0 min.

*tert-Butyl (Z)-3-(3-(4-(N′-Hydroxycarbamimidoyl)-phenoxy)phenyl)propanoate (**6d**).* The title compound was synthesized from *tert*-butyl 3-(3-(4-cyanophenoxy)phenyl)-propanoate **5d** (1.77 mmol, 570 mg) according to general procedure C and obtained as brown oil (628 mg, 100%). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.57 (br, 1H), 7.69−7.64 (m, 2H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 7.9 Hz, 1H), 6.97−6.93 (m, 2H), 6.92−6.90 (m, 1H), 6.85 (dd, *J* = 7.8, 2.1 Hz, 1H), 5.78 (br, 2H), 2.80 (t, *J* = 7.4 Hz, 2H), 2.51 (t, *J* = 6.9 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 158.2, 154.8, 150.8, 136.6, 130.5, 130.4, 128.7, 127.6, 119.5, 119.5, 117.9, 80.1, 55.4, 36.8, 30.3, 28.2. LCMS (ESI$^+$) *m/z*: 357.2 [M + H]$^+$, t_R = 5.1 min.

*tert-Butyl (Z)-2-(3-(4-(N′-Hydroxycarbamimidoyl)-phenoxy)phenoxy)acetate (**6e**).* The title compound was synthesized from *tert*-butyl 2-(3-(4-cyanophenoxy)phenoxy)-acetate **5e** (2.53 mmol, 823 mg) according to general procedure C and obtained as viscous yellow oil (906 mg, 100%). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61−7.56 (m, 2H), 7.24 (t, *J* = 8.3 Hz, 1H), 7.03−6.97 (m, 2H), 6.68−6.62 (m, 2H), 6.56 (t, *J* = 2.3 Hz, 1H), 4.93 (br, 2H), 4.47 (s, 2H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.8, 159.2, 158.6, 157.7, 130.4, 127.5, 127.4, 118.7, 112.3, 109.9, 106.1, 82.5, 65.7, 45.8, 28.0, 8.6. LCMS (ESI$^+$) *m/z*: 359.2 [M + H]$^+$, t_R = 5.0 min.

*tert-Butyl (Z)-2-(4-(4-(N′-Hydroxycarbamimidoyl)-phenoxy)phenyl)acetate (**6f**).* The title compound was synthesized from *tert*-butyl 2-(4-(4-cyanophenoxy)phenyl)-acetate **5f** (3.05 mmol, 943 mg) according to general procedure C and obtained as light yellow oil (1.04 g, 100%). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.57 (br, 1H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.28 (t, *J* = 9.2 Hz, 2H), 6.98 (t, *J* = 8.6 Hz, 4H), 5.78 (br, 2H), 3.55 (s, 2H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 159.0, 155.3, 130.7, 127.4, 119.4, 118.4, 41.8, 28.1. LCMS (ESI$^+$) *m/z*: 343.9 [M + H]$^+$, t_R = 5.1 min.

*tert-Butyl (Z)-3-(4-(4-(N′-Hydroxycarbamimidoyl)-phenoxy)phenyl)propanoate (**6g**).* The title compound was synthesized from *tert*-butyl 3-(4-(4-cyanophenoxy)phenyl)-propanoate **5g** (3.81 mmol, 1.23 g) according to general procedure C and obtained as yellow oil (1.35 g, 99%). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61−7.54 (m, 2H), 7.21−7.16 (m, 2H), 6.99−6.93 (m, 4H), 4.86 (br, 2H), 2.90 (t, *J* = 7.7 Hz, 2H), 2.54 (t, *J* = 7.7 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 159.3, 154.6, 152.3, 136.5, 129.8, 127.4, 127.0, 119.6, 118.1, 80.5, 37.1, 30.4, 28.1. LCMS (ESI$^+$) *m/z*: 357.2 [M + H]$^+$, t_R = 5.1 min.

*tert-Butyl (Z)-2-(4-(4-(N′-Hydroxycarbamimidoyl)-phenoxy)phenoxy)acetate (**6h**).* The title compound was synthesized from *tert*-butyl 2-(4-(4-cyanophenoxy)phenoxy)-acetate **5h** (2.53 mmol, 823 mg) according to general procedure C and obtained as a yellow amorphous solid (860 mg, 95%). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57−7.53 (m, 2H), 6.99−6.86 (m, 6H), 4.87 (br, 2H), 4.50 (s, 2H), 1.49 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.0, 159.9, 154.6, 150.1, 127.4, 126.6, 121.1, 117.5, 115.9, 82.5, 66.2, 28.1. LCMS (ESI$^+$) *m/z*: 359.2 [M + H]$^+$, t_R = 4.9 min.

*tert-Butyl 2-(2-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)acetate (**8a**).* The title compound was synthesized from *tert*-butyl (Z)-2-(2-(4-(N′-hydroxycarbamimidoyl)phenoxy)phenyl)acetate **6a** (1.17 mmol, 400 mg) according to general procedure D and purified using flash chromatography (15% EtOAc and 1% acetic acid in petroleum ether) to yield the product as a tan solid (169 mg, 33%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11−8.05 (m, 4H), 7.35 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.31−7.25 (m, 1H), 7.16 (t, *J* = 7.5 Hz, 1H), 7.06−7.02 (m, 2H), 7.00−6.93 (m, 3H), 5.96 (br, 1H), 3.59 (s, 2H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.6, 170.9, 168.5, 160.2, 159.8, 154.2, 131.9, 130.5, 129.4, 128.9, 127.3, 124.7, 121.7, 120.0, 118.0, 117.1, 116.2, 81.2, 37.3, 28.1. HPLC: t_R = 6.9 min, 97% at 254 nm. HRMS *m/z*: calcd for $C_{26}H_{24}N_2O_5$ + H^+ [M + H^+], 445.1758; found, 445.1751.

*tert-Butyl 3-(2-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)propanoate (**8b**).* The title compound was synthesized from *tert*-butyl (Z)-3-(2-(4-(N′-hydroxycarbamimidoyl)phenoxy)phenyl)propanoate **6b**

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(0.374 mmol, 133 mg) according to general procedure D and purified using flash chromatography (0−5% EtOAc and 1% acetic acid in petroleum ether) to yield the product as an amorphous white solid (49.0 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.57 (br, 1H), 8.08−8.03 (m, 2H), 8.03−7.99 (m, 2H), 7.38 (dd, J = 7.6, 1.6 Hz, 1H), 7.30 (td, J = 7.8, 1.7 Hz, 1H), 7.19 (td, J = 7.5, 1.2 Hz, 1H), 7.10−7.05 (m, 2H), 7.02 (dd, J = 8.1, 1.1 Hz, 1H), 7.01−6.97 (m, 2H), 2.78 (t, J = 7.6 Hz, 2H), 2.52−2.45 (m, 2H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.7, 173.2, 168.3, 160.4, 160.4, 154.0, 132.5, 130.9, 130.4, 129.4, 128.1, 124.8, 121.4, 120.3, 117.7, 116.6, 116.2, 81.1, 35.8, 28.2, 26.1. HPLC: t_R = 9.3 min, 100% at 254 nm. HRMS m/z: calcd for $C_{27}H_{26}N_2O_5$ + H$^+$ [M + H$^+$], 459.1914; found, 459.1927.

*tert-Butyl 2-(3-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)acetate (**8c**).* The title compound was synthesized from *tert*-butyl (*Z*)-2-(3-(4-(*N*′-hydroxycarbamimidoyl)phenoxy)phenyl)acetate **6c** (2.19 mmol, 750 mg) according to general procedure D and purified using flash chromatography (15% EtOAc and 1% acetic acid in petroleum ether) to yield the product as an off-white solid (153 mg, 16%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.07 (d, J = 8.6 Hz, 2H), 8.02 (d, J = 8.6 Hz, 2H), 7.40 (t, J = 7.8 Hz, 1H), 7.16 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 7.7 Hz, 1H), 7.06−6.95 (m, 4H), 3.58 (s, 2H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.8, 171.2, 168.4, 160.4, 160.1, 156.4, 136.8, 130.5, 130.1, 129.4, 125.1, 121.8, 120.7, 118.6, 118.4, 116.7, 116.3, 81.7, 42.7, 28.2. HPLC: t_R = 7.0 min, 96% at 254 nm. HRMS m/z: calcd for $C_{26}H_{24}N_2O_5$ + H$^+$ [M + H$^+$], 445.1758; found, 445.1770.

*tert-Butyl 3-(3-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl) Propanoate (**8d**).* The title compound was synthesized from *tert*-butyl (*Z*)-3-(3-(4-(*N*′-hydroxycarbamimidoyl)phenoxy)phenyl)propanoate **6d** (1.53 mmol, 543 mg) according to general procedure D and purified using flash chromatography (5−10% EtOAc and 1% acetic acid in petroleum ether) to yield the product as an off-white solid (330 mg, 47%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.59 (br, 1H), 8.08−8.04 (m, 2H), 8.04−8.00 (m, 2H), 7.36 (t, J = 7.9 Hz, 1H), 7.16−7.11 (m, 2H), 7.09 (d, J = 7.9 Hz, 1H), 7.02−6.98 (m, 3H), 6.96 (dd, J = 7.7, 2.1 Hz, 1H), 2.83 (t, J = 7.4 Hz, 2H), 2.55−2.51 (m, 2H), 1.34 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.4, 171.4, 167.5, 162.1, 159.8, 155.3, 143.3, 130.1, 130.1, 129.1, 124.6, 121.0, 119.6, 118.2, 117.4, 116.3, 114.1, 79.7, 36.0, 30.2, 27.7. HPLC: t_R = 8.2 min, 99% at 254 nm. HRMS m/z: calcd for $C_{27}H_{26}N_2O_5$ + H$^+$ [M + H$^+$], 459.1914; found, 459.1927.

*tert-Butyl 2-(3-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenoxy)acetate (**8e**).* The title compound was synthesized from *tert*-butyl (*Z*)-2-(3-(4-(*N*′-hydroxycarbamimidoyl)phenoxy)phenoxy)acetate **6e** (2.42 mmol, 867 mg) according to general procedure D and purified using flash chromatography (15% EtOAc and 1% acetic acid in petroleum ether) to yield the product as a yellow solid (312 mg, 28%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (br, 1H), 8.09−8.04 (m, 2H), 8.04−7.99 (m, 2H), 7.35 (t, J = 8.2 Hz, 1H), 7.19−7.13 (m, 2H), 7.02−6.97 (m, 2H), 6.77 (dd, J = 8.2, 2.2 Hz, 1H), 6.72 (dd, J = 8.0, 2.0 Hz, 1H), 6.65 (t, J = 2.3 Hz, 1H), 4.66 (s, 2H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.5, 167.6, 167.5, 162.1, 159.4, 159.2, 156.5, 130.8, 130.1, 129.1, 121.3, 118.5, 116.3, 114.1, 112.1, 110.8, 106.0, 81.5, 65.1, 27.6. HPLC: t_R = 6.8 min, 98% at 254 nm. HRMS m/z: calcd for $C_{26}H_{24}N_2O_6$ + H$^+$ [M + H$^+$], 461.1707; found, 461.1699.

*tert-Butyl 2-(4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)acetate (**8f**).* The title compound was synthesized from *tert*-butyl (*Z*)-2-(4-(4-(*N*′-hydroxycarbamimidoyl)phenoxy)phenyl)acetate **6f** (1.53 mmol, 522 mg) according to general procedure D and purified using flash chromatography (15% EtOAc and 1% acetic acid in petroleum ether) to yield the product as off-white crystals (316 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.58 (br, 1H), 8.06 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 8.7 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.8 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 3.58 (s, 2H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.7, 171.8, 168.4, 160.3, 160.2, 155.2, 131.0, 130.5, 130.4, 129.4, 121.7, 119.9, 118.5, 116.6, 116.3, 81.7, 42.0, 28.2. HPLC: t_R = 7.9 min, 99% at 254 nm. HRMS m/z: calcd for $C_{26}H_{24}N_2O_5$ + H$^+$ [M + H$^+$], 445.1758; found, 445.1748.

*tert-Butyl 3-(4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)propanoate (**8g**).* The title compound was synthesized from *tert*-butyl (*Z*)-3-(4-(4-(*N*′-hydroxycarbamimidoyl)phenoxy)phenyl)propanoate **6g** (2.39 mmol, 851 mg) according to general procedure D and purified using flash chromatography (15% EtOAc and 1% acetic acid in petroleum ether) to yield the product as a tan solid (303 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12−8.08 (m, 4H), 7.21 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 8.6 Hz, 2H), 6.99 (t, J = 8.4 Hz, 4H), 2.92 (t, J = 7.7 Hz, 2H), 2.57 (t, J = 7.7 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.6, 172.6, 168.5, 160.5, 160.0, 154.5, 136.8, 130.5, 130.0, 129.4, 121.6, 120.0, 118.2, 117.0, 116.2, 80.8, 37.3, 30.6, 28.2. LCMS: t_R = 7.2 min, 84% at 254 nm. HRMS m/z: calcd for $C_{27}H_{26}N_2O_5$ + H$^+$ [M + H$^+$], 459.1914; found, 459.1909.

*tert-Butyl 2-(4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenoxy)acetate (**8h**).* The title compound was synthesized from *tert*-butyl (*Z*)-2-(4-(4-(*N*′-hydroxycarbamimidoyl)phenoxy)phenoxy)acetate **6h** (2.42 mmol, 867 mg) according to general procedure D and purified using flash chromatography (15% EtOAc and 1% acetic acid in petroleum ether) to yield the product as white powder (323 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.61 (br, 1H), 8.09−7.96 (m, 4H), 7.13−7.05 (m, 4H), 7.02−6.95 (m, 4H), 4.67 (s, 2H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.4, 167.9, 167.5, 162.1, 160.7, 154.6, 148.7, 130.1, 129.1, 121.4, 120.5, 117.3, 116.3, 116.0, 114.1, 81.4, 65.4, 27.7. HPLC: t_R = 6.8 min, 99% at 254 nm. HRMS m/z: calcd for $C_{26}H_{24}N_2O_6$ + H$^+$ [M + H$^+$], 461.1707; found, 461.1720.

*2-(2-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)-phenoxy)phenyl)acetic Acid (**9a**).* The title compound was synthesized from *tert*-butyl 2-(2-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)acetate **8a** (0.225 mmol, 100 mg) according to general procedure E and obtained as white powder (78 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (br, 1H), 8.03 (t, J = 8.8 Hz, 4H), 7.42 (d, J = 7.4 Hz, 1H), 7.34 (t, J = 7.4 Hz, 1H), 7.21 (t, J = 7.3 Hz, 1H), 7.09 (d, J = 8.6 Hz, 2H), 7.00 (t, J = 8.7 Hz, 3H), 3.59 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.4, 172.0, 167.6, 162.0, 159.7, 153.6, 132.2, 130.1, 129.0, 128.8, 127.3, 124.6, 120.9, 119.8, 117.8, 116.3, 114.2, 35.4. HPLC: t_R = 5.7 min, 98% at 254 nm. HRMS m/z: calcd for $C_{22}H_{16}N_2O_5$ + H$^+$ [M + H$^+$], 389.1132; found, 389.1125.

*3-(2-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)-phenoxy)phenyl)propanoic Acid (**9b**).* The title compound

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was synthesized *tert*-butyl 3-(2-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)propanoate **8b** (0.058 mmol, 27 mg) according to general procedure E and obtained as an off-white solid (23 mg, 98%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.14 (br, 1H), 10.55 (br, 1H), 8.05 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 7.6 Hz, 1H), 7.30 (td, J = 7.8, 1.5 Hz, 1H), 7.20 (t, J = 7.1 Hz, 1H), 7.08 (d, J = 8.8 Hz, 2H), 7.03 (d, J = 8.0 Hz, 1H), 6.99 (d, J = 8.7 Hz, 2H), 2.79 (t, J = 7.7 Hz, 2H), 2.54−2.47 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.4, 173.6, 167.6, 162.1, 160.1, 152.9, 132.6, 130.7, 130.1, 129.2, 128.2, 125.1, 120.7, 120.5, 117.3, 116.3, 114.2, 33.7, 24.9. HPLC: t_R = 6.6 min, 100% at 254 nm. HRMS m/z: calcd for $C_{23}H_{18}N_2O_5$ + H$^+$ [M + H$^+$], 403.1288; found, 403.1300.

*2-(3-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)-phenoxy)phenyl)acetic Acid (**9c**).* The title compound was synthesized from *tert*-butyl 2-(3-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)acetate **8c** (0.225 mmol, 100 mg) according to general procedure E and obtained as white powder (85 mg, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (br, 1H), 8.10−8.05 (m, 2H), 8.05−7.99 (m, 2H), 7.39 (t, J = 7.8 Hz, 1H), 7.18−7.14 (m, 2H), 7.12 (d, J = 7.7 Hz, 1H), 7.06−7.02 (m, 1H), 7.02−6.97 (m, 3H), 3.61 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.5, 172.4, 167.5, 162.1, 159.6, 155.3, 137.5, 130.1, 130.0, 129.2, 125.6, 121.1, 120.7, 118.4, 117.8, 116.3, 114.2, (1 peak missing). HPLC: t_R = 5.8 min, 99% at 254 nm. HRMS m/z: calcd for $C_{22}H_{16}N_2O_5$ + H$^+$ [M + H$^+$], 389.1132; found, 389.1123.

*3-(3-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)-phenoxy)phenyl)propanoic Acid (**9d**).* The title compound was synthesized from *tert*-butyl 3-(3-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl) propanoate **8d** (0.22 mmol, 100 mg) according to general procedure E and obtained as an off-white solid (84 mg, 95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.12 (br, 1H), 10.55 (br, 1H), 8.09−8.04 (m, 2H), 8.05−8.00 (m, 2H), 7.36 (t, J = 7.9 Hz, 1H), 7.17−7.12 (m, 2H), 7.10 (d, J = 8.0 Hz, 1H), 7.04−6.97 (m, 3H), 6.97−6.92 (m, 1H), 2.84 (t, J = 7.5 Hz, 2H), 2.55 (t, J = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.4, 173.7, 167.6, 162.1, 159.7, 155.3, 143.6, 130.1, 130.1, 129.1, 124.5, 120.9, 119.6, 118.2, 117.3, 116.3, 114.1, 35.0, 30.1. HPLC: t_R = 7.2 min, 100% at 254 nm. HRMS m/z: calcd for $C_{23}H_{18}N_2O_5$+Na$^+$ [M + Na$^+$], 425.1108; found, 425.1122.

*2-(3-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)-phenoxy)phenoxy)acetic Acid (**9e**).* The title compound was synthesized from *tert*-butyl 2-(3-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenoxy)acetate **8e** (0.217 mmol, 100 mg) according to general procedure E and obtained as off-white powder (84 mg, 96%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.57 (br, 1H), 8.07 (d, J = 8.7 Hz, 2H), 8.02 (d, J = 8.7 Hz, 2H), 7.35 (t, J = 8.5 Hz, 1H), 7.17 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.81−6.75 (m, 1H), 6.73−6.67 (m, 2H), 4.70 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.5, 170.0, 167.5, 162.1, 159.4, 159.3, 156.5, 130.8, 130.1, 129.2, 121.2, 118.5, 116.4, 114.1, 112.0, 110.7, 106.2, 64.7. HPLC: t_R = 5.8 min, 99% at 254 nm. HRMS m/z: calcd for $C_{22}H_{16}N_2O_6$ + H$^+$ [M + H$^+$], 405.1081; found, 405.1067.

*2-(4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)-phenoxy)phenyl)acetic Acid (**9f**).* The title compound was synthesized from *tert*-butyl 2-(4-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)acetate **8f** (0.225 mmol, 100 mg) according to general procedure E and obtained as a white solid (78 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.38 (br, 1H), 10.56 (br, 1H), 8.09−8.05 (m, 2H), 8.04−8.00 (m, 2H), 7.34 (d, J = 8.6 Hz, 2H), 7.18−7.12 (m, 2H), 7.12−7.06 (m, 2H), 7.03−6.96 (m, 2H), 3.60 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.5, 172.7, 167.6, 162.1, 159.9, 154.0, 131.3, 130.2, 129.2, 121.0, 119.6, 118.2, 116.3, 114.2, 39.9, (1 peak missing). HPLC: t_R = 6.4 min, 99% at 254 nm. HRMS m/z: calcd for $C_{22}H_{16}N_2O_4$ + H$^+$ [M + H$^+$], 389.1132; found, 389.1146.

*3-(4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)-phenoxy)phenyl)propanoic Acid (**9g**).* The title compound was synthesized from *tert*-butyl 3-(4-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)propanoate **8g** (0.437 mmol, 200 mg) according to general procedure E and obtained as yellow powder (160 mg, 91%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (br, 1H), 8.03 (dd, J = 13.9, 8.7 Hz, 4H), 7.30 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 8.7 Hz, 2H), 7.04 (d, J = 8.4 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 2.84 (t, J = 7.5 Hz, 2H), 2.56 (t, J = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO): δ 175.4, 173.8, 167.6, 162.1, 160.0, 153.5, 137.2, 130.1, 130.1, 129.1, 120.9, 119.8, 118.0, 116.3, 114.2, 35.2, 29.7. HPLC: t_R = 5.9 min, 99% at 254 nm. HRMS m/z: calcd for $C_{23}H_{18}N_2O_5$ + H$^+$ [M + H$^+$], 403.1288; found, 403.1280.

*2-(4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)-phenoxy)phenoxy)acetic Acid (**9h**).* The title compound was synthesized from *tert*-butyl 2-(4-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenoxy)acetate **8h** (0.217 mmol, 100 mg) according to general procedure E and obtained as off-white powder (83 mg, 95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (br, 1H), 8.03 (t, J = 9.1 Hz, 4H), 7.14−7.05 (m, 4H), 7.02−6.95 (m, 4H), 4.69 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.4, 170.2, 167.6, 162.1, 160.7, 154.7, 148.6, 130.1, 129.1, 121.4, 120.5, 117.3, 116.3, 116.0, 114.2, 64.9. HPLC: t_R = 5.8 min, 99% at 254 nm. HRMS m/z: calcd for $C_{22}H_{16}N_2O_6$ + H$^+$ [M + H$^+$], 405.1081; found, 405.1073.

*(Z)-N′-Hydroxy-4-iodobenzimidamide (**10**).* The title compound was synthesized from 4-iodobenzonitrile **4** (5.24 mmol, 1.20 g) according to general procedure C and obtained as off-white crystals (1.16 g, 84%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.72 (br, 1H), 7.77−7.68 (m, 2H), 7.49−7.42 (m, 2H), 5.84 (br, 2H); spectroscopic data consistent with the literature.[43]

*4-((tert-Butyldimethylsilyl)oxy)benzoyl Chloride (**11**).* The title compound was synthesized as previously reported and used directly in the next step without further purification.[26]

*5-(4-((tert-Butyldimethylsilyl)oxy)phenyl)-3-(4-iodophenyl)-1,2,4-oxadiazole (**12**).* A suspension of 4-((*tert*-butyldimethylsilyl)oxy)benzoyl chloride **11** (1 equiv, 9.11 mmol) in toluene (30 mL) was added to a suspension of (*Z*)-*N*′-hydroxy-4-iodobenzimidamide **10** (1 equiv, 9.11 mmol, 2.39 g) in toluene (20 mL) and allowed to stir at reflux (130 °C) for 48 h. The solution was filtered while still hot, and the filtrate was concentrated in vacuo. The residue was recrystallized from EtOH to yield the product as off-white crystals (2.62 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12−8.07 (m, 2H), 7.92−7.83 (m, 4H), 7.00−6.95 (m, 2H), 1.02−0.99 (m, 9H), 0.28−0.24 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.0, 168.4, 160.2, 138.2, 130.2, 129.2, 126.9, 120.9, 117.4, 97.9, 25.7, 18.4, −4.2. HPLC: (hydrophobic PP method) t_R = 5.0 min, 92% at 254 nm. HRMS m/z: calcd for $C_{20}H_{23}IN_2O_2Si$ + H$^+$ [M + H$^+$], 479.0646; found, 479.0661.

General Procedure F. 5-(4-((*tert*-Butyldimethylsilyl)oxy)-phenyl)-3-(4-iodophenyl)-1,2,4-oxadiazole **12** (1 equiv), phe-

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nol (4−5 equiv), K_2CO_3 (2 equiv) and 1,3-diphenyl-1,3-propanedione (1 equiv) were dissolved in DMSO (0.93 M) in a sealed tube and purged with N_2 several times. Copper(II) acetylacetonate (0.5 equiv) was then added, the reaction vessel was sealed, and the solution was stirred at 90 °C for 24 h. The reaction mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with aq NH_4Cl solution and brine; then, the organic layers were dried over $MgSO_4$ and solvent was removed in vacuo. The desired product was isolated using flash chromatography eluting with 10−35% EtOAc in petroleum ether. 1,3-Diphenyl-1,3-propanedione impurities that were present with the obtained product were removed after the subsequent reaction.

General Procedure G. The boc-protected substrate was dissolved in 4 M HCl/1,4-dioxane (∼1 mL/30 mg substrate) and stirred at rt for 6 h. After reaction completion as determined by TLC, the solution was diluted with Et_2O, filtered, and further washed with Et_2O. The filtered solid was air-dried and collected to yield the HCl salt of the product.

*tert-Butyl (2-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)benzyl)carbamate (**14a**).* The title compound was synthesized from *tert*-butyl (2-hydroxybenzyl)carbamate **13a** (4 equiv, 1.6 mmol, 350 mg) according to general procedure F and purified using flash chromatography (10−25% EtOAc in petroleum ether) to yield the product as a yellow solid (48 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05−8.00 (m, 2H), 8.00−7.93 (m, 2H), 7.41 (d, J = 7.3 Hz, 1H), 7.31−7.26 (m, 1H), 7.17 (td, J = 7.5, 0.8 Hz, 1H), 7.00−6.88 (m, 5H), 5.06 (br, 1H), 4.37 (d, J = 5.8 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.8, 168.2, 160.9, 160.0, 156.5, 153.9, 130.4, 130.3, 129.9, 129.5, 129.3, 124.9, 121.9, 120.1, 117.8, 116.2, 116.2, 80.3, 40.2, 28.5. HPLC: t_R = 8.1 min, 95% at 254 nm. HRMS m/z: calcd for $C_{26}H_{25}N_3O_5$ + H^+ [M + H^+], 460.1867; found, 460.1883.

*tert-Butyl (2-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenethyl)carbamate (**14b**).* The title compound was synthesized from *tert*-butyl (2-hydroxyphenethyl)-carbamate **13b** (5 equiv, 3.0 mmol, 700 mg) according to general procedure F and purified using flash chromatography (10−25% EtOAc in petroleum ether) to yield the product as a yellow solid (78 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (t, J = 8.7 Hz, 4H), 7.31−7.25 (m, 1H), 7.23 (dd, J = 7.9, 1.8 Hz, 1H), 7.14 (td, J = 7.4, 1.2 Hz, 1H), 7.00 (d, J = 8.8 Hz, 2H), 6.98−6.92 (m, 3H), 4.75 (br, 1H), 3.41 (dd, J = 12.4, 6.1 Hz, 2H), 2.84 (t, J = 6.9 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.8, 168.4, 160.6, 160.2, 156.4, 154.3, 131.5, 130.8, 130.4, 129.5, 128.4, 124.8, 121.8, 120.1, 117.9, 116.5, 116.3, 79.8, 41.0, 30.9, 28.6. HPLC: t_R = 7.5 min, 83% at 254 nm. HRMS m/z: calcd for $C_{27}H_{27}N_3O_5$ + H^+ [M + H^+], 474.2023; found, 474.2035.

*tert-Butyl (4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)benzyl)carbamate (**14d**).* The title compound was synthesized from *tert*-butyl (4-hydroxybenzyl)carbamate **13d** (5 equiv, 2.09 mmol, 467 mg) according to general procedure F and purified using flash chromatography (10−25% EtOAc in petroleum ether) to yield the product as a flaky yellow solid (69 mg, 36%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12−8.04 (m, 4H), 7.28 (d, J = 8.5 Hz, 2H), 7.08−7.00 (m, 4H), 6.97 (d, J = 8.8 Hz, 2H), 4.98 (br, 1H), 4.33 (d, J = 5.3 Hz, 2H), 1.49 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.8, 168.4, 160.5, 160.1, 156.4, 155.6, 134.6, 130.5, 129.4, 129.2, 121.9, 120.0, 118.5, 116.7, 116.3, 80.2, 44.3, 28.6. HPLC: t_R = 6.6 min, 99% at 254 nm. HRMS m/z: calcd for $C_{26}H_{25}N_3O_5$ + H^+ [M + H^+], 460.1867; found, 460.1870.

*tert-Butyl (4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenethyl)carbamate (**14e**).* The title compound was synthesized from *N*-boc-tyramine (5 equiv, 4.71 mmol, 1.12 g) according to general procedure F (using Cs_2CO_3 instead of K_2CO_3) and purified using flash chromatography (10−35% EtOAc in petroleum ether) to yield the product as a yellow solid (319 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15−8.07 (m, 4H), 7.20 (d, J = 8.4 Hz, 2H), 7.09−7.05 (m, 2H), 7.04−6.96 (m, 4H), 6.06 (br, 1H), 4.61 (br, 1H), 3.40 (d, J = 6.1 Hz, 2H), 2.80 (t, J = 7.0 Hz, 2H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.7, 168.5, 160.3, 160.3, 156.3, 154.9, 134.8, 130.5, 130.4, 129.4, 121.8, 120.0, 118.4, 116.9, 116.3, 79.8, 42.1, 35.8, 28.6. HPLC: t_R = 7.6 min, 99% at 254 nm. HRMS m/z: calcd for 2×($C_{27}H_{27}N_3O_5$) + H^+ [2M + H^+], 947.3974; found, 947.3977.

*(RS)-tert-Butyl (2-(4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)propyl)carbamate (**14f**).* The title compound was synthesized from (*RS*)-*tert*-butyl (2-(4-hydroxyphenyl)propyl)carbamate **13f** (4.5 equiv, 1.9 mmol, 470 mg) according to general procedure F and purified using flash chromatography (10−25% EtOAc in petroleum ether) to yield the product as a yellow solid (71 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12−8.05 (m, 4H), 7.20 (d, J = 8.3 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 7.03−6.97 (m, 4H), 4.59 (br, 1H), 3.39 (dt, J = 13.0, 6.5 Hz, 1H), 3.21 (ddd, J = 13.5, 8.1, 5.5 Hz, 1H), 2.93 (dd, J = 13.6, 6.7 Hz, 1H), 1.44 (s, 9H), 1.27 (d, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.8, 168.4, 160.6, 160.2, 156.4, 154.9, 140.0, 130.5, 129.4, 128.8, 121.8, 119.9, 118.5, 116.6, 116.3, 79.9, 47.6, 39.7, 28.6, 19.3. HPLC: t_R = 7.8 min, 91% at 254 nm. HRMS m/z: calcd for 2×($C_{28}H_{29}N_3O_5$) + H^+ [2M + H^+], 975.4287; found, 975.4271.

*tert-Butyl (4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenethyl)(methyl)carbamate (**14g**).* The title compound was synthesized from *tert*-butyl (4-hydroxyphenethyl)(methyl)carbamate **13g** (4 equiv, 1.8 mmol, 450 mg) according to general procedure F and purified using flash chromatography (10−25% EtOAc in petroleum ether) to yield the product as a yellow solid (44 mg, 21%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12−8.04 (m, 4H), 7.18 (d, J = 7.8 Hz, 2H), 7.08−6.94 (m, 6H), 3.51−3.41 (m, 2H), 2.89−2.77 (m, 5H), 1.44 (s, 9H).·^{13}C NMR (101 MHz, $CDCl_3$): δ 175.8, 168.4, 160.8, 160.4, 156.1, 154.8, 135.1, 130.5, 130.4, 129.4, 121.8, 120.0, 118.3, 116.5, 116.3, 80.1, 51.1, 34.8, 33.9, 28.6. HPLC: t_R = 8.8 min, 97% at 254 nm. HRMS m/z: calcd for [M + H]$^+$ $C_{28}H_{29}N_3O_5$, 488.2180; found, 488.2190.

*tert-Butyl (3-(4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)propyl)carbamate (**14h**).* The title compound was synthesized from *tert*-butyl (3-(4-hydroxyphenyl)propyl)carbamate **13h** (4.8 equiv, 3.52 mmol, 885 mg) according to general procedure F (using Cs_2CO_3 instead of K_2CO_3) and purified using flash chromatography (10−25% EtOAc in petroleum ether) to yield the product as an amorphous yellow solid (257 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14−8.07 (m, 4H), 7.18 (d, J = 8.6 Hz, 2H), 7.09−7.04 (m, 2H), 7.02−6.95 (m, 4H), 5.69 (br, 1H), 4.56 (br, 1H), 3.18 (d, J = 6.6 Hz, 2H), 2.70−2.60 (m, 2H), 1.84 (dq, J = 14.6, 7.1 Hz, 2H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.8, 168.4, 160.7, 160.5, 156.6, 154.4, 137.4, 130.5, 129.9, 129.4, 121.6, 120.0, 118.2, 116.6, 116.3, 79.9, 40.4, 32.5, 31.8, 28.6. HPLC: t_R = 8.5 min, 92% at 254 nm.

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HRMS m/z: calcd for $C_{28}H_{29}N_3O_5$ + H^+ [M + H^+], 488.2180; found, 488.2185.

*tert-Butyl 7-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**14i**).* The title compound was synthesized from *tert*-butyl 7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate **13i** (4 equiv, 1.4 mmol, 350 mg) according to general procedure F and purified using flash chromatography (10−20% EtOAc in petroleum ether) to yield the product as a yellow solid (37 mg, 22%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (dd, J = 8.3, 4.8 Hz, 4H), 7.14 (d, J = 8.3 Hz, 1H), 7.06 (d, J = 8.0 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.90 (dd, J = 8.2, 2.0 Hz, 1H), 6.82 (d, J = 2.3 Hz, 1H), 4.57 (d, J = 11.3 Hz, 2H), 3.68 (t, J = 5.8 Hz, 2H), 2.84 (t, J = 5.8 Hz, 2H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.8, 168.4, 160.6, 160.3, 155.3, 154.7, 135.4, 130.6, 130.5, 130.3, 129.4, 121.9, 118.4, 118.3, 117.5, 116.6, 116.3, 80.5, 46.1, 42.0, 28.7, 28.5. HPLC: t_R = 8.1 min, 75% at 254 nm. HRMS m/z: calcd for $C_{28}H_{27}N_3O_5$ + Na^+ [M + Na^+], 508.1843; found, 508.1853.

*4-(3-(4-(2-(Aminomethyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol Hydrochloride (**15a**).* The title compound was synthesized from *tert*-butyl (2-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)benzyl)carbamate **14a** (0.102 mmol, 47 mg) according to general procedure G and obtained as off-white powder (30 mg, 74%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.67 (br, 1H), 8.47 (br, 3H), 8.14−8.09 (m, 2H), 8.05−7.99 (m, 2H), 7.66 (dd, J = 7.7, 1.5 Hz, 1H), 7.44 (td, J = 8.1, 1.7 Hz, 1H), 7.28 (td, J = 7.6, 1.0 Hz, 1H), 7.26−7.21 (m, 2H), 7.05−6.99 (m, 3H), 4.06 (q, J = 5.7 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.5, 169.3, 163.6, 160.2, 156.4, 132.4, 132.2, 131.2, 130.5, 125.7, 125.4, 124.3, 120.3, 119.8, 117.2, 116.4, 39.6. HPLC: t_R = 5.4 min, 98% at 254 nm. HRMS m/z: calcd for $C_{21}H_{17}N_3O_3$ + H^+ [M + H^+], 360.1343; found, 360.1347.

*4-(3-(4-(2-(2-Aminoethyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol Hydrochloride (**15b**).* The title compound was synthesized from *tert*-butyl (2-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenethyl)carbamate **14b** (0.13 mmol, 63 mg) according to general procedure G and obtained as tan powder (9.1 mg, 17%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.61 (br, 1H), 8.10−8.06 (m, 2H), 8.04−7.99 (m, 2H), 7.88 (br, 3H), 7.43 (dd, J = 7.6, 1.6 Hz, 1H), 7.37 (td, J = 7.8, 1.8 Hz, 1H), 7.25 (td, J = 7.5, 1.2 Hz, 1H), 7.15−7.11 (m, 2H), 7.05 (dd, J = 8.1, 1.0 Hz, 1H), 7.03−6.98 (m, 2H), 3.04−3.00 (m, 2H), 2.89 (dd, J = 9.6, 6.3 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.4, 169.3, 163.5, 161.4, 155.5, 132.4, 131.2, 130.4, 130.4, 129.6, 126.3, 123.3, 121.4, 118.9, 117.2, 116.4, 40.8, 29.6. HPLC: t_R = 6.0 min, 95% at 254 nm. HRMS m/z: calcd for $C_{22}H_{19}N_3O_3$ + H^+ [M + H^+], 374.1499; found, 374.1503.

*4-(3-(4-(3-(2-Aminoethyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol Hydrochloride (**15c**).* The title compound was synthesized from *tert*-butyl (3-hydroxyphenethyl)carbamate **13c** (5 equiv, 1.6 mmol, 370 mg) according to general procedure F, and flash chromatography (5−20% EtOAc in petroleum ether) was carried out to yield the impure product. The crude material was dissolved in 4 M HCl/1,4-dioxane (0.5 mL) and stirred at rt for 6 h. The solution was diluted with Et_2O, filtered, and further washed with Et_2O. The collected solid was air-dried, and the products were separated using preparative HPLC. The recovered material was treated with 1.25 M HCl/1,4-dioxane (0.50 mL) to convert the product to the HCl salt. The reaction mixture was concentrated in vacuo, diluted with Et_2O, and the resulting solid was collected and dried to yield the product as a white solid (7.2 mg, 6% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.11−8.05 (m, 2H), 8.02 (d, J = 8.8 Hz, 2H), 7.75 (br, 3H), 7.42 (t, J = 7.9 Hz, 1H), 7.21−7.16 (m, 2H), 7.14 (d, J = 7.7 Hz, 1H), 7.07 (s, 1H), 7.05−6.99 (m, 3H), 3.10−3.03 (m, 2H), 2.93−2.86 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.4, 169.4, 163.5, 161.5, 158.1, 140.4, 131.7, 131.2, 130.3, 125.7, 123.2, 121.1, 119.6, 117.2, 116.4, 41.8, 34.4 (1 peak missing). HPLC: t_R = 6.1 min, 99% at 254 nm. HRMS m/z: calcd for $C_{22}H_{19}N_3O_3$ + H^+ [M + H^+], 374.1499; found, 374.1503.

*4-(3-(4-(4-(Aminomethyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol Hydrochloride (**15d**).* The title compound was synthesized from *tert*-butyl (4-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)benzyl)carbamate **14d** (0.124 mmol, 57.0 mg) according to general procedure G and obtained as off-white powder (46 mg, 94%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.59 (br, 1H), 8.23 (br, 3H), 8.12−8.07 (m, 2H), 8.04−8.00 (m, 2H), 7.55 (d, J = 8.7 Hz, 2H), 7.23−7.18 (m, 2H), 7.18−7.13 (m, 2H), 7.03−6.97 (m, 2H), 4.05 (q, J = 5.8 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.5, 169.4, 163.6, 161.0, 158.6, 132.1, 131.2, 130.4, 130.1, 123.7, 121.0, 119.9, 117.2, 116.4, 43.8. HPLC: t_R = 5.0 min, 97% at 254 nm. HRMS m/z: calcd for $C_{21}H_{17}N_3O_3$ + H^+ [M + H^+], 360.1343; found, 360.1342.

*4-(3-(4-(4-(2-Aminoethyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol Hydrochloride (**15e**).* The title compound was synthesized from *tert*-butyl (4-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenethyl)carbamate **14e** (0.32 mmol, 149 mg) according to general procedure G and obtained as a tan solid (128 mg, 100%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.63 (br, 1H), 8.10−7.94 (m, 7H), 7.35 (d, J = 8.4 Hz, 2H), 7.13 (dd, J = 12.6, 8.7 Hz, 4H), 7.01 (d, J = 8.7 Hz, 2H), 3.11−3.00 (m, 2H), 2.95−2.86 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.5, 167.5, 162.1, 159.8, 154.1, 133.5, 130.5, 130.1, 129.1, 121.0, 119.9, 118.1, 116.3, 114.1, 32.3. HPLC: t_R = 5.2 min, 100% at 254 nm. HRMS m/z: calcd for $C_{22}H_{19}N_3O_3$ + H^+ [M + H^+], 374.1499; found, 374.1507.

*(RS)-4-(3-(4-(4-(1-Aminopropan-2-yl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol Hydrochloride (**15f**).* The title compound was synthesized from (*RS*)-*tert*-butyl (2-(4-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)-propyl)carbamate **14f** (0.094 mmol, 46 mg) according to general procedure G and obtained as tan powder (30 mg, 75%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.65 (br, 1H), 8.07 (d, J = 8.1 Hz, 5H), 8.01 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 7.16 (d, J = 8.8 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.01 (d, J = 8.7 Hz, 2H), 3.09 (dt, J = 13.4, 6.8 Hz, 1H), 3.01 (d, J = 5.5 Hz, 2H), 1.28 (d, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.4, 169.4, 163.5, 161.6, 156.9, 139.2, 131.2, 130.3, 130.0, 123.1, 121.5, 119.4, 117.2, 116.4, 46.9, 39.1, 20.0. HPLC: t_R = 5.8 min, 99% at 254 nm. HRMS m/z: calcd for $C_{23}H_{21}N_3O_3$ + H^+ [M + H^+], 388.1656; found, 388.1661.

*4-(3-(4-(4-(2-(Methylamino)ethyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol Hydrochloride (**15g**).* The title compound was synthesized from *tert*-butyl (4-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenethyl)(methyl)-carbamate **14g** (0.067 mmol, 34 mg) according to general procedure G and obtained as tan powder (23 mg, 77%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.62 (br, 1H), 8.83 (br, 2H), 8.07 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.13 (t, J = 9.0 Hz, 4H), 7.01 (d, J = 8.7 Hz, 2H),

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3.14 (s, 2H), 2.99−2.91 (m, 2H), 2.58 (s, 3H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.4, 169.4, 163.5, 161.6, 156.9, 133.5, 131.6, 131.2, 130.3, 123.1, 121.3, 119.4, 117.2, 116.4, 51.4, 33.8, 32.6. HPLC: t_R = 5.8 min, 100% at 254 nm. HRMS *m/z*: calcd for $C_{23}H_{21}N_3O_3$ + H^+ [M + H^+], 388.1656; found, 388.1658.

*4-(3-(4-(4-(3-Aminopropyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol Hydrochloride (**15h**).* The title compound was synthesized from *tert*-butyl (3-(4-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenyl)propyl)carbamate **14h** (0.032 mmol, 16 mg) according to general procedure G and obtained as tan powder (12 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.07 (d, *J* = 8.8 Hz, 2H), 8.02 (d, *J* = 8.7 Hz, 2H), 7.96−7.53 (br, 3H), 7.30 (d, *J* = 8.5 Hz, 2H), 7.11 (dd, *J* = 15.3, 8.7 Hz, 4H), 7.00 (d, *J* = 8.8 Hz, 2H), 2.83−2.78 (m, 2H), 2.67 (t, *J* = 7.6 Hz, 2H), 1.90−1.82 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.4, 169.4, 163.5, 161.9, 156.0, 137.9, 131.2, 131.0, 130.2, 122.9, 121.1, 119.1, 117.2, 116.4, 40.3, 32.8, 30.4. HPLC: t_R = 5.9 min, 100% at 254 nm. HRMS *m/z*: calcd for $C_{23}H_{21}N_3O_3$ + H^+ [M + H^+], 388.1656; found, 388.1666.

*4-(3-(4-((1,2,3,4-Tetrahydroisoquinolin-7-yl)oxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol Hydrochloride (**15i**).* The title compound was synthesized from *tert*-butyl 7-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate **14i** (0.068 mmol, 33 mg) according to general procedure G and obtained as a tan solid (21 mg, 75%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.61 (br, 1H), 9.28 (br, 2H), 8.11−8.06 (m, 2H), 8.04−8.00 (m, 2H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.17−7.12 (m, 2H), 7.10−7.04 (m, 2H), 7.03−6.98 (m, 2H), 4.26 (s, 2H), 3.42−3.34 (m, 2H), 3.01 (t, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.4, 169.4, 163.5, 161.3, 156.6, 132.0, 131.2, 131.1, 130.3, 128.5, 123.4, 120.8, 119.5, 118.7, 117.2, 116.4, 45.7, 43.0, 25.6. HPLC: t_R = 5.7 min, 100% at 254 nm. HRMS *m/z*: calcd for $C_{23}H_{19}N_3O_3$ + H^+ [M + H^+], 386.1499; found, 386.1508.

*4-((tert-Butoxycarbonyl)amino)benzoic Acid (**17a**).* 4-Aminobenzoic acid **16a** (1 equiv, 21.9 mmol, 3.00 g) was solubilized in MeOH (24 mL) and water (24 mL) and $NaHCO_3$ (2 equiv, 43.8 mmol, 3.72 g) was added. Boc_2O (1.5 equiv, 32.8 mmol, 7.15 g) dissolved in MeOH (24 mL) was added dropwise, and the solution was stirred at rt overnight. The solvent was removed in vacuo, and the residue was extracted with EtOAc and then washed with aq 1 M HCl solution and brine. The organic layers were combined, dried over $MgSO_4$, and the solvent was removed in vacuo to yield the product as a white solid (4.47 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06−8.01 (m, 2H), 7.46 (d, *J* = 8.8 Hz, 2H), 6.73 (br, 1H), 1.54 (s, 9H); spectroscopic data consistent with the literature.[44]

*4-((tert-Butoxycarbonyl)(methyl)amino)benzoic Acid (**17b**).* 4-(Methylamino)benzoic acid **16b** (1 equiv, 13.2 mmol, 2.0 g) was solubilized in MeOH (16 mL) and water (16 mL) and $NaHCO_3$ (2 equiv, 26.5 mmol, 2.25 g) was added. Boc_2O (1.5 equiv, 19.9 mmol, 4.32 g) dissolved in MeOH (16 mL) was added dropwise, and the solution was stirred at rt overnight. The solvent was removed in vacuo, and the residue was extracted with EtOAc and then washed with aq 1 M HCl solution and brine. The organic layers were combined, dried over $MgSO_4$, and the solvent was removed in vacuo to yield the product as a white solid (2.61 g, 79%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.95−7.82 (m, 2H), 7.47−7.33 (m, 2H), 3.22 (s, 3H), 1.41 (s, 9H); spectroscopic data consistent with the literature.[45]

*(Z)-N′-Hydroxy-4-phenoxybenzimidamide (**18**).* The title compound was synthesized as previously reported and obtained as a white solid (1.06 g, 80%).[26] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63−7.56 (m, 2H), 7.40−7.33 (m, 2H), 7.18−7.12 (m, 1H), 7.06−6.98 (m, 4H), 4.84 (br, 2H); spectroscopic data consistent with the literature.[26]

General Procedure H. A solution of (*Z*)-*N*′-hydroxy-4-phenoxybenzimidamide **18** (1 equiv) in DMF (0.11 M) was treated with benzoic acid (1.5 equiv), HOBt (2 equiv), and EDC·HCl (2 equiv); then, the solution was stirred at rt for 16 h. The temperature was increased to 100 °C, and the stirring was continued for 16 h. The reaction mixture was concentrated in vacuo, diluted with a 10% solution of aq Na_2CO_3, and then, EtOAc was added to extract the product. The separated organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo; then, the residue was purified using flash chromatography eluting with 5−20% EtOAc in petroleum ether to yield the product.

***tert*-Butyl (4-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)phenyl)carbamate (19a).** The title compound was synthesized from 4-((*tert*-butoxycarbonyl)amino)benzoic acid **17a** (4.22 mmol, 1.00 g) according to general procedure H and purified using flash chromatography (5−10% EtOAc in petroleum ether) to yield the product as a white solid (252 mg, 21%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17−8.09 (m, 4H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.43−7.35 (m, 2H), 7.21−7.15 (m, 1H), 7.12−7.06 (m, 4H), 6.69 (br, 1H), 1.55 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 212.8, 205.2, 197.4, 193.0, 190.1, 181.8, 167.9, 166.8, 166.6, 162.1, 158.6, 157.3, 155.9, 155.7, 154.2, 117.5, 65.6. HPLC: t_R = 8.6 min, 98% at 254 nm. HRMS *m/z*: calcd for $C_{25}H_{23}N_3O_4$ + H^+ [M + H^+], 430.1761; found, 430.1769.

*tert-Butyl Methyl(4-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)phenyl)carbamate (**19b**).* The title compound was synthesized from 4-((*tert*-butoxycarbonyl)(methyl)amino)benzoic acid (3.58 mmol, 900 mg) **17b** (4.22 mmol, 1.00 g) according to general procedure H and purified using flash chromatography (10−20% EtOAc and 1% Et_3N in petroleum ether) to yield the product as yellow crystals (216 mg, 14%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19−8.10 (m, 4H), 7.49−7.43 (m, 2H), 7.42−7.35 (m, 2H), 7.21−7.15 (m, 1H), 7.13−7.06 (m, 4H), 3.34 (s, 3H), 1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.4, 168.6, 160.3, 156.4, 154.2, 148.0, 130.1, 129.5, 128.7, 125.2, 124.3, 121.8, 120.7, 119.9, 118.6, 81.4, 37.0, 28.5. HPLC: t_R = 8.9 min, 90% at 254 nm. HRMS *m/z*: calcd for $C_{26}H_{25}N_3O_4$ + H^+ [M + H^+], 444.1918; found, 444.1927.

*N-(4-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)phenyl)-acetamide (**19c**).* The title compound was synthesized from 4-acetamidobenzoic acid **17c** (1.04 mmol, 186 mg) according to general procedure H and purified using flash chromatography (20% EtOAc in petroleum ether) to yield the product as an off-white solid (54 mg, 21%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19−8.09 (m, 4H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.54 (br, 1H), 7.42−7.35 (m, 2H), 7.21−7.14 (m, 1H), 7.11−7.04 (m, 4H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.3, 168.7, 168.6, 160.3, 156.3, 142.1, 130.1, 129.5, 129.4, 124.3, 121.7, 120.0, 119.9, 119.6, 118.5, 24.9. HPLC: t_R = 6.6 min, 99% at 254 nm. HRMS *m/z*: calcd for $C_{22}H_{17}N_3O_3$ + H^+ [M + H^+], 372.1343; found, 372.1339.

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*5-(4-Nitrophenyl)-3-(4-phenoxyphenyl)-1,2,4-oxadiazole (**19d**).* A solution of (*Z*)-*N*′-hydroxy-4-phenoxybenzimidamide **18** (1.5 equiv, 2.07 mmol, 473 mg) in DMF (5 mL) was treated with 4-nitrobenzoic acid **17d** (1 equiv, 1.38 mmol, 231 mg), HOBt (2 equiv, 2.76 mmol, 373 mg), and EDC·HCl (2 equiv, 2.76 mmol, 531 mg); then, the solution was stirred at rt for 16 h. The temperature was increased to 100 °C, and the stirring was continued for 16 h. The reaction mixture was concentrated in vacuo, diluted with a 10% solution of aq Na_2CO_3, and then, EtOAc was added to extract the product. The separated organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo; then, the residue was purified using flash chromatography (20% EtOAc in petroleum ether) to yield the product as a yellow solid (290 mg, 58%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.28−8.23 (m, 1H), 8.20−8.15 (m, 1H), 8.06 (d, J = 8.7 Hz, 2H), 8.02−7.96 (m, 2H), 7.47 (t, J = 7.7 Hz, 2H), 7.27−7.21 (m, 1H), 7.19−7.12 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.4, 167.8, 160.0, 155.3, 148.2, 134.2, 133.9, 131.6, 130.3, 129.3, 124.9, 124.5, 120.1, 119.7, 118.4, 117.5. HPLC: t_R = 6.9 min, 99% at 254 nm. HRMS m/z: calcd for $C_{20}H_{13}N_3O_4$ + H$^+$ [M + H$^+$], 360.0979; found, 360.0980.

*4-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)aniline (**20a**).* A solution of *tert*-butyl (4-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)phenyl)carbamate **19a** (0.50 mmol, 214 mg) in 4 M HCl/1,4-dioxane (4.0 mL) was stirred at rt for 6 h. After reaction completion as determined by TLC, the solution was diluted with Et_2O, filtered, and further washed with Et_2O. The recovered precipitate was suspended in saturated aq sodium bicarbonate solution and washed with EtOAc. The organic layers were combined and dried over $MgSO_4$ and then concentrated in vacuo to yield the product as yellow powder (128 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15−8.08 (m, 2H), 8.04−7.97 (m, 2H), 7.42−7.35 (m, 2H), 7.20−7.14 (m, 1H), 7.12−7.06 (m, 4H), 6.78−6.73 (m, 2H), 4.13 (br, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.1, 168.3, 160.1, 156.4, 150.8, 130.2, 130.1, 129.4, 124.2, 122.2, 119.8, 118.5, 114.7, 114.2. HPLC: t_R = 7.3 min, 99% at 254 nm. HRMS m/z: calcd for $C_{20}H_{15}N_3O_2$ + H$^+$ [M + H$^+$], 330.1237; found, 330.1245.

***N*-Methyl-4-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)aniline (20b).** A solution of *tert*-butyl methyl(4-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)phenyl)carbamate **19b** (0.23 mmol, 100 mg) in 4 M HCl/1,4-dioxane (2.0 mL) was stirred at rt for 6 h. After reaction completion as determined by TLC, the solution was diluted with Et_2O, filtered, and further washed with Et_2O. The collected precipitate was air-dried to yield the product as a white solid (66 mg, 86%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10−8.01 (m, 2H), 7.93−7.85 (m, 2H), 7.49−7.42 (m, 2H), 7.26−7.20 (m, 1H), 7.17−7.10 (m, 4H), 6.73−6.66 (m, 2H), 2.77 (s, 3H). ^{13}C NMR (101 MHz, methanol-d_4): δ 176.5, 169.7, 162.0, 157.5, 147.1, 131.2, 131.1, 130.3, 125.5, 122.6, 121.1, 120.9, 120.0, 119.3, 34.6. HPLC: t_R = 7.9 min, 99% at 254 nm. HRMS m/z: calcd for $C_{21}H_{17}N_3O_2$ + H$^+$ [M + H$^+$], 344.1394; found, 344.1399.

***tert*-Butyl (2-Methoxyethyl)(4-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)phenyl)carbamate (21).** *tert*-Butyl (4-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)phenyl)-carbamate **19a** (0.12 mmol, 50 mg) was dissolved in anhydrous DMF (3 mL) under N_2 atmosphere and NaH (60% in mineral oil, 1.2 equiv, 0.14 mmol, 5.8 mg) was added in small portions at 0 °C. The reaction mixture was stirred for 1 h at rt. 1-Bromo-2-methoxyethane (1.5 equiv, 0.18 mmol, 16 μL) was added slowly at 0 °C; then, the mixture was stirred at rt for 2 h. The reaction mixture was diluted with aq NH_4Cl solution and extracted with EtOAc. The organic layers were combined, washed with aq NH_4Cl solution and brine, then dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using flash chromatography (5−10% EtOAc in petroleum ether) to yield the product as opaque oil (16 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19−8.11 (m, 4H), 7.52−7.46 (m, 2H), 7.42−7.36 (m, 2H), 7.18 (ddd, J = 8.5, 2.2, 1.1 Hz, 1H), 7.12−7.07 (m, 4H), 3.86 (t, J = 5.7 Hz, 2H), 3.59 (t, J = 5.7 Hz, 2H), 3.34 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.4, 168.7, 160.3, 156.3, 154.2, 147.3, 130.1, 129.5, 128.8, 127.2, 124.3, 121.8, 121.4, 119.9, 118.6, 81.3, 70.56, 58.9, 49.8, 28.5. HPLC: t_R = 9.3 min, 94% at 254 nm. HRMS m/z: calcd for $C_{28}H_{29}N_3O_5$ + H$^+$ [M + H$^+$], 488.2180; found, 488.2186.

*N-(2-Methoxyethyl)-4-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)aniline (**22**).* A solution of *tert*-butyl (2-methoxyethyl)(4-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)-phenyl)carbamate **21** (0.031 mmol, 15 mg) in 4 M HCl/1,4-dioxane (1.5 mL) was stirred at rt for 6 h. After reaction completion as determined by TLC, the solution was diluted with Et_2O, filtered, and further washed with Et_2O. The obtained precipitate was dissolved in acetonitrile and washed with petroleum benzines; then, the solvent was removed in vacuo. The residue was dissolved in EtOAc and washed with water; then, the organic layer dried over $MgSO_4$ and concentrated in vacuo to yield the product as a yellow solid (7.4 mg, 87%). ^{1}H NMR (400 MHz, methanol-d_4): δ 8.10−8.05 (m, 2H), 7.95−7.90 (m, 2H), 7.41 (tt, J = 7.6, 2.2 Hz, 2H), 7.22−7.17 (m, 1H), 7.11−7.05 (m, 4H), 6.78−6.72 (m, 2H), 3.61 (t, J = 5.5 Hz, 2H), 3.39 (s, 3H), 3.37 (t, J = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.9, 169.2, 161.7, 157.6, 154.4, 131.1, 130.8, 130.2, 125.4, 123.1, 120.8, 119.3, 113.1, 112.3, 72.0, 59.0, 43.7. HPLC: t_R = 8.4 min, 95% at 254 nm. HRMS m/z: calcd for $C_{23}H_{21}N_3O_3$ + H$^+$ [M + H$^+$], 388.1656; found, 388.1656.

*tert-Butyl (4-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)-phenyl)glycinate (**23**).* The title compound was synthesized from 4-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)aniline **20a** (0.40 mmol, 130 mg) according to a previously reported procedure and obtained as a yellow solid (56 mg, 32%).[46] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, J = 8.7 Hz, 2H), 8.03 (d, J = 8.7 Hz, 2H), 7.38 (t, J = 7.9 Hz, 2H), 7.17 (t, J = 7.4 Hz, 1H), 7.08 (dd, J = 8.6, 1.8 Hz, 4H), 6.66 (d, J = 8.7 Hz, 2H), 4.82 (bt, J = 4.6 Hz, 1H), 3.88 (d, J = 4.9 Hz, 2H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.1, 169.5, 168.3, 160.0, 156.5, 150.7, 130.2, 130.1, 129.4, 124.2, 122.2, 119.8, 118.5, 113.6, 112.6, 82.8, 45.9, 28.2. HPLC: t_R = 7.4 min, 98% at 254 nm. HRMS m/z: calcd for $C_{26}H_{25}N_3O_4$ + H$^+$ [M + H$^+$], 444.1918; found, 444.1915.

*(4-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)phenyl)-glycine (**24**).* The title compound was synthesized from *tert*-butyl (4-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)phenyl)-glycinate **23** (0.047 mmol, 21 mg) according to general procedure E and obtained as a yellow solid (17 mg, 92%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.06 (d, J = 8.6 Hz, 2H), 7.89 (d, J = 8.7 Hz, 2H), 7.46 (t, J = 7.8 Hz, 2H), 7.23 (t, J = 7.4 Hz, 1H), 7.14 (dd, J = 8.2, 2.8 Hz, 4H), 6.99 (br, 1H), 6.74 (d, J = 8.7 Hz, 2H), 3.94 (d, J = 3.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 175.8, 171.9, 167.4, 159.6, 155.5, 152.5, 130.3, 129.5, 129.1, 124.5, 121.3, 119.7, 118.3, 112.2, 110.3,

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44.0. HPLC: t_R = 6.3 min, 95% at 254 nm. HRMS *m/z*: calcd for $C_{22}H_{17}N_3O_4$ + H^+ [M + H^+], 388.1292; found, 388.1279.

*4-(3-(4-(4-(2-(Dimethylamino)ethyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol (**25a**).* *tert*-Butyl (4-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)phenethyl)-carbamate **14e** (0.42 mmol, 200 mg) was dissolved in DCM (2.5 mL), and TFA (0.2 mL) was added to stir at rt for 6 h. The solvent was removed in vacuo, and the residue was treated with saturated aq sodium bicarbonate solution and then sonicated until a precipitate formed. The suspension was treated with 10% aq citric acid solution until pH 6 and then extracted with EtOAc three times. The organic layers were combined, dried over $MgSO_4$, and the solvent was removed in vacuo to yield 4-(3-(4-(4-(2-aminoethyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol **15e** as the neutral species (158 mg, 100%). ^{1}H NMR (400 MHz, methanol-d_4): δ 8.11−8.06 (m, 2H), 8.06−8.01 (m, 2H), 7.38−7.27 (m, 2H), 7.14−7.03 (m, 4H), 7.00−6.92 (m, 2H), 3.25−3.15 (m, 2H), 3.03−2.94 (m, 2H).

To a suspension of **15e** (1 equiv, 0.13 mmol, 50 mg) in acetonitrile (2 mL) and water (0.5 mL) was added 37% aq formaldehyde solution (12 equiv, 1.6 mmol, 130 μL). $NaBH(OAc)_3$ (4.8 equiv, 0.64 mmol, 140 mg) was added slowly, and the reaction was stirred at rt for 50 min. The reaction mixture was quenched with saturated aq sodium bicarbonate solution and then extracted several times with a 9:1 solution of EtOAc/isopropanol. The organic layers were combined and concentrated in vacuo. The residue was treated with small amounts of EtOAc and saturated aq sodium bicarbonate solution until a precipitate formed. A sodium citrate buffer of pH ∼ 6.5 was added slowly until the solution became neutral and the precipitate dissolved. The aqueous layer was washed with EtOAc; then, the organic layers were combined and set aside. The aqueous layer was concentrated in vacuo to obtain cruder residue. The sequential process of saturated aq sodium bicarbonate solution and sodium citrate buffer additions followed by EtOAc extraction was repeated until no further precipitation was observed. The combined organic layers were dried over $MgSO_4$, and the solvent was removed in vacuo to yield the product as an off-white solid (39 mg, 74%). ^{1}H NMR (400 MHz, methanol-d_4): δ 8.11−8.01 (m, 4H), 7.32−7.27 (m, 2H), 7.10−7.05 (m, 2H), 7.05−7.01 (m, 2H), 6.99−6.94 (m, 2H), 2.90−2.82 (m, 2H), 2.77−2.71 (m, 2H), 2.44 (s, 6H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.4, 169.5, 163.7, 162.0, 155.9, 136.8, 131.4, 131.2, 130.2, 122.8, 121.1, 119.1, 117.2, 116.4, 62.0, 45.1, 33.6. HPLC: t_R = 6.0 min, 99% at 254 nm. HRMS *m/z*: calcd for $C_{24}H_{23}N_3O_3$ + H^+ [M + H^+], 402.1812; found, 402.1812.

*2-(4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)-phenoxy)phenyl)-N,N,N-trimethylethan-1-aminium Iodide (**26a**).* 4-(3-(4-(4-(2-(Dimethylamino)ethyl)phenoxy)-phenyl)-1,2,4-oxadiazol-5-yl)phenol **25a** (1 equiv, 0.092 mmol, 37 mg) was dissolved in a 2:1 solution of DCM/MeOH (3 mL) under N_2 atmosphere, and iodomethane (5 equiv, 0.46 mmol, 29 μL) was added. The reaction mixture was stirred for 24 h at rt. The solvent was removed in vacuo, and then, the residue was suspended in Et_2O and filtered. The precipitate was collected to yield the product as pink crystals (50 mg, 100%). ^{1}H NMR (400 MHz, methanol-d_4): δ 8.15−8.10 (m, 2H), 8.10−8.04 (m, 2H), 7.42 (d, J = 8.6 Hz, 2H), 7.11 (dd, J = 9.8, 1.7 Hz, 4H), 7.01−6.96 (m, 2H), 3.65−3.59 (m, 2H), 3.26 (s, 9H), 3.22−3.15 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.4, 169.4, 163.6, 161.5, 156.9, 132.8, 131.8, 131.2, 130.3, 123.2, 121.3, 119.4, 117.2, 116.4, 68.4, 53.8, 53.8, 53.7, 29.6. HPLC: t_R = 6.1 min, 100% at 254 nm. HRMS *m/z*: calcd for $C_{25}H_{26}N_3O_3$ [M^+], 416.1969; found, 416.1981.

*4-(3-(4-(4-(3-(Dimethylamino)propyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol Hydrochloride (**25b**).* *tert*-Butyl (3-(4-(4-(5-(4-hydroxyphenyl)-1,2,4-oxadiazol-3-yl)phenoxy)-phenyl)propyl)carbamate **14h** (0.20 mmol, 100 mg) was dissolved in DCM (1 mL) and TFA (0.1 mL) was added to stir at rt for 7 h. The solvent was removed in vacuo, and the residue was treated with saturated aq sodium bicarbonate solution and then sonicated until a precipitate was formed. The suspension was treated with 10% aq citric acid solution until pH 6 and then was extracted with EtOAc three times. The organic layers were combined, dried over $MgSO_4$, and the solvent was removed in vacuo to yield 4-(3-(4-(4-(3-aminopropyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol **15h** as the neutral species (79 mg, 100%). ^{1}H NMR (400 MHz, methanol-d_4): δ 8.11−8.03 (m, 4H), 7.30 (d, J = 8.6 Hz, 2H), 7.10−7.02 (m, 4H), 7.00−6.94 (m, 2H), 2.99−2.92 (m, 2H), 2.79−2.71 (m, 2H), 1.99 (dt, J = 15.5, 7.7 Hz, 2H). To a suspension of **15h** (1 equiv, 0.052 mmol, 20 mg) in acetonitrile (0.9 mL) and water (0.1 mL) was added 37% aq formaldehyde solution (12 equiv, 0.62 mmol, 50 μL). $NaBH(OAc)_3$ (4.8 equiv, 0.25 mmol, 53 mg) was added slowly, and the reaction was stirred at rt for 50 min. The reaction mixture was quenched with saturated aq sodium bicarbonate solution and then extracted several times with a 9:1 solution of EtOAc/isopropanol. The organic layers were combined and concentrated in vacuo. The residue was treated with small amounts of EtOAc and saturated aq sodium bicarbonate solution until a precipitate formed. A sodium citrate buffer of pH ∼ 6.5 was added slowly until the solution became neutral and the precipitate dissolved. The aqueous layer was washed with EtOAc; then the organic layers were combined and set aside. The aqueous layer was concentrated in vacuo to obtain cruder residue. The sequential process of saturated aq sodium bicarbonate solution and sodium citrate buffer additions followed by EtOAc extraction was repeated until no further precipitation was observed. The combined organic layers were dried over $MgSO_4$, and the solvent was removed in vacuo to obtain the impure product as the neutral species. The residue was dissolved in 4 M HCl/1,4-dioxane (1.5 mL) and stirred at rt for 6 h. The solution was diluted with Et_2O, filtered, and the solid was further washed with Et_2O. The filtered solid was collected to yield the product as an off-white solid (2.4 mg, 10%). ^{1}H NMR (400 MHz, methanol-d_4): δ 8.14−8.04 (m, 4H), 7.33 (d, J = 8.5 Hz, 2H), 7.13−7.04 (m, 4H), 7.02−6.96 (m, 2H), 3.21−3.14 (m, 2H), 2.92 (s, 6H), 2.76 (t, J = 7.6 Hz, 2H), 2.13−2.03 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.4, 169.4, 163.6, 161.9, 156.1, 137.6, 131.2, 131.1, 130.2, 122.9, 121.2, 119.1, 117.2, 116.4, 58.6, 43.5, 32.7, 27.5. HPLC: t_R = 6.3 min, 96% at 254 nm. HRMS *m/z*: calcd for $C_{25}H_{25}N_3O_3$ + H^+ [M + H^+], 416.1969; found, 416.1979.

*3-(4-(4-(5-(4-Hydroxyphenyl)-1,2,4-oxadiazol-3-yl)-phenoxy)phenyl)-N,N,N-trimethylpropan-1-aminium Iodide (**26b**).* The neutral species of 4-(3-(4-(4-(3-(dimethylamino)-propyl)phenoxy)phenyl)-1,2,4-oxadiazol-5-yl)phenol **25b** (1 equiv, 0.072 mmol, 30 mg) was dissolved in a 2:1 solution of DCM/MeOH (2 mL) under N_2 atmosphere and iodomethane (5 equiv, 0.36 mmol, 23 μL) was added. The reaction mixture was stirred for 24 h at rt. The solvent was removed in vacuo; then, the residue was suspended in Et_2O and filtered.

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The precipitate was washed with EtOAc, air-dried, and then collected to yield the product as yellow powder (23 mg, 58%). ^{1}H NMR (400 MHz, methanol-d_4): δ 8.14−8.03 (m, 4H), 7.38−7.33 (m, 2H), 7.13−7.05 (m, 4H), 7.02−6.96 (m, 2H), 3.44−3.39 (m, 2H), 3.17 (s, 9H), 2.76 (t, J = 7.6 Hz, 2H), 2.22−2.12 (m, 2H). ^{13}C NMR (101 MHz, methanol-d_4): δ 177.4, 169.4, 163.6, 161.9, 156.1, 137.4, 131.2, 131.2, 130.2, 122.9, 121.2, 119.1, 117.2, 116.4, 67.4, 67.4, 67.4, 53.7, 53.7, 53.6, 32.5, 25.8. HPLC: t_R = 6.4 min, 95% at 254 nm. HRMS m/z: calcd for $C_{26}H_{28}N_3O_3$ [M$^+$], 430.2125; found, 430.2111.

Antimicrobial Susceptibility Testing. Antimicrobial susceptibility testing was performed using CLSI methods.[47] Specifically, MICs were determined by broth microdilution assays as described previously for *C. difficile*[48] and *E. faecium*.[31] In brief, a 2-fold dilution series (from 128 to 1 μg/mL) of 1,2,4 oxadiazole analogues, vancomycin (*C. difficile* comparator control), or daptomycin (*E. faecium* comparator control) was made in 100 μL volumes of either SDW (*C. difficile*) or cation-adjusted Mueller−Hinton broth (CAMHB) (*E. faecium*) purchased from Thermo Fisher (additionally supplemented with 50 μg/mL Ca^{2+} for daptomycin assays) in a 96-well plate (Corning) and an inoculum of 100 μL from an overnight *C. difficile* or *E. faecium* broth culture adjusted to 5×10^5 CFU/mL in supplemented, pre-reduced Brucella broth (*C. difficile*) or CAMHB (*E. faecium*) added. After 48 h incubation under anaerobic conditions (*C. difficile*) or 24 h incubation under aerobic conditions (*E. faecium*), the MIC was defined as the lowest antimicrobial concentration that inhibited visible growth. All MIC testing was performed in biological triplicate.

Time-Kill Assays. Time-kill assays were performed with *C. difficile* strain EDN0008 (NAP1/027) or *E. faecium* strain Aus0085 (ST203), using supplemented brain heart infusion broth (BHIBS) for *C. difficile* or CAMHB for *E. faecium*. BHIBS—BHIB supplemented with 5 g/L yeast extract, 0.1% (w/v) L-cysteine, and 0.3% (w/v) glucose. Broths were supplemented with either DMSO (vehicle) or with 1× MIC, 2× MIC, 4× MIC, and 8× MIC of 1,2,4-oxadiazole or vancomycin (8 μg/mL), daptomycin (16 μg/mL) (additionally supplemented with 50 μg/mL Ca^{2+} for daptomycin assays) or benzalkonium chloride (24 or 16 μg/mL) as controls. Broths were inoculated with overnight bacterial broth cultures and adjusted to 1×10^6 CFU/mL. Samples withdrawn at 0, 1, 3, 24, and 48 h post-inoculation were serially diluted in phosphate-buffered saline and plated onto either supplemented brain heart infusion agar and incubated overnight at 37 °C in a DG250 anaerobic chamber (Don Whitley Scientific) for *C. difficile* or on BHIA (Oxoid) and grown overnight at 37 °C aerobically for *E. faecium* before colony enumeration was performed. All assays were performed using three independent biological replicates, with each test strain being purity plated from frozen glycerol stocks three independent times and each bacterial culture used in the time-kill assay then being derived from a single colony picked from one of the three separate purity plates.

Haemolysis Assay. Fresh whole human blood was collected into K2-EDTA-coated Vacutainer tubes and pelleted at 500 g for 5 min. The red blood cell pellet was washed twice in saline solution followed by a third wash in phosphate-buffered saline (PBS) before being diluted 1:50 in PBS. Compound **15e** was prepared to 20× concentration in DMSO, and a 10 μL aliquot of each concentration was added to 190 μL of diluted blood in a 96-well U-bottom plate (Corning) giving a final concentration range of 0.125−128 mg/L. The plate was then incubated at 37 °C for 1 h. Negative (vehicle) controls were incubated with DMSO, and positive controls were incubated with 1% (v/v) Triton X-100. The plate was centrifuged at 500g after the incubation to pellet intact erythrocytes, and 100 μL of the supernatant from each well was then collected and transferred to a 96-well flat bottom plate (Corning). The absorbance of each supernatant sample was then measured at 450 nm using CLARIOstar plus (BMG LABTECH). All assays were performed using three independent biological replicates.

Caco-2 Cell Permeability. The apical to basolateral apparent permeability (A−B P_{app}) was assessed in Caco-2 cells (passage 39) seeded onto 0.3 cm^2 polycarbonate filter transwells at a density of 6×10^4 cells/well. Experiments were conducted 23 days post-seeding. Experiments were performed using pH 7.4 Hanks balanced salt solution (containing 20 mM N-(2-hydroxyethyl)piperazine-N'-ethanesulfonic acid) with the donor solution spiked with the test compound. Sample aliquots were taken 5−6 times over 120 min, and the compound flux were determined by assessing compound concentration using LCMS. The apical to basolateral apparent permeability coefficient (A−B P_{app}) for each compound was determined using the equation, P_{app} (cm/s) = $dQ/dt \times 1/(A \times C_{initial\ donor})$, where dQ/dt = apparent steady-state transport rate (μmol/s), A = surface area of Caco-2 monolayer (0.3 cm^2), and $C_{initial\ donor}$ = donor chamber concentration at the start of the experiment (μmol/cm^3). Transepithelial electrical resistance (TEER) readings, high and low permeability markers, and a multidrug resistance protein-mediated efflux marker were included in the study to assess the integrity of the cell monolayers. The TEER values for transwells used in the Caco-2 permeability study ranged from 457 to 551 Ωcm^2, indicating the presence of confluent monolayers. For lucifer yellow, P_{app} values of $<1 \times 10^{-6}$ cm/s were yielded in the acceptor solution. For propranolol, an A−B P_{app} value of $42 \pm 2.5 \times 10^{-6}$ cm/s was obtained and for rhodamine 123, an A−B P_{app} value of $1.6 \pm 0.13 \times 10^{-6}$ cm/s, B−A P_{app} value of $12 \pm 2.7 \times 10^{-6}$ cm/s, and an efflux ratio of 7.3 were obtained, all of which compared well with in-house validation data. Values are presented as the mean ± SD of n = 3 transwells.

Pharmacokinetic Studies. Experimental procedures are summarized in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c06294.

Syntheses and characterization of intermediates **13a−i**, example NMR spectra, HPLC profiles, and study design and formulations for the pharmacokinetic studies (PDF)

Molecular formula strings for final compounds (XLSX)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

Aq, aqueous; Boc, *tert*-butyloxycarbonyl; CDC, Centers for Disease Control and Prevention; CDI, *C. difficile* infections; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EDC·HCl, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; ESKAPE, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa*, and *Enterobacter species*; Et_3N, triethylamine; Et_2O, diethyl ether; EtOAc, ethyl acetate; equiv, equivalents; GI, gastrointestinal; HOBt, 1-hydroxybenzotriazole hydrate; MDR, multidrug-resistant; MeOH, methanol; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; rt, room temperature; SAR, structure−activity relationship; TBS, *tert*-butyldimethylsilyl; *t*-Bu, *tert*-butyl; TFA, trifluoroacetic acid; TLC, thin-layer chromatography; VREfm, vancomycin-resistant *E. faecium*

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