DOI 10.5012/bkcs.2011.32.4.1249

Synthesis and Biological Activities of (4-Arylpiperazinyl)piperidines as Nonpeptide BACE 1 Inhibitors

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Received December 28, 2010, Accepted February 14, 2011

Inhibition of BACE 1 activity is considered as a promising therapeutic target for Alzheimer's Disease (AD). Synthesis and inhibitory activities of (4-arylpiperazinyl)piperidines by bioisosteric replacement of a biaryl group with an arylpiperazine as BACE 1 inhibitors are described. The resulting (4-arylpiperazinyl)piperidines represent novel nonpeptide BACE 1 inhibitors with improved *in vitro* potency.

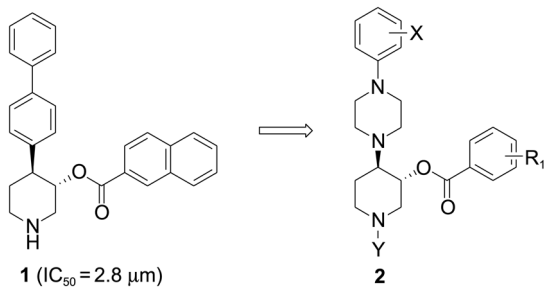
Key Words : Alzheimer's Disease (AD), BACE 1 (β-secretase), Non-peptidomimetic inhibitors, Arylpiperazinylpiperidines

Introduction

Alzheimer's disease (AD) is the most common form of dementia with clinical symptoms of memory loss and impairment on daily living activity.[1] One of the pathological hallmarks of AD is characterized by deposition of extracellular plaques composed of amyloid β peptide (Aβ),[2] which is derived from amyloid precursor protein (APP) by sequential endoproteolysis by β- and γ-secretases.[3] Although the cause of AD is still unclear, accumulation of Aβ within the brain plays an important role in the disease process.[4] Since BACE 1 (β-secretase) is a key enzyme for APP processing into Aβ and BACE 1 knockout mice are unable to generate Aβ,[5] inhibition of BACE 1 is considered as an attractive target for the treatment of AD.[6]

Peptidomimetic BACE 1 inhibitors with the transition state mimic core motifs including statine, hydroxyethylene and hydroxyethylamine backbone have been reported to possess potent activities in an enzymatic assay,[7] but their low metabolic stability, poor cell permeability and bioavailability hampered them to be developed as drug candidates. Recently, nonpeptide BACE 1 inhibitors with acylguanidine, several other cyclic amidines and guanidine isosteres as pharmacophores interacting with aspartic residues of BACE 1 have revealed high activities both in enzyme and cell assays.[8]

During the course of our program to develop novel nonpeptide BACE 1 inhibitors, 4-arylpiperidines **1** with IC_{50} of 2.87 μM was discovered.[9] Initial SAR study of **1** led us to develop (4-arylpiperazin-1-yl)piperidinyl benzoate **2** by replacement of biaryl ring with a more flexible arylpiperazine, since chair like conformation of piperazine in **2** would keep the molecular shape similar to that of **1** and the arylpiperazine group was found in many CNS drugs such as dopamine antagonists (Scheme 1).[10] Here, we report the synthesis and biological evaluation of (4-arylpiperazinyl)-piperidines as BACE 1 inhibitors.



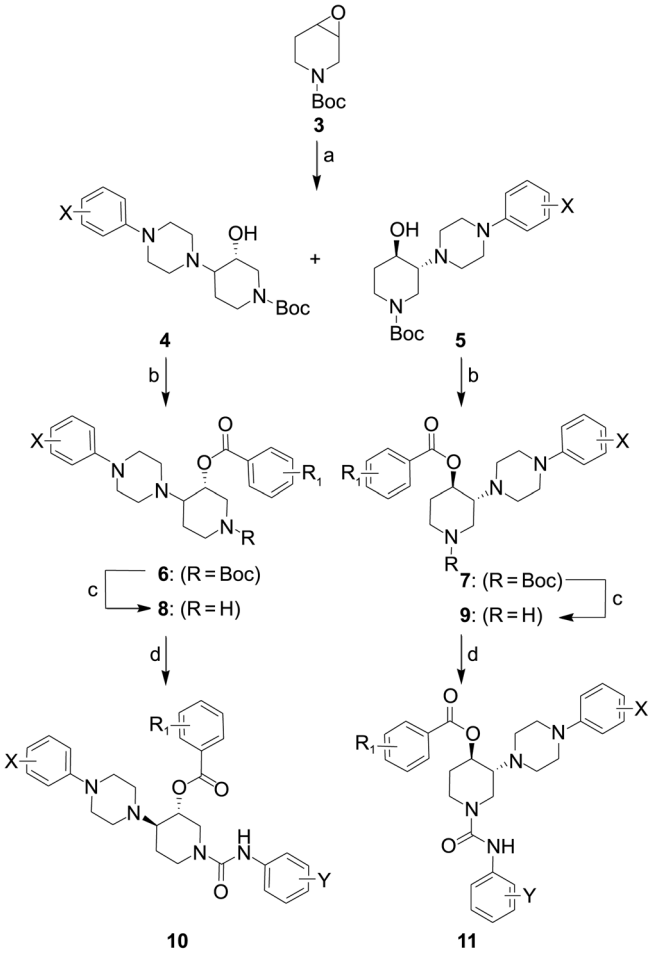
Scheme1. Bioisosteric replacement of biaryl into arylpiperazine.

Chemistry

Ring opening of *N*-Boc-piperidine oxide **3** with arylpiperazine under the microwave irradiation condition in the presence of lithium perchlorate afforded inseparable mixture of aminoalcohol **4** and **5**. The regioisomeric mixture of aminoalcohol was converted to the corresponding ester by treatment with aroyl chloride in pyridine under the microwave irradiation condition, which was then separated into resioisomer **6** and **7** by column chromatography. Microwave irradiation was highly effective for both ring opening and esterification reactions compared to the conventional thermal reaction.[11] The Boc protecting group of compound **6** was removed by TFA to afford piperdine **8**. Finally, ureidopiperidine **10** was obtained *via* a reaction with arylisocyanate. Following each chemical modification described above, compound **7** was converted to the corresponding ureidopiperidine **10** (Scheme 2).

Results and Discussion

The inhibitory activity of BACE 1 was determined at the concentration of 10, 1 and 0.1 μM of a compound by a fluorescence resonance energy transfer (FRET) assay, using a purified baculovirus-expressed BACE 1 and a specific substrate (Rh-EVNLDAEFK-Quencher). BACE 1 inhibitory activities of these (4-arylpiperazinyl)piperidines **8-11** are listed in Table 1 and 2. As we can see from Table 1, the

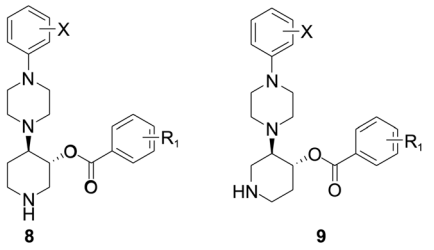


Scheme 2. Synthesis of (4-arylpiperazinyl) piperidines. [a]Reagents and conditions: (a) arylpiperazine, $LiClO_4$, CH_3CN, 150 °C, mw; (b) ArCOCl, pyridine, 130 °C, mw; (c) TFA, CH_2Cl_2; (d) ArNCO, CH_2Cl_2.

replacement of the biaryl group in **1** into (3-chlorophenyl)-piperazine in **8a** revealed the enhanced potency toward BACE 1. Regioisomer **9a** showed also a potent activity (entry 1). Modification of the 2-nathyl group was then carried out for the structural optimization. Introduction of a benzene ring with aryl and phenoxy groups resulted in further increased activities upto 8-fold compared to **1** (entry 2, 5 and 7). Substituents other than phenyl or phenoxy groups such as *tert*-butyl and nitro groups showed decreased activities, implying that the size of substituents on the benzene ring is important for the additional interaction with BACE 1 (entry 8 and 9). In comparison with activities between isomeric piperidines, piperidine **8** with arylpiperazine at 4-position was more potent than isomeric compound **9** in general.

We decided to introduce an additional carbamoyl group on nitrogen of the piperidine ring since the resulting urea could interact with aspartic residue in the catalytic site of BACE 1 (Table 2).[12] On the contrary to free piperidine compounds **8** and **9**, ureidopiperidine **11** with arylpiperazine at 3-position showed more potent activity than regioisomeric ureido-piperidine **10**. Among the ureidopiperidine tested, 3,5-di-

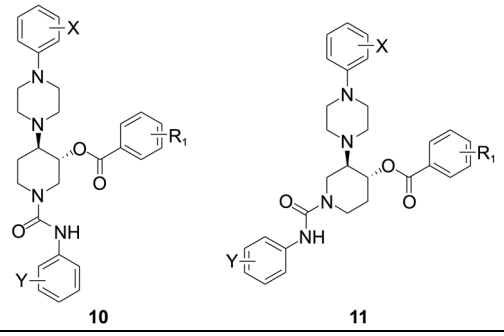
Table 1. BACE 1 inhibitory activities of **8** and **9**



Entry	Substituent		Compd. **8**	IC_{50} (μM)	Compd. **9**	IC_{50} (μM)
	R_1	X				
1	2-naph[a]	3-Cl	**8a**	1.9	**9a**	1.2
2	4-Ph	3-Cl	**8b**	0.5	**9b**	1.0
3	4-Ph	4-F	**8c**	1.4	**9c**	1.9
4	4-Ph	4-MeO	**8d**	4.5	**9d**	4.8
5	4-PhO	3-Cl	**8e**	0.6	**9e**	1.1
6	4-PhO	4-F	**8f**	2.6	**9f**	2.2
7	4-PhO	4-MeO	**8g**	0.3	**9g**	7.5
8	4-Bu-*t*	3-Cl	**8h**	7.8	**9h**	5.6
9	2-NO_2	3-Cl	**8i**	> 10	**9i**	> 10

[a]C_6H_4-R_1 : 2-naph

Table 2. BACE 1 inhibitory activities of **10** and **11**



Compd.	R_1	X	Y	IC_{50} (μM)
10a	4-Ph	3-Cl	2-F	> 10
10b	4-Ph	4-MeO	2-F	> 10
10c	4-PhO	4-MeO	3,5-Cl_2	9.6
11a	4-Ph	4-F	2-F	10
11b	4-Ph	4-MeO	2-F	2.4
11c	4-Ph	4-MeO	3-MeO	> 10
11d	4-Ph	4-MeO	3,5-Cl_2	1.9
11e	4-PhO	4-MeO	3,5-Cl_2	0.6
11f	4-PhO	4-F	3,5-Cl_2	1.1

chloro substituent resulted better activity than other substituents (**11d-f**).

In conclusion, (4-arylpiperazinyl)piperidines by bioisosteric replacement of a biaryl group with an arylpiperazine have promising *in vitro* potency as as nonpeptide BACE 1 inhibitors.

Experimental

All reagents were purchased from Aldrich Chemical Co.

and Alfa Aesar Chemical Co. and used as obtained unless otherwise noted. All microwave irradiation experiments were carried out using microwave Synthesizer Initiator instrument from Biotage. Melting points were obtained on a Thomas Scientific melting point apparatus and are uncorrected. Flash chromatography was carried out using silica gel 60 (230-400 mesh) using various solvent mixtures. NMR spectra were recorded at 200 or 300 MHz (^{1}H NMR) and 50 or 75 MHz (^{13}C NMR), referenced to an internal standard (TMS) or residual solvent protons and signals are reported in ppm (δ).

General Procedure for the Preparation of Aminoalcohol 4 and 5. A mixture of 1-*tert*-butoxycarbonyl-3,4-epoxypiperidine 3 (5.0 mmol), lithium perchlorate (5.5 mmol) and (4-aryl)-piperazine hydrochloride (5.5 mmol) in acetonitrile (10 mL) was taken in a Biotage vial containing a magnetic stirring bar. The vial was sealed and heated in the Biotage Initiator Synthesizer to 130 °C for 10-30 min. After completion of the reaction, it was diluted with ethyl acetate (50 mL) and washed with water (2 × 50 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The remaining residue was purified by flash column chromatography (EtOAc:hexane = 1:2, v/v) to give desired aminoalcohols **4** and **5** in 76-90% yield.

General Procedure for the Preparation of Ester 6 and 7. The isomeric mixture (**4** and **5**, 2 mmol) and aroyl chloride (2.5 mmol) in pyridine (5 mL) was taken in a 10 mL Biotage vial containing a magnetic stirring bar. The vial was sealed and the resulting mixture was heated in the Biotage Initiator Synthesizer at 150 °C for 3-8 min. The progress of the reaction was monitored by TLC (EtOAc: hexane = 1:2, v/v). After completion of the reaction, it was diluted with EtOAc (25 mL) and washed with cold dilute HCl (2 × 10 mL), sat'd sodium bicarbonate (2 × 10 mL) and water (10 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The isomeric products **6** and **7** were separated from the remaining residue by flash column chromatography (EtOAc:hexane = 1:3.5, v/v).

***tert*-Butyl 3-(2-naphthoyloxy)-4-(4-(3-chlorophenyl)piperazin-1-yl)piperidine-1-carboxylate (6a).** yield; 51%: mp 70-71 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.50 (s, 9H), 1.70-1.90 (m, 1H), 2.10-2.30 (m, 1H), 2.65-2.85 (m, 3H), 2.90-3.11 (m, 8H), 3.90-4.20 (m, 2H), 5.35-5.45 (m, 1H), 6.66-6.78 (m, 3H), 7.08 (t, *J* = 7.6 Hz, 1H), 7.45-7.62 (m, 2H), 7.85-7.90 (m, 2H), 7.93-7.98 (m, 1H), 8.02-8.07 (m, 1H), 8.59 (s, 1H).

***tert*-Butyl 4-(2-naphthoyloxy)-3-(4-(3-chlorophenyl)piperazin-1-yl)piperidine-1-carboxylate (7a).** yield; 47%: mp 75-76 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.46 (s, 9H), 1.60-1.80 (m, 1H), 1.90-2.00 (m, 1H), 2.60-2.75 (m, 3H), 2.80-3.15 (m, 8H), 4.00-4.15 (m, 1H), 4.20-4.35 (m, 1H), 5.15-5.30 (m, 1H), 6.66-6.79 (m, 3H), 7.09 (t, *J* = 8.2 Hz, 1H), 7.44-7.62 (m, 2H), 7.85-7.89 (m, 2H), 7.92-7.97 (m, 1H), 8.01-8.06 (m, 1H), 8.58 (s, 1H).

General Procedure for the Preparation of Piperidine 8 and 9. The Boc protected piperidine **6** (or **7**) (0.600 g) was treated with 30 mL of TFA:MC:water (70:30:2.5, v/v) at 0 °C. The reaction mixture was slowly warmed to rt and stirred for 2 h. The progress of the reaction was monitored by TLC (EtOAc:hexane = 1:2, v/v). After the completion of reaction, it was concentrated *in vacuo*, diluted with EtOAc (30 mL), washed with sat'd sodium bicarbonate solution (30 mL) and water (30 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated and purified by flash column chromatography (CH_2Cl_2:MeOH = 12:1, v/v) to afford **8** (or **9**).

4-(4-(3-Chlorophenyl)piperazin-1-yl)piperidin-3-yl 2-naphthoate (8a). yield; 99%: mp 89-90 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.60-1.85 (m, 1H), 1.90-2.20 (m, 1H), 2.60-2.72 (m, 2H), 2.75-2.84 (m, 2H), 2.85-2.95 (m, 2H), 3.00-3.10 (m, 4H), 3.15-3.35 (m, 1H), 3.40-3.65 (m, 1H), 3.70-4.05 (m, 1H), 5.25-5.40 (m, 1H), 6.66-6.77 (m, 3H), 7.08 (t, *J* = 8.0 Hz, 1H), 7.50-7.60 (m, 2H), 7.87-7.92 (m, 2H), 7.95-8.00 (m, 1H), 8.01-8.07 (m, 1H), 8.61 (s, 1H).

3-(4-(3-Chlorophenyl)piperazin-1-yl)piperidin-4-yl 2-naphthoate (9a). yield; 98%: mp 86-87 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.90-2.10 (m, 1H), 2.30-2.50 (m, 1H), 2.65-2.85 (m, 2H), 2.90-3.20 (m, 7H), 3.30-3.60 (m, 2H), 5.00-5.30 (m, 2H), 5.40-5.50 (m, 1H), 6.64-6.75 (m, 3H), 7.07 (t, *J* = 8.0 Hz, 1H), 7.50-7.65 (m, 2H), 7.84-7.87 (m, 2H), 7.96-8.00 (m, 1H), 8.02-8.07 (m, 1H), 8.61 (s, 1H).

General Procedure for the Preparation of Urea 10 and 11. To the solution of piperidine derivative **8** (0.11 mmol) and triethylamine (0.04 mL, 0.29 mmol) in dichloromethane (1.5 mL), arylisocyanate (0.13 mmol) was added. The resulting solution was stirred at rt for 3 h. The progress of the reaction was monitored by TLC (CH_2Cl_2:MeOH = 12:1, v/v). After the completion of reaction, it was washed with water (5 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated, and purified by a short gravity column (EtOAc:hexane = 2:3, v/v).

4-(4-(3Chlorophenyl)piperazin-1-yl)-1-(2-fluorophenylcarbamoyl)piperidin-3-yl biphenyl-4-carboxylate 10a). yield; 86%: mp 180-181 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.77-1.90 (m, 1H), 2.24-2.33 (m, 1H), 2.75-2.84 (m, 3H), 2.86-3.03 (m, 2H), 2.86-3.03 (m, 2H), 3.06-3.10 (m, 4H), 3.12-3.25 (m, 2H), 3.93-4.05 (m, 1H), 4.10-4.19 (m, 1H), 5.37-5.44 (m, 1H), 6.68-6.80 (m, 4H), 6.95-7.15 (m, 4H), 7.36-7.49 (m, 3H), 7.60-7.68 (m, 4H), 8.04-8.11 (m, 3H).

3-(4-(4-Fluorophenyl)piperazin-1-yl)-1-(2-fluorophenylcarbamoyl)piperidin-4-yl biphenyl-4-carboxylate (11a). yield; 95%: mp 206-207 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.73-1.88 (m, 1H), 2.00-2.10 (m, 1H), 2.70-2.78 (m, 2H), 2.80-2.98 (m, 3H), 3.00-3.10 (m, 4H), 3.11-3.28 (m, 2H), 4.03-4.13 (m, 1H), 4.21-4.26 (m, 1H), 5.25-5.29 (m, 1H), 6.74 (d, *J* = 3.8 Hz, 1H), 6.79-6.85 (m, 2H), 6.87-7.12 (m, 5H), 7.36-7.50 (m, 3H), 7.58-7.66 (m, 4H), 8.02-8.10 (m, 3H).

References

1. Mount, C.; Downton, C. *Nat. Med*. **2006**, *12*, 780.
2. (a) Shawn, J. S. *Drug Dev. Res.* **2009**, *70*, 101. (b) Selkoe, D. J. *Nature* **1999**, *399*, A23.
3. Sinha, S.; Lieberburg, I. *Proc. Natl. Acad. Sci. U.S.A*. **1999**, *96*, 11049.
4. Hsiao, K.; Chapman, P.; Nilsen, S.; Eckman, C.; Harigaya, Y.;

Younkin, S.; Yang, F.; Cole, G. *Science* **1996**, *274*, 99.
5. Luo, Y.; Bolon, B.; Damore, M. A.; Fitzpatrick, D.; Liu, H.; Zhang, J.; Yan, Q.; Vassar, R.; Citron, M. *Neurobiol. Dis.* **2003**, *14*, 81.
6. (a) Malamas, M. S.; Erdei, J.; Gunawan, I.; Turner, J.; Hu, Y.; Wagner, E.; Fan, K.; Chopra, R.; Olland, A.; Bard, J.; Jacobsen, S.; Magolda, R. L.; Pangalos, M.; Robichaud, A. *J. Med. Chem.* **2010**, *53*, 1146. (b) Citron, M. *Nature Rev. Neurosci.* **2004**, *5*, 677.
7. (a) Gosh, A. K.; Shin, D. W.; Downs, D.; Koelsch, G.; Lin, X.; Ermolieff, J.; Tnag, J. *J. Am. Chem. Soc*. **2000**, *122*, 3522. (b) Hanessian, S.; Yun, H.; Hou, Y.; Yang, G.; Bayrakdarian, M.; Therrien, E.; Moitessier, N.; Roggo, S.; Veenstra, S.; et al. *J. Med. Chem*. **2005**, *48*, 5175. (c) Yang, W.; Lu, W.; Lu, Y.; Zhong, M.; Sun, J.; Thomas, A. E.; Wilkinson, J. M.; Fucini, R. V.; Lam, M.; Randal, M.; Shi, X.-P.; Jacobs, J. W.; McDowell, R. S.; Gordon, E. M.; Ballinger, M. D. *J. Med. Chem*. **2006**, *49*, 839.
8. (a) Edwards, P. D.; Albert, J. S.; Sylvester, M.; Aharony, D.; Andisik, D.; Callaghan, O.; Campbell, J. B.; Carr, R. A.; Chessari, G.; Congreve, M.; Frederickson, M.; Folmer, R. H.; Geschwindner, S.; Koether, G.; Kolmodin, K.; Krumrine, J.; Mauger, R. C.; Murray, C. W.; Olsson, L.-L.; Patel, S.; Spear, N.; Tian, G. *J. Med. Chem.* **2006**, *49*, 6158. (b) Cole, D. C.; Manas, E. S.; Stock, J. R.; Condon, J. S.; Jennings, L. D.; Aulabaugh, A.; Chopra, R.; Cowling, R.; Ellingboe, J. W.; Fan, K. Y.; Harrison, B. L.; Hu, Y.; Jacobsen, S.; Jin, G.; Lin, L.; Lovering, F. E.; Malamas, M. S.; Stahl, M. L.; Strand, J.; Sukhdeo, M. N.; Svenson, K.; Turner, M. J.; Wagner, E.; Wu, J.; Zhou, P.; Bard, J. *J. Med. Chem.* **2007**, *50*, 5912. (c) Malamas, M. S.; Erdei, J.; Gunawan, I.; Barnes, K.; Johnson, M.; Hui, Y.; Turner, J.; Hu, Y.; Erik Wagner, E.; Fan, K.; Olland, A.; Bard, J.; Robichaud, A. J. *J. Med. Chem*. **2009**, *52*, 6314.
9. Lim, H.-J.; Jung, M. H.; Choi, I. Y.; Park, W. K. *Bull. Korean Chem. Soc*. **2006**, *27*, 1371.
10. Zhang, A.; Neumeyer, J. L.; Baldessarini, R. J. *Chem. Rev.* **2007**, *107*, 274.
11. Boja, P.; Lim, H.-J. *Z. Naturforsch.* **2010**, *65b*, 197.
12. Huang, D.; Luthi, U.; Kolb, P.; Edler, K.; Cecchini, M.; Audetat, S.; Barberis, A.; Caflisch, A. *J. Med. Chem.* **2005**, *48*, 5108.