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(54) **Title**: BACE1 INHIBITOR PEPTIDES

(57) **Abstract**: The present invention relates to dual-site BACE1 inhibitors, their manufacture, pharmaceutical compositions containing them and their use as therapeutically active substances. The active compounds of the present invention are useful in the therapeutic and/or prophylactic treatment of e.g. Alzheimer's disease.

BACE1 INHIBITOR PEPTIDES

The present invention is concerned with peptides having dual BACE1 inhibitory properties, their manufacture, pharmaceutical compositions containing them and their use as therapeutically active substances.

Technical Field

The present compounds have Asp2 (β-secretase, BACE1 or Memapsin-2) inhibitory activity and may therefore be used in the therapeutic and/or prophylactic treatment of diseases and disorders characterized by elevated β-amyloid levels and/or β-amyloid oligomers and/or β-amyloid plaques and further deposits, particularly Alzheimer's disease.

Background Art

Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system and the leading cause of a progressive dementia in the elderly population. Its clinical symptoms are impairment of memory, cognition, temporal and local orientation, judgment and reasoning but also severe emotional disturbances. There are currently no treatments available which can prevent the disease or its progression or stably reverse its clinical symptoms. AD has become a major health problem in all societies with high life expectancies and also a significant economic burden for their health systems.

The BACE1 enzyme is responsible for one of the proteolytic cleavages of the APP protein that contributes to the generation of the Alzheimer's disease-associated Aβ-peptide. Retarding or stopping the production of Aβ-peptide through inhibition of the BACE1 enzyme is a promising therapeutic concept.

Active site-directed BACE1 inhibitors are described in e.g. WO2006/002907 and exosite-directed (catalytic domain) BACE1 inhibitors are described in e.g. Kornacker et al., Biochemistry 2005, 44, 11567-73. Further Linning, Organic & Biomolecular Chemistry 10(41), 2012, p8216, WO2005097199, US2007149763 and WO2013056054 describe Bace1 inhibitors with peptidic structures.

Detailed description of the invention

Object of the present invention is dual-site BACE1 inhibitor, binding to both, the enzymatic active site and the catalytic domain of the BACE enzyme, the preparation of the above mentioned compounds, medicaments containing them and their manufacture as well as the use of the above mentioned compounds in the therapeutic and/or prophylactic treatment of diseases and disorders which are associated with inhibition of BACE1 activity, such as

Alzheimer's disease. Furthermore, the formation, or formation and deposition, of β-amyloid plaques in, on or around neurological tissue (e.g., the brain) are inhibited by the present compounds by inhibiting the Aβ production from APP or an APP fragment.

The following definitions of the general terms used in the present description apply irrespectively of whether the terms in question appear alone or in combination with other groups.

Amino Acid	3-Letter	1-Letter
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Table 1: amino acid abbreviations used herein

Table 2: amino acid abbreviations used herein

The term "Sta" stands for statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (CAS 49642-07-1).

The term "MetSta" stands for (3*S*,4*S*)-4-amino-3-hydroxy-6-methylthiohexanoic acid (CAS n/a), (CAS of Fmoc protected: 268542-18-3).

The term "PEG(4)" stands for 15-amino-4,7,10,13,tetraoxapentadecanoic acid (CAS: n/a), (CAS of Fmoc protected: 557756-85-1).

The term Leu*Ala stands for the "Tang" hydroxyethylene dipeptide isostere (reference: A. K.Ghosh, D. Shin, D. Downs, G. Koelsch, X. Lin, J. Ermolieff and J. Tang, J. Am. Chem. Soc., 2000, 122, 3522).

The term "PEG2" stands for 8-amino-3,6-dioxaoctanoic acid (CAS: n/a), (CAS of Fmoc protected: 166108-71-0)

The term "PEG3" stands for 12-amino-4,7,10-trioxadodecanoic acid (CAS: n/a), CAS of Fmoc protected: 867062-95-1)

The term "pharmaceutically acceptable salts" refers to salts that are suitable for use in contact with the tissues of humans and animals. Examples of suitable salts with inorganic and organic acids are, but are not limited to acetic acid, citric acid, formic acid, fumaric acid, hydrochloric acid, lactic acid, maleic acid, malic acid, methane-sulfonic acid, nitric acid, phosphoric acid, p-toluenesulphonic acid, succinic acid, sulfuric acid, sulphuric acid, tartaric acid, trifluoroacetic acid (TFA) and the like. A specific salt is trifluoroacetate.

The terms "pharmaceutically acceptable carrier" and "pharmaceutically acceptable auxiliary substance" refer to carriers and auxiliary substances such as diluents or excipients that are compatible with the other ingredients of the formulation.

The term "pharmaceutical composition" encompasses a product comprising specified ingredients in pre-determined amounts or proportions, as well as any product that results, directly or indirectly, from combining specified ingredients in specified amounts. Preferably it encompasses a product comprising one or more active ingredients, and an optional carrier comprising inert ingredients, as well as any product that results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients.

The term "half maximal inhibitory concentration" (IC_{50}) denotes the concentration of a particular compound required for obtaining 50% inhibition of a biological process in vitro. IC_{50} values can be converted logarithmically to pIC_{50} values (-log IC_{50}), in which higher values indicate exponentially greater potency. The IC_{50} value is not an absolute value but depends on experimental conditions e.g. concentrations employed. The IC_{50} value can be converted to an absolute inhibition constant (Ki) using the Cheng-Prusoff equation (Biochem. Pharmacol. (1973) 22:3099). The term "inhibition constant" (Ki) denotes the absolute binding affinity of a particular inhibitor to a receptor. It is measured using competition binding assays and is equal to the concentration where the particular inhibitor would occupy 50% of the receptors if no competing ligand (e.g. a radioligand) was present. Ki values can be converted logarithmically to pKi values (-log Ki), in which higher values indicate exponentially greater potency.

"Therapeutically effective amount" means an amount of a compound that, when administered to a subject for treating a disease state, is sufficient to effect such treatment for the disease state. The "therapeutically effective amount" will vary depending on the compound, disease state being treated, the severity or the disease treated, the age and relative health of the subject, the route and form of administration, the judgment of the attending medical or veterinary practitioner, and other factors.

The term "as defined herein" and "as described herein" when referring to a variable incorporates by reference the broad definition of the variable as well as preferred, more preferred and most preferred definitions, if any.

The terms "treating", "contacting" and "reacting" when referring to a chemical reaction means adding or mixing two or more reagents under appropriate conditions to produce the indicated and/or the desired product. It should be appreciated that the reaction which produces the indicated and/or the desired product may not necessarily result directly from the combination of two reagents which were initially added, i.e., there may be one or more intermediates which are produced in the mixture which ultimately leads to the formation of the indicated and/or the desired product.

The invention also provides pharmaceutical compositions, methods of using, and methods of preparing the aforementioned compounds.

All separate embodiments may be combined.

Present invention relates to a dual-site BACE1 inhibitor, binding to both, the enzymatic active site and the catalytic domain of the BACE1 enzyme.

The exosite motifs as well as the active site inhibitors containing statine-type transition state mimetic alone do not show BACE1 inhibition.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein, binding to both, the enzymatic active site and the catalytic domain of the BACE enzyme, whereby the exosite inhibitory part (A') is connected to the active-site inhibitory part (B') of said BACE1 inhibitor by a linker (L'), or a pharmaceutically acceptable salt thereof.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein, wherein L' is selected from the group consisting of

i. $-(Gly)_x-$, wherein x is 3,

ii. PEG(4), and

iii. -Gly-DLys-Gly-.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein, wherein A' is selected from the group consisting of

i. Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

ii. Tyr-Pro-Lys-Phe-Ile-Pro-Leu-,

iii. Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

iv. Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-,

v. Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-

vi. Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-,

vii. Tyr-Pro-Lys-Pro-Ala-Gln-Gly-

viii. Gly-Ala-Arg-Phe-Ile-Pro-Ala-,

ix. Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-,

x. Tyr-Pro-Tyr-Phe-Ile-Pro-DLys,

xi. DLys-Pro-Tyr-Phe-Ile-Pro-Leu-,

xii. H-Tyr-Pro-Tyr-Phe-DLys-Leu-,

xiii. H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

xiv. H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

xv. Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-.

xvi. H-Tyr-DLys-Tyr-Phe-Ile-Pro-Leu-,

xvii. H-Tyr-Pro-DLys-Phe-Ile-Pro-Leu-,

xviii. H-Tyr-Pro-Tyr-DLys-Ile-Pro-Leu-,

xix. Biotin-PEG2-Cys-PEG8-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

xx. Biotin-PEG2-Cys-PEG3-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-, and

xxi. H-Gly-Gly-Gly-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein, wherein A' is selected from the group consisting of

i. Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

ii. Tyr-Pro-Lys-Phe-Ile-Pro-Leu-,

iii. Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

iv. Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-,

v. Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-

vi. Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-,

vii. Tyr-Pro-Lys-Pro-Ala-Gln-Gly-

viii. Gly-Ala-Arg-Phe-Ile-Pro-Ala-,

ix. Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-,

x. Tyr-Pro-Tyr-Phe-Ile-Pro-DLys,

xi. DLys-Pro-Tyr-Phe-Ile-Pro-Leu-, and

xii. H-Tyr-Pro-Tyr-Phe-DLys-Leu-.

xiii. H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

xiv. H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-, and

xv. Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein, wherein B' is selected from the group consisting of

i. -Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

ii. -Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2,

iii. -Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2,

iv. -Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2, and

v. Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein, selected from the group consisting of

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA

1 H-Tyr-Pro-Lys-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
DLys-Pro-Tyr-Phe-Ile-Pro-Leu- Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Gly-Ala-Arg-Phe-Ile-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-Tyr-Pro-Tyr-Phe-DLys-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,
Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Lys-Pro-Ala-Gln-Gly-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 2TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA,
Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 2TFA,
H-Tyr-DLys-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

H-Tyr-Pro-DLys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-Tyr-DLys-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Biotin-PEG2-Cys-PEG8-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Biotin-PEG2-Cys-PEG3-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2, and
H-Gly-Gly-Gly-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 3TFA.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein, selected from the group consisting of

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA,
1 H-Tyr-Pro-Lys-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
DLys-Pro-Tyr-Phe-Ile-Pro-Leu- Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Gly-Ala-Arg-Phe-Ile-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-Tyr-Pro-Tyr-Phe-DLys-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,
Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Lys-Pro-Ala-Gln-Gly-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 2TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA,

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA, and
Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 2TFA.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein, wherein the pharmaceutically acceptable salt is trifluoroacetate.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein for use as therapeutically active substance.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein for the use as inhibitor of BACE1 activity.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein for the use as therapeutically active substance for the therapeutic and/or prophylactic treatment of diseases and disorders characterized by elevated β-amyloid levels and/or β-amyloid oligomers and/or β-amyloid plaques and further deposits, particularly Alzheimer's disease.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein for the use as therapeutically active substance for the therapeutic and/or prophylactic treatment of Alzheimer's disease.

A certain embodiment of the invention relates to a pharmaceutical composition comprising a dual-site BACE1 inhibitor as described herein and a pharmaceutically acceptable carrier and/or a pharmaceutically acceptable auxiliary substance.

A certain embodiment of the invention relates to the use of a dual-site BACE1 inhibitor as described herein for the manufacture of a medicament for the use in inhibition of BACE1 activity.

A certain embodiment of the invention relates to the use of a dual-site BACE1 inhibitor as described herein for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of diseases and disorders characterized by elevated β-amyloid levels and/or β-amyloid oligomers and/or β-amyloid plaques and further deposits, particularly Alzheimer's disease.

A certain embodiment of the invention relates to the use of a dual-site BACE1 inhibitor as described herein for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of Alzheimer's disease.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein for the use in inhibition of BACE1 activity.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein for the use in the therapeutic and/or prophylactic treatment of diseases and disorders characterized by elevated β-amyloid levels and/or β-amyloid oligomers and/or β-amyloid plaques and further deposits, particularly Alzheimer's disease.

A certain embodiment of the invention relates to a dual-site BACE1 inhibitor as described herein for the use in the therapeutic and/or prophylactic treatment of Alzheimer's disease.

A certain embodiment of the invention relates to a method for the use in inhibition of BACE1 activity, particularly for the therapeutic and/or prophylactic treatment of diseases and disorders characterized by elevated β-amyloid levels and/or β-amyloid oligomers and/or β-amyloid plaques and further deposits, Alzheimer's disease, which method comprises administering dual-site BACE1 inhibitor as described herein to a human being or animal.

Furthermore, the invention includes all optical isomers, i.e. diastereoisomers, diastereomeric mixtures, racemic mixtures, all their corresponding enantiomers and/or tautomers as well as their solvates.

The dual-site BACE1 inhibitors may be prepared as described herein. The starting material is commercially available or may be prepared in accordance with known methods.

The corresponding pharmaceutically acceptable salts with acids can be obtained by standard methods known to the person skilled in the art, e.g. by dissolving the dual-site BACE1 inhibitor in a suitable solvent such as e.g. dioxan or THF and adding an appropriate amount of the corresponding acid. The products can usually be isolated by filtration or by chromatography. The conversion of a dual-site BACE1 inhibitor into a pharmaceutically acceptable salt with a base can be carried out by treatment of such a compound with such a base. One possible method to form such a salt is e.g. by addition of 1/n equivalents of a basic salt such as e.g. $M(OH)_n$, wherein M = metal or ammonium cation and n = number of hydroxide anions, to a solution of the compound in a suitable solvent (e.g. ethanol, ethanol-water mixture, tetrahydrofuran-water mixture) and to remove the solvent by evaporation or lyophilisation.

Insofar as their preparation is not described in the examples, the dual-site BACE1 inhibitors as well as all intermediate products can be prepared according to analogous methods or according to the methods set forth herewithin. Starting materials are commercially available, known in the art or can be prepared by methods known in the art or in analogy thereto.

It will be appreciated that the dual-site BACE1 inhibitors in this invention may be derivatised at functional groups to provide derivatives which are capable of conversion back to the parent compound *in vivo*.

Pharmacological Tests

The dual-site BACE1 inhibitor and their pharmaceutically acceptable salts possess valuable pharmacological properties. It has been found that the compounds of the present invention are associated with inhibition of BACE1 activity. The compounds were investigated in accordance with the test given hereinafter.

Cellular Aβ-lowering assay:

Human HEK293 cells which are stably transfected with a vector expressing a cDNA of the human APP wt gene (APP695) were used to assess the potency of the compounds in a cellular assay. The cells were seeded in 96-well microtiter plates in cell culture medium (Iscove, plus 10% (v/v) fetal bovine serum, glutamine, penicillin/streptomycin) to about 80% confluence and the compounds were added at a 10x concentration in 1/10 volume of medium without FCS containing 8% DMSO (final concentration of DMSO was kept at 0.8% v/v). After 18-20 hrs incubation at 37 °C and 5% CO_2 in a humidified incubator the culture supernatant was harvested for the determination of Aβ40 concentrations. 96well ELISA plates (e.g., Nunc MaxiSorb) were coated with monoclonal antibody which specifically recognize the C-terminal end of Aβ40 (Brockhaus et al., NeuroReport 9, 1481-1486; 1998). After blocking of non-specific binding sites with e.g. 1% BSA and washing, the culture supernatants were added in suitable dilutions together with a horseradish peroxidase-coupled Aβ detection antibody (e.g., antibody 4G8, Senetek, Maryland Heights, MO) and incubated for 5 to 7 hrs. Subsequently the wells of the microtiter plate were washed extensively with Tris-buffered saline containing 0.05% Tween 20 and the assay was developed with tetramethylbenzidine/H_2O_2 in citric acid buffer. After stopping the reaction with one volume 1 N H_2SO_4 the reaction was measured in an ELISA reader at 450 nm wavelength. The concentrations of Aβ in the culture supernatants were calculated from a standard curve obtained with known amounts of pure Aβ peptide.

Ex.	Name	Systematic Name	MW	IC 50 (μM)
1	YPYFIPL-PEG(4)-EVN-Sta-VAEp- NH_2 x TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA	2168.4	0.0027
2	YPYFIPL-GGG-EVN-Sta-VAEp- NH_2 x TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA	2092.27	0.42
3	YPYFIPL-GkG-EVN-Sta-VAEp- NH_2 x 2TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA	2277.41	0.094

4	YPKFIPL-GkG-EVN-Sta-VAEp- NH_2 x 3TFA	Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2356.44	0.28
5	YPYFIPk-GkG-EVN-Sta-VAEp- NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2406.45	0.019
6	YPYFKPA-GkG-EVN-Sta-VAEp- NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2364.37	0.046
7	YPYFIPL-PEG(4)-EVN-MetSta-VAEf-NH_2 x TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x TFA	2236.5	0.43
8	YPYFIPL-PEG(4)-EVN-MetSta-VAEP-NH_2 x TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA	2186.44	0.017
9	YPYFIPL-GGG-EVN-MetSta-VAEP- NH_2 x TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA	2110.31	1.34
10	YPYFIPL-GkG-EVN-MetSta-VAEP- NH_2 x 2TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 2TFA	2295.45	0.47
11	YPKFIPL-GkG-EVN-MetSta-VAEP- NH_2 x 3TFA	Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA	2374.47	0.76
12	YPYFIkL-GkG -EVN-Sta-VAEp- NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2422.49	>40
13	YPYFkPL-GkG -EVN-Sta-VAEp- NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2406.45	>40
14	YPYFKPA-GkG-EVN-Sta-VAEp- NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2364.37	0.071
15	TPKPAQG-GkG-EVN-Sta-VAEp- NH_2 x 3TFA	Tyr-Pro-Lys-Pro-Ala-Gln-Gly-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2177.14	>40
16	GARFIPA-GkG-EVN-Sta-VAEp- NH_2 x 3TFA	Gly-Ala-Arg-Phe-Ile-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2210.21	0.42
17	YPKFISA-GkG-EVN-Sta-VAEp- NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2304.32	0.75
18	YPYFIPk-GkG-EVN-MetSta-VAEP- NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA	2424.49	0.015
19	YPYFIPk-GkG-EVN-MetSta-VAEf- NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x 3TFA	2474.55	0.19
20	kPYFIPLGkGEVN-Sta-VAEp- NH_2 x 3TFA	DLys-Pro-Tyr-Phe-Ile-Pro-Leu- Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 3TFA	2356.44	2.93

21	YPKFIPL-PEG(4)-EVN-Sta-VAEp- NH_2 x 3TFA	1 H-Tyr-Pro-Lys-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 3TFA	2247.43	0.052
22	YPYFIPk-PEG(4)-EVN-Sta-VAEp- NH_2 x 2TFA	H-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 2TFA	2297.44	0.045
23	YPYFkL-PEG(4)-EVN-Sta-VAEp- NH_2 x 2TFA	H-Tyr-Pro-Tyr-Phe-DLys-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 3TFA	2200.33	>40
24	YPYFkPL-PEG(4)-EVN-Sta-VAEp- NH_2 x 2TFA	H-Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 2TFA	2297.44	>40
25	YPYFIPk-PEG(4)-EVN-Leu*Ala-Aep- NH_2 x 2TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro- NH_2 x 2TFA	2226.36	0.015
26	YPYFkLGkGEVN-Leu*Ala-Aep- NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro- NH_2 x 3TFA	2238.26	0.034
27	YPYFIPk-PEG(4)-EVN-MetSta-VAEp- NH_2 x 2TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPro- NH_2 x 2TFA	2315.48	1.01
28	yPYFIPkGkGEVN-Sta-VAEp- NH_2 x 3TFA	H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 3TFA	2406.45	0.063
29	yPYFIPLGkGEVN-Sta-VAEp- NH_2 x 2TFA	H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 2TFA	2277.41	0.81
30	Ac-yPYFIPLGkGEVN-Sta-VAEp- NH_2 x TFA	Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x TFA	2205.43	0.11
31	YkYFIPLGkGEVN-Sta-VAEp-NH2.TFA	H-Tyr-DLys-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH2	2422.49	> 40
32	YPkFIPLGkGEVN-Sta-VAEp-NH2.TFA	H-Tyr-Pro-DLys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2	2356.44	29.29
33	YPYkIPLGkGEVN-Sta-VAEp-NH2.TFA	H-Tyr-Pro-Tyr-DLys-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2	2372.44	34.83
34	Btn-PEG2-C-PEG8-YPYFIPkGkGEVN-Sta-VAEp-NH2.TFA	Biotin-PEG2-Cys-PEG8-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2	3190.52	0.018
35	Btn-PEG2-C-PEG3-YPYFIPkGkGEVN-Sta-VAEp-NH2.TFA	Biotin-PEG2-Cys-PEG3-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2	2970.26	0.026
36	GGGYPYFIPkGkGEVN-Sta-VAEp-NH2.TFA	H-Gly-Gly-Gly-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2577.61	0.0064

Table 3: IC_{50} values of selected examples

The exosite motifs as well as the active site inhibitors containing statine-type transition state mimetic alone do not show BACE1 inhibition.

Ex.	Name	Systematic Name	MW	IC 50 (µM)
E1	Ac-QQYPYFKPAN-NH_2	Ac-Gln-Gln-Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Asn-NH_2	1295.6	> 40
E2	Ac-YPTFKPANGS-NH_2	Ac-Tyr-Gln-Thr-Phe-Lys-Pro-Ala-Asn-Gly-Ser-NH_2	1121.6	> 40
E3	Ac-STGARFIPAN-NH_2	Ac-Ser-Thr-Gly-Ala-Arg-Phe-Ile-Pro-Ala-Asn-NH_2	1073.6	> 40
E4	Ac-GDYPKFISAN-NH_2	Ac-Gly-Asp-Tyr-Pro-Lys-Phe-Ile-Ser-Ala-Asn-NH_2	1151.6	> 40
E5	Ac-GDYPKFIPAS-NH_2	Ac-Gly-Asp-Tyr-Pro-Lys-Phe-Ile-Pro-Ala-Ser-NH_2	1134.6	> 40
E6	Ac-GSYPKFIDAN-NH_2	Ac-Gly-Ser-Tyr-Pro-Lys-Phe-Ile-Asp-Ala-Asn-NH_2	1151.6	> 40
E7	Ac-LTTYPYFKPA-NH_2	Ac-Leu-Thr-Thr-Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-NH_2	1240.6	14.41

Table 4: IC_{50} values of selected examples of exosite motifs

Ex.	Name	Systematic Name	MW	IC 50 (µM)
A1	EVN-Sta-VAEF	Glu-Val-Asn-Sta-Val-Ala-Glu-Phe-NH_2 x TFA	961.2	> 40
A2	EVN-MetSta-VAEf	Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe- NH_2 x TFA	981.65	> 40
A3	EVN-Sta-VAEp	Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA	1041.59	> 40

Table 5: IC_{50} values of selected examples of active site motifs

Pharmaceutical Compositions

The dual-site BACE1 inhibitors and the pharmaceutically acceptable salts can be used as therapeutically active substances, e.g. in the form of pharmaceutical preparations. The pharmaceutical preparations can be administered orally, e.g. in the form of tablets, coated tablets, dragées, hard and soft gelatin capsules, solutions, emulsions or suspensions. The administration can, however, also be effected rectally, e.g. in the form of suppositories, or parenterally, e.g. in the form of injection solutions.

The dual-site BACE1 inhibitors and the pharmaceutically acceptable salts thereof can be processed with pharmaceutically inert, inorganic or organic carriers for the production of pharmaceutical preparations. Lactose, corn starch or derivatives thereof, talc, stearic acids or its salts and the like can be used, for example, as such carriers for tablets, coated tablets, dragées and hard gelatin capsules. Suitable carriers for soft gelatin capsules are, for example, vegetable

oils, waxes, fats, semi-solid and liquid polyols and the like. Depending on the nature of the active substance no carriers are however usually required in the case of soft gelatin capsules. Suitable carriers for the production of solutions and syrups are, for example, water, polyols, glycerol, vegetable oil and the like. Suitable carriers for suppositories are, for example, natural or hardened oils, waxes, fats, semi-liquid or liquid polyols and the like.

The pharmaceutical preparations can, moreover, contain pharmaceutically acceptable auxiliary substances such as preservatives, solubilizers, stabilizers, wetting agents, emulsifiers, sweeteners, colorants, flavorants, salts for varying the osmotic pressure, buffers, masking agents or antioxidants. They can also contain still other therapeutically valuable substances.

Medicaments containing a dual-site BACE1 inhibitor or a pharmaceutically acceptable salt thereof and a therapeutically inert carrier are also an object of the present invention, as is a process for their production, which comprises bringing one or more dual-site BACE1 inhibitors and/or pharmaceutically acceptable salts thereof and, if desired, one or more other therapeutically valuable substances into a galenical administration form together with one or more therapeutically inert carriers.

The dosage can vary within wide limits and will, of course, have to be adjusted to the individual requirements in each particular case. In the case of oral administration the dosage for adults can vary from about 0.01 mg to about 1000 mg per day of a dual-site BACE1 inhibitors or of the corresponding amount of a pharmaceutically acceptable salt thereof. The daily dosage may be administered as single dose or in divided doses and, in addition, the upper limit can also be exceeded when this is found to be indicated.

The following examples illustrate the present invention without limiting it, but serve merely as representative thereof. The pharmaceutical preparations conveniently contain about 1-500 mg, preferably 1-100 mg, of a dual-site BACE1 inhibitor. Examples of compositions according to the invention are:

Example A

Tablets of the following composition are manufactured in the usual manner:

ingredient	mg/tablet			
	5	25	100	500
Dual-site BACE1 inhibitor	5	25	100	500
Lactose Anhydrous DTG	125	105	30	150
Sta-Rx 1500	6	6	6	60
Microcrystalline Cellulose	30	30	30	450
Magnesium Stearate	1	1	1	1
Total	167	167	167	831

Table 6: possible tablet composition

Manufacturing Procedure

1. Mix ingredients 1, 2, 3 and 4 and granulate with purified water.
2. Dry the granules at 50°C.
3. Pass the granules through suitable milling equipment.
4. Add ingredient 5 and mix for three minutes; compress on a suitable press.

Example B-1

Capsules of the following composition are manufactured:

ingredient	mg/capsule			
	5	25	100	500
Dual-site BACE1 inhibitor	5	25	100	500
Hydrous Lactose	159	123	148	-
Corn Starch	25	35	40	70
Talk	10	15	10	25
Magnesium Stearate	1	2	2	5
Total	200	200	300	600

Table 7: possible capsule ingredient composition

Manufacturing Procedure

1. Mix ingredients 1, 2 and 3 in a suitable mixer for 30 minutes.
2. Add ingredients 4 and 5 and mix for 3 minutes.
3. Fill into a suitable capsule.

The dual-site BACE1 inhibitor, lactose and corn starch are firstly mixed in a mixer and then in a comminuting machine. The mixture is returned to the mixer; the talc is added thereto and mixed thoroughly. The mixture is filled by machine into suitable capsules, e.g. hard gelatin capsules.

Example B-2

Soft Gelatin Capsules of the following composition are manufactured:

ingredient	mg/capsule
Dual-site BACE1 inhibitor	5
Yellow wax	8
Hydrogenated Soya bean oil	8

Partially hydrogenated plant oils	34
Soya bean oil	110
Total	165

Table 8: possible soft gelatin capsule ingredient composition

ingredient	mg/capsule
Gelatin	75
Glycerol 85 %	32
Karion 83	8 (dry matter)
Titan dioxide	0.4
Iron oxide yellow	1.1
Total	116.5

Table 9: possible soft gelatin capsule composition

Manufacturing Procedure

The dual-site BACE1 inhibitor is dissolved in a warm melting of the other ingredients and the mixture is filled into soft gelatin capsules of appropriate size. The filled soft gelatin capsules are treated according to the usual procedures.

Example C

Suppositories of the following composition are manufactured:

ingredient	mg/supp.
Dual-site BACE1 inhibitor	15
Suppository mass	1285
Total	1300

Table 10: possible suppository composition

Manufacturing Procedure

The suppository mass is melted in a glass or steel vessel, mixed thoroughly and cooled to 45°C. Thereupon, the finely powdered dual-site BACE1 inhibitor is added thereto and stirred until it has dispersed completely. The mixture is poured into suppository moulds of suitable size, left to cool; the suppositories are then removed from the moulds and packed individually in wax paper or metal foil.

Example D

Injection solutions of the following composition are manufactured:

ingredient	mg/injection solution.
Dual-site BACE1 inhibitor	3
Polyethylene Glycol 400	150
acetic acid	q.s. ad pH 5.0
water for injection solutions	ad 1.0 ml

Table 11: possible injection solution composition

Manufacturing Procedure

The dual-site BACE1 inhibitor is dissolved in a mixture of Polyethylene Glycol 400 and water for injection (part). The pH is adjusted to 5.0 by acetic acid. The volume is adjusted to 1.0 ml by addition of the residual amount of water. The solution is filtered, filled into vials using an appropriate overage and sterilized.

Example E

Sachets of the following composition are manufactured:

ingredient	mg/sachet
Dual-site BACE1 inhibitor	50
Lactose, fine powder	1015
Microcrystalline cellulose (AVICEL PH 102)	1400
Sodium carboxymethyl cellulose	14
Polyvinylpyrrolidon K 30	10
Magnesium stearate	10
Flavoring additives	1
Total	2500

Table 12: possible sachet composition

Manufacturing Procedure

The dual-site BACE1 inhibitor is mixed with lactose, microcrystalline cellulose and sodium carboxymethyl cellulose and granulated with a mixture of polyvinylpyrrolidone in water. The granule is mixed with magnesium stearate and the flavoring additives and filled into sachets.

Experimental Part

The following examples are provided for illustration of the invention. They should not be considered as limiting the scope of the invention, but merely as being representative thereof.

General procedures for the CEM Liberty Microwave Peptide Synthesizer:

0.1 mMol scale:

Deprotection of Fmoc:

The washed and preswelled resin (435 mg, 0.1 mMol, TentaGel S RAM (Load: 0.23 mMol/g), (Rapp Polymere, Cat: S30023) was treated with a solution of piperidine 20% in dimethylformamid (DMF) (7.0 mL) under microwave condition at 50°C for 3 minutes for initial deprotection. The resin was washed with DMF and treated with a solution of piperidine 20% in DMF (7.0 mL) under microwave condition at 75°C for 5 minutes for deprotection.

Coupling of amino acids:

To the washed and preswelled resin was added a solution of amino acid, 0.2M in DMF (2.5 mL, 5.0 eq.) followed by a solution of (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) 0.5M in DMF (1.0 mL, 5.0 eq.), (CAS: 1075198-30-9, Iris Biotech, Cat: RL-1175.1000) followed by a solution of diisopropylethylamine (DIPEA) 2M in N-Methyl-2-pyrrolidone (NMP) (0.5 mL, 10.0 eq.). This reaction mixture was treated under microwave condition at 75°C for 5 minutes for coupling.

<u>0.25 mMol scale:</u>

Deprotection of Fmoc:

The washed and preswelled resin (1.09 g, 0.25 mMol, TentaGel S RAM (Load: 0.23 mMol/g), (Rapp Polymere, Cat: S30023) was treated with a solution of piperidine 20% in DMF (10.0 mL) under microwave condition at 50°C for 3 minutes for initial deprotection. The resin was washed with DMF and treated with a solution of piperidine 20% in DMF (10.0 mL) under microwave condition at 75°C for 5 minutes for deprotection.

Coupling of amino acids:

To the washed and preswelled resin was added a solution of amino acid, 0.2M in DMF (5.0 mL, 4.0 eq.) followed by a solution of COMU 0.5M in DMF (2.0 mL, 4.0 eq.), (CAS: 1075198-30-9, Iris Biotech, Cat: RL-1175.1000) followed by a solution of DIPEA 2M in NMP (1.0 mL, 8.0 eq.). This reaction mixture was treated under microwave condition at 75°C for 5 minutes for coupling.

General procedure for final cleavage:

0.1 mMol scale:

The resin was washed with CH_2Cl_2 and then treated with a solution of **trifluoroacetic acid** (TFA): triisopropylsilane (TIS):water 95:2.5:2.5 (5 mL) for 30 minutes at room temperature on the shaker. The resin was filtered. The crude peptide was precipitated with Et_2O (35 mL). The

suspension was centrifuged and the solvent was decanted. The solid was dissolved in acetonitrile and water and freeze-dried to get the crude peptide.

General procedure for purification:

The crude product was dissolved in acetonitrile and water (containing 0.1% TFA) and then purified by preparative HPLC. Column YMC-Actus Pro C8, 5µm, 75x30mm with a gradient of water (containing 0.1% TFA) : acetonitrile 70 : 30 to 2 : 98 and with a flow of 30 mL/min.

The examples can be prepared analogous to the general procedures described herein.

BACE1 Binder Discovery Using Comprehensive 5mer Peptide Arrays

Array Design, Synthesis and Background Control: To discover BACE1 binders, an array having 2.47M peptides (representing a comprehensive list of all possible 5-mer peptides, excluding cysteine) was designed. Each 5-mer peptide was synthesized with 3 cycles of linker synthesis in the N-term and C-term. For linker synthesis a mixture including G and S in a 3:1 ratio was used. The peptide sequence would be in the following format: ZZZ – 5mer – ZZZ, where Z is an amino-acid from a linker mixture. After peptide synthesis, arrays were pre-stained with Cy5-streptavidin for the purpose of QC and background signal measurement in 30 ml of Binding buffer (1% alkali-soluble casein, Novagen, Billerica, USA, cat#70955, 0.05% Tween 20) containing 333 ng/ml Cy5-streptavidin (Amersham, UK, cat# PA45001) for 1 h. Array were washed in Wash Buffer I (Roche/Nimblegen, Madison, USA) for 30 sec and, briefly, in 0.1 TE buffer. The arrays were dried by a spinning in a microfuge to remove traces of water and scanned using MS200 scanner (Roche/Nimblegen) at 635 nm wavelength and 2 µm resolution. After this step, the arrays were ready for binding to a target protein.

BACE1 biotinylation: BACE1 enzyme (WT1-13-2, 0.7 mg/ml) was labelled with biotin using Pierce EZ-link Micro NHS-PEG4-biotinylation kit, cat#21955. For labelling, 70 µl or 50 µg of BACE1 enzyme was mixed with 2 µl of NHS-biotin solution freshly prepared in water and incubated at RT for 1 h. Excess of free biotin was removed by 2X spin-filtration (1,000 g, 4 min) of the reaction solution using Micro Bio-Spin 30 chromatography column (BioRad, cat 732-6223). The biotinylated BACE1 was stored in aliquots at -20C.

Binding Assay: To ensure data reproducibility 5 µl of biotinylated BACE1 was mixed with 40 µl of Binding Buffer, loaded on array with attached HX1 Mixer and incubated at 4C overnight. After incubation, the mixer was removed and array was washed in Wash Buffer I for 30 sec and, briefly in 0.1 TE buffer. The array was stained at biotinylated BACE1 in 30 ml of Binding buffer with 333 ng/ml streptavidin-Cy5 (Amersham, UK, cat# PA45001) for 1 h and washed as described above. The array was dried by spinning in a microfuge to remove traces of

water and the data were acquired using MS200 scanner (Roche/Nimblegen) at 635 nm wavelength and 2 um resolution.

Data analysis and Results: Image analysis and signal extraction performed using NimbleGen DEVA software. The biotin specific fluorescent signal resulting from BACE1 binding was plotted against the background signal collected earlier (see "Array Design, Synthesis and Background Control") for all 5-mer sequences. 5-mer peptide sequences with highest BACE1-specific signal and low background have been identified from this analysis and six most common motifs shared by these sequences are listed in the first column of the table below.

Core motifs	Extension libraries	Sequences selected from extension libraries	Double substitution/deletion libraries	Sequences selected from substitution/deletion libraries
YFK	XXYFKXX	YPYFKPA	GQYPYFKPAS QYPYFKPASG	QQYPYFKPAN
PYFK	XXPYFKXX	QYPYFKPA		
YFKP	XXYFKPXX	YPYFKPAS		
TFK	XXTFKXX	YPTFKPA	GGGYPTFKPA GGYPTFKPAG GYPTFKPAGG YPTFKPAGGG	YPTFKPANGS
PKF	XXPKFXX	DYPKFIS DYPKFLP SYPKFID	GDYPKFISGG GDYPKFLPGG GSYPKFIDGG	GDYPKFISAN GDYPKFIPAS GSYPKFIDAN
ARFIP	XXARFIPXX	TGARFIPAN	GTGARFIPAN TGARFIPANG	STGARFIPAN

Table 13

BACE1 Binder Optimization Using Extension Peptide Arrays.

Array Design: The motifs identified in 5-mer array experiments are likely to represent only short versions of optimal BACE1 binders. Longer motifs can be found by designing new arrays by extending the core sequences selected from 5-mer arrays by two amino acids from both N- and C-terminus using all 20 natural amino acids shown by X in column 2 of Table 2. Each of the extension libraries includes 160,000 unique peptides synthesized in five replicates. BACE1 binding assay and image processing was performed as described in Example 1.

Data analysis and Results: For each core motif, extended sequences with the highest binding to BACE1 are shown in Table 13 (third column). Extended sequences for 'YFK', 'PYFK' and 'YFKP' motifs (top three rows in Table 13) share the same YPYFKPA sequence suggesting that they represent the same common sequence. Three sequences, DYPKFLP, DYPKFIS, SYPKFID, were selected as 'PKF' motif extensions because they had similar binding to BACE1 and diverse amino acid composition at the N- and C-terminus.

BACE1 Binder Optimization Using Double Substitution/Deletion Peptide Arrays.

Array Design: he third round of binder optimization included extension of the sequences identified in the extension array experiments with glycine (G) amino acid to make them 10-mer peptides as shown in Table 13 (forth column) followed by design of double substitution/deletion libraries that include all possible single- and double substitution/deletion variants of the reference sequence. For a 10-mer peptide, the double substitution/deletion library included approximately 16.5 thousand unique peptides. Each peptide was synthesized in replicates of seven.

BACE1 binding assay and image processing for substitution/deletion arrays was performed as described above (BACE1 Binder Discovery Using Comprehensive 5mer Peptide Arrays).

Data analysis and Results: Peptides sequences with the highest binding signal were selected after analysing each peptide library which included the outlier removal and averaging signal for each peptide over the replicates. The selected sequences are shown in Table 13 (last column).

Claims

1. A dual-site BACE1 inhibitor, binding to both, the enzymatic active site and the catalytic domain of the BACE enzyme, whereby the exosite inhibitory part (A') is connected to the active-site inhibitory part (B') of said BACE1 inhibitor by a linker (L'), or a pharmaceutically acceptable salt thereof.

2. A dual-site BACE1 inhibitor according to any one of claims 1-2, wherein L' is selected from the group consisting of

   i. $-(Gly)_x-$, wherein x is 3,

   ii. PEG(4), and

   iii. -Gly-DLys-Gly-.

3. A dual-site BACE1 inhibitor according to any one of claims 1-3, wherein A' is selected from the group consisting of

   i. Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

   ii. Tyr-Pro-Lys-Phe-Ile-Pro-Leu-,

   iii. Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

   iv. Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-,

   v. Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-

   vi. Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-,

   vii. Tyr-Pro-Lys-Pro-Ala-Gln-Gly-

   viii. Gly-Ala-Arg-Phe-Ile-Pro-Ala-,

   ix. Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-,

   x. Tyr-Pro-Tyr-Phe-Ile-Pro-DLys,

   xi. DLys-Pro-Tyr-Phe-Ile-Pro-Leu-,

   xii. H-Tyr-Pro-Tyr-Phe-DLys-Leu-,

   xiii. H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

   xiv. H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

xv. Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-.

xvi. H-Tyr-DLys-Tyr-Phe-Ile-Pro-Leu-,

xvii. H-Tyr-Pro-DLys-Phe-Ile-Pro-Leu-,

xviii. H-Tyr-Pro-Tyr-DLys-Ile-Pro-Leu-,

xix. Biotin-PEG2-Cys-PEG8-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

xx. Biotin-PEG2-Cys-PEG3-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-, and

xxi. H-Gly-Gly-Gly-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-.

4. A dual-site BACE1 inhibitor according to any one of claims 1-4, wherein A' is selected from the group consisting of

i. Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

ii. Tyr-Pro-Lys-Phe-Ile-Pro-Leu-,

iii. Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

iv. Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-,

v. Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-

vi. Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-,

vii. Tyr-Pro-Lys-Pro-Ala-Gln-Gly-

viii. Gly-Ala-Arg-Phe-Ile-Pro-Ala-,

ix. Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-,

x. Tyr-Pro-Tyr-Phe-Ile-Pro-DLys,

xi. DLys-Pro-Tyr-Phe-Ile-Pro-Leu-,

xii. H-Tyr-Pro-Tyr-Phe-DLys-Leu-,

xiii. H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

xiv. H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-, and

xv. Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-.

5. A dual-site BACE1 inhibitor according to any one of claims 1-5, wherein B' is selected from the group consisting of

i. -Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

ii. -Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2,

iii. -Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2,

iv. -Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2, and

v. Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2.

6. A compound according to any one of claims 1 to 6, selected from the group consisting of

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA
1 H-Tyr-Pro-Lys-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
DLys-Pro-Tyr-Phe-Ile-Pro-Leu- Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Gly-Ala-Arg-Phe-Ile-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-Tyr-Pro-Tyr-Phe-DLys-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,
Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Lys-Pro-Ala-Gln-Gly-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 2TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA,

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA,
Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 2TFA,
H-Tyr-DLys-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-DLys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-Tyr-DLys-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Biotin-PEG2-Cys-PEG8-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Biotin-PEG2-Cys-PEG3-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2, and
H-Gly-Gly-Gly-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 3TFA.

7. A compound according to any one of claims 1 to 7, selected from the group consisting of

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA,
1 H-Tyr-Pro-Lys-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
DLys-Pro-Tyr-Phe-Ile-Pro-Leu- Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Gly-Ala-Arg-Phe-Ile-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-Tyr-Pro-Tyr-Phe-DLys-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
H-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,
Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,
Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Lys-Pro-Ala-Gln-Gly-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPro-NH_2,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 2TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA,
Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,
H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA, and
Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro- NH_2 x 2TFA.

8. A compound according to any one of claims 1 to 5, wherein the pharmaceutically acceptable salt is trifluoroacetate.

9. A dual-site BACE1 inhibitor according to any one of claims 1-8 for use as therapeutically active substance.

10. A dual-site BACE1 inhibitor according to any one of claims 1-8 for the use as therapeutically active substance for the therapeutic and/or prophylactic treatment of diseases and disorders characterized by elevated β-amyloid levels and/or β-amyloid oligomers and/or β-amyloid plaques and further deposits, particularly Alzheimer's disease.

11. A pharmaceutical composition comprising a dual-site BACE1 inhibitor according to any one of claims 1-8 and a pharmaceutically acceptable carrier and/or a pharmaceutically acceptable auxiliary substance.

12. Use of a dual-site BACE1 inhibitor according to any one of claims 1-8 for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of diseases and disorders

characterized by elevated β-amyloid levels and/or β-amyloid oligomers and/or β-amyloid plaques and further deposits, particularly Alzheimer's disease.

13. A method for the use in inhibition of BACE1 activity, particularly for the therapeutic and/or prophylactic treatment of diseases and disorders characterized by elevated β-amyloid levels and/or β-amyloid oligomers and/or β-amyloid plaques and further deposits, Alzheimer's disease, diabetes or type 2 diabetes, which method comprises administering a dual-site BACE1 inhibitor according to any one of claims 1-8 to a human being or animal.

14. The invention as described hereinabove.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/067447

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K7/08 C12N9/64 A61K38/55
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 A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/097199 A1 (JADOLABS GMBH [DE]; MAX PLANCK GESELLSCHAFT [DE]; UNIV DRESDEN TECH [D) 20 October 2005 (2005-10-20) cited in the application	1,8-14
Y	abstract page 5 - page 6 page 13, paragraph 3 - paragraph 5 page 16, paragraph 3; examples 26,30,31	2-5
	----- -/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 September 2016	16/09/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Gurdjian, Didier

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PHILIPP LINNING ET AL: "Optimisation of BACE1 inhibition of tripartite structures by modification of membrane anchors, spacers and pharmacophores - development of potential agents for the treatment of Alzheimer's disease", ORGANIC & BIOMOLECULAR CHEMISTRY, vol. 10, no. 41, 1 January 2012 (2012-01-01), page 8216, XP055175734, ISSN: 1477-0520, DOI: 10.1039/c2ob26103k	1,2,8-14
Y	abstract; tables 2,3	3-5

X	KORNACKER ET AL.: "An Inhibitor Binding Pocket Distinct from the Catalytic Active Site on Human Beta-APP Cleaving Enzyme", BIOCHEMISTRY, vol. 44, no. 34, 2005, pages 11567-11573, XP055006425, cited in the application	1,3,4, 8-14
Y	abstract; table 1	5

X	US 2007/149763 A1 (KORNACKER MICHAEL G [US] ET AL) 28 June 2007 (2007-06-28)	1,3,4, 8-14
Y	abstract; claim 1; figure 17c; table 1	5

X	BRIDGES K G ET AL: "A novel approach to identifying beta-secretase inhibitors: Bis-statine peptide mimetics discovered using structure and spot synthesis", PEPTIDES, ELSEVIER, AMSTERDAM, NL, vol. 27, no. 7, 1 July 2006 (2006-07-01), pages 1877-1885, XP027957454, ISSN: 0196-9781 [retrieved on 2006-07-01]	1,8-14
Y	abstract; figures 1-4	2-5

A	WO 2006/002907 A1 (JADOLABS GMBH [DE]; HAFTEN E V MAX PLANCK GES ZUR [DE]; UNIV DRESDEN T) 12 January 2006 (2006-01-12) cited in the application abstract; example 14	1,2,5, 8-13

A	WO 2013/056054 A2 (GENENTECH INC [US]; LAZARUS ROBERT A [US]; ZHANG YINGNAN [US]; WANG WE) 18 April 2013 (2013-04-18) cited in the application abstract page 8, paragraph 1; figures 1a,1b page 48, paragraph 3	1,3-5, 8-13

A	WO 2004/035606 A2 (SQUIBB BRISTOL MYERS CO [US]; KORNACKER MICHAEL G [US]; COPELAND ROBER) 29 April 2004 (2004-04-29) abstract; examples 8,16; table 1	1,3,4, 6-12

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/067447

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2016/083425 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 2 June 2016 (2016-06-02) the whole document -----	1-14

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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(54)发明名称

BACE1抑制剂肽

(57)摘要

本发明涉及双位点BACE1抑制剂,它们的制备,含有它们的药物组合物以及它们作为治疗活性物质的用途。本发明的活性化合物可用于例如阿尔茨海默病的治疗性和/或预防性治疗。

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1.一种双位点BACE1抑制剂,所述抑制剂与BACE酶的酶促活性位点和催化结构域二者结合,其中通过接头(L')将外部位点抑制部分(A')与所述BACE1抑制剂的活性位点抑制部分(B')连接,

或其药用盐。

2.根据权利要求1-2中任一项所述的双位点BACE1抑制剂,其中L'选自由以下组成的组:

i.-$(Gly)_x$-,其中x是3,

ii.PEG(4),和

iii.-Gly-DLys-Gly-。

3.根据权利要求1-3中任一项所述的双位点BACE1抑制剂,其中A'选自由以下组成的组:

i.Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

ii.Tyr-Pro-Lys-Phe-Ile-Pro-Leu-,

iii.Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

iv.Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-,

v.Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-

vi.Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-,

vii.Tyr-Pro-Lys-Pro-Ala-Gln-Gly-

viii.Gly-Ala-Arg-Phe-Ile-Pro-Ala-,

ix.Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-,

x.Tyr-Pro-Tyr-Phe-Ile-Pro-DLys,

xi.DLys-Pro-Tyr-Phe-Ile-Pro-Leu-,

xii.H-Tyr-Pro-Tyr-Phe-DLys-Leu-,

xiii.H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

xiv.H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

xv.Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-.

xvi.H-Tyr-DLys-Tyr-Phe-Ile-Pro-Leu-,

xvii.H-Tyr-Pro-DLys-Phe-Ile-Pro-Leu-,

xviii.H-Tyr-Pro-Tyr-DLys-Ile-Pro-Leu-,

xix.生物素-PEG2-Cys-PEG8-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

xx.生物素-PEG2-Cys-PEG3-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,和

xxi.H-Gly-Gly-Gly-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-。

4.根据权利要求1-4中任一项所述的双位点BACE1抑制剂,其中A'选自由以下组成的组:

i.Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

ii.Tyr-Pro-Lys-Phe-Ile-Pro-Leu-,

iii.Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

iv.Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-,

v.Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-

vi.Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-,

viiTyr-Pro-Lys-Pro-Ala-Gln-Gly-

viii.Gly-Ala-Arg-Phe-Ile-Pro-Ala-,

ix.Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-,

x.Tyr-Pro-Tyr-Phe-Ile-Pro-DLys,

xi.DLys-Pro-Tyr-Phe-Ile-Pro-Leu-,

xii.H-Tyr-Pro-Tyr-Phe-DLys-Leu-,

xiii.H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

xiv.H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-,和

xv.Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-。

5.根据权利要求1-5中任一项所述的双位点BACE1抑制剂,其中B'选自由以下组成的组:

i.-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

ii.-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2,

iii.-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2,

iv.-Glu-Val-Asn-Leu*Ala-AIa-Glu-DPro-NH_2,和

v.Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2。

6.根据权利要求1至6中任一项所述的化合物,其选自由以下组成的组:

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x TFA

1H-Tyr-Pro-Lys-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

DLys-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

Gly-Ala-Arg-Phe-Ile-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

H-Tyr-Pro-Tyr-Phe-DLys-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

H-Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

H-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x 3TFA,

Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

Tyr-Pro-Lys-Pro-Ala-Gln-Gly-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

Tyr-Pro-Tyr-Phe-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,

Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2，

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPro-NH_2，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x 2TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 2TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2x TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x TFA，

Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 2TFA，

Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 2TFA，

H-Tyr-DLys-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2，

H-Tyr-Pro-DLys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2，

H-Tyr-Pro-Tyr-DLys-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2，

生物素-PEG2-Cys-PEG8-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2，

生物素-PEG2-Cys-PEG3-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,和

H-Gly-Gly-Gly-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA。

7.根据权利要求1至7中任一项所述的化合物,其选自由以下组成的组：

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x TFA，

1H-Tyr-Pro-Lys-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2，

DLys-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2，

Gly-Ala-Arg-Phe-Ile-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

H-Tyr-Pro-Tyr-Phe-DLys-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2，

H-Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2，

H-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-GIu-DPro-NH_2，

Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x 3TFA，

Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-GIu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Lys-Pro-Ala-Gln-Gly-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2，

Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-

DPhe-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2，

Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPro-NH_2，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x 2TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 2TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2x TFA，

Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x TFA，

Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA，

H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 2TFA，和

Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 2TFA。

8.根据权利要求1至5中任一项所述的化合物，其中所述药用盐是三氟乙酸盐。

9.根据权利要求1-8中任一项所述的双位点BACE1抑制剂，其用作治疗活性物质。

10.根据权利要求1-8中任一项所述的双位点BACE1抑制剂，其用作治疗活性物质，所述治疗活性物质用于治疗性和/或预防性治疗以升高的β-淀粉样蛋白水平和/或β-淀粉样蛋白低聚物和/或β-淀粉样蛋白斑以及进一步的沉积物为特征的疾病和病症，尤其是阿尔茨海默病。

11.一种药物组合物，所述药物组合物包含根据权利要求1-8中任一项所述的双位点BACE1抑制剂和药用载体和/或药用辅助物质。

12.根据权利要求1-8中任一项所述的双位点BACE1抑制剂用于制备药物的用途，所述

药物用于治疗性和/或预防性治疗以升高的β-淀粉样蛋白水平和/或β-淀粉样蛋白低聚物和/或β-淀粉样蛋白斑以及进一步的沉积物为特征的疾病和病症，尤其是阿尔茨海默病。

13.一种用于抑制BACE1活性、特别是用于治疗性和/或预防性治疗以升高的β-淀粉样蛋白水平和/或β-淀粉样蛋白低聚物和/或β-淀粉样蛋白斑以及进一步的沉积物为特征的疾病和病症、阿尔茨海默病、糖尿病或2型糖尿病的方法，所述方法包括向人类或动物施用根据权利要求1-8中任一项所述的双位点BACE1抑制剂。

14.如上描述的本发明。

BACE1抑制剂肽

[0001] 本发明涉及具有双重BACE1抑制性质的肽,它们的制备,含有它们的药物组合物以及它们作为治疗活性物质的用途。

技术领域

[0002] 本发明化合物具有Asp2(β-分泌酶,BACE1或膜天冬氨酸蛋白酶-2(Memapsin-2))抑制活性并且因此可以用于特征在于升高的β-淀粉样蛋白水平和/或β-淀粉样蛋白低聚物和/或β-淀粉样蛋白斑以及进一步的沉积物的疾病和病症、尤其是阿尔茨海默病的治疗性和/或预防性治疗。

背景技术

[0003] 阿尔茨海默病(AD)是中枢神经系统的神经变性疾病并且是老年人口中进行性痴呆的主要原因。其临床症状是记忆、认知、暂时和局部定向、判断和推理的损害,以及严重的情绪紊乱。目前还没有可以预防该疾病或其进展或稳定地逆转其临床症状的有效治疗。在所有具有高预期寿命的社会中AD已经成为主要的健康问题,并且也已经成为这些社会健康体系的显著经济负担。

[0004] BACE1酶负责APP蛋白的蛋白水解裂解中的一种,其促进了阿尔茨海默病相关的Aβ-肽的产生。通过抑制BACE1酶来减缓或停止Aβ-肽的产生是有前途的治疗概念。

[0005] 针对活性位点的BACE1抑制剂在例如WO2006/002907中描述并且针对外部位点(exosite)(催化结构域)的BACE1抑制剂在例如Kornacker等人,Biochemistry 2005,44,11567-73中描述。Linning,Organic&Biomolecular Chemistry 10(41),2012,p8216,WO2005097199,US2007149763和WO2013056054描述了具有肽结构的Bace1抑制剂。

[0006] 发明详述

[0007] 本发明的目的是双位点BACE1抑制剂(所述抑制剂与BACE酶的酶促活性位点和催化结构域二者结合),上述化合物的制备,含有它们的药物及它们的制备,以及上述化合物在治疗性和/或预防性治疗与抑制BACE1活性相关的疾病和病症(诸如阿尔茨海默病)中的用途。此外,本发明的化合物通过抑制由APP或APP片段产生Aβ来抑制β-淀粉样蛋白斑在神经组织(例如,大脑)中、上或周围的形成、或形成和沉积。

[0008] 不管被讨论的术语单独或与其他基团组合地出现,下列对本说明书中使用的一般术语的定义均适用。

[0009]

氨基酸	3-字母	1-字母
丙氨酸	Ala	A
精氨酸	Arg	R
天冬酰胺	Asn	N
天冬氨酸	Asp	D
半胱氨酸	Cys	C

谷氨酸	Glu	E
谷氨酰胺	Gln	Q
甘氨酸	Gly	G
组氨酸	His	H
异亮氨酸	Ile	I
亮氨酸	Leu	L
赖氨酸	Lys	K
甲硫氨酸	Met	M
苯丙氨酸	Phe	F
脯氨酸	Pro	P
丝氨酸	Ser	S
苏氨酸	Thr	T
色氨酸	Trp	W
酪氨酸	Tyr	Y
缬氨酸	Val	V

[0010] 表1:本文中使用的氨基酸缩写

[0011] 表2:本文中使用的氨基酸缩写

[0012] 术语“Sta”表示抑胃酶氨酸(statine),即(3S,4S)-4-氨基-3-羟基-6-甲基庚酸(CAS 49642-07-1)。

[0013] 术语“MetSta”表示(3S,4S)-4-氨基-3-羟基-6-甲基硫己酸(CAS不适用(n/a)),(Fmoc保护的CAS:268542-18-3)。

[0014] 术语“PEG(4)”表示15-氨基-4,7,10,13,四氧杂十五烷酸(CAS:不适用),(Fmoc保护的CAS:557756-85-1)。

[0015] 术语Leu*Ala表示“Tang”羟基亚乙基二肽等排体(文献:A.K.Ghosh,D.Shin,D.Downs,G.Koelsch,X.Lin,J.Ermolieff和J.Tang,J.Am.Chem.Soc.,2000,122,3522)。

[0016] 术语“PEG2”表示8-氨基-3,6-二氧杂辛酸(CAS:不适用),(Fmoc保护的CAS:166108-71-0)。

[0017] 术语“PEG3”表示12-氨基-4,7,10-三氧杂十二烷酸(CAS:不适用),Fmoc保护的CAS:867062-95-1)。

[0018] 术语“药用盐”是指适合与人类和动物的组织接触使用的盐。与无机和有机酸的合适的盐的实例为,但是不限于:乙酸、柠檬酸、甲酸、富马酸、盐酸、乳酸、马来酸、苹果酸、甲磺酸、硝酸、磷酸、对甲苯磺酸、琥珀酸、硫磺酸、硫酸、酒石酸、三氟乙酸(TFA)等。具体的盐是三氟乙酸盐。

[0019] 术语“药用载体”和“药用辅助物质”是指与制剂的其他成分相容的载体和辅助物质如稀释剂或赋形剂。

[0020] 术语“药物组合物”包括包含预定量或比例的特定成分的产品,以及通过组合特定量的特定成分直接地或间接地得到的任何产品。优选地,它包括包含一种或多种活性成分,和任选的包含惰性成分的载体的产品,以及由任何两种以上的成分的组合、复合或聚集,或者由一种或多种成分的分解,或由一种或多种成分的其他类型的反应或相互作用直接地或

间接地得到的任何产物。

[0021] 术语“半最大抑制浓度”(IC_{50})表示在体外获得生物过程的50％抑制所需的特定化合物的浓度。可以将IC_{50}值对数地转换为pIC_{50}值(-log IC_{50}),其中较大的值表示指数地增加的效力。IC_{50}值不是绝对值而依赖于试验条件例如所采用的浓度。可以将IC_{50}值使用Cheng-Prusoff公式(Biochem.Pharmacol.(1973)22:3099)转换为绝对抑制常数(Ki)。术语“抑制常数”(Ki)表示特定抑制剂对受体的绝对结合亲和性。其使用竞争结合测定测量,并且等于如果不存在竞争配体(例如放射性配体)特定抑制剂将占据受体的50％的情况下的浓度。可以将Ki值对数地转换为pKi值(-log Ki),其中较大的值表示指数地增加的效力。

[0022] “治疗有效量”意指当被施用于受试者用于治疗疾病状态时,足以实现对于疾病状态的这种治疗的化合物的量。“治疗有效量”将依赖于化合物、所治疗的疾病状态、所治疗的疾病的严重性、受试者的年龄和相对健康状况、给药的路线和形式、主治医师或兽医师的判断以及其他因素而变化。

[0023] 术语“如本文所定义的”和“如本文所描述的”当涉及变量时通过引用结合变量的宽泛定义以及如果有的话,优选的、更优选的和最优选的定义。

[0024] 当涉及化学反应时术语“处理”、“接触”和“反应”意指在合适的条件下加入或混合两种以上的试剂以制备所示和/或所需的产物。应该明白产生所示和/或所需产物的反应可能不一定直接得自最初加入的两种试剂的组合,即,在混合物中可能产生最终导致所示和/或所需产物的形成的一种或多种中间体。

[0025] 本发明还提供药物组合物、使用上述化合物的方法和制备上述化合物的方法。

[0026] 所有单独的实施方案可以进行组合。

[0027] 本发明涉及双位点BACE1抑制剂,所述抑制剂与BACE1酶的酶促活性位点和催化结构域二者结合。

[0028] 单独含有抑胃酶氨酸型过渡态模拟物的外部位点基序以及活性位点抑制剂不显示BACE1抑制。

[0029] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂或其药用盐,所述抑制剂与BACE酶的酶促活性位点和催化结构域二者结合,其中通过接头(L')将外部位点抑制部分(A')与所述BACE1抑制剂的活性位点抑制部分(B')连接。

[0030] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其中L'选自由以下组成的组:

[0031] i.-$(Gly)_x$-,其中x是3,

[0032] ii.PEG(4),和

[0033] iii.-Gly-DLys-Gly-。

[0034] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其中A'选自由以下组成的组:

[0035] i.Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

[0036] ii.Tyr-Pro-Lys-Phe-Ile-Pro-Leu-,

[0037] iii.Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

[0038] iv.Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-,

[0039] v.Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-

[0040] vi.Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-,

[0041] vii.Tyr-Pro-Lys-Pro-Ala-Gln-Gly-

[0042] viii.Gly-Ala-Arg-Phe-Ile-Pro-Ala-,

[0043] ix.Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-,

[0044] x.Tyr-Pro-Tyr-Phe-Ile-Pro-DLys,

[0045] xi.DLys-Pro-Tyr-Phe-Ile-Pro-Leu-,

[0046] xii.H-Tyr-Pro-Tyr-Phe-DLys-Leu-,

[0047] xiii.H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

[0048] xiv.H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

[0049] xv.Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-.

[0050] xvi.H-Tyr-DLys-Tyr-Phe-Ile-Pro-Leu-,

[0051] xvii.H-Tyr-Pro-DLys-Phe-Ile-Pro-Leu-,

[0052] xviii.H-Tyr-Pro-Tyr-DLys-Ile-Pro-Leu-,

[0053] xix.生物素-PEG2-Cys-PEG8-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

[0054] xx.生物素-PEG2-Cys-PEG3-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,和

[0055] xxi.H-Gly-Gly-Gly-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-。

[0056] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其中A'选自由以下组成的组:

[0057] i.Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-,

[0058] ii.Tyr-Pro-Lys-Phe-Ile-Pro-Leu-,

[0059] iii.Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

[0060] iv.Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-,

[0061] v.Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-

[0062] vi.Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-,

[0063] vii.Tyr-Pro-Lys-Pro-Ala-Gln-Gly-

[0064] viii.Gly-Ala-Arg-Phe-Ile-Pro-Ala-,

[0065] ix.Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-,

[0066] x.Tyr-Pro-Tyr-Phe-Ile-Pro-DLys,

[0067] xi.DLys-Pro-Tyr-Phe-Ile-Pro-Leu-,和

[0068] xii.H-Tyr-Pro-Tyr-Phe-DLys-Leu-.

[0069] xiii.H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-,

[0070] xiv.H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-,和

[0071] xv.Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu。

[0072] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其中B'选自由以下组成的组:

[0073] i.-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0074] ii.-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2,

[0075] iii.-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2,

[0076] iv.-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,和

[0077] v.Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2。

[0078] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其选自由以下组成的组:

[0079] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x TFA

[0080] 1H-Tyr-Pro-Lys-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0081] DLys-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0082] Gly-Ala-Arg-Phe-Ile-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

[0083] H-Tyr-Pro-Tyr-Phe-DLys-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0084] H-Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0085] H-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0086] Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,

[0087] Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

[0088] Tyr-Pro-Lys-Pro-Ala-Gln-Gly-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

[0089] Tyr-Pro-Tyr-Phe-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,

[0090] Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

[0091] Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

[0092] Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,

[0093] Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x 3TFA,

[0094] Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

[0095] Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,

[0096] Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPro-NH_2,

[0097] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 2TFA,

[0098] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA,

[0099] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x TFA,

[0100] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x TFA,

[0101] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2x TFA,

[0102] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x TFA,

[0103] Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

[0104] Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

[0105] Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

[0106] H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

[0107] H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 2TFA,

[0108] Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 2TFA,

[0109] H-Tyr-DLys-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0110] H-Tyr-Pro-DLys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0111] H-Tyr-Pro-Tyr-DLys-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0112] 生物素-PEG2-Cys-PEG8-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0113] 生物素-PEG2-Cys-PEG3-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,和

[0114] H-Gly-Gly-Gly-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA。

[0115] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其选自由以下组成的组:

[0116] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-

NH_2x TFA,

[0117] 1H-Tyr-Pro-Lys-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0118] DLys-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0119] Gly-Ala-Arg-Phe-Ile-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

[0120] H-Tyr-Pro-Tyr-Phe-DLys-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0121] H-Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0122] H-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2,

[0123] Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2x 3TFA,

[0124] Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

[0125] Tyr-Pro-Lys-Pro-Ala-Gln-Gly-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

[0126] Tyr-Pro-Tyr-Phe-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,

[0127] Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

[0128] Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 3TFA,

[0129] Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA,

[0130] Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x 3TFA,

[0131] Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

[0132] Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH_2,

[0133] Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPro-NH_2,

[0134] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 2TFA,

[0135] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA,

[0136] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA,

[0137] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA,

[0138] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x TFA,

[0139] Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA,

[0140] Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

[0141] Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

[0142] Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

[0143] H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA,

[0144] H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 2TFA,和

[0145] Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2x 2TFA。

[0146] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其中药用盐是三氟乙酸盐。

[0147] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其用作治疗活性物质。

[0148] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其用作BACE1活性的抑制剂。

[0149] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其用作治疗活性物质,所述治疗活性物质用于治疗性和/或预防性治疗以升高的β-淀粉样蛋白水平和/或β-淀粉样蛋白低聚物和/或β-淀粉样蛋白斑以及进一步的沉积物为特征的疾病和病症,尤其是阿尔茨海默病。

[0150] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂,其用作治疗活性物质,所述治疗活性物质用于治疗性和/或预防性治疗阿尔茨海默病。

[0151] 本发明的一个特定实施方案涉及药物组合物,所述药物组合物包含如本文所述的双位点BACE1抑制剂和药用载体和/或药用辅助物质。

[0152] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂用于制备药物的用途,所述药物用于抑制BACE1活性。

[0153] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂用于制备药物的用途,所述药物用于治疗性和/或预防性治疗以升高的β-淀粉样蛋白水平和/或β-淀粉样蛋白低聚物和/或β-淀粉样蛋白斑以及进一步的沉积物为特征的疾病和病症,尤其是阿尔

茨海默病。

[0154] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂用于制备药物的用途，所述药物用于治疗性和/或预防性治疗阿尔茨海默病。

[0155] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂，所述抑制剂用于抑制BACE1活性。

[0156] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂，所述抑制剂用于治疗性和/或预防性治疗以升高的β-淀粉样蛋白水平和/或β-淀粉样蛋白低聚物和/或β-淀粉样蛋白斑以及进一步的沉积物为特征的疾病和病症，尤其是阿尔茨海默病。

[0157] 本发明的一个特定实施方案涉及如本文所述的双位点BACE1抑制剂，所述抑制剂用于治疗性和/或预防性治疗阿尔茨海默病。

[0158] 本发明的一个特定实施方案涉及一种用于抑制BACE1活性，特别是用于治疗性和/或预防性治疗以升高的β-淀粉样蛋白水平和/或β-淀粉样蛋白低聚物和/或β-淀粉样蛋白斑以及进一步的沉积物为特征的疾病和病症、阿尔茨海默病的方法，所述方法包括向人类或动物施用如本文所述的双位点BACE1抑制剂。

[0159] 此外，本发明包括所有旋光异构体，即非对映异构体、非对映异构体混合物、外消旋混合物、所有它们相应的对映体和/或互变异构体以及它们的溶剂化物。

[0160] 双位点BACE1抑制剂可以如本文中描述的制备。原材料是可商购的或者可以按照已知方法制备。

[0161] 可以通过本领域技术人员已知的标准方法获得相应的与酸的药用盐，例如通过将双位点BACE1抑制剂溶解在合适的溶剂如二噁烷或四氢呋喃(THF)中并加入合适量的相应的酸获得。通常可以通过过滤或通过色谱分离产物。用碱将双位点BACE1抑制剂转化为药用盐可以通过将这种化合物用这样的碱处理而进行。形成这种盐的一种可能方法是例如通过向该化合物在合适的溶剂(例如乙醇、乙醇-水混合物、四氢呋喃-水混合物)中的溶液中，加入1/n当量的碱盐如$M(OH)_n$，其中M＝金属或铵阳离子，并且n＝氢氧根阴离子的数量，并且通过蒸发或冷冻干燥移除溶剂。

[0162] 在其制备未被描述在实施例中的情况下，可以根据类似方法或根据在此给出的方法制备双位点BACE1抑制剂以及所有中间体产物。原材料是可商购的，本领域已知的或者可以通过本领域已知方法或与其类似的方法制备。

[0163] 应理解，可以将本发明中双位点的BACE1抑制剂在官能团处进行衍生以提供能够在体内转化回母体化合物的衍生物。

[0164] 药理学测试

[0165] 双位点BACE1抑制剂和它们的药用盐拥有有价值的药理学特性。已经发现本发明的化合物与BACE1活性的抑制有关。按照在下文中给出的测试研究该化合物。

[0166] 细胞Aβ-降低测定：

[0167] 可以使用人HEK293细胞在细胞测定中对化合物的效力进行评价，所述人HEK293细胞用表达人APP野生型基因(APP695)的cDNA的载体稳定转染。将所述细胞接种在96孔微量滴定板中的细胞培养基(Iscove，加10％(v/v)胎牛血清、谷氨酰胺、青霉素/链霉素)中，至约80％会合并且将化合物以在1/10体积培养基中的10x浓度加入，所述培养基不具有FCS而含有8％DMSO(保持DMSO终浓度为0.8％v/v)。在加湿培养箱中在37℃和5％CO_2下孵育18-20

小时后,收获培养上清以确定Aβ40浓度。96孔ELISA板(例如,Nunc MaxiSorb)用单克隆抗体包被,所述单克隆抗体特异性识别Aβ40的C-末端(Brockhaus等人,NeuroReport 9,1481-1486;1998)。在用例如1%BSA封闭非特异性结合位点并洗涤之后,将培养上清加入合适的稀释液连同辣根过氧化物酶(horseradish peroxidase)偶联的Aβ检测抗体(例如,抗体4G8,Senetek,Maryland Heights,MO),并孵育5至7小时。然后将微量滴定板的孔用含有0.05%吐温20的Tris缓冲盐水充分洗涤,并且在柠檬酸缓冲液中用四甲基联苯胺/H_2O_2显色测定。在用1体积的1N H_2SO_4终止反应后,在ELISA读数器中在450nm波长处测量反应。在培养上清中Aβ的浓度由通过已知量的纯Aβ肽获得的标准曲线计算。

[0168]

实施例	名称	系统名称	MW	IC 50 (μM)
1	YPYFIPL-PEG(4)-EVN-Sta-VAEp-NH$_2$ x TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH$_2$ x TFA	2168.4	0.0027
2	YPYFIPL-GGG-EVN-Sta-VAEp-NH$_2$ x TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH$_2$ x TFA	2092.27	0.42
3	YPYFIPL-GkG-EVN-Sta-VAEp-NH$_2$ x 2TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH$_2$ x 2TFA	2277.41	0.094
4	YPKFIPL-GkG-EVN-Sta-VAEp-NH$_2$ x 3TFA	Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH$_2$ x 3TFA	2356.44	0.28
5	YPYFIPk-GkG-EVN-Sta-VAEp-NH$_2$ x 3TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH$_2$ x 3TFA	2406.45	0.019
6	YPYFKPA-GkG-EVN-Sta-VAEp-NH$_2$ x 3TFA	Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH$_2$ x 3TFA	2364.37	0.046
7	YPYFIPL-PEG(4)-EVN-MetSta-VAEf-NH$_2$ x TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH$_2$ x TFA	2236.5	0.43

[0169]

8	YPYFIPL-PEG(4)-EVN-MetSta-VAEP-NH_2 x TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA	2186.44	0.017
9	YPYFIPL-GGG-EVN-MetSta-VAEP-NH_2 x TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-Gly-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x TFA	2110.31	1.34
10	YPYFIPL-GkG-EVN-MetSta-VAEP-NH_2 x 2TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 2TFA	2295.45	0.47
11	YPKFIPL-GkG-EVN-MetSta-VAEP-NH_2 x 3TFA	Tyr-Pro-Lys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA	2374.47	0.76
12	YPYFIkL-GkG-EVN-Sta-VAEp-NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Ile-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2422.49	>40
13	YPYFkPL-GkG-EVN-Sta-VAEp-NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2406.45	>40
14	YPYFKPA-GkG-EVN-Sta-VAEp-NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2364.37	0.071

[0170]

15	TPKPAQG-GkG-EVN-Sta-VAEp-NH_2 x 3TFA	Tyr-Pro-Lys-Pro-Ala-Gln-Gly-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2177.14	>40
16	GARFIPA-GkG-EVN-Sta-VAEp-NH_2 x 3TFA	Gly-Ala-Arg-Phe-Ile-Pro-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2210.21	0.42
17	YPKFISA-GkG-EVN-Sta-VAEp-NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Ile-Ser-Ala-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2304.32	0.75
18	YPYFIPk-GkG-EVN-MetSta-VAEP-NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-Pro-NH_2 x 3TFA	2424.49	0.015
19	YPYFIPk-GkG-EVN-MetSta-VAEf-NH_2 x 3TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x 3TFA	2474.55	0.19
20	kPYFIPLGkGEVN-Sta-VAEp-NH_2 x 3TFA	DLys-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2356.44	2.93
21	YPKFIPL-PEG(4)-EVN-Sta-VAEp-NH_2 x 3TFA	1H-Tyr-Pro-Lys-Phe-Ile-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2247.43	0.052

[0171]

22	YPYFIPk-PEG(4)-EVN-Sta-VAEp-NH$_2$ x 2TFA	H-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH$_2$ x 2TFA	2297.44	0.045
23	YPYFkL-PEG(4)-EVN-Sta-VAEp-NH$_2$ x 2TFA	H-Tyr-Pro-Tyr-Phe-DLys-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH$_2$ x 3TFA	2200.33	>40
24	YPYFkPL-PEG(4)-EVN-Sta-VAEp-NH$_2$ x 2TFA	H-Tyr-Pro-Tyr-Phe-DLys-Pro-Leu-PEG(4)-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH$_2$ x 2TFA	2297.44	>40
25	YPYFIPk-PEG(4)-EVN-Leu*Ala-Aep-NH$_2$ x 2TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH$_2$ x 2TFA	2226.36	0.015
26	YPYFkLGkGEVN-Leu*Ala-Aep-NH$_2$ x 3TFA	Tyr-Pro-Tyr-Phe-DLys-Leu-Gly-DLys-Gly-Glu-Val-Asn-Leu*Ala-Ala-Glu-DPro-NH$_2$ x 3TFA	2238.26	0.034
27	YPYFIPk-PEG(4)-EVN-MetSta-VAEp-NH$_2$ x 2TFA	Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-PEG(4)-Glu-Val-Asn-MetSta-Val-Ala-Glu-DPro-NH$_2$ x 2TFA	2315.48	1.01
28	yPYFIPkGkGEVN-Sta-VAEp-NH$_2$ x 3TFA	H-DTyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH$_2$ x 3TFA	2406.45	0.063

[0172]

29	yPYFIPLGkGEVN-Sta-VAEp-NH_2 x 2TFA	H-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 2TFA	2277.41	0.81
30	Ac-yPYFIPLGkGEVN-Sta-VAEp-NH_2 x TFA	Ac-DTyr-Pro-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA	2205.43	0.11
31	YkYFIPLGkGEVN-Sta-VAEp-NH2.TFA	H-Tyr-DLys-Tyr-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH2	2422.49	> 40
32	YPkFIPLGkGEVN-Sta-VAEp-NH2.TFA	H-Tyr-Pro-DLys-Phe-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2	2356.44	29.29
33	YPYkIPLGkGEVN-Sta-VAEp-NH2.TFA	H-Tyr-Pro-Tyr-DLys-Ile-Pro-Leu-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2	2372.44	34.83
34	Btn-PEG2-C-PEG8-YPYFIPkGkGEVN-Sta-VAEp-NH2.TFA	生物素-PEG2-Cys-PEG8-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2	3190.52	0.018
35	Btn-PEG2-C-PEG3-YPYFIPkGkGEVN-Sta-VAEp-NH2.TFA	生物素-PEG2-Cys-PEG3-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2	2970.26	0.026

[0173]

36	GGGYPYFIPkGk GEVN-Sta-VAEp-NH2.TFA	H-Gly-Gly-Gly-Tyr-Pro-Tyr-Phe-Ile-Pro-DLys-Gly-DLys-Gly-Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x 3TFA	2577.61	0.0064

[0174] 表3:选择的实例的IC_{50}值

[0175] 单独含有抑胃酶氨酸型过渡态模拟物的外部位点基序以及活性位点的抑制剂不显示BACE1抑制。

[0176]

实施例	名称	系统名称	**MW**	**IC 50 (μM)**
E1	Ac-QQYPYFKPA N-NH_2	Ac-Gln-Gln-Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-Asn-NH_2	1295.6	> 40
E2	Ac-YPTFKPANGS -NH_2	Ac-Tyr-Gln-Thr-Phe-Lys-Pro-Ala-Asn-Gly-Ser-NH_2	1121.6	> 40
E3	Ac-STGARFIPAN-NH_2	Ac-Ser-Thr-Gly-Ala-Arg-Phe-Ile-Pro-Ala-Asn-NH_2	1073.6	> 40
E4	Ac-GDYPKFISAN -NH_2	Ac-Gly-Asp-Tyr-Pro-Lys-Phe-Ile-Ser-Ala-Asn-NH_2	1151.6	> 40
E5	Ac-GDYPKFIPAS-NH_2	Ac-Gly-Asp-Tyr-Pro-Lys-Phe-Ile-Pro-Ala-Ser-NH_2	1134.6	> 40
E6	Ac-GSYPKFIDAN -NH_2	Ac-Gly-Ser-Tyr-Pro-Lys-Phe-Ile-Asp-Ala-Asn-NH_2	1151.6	> 40
E7	Ac-LTTYPYFKPA -NH_2	Ac-Leu-Thr-Thr-Tyr-Pro-Tyr-Phe-Lys-Pro-Ala-NH_2	1240.6	14.41

[0177] 表4:外部位点基序的选择的实例的IC_{50}值

[0178]

实施例	名称	系统名称	MW	IC 50 (μM)
A1	EVN-Sta-VAEF	Glu-Val-Asn-Sta-Val-Ala-Glu-Phe-NH_2 x TFA	961.2	> 40
A2	EVN-MetSta-VAEf	Glu-Val-Asn-MetSta-Val-Ala-Glu-DPhe-NH_2 x TFA	981.65	> 40
A3	EVN-Sta-VAEp	Glu-Val-Asn-Sta-Val-Ala-Glu-DPro-NH_2 x TFA	1041.59	> 40

[0179] 表5:活性位点基序的选择的实例的IC_{50}值

[0180] 药物组合物

[0181] 双位点BACE1抑制剂和药用盐可以用作治疗活性物质,例如以药物制剂的形式。该药物制剂可以口服给药,例如,以片剂、包衣片剂、糖锭剂、硬和软明胶胶囊、溶液、乳剂或混悬剂形式。然而,也可以通过直肠实现给药,例如,以栓剂的形式,或者经肠胃外给药,例如,以注射液的形式。

[0182] 可以将双位点BACE1抑制剂及其药用盐与制药学惰性的、无机或有机的载体进行加工用于制备药物制剂。例如,可以使用乳糖、玉米淀粉或其衍生物、滑石、硬脂酸或其盐等作为用于片剂、包衣片剂、糖锭剂和硬明胶胶囊的载体。适用于软明胶胶囊的载体是例如植物油、蜡、脂肪、半固体和液体多元醇等。然而,取决于活性成分的性质,在软明胶胶囊的情况下经常不需要载体。用于制备溶液和糖浆的适合的载体是例如水、多元醇、甘油、植物油等。适用于栓剂的载体是,例如,天然或硬化油、蜡、脂肪、半液体或液体多元醇等。

[0183] 此外,药物制剂可以含有药用辅助物质如防腐剂、增溶剂、稳定剂、湿润剂、乳化剂、甜味剂、着色剂、增香剂、用于改变渗透压的盐、缓冲剂、掩蔽剂或抗氧化剂。它们还可以含有其它治疗上有价值的物质。

[0184] 含有双位点BACE1抑制剂或其药用盐以及治疗惰性载体的药物也是本发明的目的,用于其制备的方法一样是本发明的目的,所述方法包括将一种或多种双位点BACE1抑制剂和/或其药用盐以及,如果需要,一种或多种其他治疗上有价值的物质与一种或多种治疗惰性载体一起制成盖仑给药形式。

[0185] 剂量可以在宽范围内变化,并且当然在每个具体病例中必须根据个体需求而调节。在口服给药的情形中,用于成人的剂量可以从约0.01mg/天变化至约1000mg/天的双位点BACE1抑制剂或相应量的其药用盐。该日剂量可以以单一剂量给药或以分剂量给药,并且此外,当需要时,也可以超过该上限。

[0186] 以下实施例说明本发明,但不对其进行限制,而是仅作为其示例。药物制剂便利地含有约1-500mg、优选1-100mg的双位点BACE1抑制剂。根据本发明的组合物的实例为:

[0187] 实施例A

[0188] 以通常方式制备以下组成的片剂:

[0189]

成分	mg/片			
	5	25	100	500
双位点 BACE1 抑制剂	5	25	100	500
无水乳糖 DTG	125	105	30	150
Sta-Rx 1500	6	6	6	60
微晶纤维素	30	30	30	450
硬脂酸镁	1	1	1	1
总计	167	167	167	831

[0190] 表6:可能的片剂组成

[0191] 制备过程:

[0192] 1.将成分1、2、3和4混合,并用纯水粒化。

[0193] 2.将颗粒在50℃下干燥。

[0194] 3.使颗粒通过合适的研磨设备。

[0195] 4.加入成分5并混合三分钟;在合适的压机上压制。

[0196] 实施例B-1

[0197] 制备以下组成的胶囊:

[0198]

成分	mg/胶囊			
	5	25	100	500
双位点 BACE1 抑制剂	5	25	100	500
含水乳糖	159	123	148	-
玉米淀粉	25	35	40	70
滑石(Talk)	10	15	10	25
硬脂酸镁	1	2	2	5
总计	200	200	300	600

[0199] 表7:可能的胶囊成分组成

[0200] 制备过程:

[0201] 1.将成分1、2和3在合适的混合机中混合30分钟。

[0202] 2.加入成分4和5,并混合3分钟。

[0203] 3.填充至合适的胶囊中。

[0204] 将双位点BACE1抑制剂、乳糖和玉米淀粉首先在混合机中混合,并且之后在粉碎机中混合。将混合物送回混合机;将滑石加入其中并充分混合。将混合物通过机器填充至合适

的胶囊中，例如硬明胶胶囊。

[0205] 实施例B-2

[0206] 制备以下组成的软明胶胶囊：

[0207]

成分	mg/胶囊
双位点BACE1抑制剂	5
黄蜡	8
氢化大豆油	8
部分氢化的植物油	34
大豆油	110
总计	165

[0208] 表8：可能的软明胶胶囊成分组成

[0209]

成分	mg/胶囊
明胶	75
甘油 85 %	32

[0210]

山梨糖醇(Karion) 83	8 (干物质)
二氧化钛	0.4
氧化铁黄	1.1
总计	116.5

[0211] 表9：可能的软明胶胶囊组成

[0212] 制备过程：

[0213] 将双位点BACE1抑制剂溶解在其他成分的温暖熔体中，并将混合物填充至适当大小的软明胶胶囊中。根据通常程序处理填充的软明胶胶囊。

[0214] 实施例C

[0215] 制备以下组成的栓剂：

[0216]

成分	mg/栓剂
双位点BACE1抑制剂	15
栓剂块	1285
总计	1300

[0217] 表10：可能的栓剂组成

[0218] 制备过程：

[0219] 将栓剂块在玻璃或钢容器中熔化，充分混合并冷却至45℃。之后，将细粉化的双位点BACE1抑制剂加入其中并搅拌直至其完全分散。将混合物倒入合适大小的栓剂模具中，放置冷却；之后将栓剂从模具移出并单独地包装在蜡纸或金属箔中。

[0220] 实施例D

[0221] 制备以下组成的针剂：

[0222]

成分	mg/针剂
双位点BACE1抑制剂	3
聚乙二醇400	150
乙酸	适量至pH5.0
针剂用水	至1.0ml

[0223] 表11:可能的针剂组成

[0224] 制备过程：

[0225] 将双位点BACE1抑制剂溶解在聚乙二醇400和注射用水(部分)的混合物中。将pH通过乙酸调节至5.0。通过加入余量的水将体积调节至1.0ml。将溶液过滤,使用适当过量装入小瓶中并灭菌。

[0226] 实施例E

[0227] 制备以下组成的小药囊：

[0228]

成分	mg/小药囊
双位点BACE1抑制剂	50
乳糖,细粉	1015
微晶纤维素(AVICEL PH 102)	1400
羧甲基纤维素钠	14
聚乙烯吡咯烷酮K 30	10
硬脂酸镁	10
调味添加剂	1
总计	2500

[0229] 表12:可能的小药囊组成

[0230] 制造过程：

[0231] 将双位点BACE1抑制剂与乳糖、微晶纤维素和羧甲基纤维素钠混合,并且用聚乙烯吡咯烷酮在水中的混合物造粒。将颗粒与硬脂酸镁和调味添加剂混合,并装入小药囊中。

[0232] 实验部分

[0233] 为说明本发明提供以下实施例。它们不应被认为是限制本发明的范围,而只是作为其代表。

[0234] CEM自由微波肽合成仪(CEM Liberty Microwave Peptide Synthesizer)的一般程序：

[0235] 0.1mMol规模：

[0236] Fmoc的去保护：

[0237] 将洗涤并预先膨胀的树脂(435mg,0.1mMol,TentaGel S RAM(载荷:0.23mMol/g),(Rapp Polymere,Cat:S30023)用哌啶20％在二甲基甲酰胺(DMF)(7.0mL)中的溶液在微波条件下在50℃处理3分钟以进行初始脱保护。将树脂用DMF洗涤,并在微波条件下在75℃用哌啶20％在DMF中的溶液(7.0mL)处理5分钟以脱保护。

[0238] 氨基酸的偶联：

[0239] 向洗涤并预先膨胀的树脂中加入氨基酸0.2M在DMF(2.5mL,5.0当量)中的溶液,接着加入(1-氰基-2-乙氧基-2-氧代亚乙基氨基氧)二甲基氨基-吗啉代-碳鎓六氟磷酸盐(COMU)0.5M在DMF(1.0mL,5.0当量)中的溶液(CAS:1075198-30-9,Iris Biotech,Cat:RL-1175.1000),然后加入二异丙基乙胺(DIPEA)2M在N-甲基-2-吡咯烷酮(NMP)(0.5mL,10.0当量)中的溶液。将该反应混合物在微波条件下在75℃处理5分钟以进行偶联。

[0240] 0.25mMol规模：

[0241] Fmoc的去保护：

[0242] 将洗涤并预先膨胀的树脂(1.09g,0.25mMol,TentaGel S RAM(载荷:0.23mMol/g),(Rapp Polymere,Cat:S30023)用哌啶20％在DMF(10.0mL)中的溶液在微波条件在50℃处理3分钟以进行初始脱保护。将树脂用DMF洗涤,并在微波条件下在75℃用哌啶20％在DMF中的溶液(10.0mL)处理5分钟以脱保护。

[0243] 氨基酸的偶联：

[0244] 向洗涤并预先膨胀的树脂中加入氨基酸0.2M在DMF(5.0mL,4.0当量)中的溶液,接着加入COMU 0.5M在DMF(2.0mL,4.0当量)(CAS:1075198-30-9,Iris Biotech,Cat:RL-1175.1000)中的溶液,然后加入DIPEA2M在NMP(1.0mL,8.0当量)中的溶液。将该反应混合物在微波条件下在75℃处理5分钟以进行偶联。

[0245] 最终裂解的一般程序：

[0246] 0.1mMol规模：

[0247] 将树脂用CH_2Cl_2洗涤,然后在摇床上在室温用三氟乙酸(TFA):三异丙基甲硅烷(TIS):水95:2.5:2.5(5mL)的溶液处理30分钟。过滤树脂。将粗制肽用Et_2O(35mL)沉淀。将悬浮液离心并将溶剂滗析。将固体溶于乙腈和水中,并冻干以得到粗制肽。

[0248] 纯化的一般程序：

[0249] 将粗产物溶于乙腈和水(含有0.1％TFA)中,然后通过制备型HPLC纯化。柱YMC-Actus Pro C8,5μm,75x30mm,水(含有0.1％TFA):乙腈70:30至2:98的梯度,以及30mL/min的流速。

[0250] 类似本文所述的一般程序,可以制备实施例。

[0251] 使用综合的5-聚体肽(5-mer peptide)阵列的BACE1结合物发现

[0252] 阵列设计、合成和背景对照:为了发现BACE1结合物,设计了具有2.47M肽(代表所有可能的5-聚体肽的完全清单,不包括半胱氨酸)的阵列。每个5-聚体肽通过在N-末端和C-末端3个循环的接头合成而合成。对于接头合成,使用包含3:1比率的G和S的混合物。肽序列将为以下格式:ZZZ-5聚体-ZZZ,其中Z是来自接头混合物的氨基酸。在肽合成后,将阵列用Cy5-链霉抗生物素蛋白(Cy5-streptavidin)预染色,用于QC以及在含有333ng/ml Cy5-链霉抗生物素蛋白(Amersham,UK,cat#PA45001)的30ml结合缓冲液(1％碱溶性酪蛋白,Novagen,Billerica,USA,cat#70955,0.05％吐温20)中背景信号测量1小时。将阵列在洗涤缓冲液I(Roche/Nimblegen,Madison,USA)中洗涤30秒,并在0.1TE缓冲液中简单洗涤。通过在微型离心机中旋转将阵列干燥以除去痕迹量的水,并使用MS200扫描仪(Roche/Nimblegen)在635nm波长和2μm分辨率下进行扫描。在这一步之后,阵列准备好用于结合靶蛋白。

[0253] BACE1生物素化:使用Pierce EZ-link Micro NHS-PEG4-生物素化试剂盒,cat# 21955,将BACE1酶(WT1-13-2,0.7mg/ml)用生物素标记。为了标记,将70μl或50μg的BACE1酶与在水中新鲜制备的2μl NHS-生物素溶液混合,并在室温下温育1小时。使用Micro Bio-Spin 30色谱柱(BioRad,cat 732-6223),通过反应溶液的2X旋转-过滤(1,000g,4分钟)来除去过量的游离生物素。生物素化的BACE1在-20C等分地储存。

[0254] 结合测定:为确保数据重现性,将5μl生物素化的BACE1与40μl结合缓冲液混合,用附接的HX1混合器装载于阵列上,并在4C温育过夜。温育后,移除混合器,并将阵列在洗涤缓冲液I中洗涤30秒,并在0.1TE缓冲液中简短洗涤。将阵列在含有333ng/ml链霉抗生物素蛋白-Cy5(Amersham,UK,cat#PA45001)的30ml结合缓冲液中进行生物素化1小时,并如上所述进行洗涤。通过在微型离心机中旋转将阵列干燥以除去痕迹量的水,并使用MS200扫描仪(Roche/Nimblegen)在635nm波长和2um分辨率下获得数据。

[0255] 数据分析和结果:使用NimbleGen DEVA软件进行图像分析和信号提取。对于所有5-聚体序列,针对早期收集的背景信号(参见"阵列设计、合成和背景对照")将由BACE1结合产生的生物素特异性荧光信号绘出。已经从该分析中鉴定了具有最高BACE1特异性信号和低背景的5-聚体肽序列,并且由这些序列共享的六个最常见基序在下表的第一列中列出。

[0256]

核心基序	延伸文库	选自延伸文库的序列	双取代/缺失文库	选自取代/缺失文库的序列
YFK	XXYFKXX	YPYFKPA	GQYPYFKPAS QYPYFKPASG	QQYPYFKPAN
PYFK	XXPYFKXX	QYPYFKPA		
YFKP	XXYFKPXX	YPYFKPAS		
TFK	XXTFKXX	YPTFKPA	GGGYPTFKPA GGYPTFKPAG GYPTFKPAGG YPTFKPAGGG	YPTFKPANGS
PKF	XXPKFXX	DYPKFIS DYPKFLP SYPKFID	GDYPKFISGG GDYPKFLPGG GSYPKFIDGG	GDYPKFISAN GDYPKFIPAS GSYPKFIDAN
ARFIP	XXARFIPXX	TGARFIPAN	GTGARFIPAN TGARFIPANG	STGARFIPAN

[0257] 表13

[0258] 使用扩展肽阵列的BACE1结合物优化。

[0259] 阵列设计:在5-聚体阵列实验中鉴定的基序可能仅代表最佳BACE1结合物的较短版本。通过这样设计新的阵列可以发现更长的基序:使用表2第2列X所示的全部20种天然氨

基酸将选自5-聚体阵列的核心序列从N-端和C-端二者延伸两个氨基酸。各延伸文库包括以一式五份合成的160,000个独特的肽。如实施例1中所述进行BACE1结合测定和图像处理。

[0260] 数据分析和结果:对于每个核心基序,与BACE1结合最高的延伸序列在表13(第三列)中显示。'YFK'、'PYFK'和'YFKP'基序的延伸序列(表13中的前三行)共享相同的YPYFKPA序列,表明它们代表相同的共同序列。DYPKFLP、DYPKFIS、SYPKFID三个序列被选作'PKF'基序延伸,因为它们具有类似的与BACE1的结合以及在N-端和C-端具有不同的氨基酸组成。

[0261] 使用双取代/缺失肽阵列的BACE1结合物优化。

[0262] 阵列设计:第三轮结合物优化包括用甘氨酸(G)氨基酸延伸在延伸阵列实验中鉴定的序列以使它们成为如表13(第四列)所示的10-聚体肽,接着设计双取代/缺失文库,其包含参考序列的所有可能的单-和双-替代/缺失变体。对于10-聚体肽,双替代/缺失文库包括大约1.65万个独特的肽。每个肽以一式七份合成。

[0263] 如上所述(使用综合的5-聚体肽阵列的BACE1结合物发现)对于取代和缺失阵列进行BACE1结合测定和图像处理。

[0264] 数据分析和结果:在分析每个肽文库后选择具有最高结合信号的肽序列,其包括异常去除和每个肽的重复物间的平均信号。选择的序列在表13(最后一列)中显示。

序列表

<110> F.Hoffmann-La Roche Ldt

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<130> P32452-WO

<150> EP15178162.2
<151> 2015-07-24

<160> 71

<170> PatentIn version 3.5

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[0001]

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1 5 10 15

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Glu Pro

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Glu Pro

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Glu Pro

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<400> 13

Tyr Pro Tyr Phe Lys Pro Leu Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

Glu Pro

<210> 14
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Tyr Pro Tyr Phe Lys Pro Ala Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

Glu Pro

<210> 15
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[0009]

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<223> DPro

<400> 15

Thr Pro Lys Pro Ala Gln Gly Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

Glu Pro

<210> 16
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<223> DLys

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<223> Z=抑胃酶氨酸(Statine)

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<223> DPro

<400> 16

Gly Ala Arg Phe Ile Pro Ala Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

[0010]

Glu Pro

<210> 17
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<223> Z=抑胃酶氨酸(Statine)

<220>
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<223> DPro

<400> 17

Tyr Pro Lys Phe Ile Ser Ala Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

Glu Pro

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[0011]

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<400> 18

Tyr Pro Tyr Phe Ile Pro Lys Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

Glu Pro

<210> 19
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<400> 19

Tyr Pro Tyr Phe Ile Pro Lys Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

[0012] Glu Phe

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<223> z=抑胃酶氨酸(Statine)

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<400> 20

Lys Pro Tyr Phe Ile Pro Leu Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

Glu Pro

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<223> 1 H-Tyr

[0013]

<220>
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<223> DPro

<400> 21

Tyr Pro Lys Phe Ile Pro Leu Xaa Glu Val Asn Glx Val Ala Glu Pro
1 5 10 15

<210> 22
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<223> H-Tyr

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<223> DLys

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<220>
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<223> DPro

[0014]

<400> 22

Tyr Pro Tyr Phe Ile Pro Lys Xaa Glu Val Asn Glx Val Ala Glu Pro
1 5 10 15

<210> 23
<211> 15
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<223> DLys

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<223> X=PEG(4)

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<223> Z=抑胃酶氨酸(Statine)

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<400> 23

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1 5 10 15

<210> 24
<211> 16
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[0015]

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<223> Z=抑胃酶氨酸(Statine)

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<400> 24

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1 5 10 15

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<223> Y=LEU*ALA

<220>
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<223> DPro

<400> 25

[0016]

Tyr Pro Tyr Phe Ile Pro Lys Xaa Glu Val Asn Tyr Ala Glu Pro
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<223> Z=LEU*ALA

<220>
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<223> DPro

<400> 26

Tyr Pro Tyr Phe Lys Leu Gly Lys Gly Glu Val Asn Glx Ala Glu Pro
1 5 10 15

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[0017] <223> X=PEG(4)

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<220>
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<223> DPro

<400> 27

Tyr Pro Tyr Phe Ile Pro Lys Xaa Glu Val Asn Glx Val Ala Glu Pro
1 5 10 15

<210> 28
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<220>
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<223> DLys

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<223> DLys

<220>
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<222> (14)..(14)
<223> Z=抑胃酶氨酸(Statine)

<220>
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<223> DPro

<400> 28

[0018]

Tyr Pro Tyr Phe Ile Pro Lys Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

Glu Pro

<210> 29
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<220>
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<223> DLys

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<223> Z=抑胃酶氨酸(Statine)

<220>
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<223> DPro

<400> 29

Tyr Pro Tyr Phe Ile Pro Leu Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

Glu Pro

<210> 30
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<220>
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[0019]

<220>
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<220>
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<223> DLys

<220>
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<223> Z=抑胃酶氨酸(Statine)

<220>
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<400> 30

Ala Cys Tyr Pro Tyr Phe Ile Pro Leu Gly Lys Gly Glu Val Asn Glx
1 5 10 15

Val Ala Glu Pro
20

<210> 31
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<220>
<223> 修饰的肽(modified peptide)

<220>
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<222> (1)..(1)
<223> Ac-Gln

<400> 31

Ala Cys Gln Gln Tyr Pro Tyr Phe Lys Pro Ala Asn
1 5 10

<210> 32
<211> 12
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<220>
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[0020]

<220>
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<400> 32

Ala Cys Tyr Pro Thr Phe Lys Pro Ala Asn Gly Ser
1 5 10

<210> 33
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<220>
<223> 修饰的肽(modified peptide)

<220>
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<223> Ac-Ser

<400> 33

Ala Cys Ser Thr Gly Ala Arg Phe Ile Pro Ala Asn
1 5 10

<210> 34
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<220>
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<220>
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<400> 34

Ala Cys Gly Asp Tyr Pro Lys Phe Ile Ser Ala Asn
1 5 10

<210> 35
<211> 12
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[0021]

<220>
<223> 修饰的肽(modified peptide)

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<400> 35

Ala Cys Gly Asp Tyr Pro Lys Phe Ile Pro Ala Ser
1 5 10

<210> 36
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<220>
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<223> Ac-Gly

<400> 36

Ala Cys Gly Ser Tyr Pro Lys Phe Ile Asp Ala Asn
1 5 10

<210> 37
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<220>
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<222> (1)..(1)
<223> Ac-Leu

<400> 37

Ala Cys Leu Thr Thr Tyr Pro Tyr Phe Lys Pro Ala
1 5 10

[0022]

<210> 38
<211> 8
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<223> Z=抑胃酶氨酸(Statine)

<400> 38

Glu Val Asn Glx Val Ala Glu Phe
1 5

<210> 39
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<220>
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<222> (4)..(4)
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<220>
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<223> DPhe

<400> 39

Glu Val Asn Glx Val Ala Glu Phe
1 5

<210> 40
<211> 8
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[0023]
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<223> Z=抑胃酶氨酸(Statine)

<220>
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<400> 40

Glu Val Asn Glx Val Ala Glu Pro
1 5

<210> 41
<211> 7
<212> PRT
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<220>
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<400> 41

Tyr Pro Tyr Phe Lys Pro Ala
1 5

<210> 42
<211> 8
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<220>
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<400> 42

Gln Tyr Pro Tyr Phe Lys Pro Ala
1 5

<210> 43
<211> 8
<212> PRT
<213> 人工序列(Artificial Sequence)

<220>
<223> 修饰的肽(modified peptide)

<400> 43

Tyr Pro Tyr Phe Lys Pro Ala Ser
1 5

[0024]

<210> 44
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<213> 人工序列(Artificial Sequence)

<220>
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<400> 44

Tyr Pro Thr Phe Lys Pro Ala
1 5

<210> 45
<211> 7
<212> PRT
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<223> 修饰的肽(modified peptide)

<400> 45

Asp Tyr Pro Lys Phe Ile Ser
1 5

<210> 46
<211> 7
<212> PRT
<213> 人工序列(Artificial Sequence)

<220>
<223> 修饰的肽(modified peptide)

<400> 46

Asp Tyr Pro Lys Phe Leu Pro
1 5

<210> 47
<211> 7
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<213> 人工序列(Artificial Sequence)

<220>
<223> 修饰的肽(modified peptide)

<400> 47

Ser Tyr Pro Lys Phe Ile Asp
1 5

[0025]

<210> 48
<211> 9
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<213> 人工序列(Artificial Sequence)

<220>
<223> 修饰的肽(modified peptide)

<400> 48

Thr Gly Ala Arg Phe Ile Pro Ala Asn
1 5

<210> 49
<211> 10
<212> PRT
<213> 人工序列(Artificial Sequence)

<220>
<223> 修饰的肽(modified peptide)

<400> 49

Gly Gln Tyr Pro Tyr Phe Lys Pro Ala Ser
1 5 10

<210> 50
<211> 10
<212> PRT
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<220>
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<400> 50

Gln Tyr Pro Tyr Phe Lys Pro Ala Ser Gly
1 5 10

<210> 51
<211> 10
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<220>
<223> 修饰的肽(modified peptide)

<400> 51

Gly Gly Gly Tyr Pro Thr Phe Lys Pro Ala
1 5 10

[0026]

<210> 52
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<220>
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<400> 52

Gly Gly Tyr Pro Thr Phe Lys Pro Ala Gly
1 5 10

<210> 53
<211> 10
<212> PRT
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<220>
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<400> 53

Gly Tyr Pro Thr Phe Lys Pro Ala Gly Gly
1 5 10

<210> 54
<211> 10
<212> PRT
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<220>
<223> 修饰的肽(modified peptide)

<400> 54

Tyr Pro Thr Phe Lys Pro Ala Gly Gly Gly
1 5 10

<210> 55
<211> 10
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<220>
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<400> 55

Gly Asp Tyr Pro Lys Phe Ile Ser Gly Gly
1 5 10

[0027]

<210> 56
<211> 10
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<220>
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<400> 56

Gly Asp Tyr Pro Lys Phe Leu Pro Gly Gly
1 5 10

<210> 57
<211> 10
<212> PRT
<213> 人工序列(Artificial Sequence)

<220>
<223> 修饰的肽(modified peptide)

<400> 57

Gly Ser Tyr Pro Lys Phe Ile Asp Gly Gly
1 5 10

<210> 58
<211> 10
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<220>
<223> 修饰的肽(modified peptide)

<400> 58

Gly Thr Gly Ala Arg Phe Ile Pro Ala Asn
1 5 10

<210> 59
<211> 10
<212> PRT
<213> 人工序列(Artificial Sequence)

<220>
<223> 修饰的肽(modified peptide)

<400> 59

Thr Gly Ala Arg Phe Ile Pro Ala Asn Gly
1 5 10

[0028]

<210> 60
<211> 10
<212> PRT
<213> 人工序列(Artificial Sequence)

<220>
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<400> 60

Gln Gln Tyr Pro Tyr Phe Lys Pro Ala Asn
1 5 10

<210> 61
<211> 10
<212> PRT
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<220>
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<400> 61

Tyr Pro Thr Phe Lys Pro Ala Asn Gly Ser
1 5 10

<210> 62
<211> 10
<212> PRT
<213> 人工序列(Artificial Sequence)

<220>
<223> 修饰的肽(modified peptide)

<400> 62

Gly Asp Tyr Pro Lys Phe Ile Ser Ala Asn
1 5 10

<210> 63
<211> 10
<212> PRT
<213> 人工序列(Artificial Sequence)

<220>
<223> 修饰的肽(modified peptide)

<400> 63

Gly Asp Tyr Pro Lys Phe Ile Pro Ala Ser
1 5 10

[0029]

<210> 64
<211> 10
<212> PRT
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<220>
<223> 修饰的肽(modified peptide)

<400> 64

Gly Ser Tyr Pro Lys Phe Ile Asp Ala Asn
1 5 10

<210> 65
<211> 10
<212> PRT
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<220>
<223> 修饰的肽(modified peptide)

<400> 65

Ser Thr Gly Ala Arg Phe Ile Pro Ala Asn
1 5 10

<210> 66
<211> 18
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<223> DLys

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<223> DPro

[0030]

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<400> 66

Tyr Lys Tyr Phe Ile Pro Leu Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

Glu Pro

<210> 67
<211> 18
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<220>
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<220>
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<223> Z=抑胃酶氨酸(Statine)

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<400> 67

Tyr Pro Lys Phe Ile Pro Leu Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

[0031]

Glu Pro

<210> 68
<211> 18
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Tyr Pro Tyr Lys Ile Pro Leu Gly Lys Gly Glu Val Asn Glx Val Ala
1 5 10 15

Glu Pro

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[0032]

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<400> 69

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Asn Glx Val Ala Glu Pro
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[0033]

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<400> 70

Asx Xaa Cys Xaa Tyr Pro Tyr Phe Ile Pro Lys Gly Lys Gly Glu Val
1 5 10 15

Asn Glx Val Ala Glu Pro
20

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<220>
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[0034]

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<400> 71

Gly Gly Gly Tyr Pro Tyr Phe Ile Pro Lys Gly Lys Gly Glu Val Asn
1 5 10 15

Glx Val Ala Glu Pro
20

[0035]