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Structure–activity relationships of chiral selective norepinephrine reuptake inhibitors (sNRI) with increased oxidative stability

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ABSTRACT

The synthesis and SAR of a series of chiral heterocyclic ring-constrained norepinephrine reuptake inhibitors are described. The best compounds compare favorably with atomoxetine in potency (IC_{50}s < 10 nM), selectivity against the other monoamine transporters, and inhibition of CYP2D6 (IC_{50}s > 1 μM). In addition, the compounds are generally more stable than atomoxetine to oxidative metabolism and thus are likely to have lower clearance in humans.

Monoamine reuptake inhibition has been an effective therapeutic intervention in a variety of CNS diseases, starting with depression and recently expanding to include chronic pain, ADHD, and stress urinary incontinence.[1–4] We were interested in multiple indications in the therapeutic spectrum of sNRI compounds and initiated work directed toward finding potent and selective compounds with good pharmaceutical properties and minimal risk of drug–drug interactions. In the previous letter, a series of potent racemic sNRIs with potential for good in vivo properties were disclosed. Further characterization of the individual enantiomers is reported here.

In the previous letters,[5,6] we showed that it was possible to introduce new ring constraints into the 3-aryloxypropylamine scaffold of atomoxetine and maintain potency and selectivity for NET (Fig. 1). Using NBI 80532 (compound **1**) as a guide (Fig. 1), both chromane and isochromane compounds were exemplified. Initial characterization focused on racemic compounds and resulted in candidates with the potency and selectivity of atomoxetine. To further develop this series for IND enabling studies, it was necessary to characterize the individual enantiomers.

Racemic compounds **2**–**16** were synthesized as described previously.[6] The 4-hydroxy derivative **19** was generated from the 4-bromo derivative as shown in Scheme 1. Protection of the 4-bromo-2-methyl secondary amine as the Boc derivative, followed by palladium mediated conversion to pinacol boronate[7] **18** proceeded in good yield. Oxidation to the alcohol[8] followed by acid deprotection generated the racemic phenol **19**. The secondary amine could also be protected as the trifluoroacetate instead of Boc, and the amide cleaved at the last step with base.

Enantiomers were generated by preparative chiral HPLC for amounts <1 g and chiral preparative SFC for larger quantities. Each compound required method development, and both enantiomers were produced in >98% ee as determined by chiral HPLC. Absolute conformations were determined by correlation of optical rotation (+ for the more active enantiomer) and crystal structures of both **3-I** and **3-II**.[9] The less active isomer was available in reasonable quantity from the chiral separation during production of **3-I** for in vivo studies and was also used to provide initial measurements of non-chiral physical properties like log*D* and solubility.

All compounds were tested for their ability to inhibit norepinephrine, serotonin, and dopamine uptake in HEK cell lines that had been stably transfected with the human transporters.[10] Compounds were initially tested in two independent dose–response experiments, and those with reasonable potency at NET were retested multiple times. Atomoxetine was included on all assay plates as a standard control, and assay variability was reasonable for a functional assay with SEM values typically below 0.2 log units. Potency values are reported as IC_{50}, though with the neurotransmitter concentrations in each assay well below their respective K_m values, little difference would be expected between the measured IC_{50} and K_i of the compounds. Active

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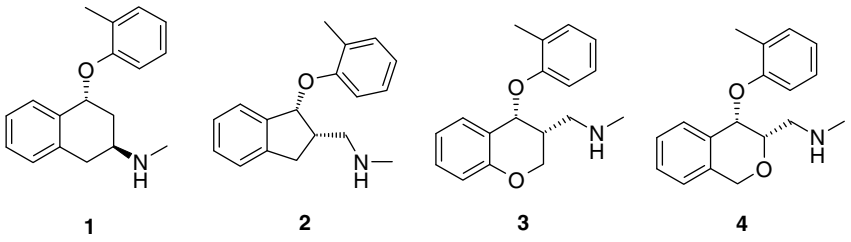
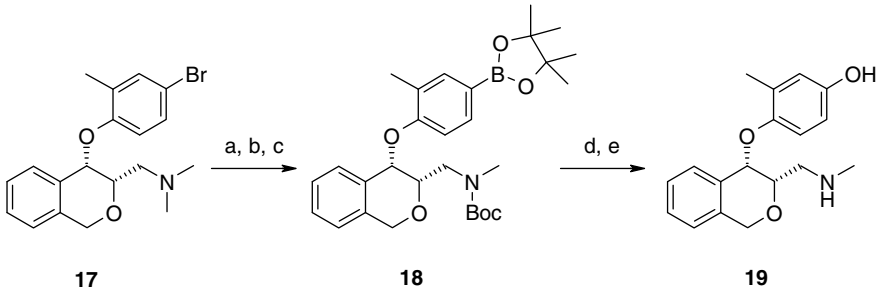


Figure 1. Structures of the four active ring constrained analogs of atomoxetine reported previously.[5,6]

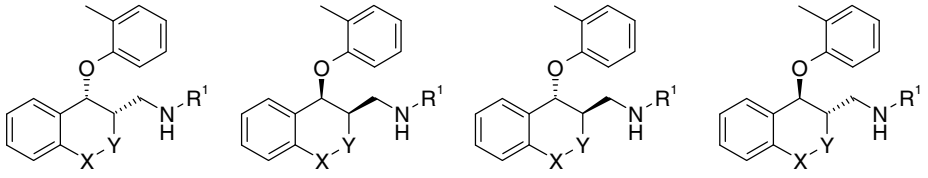


Scheme 1. Reagents and conditions: (a) ACE-Cl, diisopropylethylamine, 1,2-DCE, 1 h, 45 °C, then MeOH, rt, 51%; (b) Boc_2O, TEA, DCM, rt, 24 h, 56%; (c) bis(pinacolato)diborane, $Pd(dppf)Cl_2$, 90 °C, 3 h, 90%; (d) H_2O_2, AcOH, THF, rt, 24 h, 23%; (e) HCl, EtOH, 50 °C, 18 h, quant.

compounds were further tested in transporter binding assays by competition with the appropriate radioligand.[10] For all compounds, active functional inhibition at NET (IC_{50}) correlated well with displacement of ^{3}H-nisoxetine from its NET binding site (K_i). All compounds were tested for inhibition of CYP3A4 and CYP2D6 activity,[10,11] and the metabolic stability in human liver microsomes was also measured for NET active compounds.[10,12] No significant inhibition of CYP3A4 was observed for these compounds.

In the previous letter, multiple analogs of atomoxetine with good potency and reasonable apparent selectivity were described. The properties measured for the racemic compounds however, would not necessarily be present in a single enantiomer. Table 1 shows the characterization of all enantiomerically pure diastereomers of the indane, chromane, and isochromane cores. In all cases, the more active enantiomer had the same absolute configuration at the aryloxy chiral center, matching that of atomoxetine, though *R* and *S* designations vary with the position of the oxygen in the

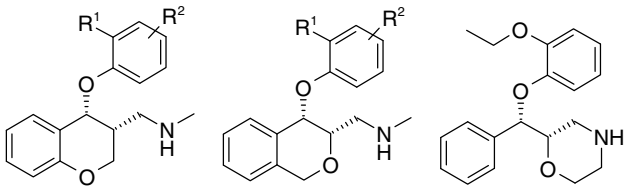
Table 1
SAR of chiral chromanes and isochromanes[a]



Compound	Isomer	X	Y	R_1	NET[b]	SERT[b]	DAT[b]	CYP2D6[c]	Clearance[d]
Atomoxetine	*R*	—		Me	5	180	3000	2000	50
2-I	*R,S*	$-CH_2-$		Me	7	1400	>10,000	800	40
2-II	*S,R*	$-CH_2-$		Me	100	4000	3800	1400	9
3-I	*R,R*	O	C	Me	40	1300	3400	1600	13
3-II	*S,S*	O	C	Me	370	2800	>10,000	180	11
4-I	*S,S*	C	O	Me	5	900	2500	800	5
4-II	*R,R*	C	O	Me	1300	7800	>10,000	4100	7
5-I	*R,R*	O	C	H	35	2100	>10,000	1600	10
5-II	*S,S*	O	C	H	3100	>10,000	>10,000	440	ND
6-I	*S,S*	C	O	H	100	4000	>10,000	4000	11
6-II	*R,R*	C	O	H	1000	>10,000	>10,000	12,000	ND
7-I	*R,S*	O	C	Me	200	1200	>10,000	210	7
7-II	*S,R*	O	C	Me	420	3000	>10,000	5800	25
8-I	*S,R*	C	O	Me	30	960	>10,000	800	$\leqslant$3
8-II	*R,S*	C	O	Me	1000	2700	>10,000	5000	ND

[a] Transporter data are the average of two or more independent measurements.
[b] IC_{50} (nM) for monoamine uptake.
[c] IC_{50} (nM) for inhibition of CYP2D6.[11]
[d] Scaled intrinsic clearance (mL/min/kg) in human liver microsomes,[12] clearance of $\leqslant$3 indicates no detectable compound loss. ND, not done.

Table 2
SAR of *cis* chromanes and isochromanes[a]



Compound	Isomer	R_1	R_2	NET[b]	SERT[b]	DAT[b]	CYP2D6[c]	Clearance[d]
9-I	*R,R*	Et		7	4600	>10,000	400	⩽3
9-II	*S,S*	Et		690	>10,000	>10,000	90	ND
10-I	*R,R*	Cl		26	1600	>10,000	1200	9
10-II	*S,S*	Cl		230	3200	>10,000	200	ND
11-I	*S,S*	Cl		8	500	>10,000	700	8
11-II	*R,R*	Cl		370	4500	7000	5700	ND
12-I	*S,S*	CF_3		3	>10,000	>10,000	370	10
12-II	*R,R*	CF_3		900	6700	10,000	1400	ND
13-I	*R,R*	Me	3-Me	27	390	2600	880	60
13-II	*S,S*	Me	3-Me	39	300	>10,000	70	35
14-I	*S,S*	Me	3-F	3	300	10,000	740	21
15-I	*S,S*	Me	6-F	4	560	>10,000	310	⩽3
16-I	*S,S*	CF_3	6-F	4	1700	>10,000	220	⩽3
19-I	*S,S*	Me	4-OH	8	35	>10,000	9000	4
20	*S,S*	—	—	3	>10,000	>10,000	—	—

[a] Transporter data are the average of two or more independent measurements.
[b] IC_{50} (nM) for monoamine uptake.
[c] IC_{50} (nM) for inhibition of CYP2D6.[11]
[d] Scaled intrinsic clearance mL/min/kg in human liver microsomes,[12] ⩽3 indicates no detectable compound loss. ND, not done.

core.[13] Although not apparent from the initial testing of the racemates, there was a consistent trend toward greater potency for the isochromane compounds, (cf the 8-fold difference between **3-I** and **4-I** and 7-fold for **7-I** and **8-I**).

Selectivity trends were consistent with the observations in the racemates, indicating that both potent NET and SERT inhibition were present in the same enantiomer. In both chromane and isochromanes, the cis diastereomer was more potent and selective than the corresponding trans diastereomer. The isochromane diastereomers exhibited a larger activity difference between enantiomers as well, 30 to 100-fold for **4-I/II** and **8-I/II** compared with 2 to 10-fold for the chromane pairs **3-I/II** and **7-I/II**. The two core structures also differed in the activity of the primary amine compounds, which by analogy with atomoxetine, were likely demethylation metabolites.[14] The active chromane **5-I** was equivalent to its methylated analog **3-I**, while **6-I** and **4-I** differed by 20-fold. Coupled with the greater potency of the isochromane analogs only at NET, this suggested a specific interaction between the isochromane oxygen and the NET transporter. Single digit nanomolar activity could only be achieved when all three key features match atomoxetine, with the additional oxygen interaction serving to counteract the less optimal 6-membered ring of the core.

Results from the separated active enantiomers of other compounds are displayed in Table 2. As described previously,[6] 2-ethyl substitution generated a potent and selective sNRI **9-I**. Other hydrophobic groups at the 2 position were also potent and selective. Methyl substitution even at the 3 position as in **13-I** resulted in a drop in selectivity to about 20-fold, and any substitution at the 4 position dramatically increased the potency at SERT; even substitution with a hydroxyl as in **19-I** was only 5-fold selective.

A comparison of the crystal conformation of **3-I** and the overlay[15] of **4-I** with **1** and (*S,S*)-reboxetine **20**, the more potent and selective enantiomer of reboxetine,[2] is shown in Figure 2. Except for the positioning of the *N*-methyl group, the overall conformations are very similar to each other. The aminomethyl sidechain is pseudoequatorial, while the aryloxy group is pseudoaxial. This puts the aryl ring and the amino group with its attached methyl at the same distance and orientation in all compounds. The position of the second aryl ring is in the same plane as the required conformation of **1**. The isochromane oxygen of **4-I** also occupies the same space as the morpholine oxygen of **20** suggesting a specific interaction with NET for that oxygen.

Two additional in vitro assays were known to be relevant for this class of compounds: inhibition of CYP2D6 activity and microsomal clearance.[16,17] Drug–drug interaction potential at CYP2D6 could be estimated from the described clinical profile of atomoxetine and duloxetine. Atomoxetine exhibited an IC_{50} of 2 μM in the *in vitro* CYP2D6 assay and showed no significant interaction with desipramine in the clinic.[14] In contrast, duloxetine had an IC_{50} of 600 nM in vitro and increased the exposure of desipramine in the clinic by 2-fold.[18] Accordingly the working threshold for further compound development was set to CYP2D6 $IC_{50} > 1$ μM to mitigate this risk. Also important at this stage was the potential for once daily dosing in humans. The terminal elimination half-life for atomoxetine in clinical studies is 3–5 h,[14] resulting in both once and twice daily dosing protocols, and this became a criteria for improvement. Because atomoxetine clearance in vivo is primarily oxidative,[14] clearance rates in human liver microsomes were used as a surrogate for overall clearance in humans.

Compared to atomoxetine, the indane **2-I** was as potent at NET and more selective versus SERT, but unfortunately was slightly more potent as a CYP2D6 inhibitor and showed no advantage over atomoxetine in microsomal clearance. In contrast, both **3-I** and **4-I** were more stable than atomoxetine in the microsomal clearance assay (3 and 10-fold, respectively). In the CYP2D6 inhibition assay, **3-I** gave similar results as atomoxetine, while **4-I** was similar to **2-I**. CYP2D6 activity ratios between enantiomers varied between the two core structures. For the chromane compounds, the more potent enantiomer at NET, for example, **3-I**, was the less potent inhibitor of CYP2D6. For the isochromanes, all activities were present in

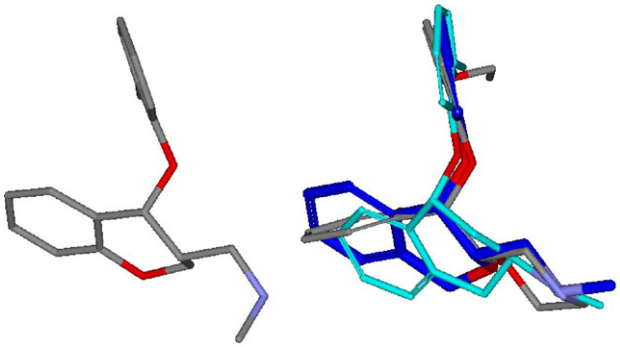


Figure 2. Comparison of the crystal structure coordinates of **3-I** and the independently generated overlay[15] of **1** (teal), **4-I** (blue), and (*S*,*S*) reboxetine **20** (gray) in the same pose to highlight the overall similarity of the conformations. Note the complete overlap of the aminopropoxyaryl chain between **4-I** and **20**, the common position of the heterocyclic oxygens and the common plane and shifted position of the second aromatic ring between **1** and **4-I**.

the more potent NET active enantiomer. Thus CYP2D6 inhibition data from the racemic compounds would only have predicted the isochromane enantiomer data correctly.

Relative CYP2D6 activity appeared to be hydrophobicity driven. Compounds with calculated log*P*s > atomoxetine were all more potent CYP2D6 inhibitors than atomoxetine. The calculated log*P* value for **2** was 0.6 log units more hydrophobic than atomoxetine,[19] and compounds with calculated log*P*s greater than that of **2** had CYP2D6 IC_{50} values significantly below 1 μM. Compounds with log*P* values between the value of **2** and atomoxetine gave acceptable inhibitory potencies. Compound **19-I** was the only compound substantially more polar than atomoxetine, and was also the only compound with a substantially improved inhibition profile. Practically this limited the range of substitutions that are acceptable for further development to methyl, chloro, and fluoro. However, within these constraints, multiple compounds were identified for further characterization in vivo, and the results of those studies will be reported in future publications.

Overall this series produced a number of potent and selective compounds with advantageous stability compared with atomoxetine. While the level of CYP2D6 inhibition was acceptable for the higher polarity compounds, the most potent and stable compounds retained a risk of drug–drug interactions with marketed drugs like desipramine. Further efforts were directed at increasing the polarity of the core structure to extend the range of substitutions with acceptable CYP2D6 properties. Initial results will be reported in the next publication of this series.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.049.

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