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ARTICLE

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Discovery of Novel Selective Norepinephrine Inhibitors: 1-(2-Morpholin-2-ylethyl)-3-aryl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxides (WYE-114152)

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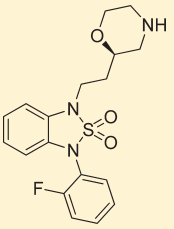
Supporting Information

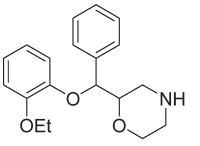
ABSTRACT: Sequential modification of the previously identified 4-[3-aryl-2,2-dioxido-2,1,3-benzothiadiazol-1(3*H*)-yl]-1-(methylamino)butan-2-ols led to the identification of a new series of 1-(2-morpholin-2-ylethyl)-3-aryl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxides that are potent and selective inhibitors of the norepinephrine transporter over both the serotonin and dopamine transporters. One representative compound **10b** (WYE-114152) had low nanomolar hNET potency (IC_{50} = 15 nM) and good selectivity for hNET over hSERT (>430-fold) and hDAT (>548-fold). **10b** was additionally bioavailable following oral dosing and demonstrated efficacy in rat models of acute, inflammatory, and neuropathic pain.



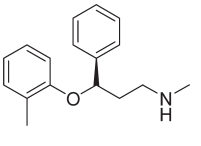
INTRODUCTION

The norepinephrine transporter (NET) is a membrane bound protein that regulates the uptake of the neurotransmitter norepinephrine (NE) from the synaptic cleft of noradrenergic neurons during synaptic transmission.[1] It therefore plays an important role in regulating the physiological functions of NE, the deficiency of which has been implicated in a number of neurological disorders. Norepinephrine reuptake inhibitors (NRIs) block the return of NE to the axon terminal, thereby increasing its synaptic concentration, resulting in a treatment for a range of CNS disorders.[2] In the past 20 years a number of monoamine neurotransmitter reuptake inhibitors have been approved for the treatment of neurological disorders such as depression and pain.[3] More recently, selective NRIs such as reboxetine **1** and atomoxetine **2** have been used clinically for the alleviation of major depressive disorder and attention deficit hyperactivity disorder, respectively.[4] There is also evidence that these compounds may have efficacy in the treatment of chronic pain including fibromyalgia and lower back pain.[5]

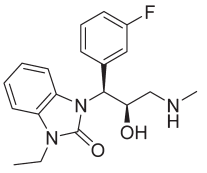
Our previous reports have detailed efforts that resulted in the discovery of selective NRIs including benzimidazol-2-ones[6] (e.g., **3**) and 4-[3-aryl-2,2-dioxido-2,1,3-benzothiadiazol-1(3*H*)-yl]-(methylamino)butan-2-ols such as compound **4**.[7]



Reboxetine **1**



Atomoxetine **2**



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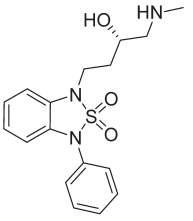

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RESULTS AND DISCUSSION

In Vitro Characterization. We have previously discussed[7] the structure−activity relationship of the chiral amino alcohols **4** and **5** and were interested in further exploring this scaffold (Table 1). Both enantiomers were potent inhibitors of the norepinephrine transporter and were selective against other human transporters.[8] Conversion of the alcohol to the methyl ether resulted in a divergence in potency with the *R*-enantiomer over 5-fold more potent at hNET than the corresponding *S*-enantiomer. Both compounds, however, demonstrated weaker hNET potency than the starting alcohol. In an effort to reduce molecular flexibility and improve compound properties,[9] the methyl groups of compounds **6** and **7** were tethered together to form morpholines **8**−**10a**. These morpholines **8**−**10a** restored the hNET activity (IC_{50} = 11−18 nM) and were equally potent and selective over hDAT; however, the *R*-enantiomer **10a** was 6-fold more selective over hSERT than the *S*-enantiomer **9**.[10]

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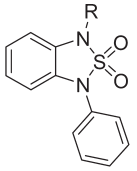
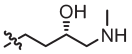
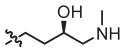
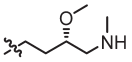
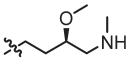
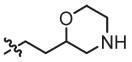
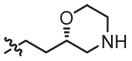
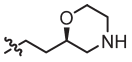
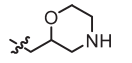
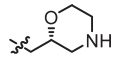
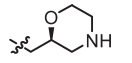
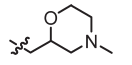
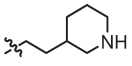
Table 1. Monoamine Reuptake Inhibition of 4−15[a]



Compd	R	hNET uptake IC_{50} (nM)[b]	hNET binding IC_{50} (nM)[c,g]	hSERT uptake %inh.@ 6 μM[d,g]	hDAT binding %inh.@ 1 μM[e,g]
4		4±0.2	2±0.2	(3380±1057)	(2113±349)
5		3±2	9	28	ND
6		151±52	103	52	(1035±195)
7		27±8	13	(5100±1008)	39
8		18±5	7	(1775±777)	(964±118)
9		13±7	9	68(1030±673)	(479±91)
10a		11±6	5	55(6770±4023)	(599±83)
11		147±59	74	0	32
12		207±88	ND	ND	(341±31)
13		199±127	ND	ND	(792±80)
14		4310±2303	2304	ND	77
15		54±22	30	(2983±728)	95

[a] Data are reported as the mean ± SEM. Compounds without standard errors are reported as having an *n* of 1. [b] Inhibition of norepinephrine uptake in MDCK-Net6 cells stably transfected with human norepinephrine transporter (hNET). Desipramine (IC_{50} = 3.4 ± 1.6 nM) was used as a standard. [c] Inhibition of [^{3}H]nisoxetine binding to MDCK-Net6 cells stably transfected with hNET. Desipramine (K_i = 2.1 ± 0.6 nM) was used as a standard. [d] Inhibition of serotonin uptake in JAR cells, natively expressing human serotonin transporter (hSERT). Fluoxetine (IC_{50} = 9.4 ± 3.1 nM) was used as a standard. [e] Inhibition of [^{3}H]WIN-35,428 binding to membranes from CHO cells expressing recombinant human dopamine transporter (hDAT). Mazindol (K_i = 22.1 ± 6.5 nM) was used as a standard. [f] Values in the parentheses are IC_{50} (nM). [g] ND: not determined.

Decreasing the length of the carbon chain between the core and the morpholine moiety, **11**−**13**, resulted in reduced hNET potency (IC_{50} = 147−207 nM). As expected, alkylation of the morpholine amine further attenuated the hNET potency (IC_{50} = 4310 nM). Conversion of racemic morpholine **8** to the corresponding piperidine **15** illustrates the impact of the morpholine oxygen on hNET potency with a decrease of 3-fold (IC_{50} = 55 nM).

The SAR of substitution around the pendent aryl ring of **10a** was also examined (Table 2). 2-F substitution, **10b**, increased hDAT selectivity (IC_{50} = 2190 nM) while maintaining hNET potency and hSERT selectivity. Addition of a 3-OMe moiety, **10c**, decreased hNET potency (IC_{50} = 89 nM) as did incorporation of a 4-F group (IC_{50} = 611 nM). 2,4-di-F substitution, **10e**, resulted in reduced potency at both hDAT and hNET. Compound **10f** containing the 2,4,6-tri-F aryl ring again showed decreased hNET potency.

Substitution on the benzo ring was investigated next (Table 3). Incorporation of a F group at positions 4−7 had little impact on hNET potency except for compound **18** (6-F) where there was a 10-fold decrease. Compound **18** also showed decreased hSERT selectivity as did compound **16** containing the 4-F group. Compounds **17** and **19** with 5-F and 7-F substitution, respectively, had a profile similar to that of desfluoro compound **10b**.

Chemistry. A synthetic route toward the preparation of the 1-(2-morpholin-2-ylethyl)-3-aryl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxides is described in Scheme 1. Commercially available (4-benzylmorpholin-2-yl)acetic acid ethyl ester **20** was debenzylated using palladium on carbon and ammonium formate, then reduced with lithium aluminum hydride to the alcohol before being protected as the *tert*-butyl carbamate **22**. Mitsonobu N-alkylation of **22** with the 2,2-dioxido-2,1,3-benzothiadiazol-1(3*H*)-yl cores **23a**−**f** followed by treatment with HCl provided racemic

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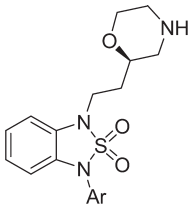
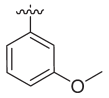
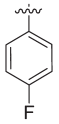
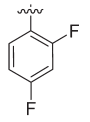
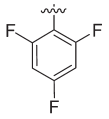
Table 2. Monoamine Reuptake Inhibition of 1-(2-Morpholin-2-ylethyl)-3-aryl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxides 10a−g[a]



Compound	R	hNET uptake IC_{50} (nM)[b]	hNET binding IC_{50} (nM)[c]	hSERT uptake %inh. @ 6 μM[d,f]	hDAT binding %inh. @ 1 μM[e,f]
10a		11±6	5	6770±4023	599±83
10b		15±7	5±0.4	6410±1777	2190±289
10c		89±59	99	749±112	ND
10d		611±437	283	ND	211±17
10e		199±143	143	4128±3273	2040±194
10f		91±27	80	1400±654	ND

[a] Data are reported as the mean ± SEM. Compounds without standard errors are reported as having an *n* of 1. [b] Inhibition of norepinephrine uptake in MDCK-Net6 cells stably transfected with human norepinephrine transporter (hNET). Desipramine (IC_{50} = 3.4 ± 1.6 nM) was used as a standard. [c] Inhibition of [^{3}H]nisoxetine binding to MDCK-Net6 cells stably transfected with hNET. Desipramine (K_i = 2.1 ± 0.6 nM) was used as a standard. Compounds without standard errors are reported as having an *n* of 1. [d] Inhibition of serotonin uptake in JAR cells, natively expressing human serotonin transporter (hSERT). Fluoxetine (IC_{50} = 9.4 ± 3.1 nM) was used as a standard. [e] Inhibition of [^{3}H]WIN-35,428 binding to membranes from CHO cells expressing recombinant human dopamine transporter (hDAT). Mazindol (K_i = 22.1 ± 6.5 nM) was used as a standard. [f] ND: not determined.

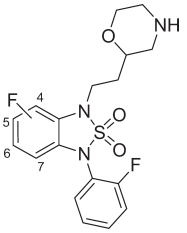
morpholines **24a**−**f**. Chiral HPLC was then utilized to furnish the desired target compounds **10a**−**f**. Compounds **6**, **7**, and **11**−**15** were prepared in a similar fashion.

In Vivo Characterization. Compound **10b** was selected based on its in vitro profile for in vivo characterization.[11] Table 4 highlights the pharmacokinetic parameters of **10b** in the male rat using both intravenous and oral administration. In general, **10b** showed high to moderate clearance, moderate volume of distribution, and moderate terminal half-life. The compound showed an oral bioavailability of 28%. In addition, **10b** had good brain penetration with a brain/plasma ratio of 3.9 in male rats. Furthermore, microdialysis studies in male Sprague−Dawley rats demonstrated a 390% increase over baseline for norepinephrine in the medial prefrontal cortex without significant effect on serotonin at 30 mg/kg po.

Compound **10b** was profiled in a series of in vivo models[12] to assess its effects on norepinephrine neurobiology. NE has been implicated in the modulation of nociceptive processing[13] which is a component of the endogenous descending pain inhibitory system. Reduced levels of NE may in part contribute to the establishment and/or maintenance of chronic pain states.[14] It was reported that by blocking reuptake of NE, NRIs increase NE levels which subsequently activate descending inhibitory pathways.[13] The rat hot plate assay of acute analgesia was used to investigate the efficacy of **10b** on alleviating pain.[15] In this assay, rats are placed on a metal plate maintained at 52 °C. The latency to exhibit a response to this thermal stimulus, such as hind paw lift, flutter, licking, or escape behavior, was measured with a cutoff of 30 s to avoid tissue damage. Upon oral administration, **10b** significantly increased latency at both 10 and 30 mg/kg 1−5 h postdosing (Figure 1). These data suggest that compound **10b** was efficacious in treating acute pain.

Compound **10b** was then evaluated in the carrageenan-induced thermal hyperalgesia model of acute inflammatory pain.[16] In this assay, inflammation is induced in the left hind paw of a rat by injection of 50 μL of 2% carrageenan. Upon oral administration **10b** again increased latency at both 10 and 30 mg/kg 4 h postdosing (Figure 2). These data suggest that compound **10b** was efficacious in treating acute inflammatory pain.

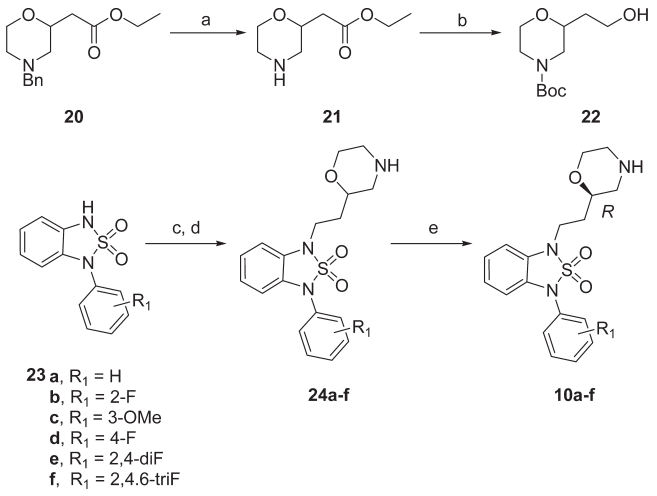
Table 3. Monoamine Reuptake Inhibition of 16−19[a]



compd	substitution	hNET uptake, IC_{50} (nM)[b]	hNET binding, IC_{50} (nM)[c]	hSERT uptake, IC_{50} (nM)[d]	hDAT binding % inh at 1 μM[e]
16	4-F	22 ± 0.4	5 ± 3	992 ± 887	50
17	5-F	14 ± 10	8	8347 ± 2447	43
18	6-F	120 ± 52	209	806 ± 409	23
19	7-F	7 ± 3	13	5790 ± 2619	20

[a] Data are reported as the mean ± SEM. Compounds without standard errors are reported as having an *n* of 1. [b] Inhibition of norepinephrine uptake in MDCK-Net6 cells, stably transfected with human norepinephrine transporter (hNET). Desipramine (IC_{50} = 3.4 ± 1.6 nM) was used as a standard. [c] Inhibition of [^{3}H]nisoxetine binding to MDCK-Net6 cells stably transfected with hNET. Desipramine (K_i = 2.1 ± 0.6 nM) was used as a standard. [d] Inhibition of serotonin uptake in JAR cells, natively expressing human serotonin transporter (hSERT). Fluoxetine (IC_{50} = 9.4 ± 3.1 nM) was used as a standard. [e] Inhibition of [^{3}H]WIN-35,428 binding to membranes from CHO cells expressing recombinant human dopamine transporter (hDAT). Mazindol (K_i = 22.1 ± 6.5 nM) was used as a standard.

Scheme 1. Synthesis of 1-(2-Morpholin-2-ylethyl)-3-aryl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxides 10a−f[a]



[a] Reagents and conditions: (a) Pd/C, NH_4HCO_2, EtOH, 23 °C; (b) $LiAlH_4$, Et_2O, 0 °C, then Boc_2O, NaOH, DCM; (c) **12**, DIAD, PPh_3, THF, 0 °C; (d) HCl (4 M in dioxane), DCM; (e) chiral HPLC.

Compound **10b** also demonstrated efficacy in the FCA-induced mechanical hyperalgesia model of chronic inflammatory pain (Figure 3).[17] In this assay, rodents were injected in the left hind paw with 50 uL of 50% FCA and were given **10b**, celecoxib, or vehicle (po) 24 h later. Threshold to paw withdrawal was measured. Upon oral administration **10b** showed efficacy at 3 mg/kg 3 and 5 h postdosing (Figure 2). These data suggest that compound **10b** was efficacious in treating chronic inflammatory pain.

Finally, the activity of compound **10b** was evaluated in a rat spinal nerve ligation (SNL) model of neuropathic pain.[18] After ligation, assessment of mechanical thresholds was measured as the hind paw withdrawal threshold to a noxious mechanical stimulus. Compound **10b** significantly and dose-dependently reversed mechanical hyperalgesia at 3, 10, and 30 mg/kg, suggesting efficacy in treating neuropathic pain (Figure 4).

CONCLUSION

Modification of the previously identified 4-[3-aryl-2,2-dioxido-2,1,3-benzothiadiazol-1(3*H*)-yl]-1-(methylamino)butan-2-ols led to the identification of a new series of 1-(2-morpholin-2-ylethyl)-3-aryl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxides that are potent and selective for the inhibition of norepinephrine transporter versus serotonin and dopamine transporters. The lead compound demonstrated good pharmacokinetic and pharmaceutical profiles. Compound **10b** was orally efficacious toward alleviating behavioral symptoms associated with NE deficiency in multiple in vivo models including acute, acute inflammatory, chronic inflammatory, and neuropathic pain. On the basis of this profile, **10b** (WYE-114152)[19] was identified as a candidate for advancement into development.

EXPERIMENTAL SECTION

^{1}H NMR spectra were recorded on a Varian INOVA 400 or Bruker AVANCE II 400 instrument. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane in $CDCl_3$ or DMSO-d_6. Electrospray (ESI) mass spectra were recorded using a Hewlett-Packard 5989B MS engine or Waters Alliance-ZMD mass spectrometer. Electron impact ionization (EI, EE = 70 eV) mass spectra were recorded on a Finnigan Trace mass spectrometer. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel, 60 F-254), and spots were visualized with UV light and stained in iodine. Solvents were purchased as anhydrous grade and were used without further purification. Crude reaction products were purified using the ISCO Rf purification system. Preparative HPLC purifications were performed on a preparative Gilson HPLC system using a CombiPrep Pro C18 column with acetonitrile (0.1% TFA) and water (0.1% TFA) as solvents at a flow rate of 20 mL/min. Chiral HPLC separations were carried out using supercritical fluid chromatography conditions using a 250 mm × 4.6 mm i.d. column at 2.0 mL/min flow rate using a Chiralpak AS-H 5 analytical supercritical fluid chromatograph (Berger Instruments, Inc. Newark, DE). Compound purity was assessed by ^{1}H NMR and analytical HPLC as described in the Supporting Information. Biological results were obtained on compounds of >95% chemical purity as determined by the above methods.

Table 4. Pharmacokinetic Parameters of Compound 10b in Male Rats after Intravenous and Oral Administrations[a]

dose (mg/kg)	Cl_p ((mL/min)/kg)	V_{ss} (L/kg)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{0-inf}(h·ng/mL)	F (%)
2 (iv)	38	5			1.8	958	
10 (po)			373	1.0	1.8	1322	28

[a] 2% Tween-80/0.5% methylcellulose in water and DMSO/80%PEG200 were used as vehicles for oral and intravenous administrations, respectively. Three rats were used in each study.

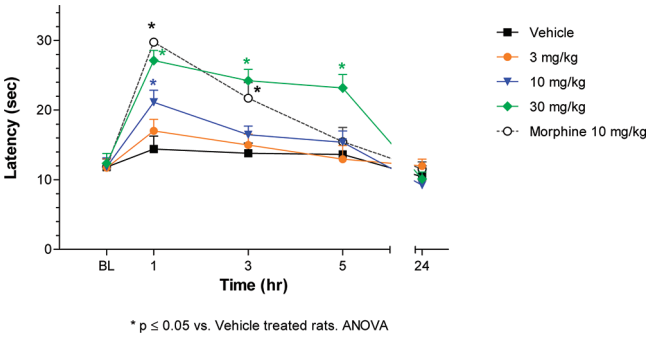


Figure 1. Oral activity of compound **10b** on hot plate latency. Male Sprague−Dawley rats, wt = 190−220 g (n = 10−11/group) were used. The hot plate was set at 52 °C and cutoff was set at 30 s. Latency to nocicfensive response was measured. **10b** was administered (po) as a solution in 2% Tween/0.5% methylcellulose (vehicle). Morphine was administered (sc) as a solution in 0.9% saline. Data shown are the mean ± SEM.

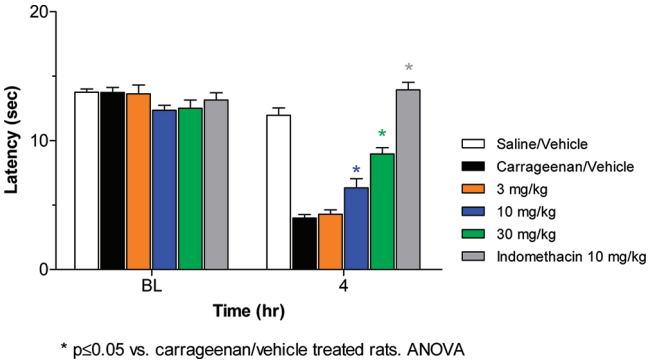


Figure 2. Oral activity of compound **10b** on carrageenan-induced thermal hyperalgesia. Male Sprague−Dawley rats, wt =197−238 g (n = 8/group) were used. Rats were injected in the left hind paw with 50 μL of 2% carrageenan. Paw withdrawal latency was measured. **10b** (po) or indomethacin (po) was administered as a solution in 0.5% methylcellulose + 2% Tween 3 h after carrageenan. Data shown are the mean ± SEM.

(2*S*)-(4-[3-(2-Fluorophenyl)-2,2-dioxido-2,1,3-benzothiadiazol-1(3*H*)-yl]-1-(methylamino)butan-2-ol (4) and (2*R*)-(4-[3-(2-Fluorophenyl)-2,2-dioxido-2,1,3-benzothiadiazol-1(3*H*)-yl]-1-(methylamino)butan-2-ol (5). These compounds were prepared as previously described.[7]

(2*S*)-4-(2,2-Dioxido-3-phenyl-2,1,3-benzothiadiazol-1(3*H*)-yl)-2-methoxy-*N*-methylbutan-1-amine (6) and (2*R*)-4-(2,2-Dioxido-3-phenyl-2,1,3-benzothiadiazol-1(3*H*)-yl)-2-methoxy-*N*-methylbutan-1-amine (7). 4-[3-(3-Phenyl)-2,2-dioxido-2,1,3-benzothiadiazol-1(3*H*)-yl]-1-(methylamino)butan-2-ol (113 mg, 0.325 mmol) and di-*tert*-butyl dicarbonate (71 mg, 0.325 mmol) were stirred in dichloromethane (5 mL) in a sealed vial at room temperature for 18 h.

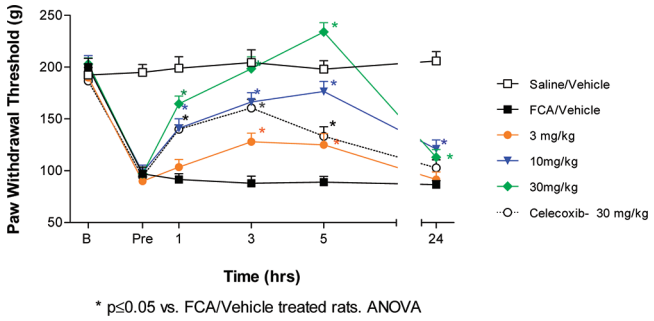


Figure 3. Oral activity of compound **10b** on FCA-induced mechanical hyperalgesia. Male Sprague−Dawley rats (210−242 g, 9−10/group) were injected in the left hind paw with 50 μL of 50% FCA and were given **10b**, celecoxib, or vehicle (po) 24 h later. Threshold to paw withdrawal (PWT) was measured. **10b** and celecoxib, the positive control, were administered (po) as suspensions in 2% Tween/0.5% methylcellulose (vehicle). Data shown are the mean ± SEM.

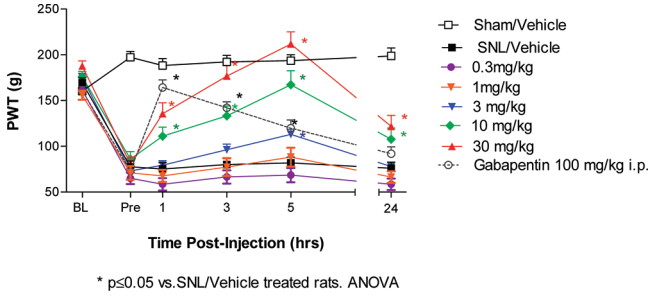


Figure 4. Oral activity of compound **10b** on SNL-induced mechanical hyperalgesia. Results are for male Sprague−Dawley rats (249−305 g, 9−10/group), 3 weeks postsurgery. Threshold to paw withdrawal (PWT) was measured. The compound was administered orally as a suspension of 0.5% methylcellulose plus 2% Tween in water. Gabapentin was used as a positive control and administered (ip) as a solution in 0.9% saline. Data shown are the mean ± SEM. The asterisk (∗) indicates $p \leq 0.05$ vs SNL/vehicle (ANOVA).

The reaction mixture was concentrated and then loaded directly onto silica gel and purified via chromatography (Redisep, silica, gradient 0−50% ethyl acetate in hexane) to afford 0.08 g of 4-[3-phenyl-2,2-dioxido-2,1,3-benzothiadiazol-1(3*H*)-yl]-1-(methylamino)butan-2-ol-carbamic acid *tert*-butyl ester as a clear oil. To this was added trimethyloxonium tetrafluoroborate (71 mg, 0.483 mmol), proton sponge (121 mg, 0.564 mmol), and 4 Å molecular sieves. This was stirred in dichloromethane (5 mL) in a sealed vial at room temperature for 18 h. The reaction mixture was concentrated and then loaded directly onto silica gel and purified via chromatography (Redisep, silica, gradient 0−50% ethyl acetate in hexane) to afford 0.065 g of (2*S*)-4-[3-phenyl-2,2-dioxido-2,1,3-benzothiadiazol-1(3*H*)-yl]-2-methoxy-*N*-methylbutan-1-aminecarbamic acid *tert*-butyl ester as a clear oil. This material was dissolved in diethyl ether/methanol, and 4 N HCl in dioxane was added. A precipitate formed and the mixture was filtered to afford 0.05 g

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of 4-(2,2-dioxido-3-phenyl-2,1,3-benzothiadiazol-1(3*H*)-yl)-2-methoxy-*N*-methylbutan-1-amine as a solid. This was dissolved in methanol. Then 200 μL of the resulting solution was repetitively injected onto the supercritical fluid chromatography instrument, and the baseline resolved enantiomers were separately collected. The chiral purity of each enantiomer was determined under the same supercritical fluid chromatography conditions using a Chiralpak AS-H 5 μm, 250 mm × 4.6 mm i.d. column at 2.0 mL/min flow rate using analytical supercritical fluid chromatography (Berger Instruments, Inc. Newark, DE). Both enantiomers were found to be >99.9% enantiomerically pure.

6: MS (ES) *m*/*z* 362.1 ([M + H]$^+$). HRMS: calcd for $C_{18}H_{23}N_3O_3S$ + H$^+$, 362.1533; found (ESI, [M + H]$^+$ calcd), 362.1533. ^{1}H NMR (DMSO-d_6) δ 8.66 (br s, 2H), 7.61 (m, 3H), 7.50 (m, 2H), 7.18 (m, 1H), 7.09 (m, 1H), 6.98 (m, 1H), 6.67 (d, *J* = 7.85 Hz, 1H), 3.92 (m, 2H), 3.71 (m, 1H), 3.38 (s, 3H), 3.20 (m, 1H), 3.09 (m, 1H), 2.56 (s, 3H), 2.07 (m, 2H).

7: MS (ES) *m*/*z* 362.1 ([M + H]$^+$). HRMS: calcd for $C_{18}H_{23}N_3O_3S$ + H$^+$, 362.1533; found (ESI, [M + H]$^+$ calcd), 362.1533. ^{1}H NMR (DMSO-d_6) δ 8.66 (br s, 2H), 7.61 (m, 3H), 7.50 (m, 2H), 7.18 (m, 1H), 7.09 (m, 1H), 6.98 (m, 1H), 6.67 (d, *J* = 7.85 Hz, 1H), 3.92 (m, 2H), 3.71 (m, 1H), 3.38 (s, 3H), 3.20 (m, 1H), 3.09 (m, 1H), 2.56 (s, 3H), 2.07 (m, 2H).

1-(2-Morpholin-2-ylethyl)-3-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (8). To a solution of 1-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide (0.2 g, 0.8 mmol) in THF (10 mL) was added triphenylphosphine (0.26 g, 1 mmol), *tert*-butyl 2-(2-hydroxyethyl)morpholine-4-carboxylate **20** (0.2 g, 0.9 mmol), and DIAD (0.2 g, 1 mmol) at 0 °C. The mixture was allowed to warm to ambient temperature overnight and then concentrated and chromatographed on silica gel (0−40% EtOAC in hexane). The resulting mostly pure carbamate was dissolved in dichloromethane (10 mL) and treated with HCL (4 mL, 4 M in dioxane). The resulting salt was chromatographed on silica (0−100% of (7 N NH_3/MeOH) in dichloromethane), giving the desired product as a clear oil (0.23 g). MS (ES) *m*/*z* 359.8. HRMS: calcd for $C_{18}H_{21}N_3O_3S$ + H$^+$, 360.1376; found (ESI, [M + H]$^+$ obsd), 360.1377 . ^{1}H NMR (DMSO-d_6) δ 9.17 (br s, 1H), 9.09 (br s, 1H), 7.61 (m, 3H), 7.50 (m, 2H), 7.11 (m, 2H), 6.98 (m, 1H), 6.67 (d, *J* = 7.57 Hz, 1H), 4.00 (m, 2H), 3.92 (m, 1H), 3.82 (m, 1H), 3.60 (m, 1H), 3.26 (d, *J* = 12.65 Hz, 1H), 3.18 (d, *J* = 12.78 Hz, 1H), 3.00 (m, 1H), 2.81 (m, 1H), 2.03 (m, 1H), 1.87 (m, 1H).

1-{2-[(2*S*)-Morpholin-2-yl]ethyl}-3-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (9) and 1-{2-[(2*R*)-Morpholin-2-yl]ethyl}-3-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (10a). **8** was dissolved in methanol, and 200 μL of the resulting solution was repetitively injected onto the supercritical fluid chromatography instrument. The baseline resolved enantiomers were separately collected. The chiral purity of each enantiomer was determined under the same supercritical fluid chromatography conditions using a Chiralpak AS-H 5 μm, 250 mm × 4.6 mm i.d. column at 2.0 mL/min flow rate using an analytical supercritical fluid chromatograp (Berger Instruments, Inc. Newark, DE). Both enantiomers were found to be >99.9% enantiomerically pure.

9: MS (ESI) *m*/*z* 359.8. HRMS: calcd for $C_{18}H_{21}N_3O_3S$ + H$^+$, 360.1376; found (ESI, [M + H]$^+$ obsd), 360.1378. δ 9.10 (br s, 2H), 7.61 (m, 3H), 7.50 (m, 2H), 7.11 (m, 2H), 6.98 (m, 1H), 6.67 (d, *J* = 7.57 Hz, 1H), 4.00 (m, 2H), 3.92 (m, 1H), 3.82 (m, 1H), 3.60 (m, 1H), 3.26 (d, *J* = 12.65 Hz, 1H), 3.18 (d, *J* = 12.78 Hz, 1H), 3.00 (m, 1H), 2.81 (m, 1H), 2.03 (m, 1H), 1.87 (m, 1H).

10a: MS (ESI) *m*/*z* 359.8. HRMS: calcd for $C_{18}H_{21}N_3O_3S$ + H$^+$, 360.1376; found (ESI, [M + H]$^+$ obsd), 360.1379. ^{1}H NMR (DMSO-d_6): δ 9.19 (br s, 2H), 7.61 (m, 3H), 7.50 (m, 2H), 7.11 (m, 2H), 6.98 (m, 1H), 6.67 (d, *J* = 7.57 Hz, 1H), 4.00 (m, 2H), 3.92 (m, 1H), 3.82 (m, 1H), 3.60 (m, 1H), 3.26 (d, *J* = 12.65 Hz, 1H), 3.18 (d, *J* = 12.78 Hz, 1H), 3.00 (m, 1H), 2.81 (m, 1H), 2.03 (m, 1H), 1.87 (m, 1H).

1-(2-Fluorophenyl)-3-{2-[(2*R*)-morpholin-2-yl]ethyl}-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (10b). Compound **10b** was prepared following the procedure for compound **8** with 1-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide being replaced with 1-(2-fluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide and was isolated via chiral HPLC as for compound **10a**. MS (ESI) *m*/*z* 378.1. HRMS: calcd for $C_{18}H_{20}FN_3O_3S$ + H$^+$, 378.1282; found (ESI, [M + H]$^+$ obsd), 378.1284. ^{1}H NMR (DMSO-d_6): δ 9.09 (br s, 2H), 7.70 (m, 1H), 7.59 (m, 2H), 7.47 (m, 1H), 7.11 (m, 2H), 6.98 (m, 1H), 6.56 (d, *J* = 7.87 Hz, 1H), 4.01 (m, 2H), 3.92 (m, 1H), 3.82 (m, 1H), 3.70 (m, 1H), 3.28 (d, *J* = 12.69 Hz, 1H), 3.17 (d, *J* = 12.96 Hz, 1H), 2.99 (m, 1H), 2.81 (m, 1H), 2.04 (m, 1H), 1.85 (m, 1H).

1-(3-Methoxyphenyl)-3-{2-[(2*R*)-morpholin-2-yl]ethyl}-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (10c). Compound **10c** was prepared following the procedure for compound **8** with 1-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide being replaced with 1-(3-methoxyphenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide and was isolated via chiral HPLC as for compound **10a**. MS (ESI) *m*/*z* 389.9. HRMS: calcd for $C_{19}H_{23}N_3O_4S$ + H$^+$, 390.1482; found (ESI, [M + H]$^+$ calcd), 390.1482. ^{1}H NMR (DMSO-d_6): δ 9.11 (br s, 2H), 7.54 (t, *J* = 8.15 Hz 1H), 7.12 (m, 4H), 6.99 (m, 2H), 6.3 (d, *J* = 7.73 Hz, 1H), 3.96 (m, 3H), 3.81 (s, 3H), 3.60 (m, 1H), 3.24 (d, *J* = 12.10 Hz, 1H), 3.16 (d, *J* = 12.91 Hz, 1H), 3.00 (m, 1H), 2.81 (m, 1H), 2.03 (m, 1H), 1.83 (m, 1H).

1-(4-Fluorophenyl)-3-{2-[(2*R*)-morpholin-2-yl]ethyl}-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (10d). Compound **10d** was prepared following the procedure for compound **8** with 1-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide being replaced with 1-(4-fluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide and was isolated via chiral HPLC as for compound **10a**. MS (ESI) *m*/*z* 379.2. HRMS: calcd for $C_{18}H_{20}FN_3O_3S$ + H$^+$, 378.12822; found (ESI, [M + H]$^+$ obsd), 378.1272. ^{1}H NMR (DMSO-d_6): δ 9.12 (br s, 2H), 7.56 (m, 2H), 7.48 (m, 2H), 7.11 (m, 2H), 6.98 (m, 1H), 6.64 (d, *J* = 7.76 Hz, 1H), 4.00 (m, 2H), 3.90 (m, 1H), 3.82 (m, 1H), 3.69 (m, 1H), 3.26 (d, *J* = 12.18 Hz, 1H), 3.18 (d, *J* = 12.54 Hz, 1H), 3.00 (m, 1H), 2.81 (m, 1H), 2.03 (m, 1H), 1.84 (m, 1H).

1-(2,4-Difluorophenyl)-3-{2-[(2*R*)-morpholin-2-yl]ethyl}-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (10e). Compound **10e** was prepared following the procedure for compound **8** with 1-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide being replaced with 1-(2,4-difluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide and was isolated via chiral HPLC as for compound **10a**. MS (ESI) *m*/*z* 395.8. HRMS: calcd for $C_{18}H_{19}F_2N_3O_3S$ + H$^+$, 396.1188; found (ESI, [M + H]$^+$ obsd), 396.1178. ^{1}H NMR (DMSO-d_6): δ 9.20 (br s, 2H), 7.69 (m, 2H), 7.36 (m, 1H), 7.11 (m, 2H), 6.98 (m, 1H), 6.61 (d, *J* = 7.82 Hz, 1H), 4.00 (m, 2H), 3.92 (m, 1H), 3.81 (m, 1H), 3.70 (m, 1H), 3.26 (d, *J* = 12.30 Hz, 1H), 3.18 (d, *J* = 12.55 Hz, 1H), 2.99 (m, 1H), 2.81 (m, 1H), 2.04 (m, 1H), 1.85 (m, 1H).

1-{2-[(2*R*)-Morpholin-2-yl]ethyl}-3-(2,4,6-trifluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (10f). Compound **10f** was prepared following the procedure for compound **8** with 1-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide being replaced with 1-(2,4,6-trifluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide and was isolated via chiral HPLC as for compound **10a**. MS (ESI) *m*/*z* 413.8. HRMS: calcd for $C_{18}H_{18}F_3N_3O_3S$ + H$^+$, 414.10937; found (ESI, [M + H]$^+$ obsd), 414.1080. ^{1}H NMR (DMSO-d_6): δ 9.14 (br s, 2H), 7.62 (m, 2H), 7.14 (m, 2H), 6.99 (m, 1H), 6.73 (d, *J* = 7.82 Hz, 1H), 4.02 (m, 2H), 3.92 (m, 1H), 3.82 (m, 1H), 3.70 (m, 1H), 3.27 (d, *J* = 13.07 Hz, 1H), 3.18 (d, *J* = 12.68 Hz, 1H), 2.98 (m, 1H), 2.81 (m, 1H), 2.05 (m, 1H), 1.85 (m, 1H).

1-(Morpholin-2-ylmethyl)-3-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (11). Compound **11** was prepared following the procedure for compound **8** with *tert*-butyl 2-(hydroxyethyl) morpholine-4-carboxylate being replaced with *tert*-butyl 2-(hydroxymethyl) morpholine-4-carboxylate. MS (ES) *m*/*z* 345.9. HRMS: calcd for $C_{17}H_{19}N_3O_3S$ + H$^+$, 346.1219; found (ESI, [M + H]$^+$), 346.1194. ^{1}H

NMR (DMSO-d_6): δ 9.38 (br s, 2H), 7.57 (m, 3H), 7.48 (m, 2H), 7.12 (d, J = 7.81 Hz, 1H), 7.03 (t, J = 7.81 Hz, 1H), 6.92 (t, J = 7.81 Hz, 1H), 6.59 (d, J = 7.81 Hz, 1H), 4.12 (m, 1H), 3.97 (m, 3H), 3.70 (m, 1H), 3.38 (d, J = 12.17 Hz, 1H), 3.13 (d, J = 12.68 Hz, 1H), 2.90 (m, 2H).

1-[(2*R*)-Morpholin-2-ylmethyl]-3-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (12). Compound **12** was prepared following the procedure for compound **8** with *tert*-butyl 2-(hydroxyethyl)morpholine-4-carboxylate being replaced with *tert*-butyl 2-(hydroxymethyl)morpholine-4-carboxylate and was isolated via chiral HPLC as for compound **10a**. MS (ES) m/z 346.2. HRMS: calcd for $C_{17}H_{19}N_3O_3S + H^+$, 346.1220; found (ESI, $[M + H]^+$), 346.1235. ^{1}H NMR (DMSO-d_6): δ 9.38 (br s, 2H), 7.57 (m, 3H), 7.48 (m, 2H), 7.12 (d, J = 7.81 Hz, 1H), 7.03 (t, J = 7.81 Hz, 1H), 6.92 (t, J = 7.81 Hz, 1H), 6.59 (d, J = 7.81 Hz, 1H), 4.12 (m, 1H), 3.97 (m, 3H), 3.70 (m, 1H), 3.38 (d, J = 12.17 Hz, 1H), 3.13 (d, J = 12.68 Hz, 1H), 2.90 (m, 2H).

1-[(2*S*)-Morpholin-2-ylmethyl]-3-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (13). Compound **13** was prepared following the procedure for compound **8** with *tert*-butyl 2-(hydroxyethyl)morpholine-4-carboxylate being replaced with *tert*-butyl 2-(hydroxymethyl)morpholine-4-carboxylate and was isolated via chiral HPLC as for compound **10a**. MS (ES) m/z 346.2. HRMS: calcd for $C_{17}H_{19}N_3O_3S + H^+$, 346.1220; found (ESI, $[M + H]^+$), 346.1229. ^{1}H NMR (DMSO-d_6): δ 9.38 (br s, 2H), 7.57 (m, 3H), 7.48 (m, 2H), 7.12 (d, J = 7.81 Hz, 1H), 7.03 (t, J = 7.81 Hz, 1H), 6.92 (t, J = 7.81 Hz, 1H), 6.59 (d, J = 7.81 Hz, 1H), 4.12 (m, 1H), 3.97 (m, 3H), 3.70 (m, 1H), 3.38 (d, J = 12.17 Hz, 1H), 3.13 (d, J = 12.68 Hz, 1H), 2.90 (m, 2H).

1-[(4-Methylmorpholin-2-yl)methyl]-3-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (14). To a solution of **11** (76 mg, 0.2 mmol) in methanol (2 mL) was added a solution of formaldehyde (37% in water, 0.15 mL), and the mixture was stirred for 30 min. Sodium cyanoborohydride (38 mg, 0.6 mmol, 3 equiv) was added portionwise, and the mixture was stirred for an additional 3 h. Saturated aqueous sodium bicarbonate (5 mL) was added slowly followed by the addition of water (5 mL). The reaction mixture was extracted with ethyl acetate (2 × 15 mL). The combined organic extracts were washed with brine, dried (anhydrous sodium sulfate), and concentrated. The crude liquid residue was purified by chromatography (silica gel, 0−10% methanol/dichloromethane) to give 70 mg (98%) of 1-[(4-methylmorpholin-2-yl)methyl]-3-phenyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide as a viscous colorless liquid. This free base was dissolved in dichloromethane (3 mL) and was treated with an ethereal solution of hydrochloric acid (1 M, 0.3 mL, 0.3 mmol). To the resulting solution was added hexane until a white powder formed, which was collected, washed with hexane, and dried in vacuo to yield 76 mg (96%) of **14**. MS (ESI) m/z 360.2 ($[M + H]^+$). HRMS: calcd for $C_{18}H_{21}N_3O_3S + H^+$, 360.1376; found (ESI, $[M + H]^+$), 360.1362. ^{1}H NMR (DMSO-d_6): δ 11.08 (br s, 1H), 7.57 (m, 3H), 7.48 (m, 2H), 7.12 (m, 1H), 7.03 (t, J = 7.82 Hz, 1H), 6.92 (t, J = 7.81 Hz, 1H), 6.59 (d, J = 7.81 Hz, 1H), 4.18 (m, 1H), 3.98 (m, 3H), 3.78 (m, 1H), 3.56 (d, J = 12.29 Hz, 1H), 3.3 (m, 1H), 2.95 (m, 2H), 2.75 (s, 3H).

1-Phenyl-3-(2-piperidin-3-ylethyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (15). Compound **15** was prepared following the procedure for compound **8** with *tert*-butyl 2-(hydroxyethyl)morpholine-4-carboxylate being replaced with 1-*N*-Boc-piperidine-3-ethanol. MS (ES) m/z 358.1. HRMS: calcd for $C_{19}H_{23}N_3O_2S + H^+$, 358.1584 found (ESI, $[M+H]^+$), 358.1587. ^{1}H NMR (DMSO-d_6): δ 8.85 (br s, 2H), 7.57 (m, 3H), 7.45 (m, 2H), 7.12 (m, 1H), 7.04 (t, J = 7.69 Hz, 1H), 6.92 (t, J = 7.68 Hz, 1H), 6.61 (d, J = 7.81 Hz, 1H), 3.82 (m, 2H), 3.25 (m, 1H), 3.13 (d, J = 12.17 Hz, 1H), 2.71 (m, 1H), 2.59 (m, 1H), 1.73 (m, 6H), 1.20 (m, 1H).

4-Fluoro-1-(2-fluorophenyl)-3-(2-morpholin-2-ylethyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (16). Compound **16** was prepared following the procedure for compound **10b** with 1-(2-fluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide being replaced with 4-fluoro-1-(2-fluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide. MS (ES) m/z 396.1. HRMS: calcd for $C_{18}H_{19}F_2N_3O_3S + H^+$, 396.1188, found (ESI, $[M + H]^+$), 396.1190. ^{1}H NMR (DMSO-d_6): δ 9.21 (br s, 2H), 7.70 (m, 1H), 7.60 (m, 2H), 7.47 (m, 1H), 7.04 (m, 2H), 6.46 (m, 1H), 4.00 (m, 3H), 3.85 (m, 1H), 3.71 (m, 1H), 3.28 (m, 1H), 3.15 (m, 1H), 2.99 (m, 1H), 2.80 (m, 1H), 2.00 (m, 6H), 1.85 (m, 1H).

5-Fluoro-1-(2-fluorophenyl)-3-(2-morpholin-2-ylethyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (17). Compound **17** was prepared following the procedure for compound **10b** with 1-(2-fluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide being replaced with 5-fluoro-1-(2-fluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide. MS (ES) m/z 396.1. HRMS: calcd for $C_{18}H_{19}F_2N_3O_3S + H^+$, 396.1188 found (ESI, $[M + H]^+$), 396.1193. ^{1}H NMR (DMSO-d_6): δ 9.38 (br s, 2H), 7.69 (m, 1H), 7.58 (m, 2H), 7.46 (m, 1H), 7.19 (dd, J = 2.52, 9.20 Hz, 1H), 6.80 (dt, J = 2.51, 9.34 Hz, 1H), 6.60 (dd, J = 4.65, 8.68 Hz, 1H), 4.00 (m, 3H), 3.83 (m, 1H), 3.72 (m, 1H), 3.26 (d, J = 12.59 Hz, 1H), 3.16 (d, J = 12.33 Hz, 1H), 2.99 (m, 1H), 2.80 (m, 1H), 2.08 (m, 6H), 1.82 (m, 1H).

5-Fluoro-3-(2-fluorophenyl)-1-(2-morpholin-2-ylethyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (18). Compound **18** was prepared following the procedure for compound **10b** with 1-(2-fluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide being replaced with 6-fluoro-1-(2-fluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide. MS (ES) m/z 396.1. HRMS: calcd for $C_{18}H_{19}F_2N_3O_3S + H^+$, 396.1188 found (ESI, $[M + H]^+$), 396.1200. ^{1}H NMR (DMSO-d_6): δ 9.24 (br s, 2H), 7.70 (m, 1H), 7.60 (m, 2H), 7.46 (m, 1H), 7.16 (dd, J = 4.63, 8.78 Hz, 1H), 6.94 (dt, J = 2.58, 9.51 Hz, 1H), 6.59 (m, 1H), 4.00 (m, 3H), 3.81 (m, 1H), 3.70 (m, 1H), 3.25 (d, J = 12.43 Hz, 1H), 3.18 (d, J = 12.31 Hz, 1H), 3.00 (m, 1H), 2.80 (m, 1H), 2.04 (m, 6H), 1.82 (m, 1H).

4-Fluoro-3-(2-fluorophenyl)-1-(2-morpholin-2-ylethyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (19). Compound **19** was prepared following the procedure for compound **10b** with 1-(2-fluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide being replaced with 7-fluoro-1-(2-fluorophenyl)-1,3-dihydro-2,1,3-benzothiadiazole 2,2-dioxide. MS (ES) m/z 396.1. HRMS: calcd for $C_{18}H_{19}F_2N_3O_3S + H^+$, 396.1188 found (ESI, $[M + H]^+$), 396.1195. ^{1}H NMR (DMSO-d_6): δ 9.04 (br s, 2H), 7.58 (m, 2H), 7.50 (m, 1H), 7.49 (m, 1H), 7.17 (m, 1H), 7.04 (d, J = 7.90 Hz, 1H), 6.60 (dd, J = 11.05, 8.65 Hz, 1H), 4.00 (m, 3H), 3.79 (m, 1H), 3.68 (m, 1H), 3.24 (m, 1H), 3.18 (m, 1H), 2.99 (m, 1H), 2.81 (m, 1H), 2.04 (m, 6H), 1.83 (m, 1H).

■ ASSOCIATED CONTENT

Supporting Information. Analytical HPLC purity data for all tested compounds; experimental procedures, including analytical and spectral data, for compounds **21** and **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

NE, norepinephrine; NET, norephinephrine transporter; NRI, norepinephrine reuptake inhibitor; CNS, central nervous system; SERT, serotonin transporter; DAT, dopamine transporter; hNET, human norepinephrine transporter; hSERT, human serotonin transporter; hDAT, human dopamine transporter; SAR, structure−activity relationship; HPLC, high performance liquid chromatography; PK, pharmacokinetics; Cl_p, clearance; V_{ss}, steady state volume of distribution; C_{max}, maximum concentration; T_{max}, time to maximum concentration; *F*, percent bioavailability; AUC, area under the curve; FCA, Freund's complete adjuvant; SNL, spinal nerve ligation

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