Bioorganic & Medicinal Chemistry Letters 19 (2009) 5807–5810

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure–activity relationships of the 1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ol series of monoamine reuptake inhibitors

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ARTICLE INFO

Article history:
Received 28 May 2009
Accepted 8 July 2009
Available online 25 August 2009

Keywords:
Vasomotor symptoms (VMS)
Norepinephrine reuptake inhibitors (NRI)
Monoamine reuptake inhibitors
Serotonin transporter

ABSTRACT

The SAR of a series of 1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ols as monoamine reuptake inhibitors, with a goal to improve both potency toward inhibiting the norepinephrine transporter and selectivity over the serotonin transporter, is reported. The effect of specific substitution on both the 3-phenyl group and the indole moiety were explored. This study led to the discovery of compound **20** which inhibited the norepinephrine transporter with an IC_{50} value of 4 nM while exhibiting 86-fold selectivity over the serotonin transporter.

The norepinephrine transporter (NET) is a protein that consists of 12-transmembrane domains and functions to sense the concentration of free norepinephrine (NE) in the synapse and transport excess NE back into presynaptic cells where it is either recycled and re-released or metabolized.[1] NE has been shown to stimulate areas of the hypothalamus that are important in temperature regulation.[2] Furthermore, depletion of estrogens as a result of menopause may produce unstable concentrations of NE resulting in fluctuations in body temperature which manifest as vasomotor symptoms (VMS) or hot flashes.[3] Previously, we disclosed that restoration of NE levels in ovariectomized rats by the administration of a non-selective norepineprine 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 group previously reported the discovery of a novel series of monoamine reuptake inhibitors, the 1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ols **2**, which were identified by combining virtual and focused screening efforts with design techniques.[9] These efforts led to the identification of Discovery lead molecule **3**, the *2R,3S*-isomer of **2**, which potently inhibited NET with an IC_{50} value of 28 nM and exhibited modest, 13-fold selectivity over the serotonin transporter (SERT). Herein, we summarize our efforts to not only optimize the potency of this series toward inhibiting NE uptake, but also and more importantly, to identify compounds with excellent selectivity (target value = 100-fold) over SERT. Compounds were screened in vitro for their ability to block NE uptake in MDCK-Net6 cells that were stably transfected with the human norepinephrine transporter (hNET). Serotonin (5-HT) uptake was measured in a similar manner using JAR cells that were stably transfected with the human serotonin transporter (hSERT). Finally, affinity for the human dopamine transporter (hDAT) was assessed via a competition radioligand-binding assay using purified CHO membranes expressing recombinant hDAT with the cocaine analog, [^{3}H]-3β-(4-fluorophenyl)tropane-2β-carboxylic acid methyl ester ([^{3}H]WIN35,428),[10] as the radioligand. All assay procedures have been formerly reported.[11]

Previously,[9] we reported two important features that were critical for optimal potency and selectivity within the 1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ol scaffold (Fig. 1). First, only secondary amines with small alkyl substituents or a primary amine were tolerated for hNET activity, with the secondary methylamine providing optimal potency. And second, of the four stereoisomers,

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0960-894X/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2009.07.050

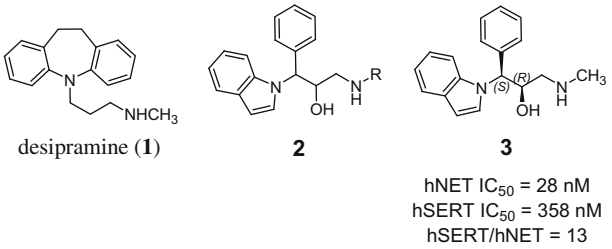
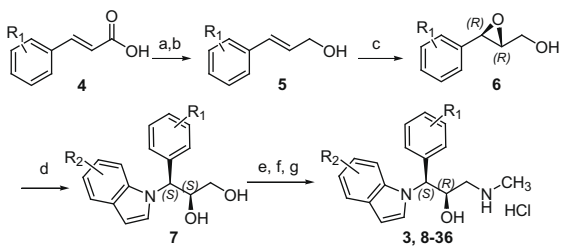


Figure 1. Structures of despiramine (**1**), the 1-amino-3-(1H-indol-1-yl)-3-phenylpropan-2-ol scaffold **2** and discovery lead compound **3**.

the *2R,3S*-stereochemistry afforded the most potent NE uptake inhibition along with the best selectivity over the hSERT. Consequently, SAR studies reported herein focus specifically on the *2R,3S*-isomer with the *N*-methyl amine moiety held constant.

(*2R,3S*)-1-Amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ol analogs **8–36** were synthesized stereospecifically as outlined in Scheme 1. The synthesis started with (*2R,3R*)-3-phenylglycidols **6** which were either obtained from commercial sources or prepared in three steps from *trans*-cinnamic acids **4**. Thus, *trans*-cinnamic acids **4** were converted to *trans*-cinnamyl alcohols **5** via a DIBAL reduction of *trans*-cinnamate esters which were obtain by O-methylation of the carboxylic acid group mediated by cesium carbonate. Subsequent Sharpless epoxidation[12] of *trans*-cinnamyl alcohols **5** afforded (*2R,3R*)-3-phenylglycidols **6** in excellent enantiomeric excess. Epoxide opening with substituted indoles occurred both regio- and stereo-selectively using sodium *tert*-butoxide in the presence of titanium *iso*-propoxide to afford propane-1,2-diols **7** which were converted in three steps to (*2R,3S*)-1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ol analogs **8–36** as previously reported.[9]

An examination of the effect of substitution on the 3-phenyl group (compounds **8–19**) revealed that, in general, substitution on the *meta* -position provided the most potent NE uptake inhibition versus either *ortho* or *para* substitution. For example, *meta*-chloro analog **9** potently inhibited NE uptake with an IC_{50} value of 16 nM versus 304 nM and 527 nM observed with *ortho*-chloro and *para*-chloro analogs **8** and **10**, respectively. Within the group of compounds that we examined, this pattern was maintained regardless of the electronic nature of the substituent. In addition, substitution on the *meta* -position with an electron-withdrawing group (analogs **9**, **12**, **18**, and **19**) improved the selectivity for NET versus SERT when compared to the unsubstituted analog **3**. Although analogs **9**, **12**, **18** and **19** exhibited similar selectivity for NET over SERT (34–40-fold), *meta*-fluoro analog **12** provided the best combination of potent NE uptake inhibition (IC_{50} = 11 nM) and drug-like properties (metabolic stability, solubility, CYP450 inhibition profile, etc.). This observation was consistent with trends observed in a related series of 3-(arylamino)-3-phenylpropan-2-olamines that were reported in an earlier communication.[13] Consequently, the *meta*-fluoro substituent was held constant when we examined the effect of substitution on the indole ring (analogs **20–36**).



Scheme 1. Synthesis of (*2R,3S*)-1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ol analogs. Reagents and conditions: (a) Cs_2CO_3, CH_3I, acetone; (b) DIBAL, CH_2Cl_2, −78°, 82–94% for two steps; (c) *D*-(−)-DIPT, *t*-BuOOH, Ti(*i*-PrO)$_4$, −25 °C, 60–72%, >99% ee; (d) indole, NaH, *t*-BuOH, Ti(*i*-PrO)$_4$, CH_2Cl_2, 25–68%; (e) TsCl, pyridine, rt; (f) CH_3NH_2, CH_3OH; (g) HCl, ethanol, iso-propyl ether, 21–52% for three steps.

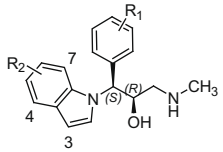
Substitution on the indole ring, in general, was well tolerated and resulted in only modest improvements or losses in inhibition of NE uptake when compared to unsubstituted analog **12**. Interestingly, substitution on any of the 4-, 5-, 6-, or 7-positions of the indole ring reduced selectivity for NET over SERT versus the unsubstituted analog **12**. Most notably, substitution on the 5-position of indole (analogs **23**, **27**, **30**, and **34**) essentially eliminated selectivity. In contrast, alkyl substitution on the indole 3-position (compounds **20** and **21**) improved selectivity for NET versus SERT compared to **12**. In fact, not only did 3-methylindole **20** nearly meet our program selectivity goal of 100-fold by exhibiting 86-fold selectivity for NET versus SERT, it was also the most potent NRI we had made to date exhibiting an IC_{50} value of 4 nM.

The pharmacokinetic profile of **20** was examined in rats after a single oral dose of 10 mg/kg versus a single intravenous dose of 2 mg/kg. Although **20** exhibited poor oral bioavailability of 6%, the brain to plasma ratio, obtained by comparing plasma area-under-the-curve (AUC) to brain AUC, was determined to be 3.5. Since the plasma concentration of **20** was significantly higher than the in vitro IC_{50} value over 8 h after oral administration (C_{max} = 87 ng/mL or 280 nM; 8 h plasma concentration = 12 ng/mL or 40 nM), it was assessed in a telemetric rat model of ovariectomy-induced thermoregulatory dysfunction using previously reported procedures.[11] The results are summarized in Table 2. Compound **20** significantly reduced tail skin temperature (TST) in a dose-dependent manner while its duration of action was consistent with pharmacokinetic findings.

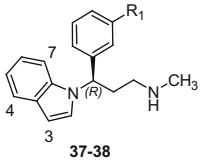
In an effort to determine the contribution of the 2-hydroxyl group toward NET potency and selectivity over SERT and DAT, data from the (*2R,3S*)-1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ols were compared to selected analogs within a series of (*3R*)-3-(1*H*-indol-1-yl)-*N*-methyl-3-phenylpropan-1-amines (compounds **37** and **38**, Table 1), a scaffold we reported previously.[14] A direct comparison of the unsubstituted analogs, compound **3** versus compound **37**, suggested that the 2-hydroxyl group does not contribute to either NE uptake inhibition or selectivity over SERT since the values were essentially identical (NET IC_{50} values of 28 nM versus 34 nM for **3** and **37**, respectively, with corresponding SERT IC_{50} values of 358 nM versus 491 nM). However, a direct comparison of the *meta*-fluoro analogs **12** and **38** revealed a different result. While compound **12**, which incorporated the 2-hydroxy group, was a potent inhibitor of NE uptake (IC_{50} = 11 nM) with good selectivity over SERT of 40-fold, *des*-hydroxy analog **38** was eightfold less potent at inhibiting NET versus **12** but was a potent inhibitor of 5-HT uptake (IC_{50} = 14 nM) with nearly sevenfold selectivity for SERT versus NET. Clearly, however, the 2-hydroxyl group provided reduced affinity for DAT versus the *des*-hydroxy analogs.

In summary, the SAR of a series of (*2R,3S*)-1-amino-3-indol-1-yl)-3-phenylpropan-2-ols was examined. In general, substitution on the *meta*-position of the 3-phenyl group with electron-withdrawing substituents afforded potent NRIs with good selectivity (34–40-fold) over SERT and only weak affinity for DAT. Alkyl substitution at the 3-position of indole further enhanced selectivity for NET versus SERT. Combining the *meta*-fluoro substituent on the 3-phenyl group with 3-methyl indole, compound **20**, provided a highly potent NRI (IC_{50} = 4 nM) that exhibited 86-fold selectivity over SERT. Compound **20** was tested in a telemetric rat model of ovariectomized-induced thermoregulatory dysfunction and reduced TST in a dose-dependent manner; however, it exhibited low oral bioavailability in rats. Efforts to improve the pharmacokinetic parameters within this series will be the subject of a future communication.

Table 1
Characterization of (*2R,3S*)-1-amino-3-(1*H*-indol-1-yl)-3-phenylpropan-2-ol analogs compared to (*3R*)-3-(1*H*-indol-1-yl)-*N*-methyl-3-phenylpropan-1-amines at the human norepinephrine, serotonin and dopamine transporters[a]



3, 8-36



37-38

Compd	R_1	R_2	hNET uptake IC_{50}[b] nM (SD)	hSERT uptake IC_{50}[c] nM (SD)	Ratio of hSERT uptake IC_{50}/hNET uptake IC_{50}[d]	hDAT binding %inhibition @ 1 μM[e] (%)
3	H	H	28 (11)[f]	358 (108)[f]	13	19
8	*o*-Cl	H	304	1333	4	7
9	*m*-Cl	H	16 (1)[f]	554 (8)[f]	34	20
10	*p*-Cl	H	527	748	1	16
11	*o*-F	H	92 (35)[f]	473	5	4
12	*m*-F	H	11 (3)[f]	441 (7)[f]	40	23
13	*p*-F	H	84	1366	16	11
14	*o*-CH_3	H	232	266	1	11
15	*m*-CH_3	H	49	377	8	8
16	*p*-CH_3	H	117	398	3	0
17	*m*-OCH_3	H	111	882	8	0
18	*m*-Br	H	14	547	39	13
19	*m*-CF3	H	54 (8)[f]	2184 (383)[f]	40	14
20	*m*-F	3-CH_3	4 (1)[f]	343 (50)[f]	86	8
21	*m*-F	3-CH_3CH_2	10 (3)[f]	834 (109)[f]	83	0
22	*m*-F	4-Cl	64	221	3	12
23	*m*-F	5-Cl	60	79	1	6
24	*m*-F	6-Cl	92	865	9	9
25	*m*-F	7-Cl	21	48	2	0
26	*m*-F	4-CH_3	15	228	15	4
27	*m*-F	5-CH_3	21	48	2	0
28	*m*-F	7-CH_3	18	527	29	7
29	*m*-F	4-F	16 (5)[f]	417 (75)[f]	26	3
30	*m*-F	5-F	22	78	4	9
31	*m*-F	6-F	20 (7)[f]	626 (45)[f]	31	15
32	*m*-F	7-F	7 (2)[f]	175 (38)[f]	25	8
33	*m*-F	4-Br	52	207	4	24
34	*m*-F	5-Br	60	52	1	10
35	*m*-F	6-Br	124	1003	8	12
36	*m*-F	7-Br	21	113	5	0
37	H	N/A	34	491	14	44
38	F	N/A	91	14	0.2	52

[a] Data is the average of at least three triplicate runs; standard error (SE) ⩽ 30% of the mean, unless otherwise indicated. NT = not tested.
[b] 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.
[c] 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.
[d] Unitless value as a ratio in which higher numbers represent relatively greater NET selectivity. A value of 1 represents no selectivity.
[e] 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.
[f] Data is the average of between 2 and 6 independent experiments, each run in triplicate.

Table 2
Oral activity of **20** in a telemetric rat model of ovariectomized-induced thermoregulatory dysfunction[a]

Dose (mg/kg)	Onset of activity (h)	Duration of action (h)	Mean reduction in TST[b] (°C)	Maximum reduction in TST[b] (°C)
10	1	5	−1.5	−2.3
30	0.5	10.5	−3.8	−7.0

[a] Compound was dosed orally (n = 16 rats) via 2% Tween-80/0.5% methylcellulose in water vehicle.
[b] TST = tail skin temperature.

Acknowledgement

The authors thank the Wyeth Discovery Analytical Chemistry group for spectral data and compound analysis.

References and notes

1. (a) Blakely, R. D.; De Felice, L. J.; Hartzell, H. *J. Exp. Biol.* **1994**, *196*, 263–281; (b) Nguyen, T. T.; Amara, S. G. *J. Neurochem.* **1996**, *67*, 645–655; (c) Lankupalle, J. D.; Annamalai, B.; Samuvel, D. J.; Gether, U.; Ramamoorthy, S. *J. Biol. Chem.* **2006**, *281*, 23326–23340.
2. (a) Kumar, V. N. *Proc. Natl. Acad. Sci., Part B: Biol. Sci.* **2003**, *69*, 507–524; (b) Riedel, W.; Dorward, P. K.; Korner, P. I. *Auton. Neurosci.* **1981**, *3*, 525–533; (c) Lahti, H.; Pyornila, A.; Hissa, R. *Experientia* **1980**, *36*, 1188.
3. Panek, D.; Dixon, W. *J. Pharmacol. Exp. Ther.* **1986**, *236*, 646–652.
4. Deecher, D.; Merchenthaler, I.; Leventhal, L.; Sipe, K.; O'Connor, L. *PCT Int. Appl.* **2004**, *58*p. WO2004035035A1. CAN140:350602.
5. (a) Dubini, A.; Bosc, M.; Polin, V. *J. Psychopharmacol.* **1997**, *11*, 17–23; (b) Ban, T. A.; Gaszner, P.; Aguglia, E.; Batista, R.; Castillo, A., et al *Hum. Psychopharmacol.* **1998**, *13*, S29–S39.
6. Bymaster, F. P.; Katner, J. S.; Nelson, D. J.; Hemrick-Luecke, S. K.; Threlkeld, P. J.; Heiligenstein, J. H.; Morin, S. M.; Gehlert, D. R.; Perry, K. W. *Neuropsychopharmacology* **2002**, *27*, 699–711.
7. Krell, H. V.; Leuchter, A. F.; Cook, I. A.; Abrams, M. *Psychosomatics* **2005**, *46*, 379–389.
8. Bergan, T. *Can. J. Psychiatry* **2004**, *49*, 499–500.
9. Kim, C. Y.; Mahaney, P. E.; McConnell, O.; Zhang, Y.; Manas, E.; Ho, D. M.; Deecher, D. C.; Trybulski, E. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5029–5032.
10. (a) Reith, M. E. A.; Meisler, B. E.; Sershen, H.; Lajtha, A. *Biochem. Pharmacol.* **1986**, *35*, 1123–1129; (b) Madras, B. K.; Fahey, M. A.; Borgman, J.; Canfield, D. R.; Spealman, R. D. J. *Pharmacol. Exp. Ther.* **1989**, *251*, 131–141.
11. Mahaney, P. E.; Vu, A. T.; McComas, C. C.; Zhang, P.; Nogle, L. M.; Watta, W. L.; Sarkahian, A.; Leventhal, L.; Sullivan, N. R.; Uveges, A. J.; Trybulski, E. J. *J. Med. Chem.* **2008**, *51*, 4038.
12. Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974–5976.

13. Vu, A. T.; Cohn, S. T.; Terefenko, E. A.; Moore, W. J.; Zhang, P.; Mahaney, P. E.; Trybulski, E. J.; Goljer, I.; Dooley, R.; Bray, J. A.; Johnston, G. H.; Leiter, J.; Deecher, D. C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2464–2467.

14. Mahaney, P. E.; Vu, A. T.; McComas, C. C.; Zhang, P.; Nogle, L. M.; Watts, W. L.; Sarkahian, A.; Leventhal, L.; Sullivan, N. R.; Uveges, A. J.; Trybulski, E. J. *Bioorg. Med. Chem.* **2006**, *14*, 8455–8466.