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ARTICLE

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Ethionamide Boosters: Synthesis, Biological Activity, and Structure−Activity Relationships of a Series of 1,2,4-Oxadiazole EthR Inhibitors

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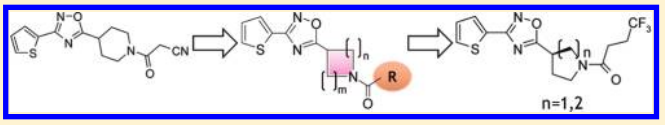
ABSTRACT: We report in this article an extensive structure−activity relationships (SAR) study with 58 thiophen-2-yl-1,2,4-oxadiazoles as inhibitors of EthR, a transcriptional regulator controling ethionamide bioactivation in *Mycobacterium tuberculosis*. We explored the replacement of two key fragments of the starting lead BDM31343. We investigated the potency of all analogues to boost subactive doses of ethionamide on a phenotypic assay involving *M. tuberculosis* infected macrophages and then ascertained the mode of action of the most active compounds using a functional target-based surface plasmon resonance assay. This process revealed that introduction of 4,4,4-trifluorobutyryl chain instead of cyanoacetyl group was crucial for intracellular activity. Replacement of 1,4-piperidyl by (*R*)-1,3-pyrrolidyl scaffold did not enhance activity but led to improved pharmacokinetic properties. Furthermore, the crystal structures of ligand−EthR complexes were consistent with the observed SAR. In conclusion, we identified EthR inhibitors that boost antibacterial activity of ethionamide with nanomolar potency while improving solubility and metabolic stability.



INTRODUCTION

Tuberculosis at a Glance. Tuberculosis (TB) is the second leading cause of death among all infectious diseases, killing an estimated 1.7 million people every year. Rates of TB continue to rise even in the 21st century, with approximately 9.4 million of new cases and 13.7 million of chronic active cases in 2009.[1] The directly observed treatment short course (DOTS), a multidrug therapy program developed by the World Health Organization, is today considered as the major weapon against the global tuberculosis epidemic. DOTS covers approximately 77% of the world's population,[2,3] and the treatment success in the 2007 DOTS campaign was 86% overall.[4] However, the regional average cure rates in some African,[5] American, and European regions remain below the 85% target.[6] In addition, the global human immunodeficiency virus (HIV) epidemic has caused an explosive escalation in TB incidence and may be contributing to the increasing emergence of multidrug resistant TB (MDR-TB). The treatment of MDR-TB is long, usually two to four years, and requires the use of second line drugs that are less effective and often badly tolerated, especially by individuals with a depressed immune system, leading to high rates of recurrence and mortality.[7] In parallel to the development of new highly active antibiotics designed to shorten treatment time, any strategy able to improve the efficacy of existing drugs should increase the

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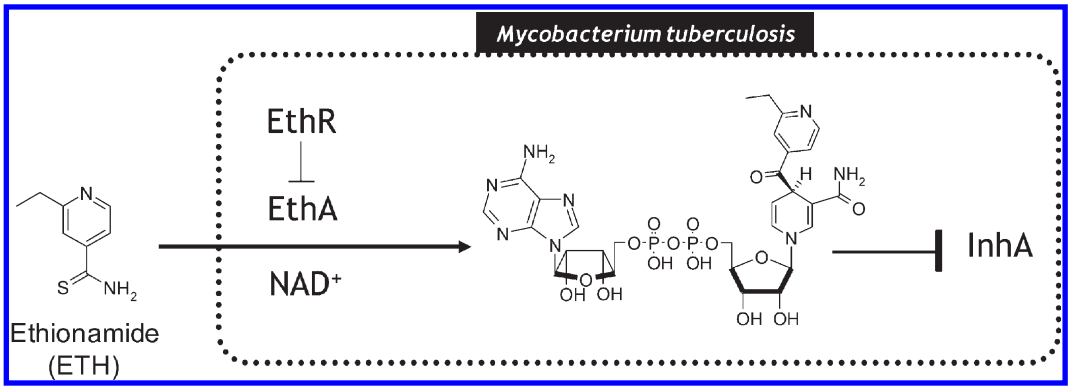

Figure 1. Structure of ETH−NADH adduct, formed after the mycobacterial activation of ETH by the monooxygenase EthA and responsible for InhA inhibition. This process is under the control of the transcriptional regulator EthR.

observance of the therapy by patients and thus would benefit the global effectiveness of DOTS.

Boosting Ethionamide Bioactivation As an Alternative Therapeutic Approach. Ethionamide is one of the drugs recommended by WHO for the treatment of MDR-TB.[8] It is a very efficient drug active against more than 80% of MDR-TB clinical strains.[9] However, it has a low therapeutic index. Indeed, the minimal dose necessary to inhibit *M. tuberculosis* growth is generally sufficient to produce serious adverse effects such as gastrointestinal disturbances, hepatitis, and mental disturbances, including depression, anxiety, and psychosis.[10] Like several other antituberculous drugs, body circulating ethionamide is inactive and needs to be enzymatically activated inside the mycobacteria to reveal its antibacterial potency. Because most of the side effects of ethionamide are dose-related,[11] we postulated that increasing bacterial bioactivation would make lower circulating doses as active against the mycobacteria but with limited dose-related toxicity of the parent compound. It has been shown that the mycobacterial flavin adenine dinucleotide-containing monooxygenase EthA is central in the bioactivation process of ethionamide.[12,13] The corresponding NAD-adduct produced eventually inhibits the *M. tuberculosis* enoyl-ACP reductase InhA involved in cell wall synthesis (Figure 1).[14] The transcription of *ethA* is under the control of the transcriptional regulator encoded by the neighboring gene *ethR*.[15,16] As EthR represses *ethA* transcription, chromosomal inactivation of *ethR* was shown to sensitize the bacteria to ethionamide.[17] On the basis of these observations, we postulated that a drug-like inhibitor of EthR would increase the production of EthA and, consequently, the sensitivity of *M. tuberculosis* to ethionamide.[17,18] The analysis of the two known crystals of liganded EthR (PDB codes: 1U9N, 1T56)[18−20] suggested that holoprotein conformations are unable to bind DNA and thus to repress transcription of *ethA*. The chemical structure of the ligands identified in these holostructures, associated to the physicochemical properties of the EthR ligand binding domain inspired the design and the synthesis of a focused library of 131 compounds. The ability of these ligands to inhibit the DNA-binding function of EthR was measured and this led to the identification of a thiophen-2-yl-1,2,4-oxadiazole family of potent inhibitors. Preliminary hit optimization led to the identification of two analogues **1** (BDM31343) and **2** (BDM31381).[21] Compound **2** was the most potent booster of ethionamide bioactivation in vitro but was rapidly cleared in mice resulting in low systemic exposure. Inversely, compound **1** was a less active compound in vitro, but showed a more favorable pharmacokinetic profile. Both compounds were evaluated in *M. tuberculosis* infected mice. The coadministration of **2** at 100 mg/kg with ethionamide resulted in a minor effect on the bacterial load in the lung of treated animals when compared to the treatment with ethionamide alone. In contrast, daily treatment with **1** at 100 mg/kg was able to triple the activity of ethionamide in the same animal model. Having demonstrated the concept that boosting ethionamide with EthR inhibitors was successful to reduce the dose of antitubercular agent while keeping the same efficacy, we report now the SAR studies of thiophen-2-yl-1,2,4-oxadiazole analogues based on ex vivo phenotypic and in vitro functional assays. In parallel, we performed cocrystallization to understand the binding mode of our compounds to the repressor EthR. Finally, physicochemical and pharmacokinetic properties of two promising compounds were measured (Figure 2).

■ CHEMISTRY

We focused chemistry at the R position by the introduction of five-membered heteroaromatic or substituted phenyl acetyl groups and alicyclic or aliphatic chains according to chemistry described in Scheme 1 (compounds **1**−**52**). In a second round of optimization, we studied the replacement of the 1,4-piperidine scaffold with smaller saturated nitrogen heterocycles (compounds **53**−**57**) or aminopropyl linker (compound **58**) substituted on nitrogen by 4,4,4-trifluorobutyryl chain.

Thiophene-2-amidoxime was obtained from commercially available thiophene-2-carbonitrile using hydroxylamine hydrochloride. Compounds **1**−**52** were obtained by reaction of thiophene-2-amidoxime with Boc-isonipecotic acid using HBTU as activating agent. The acylated product was isolated by precipitation in water prior to cyclization in DMF at 120 °C to yield the desired 1,2,4-oxadiazole ring.[22] The Boc-protecting group was then cleaved in acidic conditions, and the free piperidine was acylated with carboxylic acids, acyl chlorides, or anhydrides to yield compounds **1**−**50**. Compounds **51** and **52** were respectively obtained by sulfonylation of the deprotected piperidinyl scaffold using 3,3,3-trifluoropropane-1-sulfonyl chloride and acylation using CDI and 2,2,2-trifluoroethylamine hydrochloride under basic conditions. The synthesis of compounds **53** and **54** proceeded in three steps: thiophene-2-amidoxime was acylated with *N*-Boc-nipecotic acids in *R* or *S* configuration and cyclized to yield the desired 1,2,4-oxadiazoles as described above. Deprotection of Boc-protecting group in

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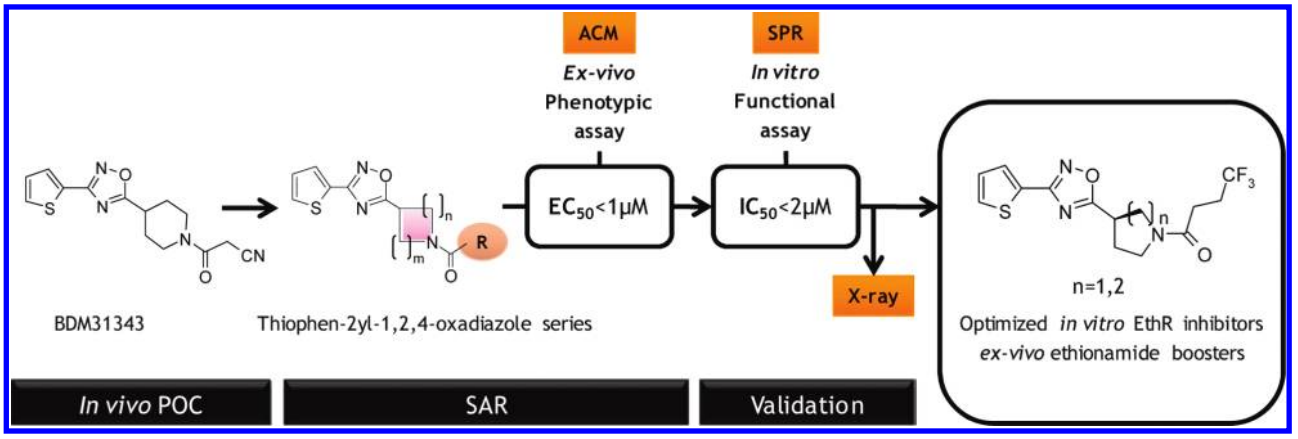
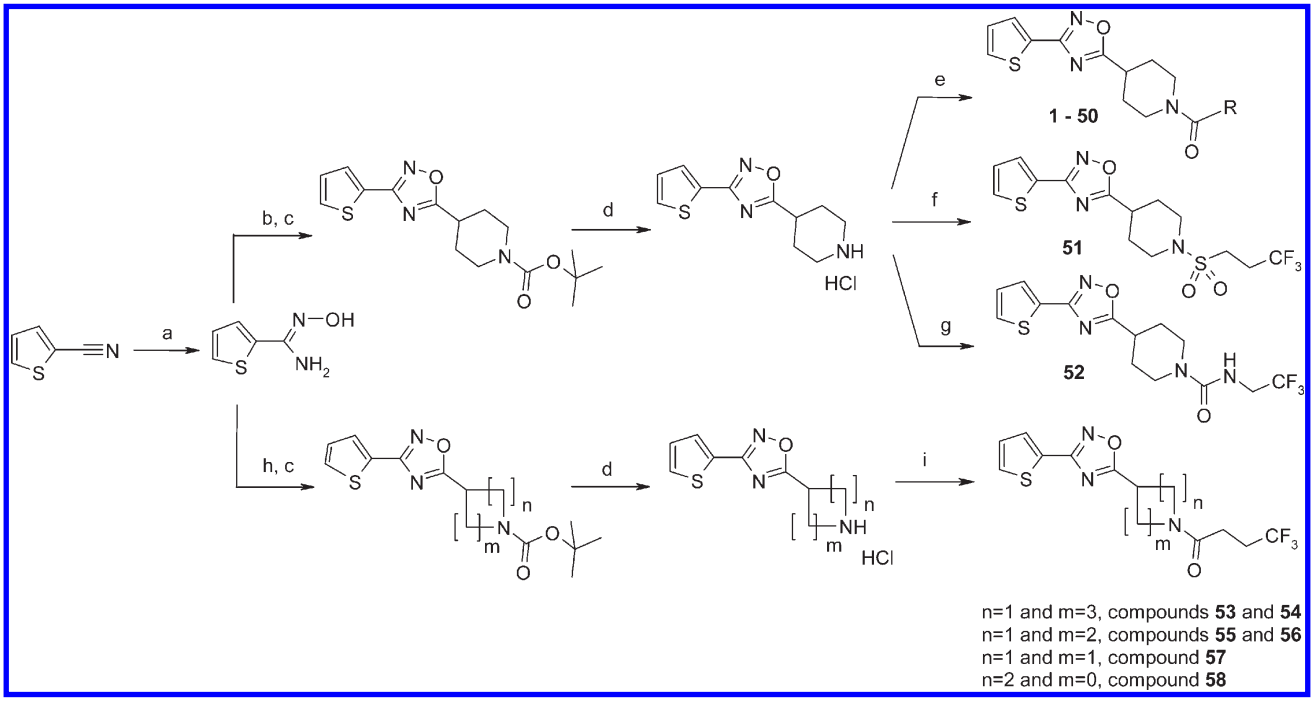


Figure 2. Screening cascade developed for SAR studies and validation of EthR inhibitors as ethionamide boosters starting from our lead compound BDM31343.

Scheme 1. Synthesis of Thiophen-2-yl-1,2,4-oxadiazole Derivatives for SAR Studies[a]



[a] Reagents and reaction conditions: (a) 1.5 equiv $H_2NOH \cdot HCl$, 1.6 equiv DIEA, EtOH, reflux, 4 h; (b) 1 equiv Boc-isonipecotic acid, 1.1 equiv HBTU, 0.2 equiv HOBt, 3 equiv DIEA, DMF, RT, 2 h; (c) DMF, 120 °C, 3 h to overnight; (d) 5 equiv HCl 4N dioxane, RT; (e) 1.5 equiv carboxylic acid, 1.5 equiv EDCI, 4.5 equiv Et_3N, CH_2Cl_2, RT, or 1.1 equiv acid chloride, 3 equiv Et_3N, CH_2Cl_2, RT, or 1.2 equiv anhydride, 3 equiv DIEA, CH_2Cl_2, RT; (f) 1.3 equiv 3,3,3-trifluoropropane-1-sulfonyl chloride, 3 equiv Et_3N, CH_2Cl_2, RT, overnight; (g) 1 equiv CDI, 1 equiv 2,2,2-trifluoroethylamine hydrochloride, 1 equiv Et_3N, THF, RT, overnight; (h) 1 equiv carboxylic acid, 1.1 equiv HBTU, 3 equiv DIEA, DMF, RT, overnight; (i) 1.3 equiv 4,4,4-trifluorobutyric acid, 1.3 equiv, EDCI, 0.3 equiv HOBt, 4 equiv DIEA, DMF, RT, overnight.

acidic conditions was followed by acylation with 4,4,4-trifluorobutyric acid using EDCI and HOBt as coupling agents. The same procedure was applied with both enantiomers of *N*-Boc-β-proline to give compounds **55** and **56**, with *N*-Boc-azetidine-3-carboxylic acid to give compound **57** and finally with *N*-Boc-γ-aminobutyric acid to yield compound **58**. Compounds with stereogenic centers were synthesized and screened as pure enantiomers.

RESULTS AND DISCUSSION

The 58 thiophen-2-yl-1,2,4-oxadiazole derivatives were screened in combination with subactive doses of ethionamide on a phenotypic assay using *M. tuberculosis* infected macrophages. Molecular composition and structural features of the mycobacterial cell envelope are thought to confer low permeability and thereby a basal resistance to most hydrophilic drugs.[23] Moreover, during the pathological course of tuberculosis, most of

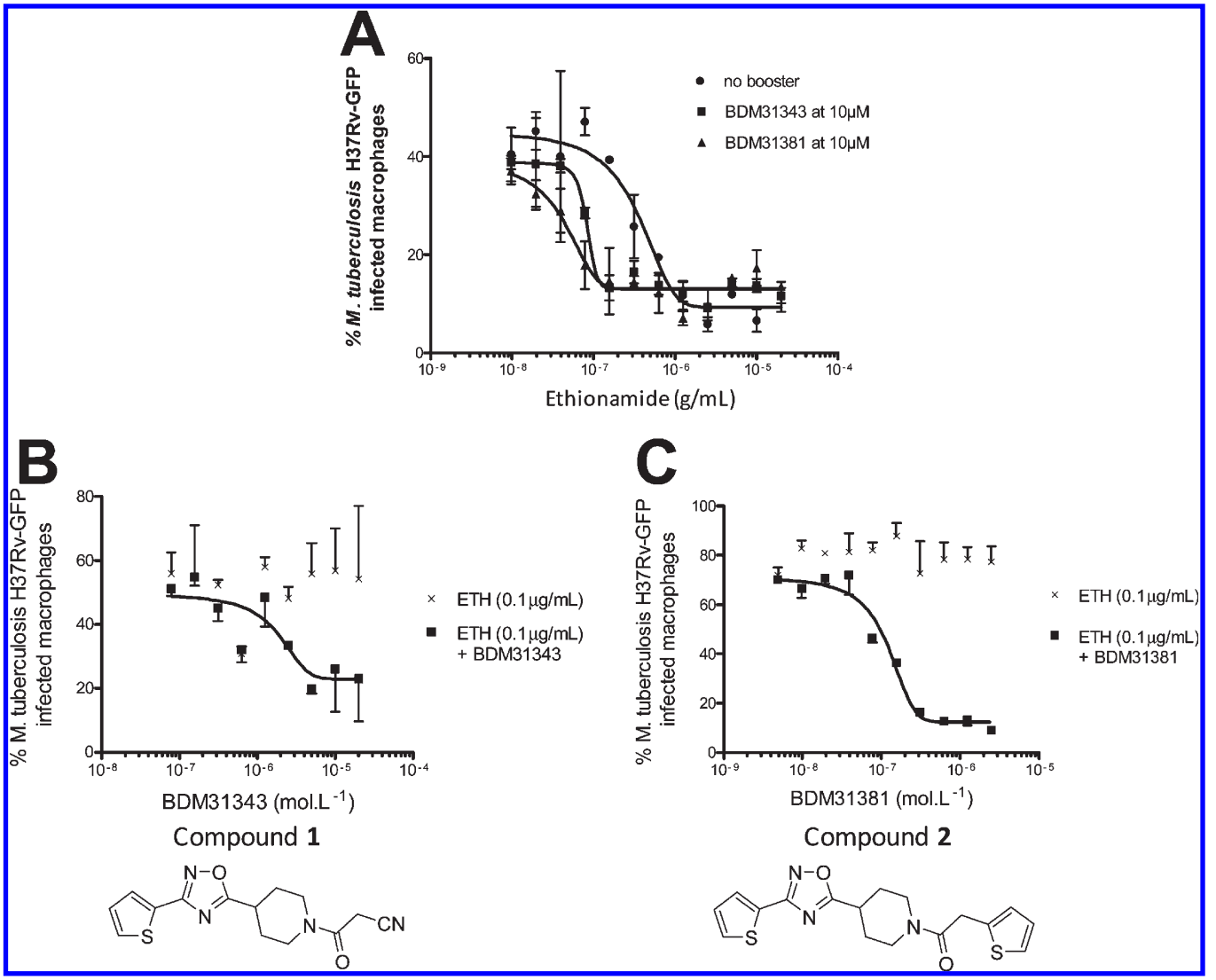


Figure 3. (A) Dose response analysis of ethionamide alone or in the presence of compound **1** (BDM31343) and compound **2** (BDM31381) at 10 μM against *M. tuberculosis* growth in Raw264.7 cells monitored by the percentage of infected cells. MIC_{99} of ethionamide alone is equal to 1 μg/mL. Compounds **1** and **2** at 10 μM reduce the MIC_{99} of ethionamide to 0.1 μg/mL. (B, C) Dose response curves showing the potency of compound **1** and compound **2** to boost ethionamide at subactive dose (0.1 μg/mL) against *M. tuberculosis* intracellular growth.

the bacilli are hidden from the immune system within macrophages, which further increases the difficulty for antibiotics to reach their target. Thus, the identification of analogues that are able to reach their mycobacterial target EthR and overcome peculiarities of intracellular *M. tuberculosis* required the use of a phenotypic ex vivo assay. This assay uses automated confocal microscopy and dedicated image analysis to allow quantification of fluorescent *M. tuberculosis* H37Rv-GFP replication inside macrophages incubated with a combination of ethionamide and thiophen-2-yl-1,2,4-oxadiazole analogues.[24,25] There are two ways to quantify the effect of an ethionamide booster. The first consists in measuring the increase of ethionamide efficacy at a saturating concentration of booster. For example, compounds **1** and **2** were tested at 10 μM in this assay and both were able to reduce the MIC of ethionamide to 0.1 μg/mL (Figure 3A). The second way consists in measuring the ability of the booster to reduce significantly the MIC_{99} of ethionamide. In our assay, several doses of boosters were given in combination with a 10 times subactive dose of ethionamide ($MIC_{99}/10 = 0.1$ μg/mL). From the dose−response curves obtained (Figure 3B,C), an EC_{50} was calculated and compounds ranked accordingly. EC_{50} for compounds **1** and **2** were respectively 1.5 and 0.1 μM (Figure 3B,C). This data are in agreement with the growth inhibition measured by the mycobacteria growth indicator tube standard technique (MGIT960).[21]

To confirm that our compounds are effective EthR inhibitors, a functional surface plasmon resonance (SPR) assay was used. SPR assay allows monitoring the potency of ligands to inhibit EthR/DNA operator interactions.[15] Dose−response curves were established, and activities were expressed as IC_{50}.

In the first round of analoguing, we investigated the introduction in R position of five- or six-membered aromatic rings. Table 1 summarizes potency of analogues of compound **2** (compounds **4**−**25**) to boost 10-fold ethionamide antibacterial activity on *M. tuberculosis* infected macrophages.

Elongation of the spacer between carbonyl group and 2-thiophene ring led to a less active booster (compound **4**), suggesting that distance between the amide function and the aryl moiety is

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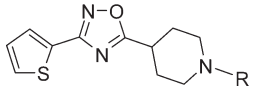
Table 1. Ex Vivo Activities of Compounds 2 and 4−25



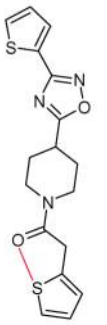
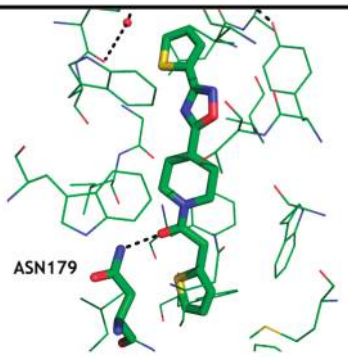
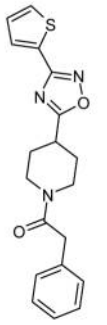
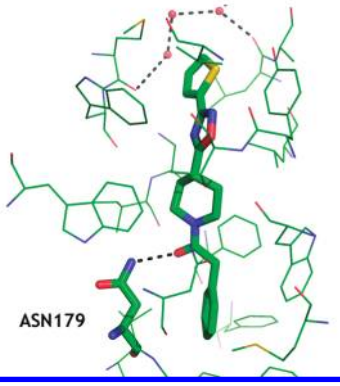
compd	R	EC_{50} (μM)[a]
2	$-COCH_2$(2-thiophene)	0.1
4	$-COCH_2CH_2$(2-thiophene)	>5.0
5	$-COCH_2$(2-imidazole)	>5.0
6	−CO-phenyl	>5.0
7	$-COCH_2$-phenyl	2.1
8	$-COCH_2$(2-F-phenyl)	>5.0
9	$-COCH_2$(3-F-phenyl)	>5.0
10	$-COCH_2$(4-F-phenyl)	0.8
11	$-COCH_2$(2-Cl-phenyl)	>5.0
12	$-COCH_2$(3-Cl-phenyl)	>5.0
13	$-COCH_2$(4-Cl-phenyl)	2.2
14	$-COCH_2$(2-CF_3-phenyl)	>5.0
15	$-COCH_2$(3-CF_3-phenyl)	>5.0
16	$-COCH_2$(4-CF_3-phenyl)	>5.0
17	$-COCH_2$(2-CH_3-phenyl)	>5.0
18	$-COCH_2$(3-CH_3-phenyl)	>5.0
19	$-COCH_2$(4-CH_3-phenyl)	>5.0
20	$-COCH_2$(2-OCH_3-phenyl)	>5.0
21	$-COCH_2$(3-OCH_3-phenyl)	>5.0
22	$-COCH_2$(4-OCH_3-phenyl)	>5.0
23	$-COCH_2$(2-pyridinyl)	>5.0
24	$-COCH_2$(3-pyridinyl)	>5.0
25	$-COCH_2$(4-pyridinyl)	>5.0

[a] EC_{50} represents the concentration of ligand that allows ethionamide at 0.1 μg/mL (normal MIC/10) to inhibit 50% of *M. tuberculosis* growth in macrophages. EC_{50} are mean of two experiments. SD was <10% in most cases.

an important criterion for the activity. Exchanging the 2-thienyl group for a five-membered aromatic heterocycle like imidazole was detrimental for activity (compound **5**). This suggested that a basic group or a hydrogen-bond donor atom is less tolerated in the hydrophobic ligand-binding cavity. The bioisosteric replacement of the thienyl core by a phenyl ring (compound **7**) reduced activity and the removal of the methylene spacer (compound **6**) strongly affected activity. Substitutions of phenyl ring in ortho, meta, or para position with electron-donating groups such as methyl and methoxy (compounds **17**−**22**) or cumbersome electron-withdrawing groups such as trifluoromethyl (compounds **14**−**16**) did not enhance the activity. Exchanging the 2-thienyl group for a pyridine led to less active compounds (**23**−**25**). Interestingly, introduction of fluorine or chlorine on the phenyl ring (compounds **8**−**13**) was tolerated only in para position. The 4-fluoro substitution (compound **10**) led to a compound with submicromolar activity (EC_{50} = 0.8 μM). In this series, the 2-thienoacetyl group (compound **2**, EC_{50} = 0.1 μM) appeared to be critical for activity compared to phenylacetyl (compound **7**, EC_{50} = 2.1 μM). Both compounds were evaluated in our functional SPR assay. With an IC_{50} equal to 0.5 μM, compound **2** revealed to be 10-fold more active than its phenyl analogue **7**, which displayed an IC_{50} equal to 5.6 μM (Table 2). According to X-ray structures of EthR complexes, both compounds were hydrogen-bonded to Asn179. The bottom of the ligand binding domain is rich in aromatic residues, showing Phe114, Trp138, Trp145, and Phe184 aromatic residues localized in a 3−5 Å sphere around the thienyl or phenyl substituents of the two boosters. On the basis of these structures, there are no obvious differences in van der Waals interactions, however, the thienyl ring is coplanar with carbonyl bond. We therefore propose that the sulfur atom could be the key feature responsible for the differences in activities. Several works demonstrated indeed that the conformation of organosulfur compounds can be influenced by intramolecular sulfur−nitrogen (or oxygen) interactions.[26] In the X-ray structure of compound **2** cocrystallized with EthR (Table 2), the distance between the sulfur atom (of the thiophene ring) and the oxygen atom (of the amide function) was equal to 3.10 Å, a distance shorter than the sum of the corresponding van der Waals radii equal to 3.35 Å. Bound to EthR, compound **2** was probably stabilized by a noncovalent 1, 5-intramolecular sulfur−oxygen interaction in a conformation more compatible with the architecture of the binding pocket while maintaining hydrogen-bond with Asn179.

To further investigate the impact of piperidine substitution, *N*-acylated analogues with aliphatic chains bearing hydrophobic or hydrophilic substituents were assessed (Table 3). The azido analogue **3** (BDM14801)[27] was as active as compound **1**. Introduction of a methyl or ethyl chain (compounds **26** and **27**) almost abolished activity. Interestingly, propyl, butyl, and isobutyl analogues (**28**−**30**) were more active than compounds **26** and **27**, suggesting that the occupancy of the bottom of EthR ligand binding domain is essential to enhance activity. Introduction of an isopentyl carbon chain (compound **32**) increased drastically the activity, leading to a submicromolar compound (EC_{50} = 0.1 μM). It is noteworthy that further methylation of compounds **30** and **32** led to less active boosters (compounds **31** and **33**). Hydrophilic groups such as hydroxyl or amino were also introduced on isobutyl and isopentyl chains (compounds **34**−**36**), but this substitution strongly reduced activity. In the same manner, replacement of a methylene spacer by an oxygen atom (compounds **37** vs **28** and **38** vs **29**) or introduction of carboxylic acid function instead of isopentyl chain (compounds **39** vs **32**) reduced potency. As it appeared clearly that hydrophilic substituents of the piperidine did not lead to potent ethionamide boosters, we introduced aliphatic rings (compounds **40**−**42**). Three-, five-, and six-membered alicyclic rings were equally efficient. Interestingly the bioisosteric replacement of the phenyl ring (compound **7**, Table 1) by a cyclopropyl (compound **40**) led to a compound 10 times more active with a submicromolar activity (EC_{50} = 0.2 μM). Also noticeable was the methyl substitution of the cyclohexyl, which strongly reduced activity (compound **43**). In the same manner, introduction of two methylene spacers between carbonyl and cyclohexyl groups (compound **44**) led to a decrease of activity suggesting that the maximum steric hindrance in the bottom of the binding pocket was reached with compound **42**. These results suggested that, in addition to the hydrophobic character of the ring, its shape and flexibility are key parameters to ensure optimal occupancy. The crystal structure of EthR in complex with compound **32** showed the molecule H-bonded to Asn179, while the isopentyl chain was engaged in hydrophobic contacts with Phe110, Phe114, and Trp145 (Figure 4). This 3D structure revealed the tightness of the bottom of the ligand-binding domain, only accessible through a narrow tunnel, which explains

Table 2. X-ray Structures of Compounds 2 (PDB ID: 3G1M) and 7 (PDB ID: 3Q0U) Co-crystallized with EthR

Compound	Structure [a]	Binding pocket	EC_{50} (μM) [b]	IC_{50} (μM) [c]
2			0.1	0.5
7			2.1	5.6

[a] For compound **2** in the left column, 1,5-intramolecular sulfur−oxygen interaction between thiophene and amide functions is symbolized with red dotted line. [b] EC_{50} represents the concentration of ligand that allows ethionamide at 0.1 μg/mL (normal MIC/10) to inhibit 50% of *M. tuberculosis* growth in macrophages, EC_{50} are mean of two experiments; [c] IC_{50} represents the concentration of ligand that inhibits 50% of the interaction of EthR with its promoter.

why linear and less hindered aliphatic substituents better accommodate this region.

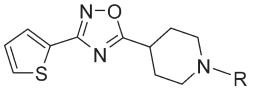
On the basis of this conclusion, we assessed the replacement of methyl groups with smaller substituents such as fluorine atoms (compounds **45**−**50**). These modifications led to an increase of the activity compared to the parent hydrogenated molecules: compound **45** (EC_{50} = 1.3 μM) was more potent than compound **27** (EC_{50} > 5.0 μM), and introduction of a 4,4,4-trifluorobutyryl substituent strongly increased activity (compound **46**, EC_{50} = 0.1 μM, compared to compound **28**, EC_{50} = 2.1 μM). Compound **46** was as potent as compound **2** in ex vivo assay, but less active in SPR assay (IC_{50} = 1.6 μM). This suggest that introduction of fluorine substituents does not improve protein−ligand interactions but possibly facilitate cell penetration, as already shown for fluoroquinolones[28,29] and enhance physicochemical properties.[30] The maximum benefit of the replacement of hydrogen by fluorine seems to have been oversteped with compound **50**, which is far less active than its analogue **32**. On the basis of van der Waals radius of 1.47 Å for fluorine atom, the volume of a trifluoromethyl group is similar to that of the ethyl group[30] and this corroborates that only limited space is available in the bottom of the ligand binding domain. Introduction of a methyl substituent (compounds **48** and **49**) led to a decrease in activity. Finally, we explored the replacement of amide bound (compound **46**) by sulfonamide (compound **51**) or urea (compound **52**) motives. These two modifications led to less active compounds and suggested that the amide function is a key parameter to ensure optimal interaction via hydrogen bond with Asn179.

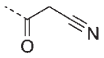
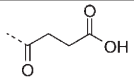
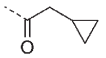
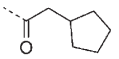
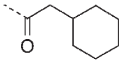
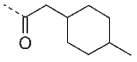
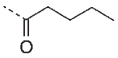
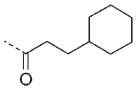
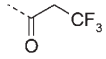
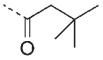
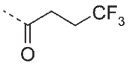
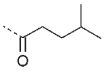
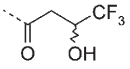
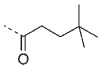
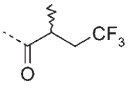
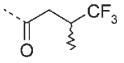
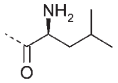
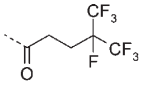
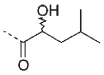
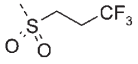
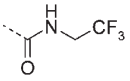
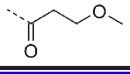
Finally, we focused on the modification of the piperidine ring by smaller cyclic or linear aliphatic amines keeping the 4,4,4-trifluorobutyryl chain. Biological data are summarized in Table 4.

Replacement of 1,4-substituted piperidine (compound **46**) by 1,3-substituted piperidine with *R* or *S* configurations (compounds **53** and **54**) strongly reduced intracellular activity. Interestingly replacement by pyrrolidine was well tolerated (compounds **55** and **56**). Compound **55** with the stereogenic center in R configuration was more active than the S stereoisomer (compound **56**). On the contrary, introduction of a smaller azetidine ring or linear chain reduced drastically activity (compounds **57** and **58**). This suggested that both rigidity of cyclic counterpart and positioning of the carbonyl group were important criteria for activity.

In conclusion it proved possible to introduce, at R position, aliphatic or fluorinated aliphatic chains, such as isopentyl and 4,4,4-trifluorobutyryl (compounds **32** and **46**), or saturated cyclic groups such as cyclopropyl, cyclopentyl, or cyclohexyl groups (compounds **40**, **41**, and **42**). Substitution of the carbon

Table 3. Biological Activities of Compounds 1, 3, and 26−52



Compound	R	EC_{50} (μM)[a]	IC_{50} (μM)[b]	Compound	R	EC_{50} (μM)[a]	IC_{50} (μM)[b]
1		1.5	-	39		>5.0	-
3		1.2	-	40		0.2	1.1
26		>5.0	-	41		0.4	1.5
27		>5.0	-	42		0.3	3.5
28		2.1	-	43		>5.0	-
29		1.1	-	44		1.8	-
30		2.5	-	45		1.3	-
31		>5.0	-	46		0.1	1.6
32		0.1	0.9	47		>5.0	-
33		>5.0	-	48		>5.0	-
34		>5.0	-	49		>5.0	-
35		>5.0	-	50		>5.0	-
36		>5.0	-	51		>5.0	-
37		>5.0	-	52		>5.0	-
38		>5.0	-				

[a] EC_{50} represents the concentration of ligand that allows ethionamide at 0.1 μg/mL (normal MIC/10) to inhibit 50% of *M. tuberculosis* growth in macrophages, EC_{50} are mean of two experiments. SD was <10% in most cases; [b] IC_{50} represents the concentration of ligand that inhibits 50% of the interaction of EthR with its promoter.

adjacent to the carbonyl group with methyl or polar groups reduced activity. Both 1,4-piperidyl and (*R*)-1,3-pyrrolidyl linkers were allowed without reducing significantly activities. Finally, the hydrogen bond with Asn179 was optimal with amide linker rather than with sulfonamide or urea (Figure 5).

Compounds displaying nanomolar intracellular activities differed by their physicochemical and biological properties. Indeed, aliphatic derivatives (compounds **32**, **41**, and **42**) are toxic for macrophages at concentrations greater than 30 μM (data not shown). In contrast, compounds **40**, **46**, and **55** are well tolerated

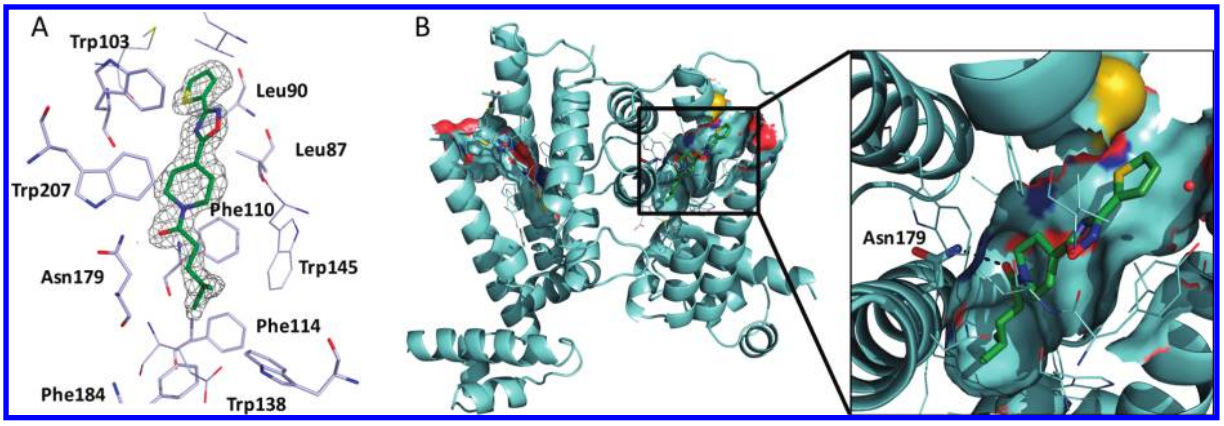
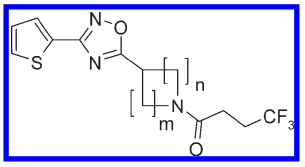
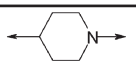
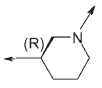
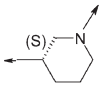
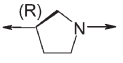
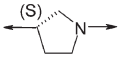
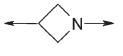
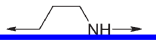


Figure 4. (A) X-ray structure representation of the ligand-binding pocket of mycobacterial transcriptional repressor EthR filled with compound **32** (PDB ID: 3Q0V) surrounded by its initial $F_o - F_c$ map at 3.0 σ contour level. $F_o - F_c$ map was calculated prior to adding the ligand to the model. Colors legend: green (compound **32**) or light-gray (EthR) = carbon, blue = nitrogen, red = oxygen, yellow = sulfur. (B) X-ray structure representation of the homodimeric conformation of EthR filled in both monomer with compound **32**. Surface of ligand binding domain is highlighted and hydrogen-bond with Asn179 is represented with dotted line.

Table 4. Biological Activities of Compounds 46 and 53−58



Compound	n	m	Scaffold	EC_{50} (μM) [a]	IC_{50} (μM) [b]
46	2	2		0.1	1.6
53	1	3		>5.0	-
54	1	3		>5.0	-
55	1	2		0.4	2.0
56	1	2		1.6	-
57	1	1		>5.0	-
58	2	0		>5.0	-

[a] EC_{50} represents the concentration of ligand that allows ethionamide at 0.1 μg/mL (normal MIC/10) to inhibit 50% of *M. tuberculosis* growth in macrophages, EC_{50} are mean of two experiments. SD was <10% in most cases.; [b] IC_{50} represents the concentration of ligand that inhibits 50% of the interaction of EthR with its promoter.

by the macrophages. Compounds **40**, **46**, and **55** comply with Lipinski and Veber's rules (data not shown),[31,32] but derivatives

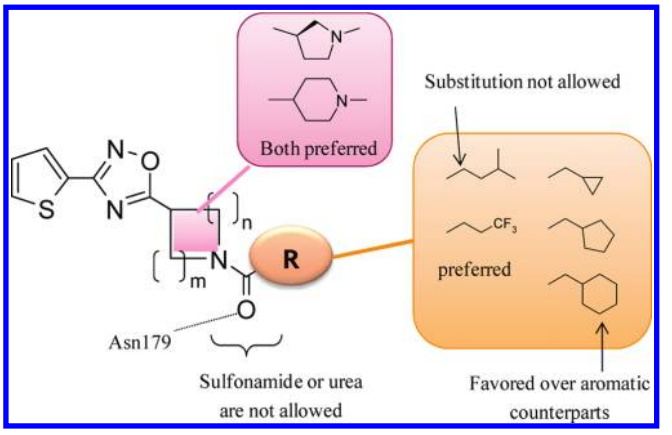


Figure 5. Summary of SAR study with thiophen-2-yl-1,2,4-oxadiazole derivatives.

46 and **55** displayed higher solubilities in PBS (150 and 80 μg/mL, respectively) than **40** (64 μg/mL). Compounds **46** and **55** were selected for further mouse liver microsome stability studies (Table 5), and compound **55** showed a better stability than its three analogues with an intrinsic clearance and a half-life equal to 4 μL/min/mg and 298 min, respectively.

CONCLUSION

In this study, we explored the structure−activity relationships of thiophen-2-yl-1,2,4-oxadiazoles as 10-fold ethionamide activity boosters. We confirmed with in vitro functional activities and X-ray crystallography that the compounds were genuine inhibitors of the mycobacterial regulator EthR. Replacement of the cyanoacetyl chain in the R position by a thienoacetyl greatly improves potency but dramatically reduces solubility and metabolic stability, jeopardizing in vivo activity.[21] Replacement of the thienoacetyl (R position) with substituted phenylacetyl or heteroarylacetyl groups did not improve potency in intracellular assay. However, introduction of aliphatic chains or rings that fit the pocket of the ligand binding domain was significantly more efficacious. At this position, a fluorinated chain (compound **46**) is equivalent to the thienoacetyl in terms of potency but not

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Table 5. Physicochemical and Stability Properties of Compounds 1, 2, 46, and 55

	physicochemical properties			intracellular anti*M. Tb* growth assay	mouse microsomal stability[c]	
compd	MW ($g \cdot mol^{-1}$)	solubility[a] ($\mu g/mL$)	clogP[b]	EC_{50} (μM)	$t_{1/2}$ (min)	CL_{int} ($\mu L/min/mg$)
1	302.3	194	1.38	4.7	91	11
2	359.5	22	2.97	0.1	3	412
46	359.4	150	2.70	0.1	5	213
55	345.3	80	2.38	0.4	298	4

[a] Solubilities were determined at pH 7.4. [b] clogP were calculated using pipeline pilot software from accelrys. [c] Propranolol known as a high hepatic clearance drug in rodents was used as reference for microsomal incubations ($t_{1/2}$ = 13 min, CL_{int} = 121 $\mu L/min/mg$). See Experimental Section for further details on all assays.

better in terms of stability, although it improves solubility. Keeping the same fluorinated chain, we managed to obtain a much more stable compound by replacing the piperidine by a pyrrolidine at a minimal cost in solubility and potency (compound **55**). Efforts to further optimize this series by the replacement of oxadiazole ring and the remaining thiophene are ongoing and will be reported in due course.

EXPERIMENTAL SECTION

Biology. *EthR−DNA Binding Assay.* SPR analysis of the molecular interactions between EthR and the *ethA* promoter region was performed using "research grades CM5 sensor chips" on a BIAcore 2000 instrument (AB, Uppsala, Sweden). Streptavidin was injected onto the CM5 sensor chips at 500 ng/mL in 10 mM sodium acetate (pH 3.5) for 12 min at a 10 μL/min flow rate. The 106 bp biotinylated DNA fragment overlapping the ethAethR intergenic region was obtained by polymerase chain reaction (PCR), purified by agarose gel electrophoresis, and immobilized onto the CM5 sensor chip. The biotinylated DNA fragment (200 ng/mL) was injected in one channel of the chip at to obtain a 50 resonance unit (RU) stable fixation to immobilized streptavidin. Another channel of the chip was loaded with a biotinylated double stranded 113 bp long irrelevant DNA fragment (+14 to +127 fragment of the *E. coli* bla gene PCR amplified using oligonucleotides O-343, TTTCCGTGTCGCCCTTATTCC, and O-344, CCACTCGTGCACCCAACTGAT, and pUC18 as substrate). Binding of EthR to the immobilized DNA was performed at 25 °C in 10 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.1 mM EDTA, 1 mM DTT, and 1% DMSO at a flow rate of 20 μL/min. Specific interaction (SI) between EthR and the 106 bp DNA fragment was defined as the signal difference between both channels. For dose response curves establishment, the test compounds were serially diluted in the binding buffer containing 540 nM EthR, incubated 5 min at 37 °C, and then injected in the BIAcore at a flow rate of 20 μL/min for 3 min. SI values were measured at the end of the injection period and used to calculate the inhibition of protein−DNA interaction. IC_{50} values were determined by using GraphPad Prism software.

Intracellular Assay. Raw264.7 macrophages (10^8 cells) were infected with H37Rv-GFP suspension at a MOI of 1:1 in 300 mL for 2 h at 37 °C with shaking (100 rpm). After two washes by centrifugation at 1100 rpm for 5 min, the remaining extracellular bacilli from the infected cells suspension were killed by a 1 h Amykacin (20 μM, Sigma, A2324−5G) treatment. After a final centrifugation step, 40 μL of *M. tuberculosis* H37Rv-GFP colonized macrophages were dispensed with the Wellmate (Matrix) into 384-well Evotec plates preplated with 10 μL of compound mixture diluted in cell medium and incubated for 5 days at 37 °C, 5% CO_2. Macrophages were then stained with SYTO 60 (Invitrogen, S11342) for 1 h followed by plate sealing. Confocal images were recorded on an automated fluorescent ultrahigh-throughput microscope Opera (Evotec). This microscope is based on an inverted microscope architecture that allows imaging of cells cultivated in 96- or 384-well microplates (Evotec). Images were acquired with a 20× water immersion objective (NA 0.70). Double laser excitation (488 and 635 nm) and dedicated dichroic mirrors were used to record green fluorescence of mycobacteria and red fluorescence of the macrophages on two different cameras, respectively. A series of four pictures at the center of each well were taken, and each image was then processed using dedicated image analysis.[24,25] The percent of infected cells and the number of cells are the two parameters extracted from images analysis as previously reported.[24] Data of two replicates are average.

Potency Assay of Test Compounds on M. tuberculosis (Ethionamide Concentration Fixed at 0.1 μg/mL, Serial Dilution of Test Compounds). Ethionamide (Sigma E6005-5G) was diluted into DMSO to 10 mg/mL, and aliquots were stored frozen at −20 °C. Test compounds were suspended in pure DMSO at a concentration of 40 mg/mL in Matrix tubes and then diluted by a 10-fold dilution to 4 mg/mL in Eppendorf tubes. Ten 2-fold serial dilutions of compounds were performed in DMSO in Greiner 384 well V-shape polypropylene plates (Greiner, no. 781280). Equal volumes (5 μL) of diluted compounds and of ethionamide were transferred to a 384-well low volume polypropylene plate (Corning, no. 3672). Two independent replicates were done for each setting. On the day of the experiment, 0.5 μL of compound-plate was first transferred by EVOBird platform (Evotec) to cell assay plates preplated with 10 μL of assay medium.

Pharmacokinetics. *Solubility/Metabolic Stability.* These experiments were analyzed using a LC-MS-MS triple-quadrupole system (Varian 1200ws) under MRM detection with the following mass parameters: mode of ionization, electrospray; declustering potential, 50 V; collision gas pressure, 1.5 mTorr; collision energy, 20 eV.

Solubility. The 10 mM solution (40 μL) in DMSO of the sample was added to 1.960 mL of MeOH or PBS at pH 7.4. The samples were gently shaken for 24 h at room temperature, then centrifuged for 5 min, and filtered over 0.45 μm filters. An amount equal to 20 μL of each solution was added to 180 μL of MeOH and analyzed by LC-MS-MS. The solubility was determined by the ratio of mass signal areas PBS/MeOH.

Metabolic Stability. We purchased male mouse (CD-1) liver microsomes from BD Gentest. We performed all incubations in duplicate in a shaking water bath at 37 °C. The incubation mixtures contained 1 μM of compound with 1% methanol used as a vehicle, mouse liver microsomes (0.6 mg of microsomal protein per mL), 5 mM $MgCl_2$, 1 mM NADP, 5 mM glucose-6-phosphate, 0.4 $U \cdot mL^{-1}$ glucose-6-phosphate dehydrogenase, and 50 mM potassium phosphate buffer (pH 7.4) in a final volume of 1.5 mL. We took samples at 5, 10, 15, 20, 25, and 30 min after microsome addition, and we terminated the reactions by adding ice-cold acetonitrile containing 1 mM internal standard (one volume). We centrifuged the samples for 10 min at 4000*g* and 4 °C to pellet precipitated microsomal proteins, and we subjected the supernatant to liquid chromatography−tandem mass spectrometry (LC-MS/MS) analysis. We performed control incubations with denaturated microsomes with acetonitrile containing 1 mM internal standard, and we took

Table 6. Data Collection Statistics

	compd **32**	compd **7**
X-ray source	ESRF ID14-2	ESRF ID14-2
temperature (K)	100	100
wavelength (Å)	0.93300	0.93300
space group	$P4_1$	$P4_12_12$
cell constants (Å)	$a = b = 121.5$ $c = 33.7$	$a = b = 122.2$ $c = 33.7$
resolution (Å)[a]	1.95 (1.95−2.0)	1.7 (1.7−1.8)
completeness (%)	99.8 (100)	99.9 (100)
R_{sym} (%)	9.5 (49.4)	9.2 (38.5)
multiplicity	7.1 (6.6)	14.0 (14.3)
$I/\sigma(I)$	17.9 (3.2)	17.3 (3.6)
total reflections	261673 (17929)	401652 (62941)
unique reflections	36781 (2692)	28678 (4406)

[a] Number in parentheses is the statistic for the highest resolution shell.

samples at the start of the incubation and 30 min later (to evaluate the chemical stability of the compounds in the experimental conditions). For LC-MS/MS, we used a Varian HPLC−MS/MS system 1200 L triple quadrupole mass spectrometer equipped with an electrospray ionization source. Analytes were separated in incubation mixtures by HPLC with a TSK gel Super-ODS C18, 2 μm, 50 mm × 4.6 mm column (Interchim). The mobile phase solvents used were 0.01% formic acid in water (A) or 0.01% formic acid in acetonitrile (B). We applied the following mobile phase gradient: 10−90% (B) for 2.30 min; hold at 90% (B) for 1.30 min; 10−90% (A) for 0.01 min; 90% (A) hold for 1.04 min. The injection volume was 10 μL, and the flow rate was 1 mL·min^{-1}. We introduced approximately 30% of the eluent into the triple quadrupole mass spectrometer source. We maintained the source temperature of the mass spectrometer at 300 °C, the declustering potential was 50 V, and the collision gas pressure was 1.5 mTorr. We optimized the collision energy and observed transitions for each compound. We quantified each compound by converting the corresponding analyte/internal standard peak area ratios to percentage drug remaining, using the initial ratio values in control incubations as 100%. We used propranolol, known as a high hepatic clearance drug in rodents, as a quality control compound for the microsomal incubations.

Crystal Structure Determination of EthR−Ligand Complexes. We produced N-terminally hexahistidine-tagged EthR in *Escherichia coli* C41 (pET-15b-ethR) and purified it as previously described.[15] The protein was buffer exchanged before crystallization against 10 mM Tris-HCl (pH 7.5), 200 mM NaCl, and concentrated to 11 mg/mL. Crystals were obtained by the vapor diffusion method using 0.17 M ammonium sulfate, 0.085 M sodium cacodylate (pH 6.5), 15% glycerol, and 25−35% polyethylene glycol 8000 as the crystallization solution. The ligands were dissolved in 100% DMSO. Before crystallization, the protein was incubated with the ligand, either compound **32** or compound **7** added at equimolar concentration. Crystals of EthR complexed with compound **7** belongs to space group $P4_12_12$, with one monomer in the asymmetric unit, while crystals of EthR complexed with compound **32** belong to space group $P4_1$, with a dimer in the asymmetric unit. In all cases, the ligands can be fitted without any ambiguity in the electron density. Data collection statistics are summarized in Table 6. The diffraction data were processed with XDS (X-ray detector software: http://xds.mpimf-heidelberg.mpg.de).[33] We refined the structures with the macromolecular refinement program REFMAC5[34] from the CCP4 suite[35] to resolutions of 1.95 and 1.7 Å for compound **32** and compound **7**, respectively. The ligand topology and parameter files were obtained from the PRODRG2 server (http://davapc1.bioch.dundee.ac.uk/prodrg/).[36] The ligands were positioned in the electron density of the crystals using the program COOT.[37] The final R (R_{free}) factors are 16.9% (22.7%) and 20.1% (23.3%) for compounds **32** and **7**, respectively. The two structures have been deposited with the Protein Data Bank (PDB) under the accession codes 3Q0V and 3Q0U.

Chemistry. *General Information.* NMR spectra were recorded on a Bruker DRX-300 spectrometer. Chemical shifts are in parts per million (ppm). The assignments were made using one-dimensional (1D) ^{1}H and ^{13}C spectra and two-dimensional (2D) HSQC and COSY spectra. Mass spectra were recorded with a LC-MSMS triple-quadrupole system (Varian 1200ws) or a LCMS (Waters Alliance Micromass ZQ 2000). LCMS analysis was performed using a C18 TSK-GEL Super ODS 2 μm particle size column, dimensions 50 mm × 4.6 mm. A gradient starting from 100% H_2O/0.1% formic acid and reaching 20% H_2O/80% CH_3CN/0.08% formic acid within 10 min at a flow rate of 1 mL/min was used. Preparative HPLC were performed using a Varian PRoStar system using an OmniSphere 10 Column C_{18} 250 mm × 41.4 mm Dynamax from Varian, Inc. A gradient starting from 20% CH_3CN/80% H_2O/0.1% formic acid and reaching 100% CH_3CN/0.1% formic acid at a flow rate of 80 mL/min or 20% MeOH/80% H_2O/0.1% formic acid reaching 100% MeOH/0.1% formic acid was used. Purity (%) was determined by reversed phase HPLC, using UV detection (215 nm), and all compounds showed purity greater than 95%. Melting points were determined on a Büchi B-540 apparatus and are uncorrected. All commercial reagents and solvents were used without further purification.

*3-Oxo-3-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-propionitrile (**1**).* Thiophene-2-carbonitrile (1 equiv), hydroxylamine hydrochloride (1.5 equiv), and diisopropylethylamine (1.6 equiv) were mixed in absolute ethanol (1M). The reaction mixture was refluxed 4 h, and then the solvent was evaporated under reduced pressure. The residue was dissolved in AcOEt and washed twice with water and once with brine. The organic layer was dried over $MgSO_4$ and then evaporated under reduced pressure to give thiophene-2-amidoxime as a white powder. Yield 95%. ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 7.47 (dd, J = 3.6 Hz, J = 0.9 Hz, 1H), 7.41 (dd, J = 5.1 Hz, J = 0.9 Hz, 1H), 7.03 (dd, J = 5.1 Hz, J = 3.6 Hz, 1H), 5.92 (brs, 2H). ^{13}C NMR (DMSO-d_6) δ 147.94, 136.97, 127.35, 126.68, 125.42. MS $[M + H]^+$ m/z 143. Boc-isonipecotic acid (10 mmol, 1 equiv), HOBt (0.2 equiv), HBTU (1.1 equiv), and diisopropylethylamine (3 equiv) were dissolved in dimethylformamide (15 mL). The solution was stirred for 5 min, and then thiophene-2-amidoxime (1 equiv) was added. The reaction mixture was stirred 2 h at room temperature and then poured in 50 mL of water. The reaction mixture produced a thick crystalline slurry. The product was recovered by filtration and washed with water. The obtained solid was dissolved in dimethylformamide (15 mL), and then the reaction mixture was heated at 120 °C overnight. The solvent was removed under vacuum, and the residue was dissolved in AcOEt. The organic layer was washed twice with HCl 1N, twice with saturated aqueous $NaHCO_3$, and once with brine and then dried over $MgSO_4$ and evaporated under reduced pressure to give 4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine-1-carboxylic acid *tert*-butyl ester as a beige powder. Yield 84%. ^{1}H NMR ($CDCl_3$) δ 7.70 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.42 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.06 (dd, J = 3.6 Hz, J = 5.0 Hz, 1H), 4.05 (m, 2H), 3.07 (m, 1H), 2.90 (m, 2H), 2.03 (m, 2H), 1.81 (m, 2H), 1.40 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 181.23, 164.23, 154.51, 129.46, 129.2, 128.32, 127.9, 79.72, 42.84, 34.38, 29.05, 28.38. $t_{R\ LCMS}$ 6.9 min. MS $[M + Na]^+$ m/z 358. Boc intermediate was dissolved in dioxane (1 M) and HCl 4N solution in dioxane (5 equiv) was added. The reaction mixture was stirred 5 h at room temperature. The product was recovered by filtration and then washed with petroleum ether to give 4-(3-thiophen-2-yl -[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride as a white powder. Yield 91%. ^{1}H NMR (DMSO-d_6) δ 9.42 (brs, 2H), 7.88 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.79 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.25 (dd, J = 5.0 Hz, J = 3.6 Hz, 1H), 3.5 (m, 1H), 3.31 (m, 2H), 3.05 (m, 2H), 2.24 (m, 2H), 2.05 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 181.36, 164.09, 131.26, 130.39,

129.03, 127.81, 42.31, 31.68, 25.91. $t_{R\ LCMS}$ 2.8 min. MS $[M + H]^+$ m/z 236. Cyanoacetic acid (2 equiv), EDCI (2 equiv), and triethylamine (4 equiv) were mixed in DCM (20 mL) for 10 min. 4-(3-Thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (5 mmol, 1 equiv) was added, and the reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure. The residue was dissolved in AcOEt, and the organic layer was washed twice with aqueous NaOH 1M, twice with aqueous HCl 1 M, and once with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (DCM/MeOH 99/1) to give compound **1** as a white powder. Yield 68%. ^{1}H NMR (CD_2Cl_2): δ 7.79 (dd, J = 3.5 Hz, J = 1.1 Hz, 1H), 7.55 (dd, J = 4.9 Hz, J = 1.1 Hz, 1H), 7.18 (dd, J = 4.8 Hz, J = 3.3 Hz, 1H), 4.41 (m, 1H), 3.73 (m, 1H), 3.58 (s, 2H), 3.28 (m, 2H), 3.03 (m, 1H), 2.20 (m, 2H), 1.86 (m, 2H). ^{13}C NMR (CD_2Cl_2): δ 180.61, 164.34, 160.12, 129.54, 129.35, 128.27, 128.05, 114.18, 45.34, 41.42, 33.84, 29.14, 28.57, 25.09. $t_{R\ LCMS}$ 4.7 min. Purity >99%. MS $[M + H]^+$ m/z 303.

General Procedure for the Synthesis of Compounds ***4***, ***8***–***25***, ***45***. The appropriate carboxylic acid (1.5 equiv), EDCI (1.5 equiv), and triethylamine (4.5 equiv) were mixed in DCM (2 mL) for 5 min. 4-(3-Thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (100 mg, 1 equiv) was added with 3 mL of DCM. The reaction mixture was stirred overnight at room temperature, and then DCM was added and the organic layer was washed with aqueous HCl 1 M (3 times), aqueous saturated $NaHCO_3$ (twice), and brine (once), dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by preparative HPLC to give the expected compound.

General Procedure for the Synthesis of Compounds ***2***, ***6***, ***7***, ***32***, ***38***, ***40***–***44***, ***46***. The appropriate carboxylic acid (2 equiv), EDCI (2 equiv), and triethylamine (4 equiv) were mixed in dried DCM (2 mL) for 5 min. 4-(3-Thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (100 mg, 1 equiv) was added, and the reaction mixture was stirred overnight at room temperature. AcOEt was added (20 mL). The organic layer was washed with aqueous NaOH 1 M (thrice, 30 mL), aqueous HCl 1 M (thrice, 30 mL) and brine (30 mL), dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by thick layer chromatography to give the expected compound.

General Procedure for the Synthesis of Compounds ***5***, ***33***, ***36***, ***47***–***50***. The appropriate carboxylic acid (1.5 equiv), EDCI (1.5 equiv), HOBt (0.3 equiv), and diisopropylethylamine (4.5 equiv) were mixed in DMF (2 mL) for 5 min. 4-(3-Thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (100 mg, 1 equiv) was added with 3 mL of DMF. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure. The residue was dissolved in AcOEt, and the organic layer was washed with aqueous HCl 1 M (3 times), aqueous saturated $NaHCO_3$ (twice), and brine (once), dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by preparative HPLC to give the expected compound.

General Procedure for the Synthesis of Compounds ***27***–***31***, ***37***. 4-(3-Thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (100 mg, 1 equiv) and triethylamine (3 equiv) were mixed in dried DCM (5 mL). The acid chloride derivative (1.1 equiv) was added dropwise, and the reaction mixture was stirred for 2 h at room temperature. DCM was added (15 mL). The organic layer was washed with aqueous NaOH 1 M (thrice, 20 mL), aqueous HCl 1 M (thrice, 20 mL), and brine (once, 20 mL), dried over $MgSO_4$, and concentrated under vacuum to give the expected compound.

General Procedure for the Synthesis of Compounds ***26***, ***39***. 4-(3-Thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (50 mg, 1 equiv) and diisopropylethylamine (3 equiv) were mixed in dried DCM (1 mL). The appropriate anhydride (1.2 equiv) was added dropwise, and the reaction mixture was stirred for 2 h at room temperature. AcOEt was added (15 mL). The organic layer was washed with aqueous NaOH 1 M (thrice, 20 mL), aqueous HCl 1 M (thrice, 20 mL), and brine (once, 20 mL), dried over $MgSO_4$, and concentrated under vacuum to give the desired compound.

*2-Thiophen-2-yl-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**2**).* Yellow oil; yield 39%. ^{1}H NMR ($CDCl_3$) δ 7.76 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.48 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.19 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.13 (dd, J = 5.1 Hz, J = 3.7 Hz, 1H), 6.94 (dd, J = 5.1 Hz, J = 3.5 Hz, 1H), 6.89 (dd, J = 3.5 Hz, J = 1.2 Hz, 1H), 4.48 (m, 1H), 3.93 (m, 1H), 3.92 (s, 2H), 3.18 (m, 2H), 3.00 (m, 1H), 2.11 (m, 2H), 1.84 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 180.71, 168.35, 164.36, 136.38, 129.62, 129.36, 128.21, 128.00, 126.92, 126.07, 124.85, 45.35, 41.05, 35.26, 34.17, 29.33, 28.72. $t_{R\ LCMS}$ 5.8 min. Purity >99%. MS $[M + H]^+$ m/z 360.

*2-Azido-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**3**).* 4-(3-Thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (2 mmol, 1 equiv) and triethylamine (2.1 equiv) were mixed in dried DCM (4 mL). The reaction mixture was cooled to −15 °C, and then chloroacetylchloride (1 equiv) was added dropwise over 10 min. The reaction mixture was stirred 2 h at room temperature, and then DCM (20 mL) was added. The organic layer was washed twice with aqueous HCl 1 M and once with brine and then dried over $MgSO_4$ and evaporated under reduced pressure to afford 2-chloro-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-ethanone. The previous compound was dissolved in DMF (3 mL), and then sodium azide (1.4 equiv) was added and the reaction mixture was stirred overnight at room temperature. The solvent was removed under vacuum, and the residue was dissolved in AcOEt. The organic layer was washed three times with water and once with brine and then dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (cyclohexane/AcOEt 7/3) to give compound **3** as a white powder. Yield 76%. ^{1}H NMR ($CDCl_3$): δ 7.76 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.48 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.13 (dd, J = 5.0 Hz, J = 3.6 Hz, 1H), 4.43 (m, 1H), 3.95 (s, 2H), 3.72 (m, 1H), 3.25 (m, 2H), 3.04 (m, 1H), 2.18 (m, 2H), 1.94 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 180.48, 165.58, 164.39, 129.68, 129.42, 128.12, 128.03, 50.77, 44.16, 41.17, 33.99, 29.68, 28.74. $t_{R\ LCMS}$ 5.2 min. Purity >99%. MS $[M + H]^+$ m/z 319.

*3-Thiophen-2-yl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-propan-1-one (**4**).* Colorless oil; yield 72%. ^{1}H NMR (MeOD) δ 7.79 (dd, J = 3.7 Hz, J = 1.3 Hz, 1H), 7.67 (dd, J = 5.1 Hz, J = 1.3 Hz, 1H), 7.17–7.22 (m, 2H), 6.87–6.92 (m, 2H), 4.46–4.51 (m, 1H), 3.97–4.01 (m, 1H), 3.23–3.40 (m, 2H), 3.17 (t, J = 7.2 Hz, 2H), 2.94–3.03 (m, 1H), 2.70–2.88 (m, 2H), 2.11–2.18 (m, 2H), 1.65–1.85 (m, 2H). $t_{R\ LCMS}$ 6.2 min. Purity >99%. MS $[M + H]^+$ m/z 374.

*2-(3H-Imidazol-4-yl)-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**5**).* Colorless oil; yield 20%. ^{1}H NMR (CD_2Cl_2) δ 8.15 (brs, NH), 7.74 (d, J = 3.6 Hz, 1H), 7.73 (s, 1H), 7.51 (d, J = 5.1 Hz, 1H), 7.14 (dd, J = 3.6 Hz, J = 5.1 Hz, 1H), 6.94 (s, 1H), 4.41–4.46 (m, 1H), 3.99–4.04 (m, 1H), 3.75 (s, 2H), 3.18–3.30 (m, 2H), 2.88–2.96 (m, 1H), 2.10–2.13 (m, 2H), 1.77–1.84 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 181.74, 168.98, 164.91, 135.15, 130.88, 130.03, 129.84, 129.07, 128.59, 118.30, 45.77, 41.58, 34.72, 32.07, 30.00, 29.37. $t_{R\ LCMS}$ 3.2 min. Purity >99%. MS $[M + H]^+$ m/z 344.

*Phenyl-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-methanone (**6**).* White powder; yield 52%. ^{1}H NMR (CD_2Cl_2) δ 7.79 (dd, J = 3.8 Hz, J = 1.2 Hz, 1H), 7.56 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.41–7.47 (m, 5H), 7.20 (dd, J = 3.8 Hz, J = 5.0 Hz, 1H), 4.50–4.58 (m, 1H), 3.84–3.91 (m, 1H), 3.28–3.35 (m, 1H), 3.15–3.23 (m, 2H), 2.15–2.22 (m, 2H), 1.93–1.99 (m, 2H). $t_{R\ LCMS}$ 5.7 min. Purity 98%. MS $[M + H]^+$ m/z 340.

*2-Phenyl-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**7**).* White powder; yield 98%. ^{1}H NMR ($CDCl_3$) δ 7.73 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.45 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.21–7.32 (m, 5H), 7.10 (dd, J = 5.1 Hz, J = 3.7 Hz, 1H), 4.47 (m, 1H),

3.86 (m, 1H), 3.73 (s, 2H), 3.13 (m, 2H), 2.94 (m, 1H), 1.6−2.12 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 180.79, 169.44, 164.30, 134.95, 129.62, 129.38, 128.82, 128.57, 128.19, 128.03, 126.90, 45.18, 41.08, 40.88, 34.15, 29.23, 28.77. $t_{R\ LCMS}$ 5.9 min. Purity 95%. MS $[M + H]^+$ m/z 354.

*2-(2-Fluoro-phenyl)-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**8**).* White powder; yield 69%. ^{1}H NMR (CD_2Cl_2) δ 7.75 (d, J = 3.7 Hz, 1H), 7.51 (d, J = 5.1 Hz, 1H), 7.23−7.27 (m, 2H), 7.02−7.17 (m, 3H), 4.46−4.50 (m, 1H), 3.93−3.98 (m, 1H), 3.71 (s, 2H), 3.17−3.29 (m, 2H), 2.90−2.98 (m, 1H), 2.10−2.16 (m, 2H), 1.78−1.88 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 183.64, 170.66, 166.86, 163.33 (d, J = 245.9 Hz), 133.72 (d, J = 4.2 Hz), 132.01, 131.81, 131.24 (d, J = 7.8 Hz), 130.93, 130.58, 126.73 (d, J = 3.2 Hz), 125.31 (d, J = 16.1 Hz), 117.68 (d, J = 21.0 Hz), 47.56, 43.47, 36.85, 35.92, 31.95, 31.46. $t_{R\ LCMS}$ 6.1 min. Purity 99%. MS $[M + H]^+$ m/z 372.

*2-(3-Fluoro-phenyl)-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**9**).* White powder; yield 66%. ^{1}H NMR (CD_2Cl_2) δ 7.74 (d, J = 3.7 Hz, 1H), 7.51 (d, J = 5.1 Hz, 1H), 7.26−7.33 (m, 1H), 7.15 (dd, J = 5.1 Hz, J = 3.7 Hz, 1H), 6.92−7.03 (m, 3H), 4.45−4.49 (m, 1H), 3.87−3.92 (m, 1H), 3.71 (s, 2H), 3.17−3.24 (m, 2H), 2.90−2.97 (m, 1H), 2.04−2.15 (m, 2H), 1.67−1.87 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 183.57, 171.04, 166.83, 165.43 (d, J = 245.1 Hz), 140.47 (d, J = 7.8 Hz), 132.62 (d, J = 7.8 Hz), 132.01, 131.82, 130.91, 130.58, 127.20 (d, J = 3.2 Hz), 118.31 (d, J = 21.0 Hz), 116.11 (d, J = 21.0 Hz), 47.68, 43.46, 42.86, 36.74, 32.02, 31.37. $t_{R\ LCMS}$ 6.1 min. Purity 99%. MS $[M + H]^+$ m/z 372.

*2-(4-Fluoro-phenyl)-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**10**).* White powder; yield 53%. ^{1}H NMR (CD_2Cl_2) δ 7.74 (d, J = 3.7 Hz, 1H), 7.51 (d, J = 5.1 Hz, 1H), 7.13−7.23 (m, 3H), 6.98−7.04 (m, 2H), 4.45−4.49 (m, 1H), 3.88−3.93 (m, 1H), 3.68 (s, 2H), 3.16−3.25 (m, 2H), 2.87−2.96 (m, 1H), 2.03−2.14 (m, 2H), 1.66−1.86 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 183.57, 171.42, 166.80, 164.25 (d, J = 245.9 Hz), 133.79 (d, J = 3.5 Hz), 132.99 (d, J = 7.6 Hz), 131.99, 131.80, 130.91, 130.56, 117.81 (d, J = 21.0 Hz), 47.57, 43.39, 42.31, 36.83, 32.02, 31.40. $t_{R\ LCMS}$ 6.1 min. Purity >99%. MS $[M + H]^+$ m/z 372.

*2-(2-Chloro-phenyl)-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**11**).* White powder; yield 56%. ^{1}H NMR (CD_2Cl_2) δ 7.79 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.56 (dd, J = 4.8 Hz, J = 1.2 Hz, 1H), 7.41−7.44 (m, 1H), 7.25−7.31 (m, 3H), 7.18−7.21 (m, 1H), 4.52−4.56 (m, 1H), 3.95−4.00 (m, 1H), 3.85 (s, 2H), 3.23−3.35 (m, 2H), 2.96−3.05 (m, 1H), 2.15−2.18 (m, 2H), 1.86−1.92 (m, 2H). $t_{R\ LCMS}$ 6.5 min. Purity 98%. MS $[M + H]^+$ m/z 388.

*2-(3-Chloro-phenyl)-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**12**).* White powder; yield 81%. ^{1}H NMR (CD_2Cl_2) δ 7.80 (d, J = 3.6 Hz, 1H), 7.57 (d, J = 5.0 Hz, 1H), 7.27−7.35 (m, 3H), 7.17−7.22 (m, 2H), 4.50−4.55 (m, 1H), 3.92−3.97 (m, 1H), 3.75 (s, 2H), 3.23−3.31 (m, 2H), 2.95−3.04 (m, 1H), 2.11−2.20 (m, 2H), 1.78−1.94 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 183.58, 170.98, 166.83, 140.07, 136.72, 132.40, 132.01, 131.82, 131.55, 130.96, 130.58, 129.78, 129.42, 47.79, 43.52, 42.69, 36.84, 32.04, 31.44. $t_{R\ LCMS}$ 6.5 min. Purity 98%. MS $[M + H]^+$ m/z 388.

*2-(4-Chloro-phenyl)-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**13**).* White powder; yield 30%. ^{1}H NMR (CD_2Cl_2) δ 7.74 (d, J = 3.6 Hz, 1H), 7.51 (d, J = 5.0 Hz, 1H), 7.28−7.31 (m, 2H), 7.13−7.19 (m, 3H), 4.44−4.49 (m, 1H), 3.87−3.91 (m, 1H), 3.68 (s, 2H), 3.16−3.24 (m, 2H), 2.89−2.96 (m, 1H), 2.04−2.14 (m, 2H), 1.68−1.88 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 183.57, 171.17, 166.88, 136.64, 134.97, 132.92, 132.02, 131.83, 131.17, 130.92, 130.59, 47.63, 43.43, 42.46, 36.79, 32.02, 31.42. $t_{R\ LCMS}$ 6.5 min. Purity >99%. MS $[M + H]^+$ m/z 388.

*1-[4-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-2-(2-trifluoromethyl-phenyl)-ethanone (**14**).* White powder; yield 84%. ^{1}H NMR (CD_2Cl_2) δ 7.80 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.58 (t, J = 7.8 Hz, 1H), 7.56 (dd, J = 4.8 Hz J = 1.2 Hz, 1H), 7.43 (t, J = 7.8 Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.20 (dd, J = 4.8 Hz J = 3.9 Hz, 1H), 4.51−4.56 (m, 1H), 3.84−3.96 (m, 1H), 3.91 (s, 2H), 3.25−3.33 (m, 2H), 2.96−3.05 (m, 1H), 2.16−2.19 (m, 2H), 1.82−1.94 (m, 2H). $t_{R\ LCMS}$ 6.8 min. Purity 98%. MS $[M + H]^+$ m/z 422.

*1-[4-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-2-(3-trifluoromethyl-phenyl)-ethanone (**15**).* White powder; yield 60%. ^{1}H NMR (CD_2Cl_2) δ 7.80 (dd, J = 3.9 Hz, J = 1.2 Hz, 1H), 7.49−7.57 (m, 5H), 7.20 (dd, J = 5.1 Hz, J = 3.9 Hz, 1H), 4.50−4.54 (m, 1H), 3.94−3.98 (m, 1H), 3.82 (s, 2H), 3.22−3.32 (m, 2H), 2.96−3.08 (m, 1H), 2.11−2.19 (m, 2H), 1.76−1.94 (m, 2H). $t_{R\ LCMS}$ 6.8 min. Purity 98%. MS $[M + H]^+$ m/z 422.

*1-[4-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-2-(4-trifluoromethyl-phenyl)-ethanone (**16**).* White powder; yield 25%. ^{1}H NMR (CD_2Cl_2) δ 7.75 (d, J = 3.6 Hz, 1H), 7.59 (d, J = 7.8 Hz, 2H), 7.51 (d, J = 5.1 Hz, 1H), 7.37 (d, J = 7.8 Hz, 2H), 7.15 (dd, J = 5.1 Hz, J = 3.6 Hz, 1H), 4.45−4.49 (m, 1H), 3.88−3.93 (m, 1H), 3.78 (s, 2H), 3.18−3.27 (m, 2H), 2.91−2.99 (m, 1H), 2.07 −2.15 (m, 2H), 1.71−1.88 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 181.51, 168.73, 164.84, 140.29, 129.99, 129.98, 129.81, 128.88, 125.90 (q, J = 3.9 Hz), 124.86 (q, J = 272 Hz), 45.61, 41.46, 40.77, 34.74, 29.97, 29.34. $t_{R\ LCMS}$ 6.8 min. Purity >99%. MS $[M + H]^+$ m/z 422.

*1-[4-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-2-o-tolyl-ethanone (**17**).* White powder; yield 50%. ^{1}H NMR (CD_2Cl_2) δ 7.80 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.56 (dd, J = 4.8 Hz, J = 1.2 Hz, 1H), 7.11−7.21 (m, 5H), 4.52−4.59 (m, 1H), 3.89−3.97 (m, 1H), 3.71 (s, 2H), 3.22−3.32 (m, 2H), 2.96−3.05 (m, 1H), 2.29 (s, 3H), 2.12−2.17 (m, 2H), 1.82−1.90 (m, 2H). $t_{R\ LCMS}$ 6.4 min. Purity 98%, MS $[M + H]^+$ m/z 368.

*1-[4-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-2-m-tolyl-ethanone (**18**).* White powder; yield 35%. ^{1}H NMR (CD_2Cl_2) δ 7.79 (dd, J = 3.3 Hz, J = 0.9 Hz, 1H), 7.55 (dd, J = 5.1 Hz, J = 0.9 Hz, 1H), 7.18−7.26 (m, 2H), 7.04−7.10 (m, 3H), 4.51−4.57 (m, 1H), 3.93−3.97 (m, 1H), 3.72 (s, 2H), 3.18−3.28 (m, 2H), 2.92−3.00 (m, 1H), 2.36 (s, 3H), 2.05−2.13 (m, 2H), 1.70−1.86 (m, 2H). $t_{R\ LCMS}$ 6.4 min. Purity 98%. MS $[M + H]^+$ m/z 368.

*1-[4-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-2-p-tolyl-ethanone (**19**).* White powder; yield 54%. ^{1}H NMR (CD_2Cl_2) δ 7.74 (d, J = 3.6 Hz, 1H), 7.51 (d, J = 5.1 Hz, 1H), 7.1 5 (dd, J = 5.1 Hz, J = 3.6 Hz, 1H), 7.08−7.13 (m, 4H), 4.49−4.54 (m, 1H), 3.87−3.93 (m, 1H), 3.67 (s, 2H), 3.14−3.21 (m, 2H), 2.87−2.95 (m, 1H), 2.30 (s, 3H), 1.99−2.13 (m, 2H), 1.66−1.82 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 181.60, 169.82, 164.82, 136.98, 132.76, 129.96, 129.78, 129.76, 129.08, 128.91, 128.55, 45.70, 41.30, 40.92, 34.92, 29.84, 29.31, 21.26. $t_{R\ LCMS}$ 6.4 min. Purity >99%. MS $[M + H]^+$ m/z 368.

*2-(2-Methoxy-phenyl)-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**20**).* White powder; yield 70%. ^{1}H NMR (CD_2Cl_2) δ 7.80 (d, J = 3.6 Hz, 1H), 7.57 (d, J = 5.1 Hz, 1H), 7.19−7.31 (m, 3H), 6.92−6.98 (m, 2H), 4.52−4.63 (m, 1H), 4.00−4.08 (m, 1H), 3.87 (s, 3H), 3.72 (s, 2H), 3.21−3.30 (m, 2H), 2.89−3.02 (m, 1H), 2.11−2.20 (m, 2H), 1.71−1.85 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 183.79, 172.09, 166.92, 159.53, 132.68, 131.97, 131.78, 131.00, 130.60, 130.57, 126.72, 123.06, 112.95, 57.92, 47.64, 43.49, 36.97, 36.85, 32.08, 31.53. $t_{R\ LCMS}$ 6.1 min. Purity 98%. MS $[M + H]^+$ m/z 384.

*2-(3-Methoxy-phenyl)-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**21**).* White powder; yield 70%. ^{1}H NMR (CD_2Cl_2) δ 7.74 (d, J = 3.6 Hz, 1H), 7.51 (d, J = 5.1 Hz, 1H), 7.13−7.25 (m, 2H), 6.76−6.81 (m, 3H), 4.46−4.50 (m, 1H), 3.88−3.93 (m, 1H), 3.77 (s, 3H), 3.69 (s, 2H), 3.15−3.22 (m, 2H), 2.88−2.96 (m, 1H), 2.00−2.13 (m, 2H), 1.63−1.86 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 183.75, 171.56, 166.90, 162.59, 139.54, 132.12, 131.99, 131.80, 131.01, 130.57, 123.51, 116.90, 114.65, 57.73, 47.73, 43.42, 43.39, 36.87, 31.93, 31.38. $t_{R\ LCMS}$ 6.1 min. Purity 98%. MS $[M + H]^+$ m/z 384.

*2-(4-Methoxy-phenyl)-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**22**).* White powder; yield 53%. ^{1}H NMR (CD_2Cl_2) δ 7.74 (d, J = 3.6 Hz, 1H), 7.51 (d, J = 5.1 Hz, 1H), 7.12−7.15 (m, 3H), 6.84 (d, J = 8.7 Hz, 2H), 4.45−4.50 (m, 1H), 3.89−3.94 (m, 1H), 3.76 (s, 3H), 3.64 (s, 2H), 3.15−3.22 (m, 2H), 2.87−2.95 (m, 1H), 2.00−2.13 (m, 2H), 1.62−1.82 (m, 2H). ^{13}C NMR (CD_2Cl_2) δ 183.65, 172.03, 166.93, 161.04, 132.31, 132.01, 131.82, 130.60, 129.90, 116.53, 57.76, 47.76, 43.40, 42.44, 36.86, 31.95, 31.49. $t_{R\ LCMS}$ 6.1 min. Purity 98%. MS $[M + H]^+$ m/z 384.

*2-Pyridin-2-yl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**23**).* Beige powder; yield 36%. ^{1}H NMR ($CDCl_3$) δ 8.53−8.58 (m, 1H), 7.78 (d, J = 3.5 Hz, 1H), 7.72−7.76 (m, 1H), 7.51 (d, J = 5.0 Hz, 1H), 7.42−7.45 (m, 1H), 7.23−7.26 (m, 1H), 7.15 (dd, J = 5.0 Hz, J = 3.5 Hz, 1H), 4.48−4.53 (m, 1H), 4.16−4.20 (m, 1H), 4.03 (s, 2H), 3.23−3.35 (m, 2H), 2.98−3.05 (m, 1H), 2.09−2.17 (m, 2H), 1.76−1.93 (m, 2H). $t_{R\ LCMS}$ 3.8 min. Purity 99%. MS $[M + H]^+$ m/z 355.

*2-Pyridin-3-yl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**24**).* Beige powder; yield 60%. ^{1}H NMR ($CDCl_3$) δ 8.97 (s, 1H), 8.64 (d, J = 5.6 Hz, 1H), 8.40 (d, J = 7.9 Hz, 1H), 7.87 (dd, J = 5.6 Hz, J = 7.9 Hz, 1H), 7.77 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.50 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.15 (dd, J = 5.1 Hz, J = 3.6 Hz, 1H), 4.40−4.45 (m, 1H), 4.06−4.11 (m, 3H), 3.26−3.46 (m, 2H), 3.02−3.10 (m, 1H), 2.16−2.28 (m, 2H), 1.90−2.07 (m, 2H). $t_{R\ LCMS}$ 3.8 min. Purity 99%. MS $[M + H]^+$ m/z 355.

*2-Pyridin-4-yl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**25**).* Beige powder; yield 65%. ^{1}H NMR (MeOD) δ 8.49 (d, J = 5.8 Hz, 2H), 7.78 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.68 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.37 (d, J = 5.8 Hz, 2H), 7.20 (dd, J = 5.1 Hz, J = 3.6 Hz, 1H), 4.47−4.51 (m, 1H), 4.04−4.09 (m, 1H), 3.92 (s, 2H), 3.35−3.42 (m, 2H), 3.02−3.10 (m, 1H), 2.15−2.22 (m, 2H), 1.75−1.85 (m, 2H). $t_{R\ LCMS}$ 3.8 min. Purity 98%. MS $[M + H]^+$ m/z 355.

*1-[4-(3-Thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-ethanone (**26**).* Beige powder; yield 98%. ^{1}H NMR ($CDCl_3$) δ 7.76 (dd, J = 3.7 Hz J = 1.1 Hz, 1H), 7.48 (dd, J = 5.0 Hz, J = 1.1 Hz, 1H), 7.13 (dd, J = 5.0 Hz, J = 3.7 Hz, 1H), 4.48 (m, 1H), 3.93 (m, 1H), 3.23 (m, 2H), 2.94 (m, 1H), 2.12 (m, 5H), 1.90 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 180.79, 169.09, 164.37, 129.61, 129.35, 128.21, 128.00, 34.25, 29.70, 29.33, 21.37. $t_{R\ LCMS}$ 4.5 min. Purity >99%. MS $[M + H]^+$ m/z 278.

*1-[4-(3-Thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-propan-1-one (**27**).* White powder; yield 56%. ^{1}H NMR ($CDCl_3$) δ 7.75 (dd, J = 3.8 Hz, J = 1.2 Hz, 1H), 7.47 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.11 (dd, J = 5.0 Hz, J = 3.8 Hz, 1H), 4.1 (m, 2H), 3.21 (m, 1H), 3.07 (m, 2H), 2.35 (q, J = 7.5 Hz, 2H), 2.13 (m, 2H), 1.88 (m, 2H), 1.13 (t, J = 7.5 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 180.88, 172.29, 164.33, 129.59, 129.33, 128.21, 127.98, 34.37, 29.33, 29.27, 26.49, 9.52. $t_{R\ LCMS}$ 4.9 min. Purity 98%. MS $[M + H]^+$ m/z 292.

*1-[4-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-butan-1-one (**28**).* White powder; yield 76%. ^{1}H NMR (CD_2Cl_2) δ 7.79 (dd, J = 3.8 Hz J = 1.2 Hz, 1H), 7.56 (dd, J = 5.0 Hz J = 1.2 Hz, 1H), 7.19 (dd, J = 5.0 Hz, J = 3.8 Hz, 1H), 4.51−4.55 (m, 1H), 3.93−3.97 (m, 1H), 3.23−3.29 (m, 2H), 2.85−2.94 (m, 1H), 2.33 (t, J = 7.5 Hz, 2H), 2.14−2.22 (m, 2H), 1.78−1.97 (m, 2H), 1.65 (sextuplet, J = 7.5 Hz, 2H), 0.98 (t, J = 7.5 Hz, 3H). $t_{R\ LCMS}$ 5.4 min. Purity >99%. MS $[M + H]^+$ m/z 306.

*1-[4-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-pentan-1-one (**29**).* Colorless oil; yield 76%. ^{1}H NMR (CD_2Cl_2) δ 7.79 (dd, J = 3.8 Hz, J = 1.2 Hz, 1H), 7.56 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.19 (dd, J = 5.0 Hz, J = 3.8 Hz, 1H), 4.50−4.55 (m, 1H), 3.93−3.97 (m, 1H), 3.23−3.30 (m, 2H), 2.85−2.94 (m, 1H), 2.36 (t, J = 7.5 Hz, 2H), 2.11−2.22 (m, 2H), 1.80−1.97 (m, 2H), 1.55−1.65 (m, 2H), 1.39 (sextuplet, J = 7.5 Hz, 2H), 0.96 (t, J = 7.5 Hz, 3H). $t_{R\ LCMS}$ 5.9 min. Purity >99%. MS $[M + H]^+$ m/z 320.

*3-Methyl-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-butan-1-one (**30**).* Colorless oil; yield 68%. ^{1}H NMR ($CDCl_3$) δ 7.76 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.48 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.12 (dd, J = 5.0 Hz J = 3.7 Hz, 1H), 4.52 (m, 1H), 3.93 (m, 1H), 3.21 (m, 2H), 2.91 (m, 1H), 2.22 (d, J = 6.4 Hz,1H), 2.12 (m, 4H), 1.87 (m, 2H), 0.96 (d, J = 6.4 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 180.86, 171.22, 164.31, 129.60, 129.33, 128.16, 127.97, 44.96, 42.06, 40.72, 34.34, 29.68, 29.01, 25.82, 22.70. $t_{R\ LCMS}$ 5.8 min. Purity >99%. MS $[M + H]^+$ 320 m/z.

*3,3-Dimethyl-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-butan-1-one (**31**).* Colorless oil; yield 62%. ^{1}H NMR ($CDCl_3$) δ 7.74 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.46 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.11 (dd, J = 5.0 Hz, J = 3.8 Hz, 1H), 4.51 (m, 1H), 3.98 (m, 1H), 3.19 (m, 1H), 3.00 (m, 2H), 2.27 (s, 2H), 2.13 (m, 2H), 1.88 (m, 2H), 1.03 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 180.90, 170.47, 164.33, 129.59, 129.31, 128.21, 127.98, 44.69, 34.35, 31.49, 30.06, 29.64, 29.37. $t_{R\ LCMS}$ 6.1 min. Purity 99%. MS $[M + H]^+$ m/z 334.

*4-Methyl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-pentan-1-one (**32**).* Colorless oil; yield 83%. ^{1}H NMR ($CDCl_3$) δ 7.72 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.45 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.09 (dd, J = 5.1 Hz, J = 3.7 Hz, 1H), 4.46 (m, 1H), 3.89 (m, 1H), 3.19 (m, 2H), 2.90 (m, 1H), 2.30 (t, J = 8.0 Hz, 2H), 2.10 (m, 2H), 1.84 (m, 2H), 1.49 (m, 3H), 0.86 (d, J = 6.4 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 180.87, 171.86, 164.30, 129.58, 129.32, 128.19, 127.98, 44.55, 40.73, 34.35, 34.21, 31.37, 29.27, 27.91, 22.39. $t_{R\ LCMS}$ 6.3 min. Purity 98%. MS $[M + H]^+$ m/z 334.

*4,4-Dimethyl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-pentan-1-one (**33**).* White powder; yield 80%. ^{1}H NMR ($CDCl_3$) δ 7.79 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.51 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.16 (dd, J = 5.1 Hz, J = 3.6 Hz, 1H), 4.52−4.57 (m, 1H), 3.91−3.96 (m, 1H), 3.22−3.29 (m, 2H), 2.90−2.98 (m, 1H), 2.30−2.36 (m, 2H), 2.15−2.22 (m, 2H), 1.86−2.01 (m, 2H), 1.53−1.58 (m, 2H), 0.93 (s, 9H). $t_{R\ LCMS}$ 6.2 min. Purity >99%. MS $[M + H]^+$ m/z 348.

*(S)-2-Amino-3-methyl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-butan-1-one Hydrochloride (**34**).* *N*-Boc-L-valine (1.2 equiv), EDCI (1.2 equiv), HOBt (0.5 equiv), and triethylamine (4 equiv) were mixed in DCM (2 mL) for 5 min, and then 4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (150 mg, 1 equiv) was added with 3 mL of DCM. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure. The residue was purified by thick layer chromatography (DCM/MeOH 98/2) to give 147 mg of the expected product (yield = 61%). Boc intermediate was dissolved in dioxane (2 mL), and HCl 4N solution in dioxane (5 equiv) was added. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure. The residue was purified by thick layer chromatography (DCM/MeOH 9/1) to give (**34**) as a white powder. Yield 66%. ^{1}H NMR (MeOD) δ 7.78 (dd, J = 3.6 Hz J = 1.2 Hz, 1H), 7.68 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.20 (dd, J = 5.1 Hz, J = 3.6 Hz, 1H), 4.40−4.59 (m, 1H), 4.23−4.30 (m, 1H), 3.99−4.09 (m, 1H), 3.41−3.46 (m, 2H), 3.00−3.18 (m, 1H), 2.13−2.29 (m, 3H), 1.81−2.01 (m, 2H), 1.10 (d, J = 7.2 Hz, 3H), 1.02 (d, J = 6.9 Hz, 3H). $t_{R\ LCMS}$ 3.9 min. Purity 99%. MS $[M + H]^+$ m/z 335.

*(S)-2-Amino-4-methyl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-pentan-1-one Hydrochloride (**35**).* *N*-Boc-L-leucine (1.2 equiv), EDCI (1.2 equiv), HOBt (0.5 equiv), and triethylamine (4 equiv) were mixed in DCM (2 mL) for 5 min, and then 4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (150 mg, 1 equiv) was added with 3 mL of DCM. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure. The residue was purified by thick layer chromatography (DCM/MeOH 98/2) to give 162 mg of the expected product (yield = 65%). Boc intermediate was dissolved in dioxane (2 mL), and HCl 4N solution in dioxane (5 equiv) was added. The reaction mixture was

stirred overnight at room temperature and then evaporated under reduced pressure. The residue was purified by thick layer chromatography (DCM/MeOH 9/1) to give (**35**) as a white powder. Yield 73%. ^{1}H NMR (MeOD) δ 7.79 (dd, J = 3.6 Hz, J = 1.1 Hz, 1H), 7.69 (dd, J = 5.0 Hz, J = 1.1 Hz, 1H), 7.21 (dd, J = 5.0 Hz, J = 3.6 Hz, 1H), 4.38−4.57 (m, 2H), 3.87−3.96 (m, 1H), 3.39−3.49 (m, 2H), 3.00−3.20 (m, 1H), 2.22−2.33 (m, 2H), 1.61−1.99 (m, 5H), 1.06 (d, J = 6.4 Hz, 3H), 1.02 (d, J = 6.4 Hz, 3H). $t_{R\ LCMS}$ 4.0 min. Purity 99%. MS $[M + H]^+$ m/z 349.

2-Hydroxy-4-methyl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-pentan-1-one (***36***). Colorless oil; yield 40%. ^{1}H NMR (CD_2Cl_2) δ 7.80 (d, J = 3.7 Hz, 1H), 7.56 (dd, J = 5.0 Hz J = 1.2 Hz, 1H), 7.20 (dd, J = 5.0 Hz, J = 3.7 Hz, 1H), 4.40−4.56 (m, 2H), 3.74−3.80 (m, 1H), 3.62 (brs, OH), 3.19−3.36 (m, 2H), 2.97−3.15 (m, 1H), 2.17−2.27 (m, 2H), 1.87−2.03 (m, 2H), 1.27−1.50 (m, 2H), 1.02 (d, J = 6.8 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H). $t_{R\ LCMS}$ 5.7 min. Purity >99%. MS $[M + H]^+$ m/z 350.

2-Methoxy-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-ethanone (***37***). Colorless oil; yield 72%. ^{1}H NMR ($CDCl_3$) δ 7.72 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.45 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.09 (dd, J = 5.0 Hz, J = 3.7 Hz, 1H), 4.41 (m, 1H), 4.07 (s, 2H), 3.90 (m, 1H), 3.38 (s, 3H), 3.20 (m, 2H), 2.94 (m, 1H), 2.12 (m, 2H), 1.87 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 180.76, 167.54, 164.32, 129.60, 129.35, 128.17, 127.98, 71.87, 59.07, 44.08, 40.94, 34.22, 29.56, 28.89. $t_{R\ LCMS}$ 4.5 min. Purity 98%. MS $[M + H]^+$ m/z 308.

3-Methoxy-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-propan-1-one (***38***). Colorless oil; yield 86%. ^{1}H NMR ($CDCl_3$) δ 7.70 (dd, J = 3.7 Hz, J = 1.1 Hz, 1H), 7.43 (dd, J = 5.0 Hz, J = 1.1 Hz, 1H), 7.07 (dd, J = 5.0 Hz, J = 3.7 Hz, 1H), 4.44 (m, 1H), 3.89 (m, 1H), 3.63 (t, J = 6.4 Hz, 2H), 3.29 (s, 3H), 3.17 (m, 2H), 2.89 (m, 1H), 2.57 (t, J = 6.4 Hz, 2H), 2.08 (m, 2H), 1.85 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 180.85, 169.44, 164.28, 129.57, 129.32, 128.19, 127.96, 68.75, 58.86, 44.81, 40.72, 34.25, 33.46, 29.52, 28.87. $t_{R\ LCMS}$ 4.7 min. Purity 98%. MS $[M + H]^+$ m/z 322.

4-Oxo-4-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-butyric Acid (***39***). Beige powder; yield 86%. ^{1}H NMR (CDCl3) δ 7.77 (d, J = 3.6 Hz, 1H), 7.50 (d, J = 4.9 Hz, 1H), 7.15 (dd, J = 4.9 Hz, J = 3.6 Hz, 1H), 4.48 (m, 1H), 3.95 (m, 1H), 3.27 (m, 2H), 3.02 (m, 1H), 2.72 (m, 4H), 2.17 (m, 2H), 1.96 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 180.77, 176.86, 170.22, 164.40, 129.68, 129.39, 128.15, 128.02, 34.19, 29.48, 28.86, 27.98, 27.96. t_R LCMS 4.3 min. Purity 98%. MS $[M + H]+$ m/z 336.

2-Cyclopropyl-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-ethanone (***40***). Colorless oil; yield 78%. ^{1}H NMR ($CDCl_3$) δ 7.80 (dd, J = 3.9 Hz J = 1.2 Hz, 1H), 7.56 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.20 (dd, J = 5.1 Hz, J = 3.9 Hz, 1H), 4.55 (m, 1H), 3.93 (m, 1H), 3.27 (m, 2H), 2.94 (m, 1H), 2.27 (d, J = 7.2 Hz, 2H), 2.21 (m, 2H), 1.91 (m, 2H), 1.06 (m, 1H), 0.58 (m, 2H), 0.20 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 180.86, 171.07, 164.32, 129.58, 129.32, 128.21, 127.98, 44.79, 40.66, 38.54, 34.35, 29.64, 29.00, 7.31, 4.57. $t_{R\ LCMS}$ 5.3 min. Purity >99%. MS $[M + H]^+$ m/z 318.

2-Cyclopentyl-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-ethanone (***41***). White powder; yield 94%. ^{1}H NMR ($CDCl_3$) δ 7.70 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.43 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.08 (dd, J = 5.0 Hz, J = 3.6 Hz, 1H), 4.47 (m, 1H), 3.89 (m, 1H), 3.17 (m, 2H), 2.86 (m, 1H), 2.32 (d, J = 7.5 Hz, 2H), 2.17 (m, 1H), 2.09 (m, 2H), 1.80 (m, 4H), 1.54 (m, 4H), 1.11 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 180.96, 171.36, 164.34, 129.63, 129.37, 128.26, 128.02, 44.88, 40.70, 39.25, 36.79, 34.41, 32.75, 29.71, 29.06, 25.00. $t_{R\ LCMS}$ 6.4 min. Purity >99%. MS $[M + H]^+$ m/z 346.

2-Cyclohexyl-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-ethanone (***42***). Colorless oil; yield 98%. ^{1}H NMR ($CDCl_3$) δ 7.69 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.42 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.06 (dd, J = 5.0 Hz, J = 3.7 Hz, 1H), 4.44 (m, 1H), 3.87 (m, 1H), 3.17 (m, 2H), 2.88 (m, 1H), 2.17 (d, J = 6.7 Hz, 2H), 2.09 (m, 3H), 1.59−1.83 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 180.84, 171.29, 164.24, 129.60, 129.34, 128.07, 127.95, 45.06, 41.97, 40.67, 35.17, 34.25, 33.30, 32.95, 29.64, 28.96, 26.03. $t_{R\ LCMS}$ 6.7 min. Purity 98%. MS $[M + H]^+$ m/z 360.

2-(4-Methyl-cyclohexyl)-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-ethanone (***43***). Colorless oil; yield 98%. ^{1}H NMR ($CDCl_3$) δ 7.70 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.42 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.07 (dd, J = 5.1 Hz, J = 3.7 Hz, 1H), 4.45 (m, 1H), 3.88 (m, 1H), 3.17 (m, 2H), 2.86 (m, 1H), 2.26 (d, J = 7.2 Hz, 2H), 2.17−1.18 (m, 17H). ^{13}C NMR ($CDCl_3$) δ 180.88, 171.14, 164.33, 129.59, 129.32, 128.21, 127.98, 44.98, 40.67, 37.97, 34.97, 34.40, 33.34, 32.67, 30.77, 28.74, 22.60, 20.06. $t_{R\ LCMS}$ 7.3 min. Purity 98%. MS $[M + H]^+$ m/z 374.

3-Cyclohexyl-1-[4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidin-1-yl]-propan-1-one (***44***). Colorless oil; yield 98%. ^{1}H NMR ($CDCl_3$) δ 7.71 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.44 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.08 (dd, J = 5.0 Hz, J = 3.7 Hz, 1H), 4.46 (m, 1H), 3.87 (m, 1H), 3.18 (m, 2H), 2.86 (m, 1H), 2.35−1.45 (m, 19H). ^{13}C NMR ($CDCl_3$) δ 180.87, 172.12, 164.28, 129.58, 129.32, 128.17, 121.97, 44.75, 40.71, 37.44, 34.33, 33.10, 32.80, 30.90, 29.63, 28.94, 26.52, 26.22. $t_{R\ LCMS}$ 7.4 min. Purity 98%. MS $[M + H]^+$ m/z 374.

3,3,3-Trifluoro-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-propan-1-one (***45***). White powder; yield 47%. ^{1}H NMR (MeOD) δ 7.79 (dd, J = 3.6 Hz J = 1.2 Hz, 1H), 7.67 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.20 (dd, J = 5.0 Hz, J = 3.6 Hz, 1H), 4.45−4.51 (m, 1H), 3.96−4.04 (m, 1H), 3.55 (q, J = 10.5 Hz, 2H), 3.35−3.42 (m, 2H), 3.01−3.10 (m, 1H), 2.15−2.25 (m, 2H), 1.78−2.00(m, 2H). $t_{R\ LCMS}$ 5.6 min. Purity 99%. MS $[M + H]^+$ m/z 346.

3,3,3-Trifluoro-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-butan-1-one (***46***). White powder; yield 76%. ^{1}H NMR ($CDCl_3$) δ 7.74 (dd, J = 3.7 Hz, J = 1.1 Hz, 1H), 7.47 (dd, J = 5.0 Hz, J = 1.1 Hz, 1H), 7.11 (dd, J = 5.0 Hz, J = 3.7 Hz, 1H), 4.45 (m, 1H), 3.86 (m, 1H), 3.23 (m, 2H), 2.96 (m, 1H), 2.52 (m, 4H), 2.13 (m, 2H), 1.88 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 180.66, 168.07, 164.34, 129.63, 129.36, 128.15, 128.00, 127.06 (q, J = 275 Hz), 44.38, 40.98, 34.15, 29.56 (q, J = 29 Hz), 29.38, 28.81, 25.87. $t_{R\ LCMS}$ 5.9 min. Purity 99%. MS $[M + H]^+$ m/z 360.

4,4,4-Trifluoro-3-hydroxy-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-butan-1-one (***47***). White powder; yield 27%. ^{1}H NMR (CD_2Cl_2) δ 7.80 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.56 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.20 (dd, J = 5.1 Hz, J = 3.6 Hz, 1H), 4.86 (brs, OH), 4.45−4.56 (m, 2H), 3.86−3.94 (m, 1H), 3.26−3.35 (m, 2H), 2.97−3.09 (m, 1H), 2.64−2.79 (m, 2H), 2.18−2.26 (m, 2H), 1.83−2.04 (m, 2H). $t_{R\ LCMS}$ 5.3 min. Purity >99%. MS $[M + H]^+$ m/z 376.

4,4,4-Trifluoro-2-methyl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-butan-1-one (***48***). Colorless oil; yield 70%. ^{1}H NMR (CD_2Cl_2) δ 7.80 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.56 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.19 (dd, J = 5.1 Hz, J = 3.7 Hz, 1H), 4.45−4.58 (m, 1H), 3.97−4.05 (m, 1H), 3.25−3.39 (m, 2H), 3.06−3.18 (m, 1H), 2.89−3.05 (m, 1H), 2.73−2.86 (m, 1H), 2.13−2.27 (m, 3H), 1.81−1.97 (m, 2H), 1.22−1.25 (m, 3H). $t_{R\ LCMS}$ 6.1 min. Purity >99%. MS $[M + H]^+$ m/z 374.

4,4,4-Trifluoro-3-methyl-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-butan-1-one (***49***). Colorless oil; yield 70%. ^{1}H NMR ($CDCl_3$) δ 7.80 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.53 (dd, J = 5.1 Hz, J = 1.2 Hz, 1H), 7.17 (dd, J = 5.1 Hz, J = 3.7 Hz, 1H), 4.47−4.59 (m, 1H), 3.91−3.97 (m, 1H), 3.25−3.35 (m, 2H), 2.90−3.09 (m, 2H), 2.67−2.74 (m, 1H), 2.30−2.39 (m, 1H), 2.16−2.23 (m, 2H), 1.85−2.03 (m, 2H), 1.20 (d, J = 7.0 Hz, 3H). $t_{R\ LCMS}$ 6.4 min. Purity >99%; MS $[M + H]^+$ m/z 374.

4,5,5,5-Tetrafluoro-1-[4-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-4-trifluoromethyl-pentan-1-one (***50***). White powder; yield 84%. ^{1}H NMR (CD_2Cl_2) δ 7.80 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.56 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.19 (dd, J = 5.0 Hz, J = 3.7 Hz, 1H), 4.49−4.54 (m, 1H), 3.89−3.93 (m, 1H), 3.24−3.34 (m, 2H),

2.94−3.02 (m, 1H), 2.50−2.66 (m, 4H), 2.17−2.24 (m, 2H), 1.81−2.00 (m, 2H). $t_{R\ LCMS}$ 7.2 min. Purity >99%. MS $[M + H]^+$ m/z 460.

*4-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-1-(3,3,3-trifluoro-propane-1-sulfonyl)-piperidine (**51**).* 4-(3-Thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (0.5 mmol, 1 equiv) and triethylamine (3 equiv) were mixed in dried DCM (5 mL). 3,3,3-Trifluoropropane-1-sulfonyl chloride (1.3 equiv) was added dropwise, and the reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure and purified by preparative HPLC. White powder; yield 70%. ^{1}H NMR (CD_2Cl_2) δ 7.81 (dd, J = 3.7 Hz, J = 1.2 Hz, 1H), 7.57 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.20 (dd, J = 5.0 Hz, J = 3.7 Hz, 1H), 3.78−3.86 (m, 2H), 3.08−3.26 (m, 5H), 2.60−2.76 (m, 2H), 2.25−2.31 (m, 2H), 2.00−2.13 (m, 2H). $t_{R\ LCMS}$ 6.5 min. Purity >99%. MS $[M + H]^+$ m/z 396.

*4-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidine-1-carboxylic Acid (2,2,2-Trifluoro-ethyl)-amide (**52**).* Carbonyldiimidazole (1 equiv) was added to a solution of 2,2,2-trifluoroethylamine hydrochloride (1 equiv) in THF (4 mL) and triethylamine (1 equiv). The reaction mixture was stirred 5 h at room temperature, and then 4-(3-thiophen-2-yl-[1,2,4]oxadiazol-5-yl)-piperidine hydrochloride (0.5 mmol, 1 equiv) was added with 4 mL of THF. The mixture was stirred overnight at room temperature and then evaporated under reduced pressure and purified by preparative HPLC. White powder; yield 5%. ^{1}H NMR (CD_2Cl_2) δ 7.80 (d, J = 3.6 Hz, 1H), 7.57 (d, J = 5.0 Hz, 1H), 7.21 (dd, J = 5.0 Hz J = 3.6 Hz, 1H), 4.93 (NH), 3.91−4.04 (m, 4H), 3.21−3.30 (m, 1H), 3.07−3.16 (m, 2H), 2.17−2.22 (m, 2H), 1.87−2.00 (m, 2H). $t_{R\ LCMS}$ 5.1 min. Purity 99%. MS $[M + H]^+$ m/z 361.

*4,4,4-Trifluoro-1-[(R)-3-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-butan-1-one (**53**).* (*R*)-Boc-nipecotic acid (4.6 mmol, 1 equiv), HBTU (1.1 equiv), and diisopropylethylamine (3 equiv) were dissolved in dimethylformamide (15 mL). The solution was stirred for 5 min, and then thiophene-2-amidoxime (1.1 equiv) was added. The reaction mixture was stirred overnight at room temperature and then heated at 120 °C for 6 h. The solvent was removed under vacuum, and the residue was dissolved in AcOEt. The organic layer was washed once with HCl 1N, once with saturated aqueous $NaHCO_3$, and once with brine, then dried over $MgSO_4$ and evaporated under reduced pressure. The obtained product was used in the next step without further purification. Boc intermediate was dissolved in dioxane (10 mL), and HCl 4N solution in dioxane (5 equiv) was added. The reaction mixture was stirred overnight at room temperature. The product was recovered by filtration and then washed with petroleum ether to give 990 mg of (*R*)-3-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidine hydrochloride as a beige powder (yield 79% over two steps). 4,4,4-Trifluorobutyric acid (1.3 equiv), EDCI (1.3 equiv), HOBt (0.4 equiv), and diisopropylethylamine (4 equiv) were mixed in DMF (2 mL) for 5 min. (*R*)-3-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidine hydrochloride (217 mg, 0.8 mmol, 1 equiv) was added with 3 mL of DMF. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure and purified by preparative HPLC to give **53** as a colorless oil; yield 56%. ^{1}H NMR ($CDCl_3$) cis/trans isomer mixture (53% isomer A/47% isomer B); isomer A δ 7.78 (d, J = 3.6 Hz, 1H), 7.50−7.53 (m, 1H), 7.14−7.18 (m, 1H), 3.79−3.90 (m, 3H), 3.44−3.53 (m, 1H), 3.15−3.25 (m, 1H), 2.84−2.95 (m, 1H), 2.49−2.70 (m, 3H), 2.13−2.23 (m, 2H), 1.61−1.77 (m, 2H); isomer B δ 7.78 (d, J = 3.6 Hz, 1H), 7.50−7.53 (m, 1H), 7.14−7.18 (m, 1H), 4.75−4.85 (m, 1H), 3.79−3.85 (m, 1H), 3.15−3.25 (m, 3H), 2.49−2.70 (m, 4H), 2.28−2.36 (m, 1H), 1.91−2.00 (m, 2H), 1.61−1.77 (m, 1H). ^{13}C NMR ($CDCl_3$) cis/trans isomer mixture (53% isomer A/47% isomer B); isomer A δ 179.07, 168.49, 164.40, 129.69, 129.55, 128.06, 127.95, 127.11 (q, J = 275 Hz), 47.41, 42.11, 34.87, 29.61 (q, J = 29 Hz), 27.96, 25.97, 23.15; isomer B δ 179.34, 168.32, 164.34, 129.64, 129.34, 128.22, 127.99, 127.05 (q, J = 275 Hz), 45.63, 44.42, 34.51, 29.61 (q, J = 29 Hz), 28.52, 25.97, 24.72. $t_{R\ LCMS}$ 5.7 min. Purity >99%. MS $[M + H]^+$ m/z 360.

*4,4,4-Trifluoro-1-[(S)-3-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-piperidin-1-yl]-butan-1-one (**54**).* Using the above procedure for **53** with (*S*)-Boc-nipecotic acid provided **54** as a colorless oil; yield 62%. ^{1}H NMR ($CDCl_3$) cis/trans isomer mixture (53% isomer A/47% isomer B); isomer A δ 7.78 (d, J = 3.6 Hz, 1H), 7.50−7.53 (m, 1H), 7.14−7.18 (m, 1H), 3.79−3.90 (m, 3H), 3.44−3.53 (m, 1H), 3.15−3.25 (m, 1H), 2.84−2.95 (m, 1H), 2.49−2.70 (m, 3H), 2.13−2.23 (m, 2H), 1.61−1.77 (m, 2H); isomer B δ 7.78 (d, J = 3.6 Hz, 1H), 7.50−7.53 (m, 1H), 7.14−7.18 (m, 1H), 4.75−4.85 (m, 1H), 3.79−3.85 (m, 1H), 3.15−3.25 (m, 3H), 2.49−2.70 (m, 4H), 2.28−2.36 (m, 1H), 1.91−2.00 (m, 2H), 1.61−1.77 (m, 1H). ^{13}C NMR ($CDCl_3$) cis/trans isomer mixture (53% isomer A/47% isomer B); isomer A δ 179.06, 168.49, 164.41, 129.70, 129.56, 128.07, 127.95, 127.12 (q, J = 275 Hz), 47.41, 42.11, 34.87, 29.61 (q, J = 29 Hz), 27.96, 25.97, 23.15; isomer B δ 179.34, 168.31, 164.34, 129.64, 129.34, 128.22, 127.99, 127.05 (q, J = 275 Hz), 45.63, 44.42, 34.51, 29.61 (q, J = 29 Hz), 28.53, 25.97, 24.72. $t_{R\ LCMS}$ 5.7 min. Purity >99%. MS $[M + H]^+$ m/z 360.

*4,4,4-Trifluoro-1-[(R)-3-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-pyrrolidin-1-yl]-butan-1-one (**55**).* (*R*)-*N*-Boc-pyrrolidine-3-carboxylic acid (4.65 mmol, 1 equiv), HBTU (1.1 equiv), and diisopropylethylamine (2.5 equiv) were dissolved in dimethylformamide (10 mL). The solution was stirred 5 min, and then thiophene-2-amidoxime (1 equiv) was added. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure. The residue was dissolved in AcOEt and washed twice with saturated aqueous $NaHCO_3$ and once with brine and then dried over $MgSO_4$ and evaporated under reduced pressure. The residue was dissolved in 10 mL of DMF and then heated at 120 °C for 9 h. The solvent was removed under vacuum, and the residue was dissolved in AcOEt. The organic layer was washed once with HCl 1N, once with saturated aqueous $NaHCO_3$, and once with brine, then dried over $MgSO_4$ and evaporated under reduced pressure. The obtained product was used in the next step without further purification. Boc intermediate was dissolved in dioxane (5 mL), and HCl 4N solution in dioxane (5 equiv) was added. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure. The residue was dissolved in water and then washed once with diethyl ether. The pH of the aqueous phase was adjusted to 8 with saturated aqueous K_2CO_3, and then the product was extracted 3 times with AcOEt. The organic phases were joined, washed once with brine, then dried over $MgSO_4$ and evaporated under reduced pressure to give 5-(*R*)-pyrrolidin-3-yl-3-thiophen-2-yl-1,2,4-oxadiazole (yield 74% over two steps). 4,4,4-Trifluorobutyric acid (1.3 equiv), EDCI (1.3 equiv), HOBt (0.4 equiv), and diisopropylethylamine (4 equiv) were mixed in DMF (2 mL) for 5 min. 5-(*R*)-Pyrrolidin-3-yl-3-thiophen-2-yl-1,2,4-oxadiazole (0.9 mmol, 1 equiv) was added with 3 mL of DMF. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure. The residue was dissolved in AcOEt, and the organic layer was washed with aqueous saturated $NaHCO_3$ (3 times), aqueous HCl 1 M (3 times) and brine (once), dried over $MgSO_4$, and concentrated under vacuum. The residue was recrystallized in diisopropyl ether and then in absolute ethanol to give **55** as white crystals; yield 62%. ^{1}H NMR ($CDCl_3$) δ 7.77−7.79 (m, 1H), 7.50−7.53 (m, 1H), 7.14−7.17 (m, 1H), 3.91−4.04 (m, 2H), 3.71−3.85 (m, 2H), 3.59−3.68 (m, 1H), 2.33−2.60 (m, 6H). ^{13}C NMR ($CDCl_3$) cis/trans isomer mixture (55% isomer A/45% isomer B); isomer A δ 179.10, 168.39, 164.55, 129.81, 129.53, 128.04, 127.95, 127.00 (q, J = 275 Hz), 49.31, 45.57, 35.04, 29.17 (q, J = 30 Hz), 28.96, 27.17; isomer B δ 178.63, 168.21, 164.55, 129.85, 129.62, 128.07, 127.82, 127.00 (q, J = 275 Hz), 49.54, 45.22, 36.85, 30.45, 29.17 (q, J = 30 Hz), 27.35. Melting point 111.8−112.4 °C; $t_{R\ LCMS}$ 5.6 min. Purity >99%. MS $[M + H]^+$ m/z 346.

*4,4,4-Trifluoro-1-[(S)-3-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-pyrrolidin-1-yl]-butan-1-one (**56**).* Using the above procedure for **55** with (*S*)-*N*-Boc-pyrrolidine-3-carboxylic acid provided **56** as a white powder after purification by preparative HPLC; yield 51%. ^{1}H NMR (CD_2Cl_2) δ 7.80−7.81 (m, 1H), 7.56−7.59 (m, 1H), 7.18−7.22 (m, 1H), 3.66−3.99 (m, 4H), 3.53−3.63 (m, 1H), 2.32−2.64 (m, 6H). ^{13}C NMR (CD_2Cl_2) cis/trans isomer mixture (55% isomer A/45% isomer B); isomer A δ 180.03, 168.63, 164.99, 130.19, 129.96, 128.70, 128.58, 127.81 (q, J = 275 Hz), 49.73, 46.09, 35.62, 29.57 (q, J = 30 Hz), 29.41, 27.53; isomer B δ 179.58, 168.40, 164.99, 130.22, 130.03, 128.70, 128.60, 127.81 (q, J = 275 Hz), 50.02, 45.62, 37.40, 30.97, 29.57 (q, J = 30 Hz), 27.70. $t_{R\ LCMS}$ 5.6 min. Purity >99%. MS $[M + H]^+$ m/z 346.

*4,4,4-Trifluoro-1-[3-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-azetidin-1-yl]-butan-1-one (**57**).* 1-Boc-azetidine-3-carboxylic acid (8.5 mmol, 1 equiv), HBTU (1.1 equiv), and diisopropylethylamine (2.5 equiv) were dissolved in dimethylformamide (15 mL). The solution was stirred for 5 min, and then thiophene-2-amidoxime (1 equiv) was added. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure. The residue was dissolved in AcOEt and washed twice with saturated aqueous $NaHCO_3$ and once with brine and then dried over $MgSO_4$ and evaporated under reduced pressure. The residue was dissolved in 15 mL of DMF and then heated at 120 °C for 3 h. The solvent was removed under vacuum, and the residue was dissolved in AcOEt. The organic layer was washed once with HCl 1N, twice with saturated aqueous $NaHCO_3$, and once with brine, then dried over $MgSO_4$ and evaporated under reduced pressure (yield 67%). The obtained product (5.5 mmol) was dissolved in dioxane (5 mL), and HCl 4N solution in dioxane (5 equiv) was added. The reaction mixture was stirred overnight at room temperature. The product was recovered by filtration and then washed with petroleum ether to give 1.23 g of 5-azetidin-3-yl-3-thiophen-2-yl-1,2,4-oxadiazole hydrochloride as a white powder (yield 91%). 4,4,4-Trifluorobutyric acid (1.3 equiv), EDCI (1.3 equiv), HOBt (0.4 equiv), and diisopropylethylamine (4 equiv) were mixed in DMF (2 mL) for 5 min. 5-Azetidin-3-yl-3-thiophen-2-yl-1,2,4-oxadiazole hydrochloride (0.5 mmol, 1 equiv) was added with 3 mL of DMF. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure and purified by preparative HPLC to give **57** as a white powder; yield 51%. ^{1}H NMR (CD_2Cl_2) δ 7.83 (dd, J = 3.6 Hz, J = 1.2 Hz, 1H), 7.59 (dd, J = 5.0 Hz, J = 1.2 Hz, 1H), 7.21 (dd, J = 5.0 Hz, J = 3.6 Hz, 1H), 4.32−4.64 (m, 4H), 4.12−4.23 (m, 1H), 2.37−2.60 (m, 4H). ^{13}C NMR (CD_2Cl_2) δ 178.74, 169.59, 164.69, 129.81, 129.61, 128.12, 127.93, 127.10 (q, J = 275 Hz), 53.20, 52.11, 28.69 (q, J = 29 Hz), 25.66, 23.90. $t_{R\ LCMS}$ 5.6 min. Purity >99%. MS $[M + H]^+$ m/z 332.

*4,4,4-Trifluoro-N-[3-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-propyl]-butyramide (**58**).* Boc-4-amino-butyric acid (10 mmol, 1 equiv), HBTU (1 equiv), and diisopropylethylamine (2.5 equiv) were dissolved in dimethylformamide (20 mL). The solution was stirred for 5 min, and then thiophene-2-amidoxime (1 equiv) was added. The reaction mixture was stirred overnight at room temperature and then poured in 50 mL of water. The reaction mixture produced a thick crystalline slurry. The product was recovered by filtration and washed with water. The solid obtained was dissolved in dimethylformamide (20 mL), and then the reaction mixture was heated at 120 °C for 4 h. The solvent was removed under vacuum, and the residue was dissolved in AcOEt. The organic layer was washed once with HCl 1N, once with saturated aqueous $NaHCO_3$, and once with brine, then dried over $MgSO_4$ and evaporated under reduced pressure (yield 90%). The obtained product (9 mmol) was dissolved in dioxane (20 mL), and HCl 4N solution in dioxane (5 equiv) was added. The reaction mixture was stirred overnight at room temperature. The product was recovered by filtration and then washed with petroleum ether to give 1.79 g of 3-(3-thiophen-2-yl-1,2,4-oxadiazol-5-yl)-propylamine hydrochloride as a white powder (yield 81%). 4,4,4-Trifluorobutyric acid (1.3 equiv), EDCI (1.3 equiv), HOBt (0.4 equiv), and diisopropylethylamine (4 equiv) were mixed in DMF (2 mL) for 5 min. 3-(3-Thiophen-2-yl-1,2,4-oxadiazol-5-yl)-propylamine hydrochloride (0.5 mmol, 1 equiv) was added with 3 mL of DMF. The reaction mixture was stirred overnight at room temperature and then evaporated under reduced pressure and purified by preparative HPLC to give **58** as a white powder; yield 54%. ^{1}H NMR (CD_2Cl_2) δ 7.74 (d, J = 3.6 Hz, 1H), 7.51 (d, J = 5.0 Hz, 1H), 7.15 (dd, J = 5.0 Hz, J = 3.6 Hz, 1H), 5.92 (brs, NH), 3.37 (q, J = 6.4 Hz, 2H), 2.95 (t, J = 7.2 Hz, 2H), 2.35−2.52 (m, 4H), 2.05 (quintuplet, J = 7.2 Hz, 2H). ^{13}C NMR (CD_2Cl_2) δ 180.48, 170.77, 165.38, 130.41, 130.29, 129.41, 129.09, 128.11 (q, J = 275 Hz), 39.80, 30.45 (q, J = 29 Hz), 29.60, 27.32, 25.13. $t_{R\ LCMS}$ 5.2 min. Purity >99%. MS $[M + H]^+$ m/z 334.

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ABBREVIATIONS USED

ACM, automated confocal microscopy; AcOEt, ethyl acetate; AcOH, acetic acid; Boc, *t*-butoxycarbonyl; CDI, *N*,*N*-carbonyldiimidazole; CH_3CN, acetonitrile; DCM, dichloromethane; DIEA, diisopropylethylamine; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DOTS, directly observed treatment short-course; EDCI, *N*-ethyl-3-(3-dimethyaminopropyl)-carbodiimide; ETH, ethionamide; Et_3N, triethylamine; EtOH, ethanol; GFP, green fluorescent protein; HBTU, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; MDR-TB, multidrug resistant tuberculosis; MeOH, methanol; MIC, minimal inhibitory concentration; PBS, phosphate buffered saline; PK, pharmacokinetic; RT, room temperature; SAR, structure−activity relationships; SPR, surface plasmon resonance; TB, tuberculosis; TFA, trifluoroacetic acid; THF, tetrahydrofuran

REFERENCES

(1) World Health Organization. *Global Tuberculosis Control*; World Health Organization: Geneva, 2010; ISBN 978 92 4 156406 9.

(2) Elzinga, G.; Raviglione, M. C.; Maher, D. Scale up: meeting targets in global tuberculosis control. *Lancet* **2004**, *363*, 814–819.

(3) Dye, C.; Maher, D.; Weil, D.; Espinal, M.; Raviglione, M. Targets for global tuberculosis control [Short Communication]. *Int. J. Tuberculosis Lung Dis.* **2006**, *10*, 460–462.

(4) Cox, H. S.; Ford, N.; Reeder, J. C. Are we really that good at treating tuberculosis?. *Lancet Infect. Dis.* **2009**, *9*, 138–139.

(5) Chaisson, R. E.; Martinson, N. A. Tuberculosis in Africa-Combating an HIV-Driven Crisis. *New England Journal of Medicine* **2008**, *358*, 1089–1092.

(6) *Global Tuberculosis Control: Epidemiology, Strategy, Financing*; World Health Organization: Geneva, 2009; publication no. WHO/HTM/TB/2009.411.

(7) Cox, H.; Kebede, Y.; Allamuratova, S.; Ismailov, G.; Davletmuratova, Z.; Byrnes, G.; Stone, C.; Niemann, S.; Rüsch-Gerdes, S.; Blok, L.; Doshetov, D. Tuberculosis Recurrence and Mortality after Successful Treatment: Impact of Drug Resistance. *PLoS Med.* **2006**, *3*, 1836–1843.

(8) WHO/HTM/TB. *Management of MDR-TB: a Field Guide: A Companion Document to Guidelines for Programmatic Management of Drug-Resistant Tuberculosis: Integrated Management of Adolescent and Adult Illness (IMAI)*; World Health Organization: Geneva, 2008; WHO/HTM/TB/2008.402a.

(9) (a) Schwartz, W. S. Comparison of ethionamide with isoniazid in original treatment cases of pulmonary tuberculosis. XIV. A report of the Veterans Administration−Armed Forces cooperative study. *Am. Rev. Respir. Dis.* **1966**, *93*, 685–692.(b) Ethionamide. *Tuberculosis* **2008**, *88*, 106−108

(10) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P, Eds.; Pergamon Press: New York, 1990; pp 1154−1155.

(11) *Martindale, the Extra Pharmacopoeia*, 29th ed.; Reynolds, J. E. F., Ed.; Pharmaceutical Press: London, 1989; pp 562−563.

(12) Fraaije, M. W.; Kamerbeek, N. M.; Heidekamp, A. J.; Fortin, R.; Janssen, D. B. The prodrug activator EtaA from *Mycobacterium tuberculosis* is a Baeyer−Villiger monooxygenase. *J. Biol. Chem.* **2004**, *279*, 3354–3360.

(13) Vannelli, T. A.; Dykman, A.; Ortiz De Montellano, P. R. The antituberculosis drug ethionamide is activated by a flavoprotein monooxygenase. *J. Biol. Chem.* **2002**, *277*, 12824–12829.

(14) Wang, F.; Langley, R.; Gulten, G.; Dover, L. G.; Besra, G. S.; Jacobs, W. R., Jr.; Sacchettini, J. C. Mechanism of thioamide drug action against tuberculosis and leprosy. *J. Exp. Med.* **2007**, *204*, 73–78.

(15) Engohang-Ndong, J.; Baillat, D.; Aumercier, M.; Bellefontaine, F.; Besra, G. S.; Locht, C.; Baulard, A. R. EthR, a repressor of the TetR/CamR family implicated in ethionamide resistance in mycobacteria, octamerizes cooperatively on its operator. *Mol. Microbiol.* **2004**, *51*, 175–188.

(16) DeBarber, A. E.; Mdluli, K.; Bosman, M.; Bekker, L. G.; Barry, C. E., III. Ethionamide activation and sensitivity in multidrug-resistant *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9677–9682.

(17) Baulard, A. R.; Betts, J. C.; Engohang-Ndong, J.; Quan, S.; McAdam, R. A.; Brennan, P. J.; Locht, C.; Besra, G. S. Activation of the Pro-drug Ethionamide Is Regulated in Mycobacteria. *J. Biol. Chem.* **2000**, *275*, 28326–28331.

(18) Frenois, F.; Baulard, A. R.; Villeret, V. Insights into mechanisms of induction and ligands recognition in the transcriptional repressor EthR from *Mycobacterium tuberculosis*. *Tuberculosis* **2006**, *86*, 110–114.

(19) Frenois, F.; Engohang-Ndong, J.; Locht, C.; Baulard, A. R.; Villeret, V. Structure of EthR in a ligand bound conformation reveals therapeutic perspectives against tuberculosis. *Mol. Cell* **2004**, *16*, 301–307.

(20) Dover, L. G.; Corsino, P. E.; Daniels, I. R.; Cocklin, S. L.; Tatituri, V.; Besra, G. S.; Futterer, K. Crystal structure of the TetR/CamR family repressor *Mycobacterium tuberculosis* EthR implicated in ethionamide resistance. *J. Mol. Biol.* **2004**, *340*, 1095–1105.

(21) Willand, N.; Dirie, B.; Carette, X.; Bifani, P.; Singhal, A.; Desroses, M.; Leroux, F.; Willery, E.; Mathys, V.; Deprez-Poulain, R.; Delcroix, G.; Frenois, F.; Aumercier, M.; Locht, C.; Villeret, V.; Deprez, B.; Baulard, A. R. Synthetic EthR inhibitors boost antituberculous activity of ethionamide. *Nature Med.* **2009**, *15*, 537–544.

(22) Poulain, R. F.; Tartar, A. L.; Deprez, B. P. Parallel synthesis of 1,2,4-oxadiazoles from carboxylic acids using an improved, uronium-based, activation. *Tetrahedron Lett.* **2001**, *42*, 1495–1498.

(23) Nguyen, L.; Thompson, C. J. Foundations of antibiotic resistance in bacterial physiology: the mycobacterial paradigm. *Trends Microbiol.* **2006**, *14*, 304–312.

(24) Christophe, T.; Jackson, M.; Jeon, H. K.; Fenistein, D.; Contreras-Dominguez, M.; Kim, J.; Genovesio, A.; Carralot, J.-P.; Ewann, F.; Kim, E. H.; Lee, S. Y.; Kang, S.; Seo, M. J.; Park, E. J.; Skovierova, H., H.; Pham, H.; Riccardi, G.; Nam, J. Y.; Marsollier, L.; Kempf, M.; Joly-Guillou, M.-L.; Oh, T.; Shin, W. K.; No, Z.; Nehrbass, U.; Brosch, R.; Cole, S. T.; Brodin, P. High Content Screening Identifies Decaprenyl-Phosphoribose 2′ Epimerase as a Target for Intracellular Antimycobacterial Inhibitors. *PLoS Pathog.* **2009**, *5*, e1000645.

(25) Christophe, T.; Ewann, F.; Jeon, H. K.; Cechetto, J.; Brodin, P. High-content imaging of *Mycobacterium tuberculosis*-infected macrophages: an in vitro model for tuberculosis drug discovery. *Future Medicinal Chemistry* **2010**, *2*, 1283–1293.

(26) Ohkata, K.; Ohsugi, M.; Yamamoto, K.; Ohsawa, M.; Akiba, K.-y. Structural Features of Tetraazathiapentalenes Fused with Pyrimidine and/or Pyridine Rings. Experimental Evaluation of the Nature of Hypervalent N−S−N Bond by Restricted Internal Rotation of the Pyrimidine Ring. *J. Am. Chem. Soc.* **1996**, *118*, 6355–6369.

(27) Willand, N.; Desroses, M.; Toto, P.; Dirie, B.; Lens, Z.; Villeret, V.; Rucktooa, P.; Locht, C.; Baulard, A.; Deprez, B. Exploring Drug Target Flexibility Using in Situ Click Chemistry: Application to a Mycobacterial Transcriptional Regulator. *ACS Chem. Biol.* **2010**, *5*, 1007–1013.

(28) Rentmeister, A.; Arnold, F. H.; Fasan, R. Chemo-enzymatic fluorination of unactivated organic compounds. *Nature Chem. Biol.* **2009**, *5*, 26–28.

(29) Domagala, J. M.; Hanna, L. D.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Solomon, M. New structure−activity relationships of the quinolone antibacterials using the target enzyme. The development and application of a DNA gyrase assay. *J. Med. Chem.* **1986**, *29*, 394–404.

(30) Müller, K.; Faeh, C.; Diederich, F. Fluorine in Pharmaceuticals: Looking Beyond Intuition. *Science* **2007**, *317*, 1881–1886.

(31) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **2001**, *46*, 3–26.

(32) Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. *J. Med. Chem.* **2002**, *45*, 2615–2623.

(33) Kabsch, W. Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. *J. Appl. Crystallogr.* **1993**, *26*, 795–800.

(34) Winn, M. D.; Isupov, M. N.; Murshudov, G. N. Use of TLS parameters to model anisotropic displacements in macromolecular refinement. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2001**, *57*, 122–133.

(35) Collaborative-Computational-Project-Number-4. The CCP4 suite: programs for protein crystallography. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1994**, *50*, 760−763.

(36) Schuettelkopf, A. W.; van Aalten, D. M. F. PRODRG—a tool for high-throughput crystallography of protein−ligand complexes. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2004**, *60*, 1355–1363.

(37) Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. Features and development of Coot. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2010**, *66*, 486–501.