Bioorganic & Medicinal Chemistry Letters 20 (2010) 3941–3945

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

2-Substituted *N*-aryl piperazines as novel triple reuptake inhibitors for the treatment of depression

David S. Carter *, Hai-Ying Cai, Eun Kyung Lee, Pravin S. Iyer, Matthew C. Lucas, Ralf Roetz, Ryan C. Schoenfeld, Robert J. Weikert

Department of Medicinal Chemistry, Roche Palo Alto LLC, 3431 Hillview Avenue, Palo Alto, CA 94304, USA

ARTICLE INFO

Article history:
Received 15 March 2010
Revised 4 May 2010
Accepted 5 May 2010
Available online 10 May 2010

Keywords:
Triple reuptake inhibitors
Reuptake inhibitors
Depression
Serotonin
Norepinephrine
Dopamine

ABSTRACT

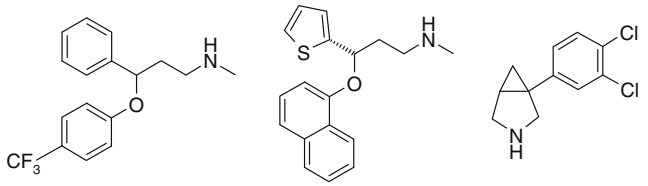
Recently a class of compounds known as triple reuptake inhibitors has emerged as a new strategy for the treatment of depression. These compounds work by simultaneously inhibiting the synaptic reuptake of serotonin, norepinephrine and dopamine. In this Letter we describe the optimization of a novel series of 2-substituted *N*-aryl piperazine based triple reuptake inhibitors.

© 2010 Elsevier Ltd. All rights reserved.

It is estimated that as much as 17% of the US population 18 and older will suffer from depression at some point in their lifetime.[1] Although any age group can be effected, depression predominately strikes 25–44 year olds. Loss of productivity from this group is estimated to be more than $31 billion per year.[2] Depression is characterized by the chronic feeling of sadness. Symptoms include depressed mood, loss of interest, disruption in sleep patterns, fatigue and sometimes suicidal tendencies. Not only can these symptoms themselves be debilitating but they often co-occur with other chronic conditions such as cancer, stroke, heart disease, pain and HIV.[3]

It is hypothesized that depression results from abnormally low levels of certain biogenic amines.[4] These small molecule monoamine neurotransmitters are responsible for relaying and modulating signals between neurons. Of all the neurotransmitters that have been identified to date, three have been identified as playing an important role in depression: serotonin, norepinephrine and dopamine.[5] Serotonin is believed to be responsible for the feeling of well being and is important in the perception of emotional cues.[6] Norepinephrine is involved in cognition, mood, and the fight or flight response.[7] Dopamine is central to the pleasure and reward system.[8]

Currently marketed treatments for depression rely on boosting the levels of one or more of these neurotransmitters by selective inhibition of transporter mediated reuptake of these amines. For example, fluoxetine (Prozac, **1**) is a selective serotonin reuptake inhibitor (SSRI). While it does demonstrate clinical efficacy, about 30% of patients don't respond to therapy. Furthermore, delayed onset of action (3–6 weeks) coupled with side effects such as insomnia and sexual dysfunction contribute to treatment cessation.[9] It has been suggested that some patients do not respond to SSRIs because they may have different symptomatic profiles (i.e., irritability or anxiety in addition to depressive symptoms).[10] These patients may see a benefit from compounds like duloxetine (Cymbalta, **2**) which is a dual serotonin and norepinephrine reuptake inhibitor (SNRI). The success of this strategy is still under investigation.



fluoxetine (1) duloxetine (2) DOV-216,303 (3)

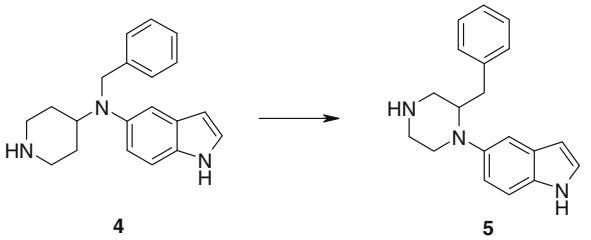
More recently, compounds such as DOV-216,303 (**3**) have emerged as an import new class of compounds for the treatment of depression.[11] The effectiveness of this class of compounds, referred to as 'triple reuptake inhibitors', is supported by clinical data which demonstrates that dopamine reuptake inhibition addresses some of the unmet needs of both SSRIs and SNRIs.[12] For example augmentation trials with buproprion, which is a NET/DAT reuptake inhibitor, and an SSRI demonstrated improved efficacy and less

* Corresponding author. Tel.: +1 650 855 5615; fax: +1 650 855 5238.
E-mail address: david.carter5@comcast.net (D.S. Carter).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.05.008

sexual side-effects over an SSRI alone.[13] Moreover, methylphenidate augmentation led to an accelerated onset of action.[14] Together these results demonstrate that compounds that act as triple reuptake inhibitors may be advantageous in treating depression.

Recently we reported on a novel C5-aminoindole series of triple reuptake inhibitors.[15] Compounds such as 4-aminopiperidine **4** were potent reuptake inhibitors at all three transporters (SERT, NET, and DAT),[16] had good drug-like properties,[17] but lacked in vivo efficacy. Compound **4** was inactive at 30 mg/kg in the locomotor assay (LMA) and the tail suspension test (TST) in mice. This was surprising since this compound was expected to have good CNS penetration.[18] Another method for predicting in vivo efficacy is the measurement of receptor occupancy. For compound **4** the percentage of occupied DAT receptors in mouse (% DAT occupancy = concentration in striatum/concentration in cerebellum) was 0%. Based on this result, the lack of in vivo activity is not surprising since human clinical data from buproprion augmentation trials demonstrated that approximately 20% DAT occupancy was required for efficacy.[19] We hypothesize that the lack of dopamine transporter occupancy was likely due to the insufficient unbound concentration required to occupy the dopamine transporters in the striatum. Although not measured directly, we expect the SERT/NET occupancy would similarly be lower than the activity threshold.



4 **5**

	SERT	NET	DAT
pKi	9.1	8.1	7.3

	SERT	NET	DAT
pKi	7.9	8.1	8.2

Utilizing a template hopping strategy, we developed a new 2-substituted piperazine-based series. We felt that the reduced pK_a of the piperazine would help to boost the brain levels of these compounds through improved permeability thereby providing in vivo efficacy. Gratifyingly, piperazine **5** was active in both the tail suspension test (TST) and locomotor assay (LMA) models (MED 10 mg/kg). Furthermore, the in vivo efficacy was confirmed by measuring high DAT occupancy (75%) in mouse. In fact the unbound brain levels[20] for piperazine **5** were determined to be five-fold higher than for piperidine **4** (206 nM vs 37 nM). Since both of these compounds were not Pgp substrates, this result suggested that the basicity of the amine played an important role in boosting the brain free fraction of piperazine **5**.

While this series generally possessed the desired level of potency[21] at all three transporters, some of the structural features (Fig. 1: basic amine, 5-aminoindole) imparted some early safety alerts. These include CYP2D6 inhibition and CYP3A4 time dependent inactivation (TDI). In addition, we needed to address the low metabolic stability and investigate the effect of the stereogenic center on the aforementioned issues. This paper describes the optimization strategy we undertook to address each of these liabilities while maintaining the desired potency profile.

Compounds such as piperazine **5** were conveniently prepared in a four step sequence outlined in Scheme 1. First, 5-bromoindole was treated with lithium bis(trimethylsilyl)amide in THF followed by TBSCl to afford the protected indole in 98% yield. The indole was coupled directly with commercially available 1-Boc-3-benzylpiperazine under Buchwald–Hartwig conditions[22] which provided the coupled product in good yield (74% yield).[23] Treatment with TBAF in THF followed by deprotection with TFA in DCM gave the final compound in 69% yield (two steps).

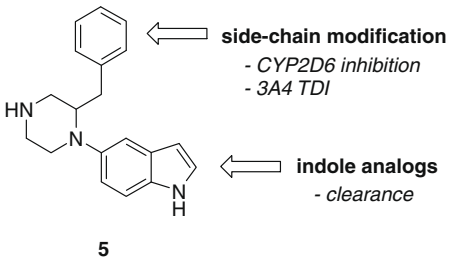
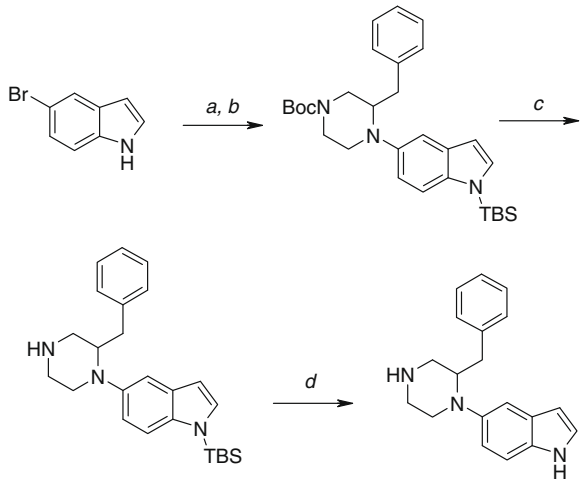


Figure 1. The optimization strategy for piperazine **5**.

Based on the SAR from an earlier template,[24] we suspected that the aminoindole moiety was responsible for the high levels of NET and DAT potency. Unfortunately, the 2,3-unsubstituted indole was most likely responsible for the low metabolic stability. We therefore prepared several analogs to determine the important features of the indole (Table 1). The phenyl analogs (**6, 7**) lacked sufficient NET and DAT activity suggesting that the 3,4-disubstituted ring system was important for activity. Furthermore, 2,3-dichlorophenyl **8** and *N*-methyl indole **9** led to a 11- and 13-fold drop in DAT potency, respectively. These results together suggested that the NET and DAT potency benefit from an important hydrogen bond interaction.

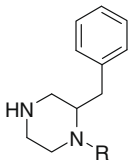
With the requirement for an indole-like heterocycle established, we turned our attention to indole analogs that were more metabolically stable since the in vitro clearance of indole **5** in human liver microsomes (HLM)[25] predicted low stability (Table 2). In order to protect the 2,3-unsubstituted indole, we prepared two amide analogs. Neither the 3-substituted amide **10** nor 2-substituted amide **11** showed significant NET activity. Interestingly, amide **11** was very potent at both SERT and DAT. Next, we prepared several 7-substituted analogs. While methoxyindole **12** lacked sufficient DAT activity, chloroindole **13** lacked the desired balance between NET and DAT. Although relatively balanced at all three transporters, fluoroindole **14** still lacked the desired high metabolic stability. Finally, we prepared two aza-indole analogs. While the 7-aza-indole **15** lacked sufficient NET and DAT activity, indazole **16** displayed good potency at all three transporters. The indazole ring also provided considerable metabolic stability as compared to the indole ring.

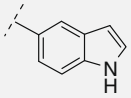
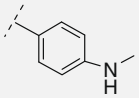
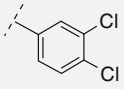
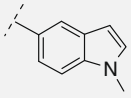
One critical liability for this series was the inhibition of CYP2D6, which is a typical issue for lipophilic basic compounds.[26] Not



Scheme 1. Synthesis of 2-substituted *N*-aryl piperazine **5.** Reagents and conditions: (a) LHMDS, THF, TBSCl; (b) $Pd(OAc)_2$, tBu_3P, NaO*t*Bu, xylenes, 1-Boc-3-benzylpiperazine, 80 °C; (c) TFA/DCM; (d) TBAF/THF.

Table 1
Key features of the indole drive NET/DAT potency



Compd	R	SERT[a] pK_i	NET[a] pK_i	DAT[a] pK_i
5		7.9	8.1	8.2
6		7.8	6.4	6.3
7		7.1	6.3	6.4
8		7.8	7.8	7.1
9		8.1	7.3	6.9

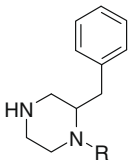
[a] pK_i (binding) values are the geometric mean of at least three experiments measured in HEK 293 cells.

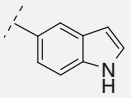
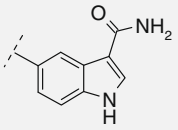
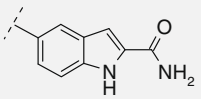
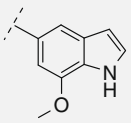
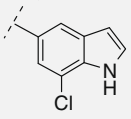
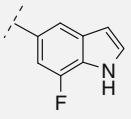
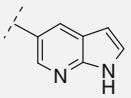
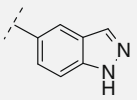
wanting to move forward with this potential drug–drug interaction (DDI) liability, we initially chose to evaluate the SAR of CYP2D6 inhibition through modifications of the benzylic side-chain. A series of indazole analogs was investigated which varied size and shape of the benzylic side-chain as well as reduced overall lipophilicity.

With very few commercial 2-substituted piperazines available,[27] we developed a flexible synthesis for these side-chain analogs (Scheme 2). Starting with 5-bromoindazole, treatment with lithium bis(trimethylsilyl)amide and triisopropylsilyl chloride in THF afforded the N-protected indazole. This indazole was coupled with 1-benzylpiperazin-2-one under Buchwald–Hartwig conditions.[22] Enolate formation was carried out with *sec*-butyllithium in THF at −78 °C.[28] Alkylation with a variety of alkyl or benzyl halides afforded 2-substituted oxo-piperazines in moderate yields (50–60%). Concomitant removal of the silyl group and reduction of the carbonyl was accomplished by treatment of the compound for 10 min with lithium aluminum hydride at 120 °C in a microwave reactor (typically 60% for two steps). Final deprotection was carried out utilizing transfer hydrogenation (yield 85–90%).

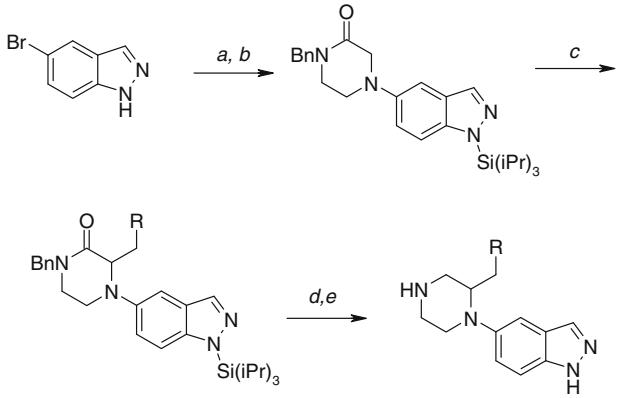
We began our search for compounds with less potency at CYP2D6[29] by preparing two aromatic side-chain analogs with varying chain length (Table 3). 2-Phenylpiperazine **17** was sixfold less potent at CYP2D6 than benzylpiperazine **16** though it lacked the desired transporter potency. Conversely, phenethylpiperazine **18** was potent at CYP2D6 without significant loss of transporter potency. Next, we prepared several aliphatic analogs (piperazines **19–22**). Although the potency of propylpiperazine **19** was 14-fold lower at SERT, it was a much weaker CYP2D6 inhibitor. Simply adding one additional carbon boosted SERT and DAT but also led to a significant increase in CYP2D6 potency (butylpiperazine **20**). Isobutylpiperazine **20** maintained transporter potency and lost some potency at CYP2D6 (as compared to butylpiperazine **19**). While methylcyclohexyl piperazine **22** lost potency at NET and DAT, it was the first analog with micromolar potency at CYP2D6, suggesting that larger branched side-chains may be a good strategy

Table 2
Indole analogs address potency[a] and microsomal stability[b]



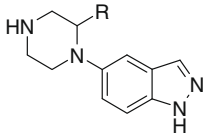
Compd	R	SERT pK_i	NET pK_i	DAT pK_i	HLM (μL/min/mg)
5		7.9	8.1	8.2	43.3
10		7.0	<5.3	6.8	—
11		8.1	6.3	8.5	—
12		7.3	7.3	5.8	—
13		8.5	7.9	7.0	—
14		7.8	8.2	7.8	16.8
15		7.2	6.6	6.3	—
16		8.9	7.6	7.8	3.8

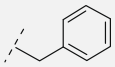
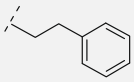
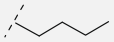
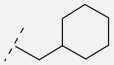
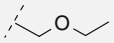
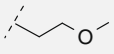
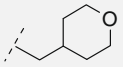
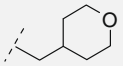
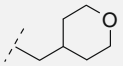
[a] pK_i (binding) values are the geometric mean of at least three experiments measured in HEK 293 cells.
[b] In vitro microsomal stability measured in human liver microsomes.



Scheme 2. A flexible synthesis for 2-substituted *N*-aryl piperazines. Reagents and conditions: (a) LHMDS, THF, TIPSCl; (b) Pd(OAc), tBu$_3$P, NaOtBu, xylenes, 1-benzylpiperazin-2-one, 80 °C; (c) *sec*-BuLi, THF, RCH$_2$Br; (d) LiAlH$_4$, THF, 120 °C, microwave 10 min; (e) Pd/C, NH$_4$OCHO, MeOH, reflux.

Table 3
Small aliphatic side-chains maintain transporter potency[a] while reducing potency at CYP2D6 and decreasing CYP3A4 TDI

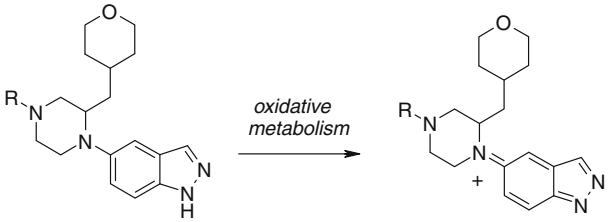


Compd	R	SERT pK_i	NET pK_i	DAT pK_i	CYP2D6 IC_{50} (μM)	3A4 TDI %
16		8.9	7.6	7.6	0.05	49
17		7.7	6.7	6.7	0.30	–
18		8.4	7.1	7.5	0.06	58
19		7.8	7.4	7.2	0.58	23
***(S)*-19**		7.5	7.5	7.6	0.35	24
***(R)*-19**		8.2	6.8	6.1	1.9	–
20		8.4	7.1	7.5	0.04	–
21		8.3	7.7	7.8	0.31	–
22		7.4	5.8	6.5	1.05	–
23		6.8	6.5	6.3	4.0	6
24		7.8	6.5	6.5	0.7	–
25		8.7	6.6	6.8	0.22	–
26		8.4	6.5	7.4	8.26	23
***(S)*-26**		7.1	7.0	7.3	9.1	–
***(R)*-26**		9.0	6.5	6.0	4.7	–

[a] pK_i (binding) values are the geometric mean of at least three experiments measured in HEK 293 cells.

to address this liability. Finally, we hypothesized that decreasing the lipophilicity through the introduction of a heteroatom might further decrease the CYP2D6 affinity. We prepared several ethers (**23–28**) that were direct comparators of the aliphatic analogs. As a general trend these ether analogs tended to be less potent at NET and DAT (typically 10-fold lower). The first compound in the series, 2-methoxymethylpiperazine **23** lost potency at each transporter but we were encouraged that such a small change let to a sevenfold reduction in CYP2D6 potency. In all cases, the additional oxygen reduced CYP2D6 potency as compared to the aliphatic analogs possible due to the lower lipophilicity (typically 15-fold lower calculated log *P*). Loss of transporter potency was ameliorated by increasing chain length or branching as exemplified by 4-pyranylpiperazine **26**. Although compound **26** was somewhat less potent at NET than our desired target profile, it was intriguing to us because it was the weakest CYP2D6 inhibitor that we investigated.

Since this piperazine series contains a stereogenic center, we wanted to know what effect stereochemistry might have on potency and metabolic profile. Propylpiperazine **19** and 4-pyranylpiperazine **26** were selected for profiling. The enantiomers of propylpiperazine **19** were initially separated via chiral SCF HPLC. Subsequently, the absolute configurations were determined via comparison of material prepared by total synthesis from commercially available amino acid precursors.[30] As we suspected, each enantiomer showed a different potency and metabolic profile.[31] The relatively balanced enantiomer ***(S)*-19** was much more potent at CYP2D6 than the SERT-selective enantiomer ***(R)*-19**. The enantiomers of piperazine **26** were also separated via super critical fluid (SCF) HPLC;[32] one enantiomer was balanced while the other was SERT-selective. In this case the balanced enantiomer ***(S)*-26** was much less potent at CYP2D6. Based on the potency profile and the low CYP2D6 DDI liability, compound ***(S)*-26** was selected for further study.



26 R = H
28 R = Ac

27 R = H
29 R = Ac

After identifying several compounds that were both potent reuptake inhibitors and weak CYP2D6 inhibitors, our attention turned to addressing the observed time dependent inhibition

(TDI) of CYP 3A4.[33] We suspected that the TDI signal was likely due to a reactive metabolite formed from the 5-aminoindole moiety. We postulated that metabolic oxidation of piperazine **26** could result in a diiminoquinone-like **27** which could be covalently trapped by CYP 3A4. Initially, we identified several indole replacements with lower TDI (7-aza-indole **15**: TDI = 10%) but none of these compounds maintained the desired potency. Fortunately, as with CYP3A4 inhibition, modifications to the benzylic side-chain were successful in decreasing TDI (Table 2). The aliphatic analog propylpiperazine **19** had much lower TDI than the aromatic analogs (**16, 18**). Analogs with lower lipophilicity, such as ether **23**, demonstrated a much lower TDI risk but were not pursued due to lack of desired transporter potency.

While attempting to moderate TDI in this series, we became aware of an additional potential liability of the piperazine-based scaffold. Specifically, if acetyl piperazine **28** formed in vivo (as a metabolite of piperazine **26**) leading to diiminoquinone **29**, it might carry a much higher risk of 3A4 TDI which could lead to drug–drug interactions. Because free piperazines are typically protonated under physiological conditions, the charged species may somewhat protect the indole ring from oxidative metabolism. If acetylated, any protective benefit from the additional charge would be lost. Although we never observed the acetyl metabolite directly, previous experience taught us to de-risk this series by preparing the purported acetyl metabolite **28**. Unfortunately, acetyl piperazine **28** was associated with a much higher TDI risk than piperazine **26** (TDI = 77%). Based on this experimental result even piperazine**s** with minimal TDI risk may generate metabolites in vivo which result in 3A4 TDI. Despite successful strategies for minimizing acetylation of piperazines by others,[34] the risk of 3A4 induced drug–drug interactions resulted in a deprioritization of this series in favor of a different template without this liability.[35]

In conclusion, we have developed a novel 2-substituted piperazine-based series of triple reuptake inhibitors. These inhibitors demonstrated activity in vivo due to their reuptake inhibitor potency and improved brain free fraction over the C5-aminoindoles. Furthermore, analogs were developed with good metabolic stability and low risk for CYP2D6 induced DDI. Although analogs with diminished risk of 3A4 TDI induced toxicity were identified, the potential for acetyl metabolite formation lent additional risk to this series. In the end, we deprioritized this series in favor of a related series that did not suffer from this liability.[35]

Acknowledgements

The authors wish to thank Marc Cummings for analytical and preparatory chiral HPLC support and R. Than Hendricks for editorial suggestions.

References and notes

1. (a) Thase, M. E. *J. Clin. Psychiatry* **2006**, *61*, 17; (b) Keesler, R. C.; Berglung, P.; Demler, O.; Jin, R.; Loretz, D.; Merikangas, K. R.; Rush, A. J.; Walters, E. E.; Wang, P. S. *J. Am. Med. Assoc.* **2003**, *289*, 3095.
2. Stewart, W. F.; Ricci, J. A.; Chee, E.; Hahn, S. R. *J. Am. Med. Assoc.* **2003**, *289*, 3135.
3. (a) Blumenthal, J. A.; Sherwood, A.; Rogers, S. D.; babyak, M. A.; Doraiswamy, P. M.; Watkins, L.; Hoffman, B. M.; O'Connell, C.; Johnson, J. J.; Patidar, S. M.; Waugh, R.; Hinderlitter, A. *Clin. Trials* **2007**, *4*, 548; (b) Moussavi, S.; Chatterji, E. M.; Tandon, A.; Patel, V.; Ustun, B. *Lancet* **2007**, *370*, 851; (c) Munce, E. E.; Stansfeld, S. A.; Blackmore, E. R.; Stewart, D. E. *J. Occup. Environ. Med.* **2007**, *49*, 1206.
4. Leonard, B. E. *Neurochem. Int.* **1982**, *4*(339), 5.
5. (a) Fava, M. *J. Clin. Psychiatry* **2003**, *64*, 26; (b) Nutt, D. J. *J. Clin. Psychiatry* **2007**, *64*, 3.
6. Harmer, C. J.; Bhagwagar, Z.; Völlm, B. A.; Cowen, P. J.; Goodwin, G. M. *Neuropsycopharmacology* **2003**, *28*, 148.
7. Dremencov, E.; el Mansari, M.; Blier, P. *Curr. Drug Targets* **2009**, *10*, 1061.
8. Esch, T.; Stefano, G. B. *Neuroendocrinol. Lett.* **2004**, *25*, 235.
9. Modell, J. G.; Katholi, C. R.; Modell, J. D.; DePalma, R. L. *J. Clin. Pharm. Ther.* **1997**, *61*, 478.
10. Sussman, N. *J. Clin. Psychiatry* **2003**, *64*, 19.
11. (a) Skolnick, P.; Kreiter, P.; Tizzano, J.; Basile, A.; Popik, P.; Czobor, P.; Lippa, A. *CNS Drug Rev.* **2006**, *12*, 123; (b) Azford, L.; Boot, J. R.; Hotten, T. M.; Keenan, M.; Martin, F. M.; Milutinovic, S.; Moore, N. A.; Oneil, M. F.; Pullar, F. M.; Tupper, D. F.; Van Belle, K.; Vivien, V. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3277.
12. Chen, Z.; Skolnick, P. *Expert Opin. Invest. Drugs* **2007**, *16*, 1365.
13. (a) Fava, M.; Rush, A. J.; Thase, M.; Clayton, A.; Stahl, S. M.; Pradko, J. F.; Johnston, J. A. *J. Clin. Psychiatry* **2005**, *7*, 106; (b) Debattista, C.; Solvanson, H. B.; Poirier, J.; Kendrick, E.; Schatzberg, A. F. *J. Clin. Psychopharmacol.* **2003**, *23*, 27.
14. (a) Kumar, L. H. *Am. J. Geriatr. Psychiatry* **2001**, *9*, 298; (b) Stoll, A. L.; Pillay, S. S.; Diamond, L.; Workum, S. B.; Cole, J. O. *J. Clin. Psychiatry* **1996**, *57*, 72.
15. Lucas, M. C.; Carter, D. S.; Cai, H.; Lee, E. K.; Schoenfeld, R. C.; Steiner, S. S.; Villa, Marzia.; Weikert, V.; Weikert, R. J.; Iyer, P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4630.
16. A scintillation proximity assay (SPA) was carried out in HEK 293 cells. In this assay, the binding affinity (pK_i) of various ligands was determined at SERT, NET and DAT by the completion with [^{3}H]-Citalopram, [^{3}H]-Nisoxetine and [^{3}H]-Vanoxerine, respectively. The specific details of this assay are reported in Carter, D. S.; Schoenfeld, R. C.; Weikert, R. J. WO 2008019971.
17. MW = 305, Clog *P* = 3.68, PSA = 27.
18. Clark, D. E. *Drug Discovery Today* **2003**, *8*, 927.
19. Meyer, J. H.; Goulding, V. S.; Wilson, A. A.; Hussey, D.; Christensen, B. K.; Houle, S. *Psychopharmacology* **2002**, *162*, 102.
20. Liu, X.; Vilenski, O.; Kwan, J.; Apparsundaram, S.; Weikert, R. *Drug Metab. Dispos.* **2009**, *37*, 1548.
21. We were interested in evaluating two potency profiles: relatively balanced (all pK_i's >7.0) similar to DOV-216,303 and SERT selective based in buproprion augmentation trials.
22. (a) Yamamoto, T.; Nishiyama, M.; Koie, Y. *Tetrahedron Lett.* **1998**, *39*, 2367; (b) Prashad, M.; mak, X. Y.; Liu, Y.; Repič, O. *J. Org. Chem.* **2003**, *68*, 1163; (c) Dubbaka, S. *Synlett* **2005**, 709.
23. The experimental procedure for a typical Buchwald coupling is as follows. To a mixture of $Pd(OAc)_2$ (0.054 g, 0.2 mmol), NaO*t*Bu (0.64 g, 6.6 mmol) in 7.0 mL xylenes in a screw capped test tube was added $t Bu_3P$ (0.049 g, 0.2 mmol). After 10 min a solution of *N*-TBS-5-bromoindole and 3-benzyl-piperazine-1-carboxylic acid *tert*-butyl ester in 15 mL xylenes was added and the mixture was warmed to 80 °C. After 30 min the mixture was cooled, taken up in EtOAc, filtered through a pad of Celite and concentrated in vacuo to afford a crude oil. Purification via flash chromatography (gradient: 2–20% EtOAc in hexanes) afforded 3-benzyl-4-[1-(*tert*-butyl-dimethyl-silanyl)-1*H*-indol-5-yl]-piperazine-1-carboxylic acid *tert*-butyl ester (1.65 g, 74%) as a pale yellow oil; ^{1}H NMR 300 MHz ($CDCl_3$) δ 7.47 (d, *J* = 9.0 Hz, 1H), 7.27–7.17 (m, 4H), 7.13–7.07 (m, 2H), 7.16 (d, *J* = 3.1 Hz, 1H), 6.96 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.56 (dd, *J* = 3.1, 0.8 Hz, 1H), 3.85–3.77 (m, 1H), 3.65–3.75 (br s, overlapping, 1H), 3.30–3.10 (m, 5H) 2.80–2.60 (br s, overlapping, 1H), 2.64–2.56 (m, 1H), 1.51 (br s, 9H), 0.93 (s, 9H), 0.63 (s, 3H), 0.60 (s, 3H).
24. Iyer, P.; Lucas, M. C.; Schoenfeld, R. C.; Villa, M.; Weikert, R. J. US 2007123527.
25. (a) Clearance measured in HT format: total incubation volume 250 μL, 0.5 mg/mL human 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 were transferred to 384 well plates for LC/MS analysis; (b) Clearance measured in human liver microsomes (μL/min/mg): low stability >35, medium stability 35–6, high stability <6.5.
26. Groot, J. M.; Wakenhut, F.; Whitlock, G.; Hyland, R. *Drug Discovery Today* **2009**, *14*, 964.
27. The piperazine starting material (1-Boc-3-phenyl piperazine) for compound **17** was commercially available.
28. Berkheij, M.; van der Sluis, L.; Sewing, C.; den Boer, D. J.; Terpstra, J. W.; Hiemstra, H.; Bakker, W. I. I.; van den Hoogenband, A.; van Maarseveen, J. H. *Tetrahedron Lett.* **2005**, 2369.
29. CYP2D6 inhibition was measured in a fluorescence based assay where CYP marker substrates become fluorescent upon CYP metabolism.
30. (a) (*R*)-2-Propylpiperazine and (*S*)-2-propylpiperazine were prepared from (*R*)-*N*-Boc-norvaline and (*S*)-*N*-Boc-norvaline, respectively by the method described in the following patent; (b) WO 03082877 A1; (c) Both piperazines were Boc-protected and converted to the final compounds ***(S)*-19** and ***(R)*-19** by the method outlined in Scheme 1.
31. ***(S)*-19** Rat SDPK (10 mpK PO): %*F* = 34, Vdss = 4.3 mL/kg, $t_{1/2}$ = 0.5 h, AUC = 406 ng-h/mL.; ***(R)*-19** Rat SDPK (10 mpK PO): %*F* = 53, $t_{1/2}$ = 0.8 h, AUC = 182 ng-h/mL.
32. (a) The absolute configurations of ***(S)*-19** and ***(R)*-19** were tentatively assigned based on comparing data from ***(S)*-19** and ***(R)*-19**.; Super critical fluid chromatography: (b) Banu, B.; Bharathi, K.; Babu, G. M. *J. Pharm. Res.* **2009**, *2*, 148.
33. (a) Zhou, S.; Chan, S. Y.; Goh, B. C.; Chan, E.; Duan, W.; Huang, M.; Mcleod, H. L. *Clin. Pharmacokinet.* **2005**, *44*, 279; (b) Kalgutkar, A. S.; Soglia, J. R. *Exp. Opin. Drug Metab. Toxicol.* **2005**, *1*, 91; (c) Human liver microsomes are preincubated with test compound and diluted at various time points (3, 6, 12 min). The remaining enzyme activity is measured (k_{obs}) and the data is plotted as a % of control (no inhibitor). In this manuscript 3A4 TDI is reported as a % of *t* = 12 min. Compounds with a greater rate of inactivation than verapamyl are categorized as *high*, while compounds with a rate less than ethynylestradiol are categorized as *low*.
34. Rawal, J.; Jones, R.; Payne, A.; Gardner, I. *Xenobiotica* **2008**, *38*, 1219.
35. Iyer, P.; Lin, C.; Lucas, M.; Lynch, S.; Madera, A. M.; Ozboya, K.; Schoenfeld, R.; Weikert, R. WO 2009153178.