Title: PEPTIDE INHIBITORS OF BACE1 FOR THE TREATMENT OF NEUROLOGICAL DISORDERS

Abstract: The present application presents novel peptide inhibitors of beta amyloid cleavage enzyme, capable to permeate the blood-brain barrier and with no cytotoxic effects. Additionally, this application relates to a new methodology to inhibit BACE1 with peptidic compounds. The peptides are incorporated in pharmaceutical compositions and applied in the treatment of Alzheimer's disease and in other neurological disorders such as Parkinson's disease, Vascular Dementia, Dementia with Lewy bodies, Amyotrophic Lateral Sclerosis, Down's Syndrome, head trauma, and stroke.
DESCRIPTION
"PEPTIDE INHIBITORS OF BACE1 FOR THE TREATMENT OF NEUROLOGICAL DISORDERS"

Technical Field
The present application relates to novel inhibitors of β-amyloid cleavage enzyme (BACE, transmembrane aspartyl protease beta-secretase, beta-site APP cleavage enzyme, memapsin2, BACE-1, EC 3.4.23.46), pharmaceutical compositions containing them, and to a new methodology to inhibit BACE1 and its use in the treatment of Alzheimer's disease (AD) and in other neurological disorders such as Parkinson's disease, Vascular Dementia, Dementia with Lewy bodies, Amyotrophic Lateral Sclerosis, Down's Syndrome, head trauma, and stroke. These pathological conditions have amyloid deposits or act as a risk factor for AD.

Background
Dementia affects a growing number of individuals, mainly aged 60 and over. The number of affected individuals is predicted to be over 100 million in 2050. Alzheimer's disease (AD) is the most common dementia worldwide. It is a chronic debilitating neurodegenerative disease of the central nervous system (specifically brain) that impairs the ability to conduct a normal life since it affects cognitive functions like short-term memory, attention, and language, and patients often show as well disorientation and behavioural problems. The neuropathological hallmarks are the amyloid plaques, which are composed of fibrillar beta amyloid peptide (Aβ), and neurofibrillary tangles mainly composed of abnormal tau that are associated with many neurological disorders commonly called tauopathies (Karren et al., 2011; Selkoe 2011). Amyloid plaques are
unique to AD, however, the presence of Aβ in the brain of patients affected by other neurological conditions namely, Parkinson's disease, Vascular Dementia, Dementia with Lewy bodies, Amyotrophic Lateral Sclerosis, and Down's syndrome has been described.

It is generally accepted that accumulation of Aβ in the brain parenchyma represents an early incident on a cascade of events that ends in neurodegeneration and dementia, and thus, Aβ is considered as the etiologic agent of the disease (Karren et al., 2011; Selkoe 2011; Yan & Vassar 2014). The Aβ has been indicated as being responsible for the hyperphosphorylation of tau, which underlies the formation of neurofibrillary tangles. The formation of Aβ requires the initial cleavage of the β-amyloid protein precursor (APP) by the β-secretase (BACE-1) enzyme followed by the activity of the γ-secretase over the ensuing transmembrane fragment. Normally, depending upon the site of C-terminal processing by γ-secretase, Aβ might have between 39-43 amino acids in length (Karren et al., 2011). These peptides have a strong propensity to adopt beta sheet structures and to oligomerize and form protein insoluble aggregates. The Aβ40 is the predominant product of the amyloidogenic APP processing, but Aβ42 tends to oligomerize and aggregate faster and is the major form of Aβ linked to AD pathogenesis, leading to synaptic and neuronal loss. The APP is also processed by α-secretase, however, the formation of Aβ is abrogated since the cleavage is between its residues 16-17 resulting in non-amyloidogenic peptides.

The BACE1 is the only β-secretase in the brain and its activity is the limiting step on the formation of Aβ (Ohno et al., 2004; Vassar et al., 2009; Luo & Yan, 2010; De
From a drug development point of view, BACE1 has the advantage of being a single molecular entity while γ-secretase is a multiple subunit aspartyl protease with a high degree of heterogeneity (De Strooper et al., 2010). BACE-1 is a type 1 transmembrane aspartic protease that preferentially localizes in acidic intracellular compartments such as the trans-Golgi network and endosomes, where it cleaves APP, a type 1 transmembrane protein as well (Karren et al., 2011; Selkoe 2011; Yan & Vassar 2014). A homologous protein, BACE-2, shares 59% homology with BACE-1 but has different cleavage specificity for APP, cleaving preferentially within the Aβ region and producing non-amyloidogenic peptides. Noteworthy, a 50% reduction of the BACE-1 gene expression was shown to diminished Aβ deposition in the brains of PDAPP;Bacel+/- mice, as well as to protect against synaptic deficits, without compromising normal brain function (McColongue et al., 2007). Also, the recently identified Ala673Thr APP variant which is less efficiently cleaved by BACE-1, leading to a decrease in Aβ production by roughly 20% in individuals that have one copy of the Ala673Thr mutation, confers protection against AD and cognitive decline in elderly individuals (Jonsson et al., 2012; Yan & Vassar 2014), pointing out that a slight BACE1 inhibition might prevent AD. Thus, a careful dosage titration of a potential BACE1 inhibitor allows the decrease in the Aβ production while minimizing mechanism-based adverse effects.

Despite the efforts of the scientific community towards the understanding of AD, at present, an effective therapy is an unmet clinical need (Karran et al., 2011; Selkoe 2011). Although several medicines are commercially available they
only offer symptomatic improvements without preventing the disease progression. Thus, a successful therapy will have an immense impact on the personal, economic and societal burden of this disease.

BACE1 is a key target in AD (Li et al., 2004; Zetterberg et al., 2008; De Strooper et al., 2010; Luo and Yan 2010; Karran et al., 2011; Selkoe 2011; Yan and Vassar, 2014). In this application we disclose new peptide inhibitors of BACE1 comprising an active peptide and a cell penetrating peptide (CPP). Cell penetrating peptides are amino acid sequences used as carriers of other molecules or pharmacological active compounds, named "cargoes". The TAT (48-57) sequence (TAT for transactivator of transcription) is a CPP enriched in positively charged residues that corresponds to the domain responsible for the cell penetrating properties of the TAT protein. The TAT positive charge is crucial to promote receptor-independent cellular uptake, mainly by the endocytic pathway (Chauhan et al., 2007; Jarver et al., 2010). The uptake of a drug by endocytosis takes particular relevance within the context of an AD therapy targeting BACE1 since this protease preferentially localizes in acidic compartments such as the endosomes. Moreover, the activation of endocytosis is a specific and early event in sporadic AD, therefore the use of TAT allows an enhancement of the drug cellular uptake and efficacy in the most common form of AD (Cataldo et al., 2000). The ability of TAT to translocate the plasma membrane facilitates blood-brain barrier (BBB) permeation and cargo delivery to the cytoplasm of cells (Aarts et al., 2002; Borsello et al., 2003; Chauhan et al., 2007; Taghibiglou et al., 2009; Ittner et al., 2010; Jarver et
The stability of CPP-delivery systems in vivo might be compromised by the action of proteolytic enzymes. To overcome proteolytic degradation the peptide sequences submitted in this application might include D-amino acids in their composition. Often, it is used the non-native D retroinverso (RI) sequence of the L-amino acid (native) peptide (Borsello et al., 2003; Snyder et al., 2004; Michod et al., 2009; Vaslin et al., 2011). This double inversion of peptide structure increases the stability and consequently the half-life of biologically active peptides, which allows a decrease of the frequency of drug administration (Michod et al., 2009).

Several BACE1 inhibitors have been developed during the last decade (Chang et al., 2004; Hussain et al., 2007; Gosh et al., 2008; Fukomoto et al., 2010; Chang et al., 2011; May et al., 2015; Thakker et al., 2015) and US 2011/0275619 A1, US 2006/0063717 A1, WO2011/119465, EP 0692490 B1, EP 2172208 A1, WO2009/131974 A1), mainly small molecules or peptides unrelated to the present application. Moreover, contrary to the molecules of the present application, these BACE1 inhibitors are not coupled to a cell penetrating peptide. In general, they have a poor performance regarding oral bioavailability, potency, selectivity and permeability across the BBB, which frequently made them unsuitable drug candidates. At present, there is no BACE1 inhibitor in clinical use although recently one BACE1 inhibitor reached the phase II/III clinical trials, the small molecule MK-8931 (Yan and Vassar, 2014). This small molecule is different from the molecules disclosed in this application,
since it is not a peptidic compound neither a molecule based on the use of peptides including cell penetrating peptide carriers.

On the other hand, there are some ongoing clinical trials with promising candidates breaking down or immunoblocking the amyloid plaques formed throughout the disease progression. However, contrary to an efficient BACE1 inhibition, these strategies target preformed amyloid plaques and do not prevent its formation, which is crucial to control AD, and therefore are not the most suitable to prevent the onset neither the progression of the disease.

Currently the symptomatic therapeutics in use for AD include acetylcholinesterase inhibitors, and a NMDA receptor antagonist (memantine), which allow a better function of the cholinergic and glutamatergic neurotransmission in AD patients. However, these compounds are not disease-modifying drugs and therefore do not prevent or delay disease progression.

Summary
The present application discloses peptide inhibitors of BACE1 comprising an APP amino acid sequence or an APP amino acid derived sequence coupled to a cell penetrating peptide sequence.

In one embodiment, the APP amino acid sequence comprises SEQ ID NO: 1 or SEQ ID NO:2.

In another embodiment, the peptide inhibitors have a homology equal or higher than 70% with the APP amino acid sequence.
In another embodiment, the APP amino acid derived sequence comprises the sequence SEQ ID NO: 3.

In a further embodiment, the cell penetrating peptide sequence comprises the peptide TAT sequence SEQ ID NO: 4 or a TAT variant sequence.

In another embodiment, the amino acids of the APP, APP derived and TAT sequences comprises D-amino acids.

In a further embodiment, the D-amino acids are in retroinverso form.

In another embodiment, the peptide inhibitor of BACE1 comprises one of the following sequences:

SEQ ID NO: 5;
SEQ ID NO: 6;
SEQ ID NO: 7;
SEQ ID NO: 8;
SEQ ID NO: 9; or
SEQ ID NO: 10.

The present application also relates to a method for the inhibition of BACE1 comprising the use of the peptide inhibitors above described.

Further disclosed in the present application is a pharmaceutical composition comprising a peptide inhibitor of BACE1 according to the herein disclosed, optionally together with one or more pharmaceutically acceptable carriers, excipients or diluents.
The present application further relates to the use of the peptides in a method for the treatment or prevention of a disorder associated with amyloid deposits or with a disorder that constitutes a risk factor for dementia.

Additionally, the present application discloses the use of the peptides in a method for the treatment or prevention of Alzheimer's disease, Parkinson's disease, Vascular Dementia, Dementia with Lewy bodies, Amyotrophic Lateral Sclerosis, Down's Syndrome, head trauma, and stroke.

**General description**

The present application discloses new drugs comprising peptides designed to inhibit BACE-1. The peptides include an active peptide (cargo) based on the APP amino acid sequence flanking Asp^{672}, as well as on innovative variations of the APP sequence which have never been used, and a cell penetrating peptide, which in a preferred embodiment is the internalization peptide TAT or a TAT variant sequence that promotes the cellular uptake of the peptide. The drugs are composed of L-amino acids or composed, in part or exclusively, of D-amino acids in retroinverso sequence (D-RI). The peptides are conceived to be used in AD, but can also be used within the context of other neurological disorders characterized by amyloid deposition or that may be a risk factor for AD. The present application provides as well a new methodology to inhibit BACE-1 based on the use of a cell penetrating peptide coupled to a peptide inhibitor of BACE1. This approach to design BACE1 inhibitors has never been addressed yet.

The approach with the peptides of the present application overcomes some of the limitations of the BACE1 inhibitors.
previously developed, since it presents the following ground-breaking features:

i) Facilitated crossing through the BBB and cellular uptake, favouring the bioavailability of the compound in the brain cells where it must act. Indeed, in vivo fluorescence imaging allowed to determine the presence of a peptide herein disclosed (peptide 6) in the mice brain after intraperitoneal administration (i.p.).

ii) Favouring the location of the BACE1 inhibitor in the endosomal compartment, where it co-localizes with BACE1, thus increasing the likelihood to inhibit BACE1.

iii) The activation of endocytosis is a specific and early event in sporadic AD, therefore we achieve an enhancement of the drug cellular uptake and efficacy. Possibly this feature also contributes to the selective targeting of the diseased neurons, decreasing potential side-effects.

iv) The use of a D-RI-peptide increases the half-life of the inhibitor, allowing for a possible reduction in the frequency of administration. Indeed, in vivo fluorescence imaging allowed to determine the presence of a peptide herein disclosed (peptide 6) in the mice brain 24 h after drug administration (i.p.).

v) A careful dosage titration of these BACE1 inhibitors allows the decrease in the Aβ production while minimizing mechanism-based adverse effects since it prevents a total BACE1 inhibition allowing the enzyme to act upon other endogenous substrates.
The above features are particularly relevant when considering a chronic treatment as in the case of AD and other neurodegenerative diseases.

The BACE1 inhibitors included in the present application aim to overcome the caveats of the existing drugs in clinical use which do not act as disease modifying therapies and only moderately improve some of the symptoms of AD. Indeed, they will allow for a delay on the onset and progression of AD since the inhibition of BACE1 will decrease Aβ production thus abrogating the amyloid pathology, which is due to Aβ accumulation in the brain parenchyma.

New peptide inhibitors of BACE1 were developed which overcome some of the limitations of previous BACE1 inhibitors that hindered their clinical use. The new peptide inhibitors of BACE1 herein disclosed comprise both active peptides (based on the molecular structure of the substrate (APP), as well as on new artificial variations of the APP sequence that have never been used) and a cell penetrating peptide, which in a preferred embodiment is the TAT(48-57) sequence (SEQ ID NO:4), or a related variant of the TAT peptide, which facilitates cellular membrane permeation and allows the inhibitor to reach effective concentrations in the central nervous system, where it must act. These new peptides constitute an innovative strategy to design an inhibitor of BACE1.

The active peptide includes the human APP sequence (SEQ ID NO:1) flanking the Met-Asp residues cleavage site. The application provides as well the human APP sequence (SEQ ID NO:2) flanking the Leu-Asp residues cleavage site, present on the APP-
Swedish mutation (APPsw) version, which has an increased affinity for BACE1. Also, it is provided the active peptide of the sequence SEQ ID NO: 3, which is a new artificial variation of the APPswe sequence. The sequences of the active peptides might include a variant sequence with more or less amino acids. The COOH-termini of all peptides are modified by amidation to increase proteolytic resistance.

The number of amino acids in the active peptides was chosen bearing in mind that the BACE1 active site pocket accommodates eight side chains, and considering three or more residues to work as a spacer between the active peptides and cell penetrating peptide sequences. The active peptides should be selectively recognized by BACE1 without interfering with the APP cleavage mediated by $\alpha$-secretase and BACE2. Indeed, it was selected the APP sequence flanking Asp$^{672}$ instead of the sequence flanking the Tyr-Glu$^{682}$ cleavage site also recognized by BACE1, in order to use an APP sequence distant from the cleavage site recognized by $\alpha$-secretase and from the in vivo preferential cleavage sites of BACE2. The preferred cell penetrating peptide sequence is a TAT sequence.

To overcome proteolytic degradation the TAT-APP peptides were designed employing protease-resistant D-amino acids and, to best mimic the structure of the natural peptide, it was considered the use of the retroinverso form (RI) of the D-peptides.

The preferred sequences of the peptides of the present application (both native L-form and D-RI-form) are:

• Peptide 1- TAT-APP: SEQ ID NO: 5;
• Peptide 2 - D-RI-TAT-APP : SEQ ID NO: 6;
• Peptide 3 - TAT-APPsw: SEQ ID NO: 7;
• Peptide 4 - D-RI-TAT-APPsw : SEQ ID NO: 8;
• Peptide 5 - TAT-artificialvariantAPPsw: SEQ ID NO: 9;
• Peptide 6 - D-RI-TAT-artificialvariantAPPsw: SEQ ID NO: 10.

These sequences of the peptides might include related variant sequences, with more or less amino acids, so that the peptides have equal or higher than 70% of homology with the sequences of the peptides included in the present application.

Brief description of drawings
Without intent to limit the disclosure herein, this application presents attached drawings of illustrated embodiments for an easier understanding.

Figure 1 illustrates the BACE1 activity in the presence of the new putative BACE1 inhibitor peptides: PEP#1 (A), PEP#2 (B), PEP#3 (C), PEP#4 (D), PEP#5 (E) and PEP#6 (F). BACE1 activity was determined using the BACE1 Activity Assay Kit (Sigma) based on a FRET assay in which the fluorescence signal enhancement is observed after BACE1 cleavage of the substrate. Briefly, recombinant BACE1 was incubated for 2 hours at 37°C with different concentrations of the inhibitors in the presence of the substrate according to the manufacturer protocol (n=3-5 independent experiments). Fluorescence was recorded in a plate reader fluorometer at 320 nm (excitation) and 405 nm (emission).
Figure 2 illustrates the effect of the new BACE1 inhibitor peptide 5 on Aβ40 and Aβ42 levels in the conditioned medium of Neuroblastoma-2A cells expressing APPswe. A and B) Effect of PEP#5 on secreted amyloid 40/42 levels. Twenty-four hours after plating, neuroblastoma-2A cells constitutively expressing the APPswe (N2A-APPswe cells) were incubated in FBS free medium with 12.5 to 300 µM of the peptide for 24 h, at 37 °C, in a humidified incubator with 5 % CO₂. At the end of the incubation period, the conditioned medium was collected and stored at -80 °C until analysis of Aβ40 and Aβ42 levels by sandwich ELISA (Invitrogen kit), according to manufacturer's protocol. Control cells were subjected to the same experimental procedures in the absence of peptide treatment. The results (pg Aβ per mL) represent the mean ± SEM of n= 2-5 independent experiments performed in duplicate, and are expressed as percentage of control. *p<0.05, ****p<0.0001, significantly different compared to control group, as determined by ANOVA, followed by Dunnet's post test.

Figure 3 illustrates the effect of the new BACE1 inhibitor peptide 6 on Aβ40 and Aβ42 levels in the conditioned medium of Neuroblastoma-2A cells expressing APPswe. A and B) Effect of PEP#6 on secreted amyloid 40/42 levels. Twenty-four hours after plating, neuroblastoma-2A cells constitutively expressing the APPswe (N2A-APPswe cells) were incubated in FBS free medium with 12.5 to 300 µM of the peptide for 24 h, at 37 °C, in a humidified incubator with 5 % CO₂. At the end of the incubation period, the conditioned medium was collected and stored at -80 °C until analysis of Aβ40 and Aβ42 levels by sandwich ELISA (Invitrogen kit), according to manufacturer's protocol. Control cells were subjected to the same experimental
procedures in the absence of peptide treatment. The results (pg Aβ per mL) represent the mean ± SEM of n= 2-5 independent experiments performed in duplicate, and are expressed as percentage of control. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 significantly different compared to control group, as determined by ANOVA, followed by Dunnet's post test.

**Figure 4** illustrates the effect of the new BACE1 inhibitors on Aβ40 and Aβ42 levels in the conditioned medium of Neuroblastoma-2A cells expressing APPswe. (A to D) Effect of PEP#5 and its D-retroinverso form peptide (PEP#6) on secreted amyloid 40/42 levels. Twenty-four hours after plating, neuroblastoma-2A cells constitutively expressing the APPswe (N2A-APPswe cells) were incubated in FBS free medium with 12.5 to 300 µM of the peptides for 24 h, at 37 °C, in a humidified incubator with 5 % CO₂. At the end of the incubation period, the conditioned medium was collected and stored at -80 °C until analysis of Aβ40 and Aβ42 levels by sandwich ELISA (Invitrogen kit), according to manufacturer's protocol. Control cells were subjected to the same experimental procedures in the absence of peptide treatment. The results (pg Aβ per mL) represent the mean ± SEM of n= 2-5 independent experiments performed in duplicate, and are expressed as percentage of control.

**Figure 5** illustrates that the new BACE1 inhibitor peptides PEP#5 and PEP#6 at the IC50 concentration do not change Neuro2A-APPswe cells viability. The cells were incubated with 50 µM (pep#5) or 75 µM (pep#6) of the BACE1 inhibitors, in FBS free culture medium for 24 h, at 37 °C, in a humidified incubator with 5 % CO₂. Untreated cells were used as control. Cell viability was assessed by
determining LDH (Cytotox 96 Non-Radioactive Cytotoxicity Assay, Promega) and Caspases 3/7 (Caspase 3/7- Glo assay, Promega) activity at the end of the incubation period. The caspase3/7 results represent the mean ± SEM of 2-4 independent experiments (n=2 for 15 min and n=4 for the 30-180 min time points) and were normalized to control cells caspase activity (A, B). The LDH results are expressed as percentage of total LDH (C, D) and represent the mean ± SEM of 4-5 independent experiments. The new BACE1 inhibitors at a concentration near the IC50 did not induce N2A-APPswe cells toxicity since no statistical significant differences between control and the experimental treatment conditions were observed, (p>0.05), as determined by ANOVA, followed by Dunnet's post test.

Figure 6 illustrates the new BACE1 inhibitor pep#6 reaches the mice brain and its levels remain high until 24 h after administration in 3xTg-AD mice. Four months old 3xTg-AD mice were treated with a single i.p. injection of 10 mg/kg of PEP#6 labelled with the fluorescent dye Cy5.5. In vivo fluorescence imaging of the brain was performed immediately before treatment and 1-48 hours post-drug administration using the Perkin Elmer IVIS Lumina XR equipment (A). For that purpose mice were anaesthetized and non-invasive in vivo brain fluorescence imaging performed in animals submitted to depilation in the brain area. Quantification of brain signal was determined by measuring radiant efficiency in a specified region of interest (ROI). For each mouse, the ROI was delimited in the brain area and the integrated density of the signal [(p/sec/cm²/sr)/µW/cm²)] determined by using the Living Image version 4.5 (Perkin Elmer) software. The graph represents the mean ± SEM of n=3 mice per group (B). Statistical analysis was performed by t
**p<0.0001 significantly different compared to time zero.

**Figure 7** illustrates that the new BACE1 inhibitors PEP#5 and PEP#6 decrease plasmatic $\alpha\beta 40/42$ levels in 3xTg-AD mice. A single i.p. treatment with the new BACE1 inhibitors PEP#5 and PEP#6 reduced plasma $\alpha\beta 40$ (A) and $\alpha\beta 42$ (B) levels in 4-month old 3xTg-AD mice. Twenty-four hours after administration, mice were sacrificed with anesthesia followed by cervical dislocation and the blood was collected into EDTA-treated tubes. After centrifugation, the plasma was collected and stored at -80 °C until analysis of $\alpha\beta 40$ and $\alpha\beta 42$ levels by sandwich ELISA (Invitrogen kit), according to manufacturer's protocol. Control mice were injected with the vehicle (saline) in the absence of peptides. The results (pg $\alpha\beta$ per mL) represent the mean ± SEM of n=3 mice per group. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 significantly different compared to control group, as determined by ANOVA, followed by Dunnet's post test.

**Figure 8** illustrates that the new BACE1 inhibitors PEP#5 and PEP#6 decrease brain-soluble $\alpha\beta 40/42$ levels in 3xTg-AD mice. A single i.p. treatment with new BACE1 inhibitors PEP#5 and PEP#6 reduced brain-soluble $\alpha\beta 40$ and $\alpha\beta 42$ levels in 4-month old 3xTg-AD mice. Twenty-four hours after administration, mice were sacrificed with anesthesia followed by cervical dislocation and the brain was collected and stored at -80 °C. Brain lysates in RIPA buffer were used to analyse $\alpha\beta 40$ and $\alpha\beta 42$ levels by sandwich ELISA (Invitrogen kit), according to manufacturer's protocol. Control mice were injected with the vehicle (saline) in the absence of peptides. Lysate
protein level was assessed by the BCA method. The results (pg Aβ per mg protein) represent the mean ± SEM of n=3 mice per group. ***p<0.001 significantly different compared to control, as determined by ANOVA, followed by Dunnet's post test.

**Figure 9** illustrates the new BACE1 inhibitor pep#5 decreases εAPPβ brain levels whereas sAPPα levels remain unchanged in 3xTg-AD mice. A single i.p. treatment with the new BACE1 inhibitors PEP#5 decreased brain εAPPβ without altering sAPPα levels in 4-month old 3xTg-AD mice. Twenty-four hours after administration, mice were sacrificed with anesthesia followed by cervical dislocation and the brain was collected and stored at -80 °C. Brain lysates in RIPA buffer were used to determine εAPPβ (A), sAPPα (B), and actin (loading control) levels through Western blotting analysis. Lysate protein was assessed by the BCA method. Control mice were injected with the vehicle (saline) in the absence of peptides. Representative Western blots for each protein are presented above the graph. The results represent the mean ± SEM of, at least, n=5-9 mice per group, and are expressed as percentage of control. Statistical analysis was performed by t test. *p<0.05 and ***p<0.001 significantly different compared to control.

**Figure 10** illustrates the new BACE1 inhibitor pep#6 decreases εAPPβ brain levels whereas sAPPα levels remain unchanged in 3xTg-AD mice. A single i.p. treatment with the new BACE1 inhibitors PEP#6 decreased brain εAPPβ without altering sAPPα levels in 4-month old 3xTg-AD mice. Twenty-four hours after administration, mice were sacrificed with anesthesia followed by cervical dislocation and the brain was collected and stored at -80 °C. Brain lysates in RIPA
buffer were used to determine εAPPβ (A), sAPPα (B), and actin (loading control) levels through Western blotting analysis. Lysate protein was assessed by the BCA method. Control mice were injected with the vehicle (saline) in the absence of peptides. Representative Western blots for each protein are presented above the graph. The results represent the mean ± SEM of, at least, n= 3-5 mice per group, and are expressed as percentage of control. Statistical analysis was performed by t test. *p<0.05 and ***p<0.001 significantly different compared to control.

Figure 11 illustrates the new BACE1 inhibitor pep#5 does not decrease brain APP and BACE1 levels in 3xTg-AD mice. A single i.p. treatment with the new BACE1 inhibitor PEP#5 does not change brain APP and BACE1 levels in 4-month old 3xTg-AD mice. Twenty-four hours after administration, mice were sacrificed with anesthesia followed by cervical dislocation and the brain was collected and stored at -80 °C. Brain lysates in RIPA buffer were used to determine APP (A), BACE1 (B) and actin (loading control) levels through Western blotting analysis. Lysate protein was assessed by the BCA method. Control mice were injected with the vehicle (saline) in the absence of peptides. Representative Western blots for each protein are presented above the graph. The results represent the mean ± SEM of, at least, n= 6-10 mice per group, and are expressed as percentage of control. Statistical analysis was performed by t test, no significant differences were found.

Figure 12 illustrates the new BACE1 inhibitor pep#6 does not decrease brain APP and BACE1 levels in 3xTg-AD mice. A single i.p. treatment with the new BACE1 inhibitor PEP#6 does not change brain APP and BACE1 levels in 4-month old
3xTg-AD mice. Twenty-four hours after administration, mice were sacrificed with anesthesia followed by cervical dislocation and the brain was collected and stored at -80 °C. Brain lysates in RIPA buffer were used to determine APP (A), BACE1 (B) and actin (loading control) levels through Western blotting analysis. Lysate protein was assessed by the BCA method. Control mice were injected with the vehicle (saline) in the absence of peptides. Representative Western blots for each protein are presented above the graph. The results represent the mean ± SEM of, at least, n= 4 mice per group, and are expressed as percentage of control. Statistical analysis was performed by t test, no significant differences were found.

**Detailed description**

In the present disclosure are described new peptide inhibitors of BACE1 which overcome some of the limitations of previous BACE1 inhibitors that hindered their clinical use.

The new peptide inhibitors of BACE1 comprise an active peptide based on the molecular structure of the substrate (APP), as well as on new artificial variations of the APP sequence that have never been used, and a cell penetrating peptide, preferably the TAT (48-57) sequence (SEQ ID NO:4), or a related variant of the TAT peptide, which facilitates cellular membrane permeation and allows the inhibitor to reach effective concentrations in the central nervous system, where it must act. These new peptides constitute an innovative strategy to design an inhibitor of BACE1 that has never been addressed before. The COOH-termini of all the peptides are modified by amidation to increase proteolytic resistance.
The active peptides include the sequence SEQ ID NO: 3, which is a new artificial variation of the APPswe sequence that has never been used before.

The active peptides include the human APP sequence (SEQ ID NO:1) flanking the Met-Asp^{672} cleavage site. The present application provides as well the human APP sequence (SEQ ID NO:2) flanking the Leu-Asp^{672} cleavage site, present on the APP-Swedish mutation (APPsw) version, which has an increased affinity for BACE1.

To overcome proteolytic degradation we designed TAT-APP peptides employing protease-resistant D-amino acids and, to best mimic the structure of the natural peptide, we considered to use the retroinverso form (RI) of the D-peptides.

The number of amino acids in the active peptide was chosen bearing in mind that the BACE1 active site pocket accommodates eight side chains, and considering three or more residues to work as a spacer between the APP and TAT sequences.

The active peptides should be selectively recognized by BACE1 without interfering with the APP cleavage mediated by a-secretase and BACE2. It was selected the APP sequence flanking Asp^{672} instead of the sequence flanking the Tyr-Glu^{682} cleavage site also recognized by BACE1, in order to use an APP sequence distant from the cleavage site recognized by a-secretase and from the in vivo preferential cleavage sites of BACE2.
Therefore, the disclosed peptides present the following functional innovative features considering previous BACE1 inhibitors:

- Facilitation of the crossing through the BBB and the cellular uptake, favouring the bioavailability of the compound in the brain cells where it must act. Indeed, in vivo fluorescence imaging allowed to determine the presence of a peptide herein disclosed (peptide 6) in the mice brain after intraperitoneal administration (i.p.).

- Favouring the location of the BACE1 inhibitor in the endosomal compartment, where it co-localize with BACE1, thus increasing the likelihood to inhibit BACE1.

- The activation of endocytosis is a specific and early event in sporadic AD, allowing for an enhancement of the peptides cellular uptake and efficacy. Possibly this feature also contributes to the selective targeting of the diseased neurons, decreasing possible side-effects.

- The use of a D-RI-peptide increases the half-life of the inhibitor, allowing a reduction in the frequency of administration. Indeed, in vivo fluorescence imaging allowed to determine the presence of a peptide herein disclosed (peptide 6) in the mice brain 24 h after drug administration (i.p.). An important feature of TAT is that it displays low toxicity. The use of TAT coupled to a BACE1 inhibitor based on the APP amino acid sequence, or in a variation of that sequence, has never been addressed in AD.
- On the other hand, a careful dosage titration of a potential BACE1 inhibitor allows decreasing Aβ production while minimizing mechanism-based adverse effects, since it will prevent a total BACE1 inhibition allowing the enzyme to act upon other cellular substrates.

The above features are particularly relevant when considering a chronic treatment as in the case of AD and other neurodegenerative diseases.

The experimental data that support the disclosed peptides as new BACE1 inhibitors is described below.

Firstly, it was determined the efficacy of the peptides to inhibit BACE1 activity using a cell free in vitro assay (BACE1 Activity Assay Kit, Sigma) following the manufacturer instructions. The in vitro assays allowed to determine the peptides IC50 (Table 1, Figure 1).

**Table 1.** Peptides IC50 determined using a cell free assay system. The IC50 refers to the peptide concentration that inhibits BACE1 activity by 50%.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IC50 (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide 1</td>
<td>8.316 x 10⁻⁷</td>
</tr>
<tr>
<td>Peptide 2</td>
<td>7.64 x 10⁻⁶</td>
</tr>
<tr>
<td>Peptide 3</td>
<td>1.485 x 10⁻⁶</td>
</tr>
<tr>
<td>Peptide 4</td>
<td>3.713 x 10⁻⁶</td>
</tr>
<tr>
<td>Peptide 5</td>
<td>9.803 x 10⁻⁶</td>
</tr>
<tr>
<td>Peptide 6</td>
<td>6.490 x 10⁻⁷</td>
</tr>
</tbody>
</table>
Thereafter, the peptides 5 and 6 were selected to initiate the studies in a cellular model of AD, the neuroblastoma cell line Neuro-2A overexpressing APPswe (N2A-APPswe), and the ability of the new BACE1 inhibitors to reduce endogenous Aβ40 and Aβ42 production, as assessed by sandwich ELISA, was determined. It was observed that, after a 24 h incubation period, 100 µM peptide 5 (Figure 2) inhibited maximally Aβ40 and Aβ42 formation by 74.0 ± 4.51 % and 84.2 ± 6.6 % while 100 µM peptide 6 (Figure 3) inhibited maximally Aβ40 and Aβ42 formation by 73.8 ± 7.7 % and 74.1 ± 5.5 %, respectively. The dose response curves (Figure 4) allowed for the determination of the IC50 for peptide 5 and peptide 6 in N2A-APPswe cells (Table 2).

Table 2. Peptides IC50 determined in a cellular assay. The IC50 refers to the peptide concentration that inhibits endogenous Aβ40 and Aβ42 production in N2A-APPswe cells by 50%.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Aβ40 - IC50 (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide 5</td>
<td>6.408 x 10⁻⁵</td>
</tr>
<tr>
<td>Peptide 6</td>
<td>7.562 x 10⁻⁵</td>
</tr>
<tr>
<td>Peptide 4</td>
<td>6.408 x 10⁻⁵</td>
</tr>
<tr>
<td>Peptide 5</td>
<td>7.823 x 10⁻⁵</td>
</tr>
</tbody>
</table>

Importantly, the incubation for 24 h with the new BACE1 inhibitors (peptide 5 and 6), at concentrations close to the IC50, did not induce caspase-3 and caspase-7 activation (Figure 5 A and B), neither increased the release of LDH (Figure 5 C and D). These results indicate that, at the IC50 concentration, peptides 5 and 6 did not cause apoptosis neither necrosis of N2A-APPswe cells. Thus, the new BACE1 inhibitors reduced N2A-APPswe cells endogenous Aβ production in the absence of a cytotoxic effect.
In vivo studies were performed using a triple transgenic mouse model of AD (3xTg-AD) harboring PS1M146V, APPswe, and TauP301L transgenes (Oddo et al., 2003) to evaluate whether the new BACE1 inhibitors, peptide 5 and peptide 6, ameliorate Aβ pathology.

To demonstrate that the peptides herein disclosed have the potential to reach the brain we labelled peptide 6 with the fluorescent dye Cy 5.5 and performed an in vivo fluorescence imaging study in 3xTg-AD mice injected (i.p.) with 10 mg/kg of the compound. As shown in Figure 6, peptide 6 has the ability to cross the blood brain barrier and to penetrate the mouse brain. Its brain levels remained high until 24 h after peptide administration and at 48 h the compound brain levels were identical to basal. Afterwards the brain and plasma levels of Aβ40 and Aβ42 were assessed 24 h after a single administration (i.p. injection) of the compounds in 4 months old 3xTg-AD mice. The results pointed out that both peptides at 1.25 mg/kg reduced plasma Aβ40 by 30% and, at a dose of 5.0 mg/kg, reduced plasma Aβ42 by at least 30 %, as assessed by sandwich ELISA (Figure 7A, B). Regarding brain soluble Aβ levels, peptide 5 and 6 at 1,25 mg/kg reduced Aβ40 by 50% and 28 %, respectively, while both peptides reduced Aβ42 by about 28 % (Figure 8A e B).

Accordingly, peptide 5 decreased the soluble APP cleavage fragment εAPPβ that ensues from APP cleavage by BACE1 by about 11 % (Figure 9A), without altering the levels of the sAPPα fragment, which arises from APP cleavage by α-secretase (Figure 9 B), as assessed by western blot. Likewise, peptide 6 decreased εAPPβ levels by about 24 %
whereas the amount of the sAPPα fragment was not significantly changed (Figure 10 A and B). These results indicate that peptides 5 and 6 selectively inhibit BACE1 activity in 3xTg-AD mice without inhibiting the activity of α-secretase.

Moreover, it was observed that the administration of peptides 5 and 6 did not decrease APP (Figure 11 A and Figure 12 A) and BACE1 protein levels (Figure 11 B and Figure 12 B), which demonstrates that the reduction in Aβ and εAPPβ levels is due to the inhibition of BACE1 activity and not because of a drop on the enzyme or its substrate levels.

REFERENCES

- Bach et al., 2012, A high-affinity, dimeric inhibitor of PSD-95 bivalently interacts with PDZ1-2 and protects against ischemic brain damage. PNAS 109: 3317-3322.
- Chang et al., 2011, Secretase inhibitor GRL-8234 rescues age-related cognitive decline in APP transgenic mice. FASEB J. 25, 775-784.

- Li et al., 2004, Amyloid β peptide load is correlated with increased β-secretase activity in sporadic Alzheimer's disease patients. PNAS 101: 3632-37.
- Plattner et al., 2014, Memory Enhancement by Targeting Cdk5 Regulation of NR2B. Neuron 81, 1070-1083
- Thakker et al., 2015, Centrally delivered BACE1 inhibitor activates microglia, and reverses amyloid pathology and cognitive deficit in aged Tg2576 mice. J Neurosci 35:6931-6936.
- Tu et al., 2010, DAPK1 Interaction with NMDA Receptor NR2B Subunits Mediates Brain Damage in Stroke, Cell 140: 222-234.

SEQUENCE LISTING

<110> Universidade de Coimbra
<120> PEPTIDE INHIBITORS OF BACE1 FOR THE TREATMENT OF NEUROLOGICAL DISORDERS
<130> PPI 51891/15
<160> 10
<170> PatentIn version 3.5

<210> 1
<211> 11
<212> PRT
<213> Homo sapiens
<400> 1
Glu Ile Ser Glu Val Asn Leu Asp Ala Glu Phe
1  5  10

<210> 2
<211> 11
<212> PRT
<213> Homo sapiens
<400> 2
Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe
1  5  10

<210> 3
<211> 11
<212> PRT
<213> Artificial Sequence
<220>
<223> Artificial sequence
<400> 3
Leu Glu He Ala Val Ser Asn Glu Phe Glu Asp
1  5  10

<210> 4
<211> 10
<212> PRT
<213> Human immunodeficiency virus
<400> 4
Gly Arg Lys Lys Arg Gin Arg Arg Arg
1 5 10

<210> 5
<211> 21
<212> PRT
<213> Artificial Sequence
<220>
<223> Artificial sequence
<400> 5
Gly Arg Lys Lys Arg Gin Arg Arg Arg Glu lie Ser Glu Val Lys
1 5 10 15
Met Asp Ala Glu Phe
20

<210> 6
<211> 21
<212> PRT
<213> Artificial Sequence
<220>
<223> Artificial sequence
<400> 6
Phe Glu Ala Asp Met Lys Val Glu Ser Ile Glu Arg Arg Arg Gin Arg
1 5 10 15
Arg Lys Lys Arg Gly
20

<210> 7
<211> 21
Artificial Sequence

Gly Arg Lys Lys Arg Arg Gin Arg Arg Arg Glu lie Ser Glu Val

Leu Asp Ala Glu Phe

Phe Glu Ala Asp Leu Asn Val Glu Ser lie Glu Arg Arg Arg Gin

Arg Lys Lys Arg Gly

Gly Arg Lys Lys Arg Arg Gin Arg Arg Arg Leu Glu lie Ala Val Ser
Asn Glu Phe Glu Asp

1 5

<210> 10

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Artificial sequence

<400> 10

Asp Glu Phe Glu Asn Ser Val Ala lie Glu Leu Arg Arg Arg Gin Arg

Arg

1 5

<210> 10

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Artificial sequence

<400> 10

Asp Glu Phe Glu Asn Ser Val Ala lie Glu Leu Arg Arg Arg Gin Arg

Arg

1 5

<210> 10

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Artificial sequence

<400> 10

Asp Glu Phe Glu Asn Ser Val Ala lie Glu Leu Arg Arg Arg Gin Arg

Arg
CLAIMS

1. A peptide inhibitor of BACE1 comprising an APP amino acid sequence or an APP amino acid derived sequence coupled to a cell penetrating peptide sequence.

2. The peptide inhibitor of BACE1 according to claim 1, wherein the APP amino acid sequence comprises SEQ ID NO: 1 or SEQ ID NO:2.

3. The peptide inhibitor of BACE1 according to the previous claim, comprising a homology equal or higher than 70% with the APP amino acid sequence.

4. The peptide inhibitors of BACE1 according to the claim 1, wherein the APP amino acid derived sequence comprises the artificial sequence SEQ ID NO: 3.

5. The peptide inhibitors of BACE1 according to claim 1, wherein the cell penetrating peptide sequence comprises the peptide TAT sequence SEQ ID NO: 4 or a TAT variant sequence or other suitable cell penetrating peptide.

6. The peptide inhibitors of BACE1 according to the previous claims, wherein the amino acids of the APP and TAT sequences comprise D-amino acids.

7. The peptide inhibitor of BACE1 according to the previous claim, wherein the D-amino acids are in retroinverso form.
8. The peptide inhibitor of BACE1 according to the previous claims, wherein it comprises one of the following sequences:
SEQ ID NO: 5;
SEQ ID NO: 6;
SEQ ID NO: 7;
SEQ ID NO: 8;
SEQ ID NO: 9; or
SEQ ID NO: 10.

9. A method for the inhibition of BACE1 comprising the use of the peptide inhibitor of claims 1-8.

10. A pharmaceutical composition comprising a peptide according to any one of claims 1 to 8 optionally together with one or more pharmaceutically acceptable carriers, excipients or diluents.

11. A peptide according to any one of claims 1 to 8 for use in a method for the treatment or prevention of a disorder associated with amyloid deposits or with a disorder that constitutes a risk factor for dementia.

12. A peptide according to any one of claims 1 to 8 for use in a method for the treatment or prevention of Alzheimer's disease, Parkinson's disease, Vascular Dementia, Dementia with Lewy bodies, Amyotrophic Lateral Sclerosis, Down's Syndrome, head trauma, and stroke.
1. A peptide inhibitor of BACE1 comprising:
   - an APP amino acid sequence, or
   - an APP amino acid sequence coupled to a cell penetrating peptide sequence,
   wherein the amino acids of the both APP sequences, isolated and coupled, comprise D-amino acids and the sequence is in the retroinverso form,
   or
   - an APP amino acid derived sequence, or
   - an APP amino acid derived sequence coupled to a cell penetrating peptide sequence.

2. The peptide inhibitors of BACE1 according to the previous claim 1, wherein the amino acids of the APP amino acid derived sequences, isolated or coupled, comprise D-amino acids.

3. The peptide inhibitors of BACE1 according to the previous claim 2, wherein the D-amino acids sequences are in the retroinverso form.

4. The peptide inhibitors of BACE1 according to claim 1, wherein the APP amino acid sequence comprises SEQ ID NO:1 or SEQ ID NO:2.

5. The peptide inhibitors of BACE1 according to the claim 1, wherein the APP amino acid derived sequence comprises the artificial sequence SEQ ID NO:3.

6. The peptide inhibitors of BACE1 according to claim 1, wherein the cell penetrating peptide sequence comprises
the peptide TAT sequence SEQ ID NO: 4 or a TAT variant sequence or other suitable cell penetrating peptide.

7. The peptide inhibitors of BACE1 according to the previous claims 1 and 6, wherein the amino acids of the TAT sequences comprise D-amino acids.

8. The peptide inhibitors of BACE1 according to the previous claim 7, wherein the D-amino acids sequence is in the retroinverso form.

9. The peptide inhibitors of BACE1 according to the previous claims, wherein it comprises one of the following sequences:
   SEQ ID NO: 5;
   SEQ ID NO: 6;
   SEQ ID NO: 7;
   SEQ ID NO: 8;
   SEQ ID NO: 9; or
   SEQ ID NO: 10.

10. The peptide inhibitors of BACE1 according to the previous claims, comprising a homology equal or higher than 70% with the APP amino acid sequences SEQ ID NO:1 to SEQ ID NO:3 and SEQ ID NO:5 to SEQ ID NO:10, wherein the amino acids comprise L-amino acids or D-amino acids.

11. The peptide inhibitors of BACE1 according to the previous claim 10, wherein the D-amino acids sequences are in the retroinverso form.

13. A pharmaceutical composition comprising a peptide according to any one of claims 1 to 11 optionally together with one or more pharmaceutically acceptable carriers, excipients or diluents.

14. The pharmaceutical composition according to the previous claim for use in a method for the treatment or prevention of a disorder associated with amyloid deposits or with a disorder that constitutes a risk factor for dementia.

15. The pharmaceutical composition according to the previous claim 13 for use in a method for the treatment or prevention of Alzheimer's disease, Parkinson's disease, Vascular Dementia, Dementia with Lewy bodies, Amyotrophic Lateral Sclerosis, Down's Syndrome, head trauma, and stroke.

16. A peptide according to any one of claims 1 to 11 for use in a method for the treatment or prevention of a disorder associated with amyloid deposits or with a disorder that constitutes a risk factor for dementia.

17. A peptide according to any one of claims 1 to 11 for use in a method for the treatment or prevention of Alzheimer's disease, Parkinson's disease, Vascular Dementia, Dementia with Lewy bodies, Amyotrophic Lateral Sclerosis, Down's Syndrome, head trauma, and stroke.
Novelty and Inventive Step (Art. 33(2) and Art. 33(3) PCT)

In view of the examiner's novelty and inventive step objection considering D1-D3, the applicant amended claim 1, limiting the APP amino acid sequences, isolated or coupled, to a sequence with D-amino acids and in the retroinverso form, which are new and inventive, since they are not mentioned or suggested in any of the prior art documents, as peptides inhibitors of BACE1.

The dependent claims 2-11 are used to define preferred embodiments of the independent claim 1, and therefore they are also new and inventive. Indeed, according to the Guidelines PCT international search and preliminary examination guidelines, Part IV, Chapter 15, point 15.23, it is recognized that if the independent claim is new and inventive, no need to investigate the novelty and inventive step of the dependent claims.

Independent claims 12, 13, 16 and 17 refer to the specific use of the peptides inhibitors of BACE1 and to a pharmaceutical compositions comprising the the peptides inhibitors of BACE1, as defined in independent claims 1 and its dependent claims 2-11, therefore these claims are also new and inventive, as their dependent claims.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
A

Soluble Hu Aβ1-40 (pg/mL)

PEP#5  PEP#6

Vehicle  1.25 mg/kg  2.50 mg/kg  5.00 mg/kg

B

Soluble Hu Aβ1-42 (pg/mL)

PEP#5  PEP#6

Vehicle  1.25 mg/kg  2.50 mg/kg  5.00 mg/kg

Figure 7
Figure 8
Figure 9
Figure 10
Figure 11
Figure 12
A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K14/47 C12N9/64

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practicable, search terms used)

EPO-Internal, Sequence Search, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>the whole document - &amp; DATABASE Geneseq [Online] 10 September 2015 (2015-09-10),</td>
<td>1-3, 5-12</td>
</tr>
<tr>
<td></td>
<td>&quot;Human APP peptidomimetics HIV-1 Tat protein transduction domain, SEQ ID 11.&quot;, XP002756102, retrieved from EBI accession no. GSP: BCC62806 Database accession no. BCC62806 sequence</td>
<td></td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search

6 April 2016

Date of mailing of the international search report

15/04/2016

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer

Mabi't, Helène
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>- &amp; DATABASE Geneseq [Online]</td>
<td>10 September 2015 (2015-09-10), &quot;Mutant human APPswe peptide-HIV-1 Tat protein transduction domain, SEQ 3.&quot;, XP002756103, retrieved from EBI access no. GSP: BCC62798 Database access no. BCC62798 sequence</td>
<td></td>
</tr>
</tbody>
</table>

6 May 2004 (2004-05-06), "N-terminal APP peptide C-terminal fragment, SEQ 1D 211.", XP002756104, retrieved from EBI access no. GSP: ADJ71548 Database access no. ADJ71548 sequence

---
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>WO 2015106098 A1</td>
<td>16-07-2015</td>
<td>NONE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2091566 A1</td>
<td>26-08-2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2010510254 A</td>
<td>02-04-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2010292157 A1</td>
<td>18-11-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2008061373 A1</td>
<td>29-05-2008</td>
</tr>
<tr>
<td>US 2010285988 A1</td>
<td>11-11-2010</td>
<td>US 2010285988 A1</td>
<td>11-11-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2011257027 A2</td>
<td>20-10-2011</td>
</tr>
<tr>
<td>WO 2004013172 A2</td>
<td>12-02-2004</td>
<td>AU 2003250102 A1</td>
<td>23-02-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2487528 A1</td>
<td>12-02-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2006513259 A</td>
<td>20-04-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2010091572 A</td>
<td>22-04-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2005175626 A1</td>
<td>11-08-2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2008299111 A1</td>
<td>04-12-2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2012258872 A1</td>
<td>11-10-2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2004013172 A2</td>
<td>12-02-2004</td>
</tr>
</tbody>
</table>