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Article

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Development of Novel Alkoxyisoxazoles as Sigma-1 Receptor Antagonists with Anti-Nociceptive Efficacy

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ABSTRACT

A novel series of sigma (σ) receptor ligands based on an alkoxyisoxazole scaffold has been designed and synthesized. Preliminary receptor binding assays identified highly potent (K_i < 1 nM) and selective σ1 ligands devoid of binding interactions with monoamine transporters DAT, NET and SERT. In particular, compound **53** was shown to possess significant anti-nociceptive activity in the mouse formalin-induced inflammation pain model when administered intraperitoneally at 40 and 80 mg/kg. Initial pharmacokinetics evaluation indicated an excellent brain exposure following oral dosing in mice, suggesting that further investigation into the use of alkoxyisoxazoles as σ1 ligands for anti-nociception is

warranted. This study supports the notion that selective σ1 antagonism could be a useful strategy in the development of novel anti-pain therapy.

INTRODUCTION

Sigma (σ) receptors belong to a unique class of receptors that is distinct from the opioid and *N*-methyl-D-aspartate/phencyclidine (NMDA/PCP) receptors. Initially classified as an opioid receptor subtype by Martin in 1976[1] and subsequent confusion with the PCP binding site of NMDA receptor, σ receptors are nowadays referred to as central nervous system (CNS) proteins with no homology to known G-protein coupled receptors but are capable of influencing opioid actions. Two types of σ receptors have been identified, namely σ1 (25–29 kDa) and σ2 (18–22 kDa), both of which have been implicated in the pathophysiology of various neurological disorders[2] and cancer[3], respectively.

The cloned σ1 receptor[4] encodes for a protein of 223 amino acid with two transmembrane domains located at the mitochondrial-associated endoplasmic reticulum membrane (MAM)[5] and regulates endoplasmic reticulum (ER)–mitochondrion calcium signaling and cell survival.[6] Sigma 1 receptor ligands trigger the translocation of σ1 receptor from MAM to other parts of the cell thereby modulating the activities of various ion channels, receptors, or kinases, thus affecting each stage of neuronal transmission. Su et al. reported that σ1 receptors modulate inositol 1,4,5-trisphosphate (IP_3) function by forming a trimeric complex with IP_3 receptor and its inhibitory protein ankyrin B.[7] Acute binding of σ1 receptor agonist was found to remove ankyrin B from IP_3 receptor thereby enhancing the calcium release from ER into the cytosol in NG-108 cells. Moreover, σ1 receptor agonists are likely to exert their effects by dissociating σ1 receptor chaperones from binding-immunoglobulin protein (BiP).[8] This is in contrast to the σ1 receptor antagonists which do not affect the σ1 receptor-BiP association and rather stabilize this complex. The σ2 receptor on the other hand has not yet been cloned but is known to be overexpressed in aggressive tumor cells. Unlike the σ1 receptor that readily translocates upon activation, σ2 receptor is localized within the progesterone receptor membrane component 1 (PGRMC1) protein known to be associated with cell proliferation.[3] The σ2 receptor is regarded as a reliable biomarker in oncology as proliferating cancer cells are found to have a 10-fold higher density of σ2 receptor compared to the

dormant cancer cells.[3] The progress towards understanding of the precise function of σ receptors however, has been hampered partly due to the lack of conclusive evidence on certain endogenous ligand(s) that selectively bind to these receptors. *N*,*N*-Dimethyltryptamine (DMT) was suggested to be a low potency endogenous ligand for the σ1 and σ2 receptors.[9] Other endogenous σ1 ligands such as D-erythro-sphingosine and sphinganine have also been studied. (+)-Pentazocine (**1**) ($K_{i,\ \sigma1}$ = 3.1 nM, $K_{i,\ \sigma2}$ = 1542 nM) and DTG (**2**, 1,3-di-*o*-tolylguanidine, $K_{i,\ \sigma1}$ = 15 nM, $K_{i,\ \sigma2}$ = 28.4 nM) are now commonly used as the radioligand of choice to study the σ1 and σ2 receptor binding.

Recent clinical and preclinical findings suggested the implication of σ1 receptor in various CNS disorders. This is best demonstrated by reports on the use of various σ1 receptor antagonists in drug addiction,[10] including alcohol, cocaine, and methamphetamine, as well as HIV-associated neurocognitive disorder and pain.[11, 12] Interestingly, σ1 receptor agonists are also reported to possess beneficial effects in neurodegenerative disorders,[13] such as stroke,[14] Alzheimer's disease,[15] pseudobulbar affect,[16] depression and anxiety disorders[17]. Of particular interest, a considerable body of evidence lends support to the hypothesis that antagonism of σ1 receptor results in antinociceptive effect. The σ1 receptor knock-out mouse showed decreased pain responses in the formalin test and no mechanical hypersensitivity following capsaicin sensitization or sciatic nerve injury.[18, 19] Pasternak group demonstrated that σ1 receptor agonist (+)-pentazocine reduced the analgesic effect of μ opiate morphine but not the inhibition of gastrointestinal transit.[20] The administration of classical σ1 receptor antagonist haloperidol (**3**) and its metabolites resulted in antinociceptive effects in the mouse formalin test.[21] The most clinically advanced selective σ1 receptor antagonist based on a morpholinyl pyrazole scaffold S1RA (E-52862, **4**) is currently in the phase II trials for treatments of diverse pain states.[22] Although inactive when administered alone, this compound enhanced the antinociceptive effect of morphine in mouse tail flick test and thus proposed as opioid adjuvants, offering novel approach in pain management.[23] Other recently reported selective σ1 receptor antagonists showing antinociceptive effects in animal models of pain include the 4-aminotriazole **5**,

hexahydro-2*H*-pyrano[3,2-c]quinoline **6**, and 5-chloro-2-(4-chlorophenyl)-4-methyl-6-(3-(piperidin-1-yl)propoxy)pyrimidine **7**.[24-26]

During our previous campaign on potent and selective α4β2 partial agonists, compounds **8** and **9** were identified as novel selective σ1 ligands resulting from a comparative analysis of the pharmacophoric elements required for σ1 and nicotinic ligands.[27] Compounds **8** and **9** displayed high affinity for the σ1 receptor (**8**: K_i, σ1 = 33 nM, K_i, σ2 = 1472 nM; **9**: K_i, σ1 = 4.1 nM, K_i, σ2 = 1312 nM) in the National Institute of Mental Health-Psychoactive Drug Screening Program (NIMH-PDSP) broad screening panel of 42 common CNS neurotransmitter transporters and receptors, while possessing good to moderate affinity for DAT (**8**: K_i, DAT = 24 nM; **9**: K_i, DAT = 373 nM) and NET (**8**: K_i, NET = 191 nM; **9**: K_i, NET = 203 nM). Considering the good preliminary ADME-Tox profile and brain uptake for this series of compounds, current efforts are undertaken to ultimately identify selective σ1 antagonists and assess their analgesic properties alone or in combination with clinically-used opioids. Herein, we report the rational design and synthesis of isoxazole-based compounds as σ1 ligands. Highly potent and selective analogues devoid of binding interactions with DAT or NET are identified, with the pharmacokinetic properties and *in vivo* anti-nociceptive efficacy assessed for the best compound.

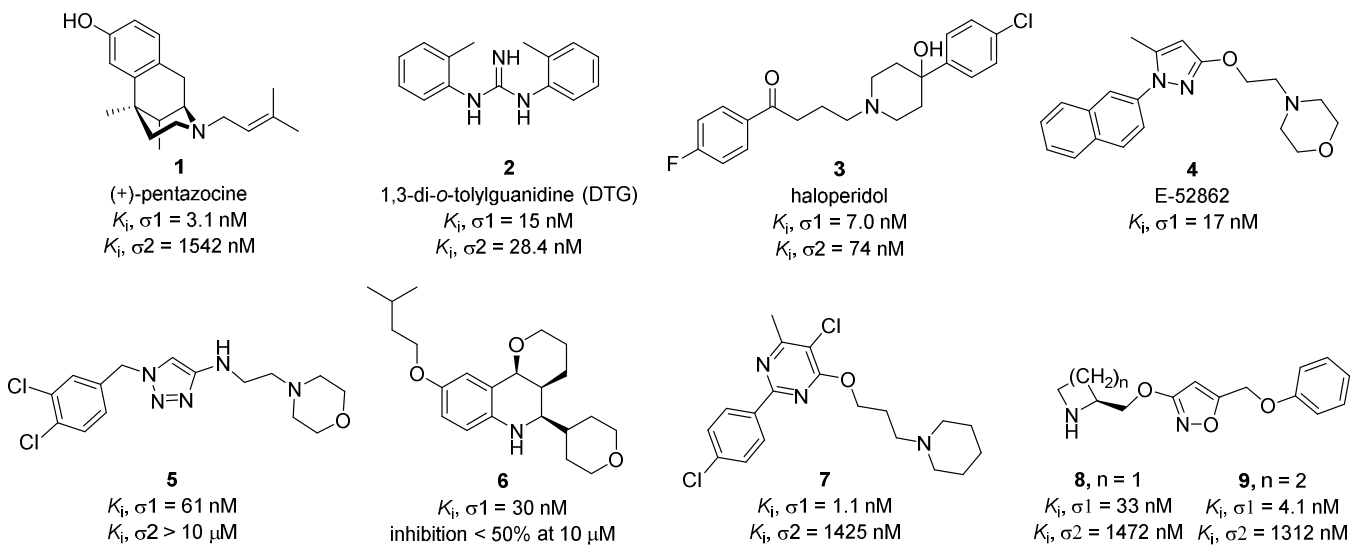


Figure 1. Selected σ receptor ligands.

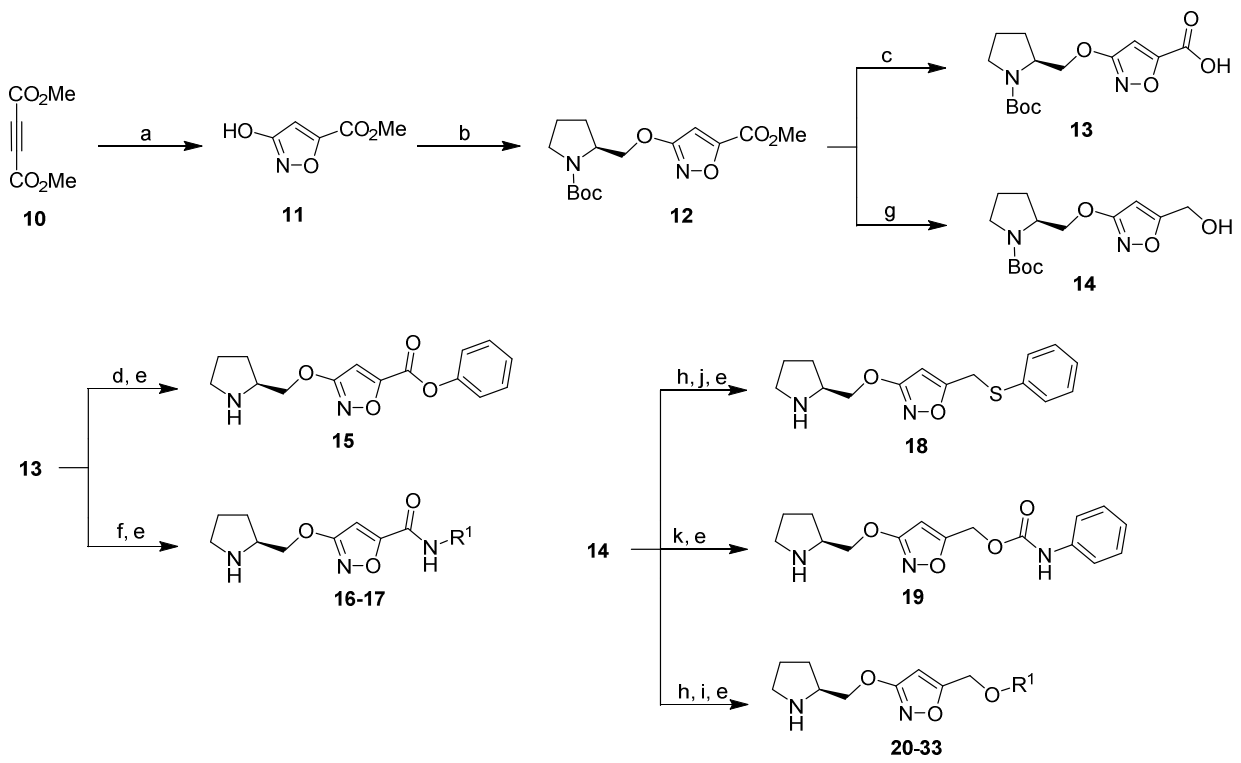
RESULTS AND DISCUSSION

Chemistry

Analogues of alkoxyisoxazoles **8** and **9** were designed based on the following principles: 1) adherence with the σ1 receptor pharmacophore requirements; 2) improve selectivity over nicotinic receptors and monoamine transporters; 3) compounds can be accessed from readily available starting materials. We previously reported that 3-alkoxyisoxazoles, originally developed for α4β2 nicotinic acetylcholine receptors, could be readily switched upon slight chemical modifications to a scaffold that is selective for σ1 receptor.[27, 28] In this study, three regions of the isoxazoles based on the previously identified selective σ1 ligand compound **9** were selected for SAR investigation: hydrophobic region, linker L, and the *N*-region (**Figure 2**), keeping the core isoxazole intact.

The 3-alkoxyisoxazoles **15–33** were synthesized starting from commercially available dimethyl 2-butynedioate (**10**) in 5–8 steps utilizing the synthetic routes shown in Scheme 1. Alkyne **10** and *N*-hydroxyurea underwent a [2+3] intermolecular cycloaddition to form isoxazole **11**, which was subsequently reacted with Boc-protected 2(*S*)-pyrrolidinylmethanol via Mitsunobu reaction to form ester **12**. Basic hydrolysis or lithium borohydride reduction of ester **12** furnished the acid **13** and primary alcohol **14** respectively. Final compounds **15–17** were synthesized through condensation of acid **13** with phenol or indoline followed by deprotection of Boc group. Alcohol **14** was initially converted to the iodide and subsequently reacted with benzenethiol or substituted phenols to form the thioether **18** and aromatic ethers **20–33**. Carbamate **19** was prepared by treatment of intermediate alcohol **14** with phenylisocyanate. After acidic deprotection final compounds **18–33** were obtained as trifluoroacetates or hydrochlorides.

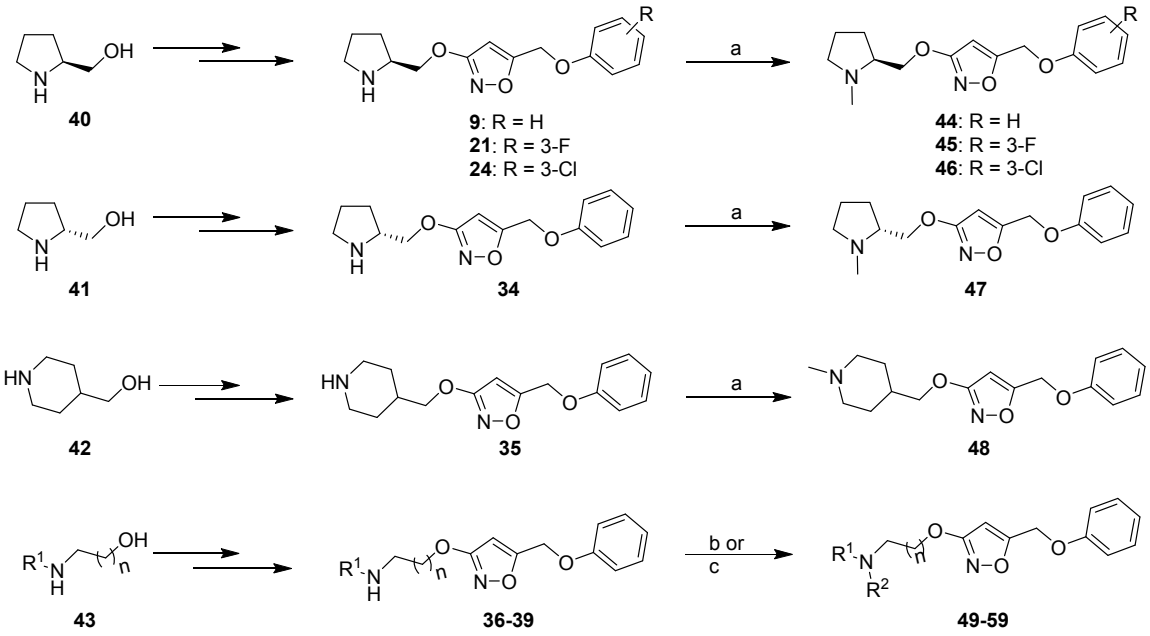
Scheme 1[a]



[a] **Reagents and conditions:** (a) *N*-hydroxyurea, 1,5-diazabicyclo[5.4.0]undec-5-ene, MeOH, 0 °C, then HCl; (b) 1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylmethanol, diisopropyl azodicarboxylate, PPh_3, THF, 0 °C to rt; (c) NaOH, THF, rt; (d) DCC, DMAP, phenol, CH_2Cl_2, rt; (e) TFA/CH_2Cl_2, rt, or HCl/EtOAc, rt; (f) EDC, DMAP, aniline or indoline, rt; (g) $LiBH_4$, THF, 0 °C to rt; (h) I_2, PPh_3, imidazole, CH_2Cl_2, 0 °C to rt; (i) phenol, or naphthalen-2-ol, or substituted phenol, K_2CO_3, DMF, rt; (j) PhSH, K_2CO_3, DMF, rt; (k) PhNCO, DMAP, CH_2Cl_2, rt.

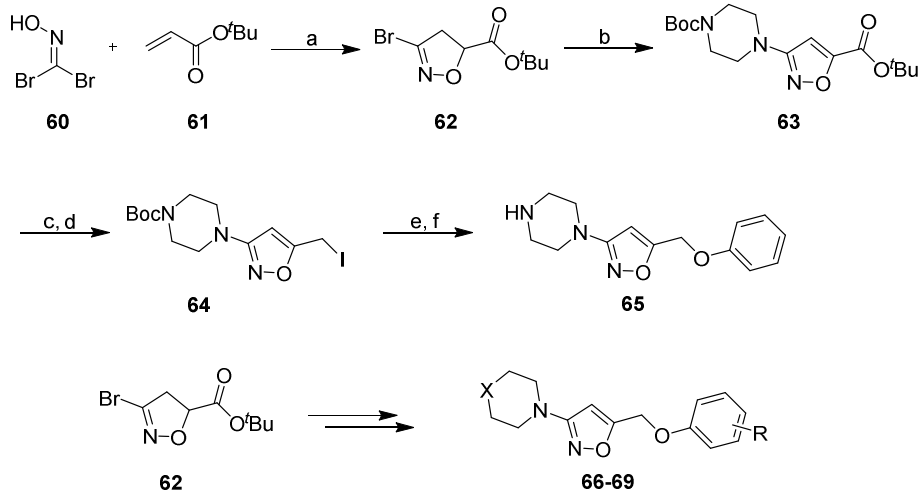
Compounds **34–39** were synthesized in a similar manner to parent compound **9**[27, 28] from methyl 3-hydroxyisoxazole-5-carboxylate **11** with corresponding alcohols **40–43** (Scheme 2). The *N*-alkylated analogues **44–59** were synthesized via reductive amination with the corresponding aldehydes or ketones. All final compounds were purified by HPLC or recrystallization as trifluoroacetates or hydrochlorides respectively.

Scheme 2[a]



[a] **Reagents and conditions:** (a) HCHO, CF_3CH_2OH, $NaBH_4$, 40 °C; (b) aldehyde or ketone, $Na(CN)BH_3$, CH_3CO_2H, CH_3OH, rt; (c) $(ClCH_2CH_2)_2O$, NaI, K_2CO_3, DMF.

Scheme 3[a]



[a] **Reagents and conditions:** (a) $KHCO_3$, DMF, -10 °C to rt then 1N HCl; (b) i. *N*-Boc piperazine, ^{t}BuOH, Na_2CO_3, reflux; ii. imidazole, I_2, PhMe, 110 °C; (c) $LiBH_4$, THF; (d) PPh_3, imidazole, I_2, rt; (e) phenol, K_2CO_3, DMF, rt; (f) HCl, EtOAc, rt.

Compounds **65–69** were synthesized by employing similar strategy as described in Scheme 3. 1,1-Dibromoformaldoxime **60** and *tert*-butyl acrylate **61** underwent intermolecular cycloaddition under basic condition to form intermediate dihydroisoxazole **62**. Addition-elimination with appropriate amine in basic condition, followed by oxidation with iodine afforded isoxazole **63**. This intermediate was reduced with lithium borohydride and reacted with iodine/triphenylphosphine to give halide **64**. Subsequent reaction with phenol in the presence of potassium carbonate and Boc deprotection gave the desired final compounds **65**–**69**.

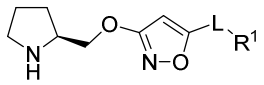
In Vitro Characterization—Radioligand Binding Studies[29]

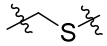
In vitro binding affinities of compounds **15–33** at σ1 and σ2 receptors were determined by the standard [^{3}H]pentazocine and [^{3}H]DTG binding assay with rat brain homogenate and P12 cells respectively (Table 1). Keeping the pyrrolidine group intact on 3-isoxazole position, we first looked at the effects of changing linker L from a methylene ether to ester (compound **15**), amide (compounds **16** and **17**), thioether **18** and carbamate **19**. Ester **15** turned out to be completely inactive both at σ1 and σ2 receptors while the amide **16** had approximately 30-fold reduction on the affinity to σ1 receptor. Substituting the benzene ring for a bulkier indoline resulted in a 9-fold improvement in σ1 binding affinity (**16** vs **17**), but still 3-fold less potent compared to the original methylene ether **9**. Thioether or carbamate linkers (**18** and **19**) also resulted in a decrease in σ1 affinity (~16- to 74-fold vs compound **9**), therefore prompted us to retain linker L as a methylene ether and investigate the effects of phenyl substitution (R^1).

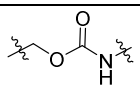
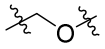
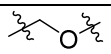
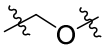
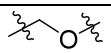
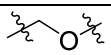
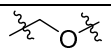
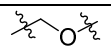
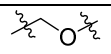
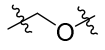
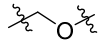
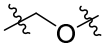
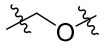
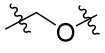
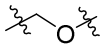
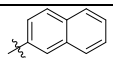
The hydrophobic region R^1 typically occupied by a benzene ring is generally deemed an important pharmacophoric element as seen in most of σ1 ligands.[30] In our previous work, we demonstrated that replacing a benzene group at R^1 with small aliphatic groups, e.g. methyl and cyclopropylmethyl, was deleterious for σ1 affinity.[27] A 5-chloropyridyl substitution also resulted in a less potent σ1 ligand (K_i σ1 103 nM, σ2 >10,000 nM). Next iteration of SAR studies was thus focused on assessing the effects of electron withdrawing group (EWG; F, Cl, Br, CF_3) and electron donating group (EDG; OMe, Me) substituents on the benzene ring. A fluoro or chloro monosubstitution (compounds **20**–**25**) generally

retained affinity at σ1 receptor, with no higher than a 3-fold decrease or increase relative to parent compound **9**. Unfortunately, this was accompanied with an unwanted improvement at σ2 receptor binding, with the single exception of 3-chloro-substituted compound **24** (K_i σ1 2.5 nM, σ2 >10,000 nM). Monobromo or monotrifluoromethyl substitution (compounds **26**–**29**) resulted in a very similar trend to those observed with monofluoro compounds. Introduction of a methoxy group at 3- or 4-position resulted in 8-fold and 51-fold reduction respectively in σ1 affinity (compounds **30** and **31**). A 3-methyl susbstitution gave an equipotent compound to parent compound **9** in terms of σ1 affinity, but a more potent binder at σ2 receptor (K_i σ2 337 nM). Substitution of the benzene ring with a sterically bulkier naphthalene yielded a more potent σ2 receptor binder and less potent affinity at σ1 (K_i σ1 36 nM, σ2 151 nM). In almost all of the tested analogues **20**–**33**, affinity at σ2 receptor was improved upon monosubstitution with an EWG or EDG, with the exception of 3-chloro-substituted compound **24** (K_i σ1 2.5 nM, σ2 >10,000 nM) and 3-methoxy-substituted compound **30 (**K_i σ1 31 nM, σ2 >10,000 nM).

Table 1. Binding affinities of 3-alkoxyisoxazole ligands at σ1 and σ2 receptors. [a]



Compound	L^1	R^1	K_i, σ1 (nM) [b]	K_i, σ2 (nM)	LogBB [d]
3	-	-	7.0 ± 1.1	19.6 ± 2.2	0.124
9		C_6H_5	4.1 ± 0.7	1312 [e]	-0.255
15		C_6H_5	NA [c]	NA	-0.390
16		C_6H_5	130 ± 18	NA	-0.618
17			13.9 ± 1.6	NA	-0.542
18		C_6H_5	65.4 ± 10.5	1033 ± 142	-0.052

19		C_6H_5	299 ± 48	NA	-0.785
20		2-F-C_6H_4	6.0 ± 0.8	396 ± 72	-0.242
21		3-F-C_6H_4	1.4 ± 0.2	277 ± 32	-0.212
22		4-F-C_6H_4	4.4 ± 0.6	471 ± 54	-0.212
23		2-Cl-C_6H_4	2.7 ± 0.4	165 ± 30	-0.072
24		3-Cl-C_6H_4	2.6 ± 0.3	NA	-0.072
25		4-Cl-C_6H_4	13.0 ± 1.5	139 ± 16	-0.072
26		3-Br-C_6H_4	3.2 ± 0.4	85.9 ± 11.8	-0.050
27		4-Br-C_6H_4	18.3 ± 1.7	137 ± 25	-0.050
28		3-CF_3-C_6H_4	6.6 ± 0.6	88 ± 12	-0.031
29		4-CF_3-C_6H_4	13.3 ± 1.2	91.7 ± 10.3	-0.031
30		3-OCH_3-C_6H_4	31.1 ± 3.6	NA	-0.325
31		4-OCH_3-C_6H_4	210 ± 19	675 ± 138	-0.325
32		3-CH_3-C_6H_4	4.0 ± 0.5	341 ± 39	-0.179
33			36.6 ± 4.1	153 ± 17	-0.077

[a]See Experimental Section. Radioligands: σ1: [^{3}H]-(+)-pentazocine; σ2: [^{3}H]DTG (ditolylguanidine). [b]The K_i values for compound **9** are cited from the literature.[27] [c] NA: not active, defined as < 50% binding in the primary assay at 10 μM. [d] LogBB values were calculated from the Clark's equation: LogBB = -0.0148×PSA + 0.152×CLogP + 0.139.[31] [e] Data shown as average of K_i values from two measurements: 665 ± 29 nM and 1958 ± 392 nM.

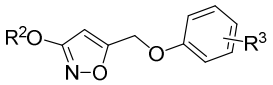
Subsequent SAR studies were focused on assessing replacement of the nitrogen-containing group R^2, keeping the linker L and hydrophobic group R^1 as methylene ether and benzene or 3-substituted benzene respectively. Compound **34,** which is an *R*-enantiomer of the parent compound **9**, was found to be ~10-

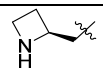
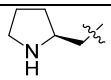
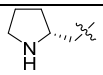
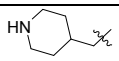
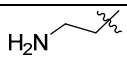
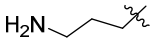
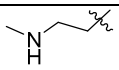
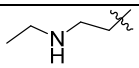
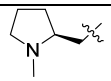
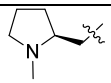
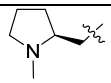
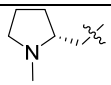
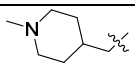
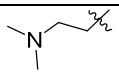
fold less potent at the σ1 receptor and 3-fold more potent at the σ2 receptor. Replacement with 4-methylpiperidine or ring-opened aminopropyl group resulted in deleterious effects on the σ1 affinity (compounds **35** and **37**), which could be explained by the non-ideal positioning of the nitrogen according to the proposed pharmacophore model shown in **Figure 2**, which consists of: 1) a substituted basic nitrogen; 2) a primary and secondary hydrophobic regions at a distance of approximately 6 to 10 Å and 2.5 to 3.9 Å from the nitrogen atom.[32] A shorter aminoethyl or *N*-methylaminoethyl substitution (compounds **36** and **38**) partially restored binding affinity at σ1 receptor, with σ1 K_i values of 63 nM and 80 nM respectively, as the nitrogen fits better with the proposed pharmacophore. *N*-Ethylaminoethyl substitution (compound **39**) resulted in a significant increase of σ1 binding affinity (K_i = 3.9 nM), while weakly binding to the σ2 receptor with a *K*i value of 975 nM.

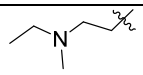
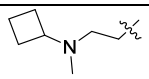
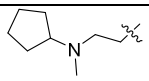
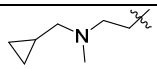
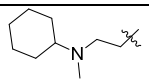
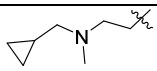
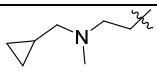
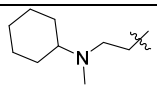
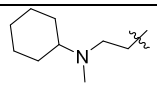
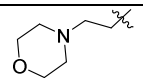
We next examined the effect of *N*-alkylation to the binding affinity profiles (**44**–**59**). *N*-Methyl pyrrolidine/piperidine analogues generally slightly improved the binding affinity at σ1 receptor relative to the corresponding unsubstituted pyrrolidines/piperidine (**44** vs **9**, **45** vs **21**, **46** vs **24**, **47** vs **34**, **48** vs **35**). Compound **47** was a less potent binder at σ1 compared to its enantiomer compound **44**, suggesting *S*-configuration of pyrrolidine moiety is favored for σ1 binding. In contrast, further *N*-methylation of compounds **38** and **39** resulted in a significant decrease of binding affinity (**49** vs **38** and **50** vs **39**). Gratifyingly, bulkier cycloaliphatic substituents (compounds **51**–**54**) resulted in compounds with low single-digit nanomolar to subnanomolar K_i values at the σ1 receptor. The cyclopropyl analogue **53** was found to possess the most superior σ2/σ1 ratio, whereas the cyclohexyl analogue **54** was the most potent binder at σ1 receptor with a K_i value of 0.3 nM. 3-Fluoro or 3-chloro substitutions at the hydrophobic region R^1 for these two compounds (**55**–**58**) yielded compounds with very similar binding profiles**.** However, binding affinity to the σ2 receptor also increased with K_i values of less than 100 nM for the cyclopentyl and cyclohexyl compounds (compounds **52** and **54**). This observation is consistent with SAR findings in the 1-arylpyrazoles series of σ ligands in which only small cyclic amines analogues possessed sufficient selectivity for σ1 vs σ2 receptor.[33] As the ethoxymorpholine group is favored in 1-arylpyrazoles

series, we attempted to attach the same appendage to our isoxazole-containing ligands resulted in compound **59**. Unfortunately the potency was found to dramatically drop by 100-fold (**59** vs **9**).

Table 2. Binding affinities of 22 isoxazole ligands at σ1 and σ2 receptors. [a]



Compound	R^2	R^3	K_i, σ1 (nM)[b]	K_i, σ2 (nM)[b]	LogBB[d]
3	-	-	7.0 ± 1.1	19.7 ± 2.2	0.124
8		H	33.0 ± 5.2	1472[e]	-0.255
9		H	4.1 ± 0.7	1312[f]	-0.255
34		H	37.4 ± 4.3	508 ± 94	-0.202
35		H	356 ± 33	284 ± 39	-0.253
36		H	63.9 ± 10.2	NA[c]	-0.617
37		H	2312 ± 318	NA	-0.556
38		H	80.8 ± 14.7	NA	-0.364
39		H	3.7 ± 0.7	987 ± 135	-0.283
44		H	3.4 ± 0.4	665 ± 76	-0.042
45		3-F	0.7 ± 0.1	687 ± 125	0.001
46		3-Cl	1.4 ± 0.1	371 ± 76	0.087
47		H	23.1 ± 2.6	411 ± 56	-0.042
48		H	96.4 ± 13.2	216 ± 35	-0.056
49		H	NA	NA	-0.143

50		H	18.4 ± 2.9	820 ± 113	-0.063
51		H	1.8 ± 0.2	307 ± 56	-0.005
52		H	0.5 ± 0.06	73.7 ± 13.4	0.080
53		H	0.8 ± 0.1	225 ± 25	0.004
54		H	0.3 ± 0.05	18.7 ± 2.1	0.165
55		3-F	0.9 ± 0.1	314 ± 57	0.048
56		3-Cl	1.1 ± 0.1	123 ± 25	0.134
57		3-F	0.5 ± 0.05	24.5 ± 5.0	0.208
58		3-Cl	0.5 ± 0.04	15.1 ± 3.1	0.295
59		H	403 ± 63	NA	-0.289

[a]See Experimental Section. Radioligands: σ1: [^{3}H]-(+)-pentazocine; σ2: [^{3}H]DTG (ditolylguanidine). [b] The K_i values for **8** and **9** are cited from the literature.[27] [c] not active, defined as < 50% binding in the primary assay at 10μM. [d] LogBB values were calculated from the Clark's equation: LogBB = -0.0148×PSA + 0.152×CLogP + 0.139.[31] [e] Data shown as average of K_i values from two measurements: 1288 ± 128 nM and 1657 ± 329 nM. [f] Data shown as average of K_i values from two measurements: 665 ± 29 nM and 1958 ± 392 nM.

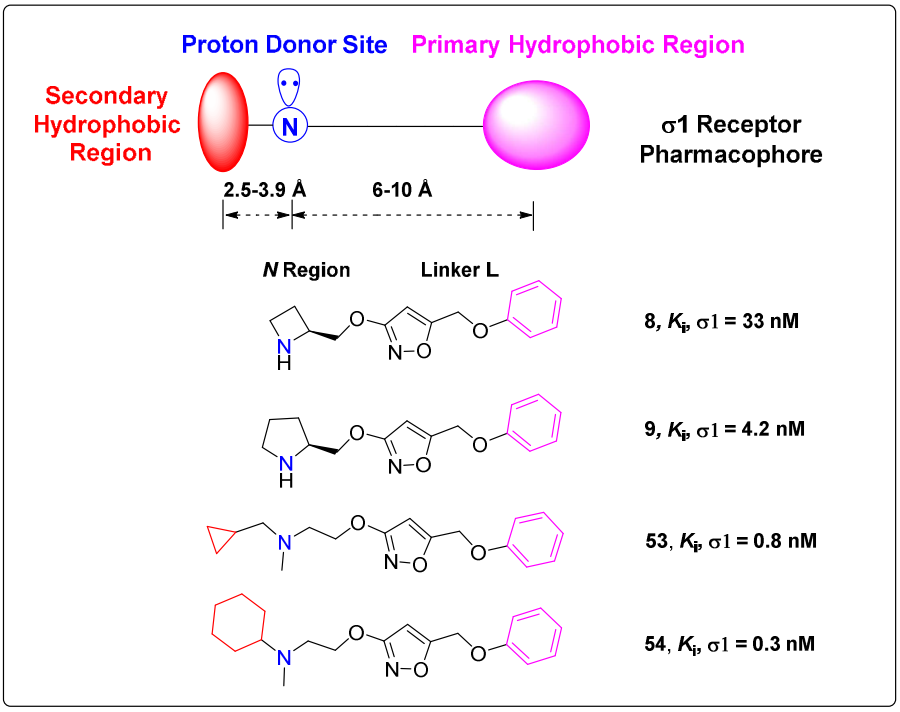
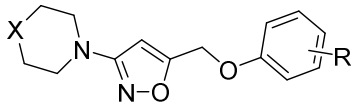


Figure 2. σ1 receptor pharmacophore model and proposed match of substructures with pharmacophore elements for compounds **8**, **9**, **53**, and **54**.

Table 3. Binding affinities of 5 isoxazole ligands at σ1 and σ2 receptors. [a]



Compound	X	R	K_i, σ1 (nM)	K_i, σ2 (nM)	LogBB[c]
3	-	-	7.0 ± 1.1	19.6 ± 2.2	0.124
65	NH	H	5.3 ± 1.0	221 ± 30	-0.272
66	NH	Cl	36.5 ± 3.3	687 ± 125	-0.142
67	NCH_3	H	63.9 ± 10.2	2202 ± 297	-0.054
68	O	H	NA[b]	NA	-0.228
69	CH_2	H	NA	NA	0.118

[a]See Experimental Section. Radioligands: σ1: [^{3}H]-(+)-pentazocine; σ2: [^{3}H]DTG (ditolylguanidine). [b] NA: not active, defined as < 50% binding in the primary assay at 10 μM. [c] LogBB values were calculated from the Clark's equation: LogBB = -0.0148×PSA + 0.152×CLogP + 0.139.[31]

We also examined briefly the effects of replacing the 3-alkyoxy groups with cyclic amines as shown in compounds **65**–**69**. Piperazine-substituted analogue **65** had a very similar K_i value to the parent compound **9** at σ1 receptor but improved binding affinity to the σ2 receptor. 3-Chloro substitution at the hydrophobic region R^1 (compound **66**) resulted in a 7-fold decrease in σ1 affinity. *N*-Methylpiperazine substitution (compound **67**) gave a much less potent σ1 binder ($K_{i,\,\sigma1}$ = 64 nM, $K_{i,\,\sigma2}$ = 2,147 nM). Morpholine, or piperidine substitution was found to be deleterious for affinity at both σ receptors (**68** and **69**).

Selectivity Studies at Selected Neurotransmitter Transporters

A broad-range screening study was carried out previously for compounds **8** and **9** to determine their global selectivity profile among 42 other CNS neurotransmitter receptors and transporters, including serotonin receptors, dopamine receptors, GABA receptors, biogenic amine transporters, adrenergic receptors, muscarinic receptors, opioid receptors, and histamine receptors.[27] We previously found that compounds **8** and **9** not only bind to σ1 receptor (K_i = 33 and 4.1 nM) but also showed moderate binding affinities at two of the biogenic amine transporters DAT (K_i = 24 and 373 nM) and NET (K_i = 191 and 203 nM), respectively. Gratifyingly, no appreciable binding was observed for DAT, NET and SERT using 10 μM concentration of compounds **53** and **54**, indicating that these two compounds have excellent selectivity for σ1 receptor over DAT, NAT and SERT. (Table 4.) The other three tested compounds **20**, **23** and **66** were found to retain some binding affinities to at least one of the three transporters tested.

Table 4. Binding affinities of selected ligands at NET, DAT, and SERT.[a]

Compound	K_i, DAT (nM)[b]	K_i, NET (nM)	K_i, SERT (nM)	K_i, σ1 (nM)
GBR12909	8.8 ± 3.8	-	-	-
Desipramine	-	2.5 ± 0.4	-	-
Amitryptiline	-	-	5.4 ± 0.9	-
8	24 ± 0.95	191 ± 10	NA[c]	33 ± 5.2
9	373 ± 24	203 ± 12	NA	4.1 ± 0.7

20	NA	1082 ± 148	NA	6.1 ± 0.8
23	NA	2109 ± 289	2946	2.7 ± 0.4
53	NA	NA	NA	0.8 ± 0.1
54	NA	NA	NA	0.3 ± 0.05
65	497 ± 297	163 ± 37	NA	5.3 ± 0.9

[a]See Experimental Section. DAT: Dopamine Transporter; NET: Norepinephrine Transporter; SERT: Serotonin Transporter. Radioligands: σ1: [^{3}H]-(+)-pentazocine; σ2: [^{3}H]DTG (ditolylguanidine); DAT: [^{3}H]WIN35428; NET: [^{3}H]nisoxetine; SERT: [^{3}H]citalopram. [b]The K_i values for **8–9** are cited from the literature.[27] [c] not active, defined as < 50% binding in the primary assay at 10 μM. K_i values were determined for those targets where the binding efficacy at 10 μM was greater than 50%.

Functional Profile

To date, there is still an unmet need of high throughput functional assays that readily identify σ1 ligands as an agonist, partial agonist or antagonist. Competition binding studies of the examined ligands with phenytoin, an allosteric modulator of σ1 receptor, has recently emerged as an accepted method[26, 33] to differentiate σ1 agonists and antagonists.[34] In this assay, phenytoin is believed to induce a conformational change in the receptor to shift the equilibrium towards the active state, thereby increasing the affinity of putative agonists but not of the antagonists.[34] Six selected compounds (**4, 9, 21, 24, 51, 53**) were subjected to this assay and found to bind less potently to the σ1 receptor in the presence of phenytoin, indicating that they behave as antagonists (See Supporting Information Table 1).

***In Vivo* Analgesic Effects—Mouse Formalin Test**

Compound **53** was further advanced to the *in vivo* analgesic studies given its *in vitro* potency, selectivity, functional profiles, as well as LogBB value. Anti-nociceptive efficacy was assessed using the mouse formalin test, a classic model in which formalin solution is injected to the hind paw of the mouse and a biphasic pain response characterized by analysing the behavior of licking and flinching of the affected

hind paw.[35, 36] The first phase (0–10 min) reflects mostly nociceptive pain whereas the second phase (11–45 min) represents the inflammatory responses. Traditional analgesics as well as σ1 receptor antagonists have been reported to attenuate the licking and flinching behavior. In this study, mice were administered compounds **53** (40 and 80 mg/kg i.p.) or selected σ1 antagonist **4** as a positive control (80 mg/kg i.p.) and the time spent licking the paw after injection was recorded as shown in **Figure 3**. Drug administration produced a reduction of paw licking behavior in mouse, suggestive of an anti-nociceptive effect. Compound **53** (80 mg/kg) or **4** (80 mg/kg) significantly attenuated paw licking behavior in both phases in mice, while 40 mg/kg of compound **53** markedly reduced paw licking in phase II but not in phase I compared to the vehicle group.

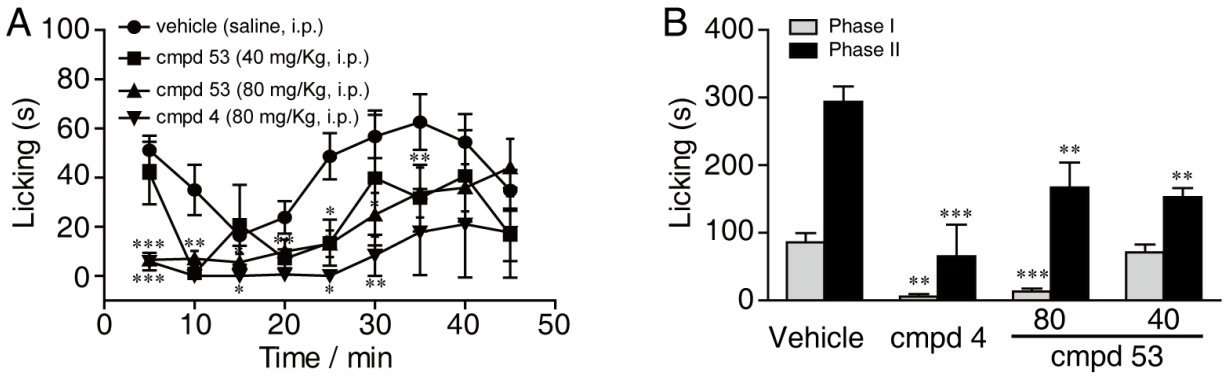


Figure 3. Analgesic effects of compound **53** and **4** in phase I (0–10 min) and phase II (11−45) of the mouse formalin test at the dose of 80 mg/kg. All drugs were administered intraperitoneally; each column and vertical line represents mean ± SEM of the values obtained in at least 6 animals. Statistically significant differences:* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; vs vehicle using unpaired, two-tailed t-test).

Rotarod Test.

The rotarod test is widely used to evaluate the motor coordination of rodents. In this study, we utilized this assay to rule out the possibility that the observed efficacy of compound **53** in the formalin test was due to the interference with motor coordination. As shown in **Figure 4**, pretreatment with pregabalin (40 mg/kg) significantly decreased the rotarod latency of mice at 60, 90, and 120 min compared to the vehicle group.

Compound **53** did not significantly alter the time mice remained on the rotarod at both 40 and 80 mg/kg suggesting no interference with motor coordination.

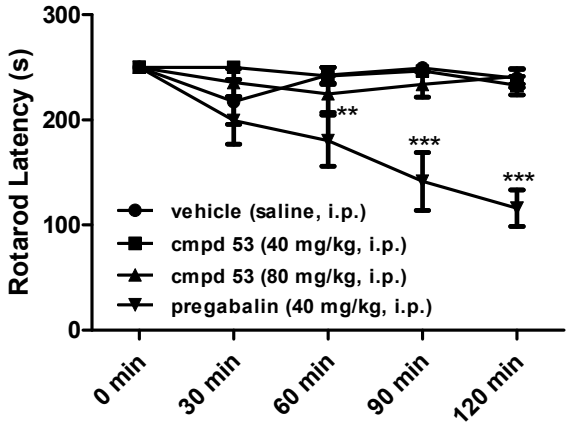


Figure 4. Dose response effect of **53** and pregabalin on rotarod test. Data obtained from 8–10 mice per group and expressed as mean ± SEM latency (s) to fall down from rod. Statistically significant differences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs vehicle (Two-Way ANOVA followed by Bonferroni test).

In Vitro PAMPA Data

The oral absorption of seven representative compounds was initially evaluated using parallel artificial membrane permeability assay (PAMPA), which is an *in vitro* model of passive, transcellular permeation widely used in the pharmaceutical industry. The *in vitro* permeability (*Pe*) of compounds **4, 9, 21, 24, 44, 53, 65** and the control drugs verapamil and atenolol through a lipid extract of porcine brain was determined using PBS as the solvent. Compounds with a *Pe* above 4.0×10^{-6} cm·s^{-1} are considered possibly penetrating into the CNS by passive diffusion (CNS+), whereas compounds with *Pe* below 2.0×10^{-6} cm·s^{-1} are considered to be impenetrant (CNS-).[37] Those with *Pe* values ranging from 2.0×10^{-6} cm·s^{-1} to 4.0×10^{-6} cm·s^{-1} are categorized as uncertain (CNS±). Compound **65** was found to possess the greatest permeability with a *Pe* value of 7.36×10^{-6} cm·s^{-1}(SI Table 2.), while all the other tested compounds have

Pe values between 2×10^{-6} cm·s^{-1} and 4×10^{-6} cm·s^{-1}, including compound **4**, which is currently in phase II clinical trial.

In Vivo Pharmacokinetic Studies of Compound 53

To determine the ability of these alkoxyisoxazole compounds to penetrate the BBB, compound **53** was selected for *in vivo* pharmacokinetic studies in mouse. Compound **53** (40 mg/kg) was orally administered to KM mice before collection of plasma and brain samples at the following time points: 0.167, 0.5, 1, 2, 4, 6, and 8 h (n = 3/group). The concentrations of compound **53** in the brain and plasma samples were determined by a developed LC−MS/MS method. The plasma and brain oral half-lives of compound **53** were found to be 2.64 and 2.52 h respectively (Table 5). The $AUC_{0-\infty}$ values were 113 h·ng·mL^{-1} and 403 h·ng·g^{-1} in the plasma and brain respectively, with the brain/plasma ratio of 3.6. These data suggest that compound **53** is absorbed rapidly following oral administration and readily crosses the BBB.

Table 5. Pharmacokinetics parameters of **53** in KM mice (40 mg/kg, po)

Plasma PK Parameters	Plasma	Brain PK Parameters	Brain	Ratio (Brain/Plasma)
K_{el}, h^{-1}	0.263	K_{el}, h^{-1}	0.276	-
$t_{1/2}$, h	2.64	$t_{1/2}$, h	2.52	-
t_{max}, h	0.167	t_{max}, h	0.167	-
C_{max}, ng·mL^{-1}	193	C_{max}, ng·g^{-1}	395	2.0
AUC_{0-8}, h·ng·mL^{-1}	108	AUC_{0-8}, h·ng·g^{-1}	373	3.6
AUC_{0-inf}, h·ng·mL^{-1}	113	AUC_{0-inf}, h·ng·g^{-1}	403	3.6
$AUMC_{0-8}$, h·h·ng·mL^{-1}	91.5	$AUMC_{0-8}$, h·h·ng·g^{-1}	578	6.3
$AUMC_{0-inf}$, h·h·ng·mL^{-1}	157	$AUMC_{0-inf}$, h·h·ng·g^{-1}	935	6.0
MRT_{PO}, h	1.39	MRT_{PO}, h	2.32	-

Conclusions

A total of 46 compounds were designed and synthesized based on the previously identified alkoxyisoxazole scaffold as potent and selective σ1 receptor ligands. Binding assays at both σ1 and σ2

receptors revealed analogues with subnanomolar potencies at the σ1 receptor. Selected compounds were tested for their binding profiles at DAT, NET and SERT transporters. Compound **53** was found to be the most promising compound overall given its *in vitro* binding profile, LogBB value and permeability in PAMPA assay. Administration of σ1 antagonist **53** at 80 mg/kg (i.p.) was found to demonstrate statistically significant analgesic effects in mouse formalin test. Combined with the acceptable pharmacokinetic parameters and its excellent brain/plasma ratio, this study overall supports the notion of the use of σ1 antagonists for novel treatment in pain.

Experimental Section

General Methods: Starting materials, reagents, and solvents were purchased from commercial suppliers and used without further purification, unless otherwise stated. Anhydrous THF and CH_2Cl_2 were obtained by distillation over sodium wire or CaH_2, respectively. All non-aqueous reactions were run under a nitrogen atmosphere with exclusion of moisture from reagents, and all reaction vessels were oven-dried. The progress of reactions was monitored by TLC on SiO_2. Spots were visualized by their quenching of the fluorescence of an indicator admixed to the SiO_2 layer, or by dipping into $KMnO_4$ solution followed by heating. SiO_2 for column chromatography (CC) was of 200–300 mesh particle size, and an EtOAc/hexane mixture or gradient was used unless stated otherwise. ^{1}H NMR spectra were recorded at a spectrometer frequency of 400 MHz, ^{13}C NMR spectra at 101 MHz. Chemical shifts are reported in δ (ppm) using the δ 0 signal of tetramethylsilane (TMS) as internal standards. High resolution mass spectra were performed using a Bruker ESI-TOF high-resolution mass spectrometer. Purities of final compounds (> 98%) were established by analytical HPLC, which was carried out on an Agilent 1200 HPLC system with a ZORBAX Eclipse XDB-C18 column, with detection at 220 or 254 nm on a variable wavelength detector G1365D; flow rate = 1.4 mL/min; gradient of 0 to 100% methanol in water (both containing 0.05 vol % of TFA) in 25 min. Final products were purified by preparative HPLC (Beckman GOLD126P) under the following conditions: column, Atlantis T3 5 μm, 250 x 4.6 mm; flow, 3 mL/min; all solvents containing 0.05 vol% TFA; UV detection at 254 nm and 220 nm; Gradient I: 8–100% MeOH in water in 30 min,

100% for 4 min, return to 8% in 20 min. The optical rotation experiments were performed using an Autopol VI polarimeter.

General Procedure for the Reduction of Isoxazolecarboxylic Acid Esters to Alcohols (Method A). To a solution of isoxazolecarboxylic acid esters (0.8 mmol) in anhydrous THF (20 mL) was added $LiBH_4$ (4 mmol) with ice cooling under Ar. After stirring overnight at rt, saturated aqueous NH_4Cl solution was added with ice cooling. Extraction with EtOAc (2×30 mL), drying over Na_2SO_4, followed by evaporation under vacuum afforded the residue which was further purified by flash chromatography to give the alcohol.

General Procedure for the Esterification of Isoxazolecarboxylic Acid with Phenol or Alcohol (Method B). To a stirred solution of isoxazole-5-carboxylic acid (0.3 mmol) and phenol (or alcohol) (0.3 mmol) in 10 mL CH_2Cl_2 was added DCC (98 mg, 0.47 mmol) and DMAP (19.2 mg, 0.16 mmol) under N_2. After stirring overnight at rt, the reaction mixture was evaporated under vacuum and the residue was purified by flash chromatography to give the ester product.

General Procedure for the Deprotection of *N*-Boc-Amines to Afford HCl Salts (Method C). To a solution of the *N*-Boc protected precursor (1 mmol) in CH_2Cl_2 (5 mL) was added HCl/EtOAc (4 mol/L, 2 mL) under N_2 with ice cooling. The mixture was stirred overnight at rt, after which the solvent was evaporated and the residue was triturated with diethyl ether (20 mL). The resultant solid was filtered off to give the HCl salt.

General Procedure for the Deprotection of *N*-Boc-Amines to Afford TFA Salts (Method D). To a solution of the *N*-Boc protected precursor (1 mmol) in CH_2Cl_2 (5 mL) was added TFA (0.5 mL) under N_2 with ice cooling. The mixture was stirred overnight at rt. After the solvent was evaporated, the residue was purified by preparative HPLC. Following evaporation under vacuum, the residue was dissolved in distilled water (about 2−3 mL) and the solution was lyophilized to obtain the TFA salt.

General Procedure for the Amidation of Isoxazole-5-Carboxylic Acid (Method E). To a stirred solution of isoxazole-5-carboxylic acid (1.6 mmol) and amine (1.6 mmol) in 20 mL CH_2Cl_2 was added

EDC·HCl (2.08 mmol) and 3-methylpyridine (2.24 mmol) under N_2. After stirring overnight at rt, the reaction mixture was evaporated and the residue was purified by flash chromatography to give the amide product.

General Procedure for the Preparation of Phenyl Ethers or Thioether from Iodides (Method F). To a stirred solution of an iodide (1 mmol) and phenol or thiophenol (2 mmol) in anhydrous DMF (4 mL) was added K_2CO_3 (6 mmol) under N_2. After stirring overnight at rt, saturated aqueous NH_4Cl solution was added. The mixture was extracted with EtOAc (2×30 mL), and the combined organic phases were washed with water (3×20 mL), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography to give the phenyl ether or thioether product.

General Procedure for the Mitsunobu Reaction of Alcohol with Hydroxyisoxazole (11) to Afford Alkoxyisoxazoles (Method G). To a stirred solution of alcohol (1.2 mmol), **11** (2.0 mmol), and PPh_3 (2.0 mmol) in anhydrous THF (20 mL) was added diisopropyl azodicarboxylate (1.5 mmol) dropwise with ice cooling under N_2. After stirring overnight at rt, the solvent was evaporated, and the residue was dissolved in EtOAc (30 mL). The solution was washed with water (20 mL) and brine (15 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography to give the alkoxyisoxazole product.

General Procedure for the Preparation of Iodides from Alcohols (Method H). To a stirred solution of crude alcohol (0.7 mmol), imidazole (1.05 mmol), and PPh_3 (1.05 mmol) in anhydrous CH_2Cl_2 (10 mL) was added I_2 (1.05 mmol) with ice cooling under N_2. After stirring overnight at rt, the solvent was evaporated and the residue was purified by flash chromatography to give the iodide.

General Procedure for the Preparation of *N*-Methyl Analogues from Primary or Secondary Amines (Method I). To a solution of primary or secondary amine (0.5 mmol) and HCHO (0.5 mL) in CF_3CH_2OH (5 mL) was added $NaBH_4$ (0.6 mmol) at 40 °C under N_2. After stirring for 2 h at 40 °C, the solvent was evaporated and extracted with EtOAc (3×10 mL). The combined organic layers were washed with water (20 mL) and brine (15 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography. To a solution of the *N*-methyl compound in CH_2Cl_2 (3 mL) was added

HCl/EtOAc (4 mol/L, 1 mL) under N_2 with ice cooling. The mixture was stirred overnight at rt and the solvent evaporated to give the HCl salt.

General Procedure for the Preparation of *N*-Alkyl Analogues from Secondary Amines (Method J). To a stirred solution of secondary amine (0.43 mmol), aldehyde or ketone (0.5 mmol) and acetic acid (0.05 mL) in CH_3OH (10 mL) was added $Na(CN)BH_3$ (0.6 mmol) at rt under N_2. After stirring overnight at rt, the solvent was evaporated and the residue was extracted with EtOAc (3×10 mL). The combined organic layers were washed with water (20 mL) and brine (15 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was then purified by flash chromatography. To a solution of the *N*-alkyl compound in CH_2Cl_2 (3 mL) was added HCl/EtOAc (4 mol/L, 1 mL) under N_2 with ice cooling. The mixture was stirred overnight at rt and the solvent evaporated to give the HCl salt.

Methyl (*S*)-3-((1-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)methoxy)isoxazole-5-carboxylate (12). This compound was obtained from 1-(*tert*-butoxycarbonyl)-2(*S*)-azetidinylmethanol and **11**[28] employing Method G. White solid; yield 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.10 (s, 1H), 4.31–4.29 (m, 1H), 4.24–4.20 (m, 1H), 4.09–3.98 (m, 1H), 3.88 (s, 3H), 3.23–3.21 (m, 2H), 2.13–1.94 (m, 1H), 1.95–1.83 (m, 2H), 1.81–1.71 (m, 1H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.9, 171.8, 160.5, 157.1, 100.7, 80.1, 70.6, 56.1, 52.8, 46.4, 28.5, 23.6, 22.6.

(*S*)-3-((1-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)methoxy)isoxazole-5-carboxylic Acid (13). To a stirred solution of **12** (326 mg, 1.0 mmol) in 10 mL THF was added NaOH (1 M, 4 mmol). After stirring for 4h at rt, the pH of the solvent was adjusted to 1 with hydrochloric acid (1 M). The reaction mixture was extracted with EtOAc (2×30 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography to give the title compound as white solid in 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 6.54 (s, 1H), 4.33 (m, 1H), 4.16 (m, 2H), 3.46 (m, 2H), 1.98 (m, 2H), 1.91 (m, 2H), 1.48 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5, 160.7, 158.2, 155.2, 100.7, 80.8, 60.6, 55.9, 41.9, 30.3, 28.5, 27.0.

***tert*-Butyl (*S*)-2-(((5-(hydroxymethyl)isoxazol-3-yl)oxy)methyl)pyrrolidine-1-carboxylate (14).** This compound was obtained from **12** employing Method A. Colorless oil; yield 91%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90 (s, 1H), 4.74 (m, 1H), 4.60 (s, 2H), 4.29 (m, 1H), 4.12 (m, 2H), 3.36 (m, 2H), 1.94 (m, 3H), 1.86 (m, 1H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 171.7, 154.6, 92.8, 79.8, 69.8, 60.4, 56.8, 56.4, 46.6, 28.4, 22.9.

Phenyl (*S*)-3-(pyrrolidin-2-ylmethoxy)isoxazole-5-carboxylate Hydrochloride (15). This compound was obtained from **13** and phenol in two steps employing Method B and C. White solid; yield 41%; purity 99.2%. $[\alpha]_D^{20}$ +18.23 (*c* = 0.13, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 7.47 (t, *J* = 8.0 Hz, 2H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.00 (s, 1H), 4.61 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.53–4.31 (m, 1H), 4.07 (m, 1H), 3.34 (t, *J* = 8.0 Hz, 2H), 2.36–2.14 (m, 1H), 2.16–1.94 (m, 2H), 1.93–1.70 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.1, 159.8, 154.6, 149.4, 129.8, 126.7, 121.5, 101.7, 69.3, 57.5, 45.4, 26.1, 23.3. HRMS (ESI): Calcd for $C_{15}H_{16}N_2O_4$ $[M+H]^+$, 289.1183; Found 289.1207.

(*S*)-*N*-Phenyl-3-(pyrrolidin-2-ylmethoxy)isoxazole-5-carboxamide Hydrochloride (16). This compound was obtained from **13** and aniline in two steps employing Method E and C. White solid; 52% yield; purity 98.6%. $[\alpha]_D^{20}$ +10.21 (*c* = 0.10, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (br s, 1H), 9.26 (br s, 1H), 8.90 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.39 (t, *J* = 8.0 Hz, 2H), 7.17 (t, *J* = 6.0 Hz, 1H), 7.09 (s, 1H), 4.54 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.47–4.32 (m, 1H), 4.14–3.83 (m, 1H), 3.24 (t, *J* = 8.0 Hz, 2H), 2.22–2.05 (m, 1H), 2.05–1.85 (m, 2H), 1.83–1.65 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.0, 164.1, 153.8, 137.5, 128.8, 124.8, 120.7, 98.0, 69.0, 57.6, 45.4, 26.0, 23.3; HRMS (ESI): Calcd for $C_{15}H_{18}N_3O_3$ $[M+H]^+$, 288.1343; Found 288.1353.

(*S*)-Indolin-1-yl(3-(pyrrolidin-2-ylmethoxy)isoxazol-5-yl)methanone Hydrochloride (17). This compound was obtained from **13** and indoline in two steps employing Method E and C. White solid; yield 78%; purity 99.0%. $[\alpha]_D^{20}$ +11.43 (*c* = 0.14 , MeOH); ^{1}H NMR(400 MHz, DMSO-d_6) δ 9.49 (br s, 2H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.25 (t, *J* = 8.0 Hz, 1H), 7.13 (t, *J* = 8.0 Hz, 1H), 7.01 (s, 1H), 4.57–4.43 (m, 2H), 4.36 (t, *J* = 8.0 Hz, 2H), 4.01–3.91 (m, 1H), 3.22 (m, 4H), 2.21–2.07 (m, 1H),

2.04–1.84 (m, 2H), 1.81–1.67 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.7, 164.1, 154.6, 142.0, 132.8, 127.2, 125.1, 125.0, 116.9, 99.0, 68.9, 57.1, 49.1, 45.0, 27.9, 26.4, 23.2; HRMS (ESI): Calcd for $C_{17}H_{20}N_3O_3$ $[M+H]^+$, 314.1499; Found 314.1522.

(*S*)-5-((Phenylthio)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Trifluoroacetate (18). This compound was obtained from **14** and benzenethiol in two steps employing Method F, D, and Gradient I. White solid; yield 24%; purity 98.4%. $[\alpha]_D^{20}$ +13.49 (*c* = 0.16, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (br s, 1H), 8.77 (s, 1H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.34 (t, *J* = 8.0 Hz, 2H), 7.24 (t, *J* = 8.0 Hz, 1H), 6.11 (s, 1H), 4.40 (m, 1H), 4.36 (s, 2H), 4.30–4.19 (m, 1H), 3.88 (m, 1H), 3.19 (m, 2H), 2.16–2.01 (m, 1H), 2.02–1.78 (m, 2H), 1.78–1.57 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ170.9, 170.6, 158.2 (TFA), 134.3, 129.1, 128.7, 126.6, 116.1 (TFA), 93.9, 68.3, 57.6, 45.3, 27.6, 26.0, 23.3; HRMS (ESI): Calcd for $C_{15}H_{19}N_2O_2S[M+H]^+$, 291.1162; Found, 291.1170.

(*S*)-(3-(Pyrrolidin-2-ylmethoxy)isoxazol-5-yl)methyl phenylcarbamate Trifluoroacetate (19). To a stirred solution of alcohol **14** (0.8 mmol) and DMAP (1.5 mmol) in 20 mL CH_2Cl_2 was added isocyanatobenzene (1.0 mmol) under N_2. After stirring for 5 h at rt, the reaction mixture was evaporated and the residue was purified by flash chromatography to give Boc-protected carbamate processor. The title compound was obtained by employing Method D and Gradient I. White solid; yield 40% in two steps; purity 99.0%. $[\alpha]_D^{20}$ +11.67 (*c* = 0.16, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.92 (br s, 1H), 9.48 (br s, 1H), 8.98 (s, 1H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.38–7.21 (m, 2H), 7.09–6.97 (m, 1H), 6.40 (s, 1H), 5.20 (s, 2H), 4.45 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.33 (dd, *J* = 12.0, 8.0 Hz, 1H), 3.94 (m, 1H), 3.22 (s, 2H), 2.25–2.03 (m, 1H), 2.04–1.81 (m, 2H), 1.82–1.63 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 168.8, 158.3 (TFA), 152.5, 138.6, 128.8, 122.8,118.3, 115.2 (TFA), 95.2, 68.5, 57.5, 56.5, 45.2, 26.0, 23.3; HRMS (ESI): Calcd for $C_{16}H_{20}N_3O_4$ $[M+H]^+$, 318.1448; Found, 318.1444.

(*S*)-5-((2-Fluorophenoxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Hydrochloride (20). This compound was obtained from **14** and 2-fluorophenol in two steps employing Method F and C. White solid; yield 37%; purity 98.0%. $[\alpha]_D^{20}$ +13.32 (*c* = 0.16, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90 (br s,

1H), 9.39 (br s, 1H), 7.37–7.19 (m, 2H), 7.14 (m, 1H), 7.00 (m, 1H), 6.47 (s, 1H), 5.27 (s, 2H), 4.42 (m, 2H), 3.89 (m, 1H), 3.17 (m, 2H), 2.22–1.99 (m, 1H), 1.97–1.73 (m, 2H), 1.78–1.54 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 168.5, 151.7 (d, $J_{C\text{-}F}$ = 248.0 Hz), 145.2 (d, $J_{C\text{-}F}$ =10.0 Hz) 124.8 (d, $J_{C\text{-}F}$ = 4.0 Hz), 122.2 (d, $J_{C\text{-}F}$ = 7.0 Hz), 116.2 (d, $J_{C\text{-}F}$ = 19.2 Hz), 115.6, 95.8, 68.4, 61.3, 57.3, 44.9, 26.1, 23.2; HRMS (ESI): Calcd for $C_{15}H_{17}FN_2O_3$ $[M+H]^+$, 293.1309; Found, 293.1311.

(*S*)-5-((3-Fluorophenoxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Hydrochloride (21). This compound was obtained from **14** and 3-fluorophenol in two steps employing Method F and C. White solid; yield 36%; purity 97.8%. $[\alpha]_D^{20}$ +9.33 (*c* = 0.10, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.05 (m, 1H), 6.78–6.58 (m, 3H), 5.99 (s, 1H), 5.00 (s, 2H), 4.39–4.24 (m, 1H), 4.22–4.15 (m, 1H), 3.71–3.56 (m, 1H), 3.16–2.95 (m, 2H), 2.02–1.94 (m, 1H), 1.93–1.76 (m, 2H), 1.70–1.52 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 168.4, 163.0 (d, $J_{C\text{-}F}$ = 136.2 Hz), 159.1 (d, $J_{C\text{-}F}$ = 10.8 Hz), 130.6 (d, $J_{C\text{-}F}$ = 9.9 Hz), 108.7(d, $J_{C\text{-}F}$ = 11.1 Hz), 102.9 (d, $J_{C\text{-}F}$ = 25.3 Hz), 95.3, 71.8, 61.8, 60.5, 57.2, 46.2, 27.6, 24.9. HRMS(ESI): Calcd for $C_{15}H_{17}FN_2O_3$ $[M+H]^+$, 293.1309; Found, 293.1311.

(*S*)-5-((4-Fluorophenoxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Hydrochloride (22). This compound was obtained from **14** and 4-fluorophenol in two steps employing Method F and C. White solid; yield 36%; purity 98.1%. $[\alpha]_D^{20}$ +10.67 (*c* = 0.10 , MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (br s, 1H), 9.26 (br s, 1H), 7.17 (m, 2H), 7.10–7.01 (m, 2H), 6.44 (s, 1H), 5.18 (s, 2H), 4.48–4.31 (m, 2H), 3.89 (m, 1H), 3.19 (m, 2H), 2.09 (m, 1H), 2.00–1.79 (m, 2H), 1.71 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 169.1, 157.0 (d, $J_{C\text{-}F}$ = 236.9 Hz), 153.7 (d, $J_{C\text{-}F}$ = 2.0 Hz), 116.3 (d, $J_{C\text{-}F}$ = 8.1 Hz), 115.9 (d, $J_{C\text{-}F}$ = 23.2 Hz), 95.6, 68.5, 61.1, 57.4, 45.1, 26.2, 23.3. HRMS (ESI): Calcd for $C_{15}H_{17}FN_2O_3$ $[M+H]^+$, 293.1309; Found, 293.1311.

(*S*)-5-((2-Chlorophenoxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Hydrochloride (23). This compound was obtained from **14** and 2-chlorophenol in two steps employing Method F and C. White solid; yield 71%; purity 98.4%. $[\alpha]_D^{20}$ +14.71 (*c* = 0.14, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (br s,

2H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.33 (m, 2H), 7.02 (m, 1H), 6.48 (s, 1H), 5.31 (s, 2H), 4.60–4.36 (m, 2H), 4.15–3.77 (m, 1H), 3.19 (m, 2H), 2.20–2.04 (m, 1H), 2.01–1.81 (m, 2H), 1.79–1.60 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 168.6, 152.7, 130.2, 128.3, 122.5, 121.5, 114.4, 95.8, 68.4, 61.3, 57.3, 45.0, 26.1, 23.2; HRMS (ESI): Calcd for $C_{15}H_{18}ClN_2O_3$ $[M+H]^+$, 309.1000; Found, 309.1014.

(*S*)-5-((3-Chlorophenoxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Trifluoroacetate (24). This compound was obtained from **14** and 3-chlorophenol in two steps employing Method F, D, and Gradient I. White solid; yield 51%; purity 98.1%. $[\alpha]_D^{20}$ +7.44 (*c* = 0.14, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (br s, 1H), 8.98 (s, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.14 (m, 1H), 7.09–6.97 (m, 2H), 6.44 (s, 1H), 5.24 (s, 2H), 4.44 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.37–4.28 (m, 1H), 3.93 (m, 1H), 3.21 (m, 2H), 2.21–2.04 (m, 1H), 2.03–1.82 (m, 2H), 1.80–1.60 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 168.7, 160.9 (TFA), 158.3, 133.8, 131.0, 121.5, 115.4 (TFA), 114.9, 113.9, 95.6, 68.5, 60.8, 57.5, 45.2, 26.0, 23.3.; HRMS (ESI): Calcd for $C_{15}H_{18}ClN_2O_3$ $[M+H]^+$, 309.1000; Found, 309.1014.

(*S*)-5-((4-Chlorophenoxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Hydrochloride (25). This compound was obtained from **14** and 4-chlorophenol in two steps employing Method F and C. White solid yield 55%; purity 97.1%. $[\alpha]_D^{20}$ +5.95 (*c* = 0.14, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.44 (s, 1H), 8.93 (s, 1H), 7.36 (d, *J* = 12.0 Hz, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 6.44 (s, 1H), 5.21 (s, 2H), 4.44 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.33 (dd, *J* = 12.0, 8.0 Hz, 1H), 3.94 (m, 1H), 2.17–2.06 (m, 1H), 2.02–1.84 (m, 2H), 1.77–1.64 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 168.9, 156.2, 129.3, 125.2, 116.6, 95.5, 68.5, 60.8, 57.6, 45.3, 26.0, 23.3; HRMS (ESI): Calcd for $C_{15}H_{18}ClN_2O_3$ $[M+H]^+$, 309.1000; Found, 309.0996.

(*S*)-5-((3-Bromophenoxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Hydrochloride (26). This compound was obtained from **14** and 3-bromophenol in two steps employing Method F and C. White solid; yield 40%; purity 98.0%. $[\alpha]_D^{20}$ +9.79 (*c* = 0.16, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.60 (s, 1H), 9.11 (s, 1H), 7.35–7.24 (m, 2H), 7.19 (d, *J* = 8.0 Hz, 1H), 7.06 (d, *J* = 8.0 Hz, 1H), 6.47 (s, 1H), 5.25 (s, 2H), 4.44 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.37 (dd, *J* = 12.0, 8.0 Hz 1H), 3.90 (m, 1H), 3.19 (m, 2H), 2.19–

2.02 (m, 1H), 2.02–1.83 (m, 2H), 1.81–1.59 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.7, 168.7, 158.2, 131.4, 124.4, 122.1, 117.5, 114.2, 95.6, 68.3, 60.6, 57.6, 45.2, 25.8, 23.2; HRMS (ESI): Calcd for $C_{15}H_{18}BrN_2O_3$ $[M+H]^+$, 353.0495; Found, 353.0516.

(*S*)-5-((4-Bromophenoxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Hydrochloride (27). This compound was obtained from **14** and 4-bromophenol in two steps employing Method F and C. White solid; yield 34%; purity 98.2%. $[\alpha]_D^{20}$ +12.41 (*c* = 0.19, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (br s, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 2H), 6.45 (s, 1H), 5.22 (s, 2H), 4.45 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.32 (dd, *J* = 12.0, 8.0 Hz, 1H), 3.93 (m, 1H), 3.21 (t, *J* = 6.0 Hz, 1H), 2.18–2.05 (m, 1H), 2.02–1.83 (m, 2H), 1.82–1.61 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 168.8, 158.3 (TFA), 156.6, 132.2, 117.1, 115.6 (TFA), 113.0, 95.5, 68.5, 60.7, 57.6, 45.3, 26.0, 23.3; HRMS (ESI): Calcd for $C_{15}H_{18}BrN_2O_3$ $[M+H]^+$, 353.0495; Found, 353.0523.

(*S*)-3-(Pyrrolidin-2-ylmethoxy)-5-((3-(trifluoromethyl)phenoxy)methyl)isoxazole Trifluoroacetate (28). This compound was obtained from **14** and 3-(trifluoromethyl)phenol in two steps employing Method F, D, and Gradient I. Pale yellow liquid; yield 30%; purity 98.5%. $[\alpha]_D^{20}$ +13.96 (*c* = 0.16, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.59 (br s, 1H), 9.06 (br s, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.37 (m, 2H), 6.47 (s, 1H), 5.32 (s, 2H), 4.45 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.34 (dd, *J* = 10.0, 4.0 Hz, 1H), 3.96 (m, 1H), 3.23 (t, *J* = 6.0 Hz, 2H), 2.18–2.06 (m, 1H), 2.04–1.81 (m, 2H), 1.81–1.61 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 168.6, 158.6 (TFA) , 157.7, 130.8, 130.2 (q, $J_{C\text{-}F}$ = 31.6 Hz), 123.8 (q, $J_{C\text{-}F}$ = 271.0 Hz), 119.0, 118.0 (q, $J_{C\text{-}F}$ = 4.0 Hz), 115.6 (TFA), 111.4 (q, $J_{C\text{-}F}$ = 4.0 Hz), 95.6, 68.4, 60.8, 57.5, 45.2, 26.0, 23.3; HRMS (ESI): Calcd for $C_{16}H_{18}F_3N_2O_3$ $[M+H]^+$, 343.1264; Found, 343.1281.

(*S*)-3-(Pyrrolidin-2-ylmethoxy)-5-((4-(trifluoromethyl)phenoxy)methyl)isoxazole Trifluoroacetate (29). This compound was obtained from **14** and 4-(trifluoromethyl)phenol in two steps as white solid in 30% yield employing Method F, D, and Gradient I. Purity 95.6%. $[\alpha]_D^{20}$ +9.24 (*c* = 0.18, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (br s, 1H), 8.96 (br s, 1H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 12.0

Hz, 2H), 6.48 (s, 1H), 5.32 (s, 2H), 4.45 (dd, J = 12.0, 4.0 Hz, 1H), 4.33 (dd, J = 12.0, 8.0 Hz, 1H), 3.94 (m, 1H), 3.22 (t, J = 8.0 Hz, 2H), 2.19–2.04 (m, 1H), 2.04–1.84 (m, 2H), 1.80–1.64 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 168.6, 163.5 (TFA), 160.2, 127.0 (q, $J_{C\text{-}F}$ = 3.5 Hz), 122.8 (q, $J_{C\text{-}F}$ = 264.4 Hz), 122.0 (q, $J_{C\text{-}F}$ = 32.1 Hz), 116.0 (TFA), 115.3, 95.7, 68.5, 60.7, 57.6, 45.3, 26.0, 23.3; HRMS (ESI): Calcd for $C_{16}H_{18}F_3N_2O_3$ $[M+H]^+$, 343.1264; Found, 343.1296.

(*S*)-5-((3-Methoxyphenoxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Hydrochloride (30). This compound was obtained from **14** and 3-methoxyphenol in two steps employing Method F and C. Pale brown solid; yield 33%; purity 95.6%. $[\alpha]_D^{20}$ +13.49 (c = 0.13, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 9.54 (s, 1H), 7.20 (t, J = 8.0 Hz, 1H), 6.61 (d, J = 8.0 Hz, 2H), 6.56 (d, J = 8.0 Hz, 1H), 6.45 (s, 1H), 5.17 (s, 2H), 4.42 (d, J = 8.0 Hz, 2H), 3.98–3.82 (m, 1H), 3.72 (s, 3H), 3.17 (m, 2H), 2.15–2.03 (m, 1H), 1.99–1.82 (m, 2H), 1.77–1.61 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 169.0, 160.5, 158.6, 130.2, 107.1, 106.8, 101.1, 95.5, 68.4, 60.5, 57.3, 55.1, 44.8, 26.2, 23.2; HRMS (ESI): Calcd for $C_{16}H_{21}N_2O_4$ $[M+H]^+$, 305.1496; Found 305.1517.

(*S*)-5-((4-Methoxyphenoxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Hydrochloride (31). This compound was obtained from **14** and 4-methoxyphenol in two steps employing Method F and C. White solid; yield 55%; purity 98.8%. $[\alpha]_D^{20}$ +10.00 (c = 0.16, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 8.94 (s, 1H), 6.97 (d, J = 12.0 Hz, 2H), 6.87 (d, J = 8.0 Hz, 2H), 6.41 (s, 1H), 5.12 (s, 2H), 4.44 (dd, J = 12.0, 4.0 Hz, 1H), 4.31 (dd, J = 12.0, 8.0 Hz, 1H), 3.94 (m, 1H), 3.70 (s, 3H), 3.21 (t, J = 8.0 Hz, 2H), 2.22–2.01 (m, 1H), 2.03–1.77 (m, 2H), 1.81–1.58 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.7, 169.5, 153.9, 151.3, 115.8, 114.6, 95.2, 68.5, 61.1, 57.6, 55.3, 45.3, 26.1, 23.3; HRMS (ESI): Calcd for $C_{16}H_{21}N_2O_4$ $[M+H]^+$, 305.1496; Found, 305.1517.

(*S*)-3-(Pyrrolidin-2-ylmethoxy)-5-((m-tolyloxy)methyl)isoxazole Hydrochloride (32). This compound was obtained from **14** and 3-methylphenol in two steps employing Method F and C. White solid yield 41%; purity 96.7%. $[\alpha]_D^{20}$ +6.00 (c = 0.10, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (br s, 2H),

7.19 (t, *J* = 8.0 Hz, 1H), 6.83 (m, 3H), 6.43 (s, 1H), 5.17 (s, 2H), 4.52–4.30 (m, 2H), 4.01–3.76 (m, 1H), 3.20 (dd, *J* = 9.4, 5.2 Hz, 2H), 2.28 (s, 3H), 2.15–2.03 (m, 1H), 1.92 (m, 2H), 1.77–1.62 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 169.3, 157.1, 139.2, 129.2, 122.2, 115.2, 111.5, 95.2, 68.2, 60.2, 57.2, 44.9, 25.9, 22.9, 20.9. HRMS (ESI): Calcd for $C_{16}H_{20}N_2O_3$ $[M+H]^+$, 289.1556; Found 289.1547.

(*S*)-5-((Naphthalen-2-yloxy)methyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Trifluoroacetate (33). This compound was obtained from **14** and naphthalen-2-ol in two steps employing Method F, D, and Gradient I. White solid; yield 33%; purity 98.2%. $[\alpha]_D^{20}$ +7.33 (*c* = 0.10, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (br s, 1H), 8.82 (br s, 1H), 7.86 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.49 (m, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 8.0 Hz, 1H), 6.51 (s, 1H), 5.33 (s, 2H), 4.45 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.32 (dd, *J* = 12.0, 8.0 Hz, 1H), 3.94 (m, 1H), 3.21 (t, *J* = 6.0 Hz, 2H), 2.11 (m, 1H), 2.03–1.79 (m, 1H), 1.71 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 169.1, 158.1 (TFA), 155.2, 134.0, 129.5, 128.8, 127.6, 126.8, 126.6, 124.0, 118.4, 113.7 (TFA), 107.5, 95.5, 68.5, 60.6, 57.6, 45.2, 25.9, 23.2. HRMS (ESI): Calcd for $C_{19}H_{20}N_2O_3$ $[M+H]^+$, 325.1565; Found 325.1547.

(*R*)-5-(Phenoxymethyl)-3-(pyrrolidin-2-ylmethoxy)isoxazole Hydrochloride (34). This compound was obtained from **41** and **11** in 5 steps employing Method G, A, H, F, and C. Pale gray solid; yields of each step: 89% (G), 35% (A and H), 60% (F and C); purity 98.8%. $[\alpha]_D^{20}$ -37.10 (*c* = 0.12, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 (t, *J* = 8.0 Hz, 2H), 7.03 (d, *J* = 8.0 Hz, 2H), 6.99 (t, *J* = 6.0 Hz, 1H), 6.45 (s, 1H), 5.19 (s, 2H), 4.53–4.36 (m, 2H), 4.03–3.72 (m, 1H), 3.16 (m, 2H), 2.14–2.02 (m, 1H), 1.99–1.79 (m, 2H), 1.76–1.61 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) 170.8, 169.1, 163.3 (TFA), 157.3, 129.6, 121.4, 115.3 (TFA), 114.7, 95.4, 68.7, 60.4, 57.2, 45.0, 26.3, 23.3. HRMS (ESI): Calcd for $C_{15}H_{18}N_2O_3$ $[M+H]^+$, 275.1390; Found 275.1411.

5-(Phenoxymethyl)-3-(piperidin-4-ylmethoxy)isoxazole Trifluoroacetate (35). This compound was obtained from **42** and **11** in 5 steps employing Method G, A, H, F, D and Gradient I. White solid; yields of each step: 85% (G), 53% (A and H), 55% (F and D); purity 97.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ

8.59 (br s, 1H), 8.27 (s, 1H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 1H), 6.38 (s, 1H), 5.16 (s, 2H), 4.09 (d, *J* = 4.0 Hz, 2H), 3.28 (m, 1H), 2.90 (t, *J* = 12.0 Hz, 2H), 2.09 (m, 1H), 1.87 (d, *J* = 12.0 Hz, 2H), 1.58–1.33 (m, 2H), 1.24 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.3, 169.0, 162.3 (TFA) 157.4, 129.6, 121.4, 114.7, 111.9 (TFA) 95.3, 73.0, 60.5, 42.7, 32.8, 24.9; HRMS (ESI): Calcd for $C_{16}H_{21}N_2O_3$ $[M+H]^+$, 289.1547; Found, 289.1567.

2-((5-(Phenoxymethyl)isoxazol-3-yl)oxy)ethan-1-amine Hydrochloride (36). This compound was obtained from *tert*-butyl (2-hydroxyethyl)carbamate and **11** in 5 steps employing Method G, A, H, F, and C. White solid; yield of each step: 60% (G), 43% (A and H), 30% (F and C) purity 95.3%. ^{1}H NMR (400 MHz, D_2O) δ 7.43–7.39 (m, 2H), 7.13 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 6.30 (s, 1H), 5.23 (s, 2H), 4.50 (t, *J* = 6.0 Hz, 2H), 3.47 (t, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, D_2O) δ 171.3, 169.6, 157.0, 129.9, 122.4, 115.2, 95.4, 66.1, 61.3, 38.6; HRMS (ESI): Calcd for $C_{12}H_{14}N_2O_3$ $[M+H]^+$, 235.1041; Found 235.1057.

3-((5-(Phenoxymethyl)isoxazol-3-yl)oxy)propan-1-amine Hydrochloride (37). This compound was obtained from *tert*-butyl (3-hydroxypropyl)carbamate and **11** in 5 steps employing Method G, A, H, F, and C. White solid; yield of each steps: 90% (G), 50% (A and H), 30% (F and C); purity 98.4%. ^{1}H NMR (400 MHz, D_2O) δ 7.32 (t, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 1H), 7.00 (d, *J* = 8.0 Hz, 2H), 6.16 (s, 1H), 5.12 (s, 2H), 4.26 (t, *J* = 4.0 Hz, 2H), 3.11 (t, *J* = 6.0 Hz, 2H), 2.15–1.96 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 171.7, 169.3, 157.0, 129.9, 122.4, 115.2, 95.4, 67.7, 61.3, 37.0, 26.2. HRMS (ESI): Calcd for $C_{13}H_{16}N_2O_3$ $[M+H]^+$, 249.1234; Found 249.1251.

***N*-Methyl-2-((5-(phenoxymethyl)isoxazol-3-yl)oxy)ethan-1-amine Hydrochloride (38).** This compound was obtained from *tert*-butyl (2-hydroxyethyl)(methyl)carbamate and **11** in 5 steps employing Method G, A, H, F, and C. White solid; yield of each steps: 60% (G), 60% (A and H), 70% (F and C); purity 99.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (br s, 2H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.03 (d, *J* = 8.0 Hz, 2H), 6.99 (t, *J* = 8.0 Hz, 1H), 6.42 (s, 1H), 5.19 (s, 2H), 4.46 (t, *J* = 4.0 Hz, 2H), 3.31–3.25 (m, 2H),

2.59 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 169.2, 157.4, 129.6, 121.5, 114.7, 95.4, 65.4, 60.4, 46.9, 32.7. HRMS (ESI): Calcd for $C_{13}H_{16}N_2O_3$ $[M+H]^+$, 249.1234; Found 249.1250.

***N*-Ethyl-2-((5-(phenoxymethyl)isoxazol-3-yl)oxy)ethan-1-amine Hydrochloride (39).** This compound was obtained from *tert*-butyl ethyl(2-hydroxyethyl)carbamate and **11** in 5 steps employing Method G, A, H, F, and C. White solid; yield of each steps: 80% (G), 50% (A and H), 70% (F and C); purity 99.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (br s, 2H), 7.32 (t, J = 8.0 Hz, 2H), 7.03 (d, J = 8.0 Hz, 2H), 6.99 (t, J = 8.0 Hz, 1H), 6.43 (s, 1H), 5.19 (s, 2H), 4.48 (t, J = 6.0 Hz, 2H), 3.13–2.89 (m, 2H), 2.52 (m, 2H), 1.21 (t, J = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 169.2, 157.4, 129.6, 121.4, 114.7, 95.4, 65.5, 60.5, 45.1, 42.3, 10.8. HRMS (ESI): Calcd for $C_{14}H_{19}N_2O_3$ $[M+H]^+$, 262.1390; Found, 263.1392.

(*S*)-3-((1-Methylpyrrolidin-2-yl)methoxy)-5-(phenoxymethyl)isoxazole Hydrochloride (44). This compound was obtained from **9** employing Method I. Pale yellow liquid; yield 80%; purity 95.6%. $[\alpha]_D^{20}$ +43.75 (c = 0.10, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (br s, 1H), 7.32 (t, J = 8.0 Hz, 2H), 7.03 (d, J = 8.0 Hz, 2H), 6.99 (t, J = 8.0 Hz, 1H), 6.45 (s, 1H), 5.20 (s, 2H), 4.53 (dd, J = 12.0, 4.0 Hz, 1H), 4.44 (dd, J = 12.0, 8.0 Hz, 1H), 3.83 (m, 1H), 3.58 (m, 1H), 3.19–3.06 (m, 1H), 2.92 (s, 3H), 2.31–2.17 (m, 1H), 2.14–2.00 (m, 1H), 1.96–1.77 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.7, 169.4, 157.4, 129.6, 121.5, 114.7, 95.3, 67.6, 66.4, 60.5, 56.5, 40.6, 26.1, 22.0. HRMS (ESI): Calcd for $C_{16}H_{21}N_2O_3$ $[M+H]^+$, 289.1547; Found, 289.1566.

(*S*)-5-((3-Fluorophenoxy)methyl)-3-((1-methylpyrrolidin-2-yl)methoxy)isoxazole Hydrochloride (45). This compound was obtained from **21** employing Method I. White solid; yield 67%; purity 95.7%. $[\alpha]_D^{20}$ +2.33 (c = 0.10, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.15 (m, 1H), 6.79–6.57 (m, 3H), 5.98 (s, 1H), 5.01 (s, 2H), 4.22 (m, 2H), 3.10 (m, 1H), 2.59 (m, 1H), 2.42 (s, 3H), 2.26 (m, 1H), 2.03–1.63 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 168.0, 163.6 (d, $J_{C\text{-}F}$ = 247.5 Hz), 159.0 (d, $J_{C\text{-}F}$ = 11.0 Hz), 130.5 (d, $J_{C\text{-}F}$ = 9.1 Hz), 110.4, 108.7 (d, $J_{C\text{-}F}$ = 21.2 Hz), 102.8 (d, $J_{C\text{-}F}$ = 25.3 Hz), 95.3, 71.7, 64.0, 61.8, 57.7, 41.4, 28.3, 22.8. HRMS(ESI): Calcd for $C_{16}H_{20}FN_2O_3$ $[M+H]^+$, 307.1452; Found, 307.1467.

(*S*)-5-((3-Chlorophenoxy)methyl)-3-((1-methylpyrrolidin-2-yl)methoxy)isoxazole Hydrochloride (46). This compound was obtained from **24** employing Method I. Colorless oil; yield 49%; purity 97.5%. $[\alpha]_D^{20}$ +5.68 (*c* = 0.10, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (t, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 6.92 (s, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 5.97 (s, 1H), 5.00 (s, 2H), 4.27–4.18 (m, 2H), 3.09 (t, *J* = 8.0 Hz, 1H), 2.61–2.56 (m, 1H), 2.41 (s, 3H), 2.26 (dd, *J* = 16.0, 8.0 Hz, 1H), 1.97–1.68 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 167.8, 158.0, 134.7, 130.3, 121.9, 115.3, 112.9, 95.1, 71.5, 63.8, 61.5, 59.2, 41.2, 27.8, 22.7. HRMS (ESI): Calcd for $C_{16}H_{20}ClN_2O_3$ $[M+H]^+$, 323.1157; Found, 323.1150.

(*R*)-3-((1-Methylpyrrolidin-2-yl)methoxy)-5-(phenoxymethyl)isoxazole Hydrochloride (47). This compound was obtained from **34** employing Method I. White solid; yield 77%; purity 98.6%. $[\alpha]_D^{20}$ -20.31 (*c* = 0.32, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 (t, *J* = 8.0 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 2H), 6.99 (t, *J* = 8.0 Hz, 1H), 6.38 (s, 1H), 5.16 (s, 2H), 4.15 (dd, *J* = 12.0, 8.0 Hz, 1H), 4.04 (dd, *J* = 12.0, 8.0 Hz, 1H), 2.99–2.87 (m, 1H), 2.61–2.52 (m, 1H), 2.32 (s, 3H), 2.18 (q, *J* = 8.6 Hz, 1H), 2.00–1.84 (m, 1H), 1.74–1.62 (m, 2H), 1.62–1.49 (m, 1H).; ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.5, 168.8, 157.4, 129.6, 121.4, 114.7, 95.3, 72.2, 63.2, 60.5, 56.9, 41.1, 28.0, 22.5. HRMS (ESI): Calcd for $C_{16}H_{21}N_2O_3$ $[M+H]^+$, 289.1547; Found, 289.1556.

3-((1-Methylpiperidin-4-yl)methoxy)-5-(phenoxymethyl)isoxazole Hydrochloride (48). This compound was obtained from **35** employing Method I. White solid; yield 66%; purity 98.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (br s, 1H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.03 (d, *J* = 8.0 Hz, 2H), 7.01–6.92 (m, 1H), 6.40 (s, 1H), 5.17 (s, 2H), 4.08 (m, 2H), 3.39 (m, 2H), 3.09–2.84 (m, 2H), 2.69 (s, 3H), 2.03 (m, 1H), 1.98–1.77 (m, 2H), 1.70–1.48 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.5, 168.8, 157.4, 129.6, 121.4, 114.7, 95.3, 74.0, 60.5, 54.7, 46.1, 34.6, 28.1. HRMS (ESI): Calcd for $C_{17}H_{23}N_2O_3$ $[M+H]^+$, 303.1703; Found, 303.1703.

***N*,*N*-Dimethyl-2-((5-(phenoxymethyl)isoxazol-3-yl)oxy)ethan-1-amine Hydrochloride (49).** This compound was obtained from **38** employing Method I. White solid; yield 68%; purity 98.4%. ^{1}H NMR

(400 MHz, $CDCl_3$) δ 10.84 (br s, 1H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.03 (d, *J* = 12.0 Hz, 2H), 6.99 (t, *J* = 8.0 Hz, 1H), 6.44 (s, 1H), 5.19 (s, 2H), 4.58 (t, *J* = 4.0 Hz, 2H), 3.52 (t, *J* = 6.0 Hz, 2H), 2.81 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 169.3, 157.4, 129.6, 114.7, 95.4, 64.4, 60.4, 54.9, 42.6. HRMS (ESI): Calcd for $C_{14}H_{19}N_2O_3$ $[M+H]^+$, 263.1390; Found 263.1393.

***N*-Ethyl-*N*-methyl-2-((5-(phenoxymethyl)isoxazol-3-yl)oxy)ethan-1-amine Hydrochloride (50).** This compound was obtained from **39** employing Method I. White solid; yield 67%; purity 98.6%. ^{1}H NMR (400 MHz, D_2O) δ 7.32 (t, *J* = 8.0 Hz, 2H), 7.03 (t, *J* = 4.0 Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 2H), 6.20 (s, 1H), 5.13 (s, 2H), 4.50 (t, *J* = 4.0 Hz, 2H), 3.70–3.56 (m, 1H), 3.53–3.41 (m, 1H), 3.36–3.24 (m, 1H), 3.23–3.10 (m, 1H), 2.85 (s, 3H), 1.25 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ 171.0, 169.7, 157.0, 129.9, 122.4, 115.2, 95.3, 63.7, 61.2, 53.7, 51.8, 39.4, 8.4. HRMS (ESI): Calcd for $C_{15}H_{21}N_2O_3$ $[M+H]^+$, 277.1547; Found, 277.1561.

***N*-Methyl-*N*-(2-((5-(phenoxymethyl)isoxazol-3-yl)oxy)ethyl)cyclobutanamine Hydrochloride (51).** This compound was obtained from **38** and cyclobutanone employing Method J. White solid; yield 54%; purity 97.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 1H), 7.36–7.28 (m, 2H), 7.03(d, *J* = 8.0 Hz, 2H), 6.99 (t, *J* = 8.0 Hz, 1H), 6.45 (s, 1H), 5.19 (s, 2H), 4.56 (m, 2H), 3.81–3.68 (m, 1H), 2.67 (s, 3H), 2.38–2.24 (m, 2H), 2.17 (m, 2H), 1.68 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.9, 169.6, 157.0, 129.9, 122.3, 115.0, 95.4, 63.6, 61.1, 59.9, 52.0, 36.8, 25.9, 12.6. HRMS (ESI): Calcd for $C_{17}H_{23}N_2O_3$ $[M+H]^+$, 303.1703; Found, 303.1711.

***N*-Methyl-*N*-(2-((5-(phenoxymethyl)isoxazol-3-yl)oxy)ethyl)cyclopentanamine Hydrochloride (52).** This compound was obtained from **38** and cyclopentanone employing Method J. White solid; yield 58%; purity 97.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.11 (s, 1H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.05–6.98 (m, 3H), 6.46 (s, 1H), 5.20 (s, 2H), 4.70–4.50 (m, 2H), 3.65–3.56 (m, 2H), 3.47 (m, 1H), 2.78 (d, *J* = 4.0 Hz, 3H), 2.03–1.97 (m, 2H), 1.82–1.71 (m, 4H), 1.55–1.53 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.6, 169.3, 157.4, 129.6, 121.4, 114.7, 95.4, 66.4, 64.3, 60.6, 52.6, 38.3, 27.7, 27.2, 23.5. HRMS (ESI): Calcd for $C_{18}H_{25}N_2O_3$ $[M+H]^+$, 317.1860; Found, 317.1856.

***N*-(Cyclopropylmethyl)-*N*-methyl-2-((5-(phenoxymethyl)isoxazol-3-yl)oxy)ethan-1-amine Hydrochloride (53).** This compound was obtained from **38** and cyclopropanecarboxaldehyde employing Method J. White solid; yield 60%; purity 97.8% ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.18 (m, 2H), 7.00 (t, *J* = 8.0 Hz, 1H), 6.94 (d, *J* = 4.0 Hz, 2H), 5.99 (s, 1H), 5.03 (s, 2H), 4.35 (t, *J* = 6.0 Hz, 2H), 2.86 (t, *J* = 4.0 Hz, 2H), 2.39 (s, 3H), 2.34 (d, *J* = 4.0 Hz, 2H), 0.88 (m, 1H), 0.59–0.47 (m, 2H), 0.11 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 168.9, 157.9, 129.8, 122.0, 115.0, 95.4, 67.8, 63.1, 61.8, 55.9, 42.9, 8.8, 4.1. HRMS (ESI): Calcd for $C_{17}H_{22}N_2O_3$ $[M+H]^+$, 303.1703; Found 303.1701.

***N*-Methyl-*N*-(2-((5-(phenoxymethyl)isoxazol-3-yl)oxy)ethyl)cyclohexanamine Hydrochloride (54).** This compound was obtained from **38** and cyclohexanone employing Method J. White solid; yield 77%; purity 99.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.27 (m, 2H), 7.00 (t, *J* = 8.0 Hz, 1H), 6.94 (d, *J* = 8.0 Hz, 2H), 5.97 (s, 1H), 5.03 (s, 2H), 4.30 (t, *J* = 4.0 Hz, 2H), 2.84 (t, *J* = 6.0 Hz, 2H), 2.44–2.37 (m, 1H), 2.35 (s, 3H), 1.84–1.75 (m, 4H), 1.62 (d, *J* = 12.0 Hz, 1H), 1.28–1.02 (m, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 168.6, 157.7, 129.6, 121.8, 114.8, 95.2, 68.4, 63.1, 61.6, 51.8, 38.7, 28.6, 26.3, 26.0. HRMS (ESI): Calcd for $C_{19}H_{26}N_2O_3$ $[M+H]^+$, 331.2016; Found 331.2028.

***N*-(Cyclopropylmethyl)-2-((5-((3-fluorophenoxy)methyl)isoxazol-3-yl)oxy)-*N*-methylethan-1-amine Hydrochloride (55).** Colorless oil; yield 50%; purity 98.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (m, 1H), 6.72 (dd, *J* = 8.0, 4.0 Hz, 2H), 6.66 (m, 1H), 5.98 (s, 1H), 5.01 (s, 2H), 4.35 (t, *J* = 6.0 Hz, 2H), 2.85 (t, *J* = 6.0 Hz, 2H), 2.38 (s, 3H), 2.33 (d, *J* = 6.0 Hz, 2H), 0.89 (m, 1H), 0.51 (m, 2H), 0.10 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 168.0, 163.5 (d, $J_{C\text{-}F}$ = 247.5 Hz), 159.0 (d, $J_{C\text{-}F}$ = 11.1 Hz), 130.4 (d, $J_{C\text{-}F}$ = 10.1 Hz), 110.4 (d, $J_{C\text{-}F}$ = 3.0 Hz), 108.7 (d, $J_{C\text{-}F}$ = 21.2 Hz), 102.8 (d, $J_{C\text{-}F}$ = 25.3 Hz), 95.4 , 67.7, 62.9, 61.8, 55.7, 42.7, 8.6, 3.9. HRMS (ESI): Calcd for $C_{17}H_{22}FN_2O_3$ $[M+H]^+$, 321.1609; Found, 321.1614.

2-((5-((3-Chlorophenoxy)methyl)isoxazol-3-yl)oxy)-*N*-(cyclopropylmethyl)-*N*-methylethan-1-amine Hydrochloride (56). Colorless oil; yield 50%; purity 98.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (t, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 2H), 6.82 (m, 1H), 5.97 (s, 1H), 5.00 (s, 2H), 4.34 (t, *J* = 6.0 Hz, 2H),

2.84 (t, J = 6.0 Hz, 2H), 2.38 (s, 3H), 2.33 (d, J = 8.0 Hz, 2H), 0.93–0.80 (m, 1H), 0.50 (m, 2H), 0.10 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 167.9, 158.4, 135.0, 130.4, 122.1, 115.4, 113.1, 95.4, 67.7, 62.9, 61.7, 55.7, 42.7, 8.6, 3.9. HRMS (ESI): Calcd for $C_{17}H_{22}ClN_2O_3$ $[M+H]^+$, 337.1327; Found, 337.1313.

***N*-(2-((5-((3-Fluorophenoxy)methyl)isoxazol-3-yl)oxy)ethyl)-*N*-methylcyclohexanamine Hydrochloride (57).** Colorless oil ; yield 50%; purity 95.7%.1H NMR (400 MHz, $CDCl_3$) δ 7.26–7.20 (m, 1H), 6.71 (dd, J = 8.0, 4.0 Hz, 2H), 6.66 (m, 1H), 5.97 (s, 1H), 5.01 (s, 2H), 4.30 (t, J = 6.0 Hz, 2H), 2.84 (t, J = 6.0 Hz, 2H), 2.43–2.37 (m, 1H), 2.34 (s, 3H), 1.81–1.78 (m, 5H), 1.62 (m, 1H), 1.25–1.18 (m, 4H), 1.13–1.00 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 168.0, 163.6 (d, $J_{C\text{-}F}$ = 247.5 Hz), 159.0 (d, $J_{C\text{-}F}$ = 11.1 Hz), 130.4 (d, $J_{C\text{-}F}$ = 10.1 Hz), 110.4, 108.7 (d, $J_{C\text{-}F}$ = 21.2 Hz), 102.8 (d, $J_{C\text{-}F}$ = 25.3 Hz), 95.3, 68.5, 63.1, 61.8, 51.8, 38.7, 28.6, 26.3, 26.0. HRMS (ESI): Calcd for $C_{19}H_{26}FN_2O_3$ $[M+H]^+$, 349.1922; Found, 349.1934.

***N*-(2-((5-((3-Chlorophenoxy)methyl)isoxazol-3-yl)oxy)ethyl)-*N*-methylcyclohexanamine Hydrochloride (58).** Colorless oil; yield 49%; purity 95.1%. ^{1}H NMR (400 MHz, D_2O) δ 7.24 (t, J = 8.0 Hz, 1H), 7.02–7.01 (m, 2H), 6.90 (d, J =8.0 Hz, 1H), 6.21 (s, 1H), 5.11 (s, 2H), 4.50 (m, 2H), 3.49 (m, 2H), 3.25 (t, J = 12.0 Hz, 1H), 2.80 (s, 3H), 1.92 (m, 2H), 1.81 (m, 2H), 1.58 (m, 1H), 1.42 (m, 2H), 1.24 (m, 2H), 1.08 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 170.9, 169.1, 157.9, 134.4, 130.8, 122.1, 115.5, 113.4, 95.5, 65.3, 64.2, 61.3, 51.3, 36.8, 26.1, 24.5, 24.4. HRMS (ESI): Calcd for $C_{19}H_{26}ClN_2O_3$ $[M+H]^+$, 365.1626; Found, 365.1627.

4-(2-((5-(Phenoxymethyl)isoxazol-3-yl)oxy)ethyl)morpholine Hydrochloride (59). To a stirred solution of **36** (100 mg, 0.45 mmol), 1-chloro-2-(2-chloroethoxy)ethane (280 mg, 2mmol) ,and NaI (130 mg, 0.86 mmol) in anhydrous DMF (5 mL) was added K_2CO_3 (500 mg , 4 mmol) at 80 °C under N_2. After stirring overnight at 80 °C, the reaction mixture was cooled to rt and 20 mL of water was added. The resulting mixture was extracted with EtOAc (3×30 mL) and the combined organic layers were washed with water (3×20 mL) and brine (15 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue

was purified by flash chromatography. To the solution of purified compound in CH_2Cl_2 (3 mL) was added HCl/EtOAc (4 mol/L, 1 mL) under N_2 with ice cooling. The mixture was stirred overnight at rt. After the solvent was evaporated, the residue was triturated with diethyl ether (10 mL). The resultant solid was filtered off to give the HCl salt. White solid; yield 31%; purity 98.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (m, 2H), 7.00 (t, *J* = 8.0 Hz, 1H), 6.94 (d, *J* = 8.0 Hz, 2H), 5.98 (s, 1H), 5.03 (s, 2H), 4.37 (t, *J* = 4.0 Hz, 2H), 3.72 (t, *J* = 4.0 Hz, 4H), 2.77 (t, *J* = 4.0 Hz, 2H), 2.53 (t, *J* = 4.0 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 168.8, 157.7, 129.6, 121.9, 114.8, 95.0, 67.1, 66.8, 61.6, 57.3, 53.9. HRMS (ESI): Calcd for $C_{16}H_{20}N_2O_4$ $[M+H]^+$, 305.1496; Found 305.1518.

***tert*-Butyl 3-bromo-4,5-dihydroisoxazole-5-carboxylate (62).** To a stirred solution of **60** (2.1 g, 10 mmol) in DMF (10 mL) was added 5 mL of *tert*-butyl acrylate (**61**) at 0 °C and subsequently 8 mL of saturated $KHCO_3$ solution at -10 °C. After stirring for 4h at rt, HCl (1 M, 20 mL) was added to the reaction mixture. The resulting mixture was extracted with MTBE (3 × 20 mL) and the combined organic layers were washed with water (20 mL) and brine (15 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography to give the title compound as a colorless oil in 46% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.97 (dd, *J* = 8.0, 8.0 Hz, 1H), 3.46 (d, *J* = 4.0 Hz, 1H), 3.44 (m, 1H), 1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8, 136.5, 83.3, 79.0, 44.7, 28.0.

***tert*-Butyl 3-(4-(tert-butoxycarbonyl)piperazin-1-yl)isoxazole-5-carboxylate (63).** To a stirred solution of **62** (1.0 g, 4 mmol) and *tert*-butyl piperazine-1-carboxylate (900 mg, 4.8 mmol) in 16 mL of *tert*-butyl alcohol was added a 10 mL aqueous solution of Na_2CO_3 (1.0 g, 9.4 mmol). After stirring overnight at 80 °C, the resulting mixture was extracted with MTBE (3×20 mL), and the combined organic layers were washed with water (20 mL) and brine (15 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography to give *tert*-butyl 3-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-4,5-dihydroisoxazole-5-carboxylate 500 mg, which was dissolved in 5 mL toluene. Imidazole (280 mg, 4.2 mmol) and I_2 (500 mg, 2.1 mmol) were then added in sequence. After stirring at 120 °C for 4 h, the reaction mixture was cooled to rt, diluted with EtOAc and treated with

$Na_2S_2O_6$ (10% aq). The aqueous layer was extracted with EtOAc (2×15 mL). The combined organic layers were washed with brine (15 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography to give the title compound as a white solid in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.49 (s, 1H), 3.53 (m, 4H), 3.27 (m, 4H), 1.58 (s, 9H), 1.48 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 161.3 , 156.0, 154.6, 99.6, 83.8, 80.2, 47.1, 28.4, 28.0.

***tert*-Butyl 4-(5-(iodomethyl)isoxazol-3-yl)piperazine-1-carboxylate (64).** This compound was obtained from **63** employing Method A and H. White solid; yield 43%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.94 (s, 1H), 4.27 (s, 2H), 3.52 (m, 4H), 3.24 (m, 4H), 1.48 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.6, 167.0, 154.6, 93.7, 80.2, 47.0, 28.4, -12.4.

5-(Phenoxymethyl)-3-(piperazin-1-yl)isoxazole Hydrochloride (65). This compound was obtained from **64** employing Method F and C. White solid; yield 45%; purity 97.0%. ^{1}H NMR (400 MHz, D_2O) δ 7.28 (t, J = 8.0 Hz, 2H), 7.09 (d, J = 8.0 Hz, 1H), 7.05 (d, J = 8.0 Hz, 2H), 6.23 (s, 1H), 5.09 (s, 2H), 3.55–3.30 (m, 4H), 3.35–3.07 (m, 4H), 1.46 (s, 1H). ^{13}C NMR (101 MHz, D_2O) δ 168.7, 166.5, 129.9, 122.4, 115.2, 100.0, 95.4, 61.2, 43.8, 42.3. HRMS (ESI): Calcd for $C_{14}H_{17}N_3O_2$ $[M+H]^+$, 260.1394; Found 260.1416.

5-((3-Chlorophenoxy)methyl)-3-(piperazin-1-yl)isoxazole Hydrochloride (66). White solid; yield 60%; purity 98.9%. ^{1}H NMR (400 MHz, D_2O) δ 7.36 (t, J = 8.0 Hz, 1H), 7.13 (m, 2H), 7.01 (d, J = 8.0 Hz, 1H), 6.37 (s, 1H), 5.20 (s, 2H), 3.58 (m, 4H), 3.39 (m, 4H). ^{13}C NMR (101 MHz, D_2O) δ 168.3, 166.0, 157.7, 134.5, 130.8, 122.3, 115.5, 113.7, 95.5, 61.4, 43.8, 42.4. HRMS (ESI): Calcd for $C_{14}H_{17}ClN_3O_2$ $[M+H]^+$, 294.1004; Found, 294.1032.

3-(4-Methylpiperazin-1-yl)-5-(phenoxymethyl)isoxazole Hydrochloride (67). This compound was obtained from **65** employing Method I. White solid; yield 65%; purity 96.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 2H), 7.00 (t, J = 8.0 Hz, 1H), 6.94 (d, J = 8.0 Hz, 2H), 5.98 (s, 1H), 5.03 (s, 2H), 3.38–3.19 (m, 4H), 2.56–2.45 (m, 4H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1, 167.2, 158.0, 129.8,

121.9, 114.9, 94.4, 61.9, 54.4, 47.3, 46.5. HRMS (ESI): Calcd for $C_{15}H_{19}N_3O_2$ $[M+H]^+$, 274.1550; Found 274.1574.

4-(5-(Phenoxymethyl)isoxazol-3-yl)morpholine Hydrochloride (68). White solid; yield 67%; purity 96.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.28 (m, 2H), 7.00 (t, *J* = 6.0 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 2H), 5.98 (s, 1H), 5.05 (s, 2H), 3.82–3.76 (m, 4H), 3.29–3.22 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 167.2, 157.8, 129.7, 121.8, 114.8, 93.9, 66.2, 61.7, 47.5. HRMS (ESI): Calcd for $C_{14}H_{16}N_2O_3$ $[M+H]^+$, 261.1234; Found 261.1239.

5-(Phenoxymethyl)-3-(piperidin-1-yl)isoxazole Hydrochloride (69). White solid; yield 68%; purity 97.1 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 2H), 7.00 (t, *J* = 6.0 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 2H), 5.97 (s, 1H), 5.03 (s, 2H), 3.24 (m, 4H), 1.67–1.60 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7, 167.5, 158.1, 129.6, 121.9, 115.0, 94.5, 62.0, 48.5, 25.2, 24.3. HRMS (ESI): Calcd for $C_{15}H_{18}N_2O_2$ $[M+H]^+$, 259.1441; Found 259.1434.

***In Vitro* Binding Studies.** Radioligand competition studies were carried out by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). For experimental details please refer to the PDSP website http://pdsp.med.unc.edu/.

General Procedures for Behavioral Studies.

Animals. Chinese Kun Ming (KM) Mice weighted 22–27 g were used in behavioral and PK studies. All animal experiments conformed to the regulations drafted by the Association for Assessment and Accreditation of Laboratory Animal Care in Shanghai and were approved by the East China Normal University Center for Animal Research and Institutional Animal Care and Use Committees. The animals were obtained from Shanghai SLAC Laboratory Animal Co, Ltd (Shanghai, China). All animal experiments were performed in a blinded manner, ie, administration of drugs and behavioral assessments were performed by different investigators.

Drugs. Compounds **53** and **4** were synthesized according to procedures described in the text and literature.[21] Pregabalin was purchased from Energy Chemical, China. All compounds were dissolved in injectable water and administrated intraperitoneally (ip) in a volume of 10 mL/kg.

Formalin Test. KM mice were randomly grouped and allowed to habituate for at least 30 minutes in a transparent observation chamber before the experiment. The animals were given a subcutaneous injection of formalin (1%, 20 μL) into the plantar of the left hind paw. Formalin-induced nociception was assessed by scoring pain behaviors and licking time during a 45-minute observation. Phases were defined as follows: phase I (0–10 minutes), and phase II (11–45 minutes). Compounds **53** or **4** was administrated intraperitoneally 15 min before the injection of formalin.

Rotarod Test. The motor performance of mice was assessed by an automated rotarod (JLBehv-RRTG, China). Mice were trained, and those which could not stay moving on the rod for 300 s in 10 rpm were omitted from the study before drug treatment. In the test, mice were required to walk against the motion of an elevated rotating drum at 10 rpm and the latencies to fall-down were recorded. With the selected animals, rotarod latencies were measured 30, 60, 90, and 120 min after ip administration of drugs.

***In vitro* PAMPA.**

The ability of compounds to cross the cell membrane was predicted and evaluated using PAMPA. Tested compounds were dissolved in DMSO at 5 mg/mL as stock solutions. A 10 μL sample of stock solution was diluted in PBS to make a secondary stock solution (final concentration 25 mg/mL). These solutions were filtered and a 300 μL sample of secondary stock solution was added to the donor well. The porcine polar brain lipid was dissolved in dodecane at 20 mg/mL. The filter membrane was coated with 4 μL of porcine polar brain lipid solution, and the acceptor well was filled with 150 μL of PBS. The acceptor filter plate was carefully put on the donor plate to form a “sandwich” which was composed of the donor with tested compounds on the bottom, artificial lipid membrane in the middle, and the acceptor on the top. The sandwich was incubated undisturbed at room temperature for 18 h. The donor plate was removed after

incubation. The concentrations of tested compounds in the acceptor and reference solutions were determined by a UV plate reader. Reference solutions were prepared by diluting the sample secondary stock solution to the same concentration as that with no membrane barrier. Every sample was analyzed at three wavelengths in three wells, and in three independent runs.

Pharmacokinetics Study in Mice. Compound **53** (40 mg/kg) was orally administered to KM mice before collection of plasma and brain samples at the following time points: 0.167, 0.5, 1, 2, 4, 6, and 8 h (n = 3/group). Aliquots of 10.0 μL of real samples, calibration curve samples and QC samples were supplemented with 200 μL of precipitant. After vortexed for 3.00 min and centrifuged at 12000 rpm for 3.00 min, the supernatant 75.0 μL was transfered into 96-well plate with equal volume water, after vortex. The mixture solution (10 μL) were injected for LC-MS/MS analysis.

Statistical Analysis. Data are expressed as the mean ± SEM. Multiple group comparisons for formalin test were performed using unpaired, two-tailed t-test. Multiple group comparisons for rotarod test were performed by two-way ANOVA followed by Bonferroni posttest. Differences were considered statistically significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

ASSOCIATED CONTENT

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all final compounds, PAMPA permeability of selected compounds, and functional profile of compound **53**.

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ABBREVIATIONS

CNS, central nervous system; AD, Alzheimer's disease; ADHD, attention deficit hyperactivity disorder; MAM, mitochondrial-associated endoplasmic reticulum membrane; ER, endoplasmic reticulum; IP_3, inositol 1,4,5-trisphosphate; BiP, binding-immunoglobulin protein; PGRMC1, progesterone receptor membrane component 1; DMT, *N*,*N*-dimethyltryptamine; NIMH-PDSP, National Institute of Mental Health-Psychoactive Drug Screening Program; ADME-Tox, absorption, distribution, metabolism, excretion, and toxicity; nAChR(s), nicotinic acetylcholine receptor(s); DAT, dopamine transporter; NET, norepinephrine transporter; SERT, serotonin transporter; SAR, structure-activity relationship; BBB, blood brain barrier; CC, column chromatography; rt, room temperature; TFA, trifluoroacetic acid.

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