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A novel class of 3-(phenoxy-phenyl-methyl)-pyrrolidines as potent and balanced norepinephrine and serotonin reuptake inhibitors: Synthesis and structure–activity relationships

Lori Jean Van Orden *, Priscilla M. Van Dyke, D. Roland Saito, Timothy J. Church, Ray Chang, Jacqueline A. M. Smith, William J. Martin, Sarah Jaw-Tsai, Eric L. Stangeland

Theravance, Inc., 901 Gateway Blvd, South San Francisco, CA 94080, USA

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ABSTRACT

A series of 3-(phenoxy-phenyl-methyl)-pyrrolidine analogues were discovered to be potent and balanced norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors. Several of these compounds were identified to have suitable in vitro pharmacokinetic properties for an orally dosed and CNS-targeted drug. Compound **39b**, in particular, was identified as a potent NET and SERT reuptake inhibitor (NSRI) with minimal off-target activity and demonstrated robust efficacy in the spinal nerve ligation model of pain behavior.

Monoamine reuptake inhibitors alleviate the symptoms associated with a variety of disorders of the central nervous system including multi-factorial depression, anxiety, and obsessive-compulsive disorder.[1] Whereas these disorders are most commonly treated by selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine[2] (**1**), some clinical studies suggest that compounds which inhibit both the serotonin transporter (SERT) and the norepinephrine transporter (NET) may be more effective in treating major depressive disorder.[3] Moreover, the clinical benefits of these dual SERT and NET reuptake inhibitors (SNRIs) may extend into other CNS-associated disorders such as chronic pain.[4] For example, meta-analyses suggest that compounds that inhibit norepinephrine uptake (such as the tricyclic antidepressants) and the dual serotonin-norepinephrine reuptake inhibitors offer greater analgesic benefit than the SSRIs.[5] In addition to analgesic activity, NET inhibition is reported to offer advantages with respect to other symptom domains relevant in chronic pain such as cognitive function and motivation/vitality.[6] Consistent with a potentially beneficial role of norepinephrine in managing pain, the SNRIs duloxetine (**2**) and milnacipran (**3**), are approved for the treatment of chronic pain syndromes such as painful diabetic peripheral neuropathy (DPNP),[7] chronic musculoskeletal pain and/or fibromyalgia.[8] Despite being termed an SNRI, duloxetine exhibits a SERT-selective profile[9] and the most common adverse events reported for duloxetine during clinical trials[10] are consistent with the most frequently observed dose-limiting side effects of the SSRIs,[11] namely nausea, somnolence, and fatigue. Milnacipran is reported to be a modestly NET-selective inhibitor[9b] and shows analgesic activity with a lower incidence of fatigue.[8,12] However, milnacipran demonstrates a propensity for efflux by the P-glycoprotein (P-gp) transporter,[13] which limits its CNS exposure and generates a requirement for relatively high concentrations in the plasma, increasing the risk for peripherally mediated adverse events.[12] Taken together, the data on duloxetine and milnacipran suggest that a highly CNS-penetrant, slightly NET-selective dual reuptake inhibitor (NSRI) may offer robust analgesic efficacy with an improved tolerability profile. Therefore, we sought to identify a novel NSRI to overcome the potential limitations of currently marketed agents.

A variety of chemotypes have been reported to inhibit monoamine reuptake. Acyclic methylaminopropane scaffolds are common in many reported SERT and NET inhibitors, such as fluoxetine (Fig. 1).[14] These chemotypes undergo metabolic N-demethylation, which complicates compound development because both the parent and demethylated compounds are active monoamine reuptake inhibitors.[15] Several cyclic amine variants have also been explored which presumably avoid this particular metabolic pathway.[16] For example, Orjales et al.[16a] and Fish et al.[16b] each described series of 3- and 4-substituted piperidinyl-derived SNRIs (Fig. 1) that are differentiated by a core phenyl (**5**

* Corresponding author.
E-mail address: lvanorden@theravance.com (L.J. Van Orden).

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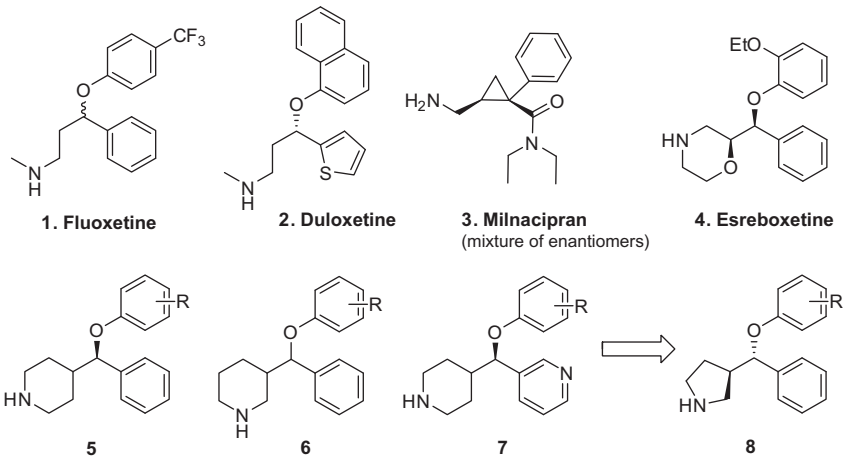
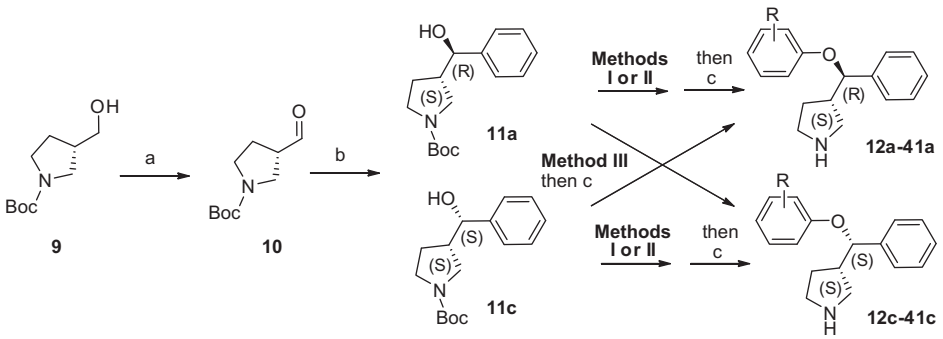


Figure 1. Selected monoamine reuptake inhibitors.

and **6)** or pyridyl moiety (**7**). The latter group reported that the pyridyl unit imparted lower lipophilicity, a design strategy used to circumvent off-target activity and microsomal instability. Our own studies on a small subset of these pyridyl-containing compounds however, revealed a high propensity for efflux by the P-gp transporter, and were therefore determined to be sub-optimal for targeting the CNS. In each of these studies, the aryl ethers were mostly limited to monosubstitution and/or 2-alkoxy-aryl ether compounds. At the time of these reports, we were concurrently developing our own series of NSRIs on a related pyrrolidine series (**8**) with a particular focus on the aryl ether substituents. Since there is generally a large degree of pharmacophore overlap between compounds which inhibit NET and SERT with those that also inhibit the dopamine reuptake transporter (DAT), our in vitro screening paradigm also included DAT. After assessing key in vitro pharmacokinetic (PK) parameters, a preclinical model of persistent pain (rat formalin model, RFM)[17] was used to investigate compounds of interest for in vivo efficacy. The spinal nerve ligation model (SNL)[18] was used as a second model for the assessment of pain reversal and also to understand the oral PK-PD of our compounds. Collectively, these assessments led to the discovery of several potent, balanced, monoamine reuptake inhibitors, the most promising of which was determined to be NSRI, **39b**.

A robust and concise asymmetric synthesis of the compounds described in this report is depicted in Scheme 1. Similar syntheses were also implemented to access some of the related small molecule scaffolds referred to above.[16] In contrast to one of these reports,[16c] we found that the commercially available *S*-alcohol **9** was only effectively oxidized to the corresponding aldehyde **10** using radical-mediated TEMPO oxidation. Alternative procedures such as the Swern or Parikh–Doering oxidation provided significant amounts of racemized material.[19] Nucleophilic addition of phenylmagnesium bromide to the chiral aldehyde **10** afforded approximately a 1:1 mixture of **11a** and **11c**, which were separable by chromatography. Each of these building blocks were subjected to either copper(I)-mediated coupling conditions[20] with aryl iodides (Method I) or nucleophilic aromatic substitution with aryl fluorides (Method II) followed by treatment with ethanolic hydrogen chloride to afford the corresponding aryl ethers **12a**–**45a** or **12c**–**45c** with retained stereochemistry. We were also able to make use of the wide variety of commercially available substituted phenols by subjecting either **11a** or **11c** to modified Mitsunobu[21] conditions (Method III), which would provide the inverted ether stereocenter. The RS (**12b**–**45b**) and *RR* (**12d**–**45d**) analogues were prepared using the same method described in Scheme 2 but with substitution of **10** with (*R*)-3-formyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester as the starting aldehyde.

Crystallization of **11c**, followed by X-ray crystal structure analysis enabled us to unambiguously assign the stereochemical configuration of this key intermediate. As shown in Figure 2, the computer-generated representation of the crystallographic information for structure **11c** confirms that our 2nd eluting intermediate, after performing reverse phase HPLC chromatography, possesses *SS* stereochemistry.[22] With this information, the stereochemistry of final compounds could then be assigned based on knowledge of the reaction conducted at the aryl ether position:



Scheme 1. Asymmetric synthesis of NSRIs. Reagents and conditions: (a) TEMPO, KBr, NACIO, DCM, 0 °C; (b) phenylmagnesiumbromide, THF, −78 °C; Method I: CuI, 1,10-phenanthroline, Cs_2CO_3, aryl iodide, toluene, 105 °C, 48 h; Method II: NaH, aryl flouride, DMF, 70 °C, 3 h; Method III: DIAD, PPh_3, phenol, sonication, THF, 15 min; (c) 1.25 M HCl in EtOH.

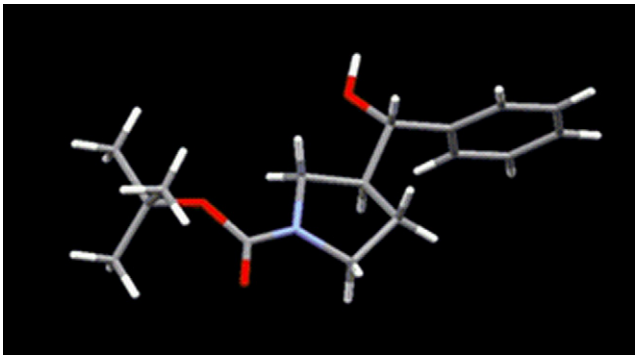


Figure 2. X-ray crystal structure of **11c**.

S_NAr and Ullmann chemistry would provide retention of the alcohol stereocenter and the Mitsunobu coupling conditions would provide the opposite stereoisomer.

Table 1 details the NET and SERT functional inhibitory potencies and the DAT binding affinities for a representative subset of 3-(phenoxy-phenyl-methyl)-pyrrolidine derivatives that were prepared.

One study on a reboxetine-like scaffold (**4**) described the effect that both stereochemistry and aryl ether substituents have on the SERT/NET potency profile.[16c] The stereocenters on our pyrrolidinyl ether structural class as well as the substituents on the aryl ether also significantly influenced NET and SERT potencies. Analysis of a variety of monosubstituted aryl ether containing compounds in the series (Table 1) revealed that compounds with the *SR*-stereochemistry (shaded sections) exhibited higher (at least 10-fold) NET potencies compared to their other stereoisomers.[24] These *SR*-compounds also tended to be more NET-selective. The *RS* compounds by contrast were more balanced at SERT and NET, due to lower potencies at NET, and higher SERT potencies. A variety of substituents provided compounds with pIC_{50} values >8.0 at NET. Substituents at the 2-position tended to provide higher NET potencies and lower SERT potencies than their 3- or 4-substituted analogs (compare **14a**–**15a** and **16a**). This observation is supported by previous studies that show the importance of substitution at the 2-position of the phenyl ring for NRI activity.[25] Compounds containing electron withdrawing substituents (**18**–**20**) were generally less tolerated at NET. There was no clear SAR trend for DAT affinity within this series with respect to stereochemistry, aryl ether substitution, or lipophilicity (*c*log*D*), but a few compounds (**12a**, **12c**, **13a**, **14a**) showed >100-fold selectivity for NET over DAT. We next investigated how combining some of these substituents into multi-substituted aryl ethers would affect potency at the three transporters.

Many of the dichlorinated compounds (**21**–**24**) were found to be potent, balanced NSRIs with pIC_{50} values >8.5 at NET (Table 2). In contrast to the monochlorinated compounds in Table 1 where the *SR* diastereomer was favored for NET potency, the *RS* diastereomers of the dihalogenated compounds seemed to be favored with some substitution patterns (**21b**, **24b**). The difluorinated compounds exhibited a decrease in potency at both transporters compared to their dichlorinated analogs thus demonstrating the importance of both electron density and lipophilicity on the aryl ring for optimal NSRI potency. Generalizations for absolute DAT affinity were difficult to draw in this series and in addition, there were varying degrees of apparent selectivity for NET over DAT. Combining a 2-methoxy functional group (which conferred NET

Table 1
SAR of monosubstituted compounds[a]

Compound	Synthetic method	R	Streochemistry[b]	NET pIC_{50}[c]	SERT pIC_{50}[d]	NET selectivity over SERT[e]	DAT pK_i[f]	NET selectivity over DAT[g]	*c*log*D*[h]
Duloxetine[i]	—	—	—	8.2	9.5	0.08	6.5	50	1.3
Milnacipran[i]	—	—	—	7.3	7.3	1	<4.5	>1000	−0.6
Esreboxetine[i]	—	—	—	9.5	5.5	>1000	4.7	>1000	2.8
12a	I	2-OMe	*SR*	9.0	6.8	200	6.0	100	0.8
12b	I	2-OMe	*RS*	7.8	7.0	6	6.3	31	0.8
12c	I	2-OMe	*SS*	8.0	6.8	20	6.0	100	0.8
12d	I	2-OMe	*RR*	7.8	6.9	8	6.6	15	0.8
13a	I	2-Me	*SR*	8.8	7.2	40	6.8	100	1.5
13b	I	2-Me	*RS*	7.8	7.9	0.8	6.9	8	1.5
14a	I	2-Cl	*SR*	9.3	6.9	300	7.2	125	1.7
14b	I	2-Cl	*RS*	8.3	8.4	0.8	7.3	10	1.7
15a	III	3-Cl	*SR*	8.4	7.5	8	6.9	31	1.8
15b	III	3-Cl	*RS*	8.3	8.6	0.5	7.5	6	1.8
16a	II	4-Cl	*SR*	8.0	8.1	0.8	7.8	1.2	2.0
16b	II	4-Cl	*RS*	7.2	8.9	0.02	6.8	2	2.0
17b	II	2-F	*RS*	8.1	7.7	2.5	6.9	15	1.1
18b	II	3-CN	*RS*	7.4	9.2	0.02	N.T.	—	0.8
19c	II	4-CF_3	*SS*	6.4	8.7	0.01	N.T.	—	2.4
20b	II	2-NO_2	*RS*	7.3	7.2	1.3	N.T.	—	0.9

[a] See Ref. 22 for details of assay conditions.
[b] When assigning stereochemistry, the first stereocenter will be assigned to the pyrrolidine center and the second assignment will be given to the ether position. For example, compound **12a** (3*S*/4*R*) is designated *SR*.
[c] Inhibition of [^{3}H]-NE uptake in HEK293 cells expressing human recombinant NET.
[d] Inhibition of [^{3}H]-5-HT uptake in HEK293 cells expressing human recombinant SERT.
[e] NET selectivity over SERT was determined as: $10^{(NET\cdot pIC_{50} - SERT\cdot pIC_{50})}$.
[f] Inhibition of [^{3}H]-WIN35428 binding to membranes prepared from HEK293 cells expressing human recombinant DAT.
[g] NET selectivity over DAT was determined as: $10^{(NET\cdot pIC_{50} - DAT\cdot pKi)}$.
[h] *c*log*D* was calculated using the Pipeline Pilot Chemistry Collection (version 7.5) from Accelrys (San Diego, California). The properties calculated were *c*log*D* (pH 7.4) using methods published here: Csizmadia, F.; Tsantili-Kakoulidou, A.; Panderi, I.; Darvas, F. *J. Pharm. Sci.* **1997**, *86*, 865.
[i] All data for duloxetine, milnacipran, and esreboxetine were acquired in house.

Table 2
SAR of multisubstituted compounds[a]

Compound	Synthetic method	R	Streochemistry	NET pIC_{50}	SERT pIC_{50}	NET selectivity over SERT	DAT pK_i	NET selectivity over DAT	*c*log*D*
21a	I	2,3-diCl	*SR*	8.8	8.0	6	7.4	25	2.6
21b	I	2,3-diCl	*RS*	9.1	9.1	1	8.5	3	2.6
22a	III	2,4-diCl	*SR*	8.7	8.5	2	7.4	19	2.8
23a	I	2,6-diCl	*SR*	9.4	8.0	30	6.9	300	2.4
23b	I	2,6-diCl	*RS*	8.7	7.4	20	6.5	150	2.4
24a	I	3,5-diCl	*SR*	8.3	8.2	1.3	6.5	63	2.9
24b	II	3,5-diCl	*RS*	8.5	9.1	0.3	6.8	50	2.9
25a	I	2,4-diF	*SR*	8.1	8.1	1	6.8	19	1.6
26a	III	2,6-diF	*SR*	9.1	6.8	200	7.2	80	1.6
27b	I	3,5-diF	*RS*	8.4	8.4	1	7.0	25	1.2
28a	I	2,F,4-Cl	*SR*	8.0	7.9	1.3	7.5	3	2.4
29a	III	2,Cl,4-F	*SR*	8.7	7.8	8	7.0	50	2.2
30a	III	2-OMe,4-Cl	*SR*	9.3	8.9	3	6.4	800	1.9
30b	II	2-OMe,4-Cl	*RS*	7.5	9.2	0.02	6.9	3	1.9
31a	I	2-OMe,4-NO_2	*SR*	8.3	8.8	0.3	5.6	500	1.1
32a	III	2-Me,4-C	*SR*	8.4	9.0	0.3	7.4	9	2.6
33a	I	2,3,5-triCl	*SR*	7.3	7.6	0.5	6.1	15	3.6
33b	I	2,3,5-triCl	*RS*	8.0	8.8	0.2	6.4	40	3.6
34a	III	2,3,6-triCl	*SR*	9.3	8.4	8	7.0	200	3.1
34b	III	2,3,6-triCl	*RS*	8.9	8.4	3	7.0	80	3.1
35a	III	2,3,6-triF	*SR*	9.2	7.2	100	6.9	200	1.8
36a	III	2,6-diCl,3,5-diF	*SR*	9.1	8.1	10	7.0	125	3.2
36b	III	2,6-diCl,3,5-diF	*RS*	9.0	8.4	4	6.5	300	3.2
37a	III	2,4-di-Cl,6-	*SR*	9.0	9.3	0.5	8.1	7	3.1
38a	III	2-Cl,3,6-diF	*SR*	9.5	7.8	50	7.9	40	2.5
38b	III	2-Cl,3,6-diF	*RS*	9.3	9.0	2	8.1	15	2.5
38c	III	2-Cl,3,6-diF	*SS*	8.4	8.3	1.3	7.2	16	2.5
38d	III	2-Cl,3,6-diF	*RR*	8.4	8.2	1.6	7.2	16	2.5
39a	III	2,3,5,6-tetraF	*SR*	9.1	7.1	100	7.0	125	2.0
39b	III	2,3,5,6-tetraF	*RS*	8.8	8.3	3.2	7.1	50	2.0
40a	II	2-Pyr,4-Cl,6-Me	*SR*	8.3	8.2	1.5	7.0	19	1.7
41a	II	2-Pyr,4,6-diCl	*SR*	8.6	7.5	13	7.0	40	1.8

[a] See Ref. 23 and footnotes on Table 1 for details of assay conditions.

selectivity on the monosubstituted aryl ether series) with a 4-chloro substituent (which conferred high SERT selectivity) provided the corresponding 2,4-disubstituted aryl ether **30a**. High potency was observed at both SERT and NET for this compound, suggesting an additive effect for the substituents. This additivity, however, was not observed with 2-Me,4-Cl substituted compound **32a** and diminished potencies were also observed for the analog with an electron-withdrawing nitro group (**31a**). A variety of the tri- and tetra-halogenated compounds **33**–**39** further demonstrated how small modifications on the aryl ether ring and slight changes in lipophilicity could profoundly affect inhibition of each of the monoamine transporters. For example, compounds with the substituents at both the 2- and 6-positions tended to have higher NET potencies than ones without both positions substituted (e.g., **33a**/**b** vs **34a**/**b**). In many cases, both the *RS* and *SR* diastereomers exhibited high NET potencies, but the *RS* diastereomer tended to have higher levels of SERT inhibition, rendering the *RS* diastereomers more SERT/NET balanced, as was also observed with the monosubstituted analogs. Again, DAT affinity did not appear to correlate with any substitution pattern, but many compounds exhibited high selectivity for NET over DAT. The *SR* diasteromers tended to be more selective for NET versus DAT due mostly to higher NET inhibition. One set of *SS* and *RR* diastereomers were also prepared (**38c** and **38d**) to ensure that with multiple substitutions, we were still focusing our efforts on the appropriate diastereomers. Pyridyl ethers such as **40a** and **41a** exhibited diminished NET potency and were not pursued further.

The in vitro PK properties of several compounds that were potent (>8.0 pIC_{50} at NET), balanced (SERT and NET potencies within 10-fold), and selective (>25-fold selective for NET vs DAT) inhibitors were evaluated (Table 3). Many SSRIs and SNRIs, including duloxetine, inhibit or are substrates for, cytochrome P450, 2D6 isoform (CYP2D6), therefore introducing the potential for drug–drug interactions.[26] To mitigate against this risk, compounds were screened for CYP2D6 inhibition. Generally, compounds with the *SR* stereochemistry tended to have higher CYP2D6 inhibition (pIC_{50} >6.0) than the *RS* diastereomers. The majority of compounds across this series were stable in human liver microsomes (HLM), but unstable in rat liver microsomes (RLM). Many of the compounds demonstrated good permeability in *MDR1*-MDCKII cells, but a few compounds (notably the 2-methoxy substituted compounds **30a** and **31a**) had a higher susceptibility to P-gp efflux (efflux ratio >5), which would be anticipated to limit the CNS exposure of the compounds.

Five compounds with acceptable in vitro PK properties (**24b**, **34a**, **38a**, **38b**, and **39b**) were tested in vivo in two preclinical models of pain. The rat formalin model[28] was selected because SNRIs such as duloxetine are active in this model. Furthermore, use of an automated system permitted rapid and objective evaluation of compound activity.[17] In this screening mode, the% inhibition of the flinching behavior induced by intra-plantar formalin was determined following a single 10 mg/kg ip dose of the test compound. From the set of five compounds tested, four of them significantly reduced flinches by >30% (Table 4). Notably, compound (**38b**), which was inactive, also had the lowest permeability and the highest efflux ratio of the set tested in RFM.

The four compounds that were active in the formalin model (**24b**, **34a**, **38a**, and **39b**) were tested in a rat model of neuropathic pain, the spinal nerve ligation model. In this model, ligation of the L5 spinal nerve increases mechanical sensitivity similar to that observed in patients with neuropathic pain.[18] Test compounds were administered orally and reversal of mechanical hypersensitivity

Table 3
In vitro PK studies

Compound	CYP2D6 pIC_{50}[a]	HLM 0.1 μM[b]	RLM 0.1 μM[b]	MDCK A to B[c]	Pgp efflux ratio
Duloxetine[d]	5.7	<5	1560	7.0	0.4
Milnacipran[d]	4.5	<5	<5	9.1	23.0
Esreboxetine[d]	5.5	570	5100	13.0	4.4
15a	6.6	<5	2910	6.9	1.0
21a	6.1	<5	N.T.	1.5	5.4
24a	6.2	<5	4530	0.2	2.8
24b	5.4	<5	21	1.3	2.5
29a	6.3	<5	185	10.0	2.1
30a	6.5	<5	1990	6.0	7.8
31a	5.3	<5	N.T.	1.21	25.0
33b	5.7	<5	100	0.04	7.5
34a	5.9	<5	4620	1.9	1.1
34b	6.0	<5	4600	0.6	3.5
38a	5.4	113	1660	2.9	1.8
38b	5.7	115	1340	1.1	3.8
39b	5.4	<5	200	16.0	2.9

[a] See the supplementary information for assay conditions. N.T. = not tested. Grey cells indicate values outside of desired range.
[b] Intrinsic clearance units: (μL/min/mg protein).
[c] Units: 1×10^{-6} cm/s.
[d] All data for duloxetine, milnacipran,[27] and esreboxetine were acquired in house.

Table 4
In vivo efficacy studies

Compound	IP RFM %inh[b]	PO SNL %reversal[c]		
		1 h	2 h	4 h
Duloxetine[a]	49	27	44	49
Milnacipran[a]	−4	2	3	2
Esreboxetine[a]	33	1	2	1
24b	35	53	69	48
34a	35	6	N.T.	N.T.
38a	38	19	15	25
38b	12	N.T.	N.T.	N.T.
39b	41	28	29	54

[a] All data for duloxetine, milnacipran, and esreboxetine were acquired in house.
[b] Rat formalin model % inhibition determined after a 10 mg/kg ip dose, *n* values between 4 and 10 animals for all compounds.
[c] Spinal nerve ligation model % reversal determined after a 30 mg/kg p.o. dose, except milnacipran which was tested at 100 mg/kg p.o., *n* values between 9 and 12 animals for all compounds.

was determined (Table 4). When dosed at 30 mg/kg, compounds **24b** and **39b** each reduced pain behavior at 4 h post dose (48% and 54%, respectively). The extent of this reversal was comparable to the response by duloxetine (49% reversal) at the same dose. Compound **38a** failed to reach 30% reversal by the 4 h timepoint and compound **34a** had no statistically significant response at the 1 h timepoint and was not tested at later timepoints. Poor in vivo oral rat pharmacokinetics (10 mg/kg) may serve as an explanation for the complete lack of oral activity observed with **34b** (6 %F and AUC of 0.07 μg h/mL)

In vivo rat CNS PK studies confirmed that following ip administration at 10 mg/kg, both **24b** and **39b** are rapidly absorbed and reached equally high concentrations in the brain after 1 h (Fig. 3). Although significant levels of **24b** were detected in the brain, only a small percentage of the compound was detected in the CSF. By contrast, the CSF concentration of compound **39b** was at least 10-fold higher, suggesting better brain penetration. Based on the pIC_{50} values for NET and SERT listed in Table 2, and the data from the PK study, the receptor occupancy at each transporter could be calculated for compounds **24b** and **39b**. The mean unbound brain concentration for **24b** at 75-min post dose was found to be 7 nM, which led to a calculated receptor occupancy of 69% and 74% for NET and SERT, respectively. The mean unbound brain concentration for compound **39b**, was found to be 207 nM at 75-min post dose, leading to a much higher calculated receptor occupancy of 99% and 96% for NET and SERT, respectively.

The favorable PK profile, potent inhibition of NET and SERT, and oral efficacy in a preclinical model of pain behavior make **39b** a compound of interest for further studies. Additional PK-PD studies and tolerability studies on this compound and other compounds in the series will be reported in due course.

In summary, a novel class of 3-(phenoxy-phenyl-methyl)-pyrrolidines have been discovered to be potent inhibitors of both NET and SERT. Despite the relative complexity of a scaffold system with two stereocenters, the SAR generated highlights the importance of spatial and electronic factors within this novel class of compounds and provides insight into how to optimize the overall properties of an NSRI.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.061.

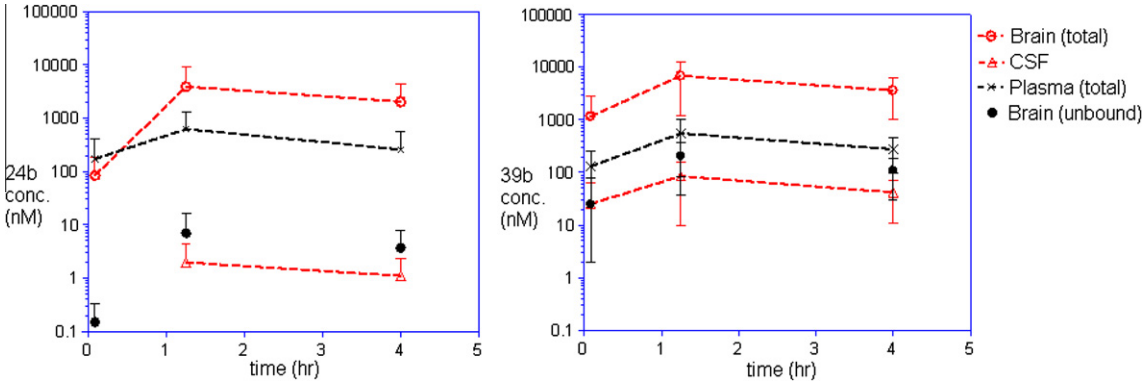

Figure 3. Rat CNS pharmacokinetics of **24b** and **39b**.

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