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Exploration of 3-Aminoazetidines as Triple Reuptake Inhibitors by Bioisosteric Modification of 3-α-Oxyazetidine

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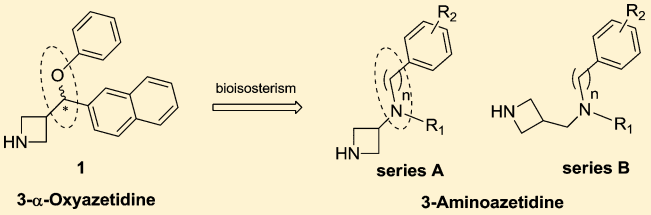
Supporting Information

ABSTRACT: For a development of broad spectrum antidepressant 3-aminoazetidine derivatives, two series of compounds were explored by bioisosteric modification of 3-α-oxyazetidine. We synthesized 166 novel 3-aminoazetidine derivatives in series A and B, starting from Boc-protected 3-azetidinone (**3**) and Boc-protected 3-azetidinal (**9**) respectively, through parallel syntheses. The inhibitory reuptake activities against serotonin (5-HT), norepinephrine (NE), and dopamine (DA) neurotransmitters were measured by the Neurotransmitter Transporter Uptake Assay Kit using the human embryonic kidney 293 (HEK293) cells stably transfected with the respective three kinds of human transporters (hSERT, hNET, and hDAT). Our study aimed to identify compounds having relative inhibitory activities against hSERT > hNET > hDAT. Lead optimization including microsomal stability, CYP, hERG assay, Ames test, BBB, and PK study resulted in the identification of compound **10dl** as a candidate for further studies.



KEYWORDS: *Depression, triple reuptake inhibitor, 3-aminoazetidines, bioisosterism*

Depression is one of the leading diseases worldwide. Epidemiological studies demonstrate that depressive disorders are highly prevalent; it is estimated that, in general, the lifetime prevalence of major depression was 20% in women and 10% in men. The incidence of depression has risen every year since the early 20th century. There are probably many reasons for this rise, though most studies point to significant socioeconomic changes and complicated circumstances experienced by the younger generation. 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.[1−4] Since the launch of the first selective serotonin reuptake inhibitors (SSRIs) in the 1980s, second generation antidepressants such as dual 5-HT and NE reuptake inhibitors (SNRIs) or NE and DA reuptake inhibitors (NDRIs) with enhanced properties have been substituted to tricyclic derivatives or monoamine oxidase inhibitors.[5,6] However, a very large percentage of patients treated with SSRIs or SNRIs show partial responses and remission rate around 30%.[7] Additionally, the associated side effects such as insomnia, sexual dysfunction, and elevated blood pressure hamper their efficacy.[8,9] In modern therapies for depression, one important 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. Recent study results support the effect of DA in depression.[10] NDRI bupropion enhanced the antidepressant actions of SSRIs and SNRIs in humans,[5,11] and suppression of DA reuptake enhances sexual function and may improve cognitive performance.[1,12] An important recent development has been achieved with the discovery of triple reuptake inhibitors (TRIs), broad spectrum antidepressants that are capable of inhibiting the reuptake of 5-HT, NE, and DA by one molecule.[13,14] Therefore, TRIs working as a single molecule are expected to become the next generation of antidepressants and offer desirable therapeutic effects. Although many of the discovered compounds such as TRI have balanced ratio of potency for inhibition of reuptake of 5-HT, NE, and DA, recent reports suggest that the potency for reuptake inhibition of 5-HT and NE should be more important than those of DA for antidepressant activity of TRI.[15−17] Thus, our goal was to identify the triple reuptake inhibitor having relative inhibitory activities in the order 5-HT transporter (SERT) > NE transporter (NET) > DA transporter (DAT).

In our previous paper, we reported the 3-substituted azetidine **1** with similar relative inhibitory activities against 5-HT, NE, and DA transporters (SERT ≅ NET ≅ DAT), which showed antidepressant effect in the forced swimming test in mice at 10 mg/kg iv or 20−40 mg/kg po.[18] On the negative side, compound **1** is a racemate, which required separation by either additional chiral chromatography or by independent asymmetric synthesis. In fact, we had been frustrated by the difficult chiral resolution and the enantioselective synthesis. As a part of our continuing efforts to advance these compounds, we focused on the bioisosterism with removal of stereogenic center of **1**.

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Bioisosterism is an approach and strategy for the rational modification of lead compounds into safer and more clinically effective agents.[19,20] The replacement carbon atom of template **1** with a nitrogen atom leads to 3-aminoazetidine **2** as shown in Figure 1. Additionally, considering the characteristic structural

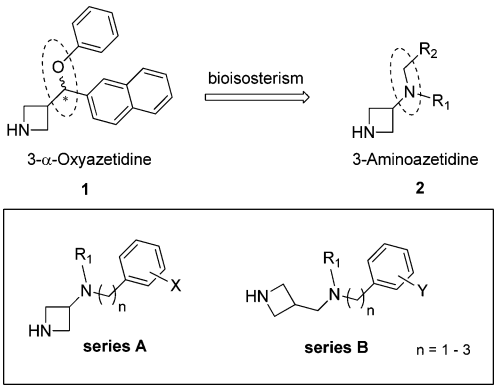
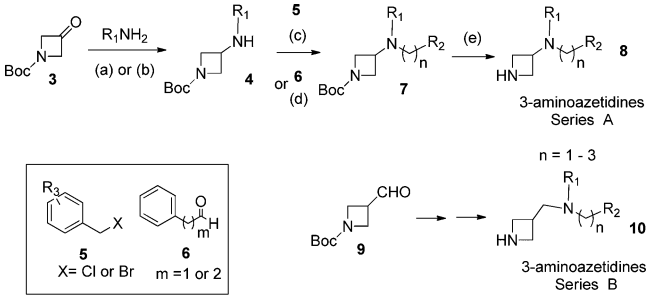


Figure 1. 3-Aminoazetidines: series A and B designed by bioisosterism of 3-α-oxyazetidine **1**.

common points of "Rule of 7" for designing new antidepressants,[21] we have designed the novel 3-aminoazetidine series A and B.

A synthetic route to 3-aminoazetidines, series A and B, is summarized in Scheme 1. Intermediates **4** were prepared by

Scheme 1[a]



[a]Reagents and reaction conditions: (a) $NaBH(OAc)_3$, AcOH, CH_2Cl_2, r.t.; (b) toluene, 110°C, and then $NaBH_4$, MeOH. r.t.; (c) K_2CO_3, DMF, 150°C; (d) $NaBH(OAc)_3$, AcOH, CH_2Cl_2, r.t.; (e) TFA, CH_2Cl_2, r.t., and then aq. 1 N NaOH or 1 N HCl, MeOH, 60°C.

reductive amination of commercially available 3-azetidinone **3** and primary amine in the presence of sodium triacetoxyborohydride ($NaBH(OAc)_3$) in methylene chloride solution at room temperature. The reaction proceeded smoothly where the R_1 is phenyl, substituted phenyl, or aryl group in moderate to high yields (49−95%). In contrast, in the case that R_1 is an alkyl, cycloalkyl, or benzyl group, the reaction required drastic conditions. Thus, heating **3** with alkyl, cycloalkyl, or benzylamine in toluene at reflux under a Dean−Stark water trap, followed by the treatment of sodium borohydride in methanol at room temperature gave **4** in good yield (74−86%). *N*-Alkylation of **4** was accomplished by the reaction using the appropriate building blocks **5** or **6**. For instance, the reaction of **4** with benzyl halide **5** in the presence of potassium carbonate in boiling dimethylformamide (DMF) gave the corresponding benzyl azetidine **7**. Otherwise, the treatment of **4** with aldehyde **6** in the presence of $NaBH(OAc)_3$ in methylene chloride gave the corresponding azetidine **7**. Deprotection of the Boc group in the azetidine by the treatment of trifluoroacetic acid (TFA) in methylene chloride at room temperature or 1 N HCl in boiling methanol gave crude product **8** as the corresponding TFA or HCl salt, respectively. This was treated with 1 N aqueous sodium hydroxide followed by purification via flash chromatography or crystallization to obtain the compounds **8** in yields ranging from 26 to 96%.

The structures of prepared compounds were confirmed by ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopies and high-resolution mass spectrometry (HRMS), and the purity was determined by high-performance liquid chromatography (HPLC). Seventy four analogues of the 3-substituted aminoazetidine derivatives **8** (series A) were synthesized in this manner. Further, 92 derivatives of 3-aminoazetidine **10** (series B) were prepared from 3-azetidinal **9** by a similar synthetic method.

The reuptake inhibitory activities against 5-HT, NE, and DA neurotransmitter were measured by the Neurotransmitter Transporter Uptake Assay Kit (Molecular Devices, Sunnyvale, CA, USA) with the FDSS6000 96-well fluorescence plate reader, a high throughput screening device (Hamamatsu Photonics, Hamamatsu, Japan).[22] In this study, the human embryonic kidney 293 (HEK293) cells stably transfected with human dopamine transporter (hDAT), human norepinephrine transporter (hNET), or human serotonin transporter (hSERT) were used for the assay. All the synthesized compounds were screened at three concentrations (10, 1.0, and 0.1 μM), and the selected compounds were further screened to obtain their IC_{50}. Fluoxetine (SSRI), nisoxetine (NRI), GBR12909 (DRI), venlafaxine (SNRI), and duloxetine (SNRI) were selected as references. Primary screening results of selected 3-aminoazetidines series A and B at a concentration of 0.1 μM are summarized in Tables 1 and 2, respectively (see the Supporting Information for complete screening results).

At a glance, the biological activities data of the compound of series A in Table 1 revealed that the reuptake inhibitory activities against hSERT were higher than those against hNET and hDAT. Comparing the reuptake inhibitory activities against hSERT where $n = 1$, the presence of a naphthyl or a 3,4-dichlorophenyl moiety in R_2 increased the activities significantly, while it is a disadvantage when these groups are present at R_1 except compound **8ax** (entry 10) (from comparison of entries 1, 2, 14−17 with entries 8, 9, and 11 in Table 1). In the case that these groups are present at both R_1 and R_2 (**8au** and **8ax**), decreased activity was shown. In addition, no elevated inhibitory activities were found when there is methylene spacer present between the tertiary nitrogen and R_2 group ($n = 2$ or 3) (compare entries 3 with 4, 5 and 9 with 6, 7 in Table 1). The screening results of 74 compounds in series A are reported in the Supporting Information. In general, the compounds of series B shown in Table 2 demonstrated relatively higher reuptake inhibitory activity against the three monoamine transporters than those of series A shown in Table 1. Most of the compounds of series B showed high reuptake inhibitory activities against hSERT and hNET and moderate activity against hDAT (Table 2). Interestingly, the bulkiness of R_1 seems to be a disadvantage for reuptake inhibitory activities. Thus, the compounds of series B when R_1 is a bulky group such as 3,4-dichlorophenyl (entries 3−5 in Table 2), naphthyl (entries 10 and 11), or 4-phenoxyphenyl (entries 12−14) showed less activity than when R_1 is a relatively small substituent such as cyclopropyl (entry 9), cyclopentyl (entries 8 and 15), cyclohexyl (entries 6, 7 and 16), or phenyl (entries 1 and 2). The screening results of 92

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Table 1. Percentage Inhibition of Activities of Selected 3-Aminoazetidine Derivatives 8 (Series A) at HEK-hSERT, HEK-hNET, and HEK-hDAT

					% reuptake inhibition[a] at 0.1 μM		
entry	compd	R_1	R_2	*n*	hSERT	hNET	hDAT
	Fluoxetine				44	5	6
	Nisoxetine				10	78	10
	GBR12909				2	−6	29
	venlafaxine				56	9	6
1	**8ad**	cyclohexyl	$C_{10}H_7$	1	95	22	5
2	**8af**[b]	C_6H_5	$C_{10}H_7$	1	99	18	5
3	**8ak**	C_6H_5	C_6H_5	1	19	30	10
4	**8al**	C_6H_5	C_6H_5	2	11	16	8
5	**8am**	C_6H_5	C_6H_5	3	53	12	8
6	**8aq**	C_6H_3(3,4-di Cl)	C_6H_5	2	29	17	5
7	**8ar**	C_6H_3(3,4-di Cl)	C_6H_5	3	30	16	13
8	**8au**	$C_{10}H_7$	$C_{10}H_7$	1	13	13	2
9	**8aw**	C_6H_3(3,4-di Cl)	C_6H_5	1	59	100	9
10	**8ax**	C_6H_3(3,4-di Cl)	$C_{10}H_7$	1	11	10	4
11	**8ay**	C_6H_3(3,4-di Cl)	C_6H_4(4-Cl)	1	11	22	4
12	**8ba**	C_6H_4(4-CH_3)	$C_{10}H_7$	1	100	12	7
13	**8bl**	$CH_2C_6H_5$	$C_{10}H_7$	1	86	57	13
14	**8bv**	cyclopropyl	$C_{10}H_7$	1	100	1	14
15	**8bz**	cyclopentyl	$C_{10}H_7$	1	100	21	21
16	**8cf**	C_6H_4(2-F)	$C_{10}H_7$	1	100	47	22
17	**8cu**	cyclopropyl	C_6H_3(3,4-di Cl)	1	40	9	12

[a]Percent values are the means obtained at least three or four times. [b]HCl salt.

Table 2. Percentage Inhibition of Activities of Selectied 3-Aminoazetidine Derivatives 10 (Series B) at HEK-hSERT, HEK-hNET, and HEK-hDAT

					% reuptake inhibition[a] at 0.1 μM		
entry	compd	R_1	R_2	*n*	hSERT	hNET	hDAT
	Fluoxetine				44	5	6
	Nisoxetine				10	78	10
	GBR12909				2	−6	29
	venlafaxine				56	9	6
1	**10ab**	C_6H_5	$C_{10}H_7$	1	95	50	4
2	**10ac**	C_6H_5	C_6H_4(4-Cl)	1	90	73	10
3	**10ai**	C_6H_3(3,4-di Cl)	C_6H_5	1	82	54	18
4	**10aj**	C_6H_3(3,4-di Cl)	$C_{10}H_7$	1	44	7	5
5	**10ak**	C_6H_3(3,4-di Cl)	C_6H_4(4-Cl)	1	43	15	12
6	**10ar**	cyclohexyl	$C_{10}H_7$	1	100	49	7
7	**10as**	cyclohexyl	C_6H_4(4-Cl)	1	63	85	1
8	**10ax**	cyclopentyl	$C_{10}H_7$	1	100	28	5
9	**10bd**	cyclopropyl	$C_{10}H_7$	1	100	57	25
10	**10bf**[b]	$C_{10}H_7$	C_6H_4(4-Cl)	1	16	40	17
11	**10bg**[b]	$C_{10}H_7$	C_6H_4(3-CH_3)	1	29	55	24
12	**10bh**[b]	C_6H_4(4-OPh)	$C_{10}H_7$	1	9	27	5
13	**10bi**[b]	C_6H_4(4-OPh)	C_6H_4(4-Cl)	1	6	7	4
14	**10bj**[b]	C_6H_4(4-OPh)	C_6H_5	1	14	5	11
15	**10 cd**	cyclopentyl	C_6H_3(3,4-di Cl)	1	93	80	30
16	**10ce**	cyclohexyl	C_6H_3(3,4-di Cl)	1	76	62	16
17	**10cl**[b]	C_6H_5	C_6H_4(4-F)	1	100	93	22
18	**10cn**[b]	C_6H_5	C_6H_4(3-CH_3)	1	100	100	22
19	**10cq**[b]	C_6H_5	C_6H_3(2,3-di Cl)	1	100	100	36
20	**10dl**[c]	isopropyl	C_6H_3(3,4-di Cl)	1	100	97	22

[a]Percent values are the means obtained at least three or four times. [b]TFA salt. [c]HCl salt.

compounds in series B are reported in the Supporting Information.

On the basis of the initial results of reuptake inhibitory activities, 45 compounds were selected for further characterization of their IC_{50} against the three monoamine transporters and the microsomal stability against human liver microsomes (percentage remaining after 0.5 h using BD Gentest assay kit). Table 3 represents the data of the 16 selected compounds, which

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Table 3. IC_{50} Values of Monoamine Reuptake Inhibitory Activities and Percentage Remaining of Microsomal Stability (M.S.) for the Selected Compounds, Series A and B

		reuptake assay (IC_{50}, nM)[a]			relative ratio of IC_{50}		
entry	compd	hSERT	hNET	hDAT	hNET/hSERT	hDAT/hSERT	M.S (% remaining) after 30 min
	Fluoxetine	150	4410	18400	29.4	122	
	Nisoxetine	700	20.0	1150	0.0	1.6	
	GBR12909	3840	1460	190	0.4	0.0	
	venlafaxine	36.1	5720	15700	158	436	
	duloxetine	10.4	515	977	49.5	93.9	
	mibefradil						64
1	**8ab**	42.2	518	533	12.3	12.6	99
2	**8ad**	6.2	269	498	43.4	80.4	26
3	**8af**	11.9	333	678	28.0	57.0	73
4	**8ba**	23.0	597	696	26.0	30.3	49
5	**8bk**	20.2	130	406	6.4	20.1	3
6	**8cg**	67.4	179	415	2.7	6.2	66
7	**8ch**	99.9	531	394	5.3	3.9	64
8	**8cu**	114	479	265	4.2	2.3	100
9	**10ab**	8.4	87.9	684	10.5	81.4	23
10	**10ac**	14.6	29.1	540	2.0	37.0	9
11	**10 cd**	28.8	44.8	150	1.6	5.2	3
12	**10ck**	5.2	1.6	441	0.3	84.8	55
13	**10cl**	17.9	34.0	187	1.9	10.4	27
14	**10cn**	11.4	24.2	327	2.1	28.8	18
15	**10cq**	11.1	17.6	118	1.6	10.6	61
16	**10dl**	7.6	45.2	330	6.0	43.8	74

[a]The values are the means obtained at least three or four times.

Table 4. IC_{50} Values of Human CYP Enzyme Activities and hERG Channel Inhibitory Activities for the Selected Compounds

		CYP450 (IC_{50}, μM)						
entry	compd	1A2	2D6	2C9	3A4	2C19	hERG (IC_{50}, μM)	hERG IC_{50} /hSERT IC_{50}
	positive control[a]	32.6	25.1	4.2	2.5	17.9		
	duloxetine	5.3	1.58	29.1	0.44	4.1		
1	**8ab**	205	0.13	6.89	0.81	1.75		
2	**8af**	12.7	0.01	3.13	1.33	0.27		
3	**8cg**	9.39	0.02	0.46	3.64	0.07		
4	**8ch**	13.9	13.7	3.34	0.5	7.06		
5	**8cu**	19.3	2.1	12.9	0.5	8.08	4.71	41
6	**10ck**	52.7	1.88	0.36	0.12	5.4		
7	**10cq**	0.57	0.62	0.44	32.7	0.8	1.88	169
8	**10dl**	10.6	2.34	32.7	1.10	1.02	5.5	733

[a]Positive control: α-naphthoflavone for 1A2, sulfaphenazole for 2C9, quinidine for 2D6, ketoconazole for 3A4, and miconazole for 2C19.

show good reuptake inhibitory potency against the three monoamine transporters in comparison with the reference standards (the data for the 45 compounds are reported in the Supporting Information). From series A, on the basis of the data of IC_{50} value, the relative ratio of IC_{50} (hNET/hSERT and hDAT/hSERT), and the microsomal stability, five compounds (**8ab**, **8af**, **8cg**, **8ch**, and **8cu**) were selected as candidates for the next experiments. Additionally, the compounds of series B (entry 9−16 in Table 3) exhibited high potency against hSERT and hNET compared with that against hDAT. Among the compounds of series B, **10ck**, **10cq**, and **10dl** showed most potent activity against hSERT and hNET as well as good microsomal stability.

The next step was to examine the selected compounds against cytochrome P450 (CYP) and human ether-a go-go-related gene (hERG) potassium channel inhibition. Having obtained the reuptake inhibitory activities (IC_{50}) and the microsomal stability profile, eight compounds were selected and screened against five isozymes of CYP and hERG channel inhibition, and the data are summarized in Table 4. Compounds **8ab**, **8af** and **8cg** are at a disadvantage for use of CYP2D6, and compound **10ck** showed low IC_{50} for CYP3A4 to rule out further study. Then, three compounds (**8cu**, **10cq**, and **10dl**) were screened against hERG channel assay. As a result, we selected compound **10dl** for blood−brain barrier (BBB) and pharmacokinetics (PK) studies for the following two reasons. First, the IC_{50} values of compound **10dl** against CYP1A2, CYP2D6, and CYP3A4 were higher than those of duloxetine.[23] Second, compound **10dl** showed the highest safety margin (733-fold) of hERG IC_{50} to target IC_{50} (hERG IC_{50}/hSERT IC_{50}).[24−26] Additionally, compound **10dl** did not have promutagenic or mutagenic effects on *Salmonella typhimurium* strains TA98 and TA100 in the bacterial reverse mutation assay (see Supporting Information).

Finally, the selected compound **10dl** showed adequate brain-to-plasma ratio (B/P = 2.09 at 3 h) in the BBB study and good PK profile (Table 5).

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Table 5. Pharmacokinetic Parameters of Compound 10dl

compd **10dl**		
parameters	po (10 mg/kg, n = 5)	iv (5.0 mg/kg, n = 5)
$AUC_{0-\infty}$ (μg·min/mL)	17.0	60.0
AUC_{last} (μg·min/mL)	16.3	55.8
$T_{1/2}$ (min)	100.2	136.1
C_{max} (μg/mL)	0.11	
T_{max} (min)	48	
CL (mL/min/kg)		168.0
MRT (min)		123.7
V_{ss} (mL/kg)		27611
Ae (%)	0.02	0.03
F (%)	28.4	

In summary, we have explored novel 3-aminoazetidine derivatives series A and B by bioisosteric modification of 3-α-oxyazetidine as a triple reuptake inhibitor for development of an antidepressant. A focused library of 3-aminoazetidines composed of 166 compounds was constructed through parallel syntheses. The synthesized compounds were screened against three kinds of human transporters (hSERT, hNET, and hDAT). Compound **10dl** was selected, having relative inhibitory activities sequentially against hSERT > hNET > hDAT through cell-based in vitro assay, microsomal stability, CYP, hERG assay, Ames test, BBB, and PK studies. Further studies are in progress and will be reported soon.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and biological screening method, yields, melting points, ^{1}H and ^{13}C NMR data for all the compounds, purity and HRMS data for the representative compounds, and 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

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; NDRI, norepinephrine dopamine reuptake inhibitor; TRI, triple reuptake inhibitor; HEK, human embryonic kidney; hSERT, human serotonin transporter; hNET, human norepinephrine transporter; hDAT, human dopamine transporter; hERG, human ether-a go-go-related gene; CYP, cytochrome P450; BBB, blood−brain barrier; PK, pharmacokinetics

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