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Novel γ-secretase inhibitors discovered by library screening of in-house synthetic natural product intermediates

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Abstract—Screening of our in-house compound library comprised of intermediates of natural product synthesis projects resulted in discovering two novel γ-secretase inhibitors, which coincidently had similar moieties, that is, cyclohexenone and two aryl groups arranged on the core six-membered ring. Structure–activity relationship studies of these compounds were also developed.

Alzheimer's disease (AD), a dementing neurodegenerative disorder, is currently a serious public health problem in aging society and is predicted to affect 20 million people worldwide within 20 years.[1] AD is characterized pathologically by neuronal loss in the cerebral cortex accompanied by massive deposition of amyloid-β peptides (Aβ) as senile plaques. A body of evidence suggests that amyloid deposits are strongly implicated in the pathogenesis of AD. Therefore, regulation of the Aβ levels is considered as a mechanism-based therapeutics for AD. Aβ is produced from amyloid precursor protein (APP) through sequential proteolytic processing by two membrane associated aspartic proteases termed β- and γ-secretases. Thus, these secretases are predicted to be prime molecular targets toward prevention and cure of AD.

A number of γ-secretase inhibitors have been reported so far.[2] In fact, some potent inhibitors (e.g., *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine *tert*-butyl ester (DAPT),[3] N^2-[(2*S*)-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-N^1-[(7*S*)-5-methyl-6-oxo-6,7-dihydro-5*H*-dibenzo[*b*,*d*]azepin-7-yl]-L-alaninamide (LY411575),[4] and 2-[(1*R*)-1-[[(4-chlorophenyl)sulfonyl](2,5-difluorophenyl)amino]ethyl]-5-fluorobenzenepropanoic acid (BMS-299897))[5] are reported to reduce Aβ levels in mouse brains and biological fluids by oral administration. However, several γ-inhibitors have been shown to affect Notch processing pathway, thereby causing unwanted side effects.[6–8] Thus, discovery and development of new inhibitors without side effects have been continuously challenging and demanding tasks for medicinal chemists. Herein we provide our new findings that cyclohexenone derivatives are novel potent γ-secretase inhibitors.

We began our search for γ-secretase inhibitors by screening our in-house compound library. The library consisted of intermediates of several natural product synthesis projects in our group (e.g., ecteinascidin 743,[9] vinblastine,[10] antascomicin,[11] etc.), and thus, high structural diversity of the members of the library is attained. The ability of compounds to inhibit Aβ formation was evaluated by an in vitro assay using recombinant C-terminal fragment of APP as a substrate.[12] After an intensive screening, we discovered two compounds with potent γ-secretase inhibitory activity among about 600 selected compounds (Fig. 1). Though these compounds were intermediates in the different targets of natural products, coincidentally they have similar structures, six-membered cyclic enone, on

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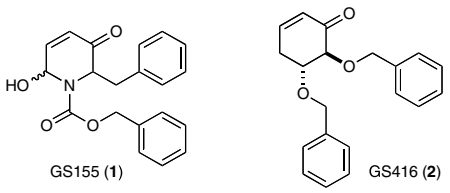
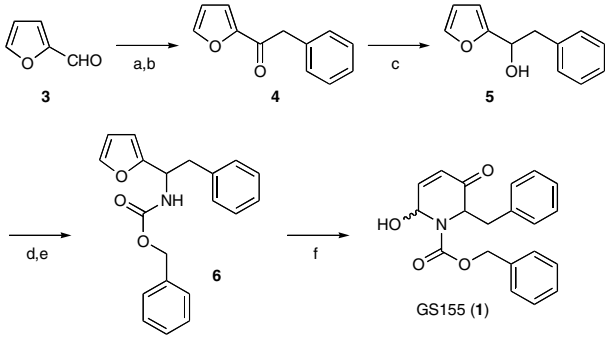


Figure 1. Compounds with potent inhibitory activity toward γ-secretase.

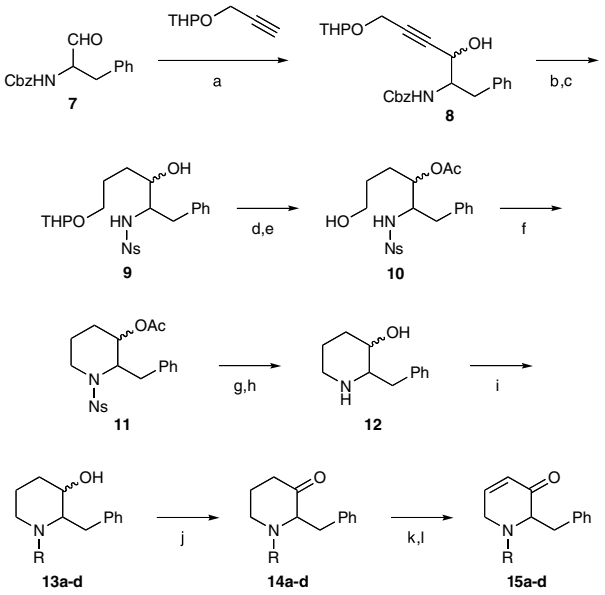
which two aromatic rings are arranged. We paid attention to the common moieties of these compounds, and synthesized various derivatives to explore the structure–activity relationships.

GS155 (**1**) had been synthesized from furfral **3** by means of aza-Achmatowicz reaction (Scheme 1). Furfral was transformed into the corresponding cyanohydrin. Alkylation followed by cleavage of the silyl ether afforded ketone **4**, which was reduced with sodium borohydride to furnish alcohol **5**. Nitrogen was introduced using *N*-benzyloxycarbonyl-2-nitorobenzenesulfonamide (NsNHCbz)[13] by means of Mitsunobu reaction,[14] and the Ns group was removed to produce **6**. Upon treatment with *m*-chloroperbenzoic acid (*m*-CPBA), **6** underwent aza-Achmatowicz reaction to form GS155 (**1**).[15]

For the derivatization of GS155, we planned to remove the hemiaminal moiety of GS155 because the hemiaminal moiety seemed labile. The derivatives were synthesized as shown in Scheme 2. Addition of alkyne to DL-phenylalaninal (**7**)[16] furnished a diastereomeric mixture of alcohol **8**. Hydrogenolysis of **8** induced saturation of the triple bond and cleavage of the benzyloxycarbonyl group (Cbz) to afford aminoalcohol. After introduction of 2-nitrobenzenesulfonyl group (Ns) onto the amino group selectively, sequential protective group manipulation furnished **10**, which was cyclized by means of Mitsunobu reaction to afford **11**. After removal of the Ns group under standard conditions, acylation of the amine with various acyl chlorides afforded **13a**–**d**. The remaining secondary alcohol was oxidized to afford ketone **14a**–**d**, which was transformed into enone **15a**–**d** according to the Ito-Saegusa's method.[17]



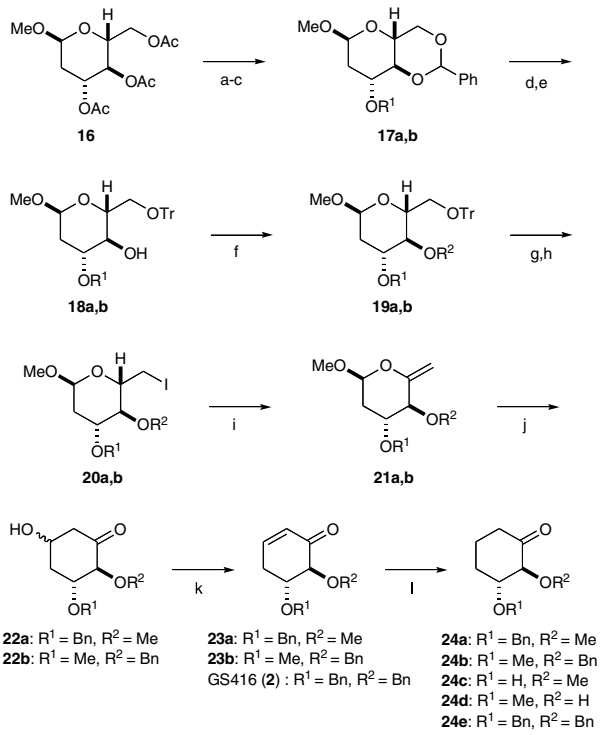
Scheme 1. Reagents and conditions: (a) TMSCN, CH_3CN, sealed tube, 120 °C, 95%; (b) LDA, BnBr, THF, −78 °C; TBAF, rt, 54%; (c) $NaBH_4$, MeOH, 0 °C to rt, 67%; (d) NsNHCbz, DEAD, Ph_3P, toluene–THF, 0 °C to rt, 68%; (e) PhSH, K_2CO_3, DMF, 53%; (f) *m*-CPBA, CH_2Cl_2, 69%.



Scheme 2. Reagents and conditions: (a) $THPOCH_2CCH$, *n*-BuLi, THF, −78 °C, 99%; (b) Pd–C, H_2, MeOH; (c) NsCl, aq Na_2CO_3, CH_2Cl_2, 0 °C, 67% (two steps); (d) Ac_2O, pyridine; (e) CSA, MeOH, 0 °C; (f) DEAD, Ph_3P, toluene, 96% (three steps); (g) K_2CO_3, MeOH, 0 °C, 96%; (h) PhSH, Cs_2CO_3, CH_3CN; (i) RCl, aq K_2CO_3, CH_2Cl_2, 84–91% (two steps); (j) DMP, CH_2Cl_2, 0 °C, 71–80%; (k) TMSCl, LHMDS, THF, −78 °C; (l) $Pd(OAc)_2$, CH_3CN.

On the other hand, GS416 (**2**) had been prepared from tri-*O*-acetyl-D-glucal via catalytic Ferrier rearrangement,[18] and the synthesis has been already reported.[11] According to the similar procedure, derivatives of **2** were synthesized as shown in Scheme 3. Deprotection of the acetyl groups of methyl glucoside **16** derived from tri-*O*-acetyl-D-glucal followed by selective acetalization of the resultant 1,3-diol moiety gave benzylidene acetal **17a**,**b**. Alkylation of the remaining 3-hydroxyl group (introduction of R^1) was followed by cleavage of the benzylidene acetal, and subsequent selective protection of the primary alcohol with trityl group afforded **18a**,**b**. The second alkyl group (R^2) was introduced onto the remaining secondary hydroxyl group. After removal of the trityl group, the resulting alcohol was converted into enol ether according to the known procedure. That is, the primary alcohol was converted into iodide **20a**,**b**, which was treated with sodium hydride to afford **21a**,**b**. The enol ether **21a**,**b** was then subjected to the conditions of catalytic Ferrier rearrangement to furnish hydroxyketone **22a**,**b**. Mesylation of the alcohol induced β-elimination to produce enone **23a**,**b**. The results of hydrogenation depended on the source of Pd–C. Using 10% Pd–C purchased from Wako Pure Chemical Industries saturated the double bond of **23a**,**b** selectively (**24a**,**b**). Similarly GS416 (**2**) was reduced to give **24e**. In contrast, 10% Pd–C purchased from Aldrich Chemical Company afforded debenzylated products (**24c**,**d**).

The results of γ-secretase inhibition assay of these derivatives are shown in Tables 1 and 2. Removal of the



Scheme 3. Reagent and conditions: (a) NaOMe, MeOH; (b) $PhCH(OMe)_2$, CSA, DMF, 73% (two steps); (c) NaH, R^1X, DMF, 0–50 °C; (d) CSA, MeOH, CH_2Cl_2, 96–97% (two steps); (e) TrCl, pyridine, CH_2Cl_2, 62–88%; (f) R^2X, NaH, DMF; (g) CSA, MeOH, 79–91% (two steps); (h) I_2, Ph_3P, imidazole, THF, 85–90%; (i) NaH, DMF, 86–91%; (j) $Hg(OCOCF_3)_2$, acetone–H_2O, 96–98%; (k) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 79–83%; (l) H_2, Pd–C, EtOAc, 95–100%.

Table 1. Inhibitory activities of GS155 (**1**) and its derivatives

Compound	R–	IC_{50} (Aβ40, μM)	IC_{50} (Aβ42, μM)
13a	BnOCO–	>100	>100
13b	MeOCO–	>100	>100
14a	BnOCO–	21% inhibition at 100 μM	>100
14b	MeOCO–	>100	>100
15a	BnOCO–	4.6	7.4
15b	MeOCO–	7.2	9.8
15c	PhCO–	1.0	2.2
15d	$PhSO_2$–	5.9	12.3
GS155 (**1**)	—	0.5	0.7

Table 2. Inhibitory activities of GS416 (**2**) and its derivatives

Compound	R^1	R^2	IC_{50} (Aβ40, μM)	IC_{50} (Aβ42, μM)
22a	Bn	Me	>100	>100
22b	Me	Bn	>100	>100
23a	Bn	Me	33.9	26.0
23b	Me	Bn	6.9	10.2
24a	Bn	Me	>100	>100
24b	Me	Bn	4.9	13.9
24c	H	Me	>100	>100
24d	Me	H	>100	>100
24e	Bn	Bn	>100	>100
GS416 (**2**)	—	—	1.6	1.1

hemiaminal moiety of **1** slightly reduced the activity (**15a**). The hydroxyl group of the hemiaminal moiety of GS155 might fix the conformation of the six-membered ring by intramolecular hydrogen bonding to increase its activity. Saturation of the double bond caused loss of the inhibitory activity (**14a**,**b**). The aryl group connected to the nitrogen atom was important for the inhibition. The methoxycarbonyl derivative **15b** had weaker activity, and benzoyl derivative **15c** was slightly stronger. In the case of GS416 (**2**) saturation of the double bond also caused a complete loss of activity, while **24b** retained activity. Though the benzyl group closer to the ketone was more important for the activity, the presence of both benzyl groups was needed for maintaining the high activity. These results were parallel to the SAR of **1**.

These results suggested that the enone moiety in each derivative is the most important structure for the γ-secretase inhibition, while some compounds such as **24b** retained activity. The enone moiety could be necessary to fix the molecule to the active conformation in the GS155 derivatives, while not necessary in the GS416 derivatives. It is known that enone moiety tends to react with enzyme nonspecifically by means of Michael addition. However, inhibitory activities of **1** and **2** against γ-secretase were retained in the presence of 2-mercaptoethanol, under which conditions Michael addition of **1** or **2** with proteins is thought to be suppressed (data not shown). Moreover, these compounds had no inhibitory effect on β-galactosidase activity (data not shown). Thus, we could rule out the possibility of nonspecific inhibition and/or crosslink of γ-secretase by these compounds.

In summary, we discovered novel cyclohexenone γ-secretase inhibitors from our in-house library comprised of intermediates of natural product synthesis projects. The SAR study of these compound suggested the enone moiety and the two aryl groups were important for the potent γ-secretase inhibitory activity. Synthesis of molecular probes for functional analysis of γ-secretase[19] as well as structural development studies using **1** and **2** are currently underway and will be reported in due course.

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