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Design and synthesis of potential inhibitors of multienzyme systems included in Alzheimer's disease

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Synthesis of a series of 6 peptides, potential inhibitors of β-secretase, analogues of the β-secretase inhibitor OM99-2 was designed and performed. Standard peptide synthesis in solution was applied by stepwise addition of amino acids from C- to N-terminus. All reactions gave very good yields (higher than 80%).

Key words: OM99-2 analogues, Alzheimer's disease, inhibitors of multienzyme systems

INTRODUCTION

Processes in the human body are usually related to the realization of a series of subsequent enzymatic reactions. For example, the blood coagulation cascade protects the organism from blood loss by thrombus formation [1,2]. Disorders of multienzyme cascade reactions in the most cases cause diseases.

Alzheimer's disease (AD) is a neurodegenerative illness, which affects millions of people worldwide. AD is characterized by progressive dementia, loss of memory, intellectual, speech and brain disturbances and inevitably leads to complete personality decay and lethal outcome. Designated neuropathological lesions associated with all forms of AD are senile plaques (SPs) and amyloid angiopathy, as well as neurofibril tangles. The amyloid hypothesis formulated in 1991 postulated that amyloid beta (Aβ) deposits, as the two dominant forms Aβ40 and Aβ42, are the fundamental reason of SPs formation and disease development [3,4]. The process of Aβ40 and Aβ42 formation includes multienzyme cascade reactions of proteolytic cleavage of wide sections from β-amyloid protein precursor (βAPP) (the 695-770 amino acids) catalyzed by various proteases known as β- and γ-secretases [5]. Once released, βAPP passes over to aggregation and SPs are obtained.

A possible approach for prevention of SPs formation is the inhibition of the multistage process of βAPP cleavage. Tung *et al*. revealed in 2002 that the shortest analogue of β-secretase inhibitor OM99-2 with saved activity is Boc-Val-Asn-Leu-Ala-OH [6]. Several years later, in 2005, Ghosh *et al*. published a series of OM 99-2 analogues which included benzylamine in their C-terminus with increased inhibition properties against β-secretase [7]. Additionally, they concluded that the presence of hydrophobic residues in P_3' and P_4' positions leads to increased activity. Herein we report on the design, synthesis and characterization of a series of new OM 99-2 mimetics including hydrophobic Ile, norvaline (Nva) and imidazol-4-acetic acid (Im-4-Ac) at P_3 position and Asn in P_2 position is replaced by Asp. Besides, both analogues with α- and β-Ala in P1 position were synthesized in order to estimate their further influence on the inhibition activity.

EXPERIMENTAL

TBTU, TCTU or DCC/HOBt used as a coupling reagent according to the schemes presented in Results and Discussion part, were purchased from IRIS Biotech GmbH, Germany. All used amino acids were purchased from IRIS Biotech GmbH, Germany.

All reactions were monitored by TLC on pre-coated TLC-sheets ALUGRAM SIL G/UV_{254} in the following chromatographic systems: **(S1)** $CHCl_3$:CH_3COOH (9:1); **(S2)** n-BuOH:CH_3COOH:H_2O (3:1:1); **(S3)** pyridine: n-BuOH:CH_3COOH:H_2O (1:1,5:0,3:1,2).

The structure of all newly synthesized compounds was proven by ES-MS (Table 1). Melting points of all compounds were determined on a Kofler apparatus. The optical rotation was measured on a Quick polarimeter Russel-Jouan Type SL1D.

General procedure for coupling reaction using TBTU or TCTU

1 mmol of the starting compound with free amino function was dissolved in a minimal amount

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of DMF. After cooling to 0°C ÷ -5°C, 1 mmol of Et_3N (till pH 7) was added to deprotect the salt obtained in a previous deprotection procedure. Further 1.2 fold excess of carboxylic component and 1.4 mmol of TBTU (TCTU) was added. The reaction mixture was stirred for 6 h and quenched by addition of water. The product was extracted with EtOAc (3 × 10 ml) and the organic layer was washed with 5% $NaHCO_3$ (3 × 10 ml), H_2O (2 × 10 ml), 10% citric acid (3 × 10 ml) and H_2O to pH 7. The solvent was dried with Na_2SO_4 and removed *in vacuo* followed by recrystallization.

General procedure for coupling reaction using DCC/HOBt

1.00 mmol of the peptide (obtained from Boc-peptide ester by treatment with 10-fold excess of TFA) was dissolved in DMF (10 ml) and, after cooling to 0÷4°C, was neutralized to pH 7–7.5 with Et_3N. 1.20 mmol of Z- or Boc- amino acid, 1.20 mmol of DCC and 1.40 mmol of 1-HOBt were added. The reaction mixture was stirred at 0÷4°C for 24 h and left at room temperature for 24 h. The obtained DC-urea was removed by filtration and then 30 ml of water was added. The product was extracted with EtOAc (3 × 10 ml) and the organic layer was washed with 5% $NaHCO_3$ (3 × 10 ml), H_2O (2 × 10 ml), 10% citric acid (3 × 10 ml) and H_2O to pH 7. The solvent was dried with Na_2SO_4 and removed *in vacuo* followed by recrystallization.

General procedure for Boc-group deprotection

1 mmol of the starting peptide was dissolved in 10-fold excess of TFA under stirring. The deblocking of the Boc-group was monitored by TLC in the system S1. At the end of the reaction TFA was evaporated *in vacuo* and the oil formed was further subjected to a condensation reaction without additional purification.

General procedure for deprotection of Z- groups by catalytic hydrogenolysis in the presence of Pd/C

1.00 mmol of the protected peptide was dissolved in MeOH and then Pd/C and 1.00 mmol of HCl or AcOH were added. Hydrogen was passed through the reaction mixture at 40 °C. The deblocking was monitored by TLC in the system S1. After the end of the reaction, Pd/C was filtered off and MeOH was evaporated *in vacuo*. The oil formed was subjected to a condensation reaction without additional purification.

General procedure for –OBzl ester group deprotection in basic conditions

1 mmol of the starting peptide was dissolved in MeOH (10 ml) and 4-fold excess of 2N NaOH was added. The reaction was monitored by TLC in the S3 system. After completion of the reaction, the pH was reduced to 7 with 10% citric acid, and a part of the solvent was removed *in vacuo*. Afterwards the pH was reduced to 4–5 with 10% citric acid and the product was extracted with EtOAc (3 × 10 ml). The following extraction of the product from the organic phase was by 5% $NaHCO_3$ (3 × 10 ml), and after reducing the pH to 4–5 with 10% citric acid, it was extracted with EtOAc (3 × 10 ml). Finally, the organic layer was washed with H_2O (3 × 10 ml), dried with Na_2SO_4 and evaporated *in vacuo*.

RESULTS AND DISCUSSION

Initially, fragment condensation using DCC/1-HOBt and/or azide method was tried. A lot of side reactions took place. Because of that, the standard peptide synthesis in solution by stepwise addition of amino acids from C-to N-terminus of the aimed peptides was chosen. Aimed compounds were synthesized according to schemes 1 and 2 (Figs. 1 and 2). TBTU or DCC/1-HOBt was used as a coupling reagent for shorter fragments and TCTU was used as a coupling reagent for the peptides.

Removal of the protecting groups was realized as follows:

- Boc-group in all intermediate compounds was removed by treatment with 10-fold excess of TFA;
- Z-group was removed by catalytic hydrogenation in the presence of HCl (molar amount) and Pd/C;
- 2N NaOH was used for all ester hydrolyses.

All final compounds were characterized by TLC, m.p. and $[\alpha]_{546}^{22}$ and their structures were proven by ES-MS. All data are presented in Table 1.

The purity of all obtained intermediate peptides was monitored in the systems S2 and S3. The deblocking procedures of Boc and Z groups were controlled in the S1 system, and final deprotection of OBzl group of Asp was monitored in the S3 system. As it can be seen from Table 1, all reactions are run with very good yields (higher than 80%).

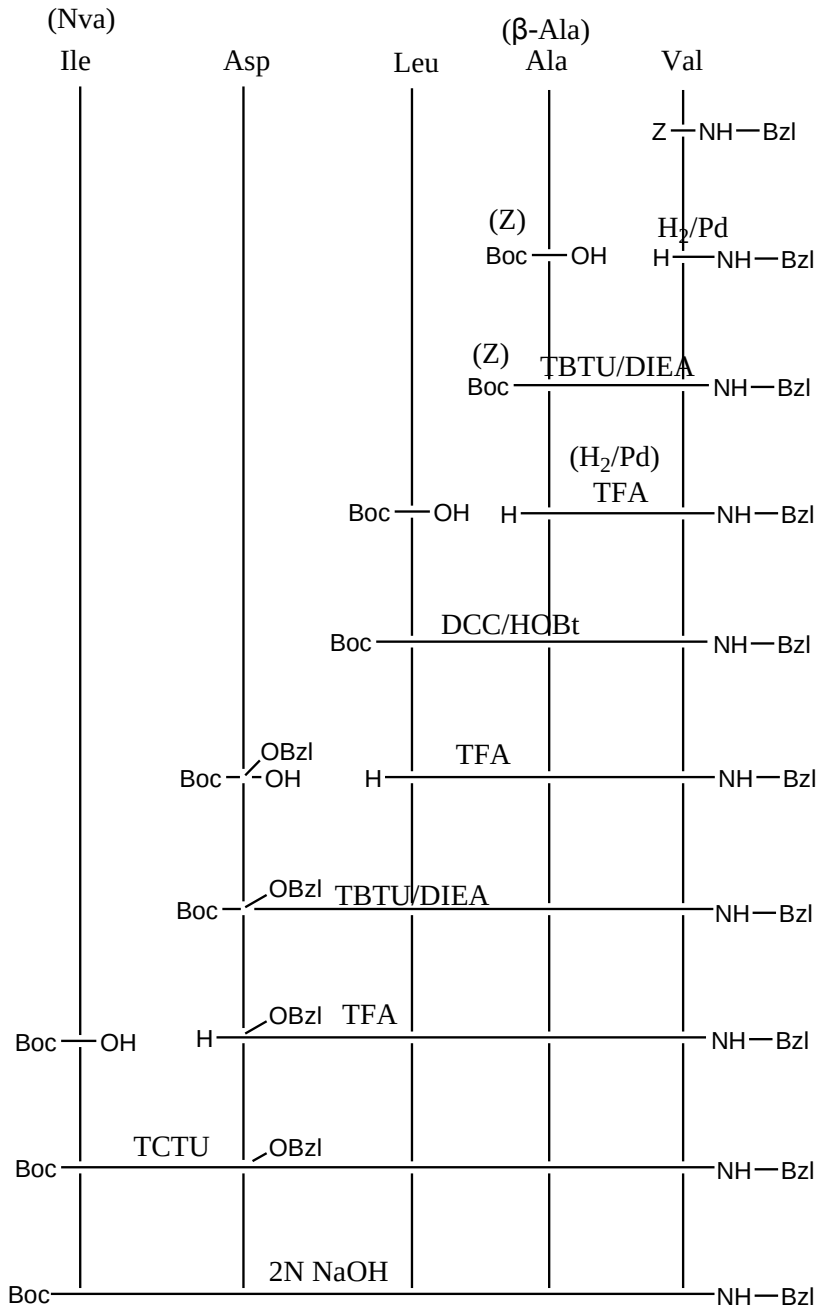

Fig. 1. Scheme of synthesis of pentapeptides Boc-Ile-Asp-Leu-Ala (β-Ala)-Val-NH-Bzl

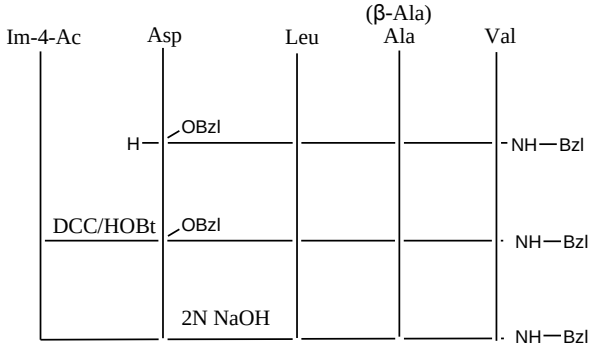

Fig. 2. Scheme of synthesis of pentapeptides Im-4-Ac-Asp-Leu-Ala (β-Ala)-Val-NH-Bzl

CONCLUSIONS

A series of 6 peptides, potential inhibitors of β-secretase, analogues of β-secretase inhibitor OM99-2 was designed. They were successfully synthesized using standard peptide synthesis in solution. All compounds were characterized by m.p., $[\alpha]_{546}^{22}$ and ESI-MS. Biological activity investigation is in a progress.

Table 1. MS data, melting points, yields and $[\alpha]_{546}^{22}$ of the newly synthesized compounds

N	Structure	MS data calc./found	M.p. [°C]	Yield [%]	*$[\alpha]_{546}^{22}$
1	Boc-Ile-Asp(OBzl)-Leu-β-Ala-Val-NH-Bzl	-	135-137	84	- 2.2
2	Boc-Ile-Asp-Leu-β-Ala-Val-NH-Bzl	718.4265/742.42 $[M^+ + Na^+]$	147-149	98	- 12.1
3	Boc-Nva-Asp(OBzl)-Leu-β-Ala-Val-NH-Bzl	-	186-189	87	- 4.4
4	Boc-Nva-Asp-Leu-β-Ala-Val-NH-Bzl	704.4109/727.42 $[M^+ + Na^+]$	210-214	97	- 14.0
5	Boc-Ile-Asp(OBzl)-Leu-Ala-Val-NH-Bzl	-	165-167	89	- 3.2
6	Boc-Ile-Asp-Leu-Ala-Val-NH-Bzl	718.4265/742.53 $[M^+ + Na^+]$	184-190	99	- 17.5
7	Boc-Nva-Asp(OBzl)-Leu-Ala-Val-NH-Bzl	-	146-148	89	- 2.0
8	Boc-Nva-Asp-Leu-Ala-Val-NH-Bzl	704.4109/727.27 $[M^+ + Na^+]$	187-189	99	- 9.2
9	Im-4-Ac-Asp(OBzl)-Leu-Ala-Val-NH-Bzl	-	161-163	79	- 1.1
10	Im-4-Ac-Asp(OBzl)-Leu-β-Ala-Val-NH-Bzl	-	192-194	77	- 2.3
11	Im-4-Ac-Asp-Leu-Ala-Val-NH-Bzl	728.4221/750.73 $[M^+ + Na^+]$	187-189	98	- 4.5
12	Im-4-Ac-Asp-Leu-β-Ala-Val-NH-Bzl	728.4221/750.73 $[M^+ + Na^+]$	198-200	99	- 28.0

* for all compounds $[\alpha]_{546}^{22}$ is determined for c = 1 in methanol

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ДИЗАЙН И СИНТЕЗ НА ПОТЕНЦИАЛНИ ИНХИБИТОРИ НА МУЛТИЕНЗИМНИ СИСТЕМИ УЧАСТВАЩИ ПРИ БОЛЕСТТА НА АЛЦХАЙМЕР

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(Резюме)

Проектирана и изпълнена беше синтеза на серия от 6 пептиди, потенциални инхибитори на β-секретаза, аналози на β-секретаза инхибитор ОМ99-2. Беше приложена стандартна пептидна синтеза в разтвор, чрез постепенно добавяне на аминокиселини от С-към N-края. Всички реакции дадоха много добри добиви (над 80%).