

New peptide mimetics with potential β -secretase inhibition activity

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Alzheimer's disease (AD) is affecting about 20 millions people worldwide. The last forecast shows that during the next 20–30 years their number will double because of the human life-span increasing. The transformation of amyloid β ($A\beta$) soluble from the insoluble β -fibers is a critical stage in the AD progression. $A\beta$ resulted from the precursor protein cleavage which is catalyzed by different proteinases named α , β and γ -secretases. That is why the inhibition of some of these enzymes is a promising alternative for AD treatment. The shortest peptide structure found with β -secretase inhibition activity is Glu-Val-Asp-Leu-Ala. A lot of investigations show the role of various amino acids in different positions for the inhibition activity and reveal important structure-activity relationships. Based on these investigations, we synthesized ten peptide mimetics with potential β -secretase inhibition activity by means of conventional peptide synthesis in solution. All newly synthesized compounds were characterized by TLC, m.p. and $[\alpha]_{546}^{22}$. The biological trials are in progress.

Key words: Alzheimer's disease; β -secretase inhibitors, peptide mimetics.

INTRODUCTION

Alzheimer's disease (AD) is affecting about 20 millions people worldwide. The last forecast shows that during the next 20–30 years their number will double because of the human life-span-increasing. The transformation of amyloid β ($A\beta$) soluble from the insoluble β -fibers is a critical stage in the AD progression. $A\beta$ resulted from the precursor protein containing 695 to 770 amino acid residues. This process starts with four cleavages of this protein catalyzed by different proteinases, named α , β and γ -secretases. The tearing in $A\beta_1$ and $A\beta_{11}$ is catalyzed by the enzyme β -secretase. There is no data in literature whether this enzyme is a part of the other life process in the organism.

Last ten years provide a number of different investigations on the β -secretase inhibition process. Tung *et al.* revealed that the shortest peptide structure with β -secretase inhibition activity is Glu-Val-Asp-Leu-Ala [1]. Their experiments show that Leu or Phe at P_1 position, Val or Leu at P_3 position and Asp or Asn at P_2 position is compulsory for inhibition activity availability. Additional investigations reveal that the minimal substrate with passable β -secretase inhibition activity is Boc-Val-Asp-Leu-Ala-OH. Based on that structure, we investigate the influence of different amino acid residues at P_4 , P_3 and P_2 positions on the inhibition activity. At some

structures the residues at P_4 and P_3 position were removed. It is well-known that the enzyme β -secretase includes high hydrophobic S_1 and S_3 -pockets [2]. That is why the availability of hydrophobic amino acids at these positions is obligatory for inhibition activity. In our new structure, we choose Leu residue for these two positions. S_2 and S_4 -pockets are hydrophilic [3]. Tung *et al.* investigated the residues Asn and Met for P_2 position. Their X-ray analysis revealed that P_2 Asn residue in the substrate made a hydrogen bond with Arg²³⁵ in the enzyme binding site [1]. In our structure, Asp in this position was used because we suppose that its hydrogen bond formation potential is better than Asn. For P_4 position Tung *et al.* choose Glu or Boc residue and they proved that $-C=O$ function participates in the strong hydrogen bond obtaining. There is no data whether the bonds made by Boc group are stronger than those obtained by the δ -COOH function of Glu [1]. We decided to investigate the length of the chain of the amino acid in this position and choose Asp.

The additional investigations on the high-potent β -secretase inhibitors conformation show that at P_2' position the chain of the compounds is turned off and the side chains of the residues at P_3' and P_4' positions do not participate in the enzyme-substrate interaction [4]. Based on the latter conclusion, Ghosh *et al.* removed and replaced the residues at P_3' and P_4' positions by different C-terminal residues [2]. They investigated the activity of derivatives based on the sequence Boc-Val-Asp-Leu-

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Ala-Val-Glu(P₃')-Phe(P₄')-OH. They succeeded to increase β -secretase inhibition activity replacing C-terminal amino acids at P₃' and P₄' positions by benzylamine. The latter results to fourfold inhibition activity increasing (IC₅₀ = 28 nM, K_i = 2.5 nM). The other potential residue they determined was piridinylmethylamine (IC₅₀ = 70 nM) [1]. Because of the better activity of the compounds with benzylamine C-terminus in our new structure the same fragment as C-terminal moiety was used. On the other hand, it is well-known that high hydrophobic moieties play a key role in the molecule for blood brain barrier passing. Based on this fact, a series of analogues with C-terminal 3,4-dimethoxybenzylamine residue was synthesized.

The X-ray investigations on the crystal structure of the enzyme-substrate complex revealed that COOH group of the residue at P₂' position made a strong hydrogen bond with OH group of Tyr¹⁹⁸ which plays key role in the enzyme-substrate interaction. That is why even in the shortest peptide structure with β -secretase inhibition activity Glu-Val-Asp-Leu-Ala(P₁') the availability of P₂' residue is obligatory. The question is: which is the best choice for amino acid residue in this position? In 2005 Hanessian *et al.* synthesized three peptides differing only by their residue at P₂' position – NH-Butyl (IC₅₀ = 1.82 μ M), Ala (IC₅₀ = 0.6 μ M) and Val (IC₅₀ = 0.19 μ M) [5]. The obtained results show that the most powerful is the analogue with Val at P₂' position. That is why our design with Val in this position was done.

EXPERIMENTAL

All newly synthesized compounds were characterized by TLC, m.p. and $[\alpha]_{546}^{22}$. Their structures were proved by ES/MS. The purity of the products was checked by TLC on precoated plates of Silica gel 60 F₂₅₄ (MERCK) with the following solvent systems: CHCl₃:AcOH 9:1 (v/v); *n*-BuOH:AcOH:H₂O 3:1:1 (v/v/v) and *n*-BuOH:AcOH:pyridine:H₂O 60:6:24:20. Spots on TLC chromatograms were detected by chlorine/*o*-tolidine reaction. The melting points were determined on a Kofler apparatus and were uncorrected. The optical rotation was measured on a Quick Russel-Jouan Type SL1D polarimeter.

General procedure for preparation by the N,N'-dicyclohexylcarbodiimid (DCC)/1-hydroxybenzotriazol (HOBt) method

1.00 mmol of the peptide (obtained from Boc-peptide ester by treatment with 10-fold excess of TFA) was dissolved in minimal amount of DMF and after cooling to –5°C neutralized to pH 7–7.5 with

Et₃N. 1.20 mmoles of Z- or Boc-amino acid, 1.20 mmoles of DCC and 1.40 mmoles of 1-HOBt were added. The reaction mixture was stirred for 24 h at 0°C and for another 24 h at room temperature. The obtained DC-urea was removed by filtration and then 30 ml of water were added. The product was extracted into EtOAc (3×10 ml) and the organic layer was washed with 5% NaHCO₃ (3×10ml), H₂O (3×10ml), 10% citric acid (3×10ml) and H₂O to pH = 7. The solvent was dried with Na₂SO₄ and removed in *vacuo* followed by recrystallization.

General procedure for preparation by the TBTU or TCTU method

1.00 mmol of the peptide (obtained from Boc-peptide ester by treatment with 10-fold excess of TFA) was dissolved in minimal amount of DMF and after cooling to –5°C neutralized to pH 7–7.5 with diisopropylethylamine (DIEA). 1.20 mmoles of Z- or Boc-amino acid, 1.20 mmoles of 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or O-(6-chloro-1-hydroxybenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TCTU) and 1.20 mmoles of DIEA were added. The reaction mixture was stirred for 24 h at room temperature. Finally, 30 ml of water were added. The product was extracted into EtOAc (3×10 ml) and the organic layer was washed with 5% NaHCO₃ (3×10ml), H₂O (3×10ml), 10% citric acid (3×10ml) and H₂O to pH = 7. The solvent was dried with Na₂SO₄ and removed in *vacuo* followed by recrystallization.

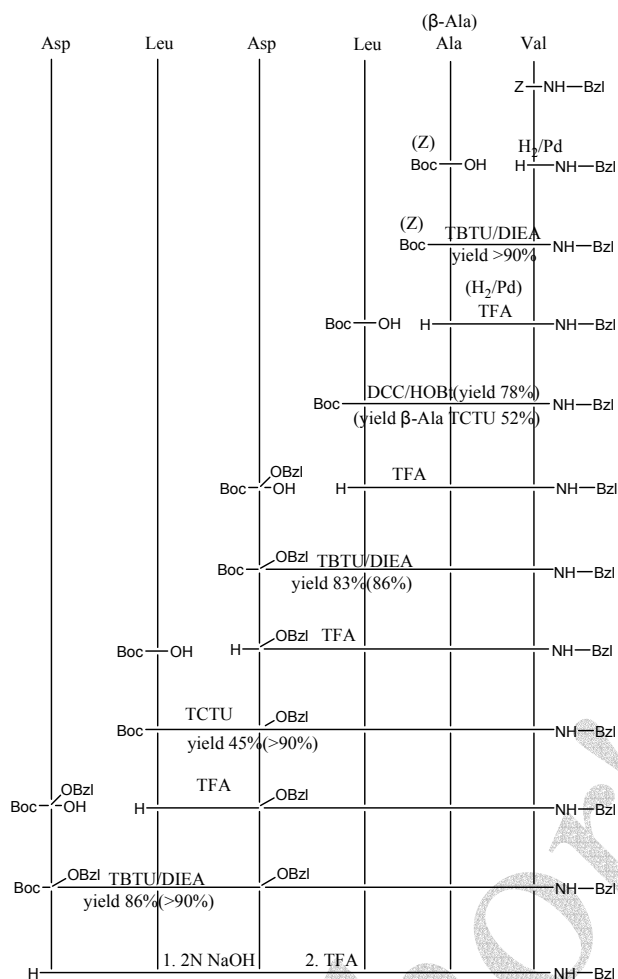
Deblocking of Z- and OBzl groups by catalytic hydrogenation in the presence of Pd/C

1.00 mmol of protected peptide was dissolved in MeOH and then Pd/C and 1.00 mmol (or catalytic amount) of HCl was added. Hydrogen was passed through the reaction mixture at room temperature. The deblocking of the protecting groups was controlled by TLC and after finishing the reaction, Pd/C was filtered out and MeOH was evaporated in *vacuo*. The formed oil was subjected to the next deblocking.

RESULTS AND DISCUSSION

Based on the information above, a design of peptide mimetics including minimal substrate subunits with C-terminal benzylamine and 3,4-dimethoxybenzylamine functions was done. Ten peptide mimetics with potential β -secretase inhibition activity were synthesized by peptide synthesis in solution according to Scheme 1–5: H-Asp-Leu-Ala-Val-NH-Bzl; H-Asp-Leu- β -Ala-Val-NH-Bzl; Boc-Leu-Asp-Leu-Ala-Val-NH-Bzl; Boc-Leu-Asp-Leu-

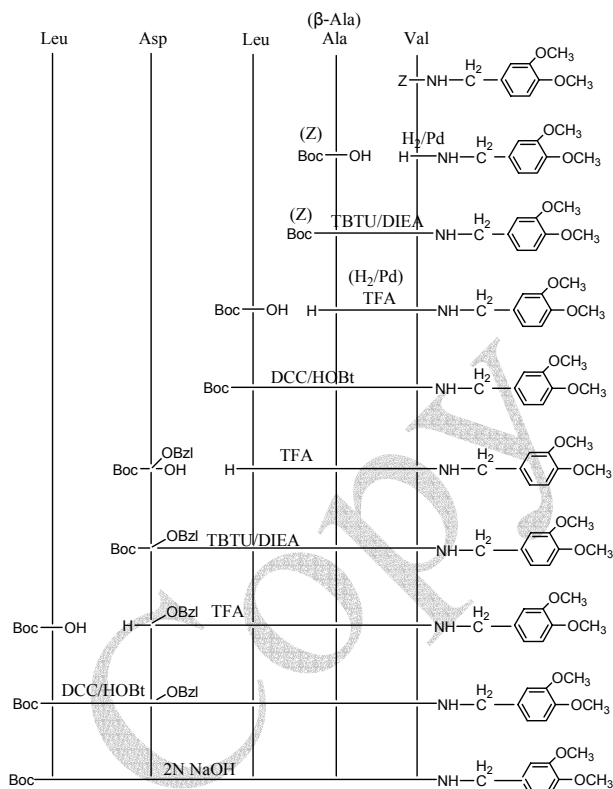
The second series of compounds include 3,4-dimethoxyphenylamine as C-terminal residue and were synthesized according to Schemes 4 and 5



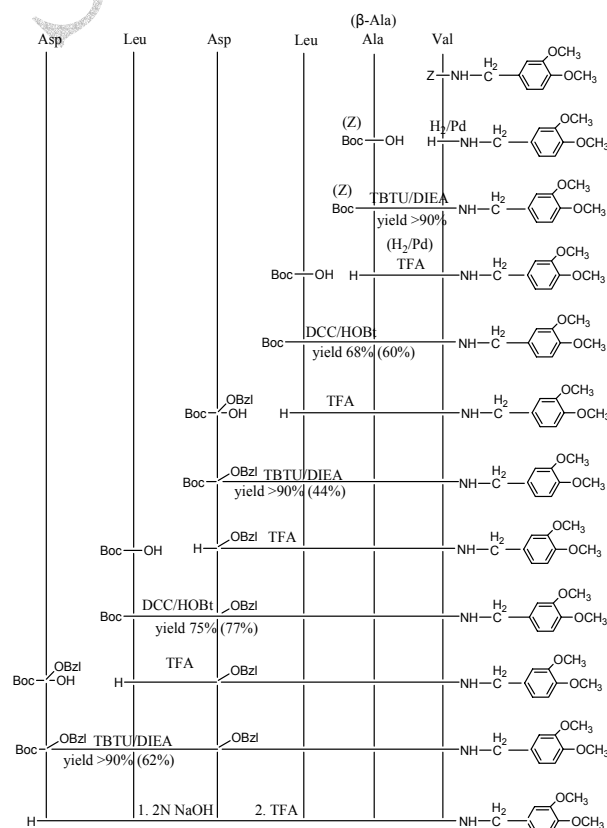
Scheme 3. Synthesis of hexapeptides H-Asp-Leu-Asp-Leu-Ala(β -Ala)-Val-NH-Bzl.

The same problems as with the first series of compounds, concerning the reactions of condensation of Leu residues were met. The reactions of condensation were carried out again at different conditions and in the presence of condensation agents with a view to get the best results with respect to yields and purity of products. Similar problems in this series of compounds were met with the reaction of condensation with participation of Boc-Asp(OBzl)-OH. The best results according to the condensation agent and yields are shown in the reaction schemes.

Boc-protecting group in all compounds was removed by treatment with 10-fold excess of TFA. Z- and -OBzl protecting groups were removed by catalytic hydrogenation in the presence of HCl (molar or catalytic amount) and Pd/C. The melting points and $[\alpha]_{546}^{22}$ are presented in Table 1:



Scheme 4. Synthesis of pentapeptides Boc-Leu-Asp-Leu-Ala(β -Ala)-Val-3,4-dimethoxybenzylamide.



Scheme 5. Synthesis of hexapeptides H-Asp-Leu-Asp-Leu-Ala(β -Ala)-Val-3,4-dimethoxybenzylamide.

Table 1. Melting points and $[\alpha]_{546}^{22}$ of the newly synthesized products.

No	Product	M.p., °C	$[\alpha]_{546}^{22}$, °
1	Z-Val-NH-Bzl	167–169	–40
2	Boc-Ala-Val-NH-Bzl	164–166	–120
3	Z- β -Ala-Val-NH-Bzl	184–186	-
4	Boc-Leu-Ala-Val-NH-Bzl	139–141	–130
5	Boc-Leu- β -Ala-Val-NH-Bzl	169–171	–40
6	Boc-Asp(OBzl)-Leu-Ala-Val-NH-Bzl	121–123	–0.2*
7	Boc-Asp(OBzl)-Leu- β -Ala-Val-NH-Bzl	124–126	+40
8	Boc-Leu-Asp(OBzl)-Leu-Ala-Val-NH-Bzl	197–199	-
9	Boc-Leu-Asp(OBzl)-Leu- β -Ala-Val-NH-Bzl	200–201	-
10	Boc-Asp(OBzl)-Leu-Asp(OBzl)-Leu-Ala-Val-NH-Bzl	193–195	-
11	Boc-Asp(OBzl)-Leu-Asp(OBzl)-Leu- β -Ala-Val-NH-Bzl	191–193	–10
12	Asp-Leu-Ala-Val-NH-Bzl	119–121	–50
13	Asp-Leu- β -Ala-Val-NH-Bzl	152–154	+30
14	Boc-Leu-Asp-Leu-Ala-Val-NH-Bzl	125–127	+30
15	Boc-Leu-Asp-Leu- β -Ala-Val-NH-Bzl	123–125	+20
16	Asp-Leu-Asp-Leu-Ala-Val-NH-Bzl	117–119	+40
17	Asp-Leu-Asp-Leu- β -Ala-Val-NH-Bzl	114–116	+60
18	Z-Val-3,4-dimethoxybenzylamide	144–146	-
19	Boc-Ala-Val-3,4-dimethoxybenzylamide	151–153	–90
20	Z- β -Ala-Val-3,4-dimethoxybenzylamide	187–189	-
21	Boc-Leu-Ala-Val-3,4-dimethoxybenzylamide	183–185	–60
22	Boc-Leu- β -Ala-Val-3,4-dimethoxybenzylamide	169–171	–20
23	Boc-Asp(OBzl)-Leu-Ala-Val-3,4-dimethoxybenzylamide	178–180	–10
24	Boc-Asp(OBzl)-Leu- β -Ala-Val-3,4-dimethoxybenzylamide	191–193	+36
25	Boc-Leu-Asp-Leu-Ala-Val-3,4-dimethoxybenzylamide	136–138	-
26	Boc-Leu-Asp-Leu- β -Ala-Val-3,4-dimethoxybenzylamide	210–212	-
27	Asp-Leu-Asp-Leu-Ala-Val-3,4-dimethoxybenzylamide	amorphous	-
28	Asp-Leu-Asp-Leu- β -Ala-Val-3,4-dimethoxybenzylamide	217–219	-

For all compounds $[\alpha]_{546}^{22}$ [°] is for C 1 MeOH except *C 0.5 MeOH.

Biological trials of all newly synthesized compounds are in progress.

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НОВИ ПЕПТИДНИ МИМЕТИЗИ С ПОТЕНЦИАЛНА β -СЕКРЕТАЗНА ИНХИБИТОРНА АКТИВНОСТ

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(Резюме)

Болестта на Алцхаймер (БА) е засегнала около 20 милиона души по целия свят. Последните проучвания показват, че през следващите 20–30 години броят им ще се удвои поради нарастващата продължителност на човешкия живот. Превръщането на разтворимия β амилоиден пептид ($A\beta$) в неговата неразтворима форма е ключов стадий в прогресията на БА. $A\beta$ се получава като резултат от разкъсване на прекурсорния β амилоиден пептид, което е катализирано от различни протеази, известни като α -, β - и γ -секретази. Ето защо инхибирането на някой от тези ензими е обещаваща алтернатива при лечението на БА. Най-късата пептидна структура с установена инхибиторна активност срещу β -секретазата е Glu-Val-Asp-Leu-Ala. В литературата са публикувани множество изследвания, показващи ролята на различните аминокиселини в този пептид за инхибиторната му активност и са изведени някои зависимости структура–активност. Основавайки се на тези литературни данни ние направихме дизайн и синтезирахме 10 пептидни миметици с потенциална β -секретазна инхибираща активност, с помощта на стандартен пептиден синтез в разтвор. Всички новосинтезирани съединения бяха охарактеризирани чрез TLC, т.т. и $[\alpha]_{546}^{22}$. Биологичните изследвания са в ход.