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Synthesis, SAR, and X-ray structure of human BACE-1 inhibitors with cyclic urea derivatives

Heuisul Park,[a,*] Kyeongsik Min,[a,*] Hyo-Shin Kwak,[a] Ki Dong Koo,[a] Dongchul Lim,[a] Sang-Won Seo,[a] Jae-Ung Choi,[a] Bettina Platt[b] and Deog-Young Choi[a]

[a]*Drug Discovery, Research & Development, LG Life Sciences, 104-1 Munji-dong, Yuseong-gu, Daejon 305-380, Republic of Korea*
[b]*School of Medical Sciences, College of Life Sciences and Medicine, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK*

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Abstract—We describe synthesis and evaluation of a series of cyclic urea derivatives with hydroxylethylamine isostere. Modification of P3, P1, and P2′ and combination of SAR display a >100-fold increase in potency with good cellular activity (IC_{50} = 0.15 μM) relative to the previously reported compound **3**.

Alzheimer's disease (AD) is the most common cause of progressive dementia characterized by pathological hallmark of intracellular neurofibrillary tangles and extracellular neuritic plaques primarily composed of amyloid beta peptide (Aβ).[1] Aβ is a 40- to 42-amino acid peptide generated by sequential proteolytic cleavage of membrane-anchored amyloid precursor protein (APP) by β-secretase (BACE-1) and γ-secretase.[2] Although the link between Aβ as its partially soluble or aggregated forms and neuronal dysfunction in AD is controversial, there are many evidences that progressive accumulation of Aβ in the brain is critical response for the neurodegeneration in AD.[3] Therefore inhibition of APP proteolysis to lower the concentration of neurotoxic Aβ is an attractive strategy for clinical intervention.

BACE-1 is a type I membrane-anchored aspartyl protease identified in 1999.[4] The proteolysis of APP by BACE-1 occurs in the luminal surface of the plasma membrane and releases the soluble ectodomain of APP. The remaining membrane-associated C-terminal domain including Aβ is subsequently cleaved to Aβ by γ-secretase. BACE-1 is highly expressed in the central nervous system. BACE-1 knockout homozygote mice showed no Aβ production and a relatively normal phenotype.[5] Furthermore, crystal structures of BACE-1 in complex with inhibitors were determined at atomic resolution,[6] which provided a powerful tool for structure-based drug design of BACE-1 inhibitors. As such, BACE-1 is an attractive therapeutic target for the treatment and prevention of AD.

In order to replace the P2 isophthalide ring, 2-oxo-heterocyclic compounds were designed and evaluated.[7] The carbonyl group of 2-oxo-heterocyclic group as in compounds **1**–**3** at P2 serves as a hydrogen acceptor of Thr232 amide NH enhancing the binding affinity. Among them, compound **3** was more potent, more selective against cathepsin D (Cat-D), and cell-permeable inhibitor (IC_{50} = 7.3 μM). In this letter, we describe optimization of compound **3** to further improve potency and selectivity (Fig. 1).

At first, we examined the hydrophobic interaction of the benzyl group at N-3 position (R^1) as well as the substituent effect of cyclic urea moiety at C-5 position (R^2). Compounds were synthesized according to Scheme 1 and the results are summarized in Table 1. Absence of the benzyl group (**4**) and replacement with the *n*-butyl group (**5**) of P3, thereby reducing van der Waals contacts, resulted in nearly 70-fold loss of potency. This reveals that the benzyl group at N-3 position (R^1) is essential for binding and the proper size is necessary to obtain high affinity for BACE-1.

Keywords: Alzheimer's disease; BACE-1; Cyclic urea.
* Corresponding authors. Tel.: +82 42 866 2240; fax: +82 42 866 5754; e-mail: renivie@lgls.com

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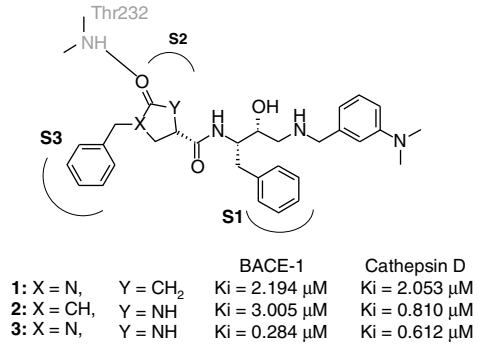
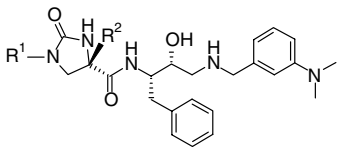


Figure 1. Structures of BACE-1 inhibitors.

We synthesized a series of aryl compounds with various lengths to optimize the phenyl ring (Scheme 2). The 3-phenyl-2-oxo-imidazoline derivatives (**10** and **11**) gave lower affinity compared with benzyl derivative (**3**). The longer chain derivatives (**6** and **7**) also gave lower affinity and increased the binding affinity for Cat-D. Although the benzyl group appears to be the best in this series, this result indicates that the proper position of the phenyl ring is important for BACE-1 affinity and selectivity.

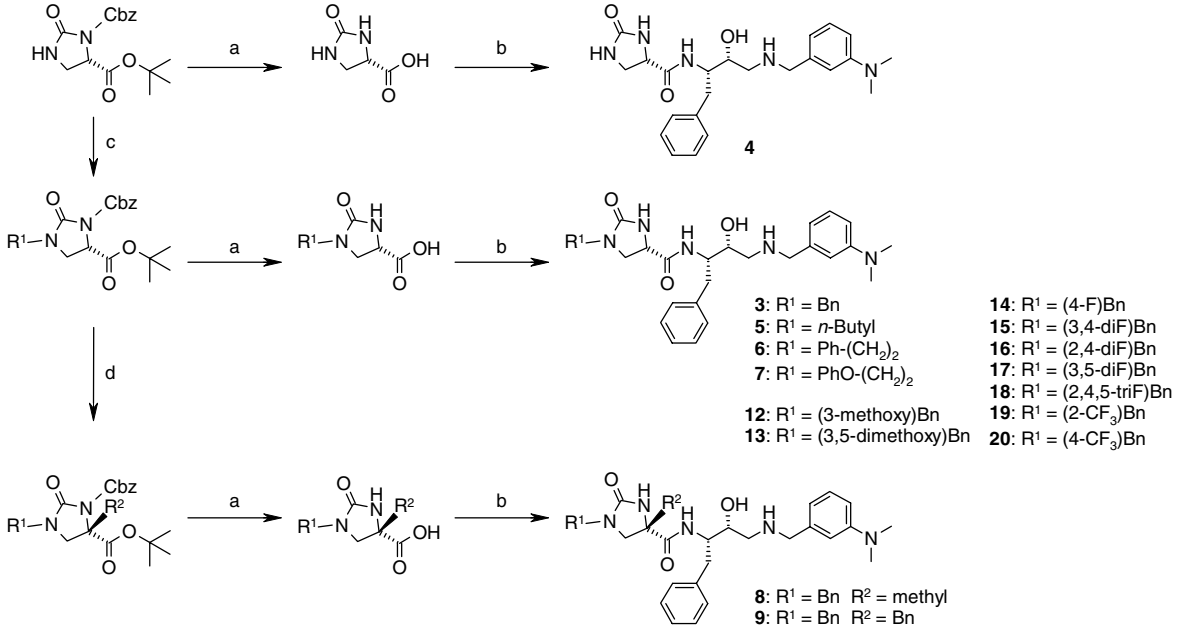
The phenyl groups of **10** and **11** might be directed to P4 position of OM99-2, a peptidomimetic inhibitor with eight residues. The longer phenethyl (**6**) and the phenoxyethyl group (**7**) were thought to be larger for the S3 site. The co-crystal structure of BACE-1 in complex with compound **7** was determined to elucidate the binding mode but the electron density for the phenoxy part was absent. However, the orientation of the ethyl part toward the P4 main-chain backbone of OM99-2 indicates that the phenoxyethyl group is not located in the binding site of the P3 benzyl group in the BACE-1/compound **3** structure.[6,7] The absence of electron density for the phenoxy part is probably related to disordered binding due to the improper binding site for the phenoxy part (data not shown).

Table 1. SAR of P2 and P3

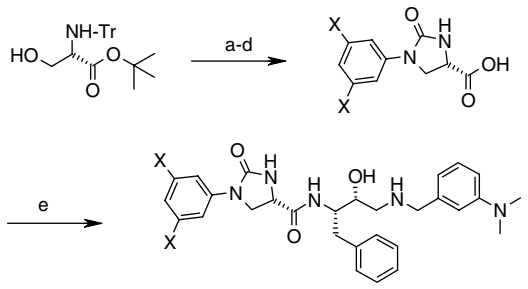


Compound	R^1	R^2	K_i (μM)	
			BACE-1	CatD
3	Benzyl	H	0.284	0.612
4	H	H	19.004	—
5	*n*-Butyl	H	39.989	—
6	Phenethyl	H	6.800	0.310
7	Phenoxy-ethyl	H	3.514	0.590
8	Benzyl	Methyl	6.768	0.431
9	Benzyl	Benzyl	5.001	0.080
10	3,5-Dibromophenyl	H	3.500	1.125
11	Phenyl	H	8.177	1.302

Filling S2 site was attempted by introducing the methyl (**8**) or the benzyl group (**9**) at C-5 position (R^2). Compounds **8** and **9** were synthesized by Scheme 1 and were selected from two kinds of isomers which showed better activity. The absolute configuration was confirmed by X-ray crystallography, respectively. S2 binding site was known to be favorable to hydrophobic side chains of Ala and Met[8] but the inhibitory activity and the selectivity of compounds **8** and **9** were approximately 20-fold decreased compared to compound **3**. To determine the binding mode of **9**, co-crystal structure was solved at 2.7 Å (Fig. 2).[13] The loss of activity is probably due to



Scheme 1. Reagents and conditions: (a) HBr, rt, 4 h; (b) (2*R*,3 *S*)-1-(3-dimethylamino)benzylamino-2-hydroxy-3-amino-4-phenyl-butane, EDC, HOBt, DMF, Et_3N, rt, 4 h; (c) NaH, R1-X, THF, 0 °C to rt, 4 h; (d) LiHMDS, R2-X, THF, 0 °C–rt, 4 h.



10: X = Br
11: X = H

Scheme 2. Reagents and conditions: (a) SO_3·Py, Et_3N, CH_2Cl_2, rt, 4 h; (b) aniline, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt, 8 h; (c) Phosgene (40% in Toluene), 0 °C–rt, 8 h; (d) HBr, rt, 4 h; (e) (2*R*, 3*S*)-1-(3-dimethylamino)benzylamino-2-hydroxy-3-amino-4-phenyl-butane, EDC, HOBt, DMF, Et_3N, rt, 4 h.

geometric differences (~1 Å) at P2 Cα position between compound **9** and OM99-2, which resulted an unfavorable interaction of the R^2 benzyl group located far away from S2 site.

Next, we introduced various R^1 substituents at the phenyl ring of compound **3** such as F, OMe, CF_3 to improve potency by enhancing hydrophobic interaction and also improve selectivity by using the S3-subpocket (S3-sub) (Scheme 1). The results are summarized in Table 2. The S3-sub was revealed by HTS hit compound/BACE-1 structure[9] and compounds with binding motif in the S3-sub showed improved potency and selectivity.[10] To access the S3-sub, we synthesized the 3-methoxy and the 3,5-dimethoxy benzyl derivatives (**12** and **13**). Contrary to our expectation, those compounds gave lower activity and selectivity than the benzyl derivative

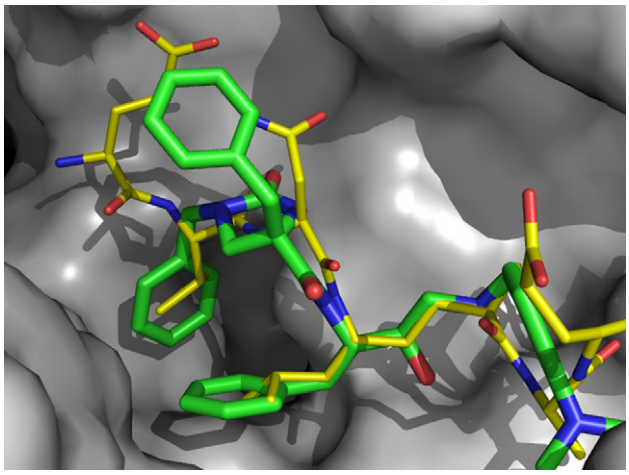
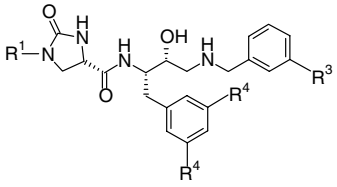


Figure 2. X-ray structure of compound **9** (green) in complex with BACE-1. Overlay with the peptidomimetic inhibitor, OM99-2 (yellow) from 1FKN. The flap region is removed to clear view of P2.

(**3**). To confirm whether the methoxy group oriented to the S3-sub or not, co-crystal structure of BACE-1 in complex with compound **12** was determined at 2.3 Å resolution (Fig. 3). BACE-1/**12** complex confirmed that the methoxy group was oriented toward the S3-sub. However, there was a subtle difference in the direction between the methoxybenzyl group of compound **12** and the S3-sub binding part of compound **27** when they were superimposed.[10,11] A close examination of the complex structure revealed that positioning of the R^3 methoxybenzyl group for optimum fit in the S3-sub was sterically hindered by the P1 benzyl group. Furthermore, this steric repulsion make the methoxy group to be unfavorable for the S3-sub. Interestingly, *ortho*-tri-

Table 2. Optimization of cyclic urea derivatives



Compound	R^1	R^3	R^4	K_i (μM)		SEAP IC_{50} (μM)
				BACE	CatD	
12	(3-Methoxy)benzyl	$-N(CH_3)_2$	H	0.449	0.195	14.22
13	(3,5-Dimethoxy)benzyl	$-N(CH_3)_2$	H	0.662	0.142	36.48
14	(4-F)Benzyl	$-N(CH_3)_2$	H	0.818	0.276	11.63
15	(3,4-diF)Benzyl	$-N(CH_3)_2$	H	0.272	0.354	5.74
16	(2,4-diF)Benzyl	$-N(CH_3)_2$	H	0.867	0.450	9.95
17	(3,5-diF)Benzyl	$-N(CH_3)_2$	H	0.053	0.417	1.97
18	(2,4,5-triF)Benzyl	$-N(CH_3)_2$	H	0.260	0.520	5.59
19	(2-CF_3)Benzyl	$-N(CH_3)_2$	H	0.267	0.190	3.12
20	(4-CF_3)Benzyl	$-N(CH_3)_2$	H	4.957	0.290	—
21	Benzyl	$-CF_3$	H	1.256	1.071	12.33
22	Benzyl	$-OCF_3$	H	1.881	0.300	21.07
23	(3,5-diF)Benzyl	*i*-Propyl	H	0.018	0.151	0.58
24	(3,5-diF)Benzyl	*t*-Butyl	H	0.009	0.135	0.43
25	Benzyl	$-N(CH_3)_2$	F	0.051	0.320	1.29
26	(3,5-diF)Benzyl	*t*-Butyl	F	0.002	0.066	0.15

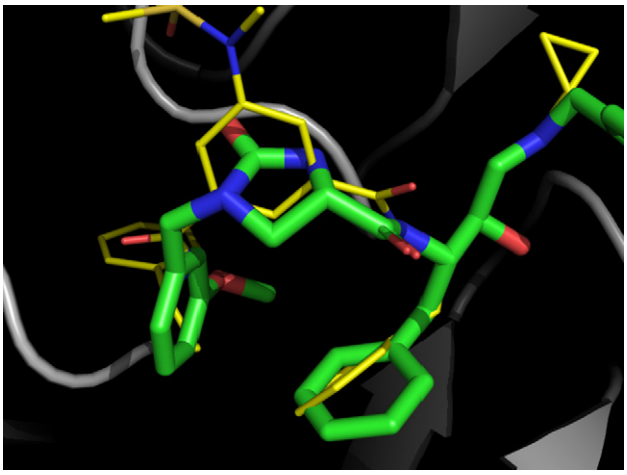
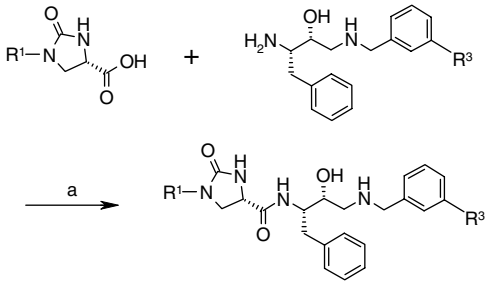


Figure 3. X-ray structure of compound **12** (green) in complex with BACE-1. Overlay with the S3-sub binding inhibitor (yellow) from 2B8L (9).

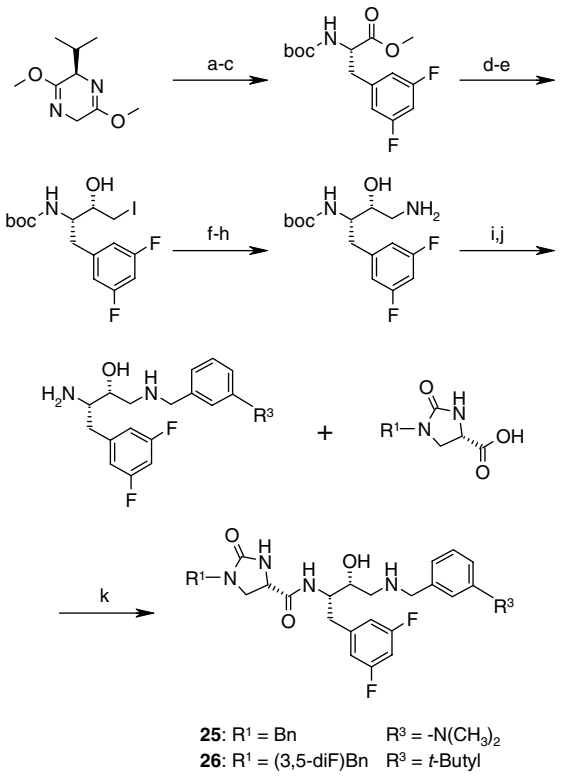


Scheme 3. Reagents and conditions: (a) EDC, HOBt, Et_3N, DMF, rt, 4 h.

fluoromethyl substitution of the R^1 benzyl group (**19**) maintained BACE-1 potency but showed a 3-fold increase in the potency for Cat-D while *para*-substitution (**20**) resulted in a 17-fold decrease of BACE-1 potency. These results suggest that the hydrophobic *ortho*-substitutent is more favorable for the potency for Cat-D and the *para*-substitutent is unfavorable for the potency for BACE-1 due to limited room for its binding.

Motivated by reports that fluorinated benzyl groups enhanced enzymatic and cellular activity,[12] a series of fluorinated benzyl compounds were investigated (**14**–**18**). Among them, the 3,5-difluorobenzyl group (**17**) is the most favorable substituent to give excellent enzyme potency, cellular activity, and selectivity over Cat-D.

We also optimized the prime site by varying R^3 substitutent at phenyl ring (Scheme 3). While substitution of the trifluoromethyl (**21**) and the trifluoromethoxy groups (**22**) at *meta* position gave low potency, simple replacement with the isopropyl (**23**) and the *tert*-butyl (**24**) groups increased a 3- and 6-fold of potency respectively and cellular activity. It is possible that those bulky hydrophobic groups could enhance the interaction between the flap and ligand. Finally, we optimized P1 site simply by introducing F at 3 and 5 positions of the phenyl ring, which was synthesized by Scheme 4. The 3,5-difluorobenzyl group at P1 site (**25** and **26**) resulted in excellent enzyme potency and cellular activity, which is presumably due to the enhanced hydrophobic interaction.



Scheme 4. Reagents and conditions: (a) LiHMDS, THF, 0 °C–rt, 4 h (92 %) ; (b) 2 N HCl, MeOH, reflux, 6 h, (92%); (c) $(BOC)_2O$, Et_3N, CH_2Cl_2, rt, 6 h (92%); (d) Chloroiodomethane, LDA, THF, 0 °C–rt, 4 h (62 %); (e) $NaBH_4$, THF, 0 °C–rt, 4 h (80 %); (f) KOH, EtOH, rt, 1 h, (88%); (g) NaN_3, NH_4Cl, H_2O, *i*-PrOH, 60 °C, 8 h (95%); (h) Pd/C, H_2 (Parr reactor, 45 psi), MeOH, rt, 4 h, (92 %); (i) Benzaldehyde, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt, 4 h (90–95%); (j) TFA, CH_2Cl_2, rt, 2 h (95%); (k) EDC, HOBt, Et_3N, DMF, rt, 4 h.

In summary, we have described the optimization and SAR of cyclic urea derivatives starting from compound **3**. Although a co-crystal structure of compound **12** bound to BACE-1 shows limits for access to the S3-sub to improve potency and selectivity, modification of P3, P1, and P2′ with hydroxyethylamine isosteres increased potency by more than 100-fold. This compound exhibited 33-fold selectivity against Cat-D and good cellular activity.

Acknowledgments

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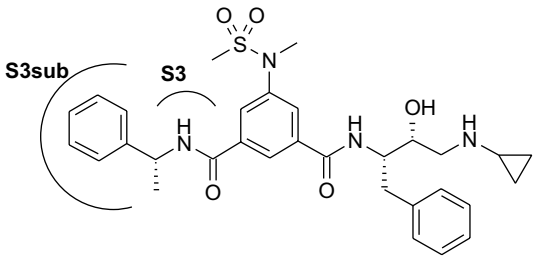
11. Compound structure in PDB ID 2B8L; refer to Ref. 9.



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12. (a) Hom, R. K.; Fang, L. Y.; Mamo, S.; Tung, J. S.; Guinn, A. C.; Walker, D. E.; Davis, D. L.; Gailunas, A. F.; Thorsett, E. D.; Sinha, S.; Knops, J. E.; Jewett, N. E.; Anderson, J. P.; John, V. *J. Med. Chem.* **2003**, *46*, 1799; (b) Pyring, D.; Lindberg, J.; Rosenquist, A .; Zuccarello, G.; Kvarnström, I.; Zhang, H.; Vrang, L.; Unge, T.; Classon, B.; Hallberg, A.; Samuelsson, B. *J. Med. Chem.* **2001**, *44*, 3083.
13. Coordinated for the BACE-1 complexes with compounds **9** and **12** have been deposited in the Protein Data Bank (www.rcsb.org) under Accession Codes 3CKP and 3CKR.