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Discovery of a new series of monoamine reuptake inhibitors, the 1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ols

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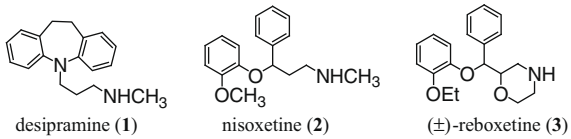
ABSTRACT

A novel series of monoamine reuptake inhibitors, the 1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ols, have been discovered by combining virtual and focused screening efforts with design techniques. Synthesis of the two diastereomeric isomers of the molecule followed by chiral resolution of each enantiomer revealed the (*2R,3S*)-isomer to be a potent norepinephrine reuptake inhibitor (IC_{50} = 28 nM) with excellent selectivity over the dopamine transporter and 13-fold selectivity over the serotonin transporter.

Vasomotor symptoms (VMS), referred to as hot flushes and night sweats, are the result of fluctuating sex steroid hormone levels and are the principal menopausal symptom[1] for which women seek medical treatment. To date, estrogens are the most effective therapeutics for the alleviation of these symptoms; however, these therapies are not acceptable or indicated for all women. Accordingly, there is a clear unmet medical need to develop effective non-hormonal therapies to expand the choice of available therapeutic options. Norepinephrine (NE) has been shown to stimulate areas of the hypothalamus that are important in temperature regulation.[2] Estrogens modulate the activity of both the norepinephrine (NE) and the serotonin (5-HT) systems thus maintaining optimal neurotransmitter levels in the brain.[3] Depletion of estrogens may produce unstable concentrations of these specific neurotransmitters resulting in fluctuations in body temperature. Previously, we disclosed that restoration of NE levels in ovariectomized rats by the administration of a non-selective norepinephrine reuptake inhibitor (NRI), desipramine (**1**) could alleviate VMS and restore normal thermoregulation.[4] Thus, the goal of this program was to identify and develop novel and selective NRIs for the treatment of temperature dysregulation associated with menopause and for evaluation toward additional conditions that have been reported to be ameliorated by NRIs including major depressive disorder (MDD),[5] attention deficit hyperactivity disorder (ADHD),[6] and certain pain disorders including fibromyalgia[7,8] and low back pain.[7]

Our search for a novel and potent NRI scaffold was initiated by screening a subset of the Wyeth compound inventory that was selected based on similarity to biogenic amines (2000 compounds). In parallel, the entire Wyeth compound collection was virtually screened using a Unity flexible 3-D search of a published 3-point pharmacophore containing a basic amine and two aromatic rings.[9] The distances were adjusted[10] to ensure the inclusion of the known NRIs desipramine (**1**), nisoxetine (**2**), and reboxetine (**3**) (Fig. 1). Filters aimed at identifying brain penetrable, lead-like compounds (MW <400, *c*Log *P* <5, rotatable bonds <9, TPSA <80) were applied



desipramine (**1**) nisoxetine (**2**) (±)-reboxetine (**3**)

Figure 1. Structures of known NRIs.

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to the list of virtual hit compounds. A selected set of compounds was screened for norepinephrine reuptake inhibition in MDCK-Net6 cells stably transfected with the human norepinephrine transporter (hNET) using previously described procedures.[11] Both screens identified β-hydroxyamines **4** and **5** (Fig. 2) that significantly inhibited NE uptake at a concentration of 1 μM. We were most interested in indoles **5** due to their potential novelty, and when the 3-phenyl group, found in known monoamine reuptake inhibitors, was incorporated, a potent, albeit large, lead molecule **(±)-6** that exhibited some selectivity over the human serotonin transporter (hSERT) was identified. Efforts to reduce the molecular weight to obtain a more lead-like molecule led to compound (±)-**7a** which maintained potent NE uptake inhibition but lost selectivity over hSERT. Compound (±)-**7a** became an early lead molecule in our program. Initial SAR studies of the phenylpropanolamine scaffold have recently been disclosed.[12]

Synthesis of the erythro diastereomeric pair of 1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ols[13] [(±)-**7**, Scheme 1] began with *trans*-1-phenylglycidol (±)-**8** which was prepared from *trans*-cinnamyl alcohol using peracetic acid.[14] Regioselective epoxide opening with indole was accomplished using either pulverized potassium hydroxide in DMSO or sodium *t*-butoxide in the presence of titanium isopropoxide. Selective conversion of the primary alcohol in compound (±)-**9** proceeded smoothly with *p*-toluenesulfonyl chloride in pyridine to form tosylate (±)-**10** which was displaced with an amine to form target compounds (±)-**7** each as an erythro diastereomeric pair. Diol (±)-**9** was also converted to the threo diastereomer (±)-**13** using the procedure[13] outlined in Scheme 2. Selective protection of the primary alcohol in diol (±)-**9** was accomplished with *p*-nitrobenzoyl chloride in pyridine at −10 °C followed by conversion of the secondary alcohol into the mesylate using methansulfonyl chloride and triethylamine to form compound (±)-**11**. Deprotection of the primary alcohol using aqueous sodium hydroxide allowed an in situ intramolecular displacement of the mesylate with inversion to form epoxide (±)-**12**. Subsequent regioselective ring opening of the epoxide with methylamine in methanol afforded target compound (±)-**13** as the threo diastereomeric pair.

Compounds **7a** and **13a** (**R**^{1} and **R^3 = H, R^2 = CH$_3$**) were separated into single enantiomers (**7a.a, 7a.b, 13a.a** and **13a.b**) using a chiral supercritical fluid chromatography (SFC) technique.[15] Subsequently, the absolute stereochemistry of each enantiomer was examined using vibrational circular dichroism (VCD), electronic circular dichroism (ECD), and NMR methods.[16] The absolute stereochemistry of eutomer **7a.a**, was confirmed by single crystal X-ray analysis[17] to possess the (*2R,3S*) configuration[18] (Fig. 3).

All target compounds were tested initially as racemates for their ability to block NE reuptake. Compounds that exhibited at least 40% inhibition at a concentration of 1 μM were tested for

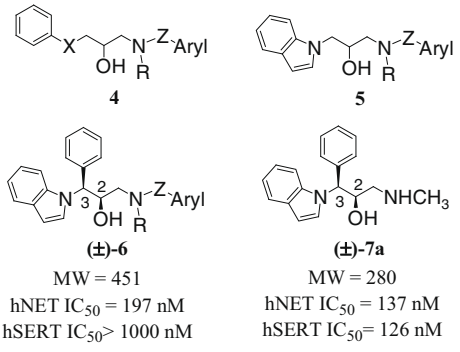
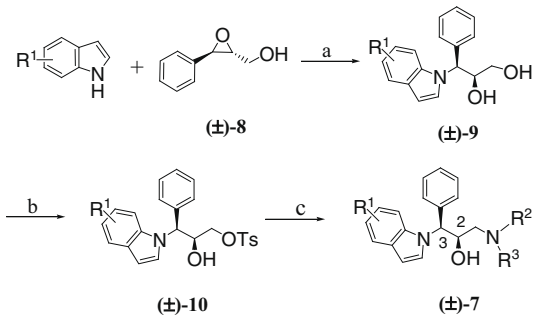
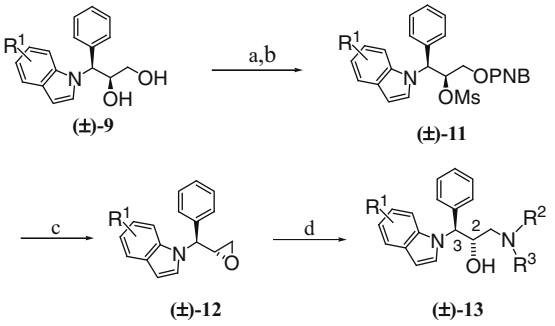


Figure 2. Conversion of NRI scaffolds identified from screening into a Discovery lead molecule.



Scheme 1. Synthesis of (±)-erythro-1-amino-3-(1*H*-indol-yl)-3-phenylpropan-2-ols. Reagents and conditions: (a) KOH, DMSO, 70 °C or NaH, *t*-BuOH, Ti(*i*-PrO)$_4$, CH_2Cl_2, rt; (b) TsCl, pyridine, rt; (c) NHR^2R^3, K_2CO_3, CH_3CN, reflux (when R^3 = H; excess NH_2Me, or NH_2Et, MeOH, rt).



Scheme 2. Synthesis of (±)-threo-1-amino-3-(1*H*-indol-yl)-3-phenylpropan-2-ols. Reagents and conditions: (a) *p*-nitrobenzoyl chloride, pyridine, −10 °C (b) MsCl, TEA, CH_2Cl_2, 0–5 °C (c) NaOH, dioxane, rt (d) excess NH_2Me, MeOH, rt.

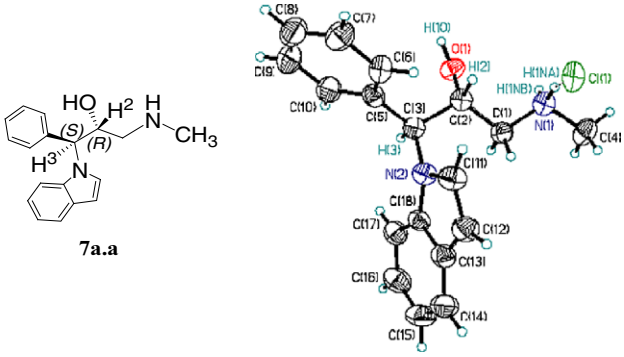
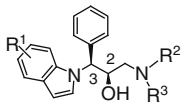


Figure 3. ORTEP view of compound **7a.a** from single crystal X-ray analysis.

selectivity over hSERT and the human dopamine transporter (hDAT). These assays were performed as previously described.[11] An examination of the effect of amine moiety size in the erythro-diastereomers (**7a–l**, Table 1) revealed that only primary amines and secondary amines with small alkyl groups were tolerated for hNET activity. Consequently, while ethylamine **7j** provided modest hNET potency (IC_{50} = 1200 nM), a drop-off in activity was observed with the *iso*-propylamine analog **7h** which exhibited only 13% inhibition of hNET at 1 μM. The secondary methylamine analog **7a** was the most potent NRI of the group (IC_{50} = 137 nM) while the primary amine **7l** was tolerated but was less effective (IC_{50} = 560 nM). When either a 3-methyl (**7n–p**) or 5-F substituent was incorporated (**7q–s**) on the indole ring, the size of the amine moiety provided an identical trend toward hNET potency that was observed in the unsubstituted analogs, that is, $NHCH_3$ preferred over NH_2 which was preferred over $NHCH_2CH_3$. Incorporation of

Table 1
Characterization of 1-amino-3-(1*H*-indol-yl)-3-phenylpropan-2-ol analogs at the human norepinephrine, serotonin and dopamine transporters



Compd	Stereochem	R^1	R^2	R^3	hNET uptake IC_{50}[a], nM (StDev)	hSERT uptake IC_{50}[b], nM (StDev)	Ratio of hSERT uptake IC_{50} /hNET uptake IC_{50}[c]	hDAT binding % inhibition @1 μM[d]
7a	(±)-Erythro	H	CH_3	H	137 (56)[e]	126 (31)[e]	1	7%
7a.a	*2R,3S*	H	CH_3	H	28 (11)[e]	358 (108)[e]	13	19%
7a.b	*2S,3R*	H	CH_3	H	104 (41)[e]	144 (50)[e]	1	18%
7b	(±)-Erythro	H	$-(CH_2)_2N(CH_3)(CH_2)_2-$		5%[f]	NT	—	NT
7c	(±)-Erythro	H	$-(CH_2)_2NH(CH_2)_2-$		34%[f]	NT	—	NT
7d	(±)-Erythro	H	$-(CH_2)_2O(CH_2)_2-$		18%[f]	NT	—	NT
7e	(±)-Erythro	H	4-PyridylCH_2	H	24%[f]	NT	—	NT
7f	(±)-Erythro	H	CyclohexylCH_2	H	41%[f]	2833 (283)[e]	—	NT
7g	(±)-Erythro	H	Benzyl	H	26%[f]	NT	—	NT
7h	(±)-Erythro	H	$(CH_3)_2CH-$	H	13%[f]	NT	—	NT
7i	(±)-Erythro	H	CH_3CH_2	CH_3	38%[f]	NT		NT
7j	(±)-Erythro	H	CH_3CH_2	H	1200[g]	298 (74)[e]	0.2	NT
7k	(±)-Erythro	H	CH_3	CH_3	25%[f]	NT	—	NT
7l	(±)-Erythro	H	H	H	560[g]	50[g]	0.1	7%
7m	(±)-Erythro	2-CH_3	CH_3	H	45%[f]	NT	—	NT
7n	(±)-Erythro	3-CH_3	CH_3CH_2	H	1100[g]	2200[g]	2	0%
7o	(±)-Erythro	3-CH_3	CH_3	H	42 (6)[e]	251 (20)[e]	6	0%
7p	(±)-Erythro	3-CH_3	H	H	321[g]	299(100)[e]	1	6%
7q	(±)-Erythro	5-F	CH_3CH_2	H	11%[f]	NT	—	NT
7r	(±)-Erythro	5-F	CH_3	H	118 (32)[e]	95[g]	1	NT
7s	(±)-Erythro	5-F	H	H	32%[f]	NT	—	NT
7t	(±)-Erythro	5-Cl	CH_3	H	49[g]	10[g]	0.2	NT
7u	(±)-Erythro	5-OCH_3	CH_3	H	230[g]	31[g]	0.1	2%
13a	(±)-Threo	H	CH_3	H	79 (32)[e]	74 (14)[e]	1	4%
13a.a	*1S,2S*	H	CH_3	H	415 (134)[e]	918[g]	2	0%
13a.b	*1R,2R*	H	CH_3	H	150 (42)[e]	161 (4)[e]	1	31%

[a] Inhibition of NE uptake in MDCK-Net6 cells, stably transfected with hNET. Desipramine (IC_{50} = 3.4 + 1.6 nM) was used as a standard.
[b] Inhibition of serotonin uptake in JAR cells, stably transfected with human SERT. Fluoxetine (IC_{50} = 9.4 + 3.1 nM) was used as a standard.
[c] Unitless value as a ratio in which higher numbers represent relatively greater NET selectivity. A value of 1 represents no selectivity.
[d] Inhibition of [^{3}H]WIN35,428 binding to membranes from CHO cells expressing recombinant hDAT. Mazindol (22.1 + 6.5 nM) was used as a standard.
[e] Data is the average of between 2 and 6 independent experiments, each run in triplicate.
[f] Percent inhibition measured at a concentration of 1000 nM.
[g] Data is the average of three triplicate runs; standard error (SE) < 30% of the mean. NT = not tested.

the 3-methyl substituent on indole (**7n**–**p**) also provided a modest improvement in selectivity for hNET over hSERT when compared to the unsubstituted analogs.

The effect of stereochemistry on hNET potency and selectivity was examined and a surprising result was observed. Diastereomeric pairs **7a** and **13a** exhibited similar potencies for both hNET and hSERT reuptake inhibition. The same was true for the threo-enantiomers **13a.a** and **13a.b**. Separation of the erythro-enantiomers **7a.a** and **7a.b**, however, revealed that although (*2S,3R*)-enantiomer **7a.b** exhibited equivalent potencies for hNET and hSERT, the (*2R,3S*)-enantiomer **7a.a** provided modest selectivity (13-fold) for hNET over hSERT. All compounds tested exhibited only weak binding to hDAT.

Compound **7a.a** was further assessed in a CNS panel for selectivity and in pharmaceutical property profiling. In a competition radioligand-binding assay in whole MDCK cells stably transfected with hNET using [^{3}H]-nisoxetine as the radioligand,[11] **7a.a** exhibited an IC_{50} value of 23 nM, similar to its functional IC_{50} of 28 nM. It also had no affinity for the 5-HT_{1a} and 5-HT_{2a} receptors or the α1-, and α2-isoforms of the adrenergic receptor. The compound also exhibited good aqueous solubility at pH 7.4 (>50 μg/mL), good PAMPA permeability (4.6×10^{-6} cm/s) and <20% inhibition of CYP3A4, CYP2D6 and CYP2C9 at 3 μM. When incubated in liver microsomes at a concentration of 1 μM, the $t_{½}$ of **7a.a** across species was 9.8 min in rat, 15.9 min in dog, 9.7 min in monkey and >60 min in human.

In summary, we have identified a series of 1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ols as NRIs. Diastereoselective synthesis and separation of enantiomers led to the finding that the (*2R,3S*)-isomer, **7a.a**, was a potent NRI with modest selectivity over the serotonin transporter. Compound **7a.a** was also selective over the dopamine transporter as well as a select panel of 5-HT and adrenergic receptors. In addition, **7a.a** possessed suitable pharmaceutical properties. Together, these data supported **7a.a** as a viable and promising Discovery lead molecule. Additional SAR toward the development of this series will be the subject of future communications.

Acknowledgements

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