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Discovery of a potent, selective, and less flexible selective norepinephrine reuptake inhibitor (sNRI)

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ABSTRACT

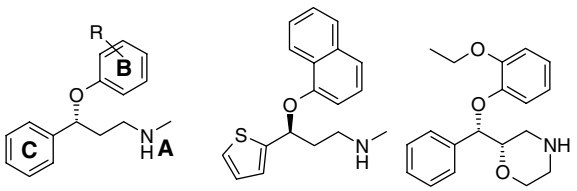
The design, synthesis, and SAR of a series of ring-constrained norepinephrine reuptake inhibitors are described. A substantially rigid inhibitor with potent functional activity at the transporter (IC_{50} = 8 nM) was used to develop a model for the distance and orientation of key features necessary for interaction with the norepinephrine transporter (NET).

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] Compounds with varied profiles have been described as active in animals and humans. The range of active compounds contains selective norepinephrine reuptake inhibitors (sNRIs) like atomoxetine **1** and nisoxetine **2**, selective serotonin (5-HT) reuptake inhibitors like fluoxetine **3**, and mixed inhibitors like duloxetine **4**.[5,6]

Interest in sNRIs as marketable drugs is relatively recent, with atomoxetine approved for ADHD in 2003 and (*S*,*S*)-reboxetine **5** reported to be effective in a phase II trial for neuropathic pain.[7] We were interested in multiple indications in the therapeutic spectrum of sNRI compounds and initiated work directed towards finding potent and selective compounds with good pharmaceutical properties and minimal risk of drug–drug interactions.

Initial criteria for target compounds were based on the properties of known reuptake inhibitors.[8] Compounds needed to be potent functional inhibitors of NET (<100 nM) and much less active as DAT (dopamine transporter) inhibitors (>1 μM) to best avoid potential dependence issues.[9] Since the relative contribution of SERT (serotonin transporter) inhibition to efficacy was not clear, we intended to produce compounds with a range of selectivities and study their properties in vivo.

Structural requirements were derived from the literature,[8] illustrated for compounds **1**–**4**, the Lilly series, in Figure 1. Three structural features were readily apparent: a basic site A, typically a secondary amine for potent NET inhibition, an aromatic ring where substitution leads to substantial changes in NET, SERT, and DAT selectivities, and an additional hydrophobe C that was aromatic for these compounds.[10] The Lilly series is maximally efficient,[11] with only four atoms separating the base features, but suffers from metabolism and clearance issues,[12,13] probably related to the overall flexibility of the molecules. Our intention was to maintain the



1 2-Me, atomoxetine **4** duloxetine **5** S,S-reboxetine
2 2-OMe, nisoxetine (rac)
3 4-CF_3, fluoxetine (rac)

Figure 1. Structure of the Lilly series with the key features labeled. (A) Amine features typically a secondary amine for NET activity; (B) aromatic ring, controls NET/SERT selectivity; (C) hydrophobic site, often aromatic.

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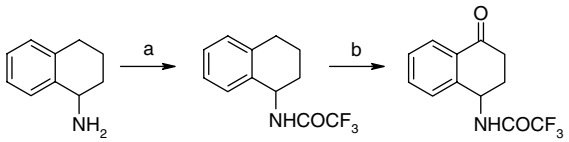
overall connectivity of the Lilly series while introducing ring constraints, aiming to improve the overall properties while maintaining potent transporter inhibition. The ring connection from C to the methylene next to A was successful in producing potent NET inhibitors.

Synthesis of the 1-amino-3-oxyarylindane core was straightforward from readily available 3-amino-3-phenylacetic acid and was initially done with racemic starting material. Following the literature procedure for the amino alcohols,[14] protection of the amino group, best as the trifluoroacetamide, followed by Friedel–Crafts cyclization gave the aminoindanone. Reduction with borane·THF produced predominantly the *cis* amino alcohol. Alternatively, reduction with sodium borohydride was less selective but proceeded in higher overall yields, and allowed for isolation of the minor *trans* amino alcohol **9t**.[15] The route used to generate the protected aryloxy intermediate was determined by the electronic properties of the aryl ring. S_NAr chemistry was employed for aryl groups with electron-withdrawing substituents like 4-chloro. The process was efficient and proceeded predominantly with retention of stereochemistry starting from either the pure *cis* or *trans* amino alcohols. Yields were lower for *ortho*-substituted aryl fluorides, especially more electron-rich aromatic rings, such as compounds **13d** and **13e**. Alternatively, the amino alcohols could be arylated with a phenol under Mitsunobu conditions.[16] This route proceeded predominantly with inversion of stereochemistry. Diastereomer assignment was verified by NOESY and coupling constant correlations for the ring hydrogens.

Substitution at the amine was achieved by basic hydrolysis to give the primary amine followed by reductive amination to give the tertiary amine. The secondary amine was generated by alkylation of the TFA-protected intermediate followed by base deprotection under forcing conditions. Final compounds were purified by preparative HPLC with mass selective collection.

Similar chemistry starting from 3-amino-4-phenyl butyric acid generated the 2,4-aminotetralin isomer. Synthesis of compounds **22**–**26** was performed with chiral starting materials and the compounds were tested individually. The 1,4-aminooxyaryltetralins were prepared from the protected intermediate **21** which in turn had been synthesized from 1-aminotetralin (Scheme 2). Following the procedure outlined in Scheme 1, we then produced compounds **27**–**29**.

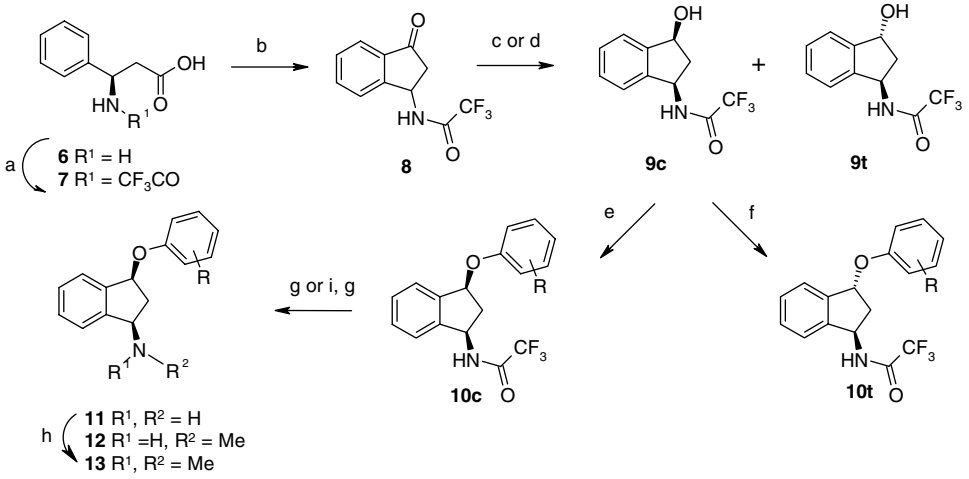
All compounds were tested for their ability to inhibit norepinephrine, serotonin and dopamine uptake in HEK cell lines which had been stably transfected with the human transporters.[17] Compounds were initially tested in two independent dose–response experiments, and those with reasonable potency at 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. A table of SEM values is included as Supplementary Material. 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 compounds were further tested in transporter binding assays by competition with the appropriate radioligand.[17]



Scheme 2. Reagents and conditions: (a) ethyl trifluoroacetate, TEA, MeOH, RT, 91%; (b) CrO_3, Ac_2O, 10 °C, 40 min, 78%.

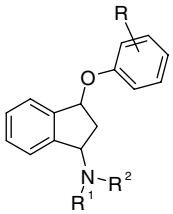
SAR was generated to determine whether the general trends previously observed in the Lilly series were present in these new compounds (Table 1). Initial experiments with the *cis* indanes indicated that the activity of amine substitution does not match that observed for the Lilly series. In particular, the most active amines were the dimethylamines **13a**, **13b**, and **13d**, followed by the monomethyl derivatives **12a**, **12b**, and **12d**. The primary amine compounds were barely active at any of the transporters. Substitutions of the amine with larger groups like ethyl (compounds **14d**, **15d**) or isopropyl (**16d**) also led to a loss of activity.

In contrast, the expected trends for aryloxy substitution were observed. Substitution at the 4 position with chloro, as in compound **13b**, led to a 40-fold increase in SERT inhibition without much activity at NET. The 4-methoxy compound **13c** was also SERT-selective but less potent. Conversely, substitution with 2-Me resulted in about a 5-fold improvement of NET inhibition, with the best compound **13d** measuring 150 nM. Interestingly, compound **13e** with 2-methoxy substitution was expected to be as potent by comparison to nisoxetine, but was much less active at 3 μM. Both enantiomers of **13d** were synthesized and the (*R*,*S*)



Scheme 1. Reagents and conditions: (a) ethyl trifluoroacetate, RT, 18 h, quantitative; (b) $SOCl_2$·DMF, DCM, dioxane, then $AlCl_3$, RT, 1 h, 92%; (c) BH_3·THF, −78 °C, 2 h, **9c** 60%; (d) $NaBH_4$, MeOH, 0 °C, **9c** 50%, **9t** 25%; (e) aryl fluoride, NaH, DMSO, 90 °C, 24 h, 30–70%; (f) Hydroxyaryl, Bu_3P, TMAD, DCM, RT, 18 h, 60–70%; (g) NaOH, EtOH, H_2O, 50 °C, 1 h, 70%; (h) HCOH, $NaBH(OAc)_3$, EtOH, RT, 50–80%; (i) CH_3I, NaH, DMF, 90–100%.

Table 1
SAR of the aryloxyindanes[a]



Compound	R^1	R^2	R	NET[b]	SERT[b]	DAT[b]
Atomoxetine, **1**	Me	H	2-Me	5	180	3000
Nisoxetine, **2**	Me	H	2-OMe	15	1400	2400
Fluoxetine, **3**	Me	H	4-CF_3	2000	47	6000
cis-**11a**	H	H	H	5000	>10,000	2000
cis-**12a**	Me	H	H	2000	>10,000	5000
cis-**13a**	Me	Me	H	800	2000	>10,000
cis-**11b**	H	H	4-Cl	4000	9000	2400
cis-**12b**	Me	H	4-Cl	4000	320	4000
cis-**13b**	Me	Me	4-Cl	1600	50	7000
cis-**13c**	Me	Me	4-OMe	6000	130	>10,000
cis-**12d**	Me	H	2-Me	660	1400	4600
cis-**13d**	Me	Me	2-Me	150	300	10,000
13d-I				60	110	8000
13d-II				350	600	3500
cis-**13e**	Me	Me	2-OMe	3000	3500	>10,000
cis-**14d**	Et	H	2-Me	3000	8000	2500
cis-**15d**	Et	Et	2-Me	2000	3000	2500
cis-**16d**	Me	iPr	2-Me	1100	>10,000	>10,000
trans-**17d**	H	H	2-Me	840	10,000	600
trans-**18d**	Me	H	2-Me	72	370	180
trans-**19d**	Me	Me	2-Me	150	150	3400

[a] Data are the average of two or more independent measurements.
[b] IC_{50} (nM) for monoamine uptake.

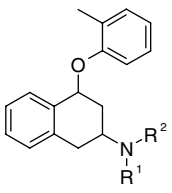
enantiomer **13d-I** is the more potent at both NET and SERT. Compared to the Lilly series, the selectivity of this series generally favored SERT. The *trans* diastereomer was slightly more potent at NET, while the most potent compound was now the secondary amine **18d**. However, these compounds showed substantial inhibition of DAT in addition to minimal selectivity versus SERT (see Table 2).

It appeared that the indane compounds could interact with the aryl pocket on the transporter, but that the amine was not able to reach the optimal position. To study this further, both tetralin regioisomers were prepared. The (2,4) isomer contained the atomoxetine functional group spacing and was expected to be more active. As shown in Table 2, all four diastereomers (**22**, **24**–**26**) were active at NET, with the best activity, almost equivalent to **1**, residing in the (*2S,4R*) isomer **22** (NBI 80532).[18] In the NET binding assay, **22** was able to compete nisoxetine binding to baseline with a K_i of 4 nM (similar to its IC_{50} of 8 nM in the NET functional assay), suggesting that its observed functional NET inhibition was due to interaction with a binding site that overlapped with nisoxetine. Amine substitution also reproduced the Lilly series, with a 50-fold drop in potency for the tertiary amine **23**. Activity of the 1,4 regioisomers **27**–**29** were ~500 nM, less than that of the indanes.

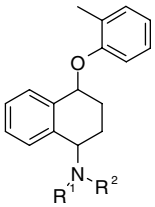
Gratifyingly, the selectivity of **22** for NET versus SERT and DAT was as good as **1**, but was achieved with only 3 rotatable bonds. The rigidity of **22** provided an opportunity to generate a model for the overlay of **1**, **5**, and **22** in the NET binding site, as shown in Figure 2.[19] Because the tetralin core is almost flat, there is little difference in the distance between the amine and the aryloxy features among the four diastereomers, but the relative orientation changes for each. Importantly, the most active isomer is one where the phenoxy group is pseudoaxial. The position of the C ring in **1** is similar to that of **1** and **3** in their crystal structures,[20] but **22** cannot reach the same conformation because of the ring constraint, consistent with the presence of an adjustable hydrophobic pocket on the transporter. This model was used to develop multiple new series because it addresses the active conformation of the aryloxy substituent and the relative positioning of the amine.

Compound **22** was evaluated further in vitro to determine its potential as a drug candidate. Selectivity against an in-house panel of receptors for amine neurotransmitters revealed no submicromolar activity except at the $5HT_{2b}$ receptor (250 nM). However, **22** was more than 5-fold more potent than **1** at inhibiting CYP2D6 activity (IC_{50} = 250 nM), and it was expected that this level of inhibition could potentially lead to drug–drug interactions with other CNS agents such as desipramine and atomoxetine itself. Accordingly, it was necessary to look for alternative ring connections with more favorable CYP interaction profiles, and the results of these studies will be reported in future publications.

Table 2
SAR of the aryloxytetralins[a]



22-26



27-29

Compound	Isomer	R^1	R^2	NET[b]	SERT[b]	DAT[b]
trans-**22**	2*S*,4*R*	Me	H	8	590	8000
trans-**23**	2*S*,4*R*	Me	Me	430	700	>10,000
trans-**24**	2*R*,4*S*	Me	H	200	3300	4500
cis-**25**	2*R*,4*R*	Me	H	190	3000	>10,000
cis-**26**	2*S*,4*S*	Me	H	160	1100	6000
cis-**27**		H	H	630	2300	1000
cis-**28**		Me	H	520	5000	1000
trans-**29**		H	H	950	>10,000	1000

[a] Data are the average of two or more independent measurements.
[b] IC_{50} (nM) for monoamine uptake.

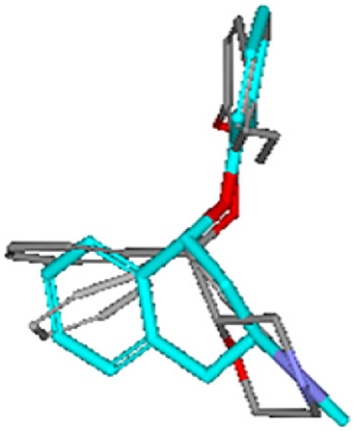


Figure 2. Overlay of **1** (light gray), **5** (dark gray), and **22** (teal) emphasizing the pseudoaxial placement of the aryloxy ring, the common overlap of the aryloxypropylamine scaffold and the different conformations of ring C.

Acknowledgments

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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.2008.05.057.

References and notes

1. Jann, M. W.; Slade, J. H. *Pharmacotherapy* **2007**, *27*, 1571.
2. Babu, R. P. K.; Maiti, S. N. *Heterocycles* **2006**, *69*, 539.
3. Zhou, J. *Drugs of the Future* **2004**, *29*, 1235.
4. Wernicke, J. F.; Iyengar, S.; Ferrer-Garcia, M. D. *Curr. Drug Ther.* **2007**, *2*, 161.
5. Walter, M. W. *Drug Dev. Res.* **2005**, *65*, 97.
6. Iversen, L. *Mol. Psychiatry* **2000**, *5*, 357.
7. Mackay, M. Pfizer R&D Analyst Meeting 2006, Neurosciences slide 9, http://www.pfizer.com/investors/presentations/presentations.jsp, web site accessed 5/2008.
8. Maxwell, R. A.; White, H. L. *Handbook Psychopharmacol.* **1978**, *14*, 83.
9. Wise, R. A. *Curr. Opin. Neurobiol.* **1996**, *6*, 243.
10. Boot, J.; Cases, M.; Clark, B. P.; Findlay, J.; Gallagher, P. T.; Hayhurst, L.; Man, T.; Montalbetti, C.; Rathmell, R. E.; Rudyk, H.; Walter, M. W.; Whatton, M.; Wood, V. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 699.
11. Reynolds, C. H.; Tounge, B. A.; Bembenek, S. D. *J. Med. Chem.* **2008**, *51*, 2432.
12. Sauer, J.-M.; Ring Barbara, J.; Witcher Jennifer, W. *Clin. Pharmacokinet.* **2005**, *44*, 571.
13. Bymaster, F. P.; Lee, T. C.; Knadler, M. P.; Detke, M. J.; Iyengar, S. *Curr. Pharm. Des.* **2005**, *11*, 1475.
14. Kinbara, K.; Katsumata, Y.; Saigo, K. *Chem. Lett.* **2002**, *31*, 266.
15. Compound **9c** was crystalline and easily separated from the *trans* oil **9t** by crystallization.
16. Tsunoda, T.; Otsuka, J.; Yamamiya, Y.; Ito, S. *Chem. Lett.* **1994**, *23*, 539.
17. Kiankarimi, M.; Hudson, S.; Dwight, W. J.; Wade, W. S. Preparation of benzofuranylmethylamines, chromanylmethylamines, and thiochromanylmethylamines as monoamine reuptake inhibitors. US 2006252818.
18. ^{1}H NMR (500 MHz, $CHCl_3$-*d*) δ ppm 9.84 (s, 1H), 9.67 (s, 1H), 7.30 (t, *J* = 7 Hz, 1H), 7.20 (m, 3H), 7.16 (d, *J* = 7 Hz, 1H), 7.15 (t, *J* = 7 Hz, 1H), 7.04 (d, *J* = 8 Hz, 1H), 6.94 (t, *J* = 7 Hz, 1H), 5.45 (s, 1H), 3.77 (s, 1H), 3.40 (dd, *J* = 15, 4 Hz, 1H), 3.05 (dd, *J* = 15, 12 Hz, 1H), 2.72 (s, 3H) 2.14 (t, *J* = 12 Hz, 1H), 2.04 (s, 3H); APCI MS *m*/*z* 268.0.
19. Molecule structures were imported into the Molecular Operating Environment (MOE) software version 2007.09 and subjected to Stochastic Conformation Search using the MMFF94x force field with GBSA implicit solvation. Low-energy conformations within 1.5 kcal/mol from the minimum that were most consistent with the known solid-state X-ray crystal structures were taken forward and aligned using the Flexible Alignment module using default field similarity terms except hydrogen-bonding terms were scaled down to 0.5. The best scoring alignment most consistent with known structure–activity patterns for NET:SERT selectivity was retained.
20. Fuller, R. W.; Wong, D. T.; Robertson, D. W. *Med. Res. Rev.* **1991**, *11*, 17.