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Synthesis and Structural Optimization of Macrocyclic BACE1 Inhibitors with a Hydrophobic Cross-Linked Structure

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Introduction

Alzheimer's disease (AD) is a brain disorder, and its pathological features are senile dementia, neurofibrillary tangles, and neuronal cell death. β-Site amyloid precursor protein cleaving enzyme 1 (β-secretase, hereafter referred to as BACE1) is a promising drug target for treating AD because it catalyzes the first step in the production of amyloid β peptide (Aβ), whose aggregation is one of the causal factors of senile dementia. To date, various BACE1 inhibitors have been reported; however, no therapeutic agents for AD have been developed.

Previously, we reported a superior BACE1 cleavage sequence (H-Ile-Ser-Glu-Ile-Thi-Thi*Nva-Ala-Glu-Phe-Arg-His-NH_2, where * denotes the cleavage site, Thi:2-thienylalanine, Nva: norvaline) that was cleaved ten times faster than wild type and Swedish mutant substrates [1]. We also reported novel BACE1 inhibitors designed by combining this sequence with hydroxyethylamine (HEA)-type transition-state mimetics [2]. X-ray crystallography of the complex formed between BACE1 inhibitor **1** and recombinant BACE1 (rBACE1) revealed a hydrophobic space between the P1 and P3 side chains (PDB ID:4TRW). In this study, based on compound **2**, which showed higher activity than inhibitor **1**, we synthesized and evaluated macrocyclic derivatives bearing a cross-linked structure between P1-P3 side chains of various lengths (Figure 1). Moreover, we introduced methyl substituents at the P3 β- position on the cross-linked structure and a 4-carboxymethylphenyl group into the P1' site.

Results and Discussion

We designed 12- to 15-membered ring macrocyclic inhibitors **9**-**16** bearing an alkane or alkane-type cross-linked structure (Scheme 1). HEA fragments **3** and **4** containing the terminal alkene were

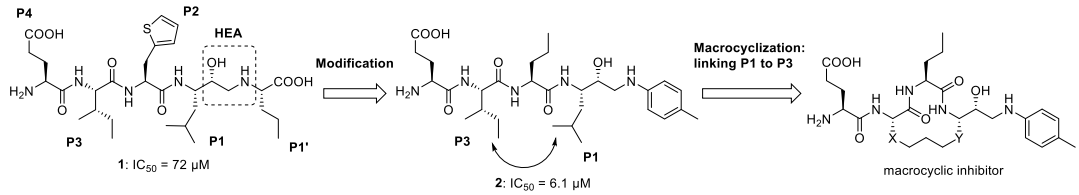
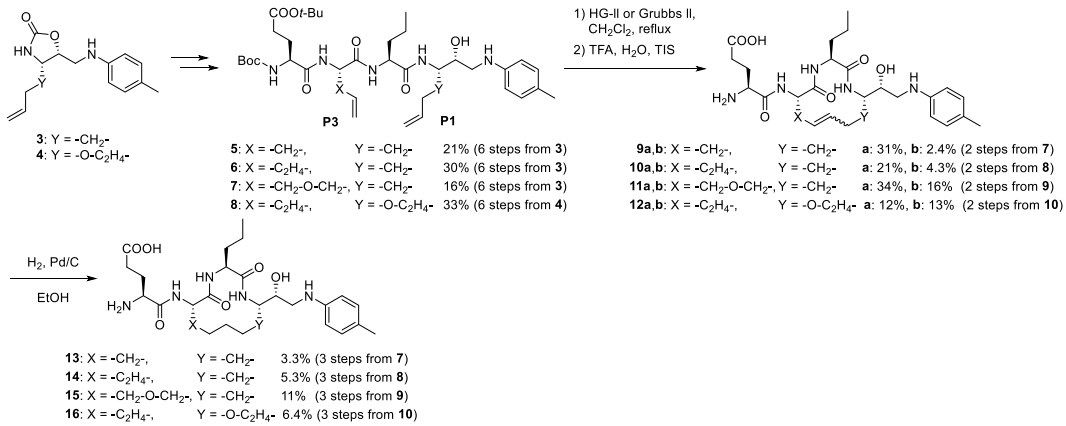


*Fig. 1. Structures of linear inhibitors **1** and **2**, and macrocyclic inhibitors.*



*Scheme 1. Synthesis of 12- to 15-membered macrocyclic inhibitors **9**-**16**.*

Table 1. Inhibitory activities of macrocyclic derivatives.

Compd.	Ring size	IC_{50} (μM)	Compd.	Ring size	IC_{50} (μM)
2	(control)	6.1			
13	12	1400	**15**	14	>1500[b]
9a[a]		>1500[b]	**11a**[a]		>1500[b]
9b[a]		1050	**11b**[a]		>1500[b]
14	13	780	**16**	15	N.D.[c]
10a[a]		380	**12a**[a]		N.D.[c]
10b[a]		1410	**12b**[a]		N.D.[c]

[a]*The alkene geometry could not be determined;* [b]*20~40% inhibition at 1500 μM;* [c]*N.D. = not detected*

prepared from commercially available Boc-Glu(OBzl)-OH or Boc-Asp-OBzl. Sequential condensation of Fmoc-Nva-OH, a P3 fragment and Boc-Glu(O*t*-Bu)-OH and Fmoc deprotection gave cyclization precursors **5-8**. Cyclization of the terminal alkenes were conducted by ring-closing metathesis (RCM) reaction. After deprotection, alkene-type macrocyclic derivatives **9-12** were obtained. Depending on the order of elution using RP-HPLC, the compounds labelled "a" or "b", which indicated (*E*)- or (*Z*)-alkene, but the alkene geometry could not be determined. They were converted to alkane-type macrocyclic derivatives **13-16** by catalytic reduction.

The inhibitory activities of macrocyclic derivatives against rBACE1 are summarized in Table 1. The activities of 12-, 14-, and 15-membered ring derivatives were markedly reduced compared to those of parent compound **2**. However, the 13-membered ring derivative, especially alkene isomer **10b**, showed weak but clear activity. These results suggest that the introduction of a simple cross-linked structure between the P1 and P3 side chains resulted in decreased activity, but that the 13-membered ring was the most suitable ring size for activity.

Docking models of **19a** and **19b** were constructed using Molecular Operating Environment (MOE) software (Figure 2). Linear inhibitor **1** complexed with rBACE1 (PDB ID:4TRW) was

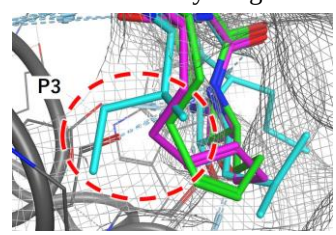
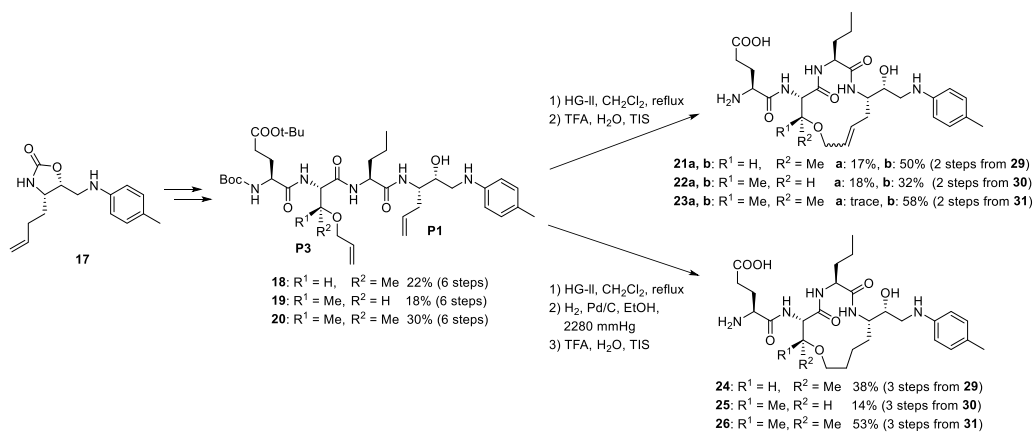


*Fig. 2. Docking models for **10a** and **10b** with rBACE1. The (Z)- and (E)-alkene derivatives and the template compound **1** are shown in green, purple and cyan, respectively. The gray mesh represents the vdW surface of rBACE1.*



*Scheme 2. Synthesis of β-branched macrocyclic inhibitors **21-26**.*

Table 2. Inhibitory activities of β-branched macrocyclic derivatives.

Compd.	R^1, R^2	IC_{50} (μM)	Compd.	R^1, R^2	IC_{50} (μM)
2	(control)	6.1	**22a**[a]	R^1 = Me, R^2 = H	1000
10a[a]	(macrocycle)	380	**22b**[a]	[(*S*)-methyl]	11.7
21a[a]	R^1 = H, R^2 = Me	1420	**25**		135
21b[a]	[(*R*)-methyl]	117	**23b**	R^1, R^2 = Me	3.9
24		1000	**26**	[dimethyl]	6.1

[a]*The alkene geometry could not be determined*

used as a template for docking. Focusing on the P3 position, the isoleucine side chain of compound **1** occupied the hydrophobic space in the S3 pocket, while the side chain of the macrocyclic derivatives occupied it insufficiently. Based on these results, we assume that the loss of hydrophobic interactions in the S3 pocket resulted in decreased activity.

To increase inhibitory activity, new inhibitors **21-26** bearing a branched structure at the P3 β-position were designed and synthesized (Scheme 2). The ring size of these compounds was fixed at 13-membered, and they contained one or two methyl groups at the P3 β-position. The alkene geometries of **21** and **22** could not be determined, whereas those of **23b** could be assigned. Another alkene isomer, **23a**, was obtained in small amounts but was not evaluated.

The inhibitory activities of β-branched macrocyclic derivatives are listed in Table 2. The introduction of the (*R*)-methyl group causes a change in activity. One of the alkene isomers, **21a**, and reductant **24** showed lower activity than the non-branched macrocyclic derivative **10a**; however, another alkene, **21b**, showed about three-fold higher activity than **10a**. This trend was also observed in the (*S*)-methyl derivatives, and the improvement rate was higher than that of the (*R*)-methyl derivatives. The activity of compound **22b** was 10-fold higher than that of compound **21b**. For the dimethyl derivatives **23b** and **26**, further improvements in activity were observed. In particular, (*Z*)-alkene **23b** showed approximately 100-fold higher activity than **10a** and approximately 1.5-fold higher activity than the parent compound **2**. These results reveal that the introduction of methyl groups on the P3 β-position improved inhibitory activity, as expected, and that the (*S*)-methyl group contributed more to activity than the (*R*)-methyl group.

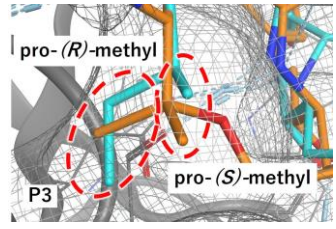


*Fig. 3. Docking model for **23b** with rBACE1. The dimethyl (Z)-alkene derivatives **23b** and the template compound **1** are shown in orange and cyan, respectively. The gray mesh represents the vdW surface of rBACE1.*

Docking simulation of (*Z*)-alkene **23b** with rBACE1 was performed using MOE software (Figure 3). Focusing on the P3 β- position, the direction of the dimethyl group of **23b** was approximately the same as that of the isoleucine side chain of compound **1**. These two methyl groups are located near the vdW surface of rBACE1, and the pro-(*S*)-methyl group is closer to the vdW surface than the pro-(*R*)-methyl group. These results suggest

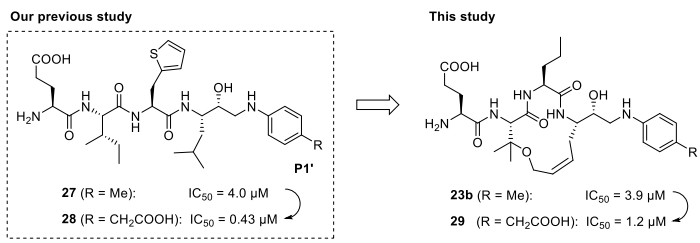


*Fig. 4. Structures and inhibitory activities of P1'-4-carboxymethylphenyl type derivatives **28** and **29**.*

that a stronger hydrophobic interaction with rBACE1 at the S3 pocket was formed by the dimethyl group of **23b** compared to the non-branched derivative **10a**, and that the additional hydrophobic interaction contributed to the enhanced activity of **23b**, which was consistent with the result that (*S*)-methyl derivatives showed higher activity than the corresponding (*R*)-methyl derivatives.

In our previous study, we reported that the introduction of a 4-carboxymethylphenyl group instead of a *p*-tolyl group increased inhibitory activity 10-fold [3]. Based on these results, the dimethyl branched macrocyclic derivative **29** containing a 4-carboxymethylphenyl group at the P1' site was synthesized (Figure 4). The activity of compound **29** was increased approximately 3.2 times from that of compound **23b**. The improvement in the activity of macrocyclic derivatives was lower than that of linear inhibitors in a previous study, but the introduction of a 4-carboxymethylphenyl group at the P1' position led to further improvement in activity.

Acknowledgments

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References

1. Kakizawa, T., et al. *Bioorg. Med. Chem.* **19**, 2785-2789 (2011), https://doi.org/10.1016/j.bmc.2011.03.056
2. Hattori, Y., et al. *Bioorg. Med. Chem.* **23**, 5626-5640 (2015), https://doi.org/10.1016/j.bmc.2015.07.023
3. Kobayashi, K., et al. *Bioorg. Med. Chem.* **50**, 116459 (2021), https://doi.org/10.1016/j.bmc.2021.116459