



(86) Date de dépôt PCT/PCT Filing Date: 2005/07/06
(87) Date publication PCT/PCT Publication Date: 2006/01/19
(85) Entrée phase nationale/National Entry: 2007/01/10
(86) N° demande PCT/PCT Application No.: EP 2005/053225
(87) N° publication PCT/PCT Publication No.: 2006/005707
(30) Priorité/Priority: 2004/07/13 (ATA 1184/2004)

(51) Cl.Int./Int.Cl. *A61K 39/00* (2006.01),
A61P 25/28 (2006.01), *G01N 33/68* (2006.01)

(71) Demandeur/Applicant:
AFFIRIS FORSCHUNGS-UND ENTWICKLUNGS
GMBH, AT

(72) Inventeurs/Inventors:
MATTNER, FRANCK, AT;
SCHMIDT, WALTER, AT

(74) Agent: OGILVY RENAULT LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : METHODE DE PREVENTION ET DE TRAITEMENT DE LA MALADIE D'ALZHEIMER
(54) Title: METHOD FOR PREVENTING AND TREATING ALZHEIMER'S DISEASE

(57) **Abrégé/Abstract:**

The invention relates to the use of a compound comprising the following amino acid sequence $X_1X_2X_3X_4X_5X_6X_7$, wherein X_1 is an amino acid, except of C, X_2 is an amino acid, except of C, X_3 is an amino acid, except of C, X_4 is an amino acid, except of C, X_5 is an amino acid, except of C, X_6 is not present or any amino acid, X_7 is not present or any amino acid, and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH, said compound having a binding capacity to an antibody being specific for the natural N-terminal A β 42 sequence DAEFRH, and 5-mers thereof having a binding capacity to said antibody being specific for the natural N-terminal A β 42 sequence DAEFRH, for the preparation of a vaccine for Alzheimer's disease.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
19 January 2006 (19.01.2006)

PCT

(10) International Publication Number
WO 2006/005707 A3

(51) International Patent Classification:

A61K 39/00 (2006.01) A61P 25/28 (2006.01)
G01N 33/68 (2006.01)

(21) International Application Number:

PCT/EP2005/053225

(22) International Filing Date: 6 July 2005 (06.07.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

A 1184/2004 13 July 2004 (13.07.2004) AT

(71) Applicant (for all designated States except US): **AFFIRIS FORSCHUNGS- UND ENTWICKLUNGS GMBH** [AT/AT]; Viehmarktgasse 2A, A-1030 Wien (AT).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **METTNER, Franck** [DE/AT]; Krottenbachstrasse 267, A-1190 Wien (AT). **SCHMIDT, Walter** [DE/AT]; Steingasse 18/1/10, A-1030 Wien (AT).

(74) Agent: **Sonn & Partner Patentanwälte**; Riemergasse 14, A-1010 Vienna (AT).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report

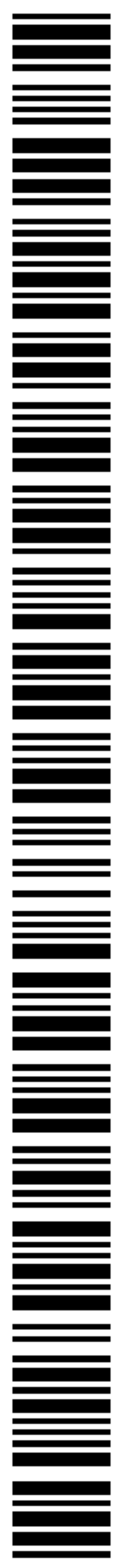
(88) Date of publication of the international search report:

17 August 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR PREVENTING AND TREATING ALZHEIMER'S DISEASE

(57) Abstract: The invention relates to the use of a compound comprising the following amino acid sequence $X_1X_2X_3X_4X_5X_6X_7$, wherein X_1 is an amino acid, except of C, X_2 is an amino acid, except of C, X_3 is an amino acid, except of C, X_4 is an amino acid, except of C, X_5 is an amino acid, except of C, X_6 is not present or any amino acid, X_7 is not present or any amino acid, and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH, said compound having a binding capacity to an antibody being specific for the natural N-terminal A β 42 sequence DAEFRH, and 5-mers thereof having a binding capacity to said antibody being specific for the natural N-terminal A β 42 sequence DAEFRH, for the preparation of a vaccine for Alzheimer's disease.



WO 2006/005707 A3

Method for preventing and treating Alzheimer's disease

The present invention relates to methods for preventing and treating Alzheimer's disease (AD).

Amyloid- β peptide (A β) plays a central role in the neuropathology of Alzheimer's disease (AD) (Roher et al 1993: "A β -Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: Implications for the pathology of Alzheimer disease" PNAS 90:10836). Familial forms of the disease have been linked to mutations in the amyloid precursor protein (APP) and the presenilin genes. Disease-linked mutations in these genes result in increased production of the 42-amino acid form of the peptide (A β 42), which is the predominant form found in the amyloid plaques of Alzheimer's disease. An animal model for the disease is commercially available. The PDAPP transgenic mouse, which over-expresses mutant human APP (in which the amino acid at position 717 is F instead of V), progressively develops many of the neuropathological hallmarks of Alzheimer's disease in an age- and brain-dependent manner (Games et al 1995: "Alzheimer-type neuropathology in transgenic mice overexpressing V717F β -amyloid precursor protein" Nature 373:523).

Vaccination studies with a "normal", not mimotope-based vaccine have already been performed. Transgenic animals were immunized with aggregated A β 42, either before the onset of AD-type neuropathologies (6 weeks) or at an older age (11 months): Immunization of young animals prevented the development of plaque formation, neuritic dystrophy and astrogliosis. Treatment of older animals markedly reduced AD-like neuropathologies. This experimental vaccination approach induced the development of antibodies against A β 42 able to cross the blood-brain barrier and attack amyloid plaques (Schenk et al 1999: "Immunization with amyloid- β attenuates Alzheimer-disease-like pathology in the PDAPP mouse" Nature 400:173). The plaques are subsequently removed by several mechanisms, including Fc-receptor mediated phagocytosis (Bard et al 2000: "Peripherally administered antibodies against amyloid β -peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease" Nature Med 6:916). This vaccine was also able to delay memory deficits

- 2 -

(Janus et al 2000: "A β peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease" Nature 408:979).

A highly promising immunization therapy for AD has been in clinical trials since late 1999. Immunization is presumed to trigger the immune system to attack the plaques and clear these deposits from the affected human brain, although the precise mechanism underlying needs to be characterized in more detail.

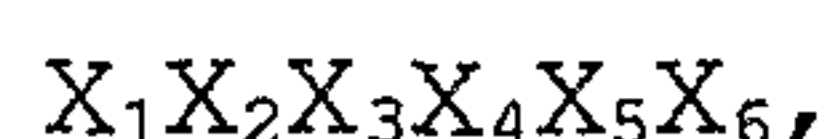
These clinical trials were conducted by the pharmaceutical company Elan in conjunction with its corporate partner, American Home Products (therapeutic vaccine AN-1792, QS21 as adjuvant). Phase I trials were successfully completed in 2000. Phase II trials were begun late 2001 to test efficacy in a panel of patients with mild to moderate AD.

Now these phase II trials have been permanently discontinued due to neuroinflammation in several patients (Editorial 2002 "Insoluble problem?" Nature Med 8:191). The symptoms included aseptic meningoencephalitis leading to the immediate halt of these world-wide trials. In the worst case scenario, affected patients will be shown to have mounted an autoimmune response - a risk inherent in many immunotherapies. Autoimmune complications could have been anticipated given the ubiquity of APP, which of course bears antigenic determinants in common with its proteolytic product. More recently, additional studies concentrated on the nature of aggregated A β 42 immunization-induced antibodies (in humans and mice) revealing that most antibodies recognize a small domain between amino acid 4 and 10 of A β 42 (A β 4-10). The mouse antibodies were able to block A β fibrillogenesis and disrupted pre-existing A β fibers (McLaurin et al 2002: "Therapeutically effective antibodies against amyloid- β peptide target amyloid- β residues 4-10 and inhibit cytotoxicity and fibrillogenesis" Nature Med 8:1263). Of note, the human antibodies do not react with APP exposed on the surface of cells or any other non-aggregated proteolytic product of the precursor (Hock et al 2002: "Generation of antibodies specific for β -amyloid by vaccination of patients with Alzheimer disease" Nature Med 8:1270). A clear difference was observed between human and mouse sera: In

- 3 -

contrast to human antibodies, mouse antibodies detect monomeric, oligomeric, and fibrillar A β . This is of importance and may be a prerequisite for the therapeutic potency since evidence is accumulating that small oligomers of A β , which are not recognized by human anti-A β , are the major toxic players in the disease (Walsh et al 2002: "Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo" Nature 416:535). Thus, a potential new strategy is the immunization with a vaccine containing β -amyloid amino acids 4-10 (instead of aggregated A β 42). Despite unknown efficacy this strategy may also face autoimmune problems since patients shall be directly immunized with a (linear B cell) "self" epitope.

Despite these disappointing developments in recent AD vaccination strategies, an A β vaccine is still regarded as the most promising way for combatting AD. However, there is an urgent need for improvements and new strategies in AD vaccination. Especially, such a vaccine should not induce autoreactive T and/or B cells. Mimotope peptides which have a binding capacity to an antibody and are specific for the natural N-terminal A β 42 sequence DAEFRH, and 5-mers thereof which have a binding capacity to said antibody being specific for the natural N-terminal A β 42 sequence DAEFRH for the preparation of a vaccine for Alzheimer's disease (AD) are described in PCT/EP04/00162 (incorporated herein by reference). Preferred mimotopes in that application comprise the following amino acid sequence



wherein

X₁ is G or an amino acid with a hydroxy group or a negatively charged amino acid, preferably E, Y, S or D,

X₂ is a hydrophobic amino acid or a positively charged amino acid, preferably I, L, V, K, W, R, Y, F or A,

X₃ is a negatively charged amino acid, preferably D or E,

X₄ is an aromatic amino acid or L, preferably Y, F or L,

X₅ is H, K, Y, F or R, preferably H, F or R, and

X₆ is S, T, N, Q, D, E, R, I, K, Y, or G, preferably T, N, D, R, I or G,

especially EIDYHR, ELDYHR, EVDYHR, DIDYHR, DLDYHR, DVDYHR,

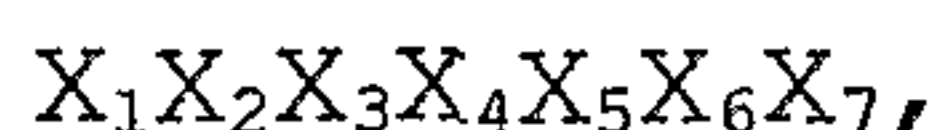
- 4 -

DIDYRR, DLDYRR, DVDYRR, DKELRI, DWELRI, YREFRI, YAEFRG, EAEFRG, DYEFRG, ELEFRG, DRELRI, DKELKI, DRELKI, GREFRN, EYEFRG, DWEFRDA, SWEFRT, DKELR or SFEFRG.

Furthermore, the natural L- or D-amino acids can be substituted by non natural L- or D-amino acids. For example, L, I or V can be substituted by Nle, Nva, Cha or alpha amino acids with other linear or cyclic aliphatic side chains, W or F by aromatic amino acids and R and K by basic amino acids like ornithin or homoarginine. Serin and threonine can be substituted by amino acids with aliphatic or aromatic side chains with a terminal OH-group.

The present invention provides further mimotope peptides (mimicking DAEFRH) to be used for vaccination for AD.

Also the compounds according to the present invention are provided by screening peptide libraries with an antibody being specific for the natural N-terminal A β 42 sequence DAEFRH. Preferably the peptides in the library have or comprise the following amino acid sequence



412

wherein X_1 is an amino acid, except of C,
 X_2 is an amino acid, except of C,
 X_3 is an amino acid, except of C,
 X_4 is an amino acid, except of C,
 X_5 is an amino acid, except of C,
 X_6 is not present or any amino acid, preferably except of C,
 X_7 is not present or any amino acid, preferably except of C,
 and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH, said peptide having a binding capacity to an antibody being specific for the natural N-terminal A β 42 sequence DAEFRH, and 5-mers thereof having a binding capacity to said antibody being specific for the natural N-terminal A β 42 sequence DAEFRH, for the preparation of a vaccine for Alzheimer's disease (AD).

According to the present invention an A β 42 mimotope is used for vaccination against AD: The mimotope induces the production of antibodies against A β 42 but not against the native APP. The

- 5 -

mimotope may be identified with a (monoclonal) antibody and (commercially available) peptide libraries (e.g. according to Reineke et al. 2002: "Identification of distinct antibody epitopes and mimotopes from a peptide array of 5520 randomly generated sequences" J Immunol Methods 267:37). A (monoclonal) antibody is used that does not recognize APP but detects only different A β species with amino-terminal aspartic acid (an example for such an antibody is described in Johnson-Wood et al 1997: "Amyloid precursor protein processing and A β 42 deposition in a transgenic mouse model of Alzheimer disease" PNAS 94:1550). Such an antibody has been proven to be an ideal tool to identify vaccine-suitable mimotopes in the course of the present invention. Although such monoclonal antibodies were shown to have beneficial effects in a mouse model of AD when directly administered to mice (Bard et al 2000: "Peripherally administered antibodies against amyloid β -peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease" Nature Med 6:916), these antibodies have never been proposed to be used as mimotope search tools for isolating AD vaccine compounds.

In the prior art, all efforts were concentrated on the naturally occurring A β peptide. As mentioned above, A β peptide vaccine clinical trials were stopped due to neuroinflammation events. ^{air} Indeed, T cell epitope prediction programs (BIMAS for class I-restricted epitopes and TEPITOPE for class II-restricted epitopes) propose high score (self) epitopes within the sequence. This could imply that the neuroinflammatory events are due to autoimmune reactions which would make such a vaccine unsuitable for a general application.

In contrast to such A β vaccines proposed by the prior art, no autoimmune reactions are expected to occur during treatment with a vaccine containing a mimotope according to the present invention, because the (monoclonal) antibody used for mimotope identification according to the present invention does not recognize APP and the mimotope sequence is different from A β 42-derived self sequences that have been used in trials so far or shall be used in future trials.

The antibody used for the mimotope identification according to

- 6 -

the present invention detects the A β -derived amino acid sequence DAEFRH (= original epitope) with a free amino terminal aspartic acid, thus it does not recognize native APP. The antibody may be a monoclonal or polyclonal antibody preparation or any antibody part or derivative thereof, the only prerequisite is that the antibody molecule specifically recognises the DAEFRH epitope, i.e. that it does not bind to the natural N-terminally prolonged forms of the amyloid precursor protein, which means that the binding capacity to the DAEFRH epitope is at least 100 times, preferably at least 1000 times, more preferred at least 10⁵ times, higher than to the APP molecule. The antibody may be an antibody showing the same or a higher binding capacity to the DAEFRH sequence as the antibody described by Johnson-Wood et al., 1997. Of course, also antibodies with a lower binding capacity may be used (>10 %, >50 % or >80 % of the binding capacity of the Johnson-Wood et al.-antibody), although the higher binding capacity is more preferred.

The compounds according to the present invention bind to those antibodies with comparable specificity as the DAEFRH sequence.

Preferably, the compound to be used according to the present invention comprises or is consisting of a peptide, wherein
X₁ is G or an amino acid with a hydroxy group or a negatively charged amino acid, preferably G, E, Y, S or D,
X₂ is a hydrophobic amino acid or a positively charged amino acid, preferably N, I, L, V, K, W, R, Y, F or A,
X₃ is a negatively charged amino acid, preferably D or E,
X₄ is an aromatic amino acid or a hydrophobic amino acid, preferably Y, F or L,
X₅ is H, K, Y, F or R, preferably H, F or R, and
X₆ is any amino acid, except P or - if X₇ is present - except C, preferably S, T, N, Q, D, E, R, I, K, Y, or G, especially T, N, D, R, I or G.

Therein, the 20 naturally occurring amino acids may be replaced by their chemical analogs or by D- amino acids; e.g. L may be replaced by Nle, Nva or Cha. Workability of such an exchange can easily be tested with the experimental model described in the example section of the present application. Steric considera-

- 7 -

tions can also be calculated via computer models for the binding of the antibody to the peptide.

Specifically preferred are peptides containing the following sequence(s): DAEFRWP, DNEFRSP, GSEFRDY, GAEFRFT, SAEFRTQ, SAEFRAT, SWEFRNP, SWEFRLY, SWELRQA, SVEFRYH, SYEFRHH, SQEFRTQ, SSEFRVS, DWEFRD, DAELRY, DWELRQ, SLEFRF, GPEFRW, GKEFRT, AYEFRH, DKE(Nle)R, DKE(Nva)R or DKE(Cha)R, especially DAEFRWP, DNEFRSP, SAEFRTQ, SAEFRAT, SWEFRNP, SWEFRLY, SWELRQA, SVEFRYH, SYEFRHH, SQEFRTQ, SSEFRVS, DWEFRD, DAELRY, DWELRQ, SLEFRF, GPEFRW or GKEFRT.

The compound (mimotope) according to the present invention has a preferred length of 5 to 15 amino acids. This compound may be provided in the vaccine in isolated (peptide) form or may be coupled or complexed to other molecules, such as pharmaceutical carrier substances or polypeptide, lipid or carbohydrate structures. Preferably, the mimotopes according to the present invention have a (minimum) length of between 5 and 15, 6 and 12 amino acid residues, specifically between 9 and 11. The mimotopes can, however, be coupled (covalently or non-covalent) to unspecific linkers or carriers, especially peptide linkers or protein carriers. Furthermore, the peptide linkers or protein carriers might consist of or contain T-cell helper epitopes.

Preferably, the pharmaceutically acceptable carrier is KLH, tetanus toxoid, albumin binding protein, bovine serum albumin, a dendrimer (MAP; Biol.Chem.358:581) as well as the adjuvant substances described in Singh et al., Nat. Biotech. 17(1999), 1075-1081 (specifically those in table 1 of this document) and O'Hagan et al., Nature Reviews, Drug Discovery 2(9)(2003), 727-735 (specifically the innate immune-potentiating compounds and the delivery systems described therein), or mixtures thereof. In addition, the vaccine composition may contain aluminium hydroxyde.

A vaccine comprising the present compound (mimotope) and the pharmaceutically acceptable carrier may be administered by any suitable application mode, e.g. i.v., i.p., i.m., intranasal, oral, subcutaneous, etc. and in any suitable delivery device (O'Hagan et al., Nature Reviews, Drug Discovery 2(9)(2003), 727-

- 8 -

735). Typically, the vaccine contains the compound according to the present invention in an amount of 0,1 ng to 10 mg, preferably 10 ng to 1 mg, especially 100 ng to 100 µg or, alternatively e.g. 100 fmole to 10 µmole, preferably 10 pmole to 1 µmole, especially 100 pmole to 100 nmole. The vaccine may also comprise typical auxiliary substances, e.g. buffers, stabilizers, etc.

Isolating suitable 5-mers according to the present invention can be achieved in the way described above, adapted to libraries with 5 amino acid variables, and may preferably be performed either by screening a library having amino acid variables X_1 to X_5 as described herein or by identifying suitable 5-mers in a positive member screened in a 6-mer-library (see: above). In the same way, also 7-mer, 8-mer, 9-mer, 10-mer, ... libraries may be applied accordingly to screen for suitable sequences which bind to the present antibody-type. Suitable antibody-binding fragments of such longer sequences can be found, e.g. by testing these fragments with a length of 5, 6, 7, 8, 9, ... amino acid residues for binding to the present antibody.

Such a method has been proven to be successful for providing Aβ mimotopes according to the present invention.

Preferably, said peptides are provided in individualised form in said library, especially immobilised on a solid surface, such as e.g. possible with the MULTIPIN™ peptide technology. The library may also be provided as a peptide mixture and the antibody:peptide complexes may be isolated after antibody binding. Alternatively, the antibody may be immobilised and the peptide library (in suspension or solution) is then contacted with the immobilised antibodies.

Preferably, the screening antibodies (or the members of the peptide library) comprise a suitable marker which allows the detection or isolation of the antibody or the antibody:peptide complex when bound to a peptide of the library. Suitable marker systems (i.a. biotinylation, fluorescence, radioactivity, magnetic markers, colour developing markers, secondary antibodies)

- 9 -

are readily available to the skilled man in the art.

The library has to be constructed to exclude the naturally occurring A β sequence (e.g. DAEFRH), since vaccination with this sequence is clearly excluded from this invention.

A further suitable technique for isolating the epitopes according to the present invention is screening in phage-peptide libraries as e.g. described in WO 03/020750.

The present invention also relates to a composition comprising an anti N-terminal A β 42-antibody-binding peptide (or, in certain cases preferred, a larger molecule comprising such a peptide (e.g. the peptide linked to a carrier or delivery molecule)) as defined herein (optionally as single effective component), preferably to a vaccine against Alzheimer's Disease comprising such an antigen. A suitable antigen includes at least one peptide selected from the group DAEFRWP, DNEFRSP, GSEFRDY, GAEFRFT, SAEFRITQ, SAEFRAT, SWEFRNP, SWEFRLY, SWELRQA, SVEFRYH, SYEFRHH, SQEFRTP, SSEFRVS, DWEFRD, DAELRY, DWELRQ, SLEFRF, GPEFRW, GKE-FRT, AYEFRH, DKE(Nle)R, DKE(Nva)R or DKE(Cha)R. These peptides are - besides the other peptides provided with the present invention specifically suited to be used for the preparation of a pharmaceutical composition, especially for AD vaccines. These sequences are purely artificial A β -mimotopes. The peptides may - for vaccination purposes - be coupled (covalently or non-covalently) to suitable carriers and may be provided as peptide compounds or complexes together with other compounds or moieties, e.g. adjuvants, peptide or protein carriers, etc. and administered in a suitable manner (as e.g. described in O'Hagan et al., Nature Reviews, Drug Discovery 2(9) (2003), 727-735).

The invention is further described in the following examples and the drawing figures, of course without being restricted thereto.

Fig. 1 shows the individualised peptide members of library 4 used for the present screening process.

Fig. 2 shows an inhibition assay with mimotopes for DAEFRH.

- 10 -

Fig. 3 shows another inhibition assay with other mimotopes for DAEFRH.

Figures 4 and 5 describe the results of inhibition assays performed with mimotope peptides according to the present invention.

Figures 6 to 9 show the results of inhibition assays performed with mimotope peptides 4011-4018, 4019-4025, 4031-4038 and 4061-4064, respectively.

EXAMPLES:

1.: Generation of monoclonal antibodies (mAb) to detect A β 42-derived peptide species with free N-terminus (free aspartic acid at the N-terminus)

Mice are vaccinated with the 6mer peptide DAEFRH (natural N-terminal A β 42 sequence) linked to the protein bovine serum albumin BSA (to make use of the hapten-carrier-effect), emulsified in CFA (first injection) and IFA (booster injections). DAEFRH-peptide-specific, antibody-producing hybridomas are detected by ELISA (DAEFRH-peptide-coated ELISA plates). Peptide SEVKMDAEFRH (natural N-terminally prolonged sequence, APP-derived, containing the A β 42-derived sequence DAEFRH) is used as negative control peptide: hybridomas recognizing the prolonged peptide are excluded because they do not distinguish between A β 42-derived peptides with free aspartic acid at the N-terminus and APP-derived peptide DAEFRH without free aspartic acid.

2.: Construction of Peptide Libraries:

The mimotopes of the present invention have been found by adapting the method of Reinke et al., 2000, by screening peptide libraries for binding to an antibody (preferably a monoclonal antibody) which is specific for A β species with amino-terminal aspartic acid. Another method is commercially available as MULTIPIN™ peptide technology.

The Multipin™ peptide technology involves synthesizing peptides

- 11 -

on to specially prepared polyethylene pins mounted on blocks in a format which is compatible with the standard 8 x 12 microtiter plate used for many biological assays. Both pin-bound (non-cleavable peptides which remain covalently bound to the pin) and solution phase peptides (those that have been cleaved off the pin surface) can be produced by this method. PepSets, based on the Multipin synthesis system, permit the simultaneous synthesis and screening of large numbers of peptides.

PepSets consist of blocks of 96 individually synthesized peptides, two of which are carefully selected control sequences. Cleaved controls are assessed for purity by reverse phase HPLC and peptide content quantitated by amino acid analysis. Positive and negative non-cleavable controls are assessed by standard ELISA techniques.

PepSet peptides are available with a variety of chemical modifications including acetylation, biotinylation, phosphorylation and cyclization. The solution phase (cleaved) peptides are shipped as lyophilized powders.

For the production of solution phase peptides there is a choice of C-terminal endings, including acid and amide, depending on the intended peptide application. The cleavable bond is incorporated onto the pin surface, either as a preformed ester derivative of the C-terminal amino acid, or onto the "Rink" amide linker. Peptides with acid or amide end groups are then released by treating the pin-bound peptide with strong acid. Options for the scale of synthesis are a nominal 1 micromole or 5 micromole scale. Factors such as hydrophobicity and cleavage efficiency will affect peptide recovery, such that the expected yield of peptide is 0.5 to 1 micromole (around 1mg of a 15mer peptide) when the peptides are synthesized on the nominal 1 micromole scale, or a yield of 2.5 to 5 micromole for peptides synthesized on the nominal 5 micromole scale.

Non-cleavable peptides remain covalently bound to the pins and can be used to rapidly screen for peptides of interest using ELISA techniques. Such peptides are useful for the purposes of antibody epitope scanning and structure-activity relationship

- 12 -

(SAR) studies. Removal of bound antibodies or other proteins regenerates the peptides and allows their reuse in further assays. PepSets are used for a variety of applications including the identification of peptide leads of biological interest from scanning through protein sequences, the optimization of peptide leads, and the development of new generations of analogs. Flexibility in terms of the overall strategy used in screening procedures is greatly enhanced through the use of a variety of synthesis designs which together provide a systematic method to fully characterize the lead candidate.

The comprehensive results obtained from systematic peptide sets not only identify peptides of interest, but also indicates critical residues, their replaceability and optimal peptide length. Consequently, a range of related peptides may be ranked as a result of such findings. Replacement of L-amino acids with D-amino acids and other unusual residues is a powerful approach to manipulate the structure and conformation of a peptide. This method is also a rapid way to discover new analogs with different pharmacological properties, such as antagonists and peptides with increased stability.

Starting with a known protein^{seq} sequence, all sequential antibody epitopes can be mapped using the Multipin approach. Several alternative procedures for mapping sequential B-cell epitopes are now possible. These include pin-bound peptides, solution phase peptides coated directly onto microtiter plates, and biotinylated peptides captured on microtiter plates previously coated with avidin or streptavidin.

For the present examples, the antibody described in example 1 is used for screening peptide libraries, however, any antibody preparation specifically recognizing the DAEFRH-sequence, but not the naturally N-terminally prolonged sequence of the A β molecule (e.g. MDAEFRH, KMDAEFRH, SEVKMDAEFRH or the complete amyloid (precursor) protein, APP), such as e.g. described by Johnson-Wood et al., 1997.

Four libraries have been constructed for this purpose:

- 13 -

2.1.: Library 1: This 6mer library contains peptides with the following sequences (amino acid positions 1 to 6):

Position 1: all natural aa except of D, K, and C (17 possibilities)

Position 2: all natural aa except of A, K, and C (17 possibilities)

Position 3: all natural aa except of E, K, and C (17 possibilities)

Position 4: all natural aa except of F, K, and C (17 possibilities)

Position 5: all natural aa except of R, K, and C (17 possibilities)

Position 6: all natural aa except of H, K, C, and P (16 possibilities)

Library 1 is a mixture of hexapeptides. Theoretically, all possible peptides containing 17 different amino acids (see below) are included. The mixture does not contain any lysine and cysteine residue. Furthermore, the mixture does not contain: aspartic acid at the specific position 1, alanine at the specific position 2, glutamic acid at the specific position 3, phenylalanine at the specific position 4, arginine at the specific position 5, and histidine at the specific position 6.

The synthesis is performed on an Applied Biosystems 431A-Synthesizer following the FastMoc protocol, with a synthesis scale of 0.25 mmol.

The synthesis starts with weighing 1 mmol of all desired amino acids (amino groups and side chains protected). Then, a mixture of Asn, Gln, Gly, Ile, Leu, Met, Pro, Ser, Thr, Trp, Tyr, Val was produced. Position-specific, the following amino acids are added:

Ala, Glu, Phe, Arg, His (position/mixture 1),
Asp, Glu, Phe, Arg, His (position/mixture 2),
Asp, Ala, Phe, Arg, His (position/mixture 3),
Asp, Ala, Glu, Arg, His (position/mixture 4),

- 14 -

Asp, Ala, Glu, Phe, His (position/mixture 5), and
Asp, Ala, Glu, Phe, Arg (position/mixture 6, without Pro).

Mixture 6 was used to load the resin (2-chloro-tritylchloride resin, 1.49 mmol/g, Alexis Germany):

1 mmol amino acid residue mixture 6
611 mg resin (= 0.91 mmol reactive groups)
15 ml dichloromethane
5.5 equivalent = 5 mmol diisopropylethylamine (871 μ l).

The mixture is shaken in a flask for 1 h. Then, 1 ml methanol is added and the mixture is shaken for an additional 10 min. The loaded resin is extracted via a frit and washed twice with dimethylformamide, dichloromethane, isopropanol, methanol, and ether (30 ml of each). The drying is performed overnight in a high vacuum. The weigh-out quantity is 737 mg.

An aliquot of 5.66 mg is treated for 30 min with 1 ml of 20% piperidine in DMF to define the density of the resin. Then, the mixture is centrifuged. The free Fmoc protective group is photometrically measured in the supernatant (301 nm, coefficient of extinction = 7800 M (e-1)). Accordingly, the density of the resin is 0.49 mmol/g.

All following steps are performed at the synthesizer, using the other mixtures (put in 5 different cartridges). 515 mg of loaded resin are used (corresponding to 0.25 mmol: amino acid mixtures are used in 4-times excess). The N-terminal Fmoc protective group is cleaved at the end of the synthesis. After washing with ethanol and drying overnight, cleavage of the peptides from the resin is accomplished by TFA/H₂O (95:5, v:v). The TFA solution is concentrated in a Speed Vac to 1/5 volume and precipitated and washed in diethylether and lyophilized.

The 6mer peptides EIDYHR, ELDYHR, and EVDYHR are examples for mimotopes that can be detected by the monoclonal antibody produced according to example 1. above.

2.2.: Library 2: This 6mer library contains peptides with the following sequences (amino acid positions 1 to 6):

- 15 -

Position 1: D (fixed)

Position 2: all natural aa except of A, K, and C (17 possibilities)

Position 3: all natural aa except of E, K, and C (17 possibilities)

Position 4: all natural aa except of F, K, and C (17 possibilities)

Position 5: all natural aa except of R, K, and C (17 possibilities)

Position 6: all natural aa except of H, K, C, and P (16 possibilities).

Peptide library 2 was constructed according to the method described above (under 2.1) for library 1.

The 6mer peptides DIDYHR, DLDYHR, and DVDYHR are examples for mimotopes that can be detected by the monoclonal antibody produced according to 1. above.

2.3.: Library 3: A third peptide library is used in an additional approach to define mimotope sequences. This library contains the original sequence, and allows the detection of mimotopes more closely related to the original epitope.

This 6mer library contains peptides with the following sequences (amino acid positions 1 to 6):

Position 1: all natural aa except of K, and C (18 possibilities)

Position 2: all natural aa except of K, and C (18 possibilities)

Position 3: all natural aa except of K, and C (18 possibilities)

Position 4: all natural aa except of K, and C (18 possibilities)

Position 5: all natural aa except of K, and C (18 possibilities)

Position 6: all natural aa except of K, C, and P (17 possibilities).

Peptide library 3 was constructed according to the method described above (under 2.1) for library 1.

The 6mer peptides DIDYRR, DLDYRR, and DVDYRR are examples for

- 16 -

mimotopes that can be detected by the monoclonal antibody produced according to 1. above (D in position 1 and R in position 5 are identical with the original epitope).

2.4.: Library 4: This peptide library 4 consists of $5 \times 18 = 90$ peptides, is commercially available from Mimotopes Ltd. (Paris, France; see manufacturer's guidelines) and is designed according to the natural N-terminal A β 42 sequence DAEFRH.

Position 1: D (fixed)

Position 2: all natural amino acids except of K and C (18 different peptides)

Position 3: all natural amino acids except of K and C (18 different peptides)

Position 4: all natural amino acids except of K and C (18 different peptides)

Position 5: all natural amino acids except of K and C (18 different peptides)

Position 6: all natural amino acids except of K and C (18 different peptides).

The individualised peptide members of library 4 are depicted in fig. 1. Peptides no. 1, 24, 48, 56 and 80 have the original sequence of the A β 42 N-terminal sequence. All other peptides are candidate peptides which are tested with respect to their binding capacity to a DAEFRH-binding antibody.

2.5.: ELISA with peptide libraries:

As mentioned above, peptide libraries 1, 2, and 3 are generated with an Applied Biosystems 431A peptide synthesizer following classical Fmoc-chemistry. The commercially available peptide library 4 is generated according to the manufacturer's description (see above and under www.mimotopes.com). The 90 peptides are C-terminally linked to a pin.

The ELISA with each of the peptide libraries have been carried out following standard protocols:

The peptide library is dissolved in 100% DMSO (final concentra-

- 17 -

tion 10 mg/ml).

The peptide solution is further diluted in PBS.

The peptide mixture is coated overnight (4 °C) onto ELISA plates (Nunc Maxisorp, Germany), starting with 500 µg/well, and titrated to 100 ng/well.

The plates are washed 3x times with PBS/Tween 20 (0.1% v/v).

The plates are blocked with PBS/BSA (2 h at room temperature).

The plates are washed 3x times with PBS/Tween.

The plates are incubated with biotinylated DAEFRH-specific mAb (10 µg/ml in PBS) for 4 h at room temperature.

The plates are washed 3x times with PBS/Tween.

The plates are incubated with streptavidin-horseradish-peroxidase (30 min at room temperature).

The plates are washed 5x times with PBS/Tween.

The plates are incubated with ABTS + H₂O₂ (0.1 % w/v; 10 to 45 min) and the reaction is stopped with citric acid followed by photometric evaluation (wavelength 405 nm).

3.: Verification of Mimotopes by Inhibition Assay

3.1. Additional library

In addition to the 4 libraries described above (see 2.1., 2.2., 2.3., and 2.4.) a fifth library is used to define mimotope sequences. This 6mer library is commercially available at EMC microcollections (Tübingen Germany) and contains 114 different hexapeptide mixtures, one position per mixture is defined by one of all natural aa except of C (19 possibilities), the remaining 5 positions are variable:

Mixtures 01 to 06 (one position fixed, alanine A, remaining 5 variable, X):

Mixture 01: AXXXXX

Mixture 02: XAXXXX

Mixture 03: XXAXXX

Mixture 04: XXXAXX

Mixture 05: XXXXAX

Mixture 06: XXXXXA

- 18 -

Mixtures 07 to 12 (one position fixed, arginine R, remaining 5 variable, X):

Mixture 07: RXXXXX

Mixture 08: XRXXXX

Mixture 09: XXRXXX

Mixture 10: XXXRXX

Mixture 11: XXXXR

Mixture 12: XXXXXR

Accordingly, mixtures 13 to 114 are designed using all natural aa except of C.

3.2. Inhibition assay

Figures 2 and 3 describe the results of inhibition assays performed with mimotope peptides included in and obtained from the 5 libraries (as described). The mimotope peptides compete with the original epitope for recognition by the monoclonal antibody. Original epitope and mimotope peptides contain an additional C at the C-terminus for coupling to a protein carrier (if desired).

The following peptides are used:

Peptide 1737 DAEFRH

Peptide 3001 DKELRI

Peptide 3002 DWELRI

Peptide 3003 YREFFI

Peptide 3004 YREFRI

Peptide 3005 YAEFRG

Peptide 3006 EAEFRG

Peptide 3007 DYEFRG

Peptide 3008 ELEFRG

Peptide 3009 SFEFRG

Peptide 3010 DISFRG

Peptide 3011 DIGWRG

Procedure:

ELISA plates (Nunc Maxisorp) are coated with the original pep-

- 19 -

tide epitope DAEFRH (C-terminally prolonged with C and coupled to bovine serum albumin BSA) at a concentration of 0.1 µg/ml peptide-BSA (100µl/well, 12h, 4°C). After blocking with PBS/BSA 1% (200µl/well, 12h, 4°C), the plates are washed 3x times with PBS/Tween. Then, biotinylated monoclonal antibody (1:2000, 50µl/well) and peptides (50µl/well) at 50, 5, 0.5, 0.05, 0.005, and 0.0005 µg/ml are added for 20 min. at 37°C. The plates are washed 3x times with PBS/Tween and are incubated with horseradish peroxidase (HRP)-labeled streptavidin (100µl/well, 30 min, RT). The plates are washed 5x times with PBS/Tween and are incubated with ABTS + H₂O₂ (0.1% w/v, 10 to 45 min) and the reaction is stopped with citric acid followed by photometric evaluation (wavelength 405 nm).

As expected and seen in Fig.2, peptide 1737 DAEFRH can compete with BSA-coupled, plate-bound peptide DAEFRH and thus inhibits recognition by the monoclonal antibody. Furthermore, it is shown that peptide 3003 is not able to inhibit binding of the monoclonal antibody to the original epitope. In contrast, peptides 3001, 3002, 3004, 3005, 3006, and 3007 (to a different extent) block epitope recognition. Whereas peptide 3004 is only inhibitory at a high concentration (50 µg/ml), peptides 3001, 3006, and 3007 are strongly inhibitory with an IC₅₀ of less than 0.5 µg/ml. Peptides 3002 and 3005 are "intermediate" inhibitors with an IC₅₀ of more than 0.5 µg/ml.

As expected and seen in Fig.3, peptide 1737 DAEFRH can successfully compete with BSA-coupled, plate-bound peptide DAEFRH for monoclonal antibody recognition in an additionally performed, independent experiment. Furthermore, it is shown that peptides 3010 and 3011 are not inhibitory at the concentrations tested, whereas peptides 3008 and 3009 are (relatively) weak inhibitors with an IC₅₀ of less than 5 µg/ml.

Table 1 briefly summarizes the inhibitory capacity of mimotopes included in and obtained from libraries (as described):

Table 1: Inhibitory capacity of mimotopes:

Peptide 3001 DKELRI strong

- 20 -

Peptide 3002 DWELRI intermediate
Peptide 3003 YREFFI none
Peptide 3004 YREFRI weak
Peptide 3005 YAEFRG intermediate
Peptide 3006 EAEFRG strong
Peptide 3007 DYEFRG strong
Peptide 3008 ELEFRG weak
Peptide 3009 SFEFRG weak
Peptide 3010 DISFRG none
Peptide 3011 DIGWRG none

4. Inhibition Assay for Additional Mimotopes Screened According to the Present Invention

Inhibition assay

Figures 4 and 5 describe the results of inhibition assays performed with mimotope peptides included in and obtained from the 5 libraries (as described). The mimotope peptides compete with the original epitope for recognition by the monoclonal antibody. Original epitope and mimotope peptides contain an additional C at the C-terminus (position 7) for coupling to a protein carrier (if desired).

The following peptides are used:

Peptide 1737 DAEFRH (original epitope + C)
Peptide 1234 KKELRI
Peptide 1235 DRELRI
Peptide 1236 DKELKI
Peptide 1237 DRELKI
Peptide 1238 DKELR
Peptide 1239 EYEFRG
Peptide 1241 DWEFRDA
Peptide 4002 SWEFRT
Peptide 4003 GREFRN
Peptide 4004 WHWSWR

Procedure:

- 21 -

ELISA plates (Nunc Maxisorp) are coated with the original peptide epitope DAEFRH (C-terminally prolonged with C and coupled to bovine serum albumin BSA) at a concentration of 0.1 µg/ml peptide-BSA (100µl/well, 12h, 4°C). After blocking with PBS/BSA 1% (200µl/well, 12h, 4°C), the plates are washed 3x times with PBS/Tween. Then, biotinylated monoclonal antibody (1:2000, 50µl/well) and peptides (50µl/well) at different concentrations are added for 20 min. at 37°C. The plates are washed 3x times with PBS/Tween and are incubated with horseradish peroxidase (HRP)-labeled streptavidin (100µl/well, 30 min, RT). The plates are washed 5x times with PBS/Tween and are incubated with ABTS + H₂O₂ (0.1% w/v, 10 to 45 min) and the reaction is stopped with citric acid followed by photometric evaluation (wavelength 405 nm).

As expected and seen in Fig.4, peptide 1737 DAEFRH can compete with BSA-coupled, plate-bound peptide DAEFRH and thus inhibits recognition by the monoclonal antibody. Furthermore, it is shown that peptide 4004 is not able to inhibit binding of the monoclonal antibody to the original epitope. In contrast, peptides 4002 and 4003 (to a different extent) block epitope recognition. Whereas peptide 4003 is only inhibitory at a relatively high concentration (10 µg/ml), peptide 4002 is strongly inhibitory with an IC₅₀ of less than 0.4 µg/ml.

As expected and seen in Fig.5, peptide 1737 DAEFRH can successfully compete with BSA-coupled, plate-bound peptide DAEFRH for monoclonal antibody recognition in an additionally performed, independent experiment. Furthermore, it is shown that peptide 1234 is hardly inhibitory at the concentrations tested, whereas peptides 1235, 1236, 1237, 1238, 1239 and 1241 (to a different extent) block epitope recognition. Peptides 1235, 1238 and 1241 are strong inhibitors with an IC₅₀ of less than 0.5 µg/ml, whereas peptides 1236 and 1237 are (relatively) weak inhibitors with an IC₅₀ of more than 5 µg/ml. Peptide 1239 is an intermediate inhibitor with an IC₅₀ of more than 0.5 µg/ml.

Table 2 briefly summarizes the inhibitory capacity of mimotopes included in and obtained from libraries (as described):

- 22 -

Table 2: Inhibitory capacity of mimotopes:

Peptide 1234	KKELRI	none
Peptide 1235	DRELRI	strong
Peptide 1236	DKELKI	weak
Peptide 1237	DRELKI	weak
Peptide 1238	DKELR	strong
Peptide 1239	EYEFRG	intermediate
Peptide 1241	DWEFRDA	strong
Peptide 4002	SWEFRT	strong
Peptide 4003	GREFRN	weak
Peptide 4004	WHWSWR	none

The results presented in Figures 4 and 5 show that in addition to various 6mer peptides (as shown here and before), 5mer peptides (namely peptide 1238 DKELR) and 7mer peptides (namely peptide 1241 DWEFRDA) may be used as epitopes in a mimotope-based Alzheimer vaccine.

5. Independent new round for screening

Libraries:

The mimotope has a preferred length of 5 to 15 amino acids. Two different libraries are used in ELISA assays to define mimotope sequences.

Library 1: This 6mer library contains peptides with the following sequences (amino acid positions 1 to 6):

Position 1: all natural aa except of C (19 possibilities)
 Position 2: all natural aa except of C (19 possibilities)
 Position 3: all natural aa except of C (19 possibilities)
 Position 4: all natural aa except of C (19 possibilities)
 Position 5: all natural aa except of C (19 possibilities)
 Position 6: all natural aa except of C (19 possibilities)

Library 2: This 7mer library contains peptides with the following sequences (amino acid positions 1 to 7):

Position 1: all natural aa except of C (19 possibilities)
 Position 2: all natural aa except of C (19 possibilities)

- 23 -

Position 3: all natural aa except of C (19 possibilities)
 Position 4: all natural aa except of C (19 possibilities)
 Position 5: all natural aa except of C (19 possibilities)
 Position 6: all natural aa except of C (19 possibilities)
 Position 7: all natural aa except of C (19 possibilities)

Inhibition assay

Figures 6 to 8 describe the results of inhibition assays performed with mimotope peptides included in and obtained from the 2 libraries (as described above). The mimotope peptides compete with the original epitope for recognition by the monoclonal antibody. Original epitope and mimotope peptides contain an additional C at the C-terminus (position 7 or position 8, respectively) for coupling to a protein carrier (if desired).

The following peptides are used:

Peptide 1737	DAEFRH	original epitope	
Peptide 4011	DAEFRWP	7mer	s
Peptide 4012	DNEFRSP	7mer	s
Peptide 4013	GSEFRDY	7mer	m
Peptide 4014	GAEFRFT	7mer	m
Peptide 4015	SAEFRTQ	7mer	s
Peptide 4016	SAEFRAT	7mer	s
Peptide 4017	SWEFRNP	7mer	s
Peptide 4018	SWEFRLY	7mer	s
Peptide 4019	SWFRNP	6mer	-
Peptide 4020	SWELRQA	7mer	s
Peptide 4021	SVEFRYH	7mer	s
Peptide 4022	SYEFRHH	7mer	s
Peptide 4023	SQEFRTF	7mer	s
Peptide 4024	SSEFRVS	7mer	s
Peptide 4025	DWEFRD	6mer	s
Peptide 4031	DAELRY	6mer	s
Peptide 4032	DWELRQ	6mer	s
Peptide 4033	SLEFRF	6mer	s
Peptide 4034	GPEFRW	6mer	s
Peptide 4035	GKEFRT	6mer	s
Peptide 4036	AYEFRH	6mer	m

- 24 -

Peptide 4037 VPTSALA	7mer	-
Peptide 4038 ATYAYWN	7mer	-

Furthermore, the following 5mer peptides (with non natural amino acids) are used for inhibition assays:

Peptide 4061 DKE(tBuGly)R	5mer	-
Peptide 4062 DKE(Nle)R	5mer	m
Peptide 4063 DKE(Nva)R	5mer	m
Peptide 4064 DKE((Cha)R	5mer	m

(s: strong inhibition, m: moderate inhibition; -: no inhibition)

Procedure:

ELISA plates (Nunc Maxisorp) are coated with the original peptide epitope DAEFRH (C-terminally prolonged with C and coupled to bovine serum albumin BSA) at a concentration of 0.1 µg/ml peptide-BSA (100µl/well, 12h, 4°C). After blocking with PBS/BSA 1% (200µl/well, 12h, 4°C), the plates are washed 3x times with PBS/Tween. Then, biotinylated monoclonal antibody (1:2000, 50µl/well) and peptides (50µl/well) at different concentrations are added for 20 min. at 37°C. The plates are washed 3x times with PBS/Tween and are incubated with horseradish peroxidase (HRP)-labeled streptavidin (100µl/well, 30 min, RT). The plates are washed 5x times with PBS/Tween and are incubated with ABTS H₂O₂ (0.1% w/v, 10 to 45 min) and the reaction is stopped with citric acid followed by photometric evaluation (wavelength 405 nm).

As expected and seen in Figure 6 (showing peptides 4011-4018), peptide 1737 DAEFRH can compete with BSA-coupled, plate-bound peptide DAEFRH and thus inhibits recognition by the monoclonal antibody. Furthermore, it is shown that peptides 4012 DNEFRSP, 4013 GSEFRDY, and 4014 GAEFRFT are able to moderately inhibit binding of the monoclonal antibody to the original epitope. In contrast, peptides 4011 DAEFRWP, 4015 SAEFRITQ, 4016 SAEFRAT, 4017 SWEFRNP, and 4018 SWEFRLY (to a different extent) strongly block epitope recognition.

As expected and presented in Figure 7 (showing peptides 4019-

- 25 -

4025), peptide 1737 DAEFRH can successfully compete with BSA-coupled, plate-bound peptide DAEFRH for monoclonal antibody recognition in an additionally performed, independent experiment. Furthermore, it is shown that peptide 4019 SWFRNP is not inhibitory at the concentrations tested, whereas peptides 4020 SWELRQA, 4021 SVEFRYH, 4022 SYEFRHH, 4023 SQEFRTP, 4024 SSERFVS and 4025 DWEFRD (to a different extent) block epitope recognition. Peptides 4021, 4022, 4023, 4024 and 4025 are strong inhibitors with an IC₅₀ of less than 0.5 µg/ml, whereas peptide 4020 is an intermediate inhibitor with an IC₅₀ of more than 0.5 µg/ml.

As expected and seen in Figure 8 (peptides 4031-4038), peptide 1737 DAEFRH can successfully compete with BSA-coupled, plate-bound peptide DAEFRH for monoclonal antibody recognition in a 3rd independent experiment. Furthermore, it is shown that peptides 4037 VPTSALA and 4038 ATYAYWN are not inhibitory at the concentrations tested, whereas peptides 4031 DAELRY, 4032 DWELRQ, 4033 SLEFRF, 4034 GPEFRW, 4035 GKEFRT and 4036 AYEFRH (to a different extent) block epitope recognition. Peptides 4031, 4032, 4033, 4034 and 4035 are relatively strong inhibitors with an IC₅₀ of less than 0.5 µg/ml, whereas peptide 4036 is a (relatively) weak inhibitor with an IC₅₀ of more than 0.5 µg/ml.

6. Inhibition assay with defined 5mer peptides: non-natural amino acids

It has been shown previously that the 5mer peptide 1238 DKELR may be used as epitope in a mimotope-based Alzheimer vaccine (see PCT/EP04/00162). In the following, amino acids of the original 5mer epitope are replaced by non-natural amino acids: L is replaced by the non-natural amino acids tBuGly, Nle, Nva, or Cha.

As expected and presented in Figure 9 (peptides 4061-4064 DKELR variants), peptide 1737 DAEFRH can successfully compete with BSA-coupled, plate-bound peptide DAEFRH for monoclonal antibody recognition in a 4th independent experiment. Furthermore, it is shown that peptide 4061 DKE(tBuGly)R is not inhibitory at the concentrations tested. Interestingly, peptides 4062 DKE(Nle)R,

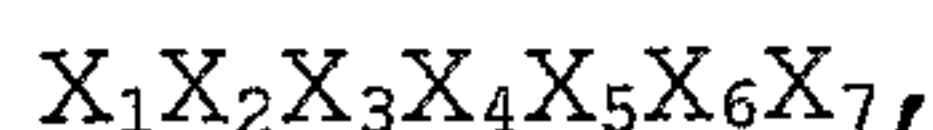
- 26 -

4063 DKE((Nva)R, and 4064 DKE(Cha)R (to a different extent) block epitope recognition. Peptides 4062, 4063, and 4064 are relatively weak inhibitors with an IC50 of more than 0.5 µg/ml.

- 27 -

Claims:

1. Use of a compound comprising the following amino acid sequence



wherein X_1 is an amino acid, except of C,

X_2 is an amino acid, except of C,

X_3 is an amino acid, except of C,

X_4 is an amino acid, except of C,

X_5 is an amino acid, except of C,

X_6 is not present or any amino acid,

X_7 is not present or any amino acid,

and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH, said compound having a binding capacity to an antibody being specific for the natural N-terminal A β 42 sequence DAEFRH, and 5-mers thereof having a binding capacity to said antibody being specific for the natural N-terminal A β 42 sequence DAEFRH, for the preparation of a vaccine for Alzheimer's disease (AD).

2. Use according to claim 1 characterised in that said compound comprises or is consisting of a peptide, wherein

X_1 is G or an amino acid with a hydroxy group or a negatively charged amino acid, preferably G, E, Y, S or D,

X_2 is a hydrophobic amino acid or a positively charged amino acid, preferably N, I, L, V, K, W, R, Y, F or A,

X_3 is a negatively charged amino acid, preferably D or E,

X_4 is an aromatic amino acid or a hydrophobic amino acid, preferably Y, F or L,

X_5 is H, K, Y, F or R, preferably H, F or R, and

X_6 is any amino acid, except P or - if X_7 is present - except C, preferably S, T, N, Q, D, E, R, I, K, Y, or G, especially T, N, D, R, I or G,

especially DAEFRWP, DNEFRSP, GSEFRDY, GAEFRFT, SAEFRTQ, SAEFRAT, SWEFRNP, SWEFRLY, SWELRQA, SVEFRYH, SYEFRHH, SQEFRTP, SSEFRVS, DWEFRD, DAELRY, DWELRQ, SLEFRF, GPEFRW, GKEFRT, AYEFRH, DKE(Nle)R, DKE(Nva)R or DKE(Cha)R.

3. Use according to claim 1 or 2 characterised in that the com-

- 28 -

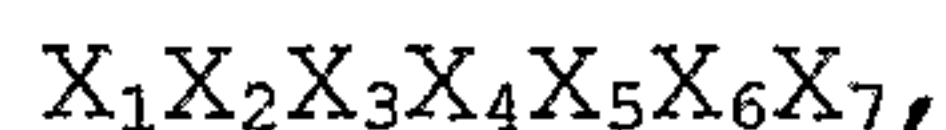
pound is a polypeptide comprising 5 to 15 amino acid residues.

4. Use according to any one of claims 1 to 3 characterised in that the compound is coupled to a pharmaceutically acceptable carrier, preferably KLH, and optionally aluminium hydroxide.

5. Use according to any one of claims 1 to 4 characterised in that it contains the compound in an amount of 0,1 ng to 10 mg, preferably 10 ng to 1 mg, especially 100 ng to 100 µg.

6. Method for isolating a compound binding to an antibody being specific for the natural N-terminal Aβ42 sequence DAEFRH comprising the steps of

- providing a peptide compound library comprising peptides containing the following amino acid sequence



wherein X_1 is an amino acid, except of C,

X_2 is an amino acid, except of C,

X_3 is an amino acid, except of C,

X_4 is an amino acid, except of C,

X_5 is an amino acid, except of C,

X_6 is not present or any amino acid,

X_7 is not present or any amino acid,

and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH,

- contacting said peptide library with said antibody and

- isolating those members of the peptide library which bind to said antibody.

7. Method according to claim 6, characterised in that said peptides are provided in individualised form in said library, especially immobilised on a solid surface.

8. Method according to claim 6 or 7, characterised in that said antibody comprises a suitable marker which allows its detection or isolation when bound to a peptide of the library.

- 29 -

9. Vaccine against Alzheimer's Disease comprising an antigen which includes at least one peptide selected from the group DAEFRWP, DNEFRSP, GSEFRDY, GAEFRFT, SAEFRTQ, SAEFRAT, SWEFRNP, SWEFRLY, SWELRQA, SVEFRYH, SYEFRHH, SQEF RTP, SSEFRVS, DWEFRD, DAELRY, DWELRQ, SLEFRF, GPEFRW, GKEFRT, AYEFRH, DKE(Nle)R, DKE(Nva)R or DKE(Cha)R.

1/7

Fig.1A

1	alanine	ala	A
2	arginine	arg	R
3	asparagine	asn	N
4	aspartic acid	asp	D
5	cysteine	cys	C
6	glutamine	gln	Q
7	glutamic acid	glu	E
8	glycine	gly	G
9	histidine	his	H
10	isoleucine	ile	I
11	leucine	leu	L
12	lysine	lys	K
13	methionine	met	M
14	phenylalanine	phe	F
15	proline	pro	P
16	serine	ser	S
17	threonine	thr	T
18	tryptophan	trp	W
19	tyrosine	tyr	Y
20	valine	val	V

2/7

Fig.1B

No.	D	A	E	F	R	H	
1	D	A	E	F	R	H	positive control
2	D	R	E	F	R	H	
3	D	N	E	F	R	H	
4	D	D	E	F	R	H	
5	D	Q	E	F	R	H	
6	D	E	E	F	R	H	
7	D	G	E	F	R	H	
8	D	H	E	F	R	H	
9	D	I	E	F	R	H	
10	D	L	E	F	R	H	
11	D	M	E	F	R	H	
12	D	F	E	F	R	H	
13	D	P	E	F	R	H	
14	D	S	E	F	R	H	
15	D	T	E	F	R	H	
16	D	W	E	F	R	H	
17	D	Y	E	F	R	H	
18	D	V	E	F	R	H	
19	D	A	A	F	R	H	positive control
20	D	A	R	F	R	H	
21	D	A	N	F	R	H	
22	D	A	D	F	R	H	
23	D	A	Q	F	R	H	
24	D	A	E	F	R	H	
25	D	A	G	F	R	H	
26	D	A	H	F	R	H	
27	D	A	I	F	R	H	
28	D	A	L	F	R	H	
29	D	A	M	F	R	H	
30	D	A	F	F	R	H	
31	D	A	P	F	R	H	
32	D	A	S	F	R	H	
33	D	A	T	F	R	H	
34	D	A	W	F	R	H	
35	D	A	Y	F	R	H	
36	D	A	V	F	R	H	
37	D	A	E	A	R	H	positive control
38	D	A	E	R	R	H	
39	D	A	E	N	R	H	
40	D	A	E	D	R	H	
41	D	A	E	Q	R	H	
42	D	A	E	E	R	H	
43	D	A	E	G	R	H	
44	D	A	E	H	R	H	
45	D	A	E	I	R	H	
46	D	A	E	L	R	H	
47	D	A	E	M	R	H	
48	D	A	E	F	R	H	
49	D	A	E	P	R	H	
50	D	A	E	S	R	H	
51	D	A	E	T	R	H	
52	D	A	E	W	R	H	
53	D	A	E	Y	R	H	
54	D	A	E	V	R	H	

3/7

Fig.1C

55	D	A	E	F	A	H	positive control
56	D	A	E	F	R	H	
57	D	A	E	F	N	H	
58	D	A	E	F	D	H	
59	D	A	E	F	Q	H	
60	D	A	E	F	E	H	
61	D	A	E	F	G	H	
62	D	A	E	F	H	H	
63	D	A	E	F	I	H	
64	D	A	E	F	L	H	
65	D	A	E	F	M	H	
66	D	A	E	F	F	H	
67	D	A	E	F	P	H	
68	D	A	E	F	S	H	
69	D	A	E	F	T	H	
70	D	A	E	F	W	H	
71	D	A	E	F	Y	H	
72	D	A	E	F	V	H	
73	D	A	E	F	R	A	positive control
74	D	A	E	F	R	R	
75	D	A	E	F	R	N	
76	D	A	E	F	R	D	
77	D	A	E	F	R	Q	
78	D	A	E	F	R	E	
79	D	A	E	F	R	G	
80	D	A	E	F	R	H	
81	D	A	E	F	R	I	
82	D	A	E	F	R	L	
83	D	A	E	F	R	M	
84	D	A	E	F	R	F	
85	D	A	E	F	R	P	
86	D	A	E	F	R	S	
87	D	A	E	F	R	T	
88	D	A	E	F	R	W	
89	D	A	E	F	R	Y	
90	D	A	E	F	R	V	

4/7

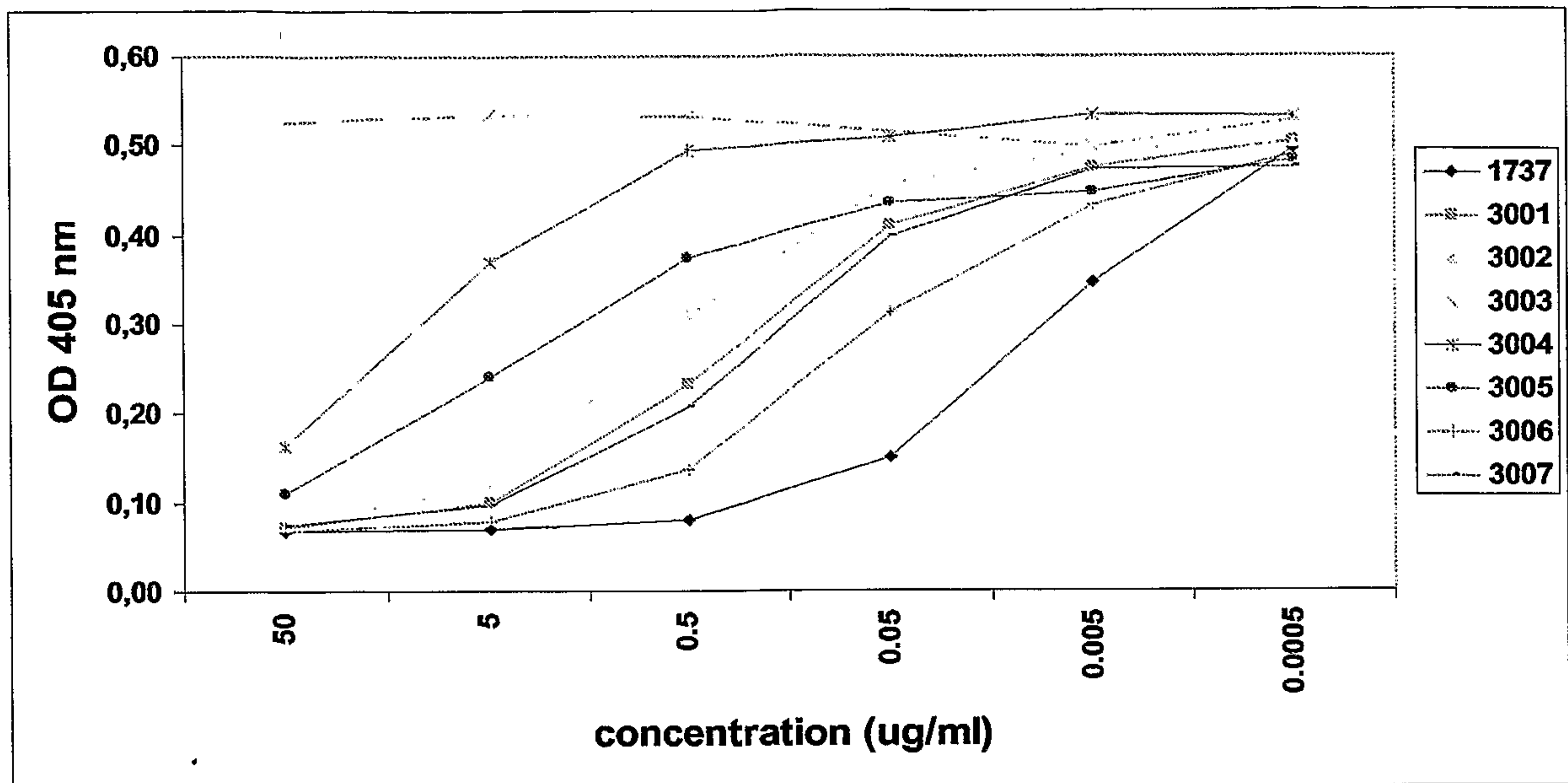


Fig. 2

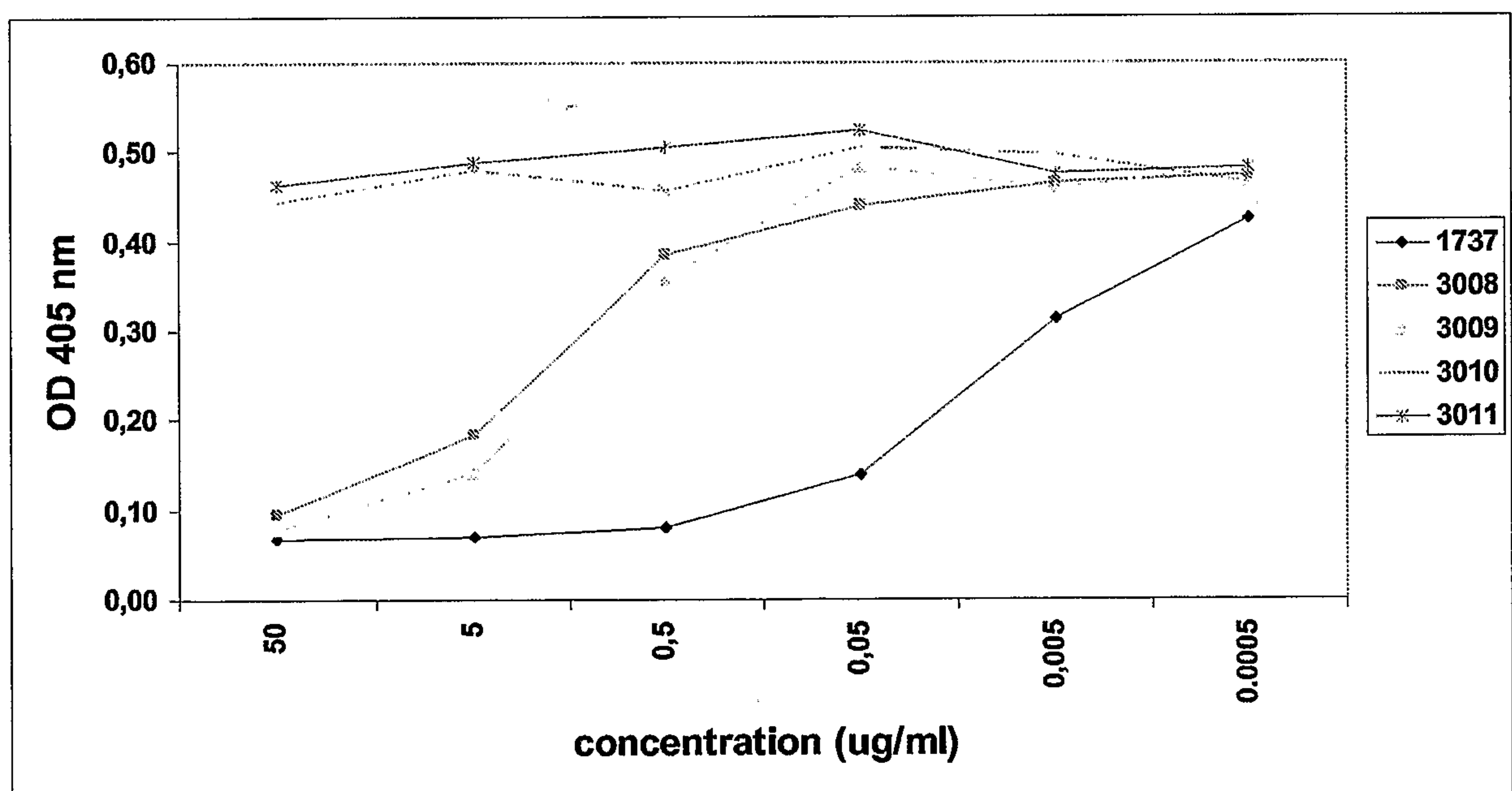


Fig. 3

5/7

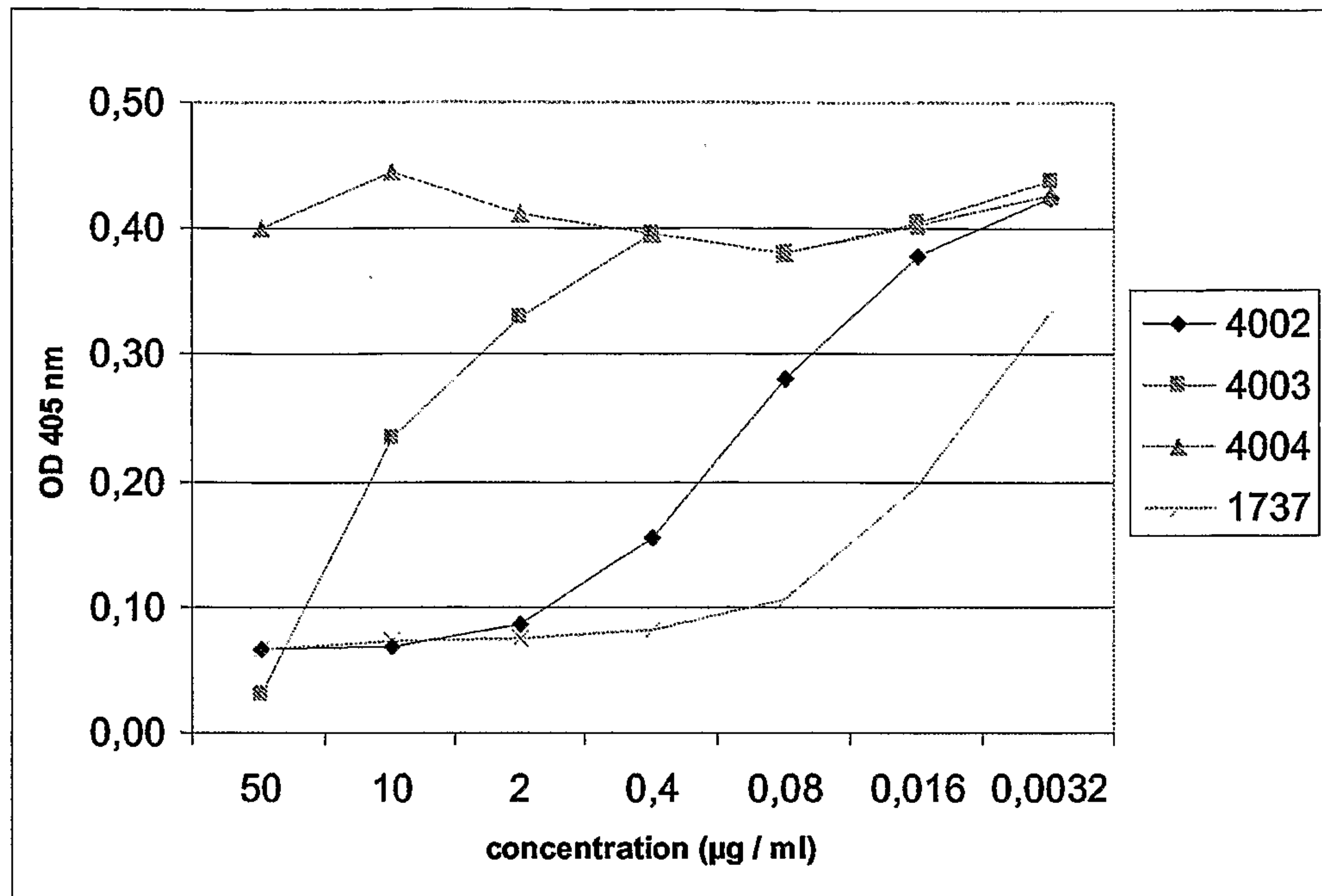


Fig. 4

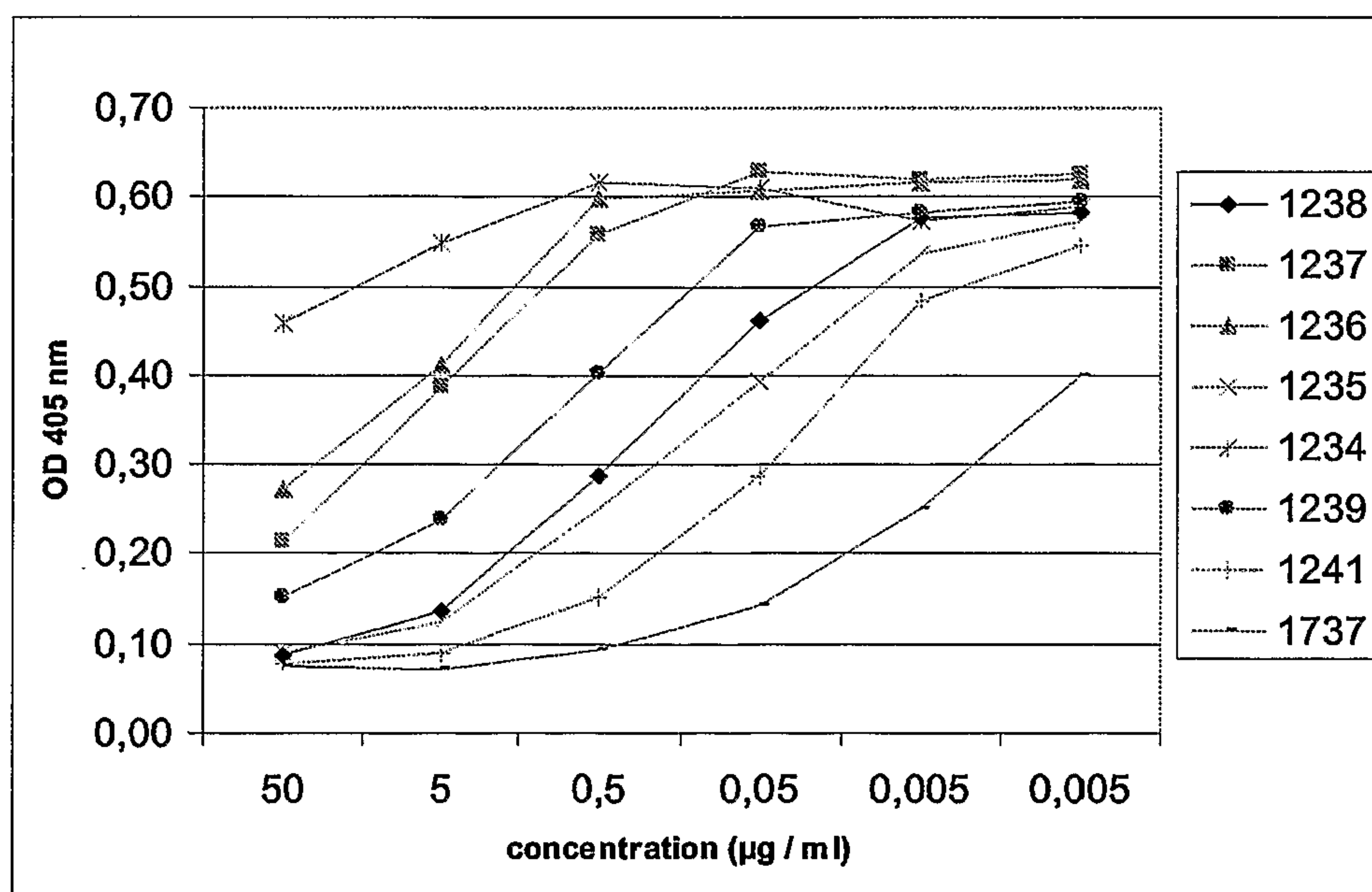


Fig. 5

6/7

Figure 6: Peptides 4011-4018

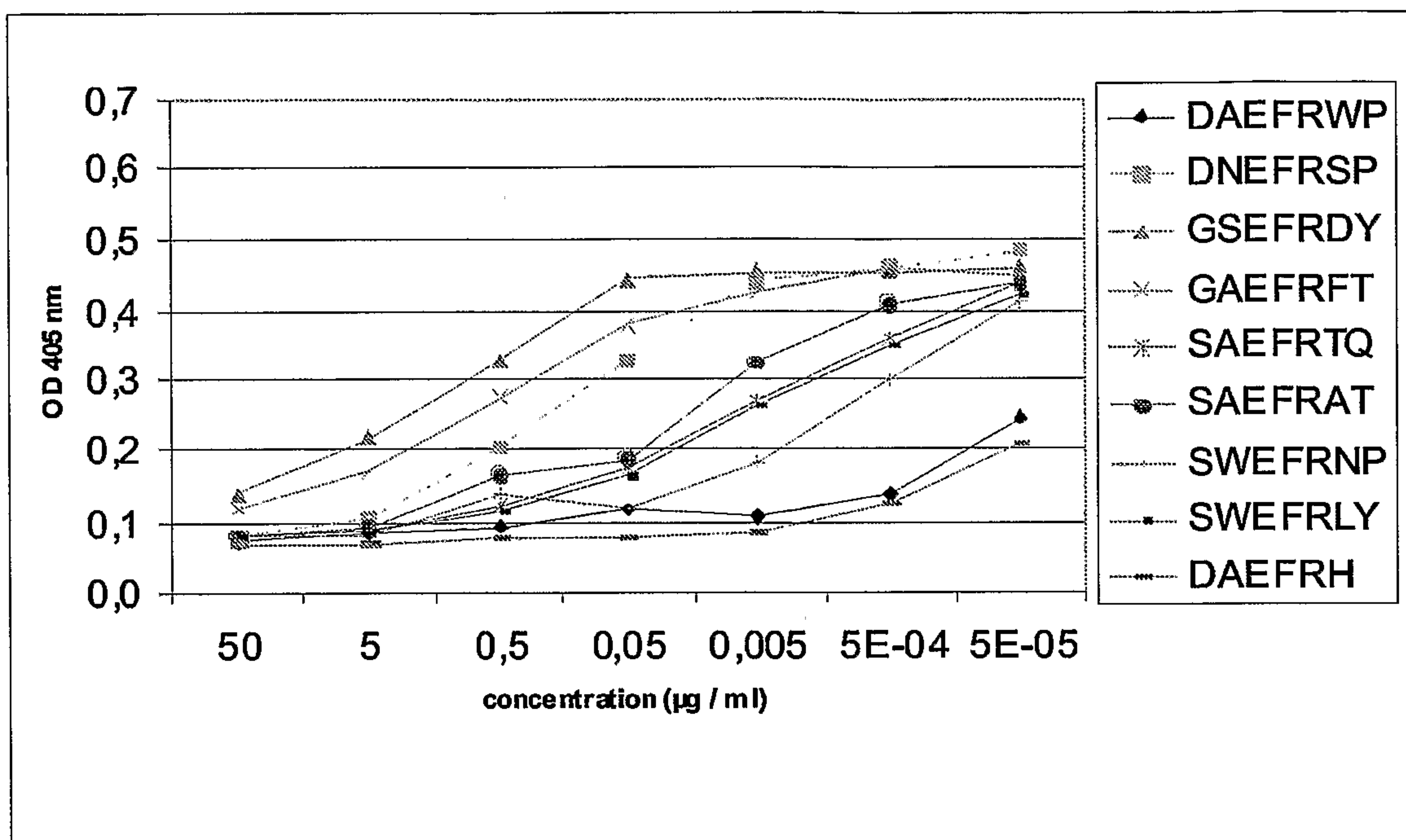


Figure 7: Peptides 4019-4025

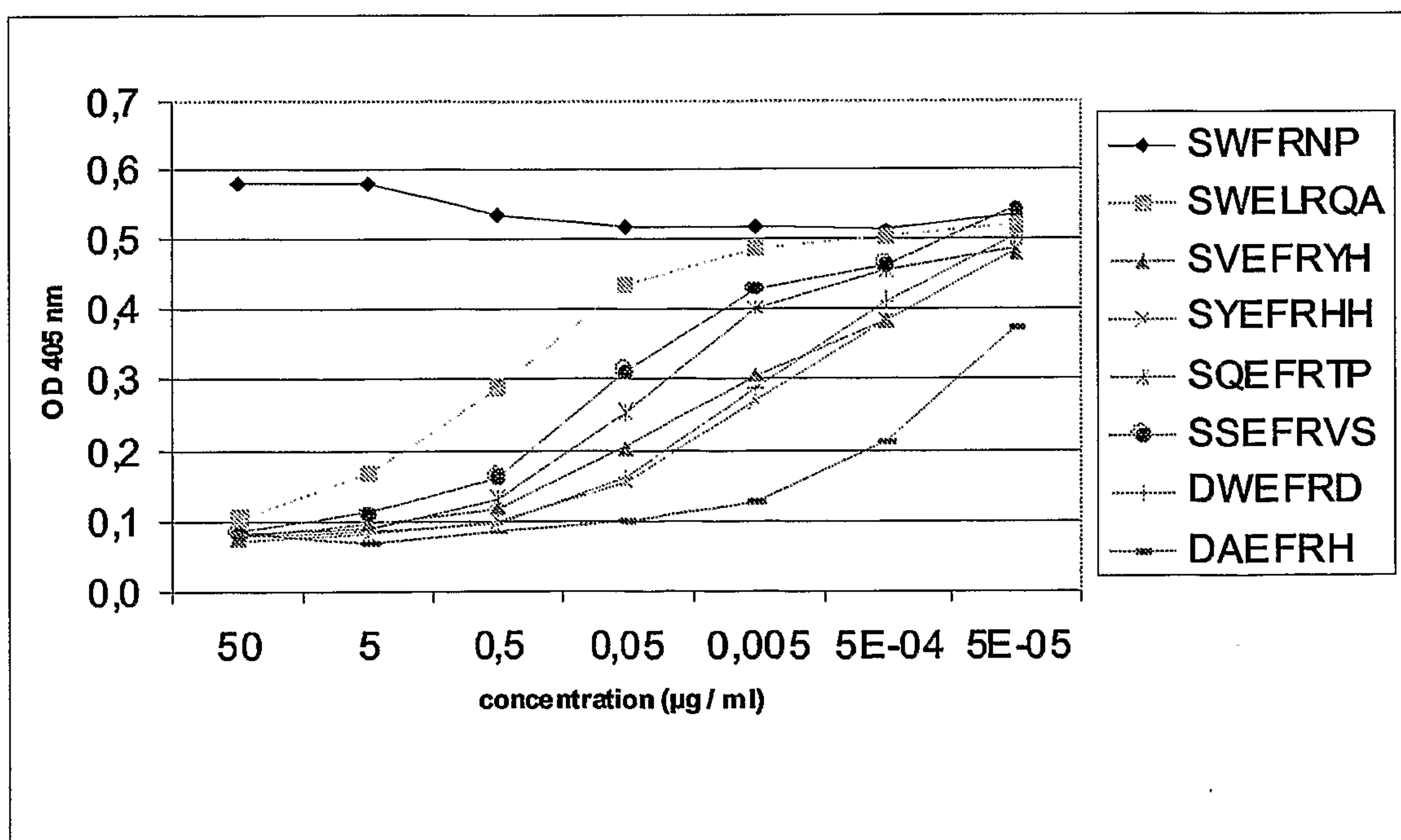


Figure 8: Peptides 4031-4038

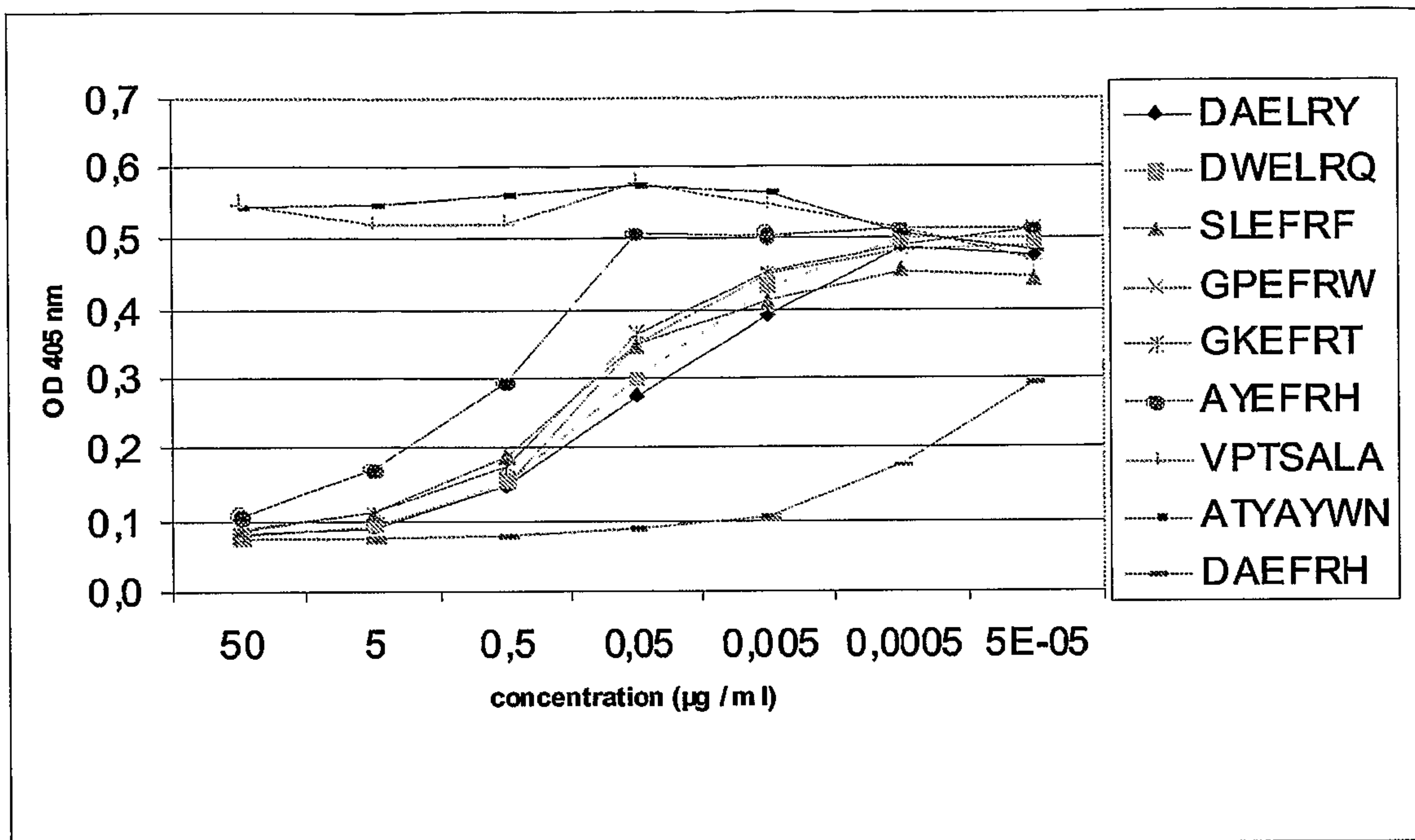


Figure 9: Peptides 4061-4064 DKELR variants

