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Design, Synthesis and Enzymatic Inhibition of Novel Unusual Amino Acids as a Transition State Analogue of Amyloid Precursor Protein Peptide

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Abstract

β-secretase 1 (BACE1) plays a pivotal role in the pathology of Alzheimer's disease via accumulation beta amyloid in the brain. In this context, identifying new scaffolds that block BACE1 is of great importance despite all pharmacokinetic drawbacks that peptide-like structures have. Here, we report a new core structure based on novel unusual amino acids by substituting phenyl amide group in the P1 position and small alkyl groups in the P1′ site that results in the formation of new biological active peptides in micromolar level. Three different scaffolds were designed based on docking studies to efficiently interact with critical Asp32 residue in the active site of BACE1 and incorporated in peptides synthesis by Fmoc solid-phase peptide synthesis (SPPS) methodology to achieve desired compounds in good yield. The inhibitory activity of all synthesized peptides was examined by FRET-based enzymatic assay. The peptide 7 showed the best inhibitory activity with IC50 = 98.14 μM. Results of this investigation revealed that utilizing unusual amino acids as building blocks for the synthesis of peptidomimetics would be an option for the development and optimization of pharmaceutical structures.

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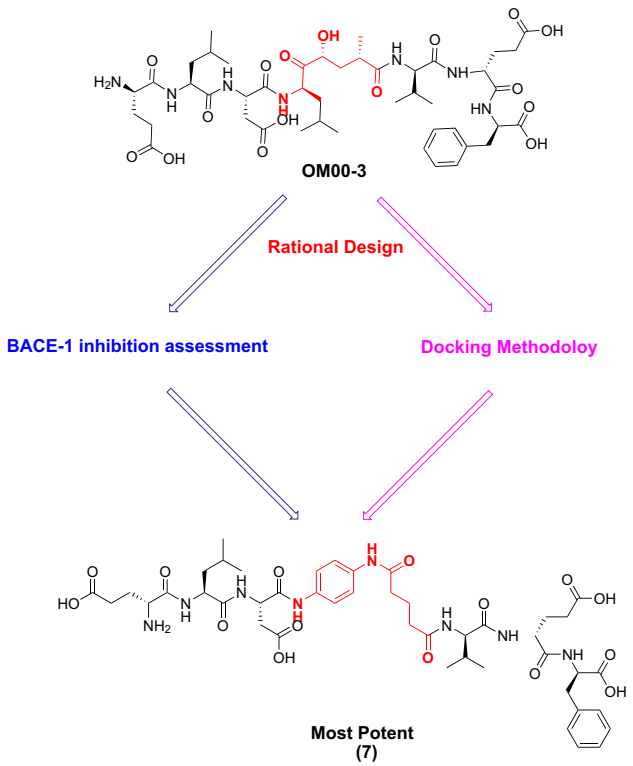
Graphic Abstract



The inhibition of β-secretase 1 (BACE1) is potentially important approach to treatment of Alzheimer disease (AD). Novel series of peptides coupled to the new unusual amino acids scaffold were investigated as BACE1 inhibitors in this study. The design of these peptides was mostly affected by OM00-3. Based on observations, in good agreement between BACE-1 inhibition assessment and docking methodology, peptide 7 was the most potent compound between all and could be the basis of future studies.

Keywords BACE-1 · Synthesis · Peptidomimetic · Unusual amino acids · OM00-3

Introduction

Among the many types of dementia, Alzheimer's disease (AD) is considered one of the most common diseases that mainly arises in the elderly. It deteriorates the nervous system, negatively affects memory, cognitive abilities and eventually leads to death (Nussbaum and Ellis 2003; Cummings 2004). In a person's brain with Alzheimer's diseases, the presence of amyloid plaques and neurofibrillary tangles are regarded as the main markers of the diseases. The former is formed by the accumulation of amyloid beta ($A\beta$), the peptides produced as a result of successive proteolysis of amyloid beta precursor protein (APP), and the latter is formed by tau protein filaments aggregated through a hyperphosphorylation mechanism (Nguyen et al. 2006; Medeiros et al. 2011). Consecutive cleavage of APP by beta and gamma secretase enzymes ends in the production of amyloid plaques therefore synthesis of any compounds that could inhibit such enzymes would help to slow the progression of the disease.

In recent years, the design of non-peptidic β-secretase 1 (BACE1) inhibitors as anti- Alzheimer's agents has been considered, in this regard, several BACE1 inhibitors have been introduced to clinical trial, unfortunately, until now, none of them can successfully pass the trial phase (Volloch and Rits 2018; Moussa 2017). So, these challenges of non-peptidic BACE1 inhibitors might drown keen attention of medicinal chemists to modify and develop the rational design of peptidic inhibitors to reach acceptable results.

The majority of peptidomimetic BACE1 inhibitors are merged into a non-cleavable transition-state isostere motif, which is the key binding element. Some of these essential motifs include hydroxyethylene (HE), hydroxyethylamine (HEA), statin and reduced amide pseudopeptide backbones (Ghosh and Osswald 2014). Many efforts have been done to obtain efficient inhibitors of BACE1 via designing new scaffolds that could be replacing the non-cleavable isostere intermediate (Verdie et al. 2007).

Recently, synthesis of unusual amino acids (UAAs) and utilizing them as building blocks have attracted considerable attention in the field of drug discovery that would benefit in function of therapeutic agents and characteristics improvement (Cardillo et al. 2006; Stevenazzi et al. 2014). In this way, a range of approved and clinical stage drugs that contain unusual amino acids have been reported (Blaskovich 2016). Peptides containing UAAs show more conformation limitation and enhanced rigidity of peptide that result in more resistance to proteasome-mediated degradation, more selectivity and affinity to receptor in contrast to peptides containing usual amino acids.

The peptide OM00-3 (Fig. 1) offers high efficiency as a peptidomimetic BACE1 inhibitor (Hong et al. 2002). This inhibitor contains a non-cleavable hydroxyethylene isostere at the cleavage site. The Ki of OM00-3 was determined 0.3 nM and it is mainly used as a lead compound for designing other effective inhibitors.

Here, we report the synthesis of the new structural analog of OM00-3 containing novel unusual amino acids (Fig. 2) as a substitution of Leu-Ala hydroxyethylene isostere in the OM00-3 backbone (Fig. 3).

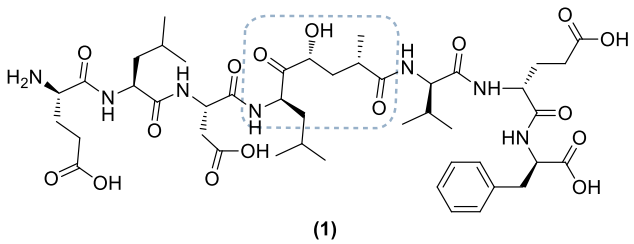
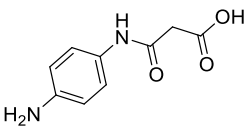
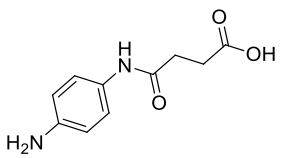


Fig. 1 Structure of OM00-3

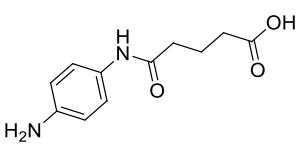
Fig. 2 Structures of synthesized UAAs **2–4**



UAA(2)
3-((4-aminophenyl)amino)-3-oxopropanoic acid



UAA(**3**)
4-((4-aminophenyl)amino)-4-oxobutanoic acid



UAA(4)
5-((4-aminophenyl)amino)-5-oxopentanoic acid

Materials and Methods

General Chemistry

Solvents that have been used during peptide synthesis including trifluoroacetic acid (TFA), methanol, dichloromethane (DCM), acetonitrile (ACN) and dimethylformamide (DMF) were provided from Merck (Germany). Triethylsilane (TES), (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), *N,N*-diisopropylethylamine (DIPEA), and benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorphosphate (PyBOP) and N-(9H-Fluoren-9-ylmethoxycarbonyloxy)succinimide (Fmoc-OSu) were purchased from Sigma-Aldrich (USA). Besides, 2-chlorotritylchloride (2-CTC) resin and 9-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids were bought from Bachem (Switzerland), Fmoc-Glu(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Val-OH, Fmoc-Phe-OH are the amino acids in this work. All other reagents were in analytical grade or higher purity from Sigma-Aldrich or Merck.

Melting points were measured by a Kofler hot stage apparatus. ^{1}H and ^{13}C NMR spectra were obtained by Bruker FT-500 and Bruker FT-300 using tetramethylsilane (TMS) as the internal standard. IR spectra were acquired using a Nicolet Magna FT-IR 550 spectrophotometer (KBr disks) and Mass-ESI-POS (Apex Qe-FT-ICR instrument) spectrometer was used to gain high-resolution mass spectra of the components. The compounds' purity was determined by HPLC (Column C-18, Eurospher 100, 7 µm).

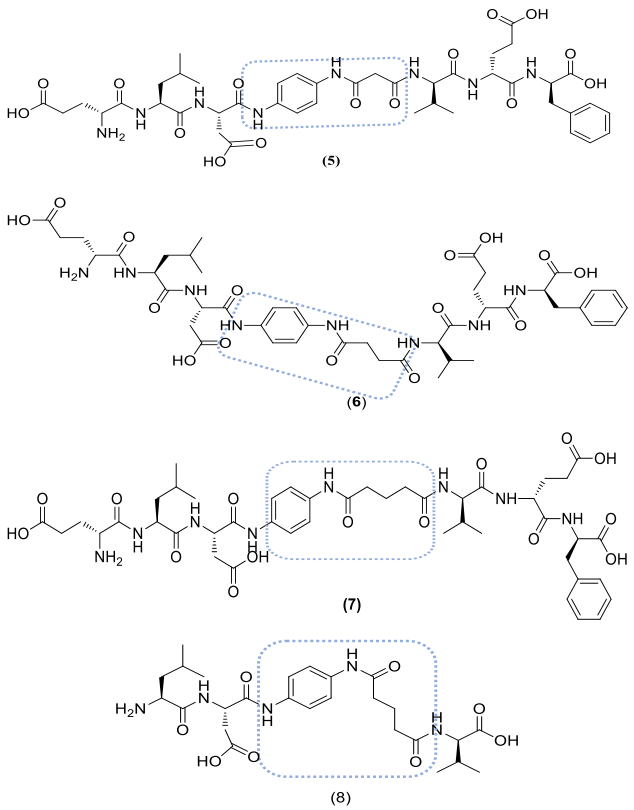


Fig. 3 BACE1 peptide inhibitor containing the proposed UAAs **2–4**

Synthesis of Fmoc-Diamine (10)

To a solution of 1, 4-phenylenediamine (1 mmol, 0.1 g) in ACN (5 ml), fmoc-OSu (1 mmol, 0.33 g) was added dropwise. The mixture was stirred at room temperature for 1 h. The progress of the reaction was monitored using TLC. After completion the reaction, the resultant precipitate was filtered and washed with ACN. White powder; Yield: > 90%; M.P. 201 ºC; IR (KBr, cm^{-1}): 3391, 3311, 3204, 3042, 2947, 1688. ^{1}H-NMR (500 MHz, DMSO-d_6): 4.29 (t, j = 6.5 Hz, 1H, CH), 4.41 (d, j = 6.2, 2H, CH_2), 5.1 (brs, 2H, NH_2), 6.53 (d, j = 6.2 Hz, 2H, Ar–H), 7.1 (d, j = 6.2 Hz, 2H, Ar–H), 7.36 (t, j = 7.0 Hz, 2H, Ar–H), 7.44 (t, j = 7.2 Hz, 2H, Ar–H), 7.76 (d, j = 7.0, 2H, Ar–H), 7.92 (d, j = 7.4 Hz, 2H, Ar–H), 9.6 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 46.7, 65.3, 114.4, 120.2, 120.3, 125.2, 127.1, 127.7, 140.8, 143.9, 154.0.

Synthesis of Protected UAA2: (11a)

The mixture of Fmoc-protected diamine (1 mmol, 0.31 g) and Meldrum's acid (1 mmol, 0.1 g) were refluxed overnight in THF/DCM (1:1 mL). The mixture was concentrated and the residual was washed with ethyl acetate and filtered to give pure product. White powder, Yield: > 80%; M.P. 231 ºC IR (KBr, cm^{-1}): 3326, 2970, 1729, 1699, 1665 ^{1}H-NMR (500 MHz, DMSO-d_6): 3.32 (s, 2H, CH2), 4.30 (t, j = 6.5 Hz, 1H, CH), 4.46 (d, j = 6.2 Hz, 2H, CH2), 7.33–7.47 (m, 8H, Ar–H), 7.75 (d, j = 7.2 Hz, 2H, Ar–H), 7.90 (d, j = 7.6 Hz, 2H, Ar–H), 9.64 (s, 1H, NH), 10.03 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSOd_6): 43.8, 46.6, 65.5, 118.7, 119.6, 120.2, 125.1, 127.1, 127.7, 133.8, 140.8, 143.8, 153.4, 164.2, 169.3.

Synthesis of Protected UAA3: (11b)

Fmoc-protected diamine (1 mmol, 0.31 g) and succinic anhydride (1 mmol, 0.11 g) were dissolved in dry Acetone (5 mL). The mixture was stirred overnight. After completion of the reaction (checked by TLC), the mixture was filtered and washed with ethyl acetate to give pure product. White powder. Yield: > 80%; M.P. 240 ºC. IR (KBr, cm^{-1}): 3312, 3054, 2936, 1700, 1663, 1H-NMR (500 MHz, DMSO-d_6): 2.49–2.52 (m, 4H, CH2), 4.29 (t, j = 6.5 Hz, 1H, CH), 4.46 (d, j = 6.0 Hz, 2H, CH2), 7.31–7.49 (m, 10H, Ar–H), 7.73 (d, j = 7.2, 2H, Ar–H), 7.88 (d, j = 7.35, 2H, Ar–H), 9.60(s, 1H, NH), 9.86 (S, 1H, NH), 11.9 (brs, 1H, COOH). ^{13}CNMR (125 MHz, DMSO-d_6): 28.9, 30.9, 46.7, 65.5, 118.7, 119.4, 120.2, 125.1, 127.1, 127.7, 134.1, 134.3, 140.8, 143.9, 153.5, 169.7, 173.9.

Synthesis of Protected UAA4: (11c)

Protected unusual **11c** was synthesized in more than 80% yield according to the synthesis method of compound **11b** using glutamic anhydride instead of succinic anhydride. White powder. Yield: > 80%; M.P. 247 ºC. IR (KBr, cm^{-1}): 3321, 3021, 2938, 1701, 1665 ^{1}H-NMR (500 MHz, DMSO-d_6):1.72–1.84 (m, 2H, CH2), 2.24–2.34 (m, 4H, CH2), 4.27 (t, j = 6.9 Hz, 1H, CH), 4.45 (d, j = 6.7 Hz, 2H, CH2), 7.31–7.48 (m, 10H, Ar–H), 7.73 (d, j = 7.1 Hz, 2H, Ar–H), 7.89 (d, j = 7.3 Hz, 2H, Ar–H), 9.59 (s, 1H, NH), 9.79 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 20.6, 33.1, 35.3 46.7, 65.6, 118.7, 119.7, 119.9, 120.3, 125.2, 127.2, 127.8, 134.3, 140.9, 143.9, 144, 153.5, 170.5, 174.3.

Procedure for the Synthesis of Peptide Sequence

The details of the practical procedure for the synthesis of peptides (H-Glu-Leu-Asp-UAAs-Val-Glu-Phe-OH) is described here as an example. The first amino acid Fmoc-Phe-OH (1 mmol) was attached to the 2-CTC resin (1.0 g) using DIPEA (8 mmol) in DMF (10 ml). After incubation for 2 h at room temperature, the mixture was filtered and washed with DMF (3 × 10 ml), and the remaining trityl chloride groups of the resin were capped during the reaction with solution of DCM/MeOH/DIPEA (20:2.4:1.2 ml) in a 30-min shake. The Fmoc group in each step of coupling

was removed by adding 25% piperidine in DMF (14 ml) to the vessel and shaking the mixture for 30 min. A solution of Fmoc-Glu-OH (3 mmol), TBTU (3 mmol), and DIPEA (3.5 mmol) in 10 ml of DMF were added to the vessel to attach the second amino acid (Glu) to the peptide chain. After 2 h, the presence of free primary amino groups was checked by Kaiser testing, other amino acids were coupled to the chain in the same procedure respectively. For the coupling of aromatic amine Fmoc-UAAs-OH the coupling reagent PyBOP (3 mmol) was used with DIPEA (8 mmol) in anhydrous DCM/DMF (10 ml, 1:1).

Chloranil is used to test for if there is any free aromatic amine in the mixture. For cleavage of peptide from solid phase, solution of 1% TFA in DCM (99 ml) was used which was neutralized by 4% pyridine in MeOH (46 ml). After solvent evaporation under reduced pressure in a rotary evaporator, the peptide was precipitated in distilled water and was filtered. Finally, cocktail TFA/TES/H_2O/MeOH (90:5:2.5:2.5) (10 ml) was used to remove all the protecting groups. In this step, after the evaporation of the solvent, the final peptide was precipitated in cold diethyl ether. Only for peptide **8**, cleavage and final deprotection were performed simultaneously in a cocktail container followed by the coupling steps.

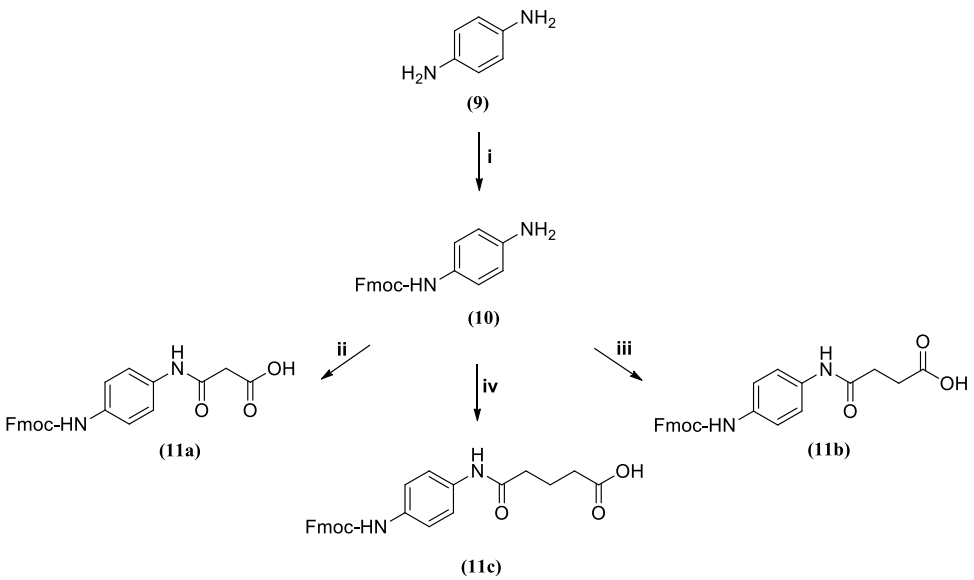
Results and Discussion

Chemistry

General procedure for the synthesis of N-protected UAAs **2–4** is summarized in Scheme 1. 1,4-phenylendiamine **(9)** was used as the starting material for the synthesis of a novel scaffold in this study. Dropwise addition of Fmoc-OSu to 1,4-phenylendiamine solution in dry ACN results in the immediate formation of a white solid with more than 90% yield **(10)** that despite its simple structure its synthesis has not yet been reported. Subsequently, nucleophilic addition of the primary amine group in Fmoc-protected diamine **(10)** in the presence of Meldrum's acid, succinic anhydride or glutamic anhydride provided a series of novel unusual protected amino acids **(11a)**, **(11b)** and **(11c)** in high yields.

The final peptidic compounds (Fig. 3) containing unusual amino acids were synthesized through solid-phase peptide synthesis (SPPS) methodology on the surface of 2-CTC resin (Golmohammadi et al. 2018). All Fmoc-L-amino acids were coupled to the peptide chain using TBTU and DIPEA in DMF. PyBOP was the coupling reagent that has been used for the coupling aromatic amine of Fmoc-UAAs-OH to the peptide sequence. Deprotection of Fmoc groups in each step was done in the presence of piperidine in DMF. TFA was used for cleavage of the side chain-protected peptides from the resin and all protecting groups were removed with a mixture of TFA/(TES)/H_2O/MeOH. The crude peptides were precipitated in diethyl ether. The purification and identification of each peptide chain were analyzed via preparative HPLC and ESI–MS as shown in Table 1. All the peptides were purified to achieve a purity > 90%.

It is worth mentioning that in the present study, in synthesizing the UAAs **2–4**, acylation of amine groups was performed with high yields through free coupling reagent reaction with no needs for recrystallization or chromatographic methods to obtain purified compounds. In the next step, the substitution was replaced on the modified Leu-Ala scaffold (OM00-3) and all peptides **5–8** were synthesized by Fmoc solid-phase peptide synthesis as described in previous sections. To establish the optimal coupling condition of Fmoc-UAAs–OH with Fmoc-Asp –OH, we investigated



Scheme 1 Reagents and conditions: (i) Fmoc-OSu, ACN, rt, 1 h, 90–95%; (ii) Meldrum's acid, THF/ DCM, reflux, overnight, > 80% (iii) Succinic anhydride, dry acetone, RT, overnight, > 80%; (iv) Glutamic anhydride, dry acetone, rt, overnight, > 80%

Table 1 Physico-chemical parameters of synthesized peptide

Peptide	Peptide sequence	Yield (%)	MW	RT (min)
5	NH_2-Glu-Leu-Asp-UAA6-Val-Glu-Phe-OH	67	926.97	18.427
6	NH_2-Glu-Leu-Asp-UAA7-Val-Glu-Phe-OH	64	940.99	26.328
7	NH_2-Glu-Leu-Asp-UAA8-Val-Glu-Phe-OH	62	955.02	26.003
8	NH_2-Leu-Asp-UAA8-Val-OH	65	549.28	15.983

Table 2 Coupling reagents for coupling aromatic amines of UAA **2–4**

Entry	Coupling reagents	Time (h)	Yield (%)
1	HATU	24	–
2	TBTU	24	–
3	DIC	24	–
4	PyBOP	2	60–70

using different coupling reagents such as HATU, DIC, and TBTU in the DCM/DMF (1:1) as a solvent.

Aryl amine group of proposed UAAs are weakly nucleophilic amine and our result indicated that N-acylation had to be done in the presence of 8 equivalents of DIPEA when PyBOP was used as coupling reagent in manual coupling for 2 h (Entry **4** in Table 2). On the other hand, the reaction totally failed when HATU or TBTU or DIC were used as activating agents.

As sites, located near the transition-state isostere (P1 and P1′), on both P and P' sides are more effective than the distal sites (P4 and P4′) Therefore, we also presented the design and synthesis of short peptide **8** as an analog of OM00-3 by removing positions P3, P4, and P4′ to determine if P1 and P1′ new scaffolds are selected properly or the chain length is responsible for the seen effects. The achievement of the least difference in comparison with peptide **7** revealed the designed structures for S1–S1′ enzymatic sites has been correctly selected that could have relatively effective interactions with the enzyme.

BACE-1 Inhibition Assessment

The BACE1 fluorescence resonance energy transfer (FRET) assay was carried out according to manufacturers' instructoins (Invitrogen. https://tools.invitrogen.com/content/sfs/manuals/L0724.pdf).

To obtain 3 × solutions, both BACE-1(provided by Baculovirus expression) and Rh-EVNLDAEFK-Quencher as substrate were diluted by BACE-1 assay buffer (50 mM sodium acetate, pH 4.5). Besides, 3 × solutions of the different concentrations of test compounds were acquired through diluting stock solutions of inhibitors in DMSO using assay buffer (final concentration of DMSO in the test plate was 6%). Reaction was incubated at room temperature for 90 min under dark condition with a mixture of test samples (10 µl of each), BACE1 enzyme (10 µl) and BACE1 substrate (10 µl) and then stopped with 2.5 M sodium acetate. Spectrophotometer recorded fluorescence at 545 nm for excitation and 585 nm for emission into 96-well plate to monitor the hydrolysis of the substrate (BMG Labtech). OM99-2 (H-Glu-Val-Asn-Leu-Ψ-Ala-Glu-Phe–OH) was employed as a reference inhibitor. Hence in each concentration of test compounds the percentages of enzyme inhibition were calculated regarding maximum enzyme activity wells (containing substrate plus enzyme) and baseline wells (containing substrate). IC_{5o} concentrations were determined by Expert software version 1.34 for Windows. Each experiment was repeated three times.

Table 3 BACE1inhibitory activity peptides **5–8**

Peptide	Inhibition (%)			IC50 (µM)
	(200 µM)	(100 µM)	(50 µM)	
5	22.98 ± 1.99	16.32 ± 2.38	7.64 ± 7.28	> 200
6	84.00 ± 18.44	46.11 ± 6.49	28.29 ± 6.13	111.80 ± 20.51
7	97.06 ± 4.58	53.87 ± 6.73	40.57 ± 3.06	98.14 ± 2.12
8	62.73 ± 3.92	44.49 ± 7.69	21.78 ± 3.54	167.00 ± 32.90
OM99-2	–	–	–	14.70 nM

Data are expressed as Mean ± SE (three independent experiments)
OM99-2 was tested at 10, 1 and 0.1 nM

Biological Activity

BACE1 inhibitory activity of peptides containing UAAs **2–4** was examined in vitro using FERT assay. Positive controls were performed using a known potent peptidomimetic inhibitor (OM99-2) and the BACE1 kit employed contained a particular APP-based peptide substrate (Rh-EVNLDAEFK quencher) and a BACE1 enzyme. The results are shown in Table 3. All the peptides synthesized exhibited fair to good BACE1 inhibitory activities. The highest potency was represented by the peptide **7**, which had the largest group in P1′site and the most flexible core.

Docking Study

To explore the mode of interactions of synthesized peptides with critical Asp32 residue in active site of BACE1, docking simulations were performed using the AutoDock Vina

(Ver. 1.1.0) (Trott and Olson 2010). The ability of software to predict the best docking pose and docking accuracy was determined with re-docking of co-crystallized inhibitor (OM00-3) with pdb ID: 1M4H. For this purpose, the ligand has been removed from crystallographic structure and then, has been docked in the active site of the protein. Calculated RMSD value was less than 2 Å. The result of this protocol and superimposition of docked structure and crystallographic ligand shown in Fig. 4 and revealed that the binding orientation and interaction of both structures are similar.

The structures of all compounds have sketched by MarvinSketch applet (Marvin package, ChemAxon Company). AutoDockTools (ADT, Ver. 1.5.6) (Morris et al. 2009) was used for preparing input files and analyzing docking results. Polar hydrogens and rotatable bonds were assigned by ADT. Docking with a maximum number of 25×10^6 energy evaluations and 50 runs per simulation using the Lamarckian Genetic Algorithm (LGA) were performed. All other parameters were set to their default values. The docked conformations of each ligand were ranked into clusters based on the binding energy. After clustering analysis, conformation with the most favorable binding energy was selected.

Reliability of the Docking Protocol

The main approach to achieve new peptidomimetic β-secretase inhibitors is to create structures that mimic transition-state isostere. The S1 site of BACE1 is large hydrophobic pocket and groups like cyclohexyl and benzyl at the P1 position can efficiently fill the S1 site, so phenyl amide was used as benzyl mimic in the P1 position. In addition, because the S1′ position accommodates hydrophobic groups properly, replacement of small alkyl groups in the P1′ site is considered to be efficient (Ghosh and Osswald 2014; Silvestri 2009) to confirm design rationale, docking was performed on all designed peptides. Due to the high flexibility of peptides, several docking programs have been used with different levels of prediction accuracy to predict protein–peptide mode of interaction (Ciemny et al. 2018). Between these applications, AutoDock Vina shows the best docking performance and scoring power on short peptides up to four residues (Rentzsch and Renard 2015). The results of our work showed that AutoDock Vina is still suitable for peptides up to seven residues (Figs. 4, 5, 6).

Investigating Binding Model of Synthesized Peptides Over BACE1

Docking results indicated that all the designed peptides bind to the active site with negative binding energy in the range of − 9.0 kcal/mol. As shown in Fig. 5 the new scaffold represented identical binding modes with other transition-state analogs of BACE1 inhibitors. The nitrogen atom of phenyl amide substitution in the novel synthesized scaffold plays the same role as the hydroxyl group in OM00-3 does. The flap residues Thr72 binds with another nitrogen in the new scaffold. The key H-bonding interaction between the new scaffold and Asp 32 was observed during the docking and the amide backbone of the inhibitor made hydrogen bonds with Arg128, Tyr198, Thr232, Thr72 and Gly230 while the side chain Glu and Asp formed hydrogen bonds with Thr231, Thr72, Thr329 and Gly34. Docking pose of peptide **7** was shown in Fig. 6

Conclusion

In this study, an efficient approach is described for the synthesis of unusual amino acids as new scaffolds that could replace the HE isostere of OM00-3 forming analogs of

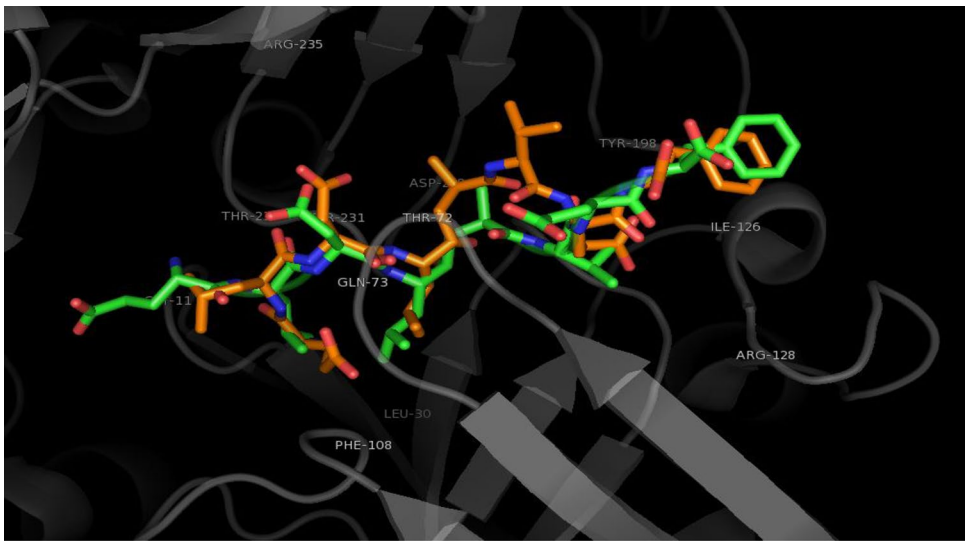


Fig. 4 Superimpose of redocked pose (orange) with crystallographic structure (green) (Color figure online)

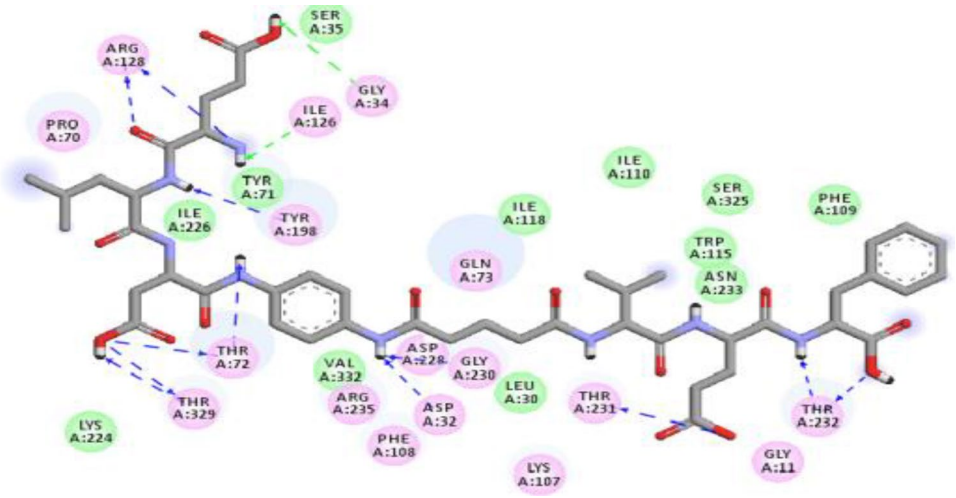


Fig. 5 Representation of binding modes of the most active peptide **7** in the active sites of BACE1

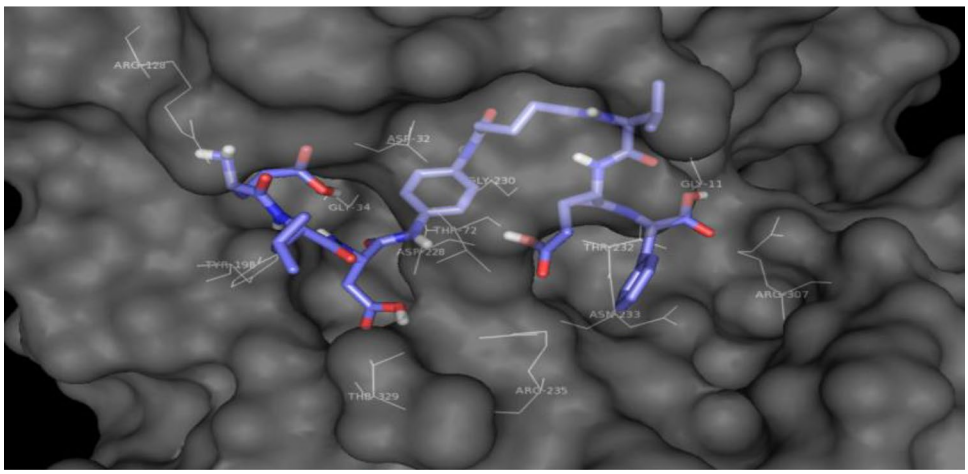


Fig. 6 Docking pose of peptide **7** in the active sites of BACE1

tetra and heptapeptides. The key component of new scaffolds consists of a cyclic amide group that interacts with catalytic Asp side chain. This novel scaffold also includes an alkyl moiety which easily fits hydrophobic S1′ pocket.

The most potent peptide **7** exhibited 98 μM BACE-1 inhibitory activity using FRET assay. SAR study of presented peptidic BACE-1 inhibitors emphasizes the important role of amide NH of unusual amino acids in key H-bonds with Asp32 and Gly230 residues. PyBOP was used in the presence of 8 eq of DIPEA to couple aromatic amine group of UAAs to peptide sequences that afforded the product in good yield and could be utilized in amidation of other weakly nucleophilic aromatic amines. Besides, the same connections and bonds between peptide **7** and OM003 that reported above, may well justify the in vitro inhibitory ability of peptide **7**.

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Compliances with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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