Bioorganic & Medicinal Chemistry Letters 24 (2014) 3234–3237

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological evaluation of 3-phenethylazetidine derivatives as triple reuptake inhibitors

Jun Yun [a,c], Minsoo Han [a], Chiman Song [a], Seung Hoon Cheon [b], Kihang Choi [c], Hoh-Gyu Hahn [a,*]

[a] Chemical Kinomics Research Center, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea
[b] College of Pharmacy, Chonnam National University, Gwangju 500-757, Republic of Korea
[c] Department of Chemistry, Research Institute for Natural Sciences, Korea University, Seoul 136-701, Republic of Korea

ARTICLE INFO

Article history:
Received 16 April 2014
Revised 29 May 2014
Accepted 10 June 2014
Available online 18 June 2014

Keywords:
Depression
Antidepressant
Triple reuptake inhibitor
3-Phenethylazetidines

ABSTRACT

We report the synthesis of 3-phenethylazetidine derivatives **2** and their biological activities against 5-HT, NE and DA transporters as well as microsomal stability, CYP inhibition, and hERG inhibition profiles. Compound **2at** showed most potent triple reuptake inhibitor with good selectivity as a candidate for depression.

Depression is a common and extremely serious disease affecting about 121 million people worldwide.[1] The most commonly prescribed antidepressants, selective serotonin (5-HT) reuptake inhibitors (SSRIs) and 5-HT norepinephrine (NE) reuptake inhibitors (SNRIs), may take several weeks of treatment to affect any improvement in symptoms, and some present side effects such as insomnia and sexual dysfunction.[2,3] Recent studies show that bupropion (NE dopamine (DA) reuptake inhibitor) enhanced the antidepressant actions of SSRIs and SNRIs and reduced sexual side effect associated with SSRIs in humans.[4,5] Therefore triple reuptake inhibitors (TRIs), 'broad spectrum' antidepressants that are capable of inhibiting the reuptake of 5-HT, NE, and DA by one molecule is an attractive strategy in modern therapy for depression.[6] There are several classes of TRIs, including DOV 21,947, SEP-225289, Lu-AA42202, NS-2359 and RG-7166, in preclinical or early clinical development stages.[7–10] However, no TRI is yet available in the market. TRIs are expected to become the next generation of antidepressants and remain desirable.

In our previous papers, we reported 3-substituted azetidine **1** with triple reuptake inhibitory activities against 5-HT, NE, and DA transporters, which showed antidepressant effect in the forced swimming test in mice at 10 mg/kg IV or 20–40 mg/kg PO.[11] As a part of our continuing efforts to develop novel TRI, we have designed the 3-phenethylazetidines **2**, which are methylene homologues of 3-substituted azetidines **1**, by inserting a methylene unit between the azetidine ring and the stereogenic center in the 3-arylmethylazetidine (Fig. 1). Homologation is an approach and strategy for the rational modification of lead compounds to improve the potency and pharmacological activity.[12] Herein we report the synthesis of 3-phenethylazetidine derivatives and their biological activities against 5-HT, NE and DA reuptake inhibition in addition to microsomal stability, cytochrome P450 (CYP) inhibition and human ether-a go-go-related gene (hERG) inhibition profiles.

An overall synthetic route of 3-phenethylazetidines **2** is depicted in Scheme 1. Aldehyde **4** was obtained in 95% yield from commercially available 3-azetidine methyl ester **3** by the treatment with lithium aluminium hydride ($LiAlH_4$) in tetrahydrofuran (THF) at −78 °C, followed by Swern oxidation. Grignard reaction of **4** with aryl magnesium halide in THF at 0 °C gave an intermediate, secondary alcohol **5**. Azetidinyl β-phenoxy compound **7** was obtained from either Mitsunobu reaction of **5** with substituted phenol in the presence of triphenylphosphine and diisopropyl azodicarboxylate in THF at room temperature or by the reaction of **5** with methanesulfonyl chloride (MsCl) in the presence of triethylamine in methylene chloride at 0 °C, followed by the treatment with substituted phenol in the presence of sodium hydride in THF at ambient temperature. Deprotection of the Boc group in **7** by the treatment of 1 N HCl in boiling methanol gave the crude prod-

Abbreviations: 5-HT, serotonin; NE, norepinephrine; DA, dopamine; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin norepinephrine reuptake inhibitor; TRI, triple reuptake inhibitor; CYP, cytochrome P450; hERG, human ether-a go-go-related gene; HEK, human embryonic kidney; hSERT, human serotonin *trans*-porter; hNET, human norepinephrine transporter; hDAT, human dopamine transporter.

* Corresponding author. Tel.: +82 2 958 5139; fax: +82 2 958 5189.
E-mail address: hghahn@kist.re.kr (H.-G. Hahn).

http://dx.doi.org/10.1016/j.bmcl.2014.06.026
0960-894X/

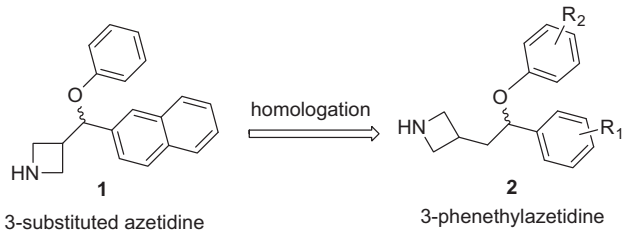


Figure 1. Design of 3-phenethylazetidines by homologation.

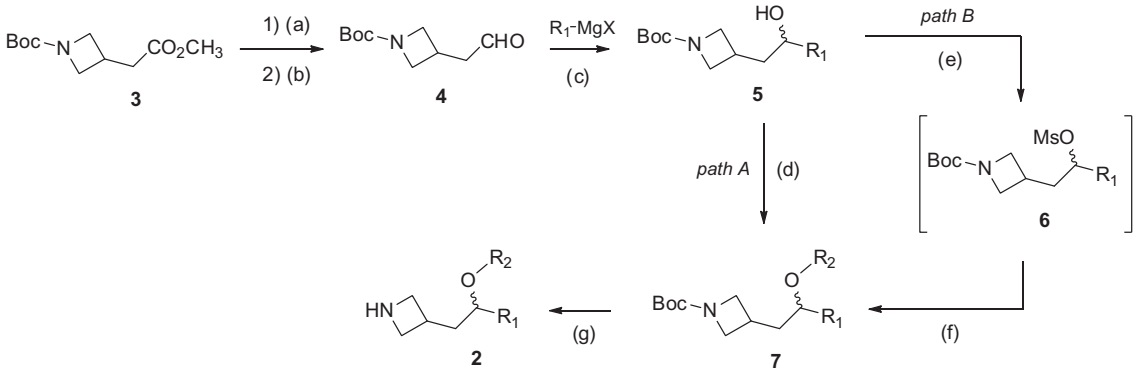
uct **2** as corresponding HCl salt. This was treated with 1 N aqueous sodium hydroxide solution followed by purification through flash chromatography to obtain the corresponding 3-phenethylazetidines **2** in 23–65% isolated yield. The structures of prepared compounds were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopies. A total of 49 analogues of 3-phenethylazetidines **2** were synthesized in this manner.

The reuptake inhibitory activities against DA, NE, and 5-HT 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).[13] In this study, the human embryonic kidney 293 (HEK293) cells stably transfected with human dopamine transporter (HEK-hDAT), human norepinephrine transporter (HEK-hNET), or human serotonin transporter (HEK-hSERT) were used for the assay. All the synthesized compounds were assayed at three different concentrations (10 μM, 1.0 μM and 0.1 μM). Some compounds were selected to obtain their IC_{50} values based on the initial screening result. The primary screening results of all the compounds are reported in Supplementary data. We used fluoxetine (SSRI), nisoxetine (selective NE reuptake inhibitor), GBR12909 (selective DA reuptake inhibitor), venlafaxine (SNRI), duloxetine (SNRI) and DOV 216,303 (TRI) as reference compounds.

The primary screening results of 26 selected compounds at a concentration of 0.1 μM are summarized in Table 1. Some compounds with 3,4-dichlorophenyl or 4-chloro-3-fluorophenyl moiety at R_1 showed good inhibitory activities against three monoamine transporters (entries 1, 2, 8, 12–20) while a 2-naphthyl group at R_1 showed low inhibitory activities (entries 25–26). In general, the compounds with 4-chloro-3-fluorophenyl moiety at R_1 showed better inhibitory activities against three monoamine transporters than those of the compounds with 3,4-dichlorophenyl moiety at the same position (compare entries 1–11 with entries 12–21). In case of the compounds in which fluorine is substituted in phenyl at R_2 showed a tendency good activities (entry 5 against hDAT, entry 8 against hNET, entries 14–16 against three monoamine transporters). In contrast, the substitution of trifluoromethoxy group at R_2 is a disadvantage to the inhibitory activities against three monoamine transporters (entries, 9–11, 21, 22). An interesting result was that some of the compounds with 2,4-difluorophenyl moiety at R_1 showed less inhibitory activities against hNET in comparison with those against hSERT and hDAT (entries 23, 24, and see example 28, 30–35 in the Supplementary material). Based on the initial results of reuptake inhibitory activities, 7 compounds were selected to determine their IC_{50} values against three monoamine reuptake transporters and for further testing of the microsomal stability against human liver microsomes (percent remaining after 0.5 h incubation using BD Gentest assay kit).

Table 2 lists the IC_{50} values and the microsomal stability of the selected 7 compounds (detailed results were reported in Supplementary data). Comparing the IC_{50} values of the selected 3-phenethylazetidines (entries 1–7) with six reference compounds for reuptake assay, the selected compounds showed better reuptake inhibitory potency than fluoxetine, GBR12909, DOV 216,303 against all the three transporters. Two compounds (entries 5 and 6) showed higher potency than nisoxetine against NE reuptake transporter. Among the 7 compounds, none of them displayed better activity than venlafaxine or duloxetine against serotonin reuptake transporter but all of them showed more than 7 times better activities than venlafaxine or duloxetine against NE and DA reuptake transporters. Some compounds (entries 3–5, 7) showed relative potency among the three transporters in the order of hNET ≳ hSERT ≳ hDAT, while the other compounds (entries 1, 2, 6) displayed relative potency among the three transporters in the order of hNET ≳ hDAT ≳ hSERT. In general the 4-chloro-3-fluorophenyl derivatives (entry 5 and 6) exhibited higher potency against all three monoamine transporter (hSERT, hNET and hDAT) than 3,4-dichlorophneyl derivatives. Microsomal stability data can be beneficial in optimizing in vivo pharmacokinetic performance. Most of the compounds were stable compared to mibefradil except compound **2aq** in the microsomal stability test.

Based on reuptake inhibition assay and microsomal stability test, four compounds (**2aq**, **2as**, **2at**, **2au**) were selected for further studies of CYP inhibition and hERG inhibition. CYP and hERG inhibition tests play important role in the preclinical studies because they can reduce attrition rate in later stage of drug development process. Similar to microsomal test, CYP and hERG inhibition assays can reduce dropout rate of drug candidate due to poor pharmacokinetic and toxicity performance in clinical studies. The four selected compounds were screened using five different isozyme of CYP (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) and hERG channel. In particular, the data of 1A2, 2D6 and 3A4 can be considered significant in this study because CYP2D6 and CYP3A4 are responsible for metabolism of a number of drugs (more than



Scheme 1. Reagents and reaction conditions: (a) $LiAlH_4$, THF, −78 °C; (b) $(COCl)_2$, DMSO, then TEA, CH_2Cl_2, −78 °C to 0 °C; (c) PhMgBr, THF, 0 °C; (d) substituted phenol, PPh_3, DIAD, THF, rt; (e) MsCl, TEA, CH_2Cl_2, 0 °C; (f) substituted phenol, NaH, THF, reflux; (g) 1 N HCl, MeOH, 60 °C and then aq 1 N NaOH.

Table 1
Activities of the selected 3-phenethylazetidines presented as percent reuptake inhibition against HEK-hSERT, HEK-hNET and HEK-hDAT

Entry	Compound	R_1	R_2	% Reuptake inhibition,[a] at 0.1 μM		
				hSERT	hNET	hDAT
	Fluoxetine			44	5	6
	Nisoxetine			10	78	10
	GBR12909			2	−6	29
	Venlafaxine			56	9	6
1	**2ab**	C_6H_3(3,4-di Cl)	C_6H_4(2-F)	59	90	77
2	**2ac**	C_6H_3(3,4-di Cl)	C_6H_5	50	86	77
3	**2ad**	C_6H_3(3,4-di Cl)	C_6H_4(3-CH_3)	21	50	40
4	**2ae**	C_6H_3(3,4-di Cl)	C_6H_4(2-CH_3)	25	39	15
5	**2ai**	C_6H_3(3,4-di Cl)	C_6H_4(3-F)	26	70	100
6	**2aj**	C_6H_3(3,4-di Cl)	C_6H_3(3,4-di Cl)	21	50	12
7	**2ak**	C_6H_3(3,4-di Cl)	C_6H_3(3,4-di F)	31	56	45
8	**2al**	C_6H_3(3,4-di Cl)	C_6H_4(4-F)	64	100	33
9	**2am**	C_6H_3(3,4-di Cl)	C_6H_4(2-OCF_3)	16	13	14
10	**2an**	C_6H_3(3,4-di Cl)	C_6H_4(3-OCF_3)	9	32	37
11	**2ao**	C_6H_3(3,4-di Cl)	C_6H_4(4-OCF_3)	16	10	13
12	**2ap**	C_6H_3(3-F,4-Cl)	C_6H_5	80	100	98
13	**2aq**	C_6H_3(3-F,4-Cl)	C_6H_4(3-Cl)	87	100	87
14	**2as**	C_6H_3(3-F,4-Cl)	C_6H_4(2-F)	77	100	68
15	**2at**	C_6H_3(3-F,4-Cl)	C_6H_4(3-F)	98	100	100
16	**2au**	C_6H_3(3-F,4-Cl)	C_6H_4(4-F)	72	100	40
17	**2av**	C_6H_3(3-F,4-Cl)	C_6H_4(2-Cl)	82	100	30
18	**2aw**	C_6H_3(3-F,4-Cl)	C_6H_4(2-CH_3)	94	87	19
19	**2ax**	C_6H_3(3-F,4-Cl)	C_6H_4(3-CH_3)	100	100	58
20	**2ay**	C_6H_3(3-F,4-Cl)	C_6H_4(4-CH_3)	97	100	41
21	**2ba**	C_6H_3(3-F,4-Cl)	C_6H_4(3-OCF_3)	25	37	33
22	**2bl**	C_6H_3(3,4-di F)	C_6H_4(2-OCF_3)	19	22	12
23	**2bd**	C_6H_3(3,4-di F)	C_6H_3(3-Cl)	77	10	77
24	**2bf**	C_6H_3(3,4-di F)	C_6H_3(2-F)	61	30	61
25	**2bq**	$C_{10}H_7$	C_6H_5	56	37	39
26	**2br**	$C_{10}H_7$	C_6H_4(2-Cl)	25	23	15

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

Table 2
IC_{50} values of monoamine reuptake inhibitory activities and microsomal stability for the selected 3-phenethylazetidines

Entry	Compound	Reuptake assay (IC_{50}, nM)[a]			Microsomal stability (% remaining after 30 min)
		hSERT	hNET	hDAT	
	Fluoxetine	150	4410	18400	
	Nisoxetine	700	20	1150	
	GBR12909	3840	1460	190	
	Venlafaxine	36.1	5722	15767	
	Duloxetine	10.4	515	977	
	DOV216,303	723	143	394	67
	Mibefradil	—	—	—	64
1	**2ab**	73.8	33.5	53.6	—
2	**2ac**	83.4	37.2	52.1	67
3	**2al**	71.9	33.7	129	—
4	**2aq**	47.1	30.0	82.5	57
5	**2as**	46.3	15.9	63.9	77
6	**2at**	36.6	14.6	35.6	78
7	**2au**	58.1	32.5	143	85

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

Table 3
IC_{50} values of the selected compounds against human CYP and hERG channel

Entry	Compound	CYP450 (IC_{50}, μM)					hERG (IC_{50}, μM)	Relative ratio of IC_{50}		
		1A2	2D6	2C9	3A4	2C19		hERG/hSERT	hERG/hNET	hERG/hDAT
	Positive control[a]	32	25	4.2	2.5	18	—			
	Duloxetine	5.3	1.6	29	0.44	4.1	—			
	DOV 216,303	0.8	0.78	99	1.3	1.6	—			
1	**2aq**	15	0.53	1.1	0.6	0.25	—			
2	**2as**	44	8.7	2.2	0.71	0.25	1.41	31	88	22
3	**2at**	13	4.0	3.1	0.51	0.21	1.46	39	97	41
4	**2au**	19	1.0	2.4	0.4	1.3	—			

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

80%), and many antidepressant drugs are metabolized by CYP1A2. The results are summarized in Table 3.

Comparing to duloxetine and DOV 216,303 in CYP inhibition studies, **2as** and **2at** exhibited a lot lower potency than the reference compounds against CYP1A2, CYP2D6. Especially **2as** has 8–50 times less potency than the reference compounds against CYP1A2 and 5 to 10 times less potency than the reference compounds against CYP2D6. Based on the overall performance in CYP tests, **2as** and **2at** were selected for hERG channel assay. As shown in Table 3, **2as** and **2at** showed 22–97-fold selectivity between hERG channel and the three monoamine transporters.

In summary, we report the design and synthesis of 3-phenethylazetidine derivatives **2** and their biological activities against 5-HT, NE and DA transporters as well as microsomal stability, CYP inhibition, and hERG inhibition profiles. Compound **2at** showed most potent triple reuptake inhibitor with good selectivity over hERG channel. Further preclinical studies of **2at** are in progress and will be reported soon.

Acknowledgments

This work was supported by Korea Drug Development Fund and Korea Institute of Science and Technology.

Supplementary data

Supplementary data (experimental procedures and biological screening methods, yields, melting points, ^{1}H and ^{13}C NMR data for all the compounds and detailed results of the biological assays) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.06.026.

References and notes

1. (a) Kulkarni, S. K.; Dhir, A. *Expert Opin. Investig. Drugs* **2009**, *18*, 767; (b) Millan, M. J. *Pharmacol. Ther.* **2006**, *110*, 135; (c) Perović, B.; Jovanović, M.; Miljković, B.; Vezmar, S. *Neuropsychiatr. Dis. Treat.* **2010**, *6*, 343.
2. (a) Prins, J.; Olivier, B.; Korte, S. M. *Expert Opin. Investig. Drugs* **2011**, *20*, 1107; (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. *Depression Anxiety* **2006**, *23*, 178.
3. (a) Daws, L. C. *Pharmacol. Ther.* **2009**, *121*, 89; (b) Costagliola, C.; Parmeggiani, F.; Semeraro, F.; Sebastiani, A. *Curr. Neuropharmacol.* **2008**, *6*, 293; (c) Vaswani, M.; Linda, F. K.; Ramesh, S. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2003**, *27*, 85.
4. Zisook, S.; Rush, A. J.; Haight, B. R.; Clines, D. C.; Rockett, C. B. *Biol. Psychiatry* **2006**, *59*, 203.
5. Prica, C.; Hascoet, M.; Bourin, M. *Behav. Brain Res.* **2008**, *194*, 92.
6. (a) Chen, Z.; Skolnick, P. *Expert Opin. Investig Drugs* **2007**, *16*, 1365; (b) Millan, M. J. *Neurotherapeutics* **2009**, *13*, 53.
7. Liang, Y.; Richelson, E. *Primary Psychiatry* **2008**, *15*, 50.
8. (a) Skolnick, P.; Popik, P.; Janowsky, A.; Beer, B.; Lippa, A. S. *Life Sci.* **2003**, *73*, 3175; (b) Skolnick, P.; Krieter, P.; Tizzano, J.; Basile, A.; Popik, P.; Czobor, P.; Lippa, A. *CNS Drug Rev.* **2006**, *12*, 123.
9. Skolnick, P.; Popik, P.; Janowsky, A.; Beer, B.; Lippa, A. S. *Eur. J. Pharmacol.* **2003**, *461*, 99.
10. Shao, L.; Li, W.; Xie, Q.; Yin, H. *Expert Opin. Ther. Pattents* **2014**, *24*, 131.
11. Han, Y.; Han, M.; Shin, D.; Song, C.; Hahn, H.-G. *J. Med. Chem.* **2012**, *55*, 8188.
12. (a) Duranti, A.; Franchini, C.; Lentini, G.; Loiodice, F.; Tortorella, V.; Luca, A. D.; Pierno, S.; Camerino, D. C. *Eur. J. Med. Chem.* **2000**, *30*, 147; (b) Collin, D. T.; Hartley, D.; Jack, D.; Lunts, L. H. C.; Press, J. C.; Ritchie, A. C.; Toon, P. *J. Med. Chem.* **1970**, *13*, 674.
13. Jørgensen, S.; Nielsen, E. Ø.; Peters, D.; Dyhring, T. *J. Neurosci. Methods* **2008**, *169*, 168.