NEW DERIVATIVES OF GALANTHAMINE CONTAINING PEPTIDE FRAGMENT

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ABSTRACT

New derivatives of galanthamine containing a peptide fragment with an antiaggregation activity are synthesized. Herein the process of synthesis, purification and characterization of the newly obtained compounds is described. Syntheses were performed by consecutive attachment of Boc-protected amino acids to either norgalanthamine or H-Gly-Ogal using TBTU/DIPEA and TCTU/DIPEA methods. The purification of final products was realized by treating with EtOAc or by HPLC. The newly compounds were characterized by TLC, NMR, m.p. and MS. Two pharmacological effects are expected to be combined in a single molecule: the anticholinesterase and the antiaggregation ones.

Keywords: Alzheimer’s disease, acetylcholinesterase, galanthamine, norgalanthamine, antiaggregation activity.

INTRODUCTION

The Alzheimer’s disease (AD) is a neurodegenerative illness, which affects millions of people worldwide. According to the World Health Organization about 26.6 million people worldwide suffered from AD in 2006 [1]. This number may quadruple by 2050 [2] because of the dynamic development of the disease. There are several drugs approved for the treatment of AD suffering patients, among which is galanthamine. It is an alkaloid, which is acting on the central nervous system with a moderated acetylcholinesterase (AChE) and a low butyrylcholinesterase inhibition activity. The reaction on the biochemical level is competitive and reversible. An important attribute of the galanthamine is its ability to inhibit the amyloid aggregation and the toxicity of the beta amyloid peptide (Aβ) [3]. Many authors demonstrated that the galanthamine induces significant improvement of the cognitive manifestations of AD patients [4 - 6].

Aβ polymerization process is a phenomenon related to the process of AD. It is shown that the aggregation and the subsequent amyloid toxicity are related to the ability of the peptide to form a β-sheet conformation. According to some authors, this phenomenon can be in vivo catalyzed [7] by proteins which are in the senile plaques. The Aβ peptide polymerizes, which leads to the formation of fibrils. The importance of AD pathogenesis refers to the fact that the whole amyloid fibrilogenesis process is significant, but not only the fibrils themselves [7]. One of the approaches aiming to block the neurotoxic activity of Aβ refers to the inhibition of the amyloid polymerization [7].

Penke has designed and synthesized neuroprotective
substances of peptide and non-peptide structures applying the „AutoDock“ simulation program. He has found a leader compound, namely the pentapeptide Leu-Pro-Tyr-Phe-Asp-NH$_2$ of ASBIM (Amyloid Surface Binding Molecule) properties. He has also verified, in in vitro and in vivo studies, that this compound prevents completely Aβ neurotoxicity at micromolar concentrations [8].

New hybrid structures are synthesized on the ground of the information presented above by introducing Leu-Pro-Tyr-Phe-Asp to positions 6 or 11 of the galanthamine molecule. The present communication describes the process of synthesis, purification and characterisation of the newly obtained compounds.

EXPERIMENTAL
Reagents
Galanthamine was provided by Sopharma Pharmaceuticals AD, Bulgaria. The amino acids and the reagents DIPEA (N,N-Diisopropylethylamine), TBTU (2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate) and TCTU (O-(6-Chloro-1-hydrocibenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate) were purchased from Sigma-Aldrich-Germany, Alfa Aesar and Novabiochem. The solvents were purchased from Merck and used without further purification.

Analysis
$^1$H and $^{13}$C spectra were recorded on Bruker Avance AV-II+-600 MHz spectrometer. The $^1$H and $^{13}$C NMR chemical shifts are given relative to TMS (Tetramethylsilane). The chemical shifts are expressed in ppm, while the coupling constants - in Hz. The precise assignment of the $^1$H and $^{13}$C NMR spectra was accomplished by measurement of 2D homonuclear correlation (COSY), DEPT-135 and 2D invers detected heteronuclear (C-H) correlations (HMQC and HMBC). ESI/MS analyses were recorded on ThermoFinnigan LCQ advantage ion trap mass spectrometer with constant infusion of the methanol solution (10 µg/mL) at a flow rate of 20 µL/min. The purity of the products and the reaction proceeding were checked by TLC on precoated plates of Silica gel 60 F254 (Merck) with the following solvent systems: CHCl$_3$:MeOH = 9:1 (S1), n-BuOH:CH$_3$COOH:H$_2$O = 3:1:1 (S2). The spots on the TLC chromatograms were detected by the chlorine/o-tolidine reaction. The melting points were determined on a Kofler apparatus and were used in absence of a correction.

A General Procedure of Galanthamine amide synthesis by TBTU or TCTU Methods
1 eq of 11-N-Demethyl-N-[Boc-AA]-Galanthamine or 11-N-Demethyl-N-[Boc-Peptide]-Galanthamine was dissolved in 10-fold excess of trifluoroacetic acid (TFA) at a room temperature. The reaction mixture was stirred until Boc group running out (the chromatographic control was carried out in systems S2). The solvent was evaporated and the residue was dissolved in a minimal amount of DMF and neutralized with DIPEA to pH = 7 - 8. The corresponding amino acids, Boc-AA-OH (1.3 eq), TBTU or TCTU (1.3 eq) and DIPEA (1.3 eq), were added to this mixture. The reaction mixture was stirred for 24 h at a room temperature. 24 h later 5 % NaHCO$_3$ was added to the reaction mixture and the precipitate was filtered and washed consecutively by 5 % NaHCO$_3$ and water to pH = 7. The residues were dried over Na$_2$SO$_4$ and recrystallized from EtOAc/petroleum ether. The yields of the products were 56 % - 93 % by the TBTU method and 90 % - 93.3 % by the TCTU method.

A general procedure for synthesis of 6-O-galanthamine esters by TBTU or TCTU Methods
1 eq. of 6-O-[Boc-AA]-Galanthamine or 6-O-[Boc-Peptide]-Galanthamine was dissolved in 10-fold excess of TFA at a room temperature. The reaction
mixture was stirred until the Boc group running out (the chromatogram control was carried out in systems S2 3:1:1). The reaction mixture was evaporated and dissolved in a minimal amount of DMF and neutralized with DIPEA to pH = 7 - 8. The corresponding amino acid Boc-AA-OH (1.3 eq), TBTU or TCTU (1.3 eq.) and DIPEA (1.3 eq) were added. The reaction mixture was stirred for 24 h at a room temperature. 5 % NaHCO$_3$ was added at the end of the reaction and the product was extracted with 3x25 ml of EtOAc. The organic layer was washed consecutively with 5 % NaHCO$_3$ (2x25ml) and H$_2$O to pH = 7. The organic layers were dried by Na$_2$SO$_4$, while the solvent was removed under vacuum. The yield of the crude product was obtained quantitatively. The product was recrystallized from EtOAc/Petroleum ether.

RESULTS AND DISCUSSION

The synthesis of galanthamine ester and amide linked with the antiaggregation peptide Leu-Pro-Tyr-Phe-Asp at position 6 or 11 is described. The peptide amide of Gal (11-N-Demethyl-N-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)]-Galanthamine) was synthesized by consecutive addition of Boc-Asp(OBzl)-OH, Boc-Phe-OH, Boc-Tyr-OH, Boc-Pro-OH, Boc-Leu-OH to N-demethylgalantamine (norgalantamine)
Norgalanthamine was prepared in this study following recipe described in ref. [9]. The syntheses were completed applying the TBTU or/and the TCTU methods in DMF in presence of the base DIPEA (N,N-Diisopropylethylamine). The crude products were obtained with high yields and recrystallized from EtOAc/petroleum ether. The intermediate derivatives of galanthamine: 11-N-Demethyl-N-[Boc-Asp(OBzl)]-Galanthamine, 11-N-Demethyl-N-[Boc-Phe-Asp(OBzl)]-Galanthamine, 11-N-Demethyl-N-[Boc-Tyr-Phe-Asp(OBzl)]-Galanthamine, 11-N-Demethyl-N-[Boc-Pro-Tyr-Phe-Asp(OBzl)]-Galanthamine were characterized by TLC, NMR and melting points determination. The final product, i.e. 11-N-Demethyl-N-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)]-Galanthamine was recrystallized and additionally purified by preparative HPLC. Its structure was proven by MS and NMR (Fig. 3).

The peptide ester of Gal, namely (6-O-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)-Gly]-Galanthamine), was synthesized applying the same approach (see Scheme 2). This synthesis was realized by using the amino acid Gly as a linker [10]. The deprotection of the Boc-group of the protected compound, 6-O-[Boc-Gly]-Galanthamine, was done by its treatment with 10 fold excess of TFA. 6-O-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)-Gly]-Galanthamine was synthesized by consecutive addition of Boc-Asp(OBzl-OH, Boc-Phe-OH, Boc-Tyr-OH, Boc-Pro-OH, Boc-Leu-OH to 6-O-[H-Gly]-Galanthamine. The crude products obtained with high yields in the course of the reaction were recrystallized from EtOAc/petroleum ether. The intermediate derivatives of galanthamine 6-O-[Boc-Asp(OBzl)-Gly]-Galanthamine, 6-O-[Boc-Phe-Asp(OBzl)-Gly]-Galanthamine, 6-O-[Boc-Tyr-Phe-Asp(OBzl)-Gly]-Galanthamine, 6-O-[Boc-Pro-Tyr-Phe-Asp(OBzl)-Gly]-Galanthamine were characterized by TLC, NMR and melting points determination. The final product, 6-O-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)-Gly]-Galanthamine, contained an impurity visible by the MS analysis (Fig. 4). A high level of purification was achieved by treatment of the crude peptide with a minimal quantity of EtOAc and further filtration. As the pure product was insoluble in EtOAc, it precipitated in the form of white crystals, while the impurities remained in the mother liquor. The data referring to the purified product is presented on Fig. 5.

11-N-Demethyl-N-[Boc-Phe-Asp(OBzl)]-Galanthamine. Yield 56 % (TBTU), 91.65 % (TCTU), mp 82 - 85°C. \( ^1H \) NMR (600 MHz, CDCl\(_3\)): \( \delta = 1.40(s, 9H, CH_3), 1.82-2.07 (m, 3H, H-5 \) and H-9), 2.98 (dd, \( J=3.8, 17.1 \) Hz, 1H, Phe-CH\(_2\)), 3.19 (d, \( J=13.6 \) Hz, 1H, H-10), 3.04 (dd, \( J=4.4, 17.1 \) Hz, 1H, Phe-CH\(_2\)), 3.55 (t, \( J=13.6 \) Hz, 1H, H-10), 3.73 (m, 1H, H-12), 3.85 (s, 3H, OCH\(_3\)), 4.18 (m, 1H, H-6), 4.50 (m, 1H, Phe-CH), 4.60 (m, 1H, H-12), 4.72 (m, 1H, H-4a), 5.18 (s, 2H, CH\(_2\)), 5.20 (m, 1H, Asp-CH), 5.97 (d, \( J=9.9 \) Hz, 1H, H-7), 6.07 (d, \( J=9.9 \) Hz, 1H, H-8), 6.46 (s, 3H, OCH\(_3\)), 6.81 (d, \( J=8.8 \) Hz, 1H, H-2), 6.88 (bs, 1H, NH), 6.89 (bs, 1H, NH), 7.11 - 7.42 (m, 10H, Ar).

\( ^{13}C \) NMR (150 MHz, CDCl\(_3\)): \( \delta = 28.2 \) (CH\(_3\)), 29.7 (5), 33.6 (9), 36.2 (Asp-C-2), 36.2 (Phe-C-3), 46.5 (10), 46.8 (Asp-C-3), 48.0 (8a), 49.0 (12), 55.4 (OCH\(_3\)), 61.8

**Fig. 3.** MS and chromatogram of purified 11-N-Demethyl-N-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)]-Galanthamine.
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Fig. 4. MS of 6-O-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)-Gly]-Galanthamine - crude product.

11-N-Demethyl-N-[Boc-Tyr-Phe-Asp(OBzl)]-Galanthamine. Yield 68.60 % (TBTU), 90.00 % (TCTU), mp 93 – 97°C. 1H NMR (600 MHz, CDCl3): δ = 1.39 (s, 9H, CH3), 1.72-2.10 (m, 3H, H-5 and H-9), 3.00 (dd, J=3.8, 17.1 Hz, 1H, Phe-CH2), 3.19 (d, J=13.6 Hz, 1H, H-10), 3.04 (dd, J=3.8, 17.1 Hz, 1H, Phe-CH2), 3.50 (m, 2H, Pro-H-5), 3.55 (t, J=13.6 Hz, 1H, H-10), 3.72 (m, 1H, H-12), 3.82 (s, 3H, OCH3), 4.19 (m, 1H, H-6), 4.51 (m, 1H, Phe-CH2), 4.60 (m, 1H, H-12), 4.72 (m, 1H, H-4a), 4.98 (m, 1H, Tyr-CH2), 5.12 (s, 2H, CH2), 5.20 (m, 1H, Asp-CH), 5.97 (d, J=9.9 Hz, 1H, H-7), 6.07 (d, J=9.9 Hz, 1H, H-8), 6.70 (d, J=8.8 Hz, 1H, H-1), 6.98 (d, J=8.8 Hz, 1H, H-2), 7.05 (bs, 1H, NH), 7.14 (bs, 1H, NH), 7.20– 7.40 (m, 14H, Ar), 11.04 (bs, 1H, OH). 13C NMR (150 MHz, CDCl3): δ = 28.2 (CH3), 29.7 (5), 33.6 (9), 36.2 (Asp-C-2), 36.2 (Phe-C-3), 46.5 (10), 46.8 (Asp-C-3), 48.0 (8a), 49.0 (12), 55.4 (OCH3), 61.8 (Phe-C-3), 61.9 (6), 66.8 (PhCH3), 80.1 (C), 88.2 (4a), 111.0 (7), 121.8 (2), 126.7 (1), 126.9 (Ar), 128.1 (Ar), 128.3 (Ar), 128.4 (Ar), 128.6 (Ar), 128.7 (Ar), 129.2 (13), 129.3 (Ar), 132.8 (14), 135.1 (i-Ar), 136.3 (i-Ar), 143.8 (4), 145.6 (3), 155.2 (Boc-O-C=O), 155.4 (p-Ar), 169.9 (NHC=O), 170.1 (NHC=O), 171.0 (O-C=O).

11-N-Demethyl-N-[Boc-Pro-Tyr-Phe-Asp(OBzl)]-Galanthamine. Yield 70.00 % (TBTU), 90.00 % (TCTU), mp 105 - 107°C. 1H NMR (600 MHz, CDCl3): δ = 1.39 (s, 9H, CH3), 1.72-2.30 (m, 7H, H-5, H-9, Pro-H-3 and Pro-H-4), 3.00 (dd, J=3.8, 17.1 Hz, 1H, Phe-CH2), 3.19 (d, J=13.6 Hz, 1H, H-10), 3.04 (dd, J=4.4, 17.1 Hz, 1H, Phe-CH2), 4.17 (d, J=13.6 Hz, 1H, Pro-H-5), 3.55 (t, J=13.6 Hz, 1H, Pro-H-5), 3.72 (m, 1H, H-12), 3.82 (s, 3H, OCH3), 4.19 (m, 1H, H-6), 4.42 (m, 1H, Pro-CH), 4.51 (m, 1H, Pro-CH).
Phe-CH), 4.60 (m, 1H, H-12), 4.72 (m, 1H, H-4a), 4.98 (m, 1H, Tyr-CH), 5.12 (s, 2H, CH$_2$), 5.97 (d, J=9.9 Hz, 1H, H-7), 6.07 (d, J=9.9 Hz, 1H, H-8), 6.70 (d, J=8.8 Hz, 1H, H-1), 6.98 (d, J=8.8 Hz, 1H, H-2), 7.05 (bs, 1H, NH), 7.14 (bs, 1H, NH), 7.20–7.40 (m, 14H, Ar), 11.04 (bs, 1H, OH).

$^{13}$C NMR (150 MHz, CDCl$_3$); δ = 24.4 (Pro-C-4), 28.3 (CH$_3$), 28.9 (Pro-C-3), 29.7 (5), 33.6 (9), 36.2 (Asp-C-2), 36.2 (Phe-C-3), 46.5 (10), 46.8 (Asp-C-3), 46.9 (Pro-C-5), 48.0 (8a), 49.0 (12), 55.4 (OCH$_3$), 60.4 (Pro-C-2), 61.8 (Phe-C-3), 61.9 (6), 66.8 (PhCH$_2$), 80.1 (C), 88.2 (4a), 111.0 (7), 121.8 (2), 126.7 (1), 127.0 (Ar), 127.6 (8), 128.1 (Ar), 128.3 (Ar), 128.4 (Ar), 128.6 (Ar), 128.7 (Ar), 129.1 (13), 129.3 (Ar), 130.2 (Ar), 130.3 (Ar), 132.8 (14), 135.2 (i-Ar), 135.9 (i-Ar), 143.8 (4), 145.6 (3), 155.1 (Boc-O-C=O), 155.3 (p-Ar), 169.9 (NHC=O), 170.1 (NHC=O), 170.7 (NHC=O), 172.8 (O-C=O).

**11-N-Demethyl-N-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)]-Galanthamine.** Yield 55 % (TBTU), 63.3 % (TCTU), mp 105 - 108°C. $^1$H NMR (600 MHz, CDCl$_3$); δ = 0.88 (d, J=6.7 Hz, 3H, CH$_3$), 0.89 (d, J=6.7 Hz, 3H, CH$_3$), 1.44 (s, 9H, CH$_3$), 1.72-2.30 (m, 9H, H-5, H-9, Leu-H-3, Pro-H-3 and Pro-H-4), 3.00 (dd, J=3.8, 17.1 Hz, 1H, Phe-CH$_2$), 3.19 (d, J=13.6 Hz, 1H, H-10), 3.04 (dd, J=4.4, 17.1 Hz, 1H, Phe-CH$_2$), 3.50 (m, 2H, Pro-H-5), 3.55 (s, 3H, OCH$_3$), 4.19 (m, 1H, H-6), 4.42 (m, 1H, Phe-CH$_2$), 4.51 (m, 1H, Phe-CH), 4.60 (m, 1H, H-12), 4.65 (m, 1H, Leu-H-2), 4.72 (m, 1H, H-4a), 4.98 (m, 1H, Tyr-CH), 5.12 (s, 2H, CH$_2$), 5.97 (d, J=9.9 Hz, 1H, H-7), 6.07 (d, J=9.9 Hz, 1H, H-8), 6.70 (d, J=8.8 Hz, 1H, H-1), 6.98 (d, J=8.8 Hz, 1H, H-2), 7.05 (bs, 1H, NH), 7.14 (bs, 1H, NH), 7.20–7.40 (m, 14H, Ar), 11.04 (bs, 1H, OH).

![Fig. 5. MS of purified 6-O-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)]-Galanthamine.](image-url)
1H, OH. $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta = 21.5$ (Leu-CH$_3$), 23.4 (Leu-CH$_3$), 24.4 (Pro-C-4), 24.7 (Leu-C-4), 28.3 (CH$_3$), 28.9 (Pro-C-3), 29.7 (5), 33.6 (9), 36.2 (Asp-C-2), 36.2 (Phe-C-3), 40.5 (Leu-C-3), 46.5 (10), 46.8 (Asp-C-3), 46.9 (Pro-C-5), 48.0 (8a), 49.0 (12), 54.0 (Leu-C-2), 55.4 (OCH$_3$), 60.4 (Pro-C-2), 61.8 (Phe-C-3), 61.9 (6), 66.8 (PHCH$_2$), 80.1 (C), 88.2 (4a), 111.0 (7), 121.8 (2), 126.7 (1), 127.0 (Ar), 127.6 (8), 128.1 (Ar), 128.3 (Ar), 128.4 (Ar), 128.6 (Ar), 128.7 (Ar), 129.1 (13), 129.3 (Ar), 130.2 (Ar), 130.3 (Ar), 132.8 (14), 135.2 (i-Ar), 135.9 (i-Ar), 143.8 (4), 145.6 (3), 155.1 (Boc-O-C=O), 155.3 (p-Ar), 168.1 (NHC=O), 169.9 (NHC=O), 170.1 (NHC=O), 170.6 (NHC=O), 172.7 (O-C=O).

Scheme 2. Synthesis of 6-O-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)-Gly]-Galanthamine.
6-O-[Boc-Asp(OBzI)-Gly]-Galanthamine. Yield 60 % (TBHU), 84 % (TCTU), mp 60 - 62 °C. 1H-NMR (600 MHz, CDCl₃): δ = 1.46 (s, 9H, CH₉), 1.58 (dd, J=2.2, 13.4 Hz, 1H, 9), 2.08 (ddd, J=3.2, 5.4, 16.4 Hz, 1H, 5), 2.14 (dt, J=2.4, 13.2 Hz, 1H, 9), 2.39 (s, 3H, NCH₃), 2.69 (tdd, J=1.2, 2.4, 16.3 Hz, 1H, 5), 2.75 (dd, J=5.8, 17.1 Hz, 1H, Asp-H-2), 3.01 (dd, J=4.2, 17.0 Hz, 1H, Asp-H-2), 3.07 (dd, J=14.3 Hz, 1H, 10), 3.30 (t, J=13.4 Hz, 1H, 10), 3.67 (d, J=15.2 Hz, 1H, 12), 3.85 (s, 3H, OCH₃), 4.00 (m, 2H, Gly-CH₂), 4.11 (d, J=15.1 Hz, 1H, 12), 4.55 (bs, 1H, 4a), 4.58 (bs, 1H, Asp-H-3), 5.14 (AB system, J=12.1 Hz, 2H, CH₂), 5.37 (t, J=5.0 Hz, 1H, 6), 5.72 (d, J=8.4 Hz, 1H, Asp-NH), 5.89 (dd, J=4.8, 10.4 Hz, 1H, 7), 6.31 (d, J=10.3 Hz, 1H, 8), 6.59 (d, J=8.2 Hz, 1H, 1), 6.66 (d, J=8.2 Hz, 1H, 2), 6.94 (bs, 1H, Gly-NH), 7.32-7.38 (m, 5H, Ar). 13C-NMR (150 MHz, CDCl₃): δ = 27.56 (5), 28.31 (CH₂), 34.17 (9), 36.13 (Asp-C-2), 41.66 (NCH₃), 41.73 (Gly-C-2), 47.97 (C), 50.54 (Asp-C-3), 53.68 (10), 55.98 (OCH₃), 60.37 (12), 64.68 (6), 66.85 (OCH₃), 80.49 (C), 86.02 (4a), 111.44 (2), 121.56 (1), 122.07 (7), 128.34 (Ph), 128.39 (p-Ph), 128.61 (Ph), 131.78 (12b), 135.42 (i-Ph), 143.97 (4), 146.51 (3), 155.50 (OC=ONH), 169.21 (NHC=O), 170.90 (OC=O), 171.68 (OC=O).

6-O-[Boc-Phe-Asp(OBzI)-Gly]-Galanthamine. Yield 83 % (TBHU), 88 % (TCTU), mp 81-85 °C. 1H-NMR (600 MHz, CDCl₃): δ = 1.39 (s, 9H, CH₉), 1.60 (m, 1H, 9), 2.08 (ddd, J=3.2, 5.4, 16.4 Hz, 1H, 5), 2.14 (t, J=13.2 Hz, 1H, 9), 2.46 (s, 3H, NCH₃), 2.66 (tdd, J=1.2, 2.4, 16.3 Hz, 1H, 5), 2.78 (dd, J=5.8, 17.1 Hz, 1H, Asp-H-2), 3.00–3.12 (m, 6H, Asp-H, 10, Phe-CH₂ and Tyr-CH₂), 3.34 (t, J=13.4 Hz, 1H, 10), 3.72 (d, J=15.2 Hz, 1H, 12), 3.85 (s, 3H, OCH₃), 3.93 (d, J=5.6, 11.3 Hz, 2H), 4.00 (m, 2H, Gly-CH₂), 4.16 (d, J=15.1 Hz, 1H, 12), 4.32 (m, 1H, Phe-CH), 4.55 (bs, 1H, 4a), 4.58 (bs, 1H, Asp-H-3), 4.93 (bs, 1H, Phe-NH), 5.11 (AB system, J=12.1 Hz, 2H, CH₂), 5.35 (t, J=4.8 Hz, 1H, 6), 5.72 (d, J=8.4 Hz, 1H, Asp-NH), 5.91 (dd, J=4.8, 10.2 Hz, 1H, 7), 6.28 (d, J=10.3 Hz, 1H, 8), 6.59 (d, J=8.0 Hz, 1H, 1), 6.66 (d, J=8.0 Hz, 1H, 2), 6.72 (d, J=7.8 Hz, 2H, Tyr-Ph), 6.76 (bs, 1H, Gly-NH), 6.96 (d, J=7.8 Hz, 2H, Tyr-Ph), 7.12 (d, J=7.6 Hz, 1H, Phe-NH), 7.19–7.38 (m, 10H, Ar). 13C-NMR (150 MHz, CDCl₃): δ = 27.60 (5), 28.32 (CH₂), 32.80 (9), 35.38 (Asp-C-2), 36.87 (Phe-CH₃), 37.35 (Tyr-CH₃), 41.50 (NCH₃), 41.74 (Gly-C-2), 47.85 (C), 49.18 (Asp-C-3), 55.10 (10), 55.99 (OCH₃), 56.33 (Tyr-CH), 61.42 (12), 64.62 (6), 66.82 (OCH₃), 80.65 (C), 86.00 (4a), 111.67 (2), 115.92 (Tyr-Ph), 121.76 (1), 122.57 (7), 122.75 (p-Ph), 123.82 (Ph), 128.40 (p-Ph), 128.63 (Ph), 128.87 (Ph), 129.23 (Ph), 130.21 (Tyr-Ph), 130.40 (Tyr-Ph), 131.38 (8), 131.78 (12b), 135.47 (i-Ph), 135.94 (i-Ph), 143.97 (4), 146.60 (3), 155.50 (OC=ONH), 155.80 (Tyr-Ph), 167.67 (NHC=O), 168.88 (NHC=O), 170.15 (OC=O), 171.34 (OC=O), 171.68 (OC=O).

6-O-[Boc-Pro-Tyr-Phe-Asp(OBzI)-Gly]-Galanthamine. Yield 90 % (TBHU), 87.3 % (TCTU), mp 114 - 116 °C. 1H-NMR (600 MHz, CDCl₃): δ = 1.40 (s, 9H, CH₉), 1.60 (m, 1H, 9), 1.95 (m, 2H, Pro-H-4), 2.08 (ddd, J=3.2, 5.4, 16.4 Hz, 1H, 5), 2.14 (t, J=13.2 Hz, 1H, 9), 2.16 (m, 2H, Pro-H-3), 2.46 (s, 3H, NCH₃), 2.66 (tdd, J=1.2, 2.4, 16.3 Hz, 1H, 5), 2.78 (dd, J=5.8, 17.1 Hz, 1H, Asp-H-2), 3.00–3.12 (m, 6H, Asp-H, 10,
Phe-CH$_2$ and Tyr-CH$_3$), 3.34 (t, J=13.4 Hz, 1H, 10), 3.40 (m, 2H, Pro-H-5), 3.72 (d, J=15.2 Hz, 1H, 12), 3.85 (s, 3H, OCH$_3$), 3.89 (d, J=5.6, 11.3 Hz, 2H), 4.00 (m, 2H, Gly-CH$_2$), 4.16 (d, J=15.1 Hz, 1H, 12), 4.32 (m, 1H, Phe-CH), 4.48 (m, 1H, Pro-CH), 4.50 (m, 1H, Tyr-CH), 4.54 (bs, 1H, 4a), 4.58 (bs, 1H, Asp-H-3), 4.93 (bs, 1H, Phe-NH), 5.11 (AB system, J=12.1 Hz, 2H, CH$_2$), 5.35 (t, J=4.8 Hz, 1H, 6), 5.72 (d, J=8.4 Hz, 1H, 1), 6.66 (d, J=8.0 Hz, 1H, 2), 6.72 (d, J=7.8 Hz, 2H, Tyr-Ph), 6.76 (bs, 1H, Gly-NH), 6.96 (d, J=7.8 Hz, 2H, Tyr-Ph), 7.12 (d, J=7.6 Hz, 1H, Phe-NH), 7.18-7.40 (m, 10H, Ar).

6-O-[Boc-Leu-Pro-Tyr-Phe-Asp(OBzl)-Gly]-Galanthamine. Yield 51% (TBTU), 59.9% (TCTU), mp 112 - 114°C. 1H-NMR (600 MHz, CDCl$_3$): $\delta$ = 0.94 (d, J=6.6 Hz, 3H, Leu-CH$_3$), 0.97 (d, J=6.6 Hz, 3H, Leu-CH$_3$), 1.33 (m, 2H, Leu-CH$_2$), 1.41 (s, 9H, CH$_3$), 1.60 (m, 1H, 9), 1.75 (m, 1H, Leu-CH), 1.95 (m, 2H, Pro-H-4), 2.08 (ddd, J=3.2, 5.4, 16.4 Hz, 1H, 5), 2.14 (t, J=13.2 Hz, 1H, 9), 2.16 (m, 2H, Pro-H-3), 2.42 (s, 3H, NCH$_3$), 2.66 (tdd, J=1.2, 2.4, 16.3 Hz, 1H, 5), 2.78 (dd, J=5.8, 17.1 Hz, 1H, Asp-H-2), 3.00–3.12 (m, 6H, Asp-H-2), 10, Phe-CH$_3$ and Tyr-CH$_3$), 3.34 (t, J=13.4 Hz, 1H, 10), 3.40 (m, 2H, Pro-H-5), 3.72 (d, J=15.2 Hz, 1H, 12), 3.85 (s, 3H, OCH$_3$), 3.89 (d, J=5.6, 11.3 Hz, 2H), 4.00 (m, 2H, Gly-CH$_2$), 4.16 (d, J=15.1 Hz, 1H, 12), 4.32 (m, 1H, Phe-CH), 4.48 (m, 2H, Pro-CH and Leu-CH), 4.50 (m, 1H, Tyr-CH), 4.54 (bs, 1H, 4a), 4.58 (bs, 1H, Asp-H-3), 4.93 (bs, 1H, Phe-NH), 5.09 (AB system, J=12.1 Hz, 2H, CH$_2$), 5.35 (t, J=4.8 Hz, 1H, 6), 5.72 (d, J=8.4 Hz, 1H, Asp-NH), 5.91 (dd, J=4.8, 10.2 Hz, 1H, 7), 6.28 (d, J=10.3 Hz, 1H, 8), 6.59 (d, J=8.0 Hz, 1H, 1), 6.66 (d, J=8.0 Hz, 1H, 2), 6.72 (d, J=7.8 Hz, 2H, Tyr-Ph), 6.76 (bs, 1H, Gly-NH), 6.96 (d, J=7.8 Hz, 2H, Tyr-Ph), 7.12 (d, J=7.6 Hz, 1H, Phe-NH), 7.18–7.40 (m, 10H, Ar).

CONCLUSIONS

Nine new derivatives of galanthamine are synthesized and characterized. Two of them are final compounds with expected combined activities in respect to β-amiloid peptide aggregation and acetylcholinesterase inhibition. Their activities will be tested by means of in vitro experiments. These compounds are planned to be used as precursors of carbohydrate derivatives.

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