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Exploration of Novel 3-Substituted Azetidine Derivatives As Triple Reuptake Inhibitors

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Supporting Information

ABSTRACT: Novel azetidines based on the 3-aryl-3-oxypropylamine scaffold were designed, synthesized, and evaluated as TRIs. Reduction of **1** followed by Swern oxidation and then Grignard reaction gave **3**. The alkylation of **3** provided the corresponding azetidine derivatives **6**, of which the two most promising, **6bd** and **6be**, were selected from 86 prepared analogues based on their biological profiles. Compound **6be** showed activity in vivo in FST at 10 mg/kg IV or 20−40 mg/kg PO.

INTRODUCTION

Major depressive disorder (MDD) is a common and serious illness with the potential to become the leading cause of disability worldwide. Pathophysiologically, the cause of depression is commonly associated with a deficiency of monoamine neurotransmitters (serotonin (5-HT), norepinephrine (NE), and dopamine (DA)) in the brain, and a number of antidepressants aim to increase the levels of these neurotransmitters in the synapses. Among various monoaminergic strategies for maintaining the concentration of neurotransmitters, blocking the reuptake of neurotransmitters by presynaptic 5-HT, NE and DA transporters (SERT, NET, and DAT) has been an important strategy in modern antidepressant therapy.[1] Although many kinds of reuptake inhibitor such as selective serotonin reuptake inhibitors (SSRIs) and serotonin NE reuptake inhibitors (SNRIs) are commercially available for the treatment of major depression, they may take several weeks of treatment to affect any improvement in symptoms, and some inhibitors present side effects such as insomnia and sexual dysfunction.[2] One strategy to improve the efficacy and/or reduce the delay in the onset of their action is the addition of a DA component to SSRIs or SNRIs. This is the concept of triple reuptake inhibition, which blocks the synaptic reuptake to all of 5-HT, NE, and DA. Recent study results supported the effect of DA in depression.[3] For example, D_3-preferring DA receptor agonists such as pramipexole showed therapeutic efficacy in major depression.[1a,4] DA reuptake inhibitors such as bupropion enhanced the antidepressant actions of SSRIs and SNRIs in humans.[5] A few classes of triple reuptake inhibitor (TRI), including DOV 216,303[6] and GSK 372,475,[7] showed positive phase II clinical effect. However, no TRI is yet available in the market. Combination- and multiple-drug therapy to inhibit the reuptake of 5-HT, NA, and DA may raise some problems of pharmacokinetics (PK). Therefore, TRIs as a single molecule are expected to become the next generation of antidepressants and remain desirable.

DESIGN

Our initial effort to explore target compounds for the development of a novel TRI as an antidepressant focused on the design of novel compounds through the structure analysis and molecular modification of the marketed reuptake transporter-based antidepressants. We selected fluoxetine, atomoxetine, reboxetine, and duloxetine, which have the 3-aryl-3-oxypropylamine scaffold in the structures. These compounds significantly affect the central nervous system (CNS) by monoamine reuptake inhibitory mechanism. For instance, fluoxetine was the first selective serotonin uptake inhibitor to reach the market with far fewer side effects than other antidepressants.[8] Atomoxetine and reboxetine are selective noradrenaline reuptake inhibitors developed for the treatment of either attention deficit and hyperactivity disorder or MDD.[9] Furthermore, duloxetine has emerged as a dual acting SNRI that offers improved efficacy and faster onset of action than SSRIs.[10] These compounds have the 3-aryl-3-oxypropylamine scaffold and rotatable bonds, except reboxetine, in which the corresponding bonds are locked into a cyclic ring (see Figure 1). On the basis of the characteristic common points of the structures, we have designed novel 3-substituted azetidine derivatives by modifying the 3-aryl-3-oxypropylamine scaffold. As shown in Figure 1, rigidification of two bonds (α and β) of the 3-aryl-3-oxypropanamine moiety via the formation of a new cyclic scaffold that included these rotatable bonds afforded a four-membered azetidine skeleton. Recently, azetidine substituted diphenyl ether derivatives with combined NE reuptake inhibitor and 5-HT_{1A} partial agonist pharmacology were

Figure 1. Design of 3-azetidine derivatives by rigidification of rotatable bonds.

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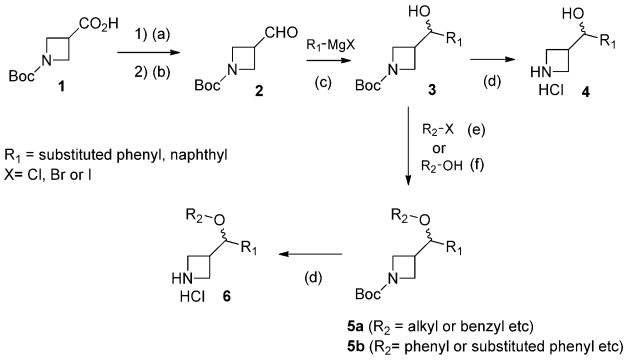
reported.[11] Additionally, the four characteristic structural common points of five compounds[6a,12−15] that show TRI activity are: (a) they are basic due to the 2° or 3° amino moiety, (b) the amino nitrogen atom presents in the cyclic ring, (c) one or two aromatic groups are present, and (d) they have one or more hydrogen bonding acceptors or donors.

SYNTHESES

The overall synthetic route of azetidine derivatives **6** is depicted in Scheme 1. Aldehyde **2** was obtained in 80% yield from the

Scheme 1[a]



[a]Reagents and reaction conditions: (a) BH_3·SMe, THF, 0°C; (b) $(COCl)_2$, DMSO, then TEA, CH_2Cl_2, −78 °C to 0 °C; (c) THF, 0°C; (d) 1N HCl, MeOH, 60°C; (e) NaH, THF, reflux; (f) PPh_3, DIAD, THF, rt.

reduction of commercially available **1** by the treatment of borane−dimethyl sulfide complex in tetrahydrofuran (THF) at 0 °C, followed by Swern oxidation. Grignard reaction of **2** with aryl magnesium halide in THF at 0 °C gave an intermediate, secondary alcohol **3**. Azetidinyl alkyl ether **5a** (R_2 = alkyl, benzyl etc) was prepared by two synthetic methods. In the case of R_2 = alkyl, the secondary alcohol **2** was treated with either alkyl halide or benzyl halide in the presence of sodium hydride in THF at ambient temperature to give the corresponding alkyl or benzyl ether **5a** in high yields (48−87%). In the case of R_2 = phenyl or substituted phenyl, azetidinyl phenyl ether derivatives **5b** were prepared successfully by Mitsunobu reaction. The reaction of **3** with substituted phenol in the presence of triphenylphosphine and diisopropyl azodicarboxylate in THF at room temperature afforded the corresponding ether **5b** in high yields (75−91%). Deprotection of the Boc group in **5a** or **5b** by the treatment of 1N HCl in boiling methanol gave the corresponding **6**. The products **6** could be simply obtained by filtration from the reaction mixture with isolated yields ranging from 12% to 99%. The structures were confirmed by ^1H and ^{13}C NMR spectroscopy and HRMS. The obtained solids were the corresponding hydrogen chloride salts of azetidine, which could be used directly for TRI screening. We synthesized 86 analogues of 3-substituted azetidine derivatives in this manner. The selected compounds are listed in Table 1, along with the results of their biological activity tests (see Supporting Information (SI) for all the prepared compounds).

BIOLOGICAL SCREENING

DA, NE, and serotonin neurotransmitter uptake activities were measured using the Neurotransmitter Transporter Uptake Assay Kit (Molecular Devices, Sunnyvale, CA, USA) with the

Table 1. Percentage Inhibition of Activities of 3-Substituted Azetidines 4 and 6 against HEK-hSERT, HEK-hNET, and HEK-hDAT

				% reuptake inhibition, at 0.1 μM		
entry	compd	R_1	R_2	SERT	NET	DAT
	fluoxetine			44	4.7	5.8
	nisoxetine			9.9	78	9.8
	GBR12909			1.9	−5.9	29
1	**4a**	C_6H_5	H	1.7	0.2	3.9
2	**4b**	C_6H_4(4-Cl)	H	2.0	3.5	2.1
3	**6aa**	C_6H_5	CH_3	3.3	1.5	2.2
4	**6ab**	C_6H_5	CH_2CH_3	4.0	4.5	3.0
5	**6ac**	C_6H_4(4-Cl)	CH_3	3.9	7.7	2.6
6	**6ad**	C_6H_4(4-Cl)	CH_2CH_3	4.6	10	7.3
7	**6ae**	C_6H_3(3,4-di Cl)	CH_3	16	47	22
8	**6af**	C_6H_3(3,4-di Cl)	CH_2CH_3	28	49	30
9	**6ah**	C_6H_5	C_6H_5	25	18	9.3
10	**6ai**	C_6H_4(4-Cl)	C_6H_5	22	68	36
11	**6aj**	C_6H_3(3,4-di Cl)	C_6H_5	16	89	58
12	**6ak**	C_6H_5	$CH_2C_6H_5$	12	9.4	2.0
13	**6al**	C_6H_4(4-Cl)	$CH_2C_6H_5$	12	16	5.7
14	**6am**	C_6H_3(3,4-di Cl)	$CH_2C_6H_5$	12	30	12
15	**6aq**	C_6H_3(3,4-di Cl)	$CH_2CH_2CH_3$	39	87	74
16	**6bd**	$C_{10}H_7$	$CH_2CH_2CH_3$	77	87	48
17	**6be**	$C_{10}H_7$	C_6H_5	64	90	64
18	**6bs**	$C_{10}H_7$	C_6H_4(3,4-di Cl)	28	79	68
19	**6bu**	C_6H_3(3-F, 4-Cl)	C_6H_5	49	76	30
20	**6bv**	C_6H_3(3-F, 4-Cl)	C_6H_4(2-F)	41	81	29

FDSS6000 96 well fluorescence plate reader, which is a high throughput screening device (Hamamatsu Photonics, Hamamatsu, Japan).[16] Human embryonic kidney 293 (HEK293) cells stably transfected with human DA transporter (HEK-hDAT), human NE transporter (HEK-hNET), or human serotonin transporter (HEK-hSERT) were used for the assay. All the synthesized compounds were screened at three concentrations (10, 1, and 0.1 μM), and the selected compounds were further screened to obtain their IC_{50} values. The primary screening results of all the compounds are reported in SI. To support the following discussion on the structure−activity relationship of the compounds, the primary screening results of 20 selected compounds at a concentration of 0.1 μM are summarized in Table 1.

We used fluoxetine, nisoxetine, and GBR12909 that are selective SERT, NET, and DAT reuptake inhibitors, respectively, as references. The biological activities data in Table 1 revealed that the presence of an *O*-substituent such as an alkyl, phenyl, or substituted phenyl group of the alcoholic moiety increased the activities (from comparison of **4b** with **6ac**, **6ad**, and **6ai**). The azetidinyl ethers **6** showed significantly elevated activities against three monoamines, where R_1 is the naphthyl moiety (**6bd**, **6be**, **6bs**). Another substituent effect on the biological activities of the azetidinyl ethers **6** was the bulkiness of R_2. Thus, the azetidine derivative in which R_2 is a bulky group such as *n*-propyl or aryl showed higher activity

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than that in which R_2 is a small substituent such as methyl (**6ae** (R_2 = methyl) < **6af** (R_2 = ethyl) < **6aq** (R_2 = *n*-propyl)). In contrast, the benzyl substituents in R_2 decreased the activity against the three transporters (compare **6ah** with **6ak**, **6ai** with **6al**, **6aj** with **6am**). On the basis of the initial results of reuptake inhibition, 11 compounds were selected for further characterization of their IC_{50} against three monoamine reuptake and human ether-a go-go-related gene (hERG) channel inhibitory activities (IC_{50}).[17] Table 2 showed the data of five selected compounds (detailed results are reported in SI).

Table 2. IC_{50} Values of Monoamine Reuptake and hERG Channel Inhibitory Activities of the Selected Azetidines

		reuptake assay (IC_{50}, nM)			
entry	compd	hSET	hNET	hDAT	hERG (IC_{50}, μM)
	fluoxetine	150	4410	18400	
	nisoxetine	700	18.0	1150	
	GBR12909	3840	1460	190	
1	**6aq**	44	10	32	2.2
2	**6bd**	6.8	12	72	0.98
3	**6be**	8.3	3.1	35	0.83
4	**6bs**	35	16	54	0.18
5	**6bu**	142	21	148	1.56

The IC_{50} values presented in Table 2 revealed the superiority of the azetidine derivatives **6** as a superior novel scaffold for application as three monoamine reuptake inhibitors in comparison with that of three reference compounds (fluoxetine, nisoxetine, and GRB12909). Comparing **6aq** (entry 1) with **6bd** (entry 2), the naphthyl substituent increased the hSET inhibitory activity approximately 6-fold. In general, the naphthyl derivatives **6bd**, **6be**, and **6bs** (entry 2, 3, and 4, respectively) showed good potency against all three monoamines. The activity of **6bd** (entry 2) decreased in the order hSET > hNET > hDAT, while the others (entries 3, 4) decreased in the order hNET > hSET ≈ hDAT. In addition, compounds **6aq**, **6bd**, and **6be** showed excellent biological activities against three monoamine uptake inhibition as well as hERG inhibition profiles. Having obtained the three monoamine uptake inhibitory profiles, we carried out human cytochrome P450 (CYP) enzyme assay (percentage inhibition at 10 μM for CYP1A2, CYP2D6, CYP2C9, and CYP3A4 using VIVID CYP enzymes assay kit) and investigated the metabolic stability at human liver microsomes (percentage remaining after 0.5 h using BD Gentest assay kit) of 37 compounds (detailed results are reported in SI). Compounds **6be** and **6bs** in which the naphthyl moiety is substituted in the azetidine scaffold showed relatively low percentage inhibitory value for CYP2D6 and CYP3A4. Most of the compounds were stable against human liver microsomes.

In the next step of the characterization, the drug development of a novel scaffold was related to the in vivo behavior activity of the compounds using an animal model. On the basis of their overall balanced profiles in terms of IC_{50} values against three monoamine uptake inhibitory activity, CYP assay data, metabolic stability, and hERG inhibition data, we chose **6bd** and **6be** for further brain–blood barrier (BBB) study and pharmacokinetic (PK) investigation. Because BBB permeability[18] is one of the factors relevant to the success of CNS-targeted drugs, brain penetration was also evaluated. Compounds **6bd** and **6be** showed adequate brain to plasma ratio of 0.86 and 2.70, respectively (see SI for detailed results).

The PK profiles of **6bd** and **6be** in rats were evaluated and the PK parameters are shown in Table 3. Compounds **6bd** and **6be** exhibited a moderate bioavailability (F) of 5.7 and 5.8, respectively.

Table 3. Pharmacokinetic (PK) Parameters of Compounds 6bd and 6be

	6bd		6be	
parameters	PO[a]	IV[b]	PO[a]	IV[b]
C_{max} (ng/mL)	7.3	198.2	5.6	112.1
T_{max} (h)	1.0		1.3	
$t_{1/2z}$ (h)	4.8	0.7	3.3	1.4
AUC_{all} (ngh/mL)	23.9	210.7	17.9	153.3
AUC_{inf} (ngh/mL)	49.4	214.6	41.7	159.7
$CL_{z/F}$ (L/h/kg)	46.5	4.7	48.7	6.4
$V_{z/F}$ (L/kg)	248.9	4.8	241.0	13.1
MRT ($0 - t$)	2.2	0.9	2.0	1.73
F (%)	5.7		5.8	

[a]PO dose: 2 mg/kg. [b]IV dose: 1 mg/kg.

To verify the potential antidepressant effect in vivo of these novel azetidine derivatives, we selected compound **6be** for profiling in the forced swimming test (FST) in mice, which is sensitive to known antidepressant drugs. The FST was carried out on mice according to the method of Porsolt.[19] Compound **6be** was administrated intravenously (IV) 0.5 h before the test in mice at 2.5, 5.0, and 10.0 mg/kg. As clearly seen in the Figure 2, **6be** showed a dose-dependently reduced immobility

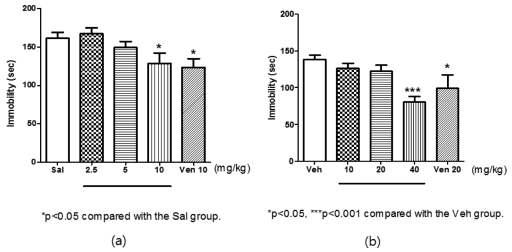

Figure 2. Effect of compound **6be** on immobility in the forced swimming test (FST) model in ICR mice (pretreatment time = 30 min). (a) IV dose: 2.5, 5.0, and 10.0 mg/kg. (b) PO dose: 10, 20, and 40 mg/kg.

time at 5.0 and 10.0 mg/kg, which was statistically significant compared to the vehicle. (Figure 2a). The positive control, venlafaxine, showed a similar reduction in immobility with that of **6be** at 10 mg/kg dosing. Oral administration (PO) of **6be** also showed dose-dependent reductions in immobility at 20 and 40 mg/kg (Figure 2b) (see SI for the detailed results).

SUMMARY

We designed a novel class of 3-substituted azetidine derivatives **6** and thereby achieved the study goal of creating a single molecular entity with triple activities as 5-HT, NE, and DA reuptake transporters. The bulkiness of the substituent at oxygen (R_2) and the presence of the naphthyl moiety at R_1 were clearly important for these activities. To verify the potential antidepressant effect in vivo of these novel azetidine derivatives, we selected compound **6be** for FST profiling in mice. This compound showed a dose dependent reduction of immobility at 5 and 10 mg/kg IV. A similar result was obtained

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by oral administration of **6be**. These 3-substituted azetidine derivatives therefore may offer an important new scaffold to act as single-molecule TRIs for application as the next generation of antidepressants.

EXPERIMENTAL SECTION

Preparation of the 3-Substituted Azetidines 6. *Synthesis of tert-Butyl 3-Formylazetidine-1-carboxylate (2).* To a solution of *tert*-butyl-3-(hydroxymethyl)azetidine-1-carboxylic acid (**1**) (1.0 g, 5.0 mmol) in THF (20 mL) at 0 °C was added borane−dimethyl sulfide complex (10 M solution in THF, 1.5 mL, 15 mmol) under N_2 atmosphere. The reaction mixture was stirred at the same temperature for 4 h. 1N HCl (20 mL) was added to the mixture while stirring, and then the reaction mixture was extracted with methylene chloride. The organic extract was washed with brine and dried over $MgSO_4$. The solvent was removed by evaporation, and the residue was used immediately in the next step. To a solution of dimethyl sulfoxide (1.1 mL, 15 mmol) dissolved in methylene chloride (20 mL) at −78 °C was added oxalyl chloride (1.3 mL, 15 mmol) under N_2 atmosphere. The reaction mixture was stirred for 10 min, and then a solution of the prepared primary alcohol above, dissolved in methylene chloride (2.0 mL), was added. After the resulting mixture was stirred for an additional 30 min at the same temperature, triethylamine (6.3 mL, 45 mmol) was added. The reaction mixture was stirred for 1 h and allowed to warm to reach room temperature. The resulting reaction mixture was washed with brine and then dried over $MgSO_4$. The solvent was removed, and the crude product was purified by flash chromatography on silica gel (methylene chloride:methyl alcohol = 9:1) to obtain **2** (0.74 g). Yield 80%; yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9H, $CO_2C(CH_3)_3$), 3.39−3.30 (m, 1H, $CH(CH_2)_2$), 4.11−4.05 (m, 4H, $CH(CH_2)_2$), 9.85 (s, 1H, CHO).

*Synthesis of the Azetidine Derivatives **3** (General Procedure).* To a solution of phenylmagnesium bromide (0.5 M solution in THF, 42 mL, 21 mmol) in THF (22 mL) at 0 °C was added a solution of the aldehyde **8** (1.3 g, 7.0 mmol) dissolved in freshly distilled THF (15 mL) under N_2 atmosphere. The reaction mixture was sirred for 3 h, poured onto aqueous NH_4Cl solution (15 mL), and stirred at room temperature for 0.5 h and then extracted with methylene chloride. The organic extracts were washed with brine and then dried over $MgSO_4$. The solvent was removed, and the crude product was purified by flash chromatography on silica gel (*n*-hexane:ethyl acetate = 2:1) to obtain **3** (57−86% yields).

Synthesis of the Azetidine Derivatives 4a and 4b (General Procedure). *Synthesis of **5a** (by the Reaction of **3** with Alkyl or Benzyl Halide).* To a suspension of sodium hydride (1.2 mmol) in THF (15 mL) at 0 °C was added **10** (0.60 mmol) dissolved in THF (5.0 mL). The alkyl halide or benzyl bromide (1.20 mmol) was added to the reaction mixture and then heated to reflux for 18 h. The reaction mixture was cooled, poured onto water (5.0 mL), and then extracted with methylene chloride. The organic extract was washed with brine and dried over $MgSO_4$. The solvent was removed, and the residue was purified by flash chromatography on silica gel (*n*-hexane:ethyl acetate = 1:1) to obtain the corresponding alkyl or benzyl ether **5a** (48−87% yields).

*Synthesis of **5b** (by the Reaction of **3** with Phenol Derivatives).* To a solution of **3** (0.45 mmol) in THF (15 mL) at 0 °C was added sequentially triphenylphosphine (0.90 mmol), phenol (0.90 mmol), and diisopropyl azodicarboxylate (0.90 mmol). The cooling bath was removed, and the reaction mixture was stirred at room temperature for 2 h. The solvent was removed by evaporation, and the residue was purified by flash chromatography on silica gel (*n*-hexane:ethyl acetate = 4:1) to obtain the corresponding phenyl ether **5b** (75−91% yields).

Synthesis of the Azetidine Derivatives 6(or 4) (General Procedure). To a solution of **5** (or **3**) (0.35 mmol) in methanol (15 mL) was added aqueous 1N HCl (5 mL). The reaction mixture was stirred at 60 °C for 12 h. The solvent was removed, and the resulting solid was washed with methylene chloride and ethyl acetate and then dried in air to afford the corresponding **6** (or **4**) (12−99% isolated yields).

ASSOCIATED CONTENT

Supporting Information

Additional experimental procedures and biological screening method, the yields, melting point, purity, ^{1}H and ^{13}C NMR data for all the compounds, HRMS data for the representative compounds, and the detailed results of biological assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

MDD, major depressive disorder; 5-HT, serotonin; NE, norepinephrine; DA, dopamine; SERT, serotonin transporter; NET, norepinephrine transporter; DAT, dopamine transporter; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin norepinephrine reuptake inhibitor; TRI, triple reuptake inhibitor; CNS, central nervous system; HEK, human embryonic kidney; hERG, human ether-a go-go-related gene; CYP, cytochrome P450; BBB, brain−blood barrier; PK, pharmcokinetics; FST, forced swimming test; IV, intravenous injection; PO, per oral

REFERENCES

(1) (a) Prins, J.; Olivier, B.; Korte, S. M. Triple reuptake inhibitors for treating subtypes of major depressive disorder: the monoamine hypothesis revisited. *Expert Opin. Invest. Drugs* **2011**, *20*, 1107−1130. (b) Kulkarni, S. K.; Dhir, A. Current investigational drugs for major depression. *Expert Opin. Invest. Drugs* **2009**, *18*, 767−788. (c) Millan, M. J. Dual- and triple-acting agents for treating core and co-morbid symptoms of major depression: novel concepts, new drugs. *Neurotherapeutics* **2009**, *6*, 53−77. (d) Skolnick, P.; Basile, A. S. Triple reuptake inhibitors as antidepressants. *Drug Discovery Today: Ther. Strategies* **2006**, *3*, 489−494. (e) Holtzheimer, P. E., III; Nemeroff, C. B. Advances in the treatment of depression. *Neurotherapeutics* **2006**, *3*, 42−56.

(2) (a) Zisook, S.; Rush, A. J.; Haight, B. R.; Clines, D. C.; Rockett, C. B. Use of bupropion in combination with serotonin reuptake inhibitors. *Biol. Psychiatry* **2006**, *59*, 203−210. (b) Papakostas, G. I.; Worthington, J. J., III; Iosifescu, D. V.; Kinrys, G.; Burns, A. M.; Fisher, L. B.; Homberger, C. H.; Mischoulon, D.; Fava, M. The combination of duloxetine and bupropion for treatment-resistant major depressive disorder. *Depression Anxiety* **2006**, *23*, 178−181.

(3) (a) Millan, M. J. Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol. Ther.* **2006**, *110*, 135−370. (b) Skolnick, P. Dopamine and Depression. In *Dopamine and Glutamate in Psychiatric Disorders*;

Schmidt, W. J., Reith, M. E. A., Eds.; Humana Press Inc.: Totowa, NJ, 2005; pp 199−214.
(4) Fava, M. Augmentation and combination strategies in treatment resistant depression. *J. Clin. Psychiatry* **2001**, *62*, 4−11.
(5) Prica, C.; Hascoet, M.; Bourin, M. Is co-administration of bupropion with SSRIs and SNRIs in forced swimming test in mice, predictive of efficacy in resistant depression? *Behav. Brain Res.* **2008**, *194*, 92−99.
(6) (a) Beer, B.; Stark, J.; Krieter, P.; Czobor, P.; Beer, G.; Lippa, A.; Skolnick, P. DOV 216,303, a "Triple" reuptake inhibitor: safety, tolerability, and pharmacokinetic profile. *J. Clin. Pharmacol* **2004**, *44*, 1360−1367. (b) Skolnick, P.; Popik, P.; Janowsky, A.; Beer, B.; Lippa, A. S. "Broad spectrum" antidepressants: is more better for the treatment of depression? *Life Sci.* **2003**, *73*, 3175−3179.
(7) Chen, Z.; Skolnick, P. Triple uptake inhibitors: therapeutic potential in depression and beyond. *Expert Opin. Invest. Drugs* **2007**, *16*, 1365−1377.
(8) Wong, D. T.; Perry, K. W.; Bymaster, F. P. The discovery of fluoxetine hydrochloride (Prozac). *Nature Rev. Drug Discovery* **2005**, *4*, 764−774.
(9) (a) 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. Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology* **2002**, *27*, 699−711. (b) Montgomery, S.; Ferguson, J. M.; Schwartz, G. E. The antidepressant efficacy of reboxetine in patients with severe depression. *J. Clin. Psychopharmacol.* **2003**, *23*, 45−50.
(10) Schueler, Y.-B.; Koesters, M.; Wieseler, B.; Grouven, U.; Kromp, M.; Kerekes, M. F.; Kreis, J.; Kaiser, T.; Becker, T.; Weinmann, S. A systematic review of duloxetine and venlafaxine in major depression, including unpublished data. *Acta Psychiatr. Scand.* **2011**, *123*, 247−265.
(11) Pettersson, M.; Campbell, B. M.; Dounay, A. B.; Gray, D. L.; Xie, L.; O'Donnell, C. J.; Stratman, N. C.; Zoski, K.; Drummond, E.; Bora, G.; Probert, A.; Whisman, T. Design, synthesis, and pharmacological evaluation of azetedine and pyrrolidine derivatives as dual norepinephrine reuptake inhibitors and 5-HT_{1A} partial agonists. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 865−868.
(12) Beadle, C. D.; Cases-Thomas, M. J.; Clark, B. P.; Callagher, P. T.; Masters, J. J.; Timms, G. H.; Walter, M. W.; Whatton, M. A.; Wood, V. A.; Gilmore, J. 3-Aminopyrrolidines as inhibitors of monoamine uptake. PCT Int. Appl. WO 2005000811 A1, 2005.
(13) Enyedy, I. J.; Zaman, W. A.; Sakamuri, S.; Kozikowski, A. P.; Johnson, K. M.; Wang, S. Pharmacophore-based discovery of 3,4-disubstituted pyrrolidines as a novel class of monoamine transporter inhibitors. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1113−1118.
(14) Bennett, D. A.; Wichems, C. H.; Hollingsworth, C. K.; Davies, H. M. L.; Thornley, C.; Sexton, T.; Childers, S. R. Novel 2-substituted cocaine analogs: uptake and ligand binding studies at dopamine, serotonin and norepinephrine transport sites in the rat brain. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 1176−1186.
(15) Zhou, J.; He, H.; Johnson, K. M.; Ye, Y.; Kozikowski, A. P. Piperidine-based nocaine/modafinil hybrid ligands as highly potent monoamine transporter inhibitors: efficient drug discovery by rational lead hybridization. *J. Med. Chem.* **2004**, *47*, 5821−5824.
(16) Jørgensen, S.; Nielsen, E. Ø.; Peters, D.; Dyhring, T. Validation of a fluorescence-based high-throughput assay for the measurement of neurotransmitter transporter uptake activity. *J. Neurosci. Methods* **2008**, *169*, 168−176.
(17) Choi, K. H.; Song, C.; Shin, D.; Park, S. hERG channel blockade by externally applied quaternary ammonium derivatives. *Biochim. Biophys. Acta* **2011**, *1808*, 1560−1566.
(18) Reichel, A. The role of blood−brain barrier studies in the pharmaceutical industry. *Curr. Drug Metab.* **2006**, *7*, 183−203.
(19) Porsolt, R. D.; Pichon, M. L.; Jalfre, M. Depression: a new animal model sensitive to antidepressant treatments. *Nature* **1977**, *266*, 730−732.