



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

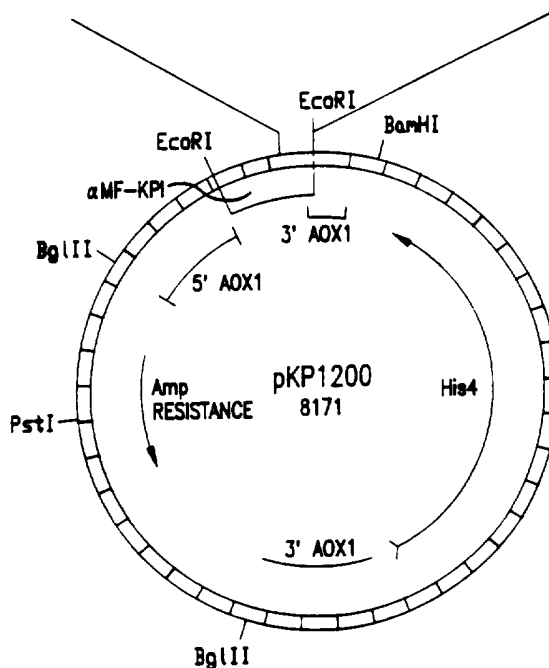
<p>(51) International Patent Classification <sup>5</sup> : C12N 15/15, 15/81, 15/62 C07K 13/00, C12P 21/02 A61K 37/64, C12N 1/19</p>	<p>A3</p>	<p>(11) International Publication Number: <b>WO 93/09233</b>  (43) International Publication Date: 13 May 1993 (13.05.93)</p>
<p>(21) International Application Number: PCT/US92/09400 (22) International Filing Date: 30 October 1992 (30.10.92) (30) Priority data: 785,638 31 October 1991 (31.10.91) US (60) Parent Application or Grant (63) Related by Continuation US 785,638 (CIP) Filed on 31 October 1991 (31.10.91) (71) Applicant (for all designated States except US): THE SALK INSTITUTE BIOTECHNOLOGY/INDUSTRIAL ASSOCIATES INC. [US/US]; 505 Coast Blvd. So., San Diego, CA 92186 (US).</p>	<p>(72) Inventors; and (75) Inventors/Applicants (for US only) : WAGNER, Steven, L. [US/US]; 1295 Prospect Street, Unit D, La Jolla, CA 92037 (US). SIEGEL, Robert [US/US]; 4360 Huggins Street, San Diego, CA 92122 (US). THILL, Gregory, P. [US/US]; 75 Donald Street, Apartment 511, Weymouth, MA 02188 (US). HARPOLD, Michael, M. [US/US]; 1341 29th Street, San Diego, CA 92102 (US). COMER, William, T. [US/US]; 5460 Calzada Del Bosque, Rancho Santa Fe, CA 92067 (US). (74) Agent: MISROCK, S., Leslie; Fennie &amp; Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US). (81) Designated States: AU, BB, BG, BR, CA, CS, FI, HU, JP, KR, LK, MG, MN, MW, NO, PL, RO, RU, SD, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 5 August 1993 (05.08.93)</p>	

(54) Title: RECOMBINANT AMYLOID PRECURSOR PROTEIN INHIBITOR DOMAIN AND TREATMENT OF VARIOUS DISEASE STATES

(57) Abstract

The present invention relates to the production of a Kuniz type protease inhibitor (KPI) peptide, and its use in regulating protease activity associated with amyloidosis. This invention is further directed to *in vivo* and *in vitro* models of amyloidosis involving the KPI peptide. The KPI peptide can also be used to treat coagulation disorders by regulating proteases in the coagulation pathway. KPI is also used to inhibit or ameliorate protease-associated damage following injury, especially head injury. Nucleic acid sequences encoding the protease inhibitor and expression systems for high level expression of the active inhibitor are provided.

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GAGGTTGTTAGACAGGTTTGTCTCAGCAAGCTGAGACTGGTCCATGTAGAGCTATGATTTCT
E V V R E Y C S E Q A E T G P C R A M I S
AGATGGTACTTCGACGTTACTGAGGTAAGTGTCTCCATCTTCTACGGTGGTGTGGTGGT
R V Y F D Y T E G K C A P F F Y G G C G G
AACAGAACCACTTCGACACTGAGGACTGTATGCTGTGTGGTCTGCTATTMA
N R N N F D T E E Y C H A V C G S A I
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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/09400

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.C1.5	C 12 N 15/15	C 12 N 15/81
C 07 K 13/00	C 12 P 21/02	A 61 K 37/64
		C 12 N 15/62
		C 12 N 1/19
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.C1.5	C 07 K	C 12 N                      A 61 K
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>o</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	WO,A,9014840 (CALIFORNIA BIOTECHNOLOGY INC.) 13 December 1990	4,60,62,63
Y	see claims 1,2,4-7,10,13,15,20,27; examples 8-10	1-3,5-52,60
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X	WO,A,9014841 (CALIFORNIA BIOTECHNOLOGY INC.) 13 December 1990 cited in the application	4,60,62,63
Y	see claims 4,5,9,10,13,15,16,24-26	1-3,5-52,60
	---                      -/-	
<p><sup>o</sup> Special categories of cited documents : <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"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</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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.</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
23-02-1993	28 -06- 1993	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	THIELE U.H. -C.H.	

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	<p>J. IND. MICROBIOL. vol. 7, no. 3, April 1991, pages 197 - 202 T. VEDVICK ET AL. 'HIGH-LEVEL SECRETION OF BIOLOGICALLY ACTIVE APROTININ FROM THE YEAST PICHIA-PASTORIS'</p> <p>see abstract see page 198, left column, paragraph 3 -paragraph 4</p> <p>---</p>	1-3,5-52
Y	<p>JOURNAL OF BIOLOGICAL CHEMISTRY. (MICROFILMS) vol. 265, no. 17, 15 June 1990, BALTIMORE, MD US pages 9591 - 9594 W.E. VAN NOSTRAND ET AL. 'Immunopurification and Protease Inhibitory Properties of Protease Nexin-2/Amyloid beta-Protein Precursor' cited in the application see page 9594, left column, line 27 - line 29; table II</p> <p>---</p>	60
X	<p>EP,A,0393431 (BAYER AG) 24 October 1990 cited in the application</p>	4,62,63
A	<p>see page 12, paragraph 1 -paragraph 3; claims 1-7</p> <p>----</p>	1-3,5-52
X	<p>FEBS LETTERS vol. 267, no. 2, July 1990, AMSTERDAM NL pages 207 - 212 M. CASTRO ET AL. 'Does the Kunitz domain from the Alzheimer's amyloid beta protein precursor inhibit a kallikrein responsible for post-translational processing of nerve growth factor precursor' see figure 1</p> <p>---</p>	4
P,X	<p>BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS vol. 186, no. 2, 31 July 1992, DULUTH, MINNESOTA US pages 1138 - 1145 S.L. WAGNER ET AL. 'high level expression, purification, and characterization of the kunitz-type protease inhibitor domain of protease nexin-2/amyloid beta-protein precursor' see the whole document</p> <p>---</p>	1-5,7-13,15-18,20-34,36-41,43-52
A	<p>NEUROCHEMICAL RESEARCH vol. 14, no. 10, 1989, NEW YORK, US G.M. COLE ET AL. 'Evidence for Lysosomal Processing of Amyloid beta-Protein Precursor in Cultured Cells' see page 934, left column, paragraph 3 -paragraph 4</p> <p>---</p>	54,55,59,64-69
	-/-	

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	BIOL. CHEM. HOPPE-SEYLER, VOL. 369 SUPPL. May 1988, BERLIN, FRG pages 149 - 152 M. LARVIN ET AL. 'Intra-Peritoneal Aprotinin Therapy for Acute Pancreatitis' ---	
A	ANN. REV. BIOCHEM. vol. 49, 1980, PALO ALTO, CA, US pages 593 - 626 M. LASKOWSKI, JR. ET I. KATO 'PROTEIN INHIBITORS OF PROTEINASES' -----	

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 92/09400

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
**Remark : Although claims 54-55, 59-60 are directed to a method of treatment of the human body (rule 39.1 IV PCT) the search has been carried out and based on the alleged effects of the compound/composition.**
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see PCT/ISA 206 mailed on 01/04/93

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-55, 59-60, 62-69.

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9209400  
SA 67121

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 09/06/93. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 9014840	13-12-90	CA-A- 2057053	07-12-90
		CA-A- 2058935	07-12-90
		EP-A- 0476015	25-03-92
		EP-A- 0477269	01-04-92
		JP-T- 5501796	08-04-93
		JP-T- 5500801	18-02-93
		WO-A- 9014841	13-12-90
		US-A- 5187153	16-02-93
WO-A- 9014841	13-12-90	US-A- 5187153	16-02-93
		CA-A- 2057053	07-12-90
		CA-A- 2058935	07-12-90
		EP-A- 0476015	25-03-92
		EP-A- 0477269	01-04-92
		JP-T- 5501796	08-04-93
		JP-T- 5500801	18-02-93
		WO-A- 9014840	13-12-90
EP-A- 0393431	24-10-90	DE-A- 3912638	31-10-90
		JP-A- 3041095	21-02-91