Bioorganic & Medicinal Chemistry Letters 18 (2008) 6062–6066

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel 3,3-disubstituted pyrrolidines as selective triple serotonin/norepinephrine/dopamine reuptake inhibitors

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ARTICLE INFO

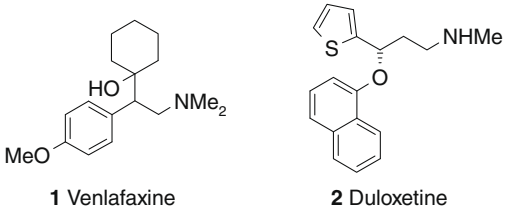
Article history:
Received 19 September 2008
Revised 3 October 2008
Accepted 7 October 2008
Available online 11 October 2008

Keywords:
Triple reuptake inhibitor
Anti-depressant
Serotonin
Norepinephrine
Dopamine
Monoamine transporter
Pain

ABSTRACT

A series of 3,3-disubstituted pyrrolidine monoamine triple reuptake inhibitors were discovered. Analogues with low nanomolar potency, good human in vitro microsomal stability and in vitro permeability, and low drug–drug interaction potential are described. One example showed in vivo anti-depressant-like effects in the mouse tail suspension assay with a minimum effective dose of 30 mg/kg ip.

Nearly all clinical antidepressants act by elevating levels of one or more of the monoamine neurotransmitters serotonin (5-HT), norepinephrine (NE) and dopamine (DA).[1] Selective inhibitors of 5-HT and NE reuptake (SNRIs) have been shown to be effective in the treatment of major depressive disorders (MDD). Venlafaxine (Effexor®) **1** and duloxetine (Cymbalta®) **2** are both Food and Drug Administration (FDA) approved SNRI drugs for the treatment of MDD. Venlafaxine **1** has been shown to have a higher remission rate than the class of selective serotonin reuptake inhibitors (SSRIs) (Fig. 1).[2]



1 Venlafaxine **2** Duloxetine

Figure 1. Marketed SNRIs.

While the SSRI/SNRI classes of drugs have been shown to provide a safe and effective treatment for depression there is still a need for improvement. Triple reuptake inhibitors (TRIs) increase DA levels in addition to 5-HT and NE.[3] Elevating CNS levels of dopamine through inhibition of the dopamine transporter (DAT) may address the anhedonic component of depression as well as shorten the time to onset. This could result in improved efficacy toward a broader range of the depressed population.[4] Duloxetine has also been approved for the treatment of diabetic neuropathic pain and preclinical data suggest that dopamine transporter (DAT) inhibition may bring additional efficacy.[5] Thus, TRIs have the potential to be more effective in the management of depression and neuropathic pain, and to clearly differentiate from the current treatments. Our aim, therefore, was to generate novel small molecule inhibitors of all three monoamine transporters that would address the need for improved therapies and also act as tool compounds to permit greater understanding of the role of the DAT in MDD.

We recently disclosed a series of indolyl phenyl propylamines as SNRIs, exemplified by **3**.[6] Our knowledge of the structure–activity relationships (SAR) around this template combined with that of DOV-21947 **4**, a weakly active TRI that is currently undergoing Phase II clinical trials by DOV Pharmaceuticals for the treatment of depression,[7] led us to design a series of novel inhibitors based

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doi:10.1016/j.bmcl.2008.10.025

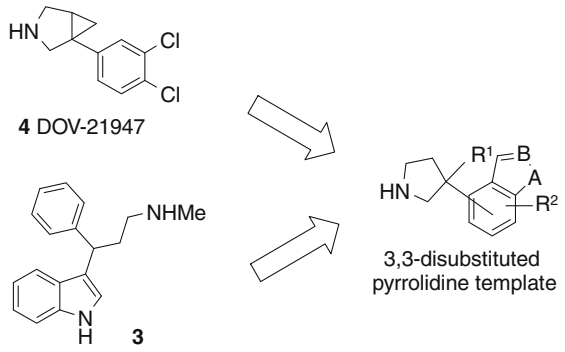


Figure 2. Design of novel triple reuptake inhibitors.

Table 1
In vitro inhibition of monoamine reuptake.[a]

Compd	SERT	K_i(nM) NET	DAT
3	9	89	>1400
4	63	63	251

[a] Monoamine reuptake K_i values are the geometric mean of at least three experiments.

on a 3,3-disubstituted pyrrolidine motif (Fig. 2 and Table 1). Herein we describe the synthesis and SAR for this series of triple reuptake inhibitors.

The target compounds were all prepared using rhodium catalyzed conjugate-addition of aryl boronic acids to maleimides as the key bond forming step.[8] Representative synthetic schemes are shown (Schemes 1–7).[9] Thus, the appropriate boronic acid **5** was coupled with *N*-benzylmaleimide to generate 1-benzyl-3-substituted pyrrolidine-2,5-diones **6** as outlined in Scheme 1. Protection of the indole under phase transfer conditions, followed by alkylation under mild conditions furnished the 3,3-disubstituted pyrrolidine-2,5-diones **7**. Deprotection of **7** followed by reduction with lithium aluminum hydride yielded the corresponding 1-benzylpyrrolidine **9**. Finally, a hydrogenolysis yielded the desired pyrrolidines **10** and **11**.

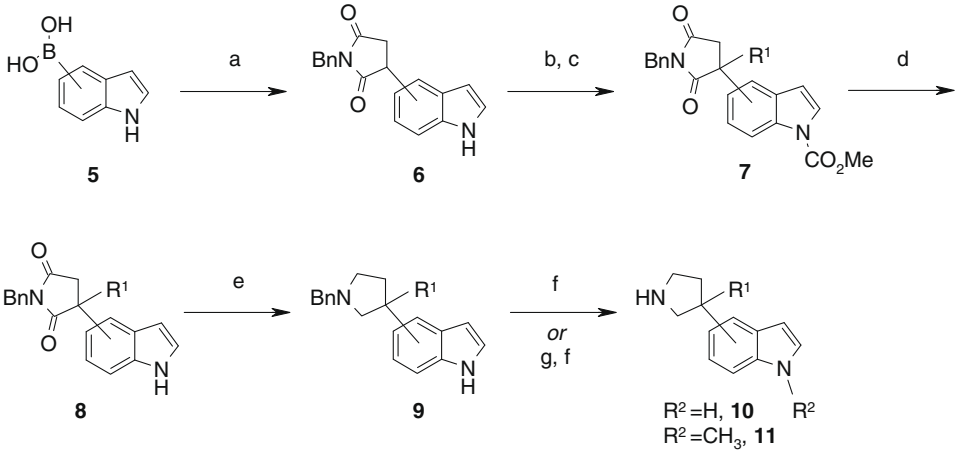
The first compound synthesized, **16**, was a potent inhibitor at all three of the monoamine reuptake transporters (Table 2). We were excited to find that it exhibited significantly higher potency than DOV-21947.[10] In addition, it showed favorable in vitro permeability and hERG inhibition (Table 6).[11,12] Liabilities included low human in vitro microsomal stability and strong CYP2D6 inhibition. When the enantiomers were separated most of the potency was found to reside in a single enantiomer, (+)-**16**. When tested in vivo in the mouse tail suspension assay, an assay used to evaluate anti-depressant agents, activity was observed at 30 mg/kg, ip.[13] Comparative data for Duloxetine showed anti-depressant like activity in the same assay with a minimum effective dose of 10 mg/kg ip.

Initial SAR investigations focused on replacing the indole ring (Table 2 and Schemes 2, 6 and 7). Many of the replacements were essentially equipotent with the indole, but maintaining acceptable microsomal stability,[14] permeability, and CYP2D6[15] and hERG inhibition levels proved challenging (Table 6). Indole regioisomer **20** retained similar potency but offered no notable benefits. Benzothiophene **18** was balanced and potent at all three SERT/NET/DAT transporters but unfortunately also showed poor in vitro stability. Introduction of polarizing groups to increase polar surface area resulted in stabilization towards microsomal clearance. Indazole **17** showed a threefold improvement in metabolic stability but remained a potent CYP2D6 inhibitor. 7-Azaindole **19** gave only a moderate improvement to stability and lost significant potency.

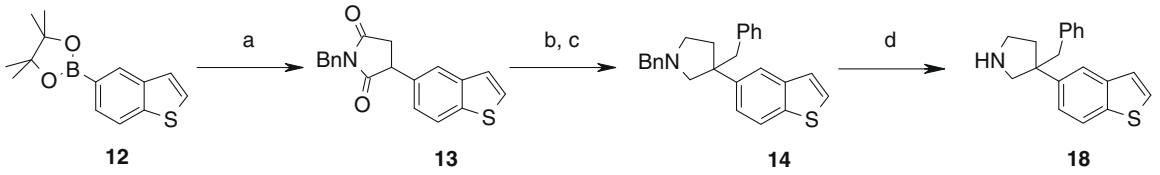
We next studied the SAR around the hydrophobic aromatic group (Table 3). In general, substitution was tolerated for potency but there was no improvement in stability towards human liver microsomes (Table 6). Replacement of the phenyl ring with a pyridyl group (e.g., **22**) did result in an improved CYP2D6 and hERG profile, but the additional hydrogen bond acceptor also resulted in P-gp mediated efflux.[16] Since minor modifications of the benzyl group appeared unlikely to yield metabolically stable compounds, we turned our focus back to the indole ring (Schemes 3–5).

Introduction of substituents in the 2- or 3-position of the indole ring reduced microsomal clearance and therefore increased metabolic stability (Tables 4 and 6). Inhibition of the hERG channel was notably worse in the case of 2-cyanoindole **28** despite its increased polar surface area, and CYP2D6 inhibition remained high. Amide **29** was potent and quite balanced at all three transporters, and showed a marked reduction in affinity to CYP2D6 and hERG. However, the two additional hydrogen bond donors resulted in strong P-gp mediated efflux. The efflux ratio was reduced by successive replacement of the hydrogen atoms with methyl groups, but could not be entirely avoided (see **30** and **31**). 3-Cyanoindole **32** lost potency and also suffered high P-gp mediated efflux.

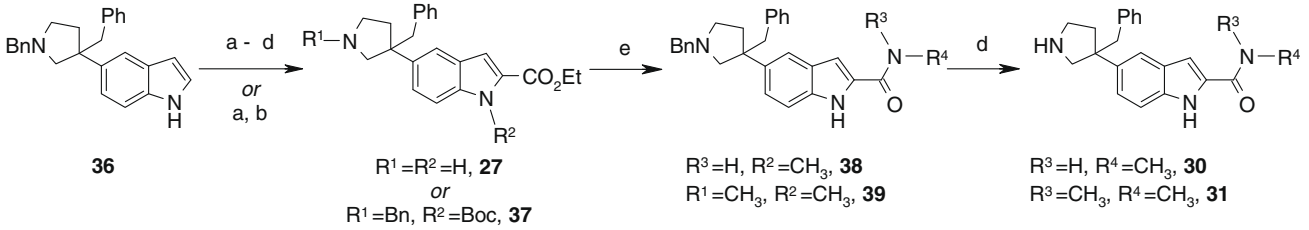
We next investigated replacement of the aromatic hydrophobe portion of our pharmacophore with an aliphatic one (Tables 5 and 6). Increasing metabolic stability by the introduction of polarizing



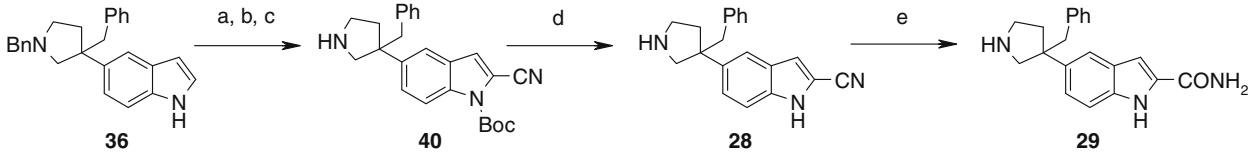
Scheme 1. Reagents and conditions: (a) *N*-benzylmaleimide, [RhOH(cod)]$_2$ Et_3N], dioxane, H_2O, rt, 99%; (b) $BnNEt_3Br$, $ClCO_2Me$, 30% NaOH, 0 °C, 80%; (c) RX, K_2CO_3, DMF, rt, 77%; (d) LiOH, THF, 80 °C, 91%; (e) $LiAlH_4$, THF, 80 °C, 90%; (f) H_2, 20% $Pd(OH)_2$/C, MeOH, 60 psi, 54%; (g) NaH, DMF, rt, then MeI, rt, 62%.



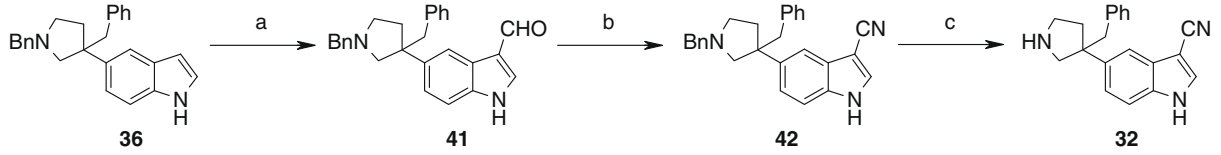
Scheme 2. Reagents and conditions: (a) *N*-benzylmaleimide, $[RhOH(cod)]_2$ Et_3N], dioxane, H_2O, rt, 97%; (b) BnBr, K_2CO_3, DMF, 45 °C; (c) $LiAlH_4$, THF, 80 °C, 61% (2 steps); (d) α-chloroethyl chloroformate, Et_3N, 1,2-DCE then MeOH, 80 °C, 15%.



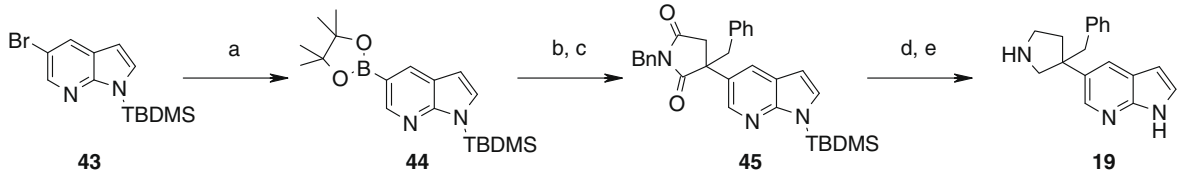
Scheme 3. Reagents and conditions: (a) Boc_2O, DMAP, Et_3N, CH_2Cl_2, 76%; (b) *t*-BuLi, THF, −78 °C, 1 h then $ClCO_2Et$, 75%; (c) TFA, CH_2Cl_2, rt, 100%; (d) H_2, 20% $Pd(OH)_2$/C, MeOH, 60 psi, 54% for compound **30**; (e) R1R2NH.HCl, $AlMe_3$, PhH, 80 °C, 32% for compound **38**.



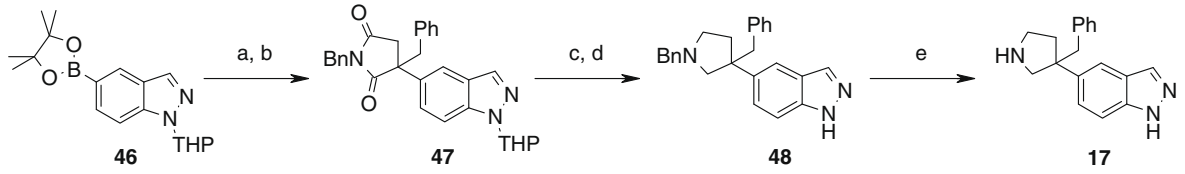
Scheme 4. Reagents and conditions: (a) Boc_2O, DMAP, Et_3N, CH_2Cl_2, 76%; (b) *t*-BuLi, THF, −78 °C, 1h then PhOCN, 53%; (c) α-chloroethyl chloroformate, Et_3N, 1,2-DCE then MeOH, 76%; (d) KOH, 100 °C, 18 h, 48%; (e) KOH, 100 °C, 24 h, 34%.



Scheme 5. Reagents and conditions: (a) $(COCl)_2$, DMF, 0 °C then NaOH, 18%; (b) MnO_2, NH_3, iPrOH, $MgSO_4$, THF, 50 °C, 15 h, 73%; (c) H_2, 20% $Pd(OH)_2$/C, MeOH, 60 psi, 67%.



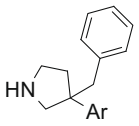
Scheme 6. Reagents and conditions: (a) bis(pinacolato)diborane, $Pd(dppf)Cl_2$. KOAc, dioxane, 95 °C, 90 min 47%; (b) *N*-benzylmaleimide, $[RhOH(cod)]_2{\cdot}Et_3N$], dioxane, H_2O, rt, 52%; (c) BnBr, K_2CO_3, DMF, rt, 67%; (d) $LiAlH_4$, THF, 80 °C, 67%; (e) H_2, 20% $Pd(OH)_2$/C, MeOH, 60 psi, 76%.



Scheme 7. Reagents and conditions: (a) *N*-benzylmaleimide, $[RhOH(cod)]_2$ Et_3N], dioxane, H_2O, rt, 80%; (b) BnBr, K_2CO_3, DMF, rt, 77%; (c) $LiAlH_4$, THF, 80 °C, 63%; (d) 2 N HCl, MeOH, 50 °C, 63%; (e) H_2, 20% $Pd(OH)_2$/C, MeOH, 60 psi, 53%.

groups had always come at the expense of in vitro permeability (vide supra). Our earlier discovery that replacing the benzyl group with a pyridine had minimized CYP2D6 and hERG binding led us to infer that the benzyl group played a major role in binding to these 'off-targets'. Loss of potency was observed with methyl compound **33**, indicating that the pharmacophore required a significant

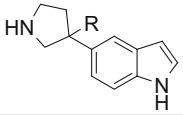
Table 2
In vitro inhibition of monoamine reuptake.[a]



Compd	Ar	K_i (nM)		
		SERT	NET	DAT
16	5-indolyl	10	1	11
(+)-16	5-indolyl	6	1	11
(-)-16	5-indolyl	54	17	81
17	5-indazolyl	1	5	115
18	5-benzothiophenyl	7	3	16
19	5-(7-azaindolyl)	41	41	741
20	6-indolyl	13	1	43
(+)-20	6-indolyl	8	1	21
(-)-20	6-indolyl	190	78	447
21	3-indolyl	23	60	1260

[a] Monoamine reuptake K_i values are the geometric mean of at least three experiments.

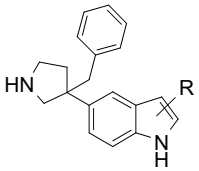
Table 3
In vitro inhibition of monoamine reuptake.[a]



Compound	R	K_i (nM)		
		SERT	NET	DAT
22	Pyridin-2-ylmethyl	53	8	62
23	4-F-Bn	15	7	79
24	3-MeO-Bn	34	11	39
25	CH_2CH_2Ph	13	16	58

[a] Monoamine reuptake K_i values are the geometric mean of at least three experiments.

Table 4
In vitro inhibition of monoamine reuptake.[a]

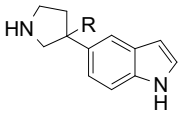


Compound	R	K_i (nM)		
		SERT	NET	DAT
26	1-(*N*)-Me	4	1	10
27	2-CO_2Et	4	19	38
28	2-CN	0.4	10	83
29	2-$CONH_2$	2	9	18
30	2-$CONHCH_3$	13	151	331
31	2-$CON(CH_3)_2$	22	10	104
32	3-CN	66	5	724

[a] Monoamine reuptake K_i values are the geometric mean of at least three experiments.

hydrophobe for tight binding to the monoamine reuptake transporters. The propyl-substituted compound **34** recovered potency and demonstrated a 40-fold increase in stability upon incubation with human liver microsomes (HLMs). CYP2D6 inhibition also concurrently improved approximately 15-fold. We were also able to increase potency at the target transporters further with the butyl compound **35**, and although CYP2D6 binding and microsomal stability suffered slightly, the overall in vitro profile of this compound was significantly improved.

Table 5
In vitro inhibition of monoamine reuptake.[a]



Compound	R	K_i (nM)		
		SERT	NET	DAT
33	Me	871	288	550
34	^{n}Pr	66	71	363
35	^{n}Bu	16	9	60

NT denotes not tested.
[a] Measured at 30 °C.

Table 6
In vitro human microsomal stability, CYP2D6 inhibition, CACO-2 efflux ratio, and hERG inhibition[a] for selected compounds.

Compound	HLM (μL/min/mg)	CYP 2D6 IC_{50} (μM)	ER (BA/AB) (cm/s $\times 10^{-6}$)	hERG IC_{50} (μM)
3	915	3.1	NT	1
4	47	7	0.7 (2.1/2.9)	10
16	100	0.5	0.8 (14/17)	9
(+)-**16**	154	0.3	0.5 (9.9/21)	NT
17	28	0.4	2.2 (22/10)	NT
18	198	NT	NT	11
19	69	11.5	NT	NT
20	NT	2.6	NT	NT
(+)-**20**	NT	0.9	NT	NT
(−)-**20**	NT	1.6	NT	NT
21	NT	1.8	NT	NT
22	86	3.5	5.6 (18/3.2)	>30
23	84	0.1	NT	9
24	77	0.1	NT	NT
25	106	0.3	0.4 (4.5/11.4)	NT
26	106	0.1	NT	7
27	47.8	0.6	1.0(3.4/3.5)	NT
28	0	0.1	1.0 (7.8/7.9)	1.4
29	1.4	4.1	28.3 (17/0.6)	>10
30	11.3	4.8	16.0 (19/1.4)	>10
31	12.8	2.9	5.9 (24/4.0)	NT
32	18.8	0.8	11.3 (25/2.2)	NT
33	NT	8.7	NT	NT
34	2.3	7.5	1.5 (24/16.5)	NT
35	38	3.4	2.0 (19/9.4)	4.4

NT denotes not tested.
[a] Measured at 30 °C.

In summary, we were able to combine two discrete sub-structures to arrive at a novel series of potent triple serotonin, norepinephrine and dopamine reuptake inhibitors. We were not only able to optimize this novel chemical series for both potency and selectivity, but also we modulated CYP2D6 and successfully improved metabolic stability in human liver microsomes. Overall, we have discovered that subtle chemical modifications in the structure lead to an enormous effect on these properties. Additional information on the progress of this scaffold will be reported in due course.

Acknowledgments

The authors thank in particular Marquis Cummings Jr. of the Analytical Separations Group for the timely separation of enantiomers and Francisco Talamas for his invaluable contributions to this work.

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9. Yields shown in Scheme 1 are for compound (±)-**16** and are representative for the compound set.
10. DOV-21947 was prepared in house and submitted to assay: pK_i (nM) SERT/NET/DAT = 63/63/251.
11. Permeability was measured using Caco-2 cells at 21 days of culture. Efflux ratios are measured using the bi-directional (P_{app} A to B and B to A) values. Substrate concentration 10 μM.
12. hERG IC_{50} values calculated using the PatchXpress 7000A (MDS, Inc., Toronto, Canada) to carry out electrical recording of K^+ currents in CHO cells expressing the human ERG channel. Recordings were made at 30 °C with test compounds at two concentrations (1 μM, 10 μM).
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14. CL_{int} measured in HT format: total incubation volume 250 μL, 0.5 mg/mL microsomal protein, reaction started by adding NADPH and performed at 37 °C with no shaking. 40 μL aliquots at four time-points (0.1, 10, 20 and 30 min) were taken then the samples transferred to 384 well plates for LC/MS analysis.
15. CYP2D6 inhibition was measured in a fluorescence based assay where CYP marker substrates become fluorescent upon CYP metabolism.
16. P-gp inhibition was confirmed by addition of 2 μM elacridar as P-gp inhibitor in the Caco-2 permeability assay.