ACS Medicinal Chemistry Letters

Letter

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Discovery of a Potent, Dual Serotonin and Norepinephrine Reuptake Inhibitor

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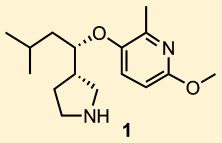
Supporting Information

ABSTRACT: The objective of the described research effort was to identify a novel serotonin and norepinephrine reuptake inhibitor (SNRI) with improved norepinephrine transporter activity and acceptable metabolic stability and exhibiting minimal drug−drug interaction. We describe herein the discovery of a series of 3-substituted pyrrolidines, exemplified by compound **1.** Compound **1** is a selective SNRI in vitro and in vivo, has favorable ADME properties, and retains inhibitory activity in the formalin model of pain behavior. Compound **1** thus represents a potential new probe to explore utility of SNRIs in central nervous system disorders, including chronic pain conditions.



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KEYWORDS: *SERT, NET, dual, reuptake inhibitor, SNRI, SERT RO, α-MMT, pain*

Manipulation of central nervous system (CNS) levels of the neurotransmitters serotonin (5-HT) and norepinephrine (NE), through inhibition of the corresponding reuptake transporters SERT (serotonin transporter) and NET (norepinephrine transporter), has been a successful strategy for treating several CNS disorders including depression, generalized anxiety disorder, and several chronic pain conditions.[1−6]

Several compounds that selectively inhibit these transporters (known as serotonin norepinephrine reuptake inhibitors or SNRIs) are available on the market (compounds **5**−**8**, see Figure 1) and have proven to be safe and effective drugs for the treatment of pain and/or mood disorders. However, these molecules often show more potent inhibition of SERT than NET in vitro (Table 1). Published studies have demonstrated that selective SERT and NET inhibitors can show additive or synergistic analgesic efficacy.[7,8] For our targeted indication of pain, we hypothesized that a SNRI that inhibited SERT and NET with comparable potency would lead to a compound with a superior efficacy and safety profile. Therefore, we undertook to develop a new SNRI that, in a single molecule, improved NET activity versus current SNRIs, retained potent and balanced in vivo activity at both transporters, had good brain exposure, was metabolically stable, and provided minimal drug−drug interaction (DDI) risk to patients on other therapies.

Our search for a balanced SNRI led to the discovery of a series of 3-substituted pyrrolidines, exemplified by compound **1**. Amine containing compounds have long been a fertile source of reuptake inhibitors with a variety of profiles.[9] SNRIs **5**−**8** all show a similar pharmacophore, with an amine and an aryl group separated by 2−4 sp^3 hybridized atoms. The pyrrolidines we synthesized and tested represent a conformationally constrained version of this general pharmacophore that maintains potent transporter inhibition.[10]

Compound **1** contains several innovative features that make it an improvement over earlier scaffolds. The pyrrolidine ring provided the secondary amine that is common to many reuptake inhibitors, but in a novel constrained geometry. Introduction of the pyridine ring, in place of a phenyl ring, was a key change that delivered both our desired pharmacological profile and the ADME properties. Compound **1** displayed reasonable projected human clearance and low potential for

Received: February 5, 2013
Accepted: May 7, 2013
Published: May 7, 2013

   dx.doi.org/10.1021/ml400049p | *ACS Med. Chem. Lett.* 2013, 4, 560−564

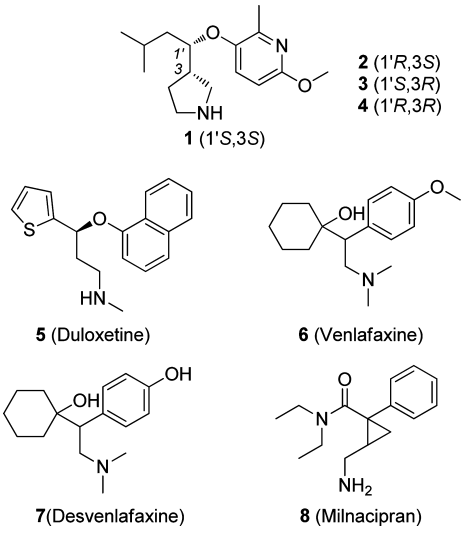


Figure 1. Compound **1**, isomers (**2**–**4**), and marketed SNRI compounds (**5**–**8**).

DDI (data not shown) while maintaining the good in vitro and in vivo activity at the transporters. The C1′ substituent generally did not tolerate the introduction of polar groups. Hydrocarbon groups were preferred, and an isobutyl group at this position provided the optimal balance of potency at SERT and/or NET, with appropriate in vivo properties.

Compound **1** was efficiently synthesized in a manner that allowed facile access to all of the stereoisomers as outlined in Scheme 1. Key intermediate **9** was prepared in four steps on the kilogram scale using a literature route.[11] The stereochemistry at C3 was set via an enzymatic resolution of the racemic ester **9** to give the (*S*)-methyl ester **11**. Lithium hydroxide hydrolysis of the methyl ester gave acid **12**. The isobutyl chain was introduced by converting acid **12** to the Weinreb amide **13**, followed by the addition of isobutylmagnesium chloride to give ketone **14** with an overall yield of 86% over two steps. Ketone **14** was reduced to the alcohols (**15**) by treatment with sodium borohydride. The resulting diastereoisomeric alcohols were then separated using flash chromatography to give (*S*,*S*)-**16**. Introduction of the 2-methyl-6-chloro-pyridine was accomplished through a nucleophilic aromatic substitution reaction to give **17**. Deprotection of the Boc-group followed by methoxide addition gave compound **1** in 79% yield over two steps and with >98% ee and de as determined by chiral HPLC.

The isomeric compounds **2**–**4** were prepared using the same route as for the preparation of **1**: isomer **2** was prepared from the 1′*R*,3*S* isomer of **16**, and isomers **3** and **4** were prepared from **10**.

A single-crystal X-ray of **1** was obtained to allow unambiguous structure determination (Figure 2). The X-ray structure confirmed the *S*,*S* configuration at the two stereogenic centers, C3 and C1′, of compound **1**.

Generally, the SAR was conducted using the L-tartrate salts. These compounds are well-behaved solids with good solubility. However, an investigation of alternative salt forms of compound **1** at a fairly advanced stage of evaluation found that the D-tartrate provided superior crystallinity over the L-tartrate form. In comparative studies, the two tartrate salts of compound **1** performed identically in our in vitro and in vivo assays.

The presence of two chiral centers proved to be a subtle probe of the transporters and allowed us to fine-tune the activities. The SERT activity was fairly consistent among the isomers except for the 3*R* isomers (compounds **3** and **4**), which showed 3–4-fold loss in potency. However, the NET activity was markedly attenuated in all but the 1′*S*,3*S* isomer, compound **1**. The ratio of SERT to NET activity in this in vitro setting was 5 to 7 in compound **1** and compares favorably to the ratios for the marketed SNRIs, **5**–**8**.

Comparative in vitro assays across species confirmed that compound **1** maintained potent and balanced binding at rat SERT and NET suggesting that the rat would be an appropriate species to investigate the in vivo performance of the molecule. Furthermore, compound **1** was tested in a functional assay of neurotransmitter uptake. Consistent with the binding results, compound **1** potently inhibited the transport of both 5-HT and NE through functional transporters. Compound **1** was also found to be selective for transporters relative to biogenic amine receptors recognizing serotonin, norepinephrine, and dopamine (data not shown).

Given the less than optimal ADME properties of some SNRIs, we carefully studied this aspect of our pyrrolidine series. Evaluation in vitro of compound **1** in microsomal stability studies indicated that the compound was highly metabolized in the rat (92%) but was considerably more stable in dog (57%) and human (28%). Consistent with this finding, high intravenous (IV) clearance, which exceeded hepatic blood flow, was observed in the rat, while the dog was observed to be lower (~30% hepatic blood flow) (Table 3). In vitro–in vivo extrapolation of the microsomal stability data suggested that the

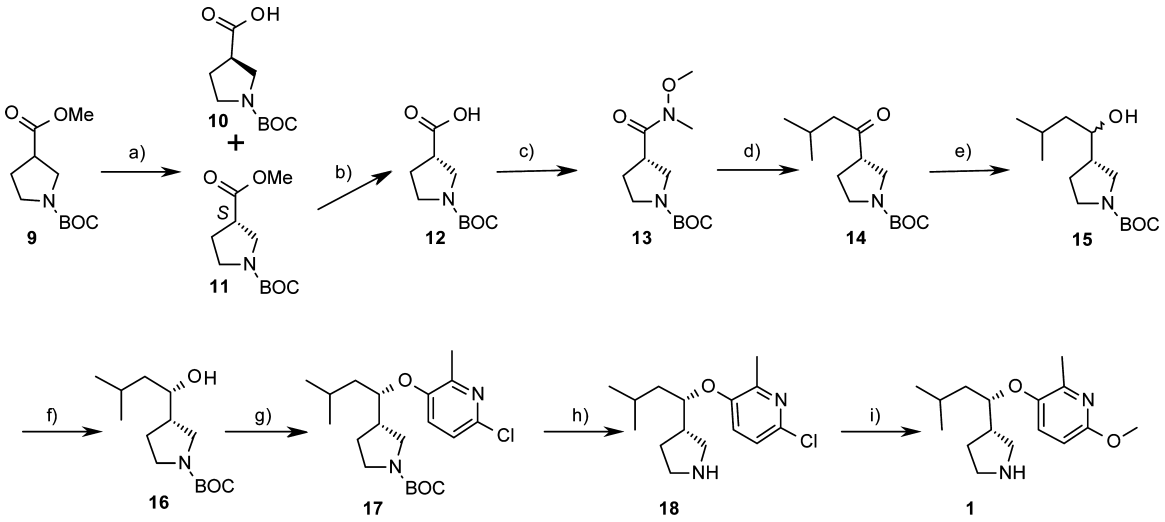
Table 1. In Vitro Binding Affinities at Human Cloned Serotonin, Norepinephrine, and Dopamine Transporters Expressed in HEK293 Cells[a]

		K_i (nM) ± SEM			
compd	salt	hSERT	hNET	hDAT	hSERT/hNET
1	L-tartrate	0.22 ± 0.025	1.2 ± 0.31	475 ± 55	5.5
1	D-tartrate	0.21 ± 0.025	1.5 ± 0.20	354 ± 24	7.1
2		0.23 ± 0.034	30 ± 6.6	>890	130
3		0.84 ± 0.60	438 ± 66	>890	521
4		0.75 ± 0.063	177 ± 19	>890	236
5		0.24 ± 0.06	6.7 ± 0.98	484 ± 46	28
6		11 ± 0.29	>553	>890	>50
7		15 ± 1.8	>650	>854	>43
8		6.5 ± 1.5	85 ± 9.8	>854	13

[a] K_i and ±SEM values were calculated on the basis of at least three independent experiments.

dx.doi.org/10.1021/ml400049p | ACS Med. Chem. Lett. 2013, 4, 560–564

Scheme 1. Synthesis of Compound 1[a]



[a]Reagents and conditions: (a) (i) lipase AS, buffer phosphate, K_2CO_3 (**10**, 55%, and **11**, 43%, 98% ee); (b) LiOH, THF, (74%); (c) *N,O*-dimethylhydroxyl-amine HCl, CDI, DCM, (92%); (d) iBuMgCl, THF, (94%); (e) $NaBH_4$, MeOH, (98%); (f) flash chromatography biotage 150 M, heptane/IPA 95:5, (45%, 96% de); (g) NaH, 6-chloro-3-fluoro-2-methyl-pyridine, DMA, (87%); (h) TFA, anisole, (quantitative yield); (i) NaOMe, DMSO, (90%).

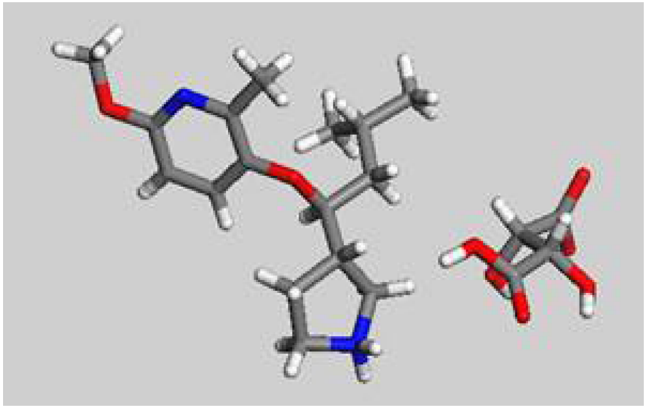


Figure 2. X-ray structure of **1**·D-tartrate.

human clearance may be moderate as well, assuming CYP-mediated oxidative clearance. Metabolic profiling of compound **1** in rat, dog, monkey, and human cryopreserved hepatocytes showed the primary route of clearance to be the result of *O*-demethylation. In vivo profiling of plasma and urine from rats and dogs were consistent with the in vitro profiling work but also showed the formation of a carbamic acid glucuronide, which was not observed in vitro. At 10 μM, compound **1** showed minimal inhibition of CYP3A4, CYP2D6, and CYP2C9 (<30%) in preliminary screening assays. Using an 8-point concentration response assay, the IC_{50} for inhibition of CYP3A4, CYP1A2, CYP2C9, and CYP2D6 was >10 μM, suggesting a low risk for drug−drug interactions.

To assess the in vivo performance of compound **1**, we leveraged separate assays to show engagement at SERT and NET individually. Despite high rat clearance, our studies demonstrated to us that compound **1** reached sufficient exposure after oral dosing to enable its evaluation in rat assays of target engagement and analgesic efficacy. SERT target engagement was evaluated in a receptor occupancy (RO) assay using unlabeled DASB[12] (3-amino-4-[2-[(di(methyl)amino)-methyl]phenyl]sulfanyl-benzonitrile) as the SERT tracer. Compound **1** blocked DASB occupancy in a dose-dependent manner resulting in an absolute ED_{80} of 10.6 mg/kg (Figure 3 and Table 4). The measured brain and plasma exposure from these studies showed an average total brain/plasma ratio of around 3 (average brain and plasma concentrations at 10 mg/kg were 450 and 146 nM, respectively). Upon comparison to the binding affinity, this brain exposure was approximately 100-fold greater than the K_i, which correlates to the good activity in the SERT RO assay.

As the lack of a suitable tracer precludes a NET RO assay, we evaluated the NET activity of **1** by measuring the ability of the compound to antagonize the α-methyl-*m*-tyramine (α-MMT)-induced depletion of catecholamines.[13] In this assay, compound

Table 2. Binding Affinities of Serotonin and Norepinephrine Transporters in Rat Brain Homogenate and Inhibition of Reuptake from Human Cloned Serotonin and Norepinephrine Transporters Expressed in HEK293 Cells[a]

	rat binding K_i (nM) ± SEM			human uptake IC_{50} (nM) ± SEM		
compd	rSERT	rNET	rSERT/rNET	hSERT	hNET	hSERT/hNET
1	0.16 ± 0.077	0.23 ± 0.026	1.3	1.9 ± 0.82	6.9 ± 1.7	3.6
5	0.082 ± 0.066	1.24	15	2.3 ± 0.6	29 ± 5.0	13
6	25 ± 3.5	>500	>20	20 ± 3.4	680 ± 110	34
7	>324	>316	NA	17 ± 1.9	1500 ± 340	88
8	27 ± 6.9	16 ± 2.6	0.6	22 ± 1.3	41 ± 5.7	1.9

[a]K_i, IC_{50}, and ±SEM were calculated on the basis of at least two independent experiments. Data without ±SEM are *n* of 1. NA = not available.

dx.doi.org/10.1021/ml400049p | ACS Med. Chem. Lett. 2013, 4, 560−564

Table 3. Single-Dose Pharmacokinetic Parameters of 1 in Fasted Rats[a] and Dogs[a]

species	route	dose (mg/kg)	C_{max} (nM)	t_{max} (h)	AUC (0−∞ h) (nM h)	$t_{1/2}$ (h)	CL (mL/min/kg)	V_{ss} (L/kg)	F (%)
rat (male)	IV	1			600 ± 333	0.84 ± 0.24	120 ± 50	7.6 ± 4.9	
	PO	1	65 ± 86	1.3 ± 0.58	251 ± 230				31
dog (female)	IV	1			8088 ± 3408	4.6 ± 0.97	8.2 ± 2.8	1.8 ± 0.80	
	PO	1	503 ± 327	1.3 ± 0.58	4134 ± 3055				47

[a]Mean ± SD. Compound **1** was approximately 57% bound to human serum proteins and 96% bound to serum proteins in rodent species studied.

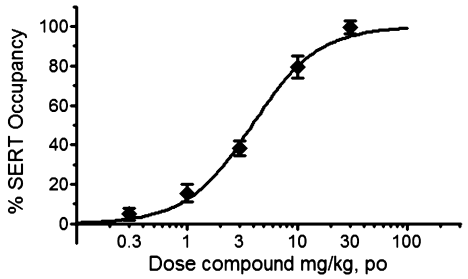


Figure 3. In vivo SERT RO from rat prefrontal cortex by compound **1** (n = 3/dose group) dosed PO two hours prior to the tracer DASB. Mean ± SEM for each dose is shown.

Table 4. SERT RO and α-MMT in Vivo Data for Compounds 1 and 5

	SERT RO	α-MMT
compd	ED_{80} (mg/kg)	ED_{80} (mg/kg)
1	10.6[a]	6.8[a]
5	21.5	47.2

[a]Geometrical mean of three individual experiments.

1 blocked the α-MMT-induced depletion in a dose dependent manner with an ED_{80} of 6.8 mg/kg (Figure 4 and Table 4). Importantly, this result demonstrated the close correlation between the rat binding data and the in vivo activity.

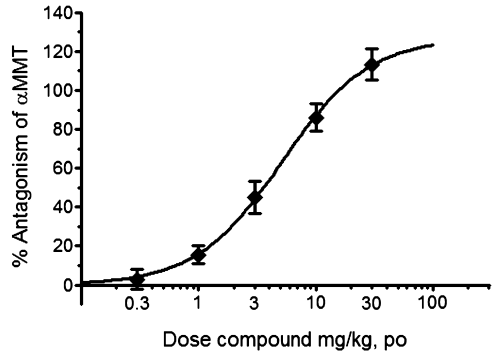


Figure 4. In vivo antagonism of α-MMT induced depletion of rat cortical norepinephrine by compound **1** (n = 3/dose group) dosed PO two hours prior to α-MMT. Mean ± SEM for each dose is shown.

As a primary goal of this effort was to characterize the compound for efficacy against pain, we tested compound **1** in the formalin pain model, which has been shown to be a robust test of the SNRI mechanism.[8] In agreement with the SERT RO and α-MMT results, we observed a reversal in the formalin-induced pain behaviors with an absolute ED_{50} of 13.4 mg/kg PO (Figure 5). Importantly, no neurological effects as measured by the rotorod assay were evident up to the highest dose tested (100 mg/kg) (data not shown).

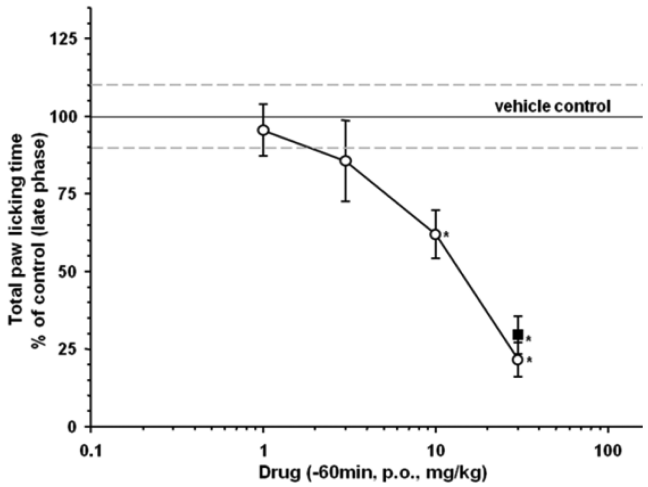


Figure 5. Reversal of rat formalin-induced pain behavior by compound **1** (○) (1, 3, 10, and 30 mg/kg, n = 8−9) dosed PO one hour prior to formalin. Data expressed as mean ± SEM. Abs ED_{50} = 13.4 mg/kg. Compound **5** (■) (duloxetine, 30 mg/kg, n = 9, PO one hour prior to formalin) is the positive control. *p < 0.05.

In summary, for compound **1**, the in vitro binding and functional data and the in vivo SERT RO, αMMT, and formalin pain behavior data compare favorably overall with the clinical comparator, compound **5** (Tables 1, 2, and 4 and Figure 5). Thus, compound **1** represents a selective serotonin and norepinephrine reuptake inhibitor in vitro and in vivo that retains pain inhibiting activity in a model of pain behavior that has previously proven responsive to clinically used SNRIs.[8] Pain inhibitory effects of SNRIs[7,8,14] are consistent with the proposed involvement of serotonin and norepinephrine in the modulation of endogenous pain transmitting systems.[15] In addition to its properties as a potent and balanced SNRI, compound **1** possesses favorable ADME properties, which may suggest its use in treating various pain conditions.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization data of compound **1**, X-ray data, and assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Ming-Shang Kuo and Philip E. Sanders for analytical support.

REFERENCES

(1) Stahl, S. M.; Grady, M. M.; Moret, C. M.; Briley, M. SNRIs: Their Pharmacology, Clinical Efficacy and Tolerability in Comparison with Other Classes of Antidepressants. *CNS Spectrums* **2005**, *10* (9), 732−747.

(2) Zajecka, J. M.; Albano, D. SNRIs in the Management of Acute Major Depressive Disorder. *J. Clin. Psy.* **2004**, *65* (suppl 17), 11−18.

(3) Marks, D. M.; Shah, M. J.; Patkar, A. A.; Masand, P. S.; Park, G.; Pae, C. Serotonin−Norepinephrine Reuptake Inhibitors for Pain Control: Premise and Promise. *Curr. Neuropharm.* **2009**, *7*, 331−336.

(4) Smith, H. S.; Bracken, D.; Smith, J. M. Duloxetine: A Review of its Safety and Efficacy in the Management of Fibromyalgia Syndrome. *J. Cent. Nerv. Syst. Dis.* **2010**, *2*, 57−72.

(5) Depoortère, R.; Meleine, M.; Bardin, L.; Aliaga, M.; Muller, E.; Ardid, D.; Newman-Tancredi, A. Milnacipran Is Active in Models of Irritable Bowel Syndrome and Abdominal Visceral Pain in Rodents. *Eur. J. Pharmacol.* **2011**, *672*, 83−87.

(6) Yucel, A.; Ozyalcin, S.; Koknel Talu, G.; Kiziltan, E.; Yucel, B.; Anderson, O. K.; Arendt-Nielsen, L.; Disci, R. The Effect of Venlafaxine on Ongoing and Experimentally Induced Pain in Neuropathic Pain Patients: a Double Blind, Placebo Controlled Study. *Eur. J. Pain* **2005**, *9*, 407−416.

(7) Jones, C. K.; Eastwood, B. J.; Need, A. B.; Shannon, H. E. Analgesic Effects of Serotonergic, Noradrenergic or Dual Reuptake Inhibitors in the Carrageenan Test in Rats: Evidence for Synergism between Serotonergic and Noradrenergic Reuptake. *Neuropharmacology* **2006**, *51*, 1172−1180.

(8) Iyengar, S.; Webster, A. A.; Hemrick-Luecke, S. K.; Xu, J. Y.; Simmons, R. M. A. Efficacy of Duloxetine, a Potent and Balanced Serotonin-Norepinephrine Reuptake Inhibitor in Persistent Pain Models in Rats. *J. Pharmacol. Exp. Ther.* **2004**, *311*, 576−584.

(9) Walter, M. W. Monoamine Reuptake Inhibitors: Highlights of Recent Research Developments. *Drug Dev. Res.* **2005**, *65*, 97−118.

(10) Van Orden, L. J.; Van Dyke, P. M.; Saito, D. R.; Church, T. J.; Chang, R.; Smith, J. A. M.; Martin, W. J.; Jaw-Tsai, S.; Stangeland, E. L. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1456−1461.

(11) Mendiola, J.; Garcia-Cerrada, S.; de Frutos, O.; de la Puente, M.; Gu, R. L.; Khau, V. V. Enzymatic Resolution of *N*-Substituted-*β*-prolines. *Org. Process Res. Dev.* **2009**, *13*, 292−296.

(12) Wilson, A. A.; Ginovart, N.; Schmidt, M.; Meyer, J. H.; Threlkeld, P. G.; Houle, S. Novel Radiotracer for Imaging the Serotonin Transporter by Positron Emission Tomography: Synthesis, Radiosynthesis, and in Vitro and ex Vivo Evaluation of ^{11}C-Labeled 2-(Phenylthio)araalkylamines. *J. Med. Chem.* **2000**, *43*, 3103−3110.

(13) Cases-Thomas, M. J.; Masters, J. J.; Walter, M. W.; Campbell, G.; Haughton, L.; Gallagher, P. T.; Dobson, D. R.; Mancuso, V.; Bonnier, B.; Giard, T.; Defrance, T.; Vanmarsenille, M.; Ledgard, A.; White, C.; Ouwerkerk-Mahadevan, S.; Brunelle, F. J.; Dezutter, N. A.; Herbots, C. A.; Lienard, J. Y.; Findlay, J.; Hayhurst, L.; Boot, J.; Thompson, L. K.; Hemrick-Luecke, S. Discovery of Novel and Selective Tertiary Alcohol Containing Inhibitors of the Norepinephrine Transporter. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2022−2025.

(14) Jones, C. K.; Peters, S. C.; Shannon, H. E. Efficacy of Duloxetine, a Potent and Balanced Serotonergic and Noradrenergic Reuptake Inhibitor in Inflammatory and Acute Pain Models in Rodents. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 1055−63.

(15) Fields, H. L.; Basbaum, A. I.; Heinricher, M. M. Central Nervous System Mechanisms of Pain Modulation. In *Wall and Melzack's Textbook of Pain*, 5th ed.; McMahon, S. B., Koltzenberg, M., Eds.; Elsevier, Churchill Livingstone: London, UK, 2006; pp 125−142.