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(54) Title: A CONSERVED XIAP-INTERACTION MOTIF IN CASPASE-9 AND SMAC/DIABLO FOR MEDIATING APOPTOSIS

(57) Abstract: The invention provides caspase-9-related peptides and polypeptides capable of binding to an Inhibitor of Apoptosis Protein (IAP), as well as caspase-9 mutant that fail to undergo normal processing and fail to bind to an IAP. Nucleic acid molecules, including expression vectors, encoding such peptides and polypeptides are also provided. Such peptides and polypeptides, are useful for inducing apoptosis and identifying inhibitors and enhancer of apoptosis.

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International application No.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : CO7H 21/04; CO7K5/00,14/00;C12N 15/63,15/85, 15/86

US CL : 435/320.1,325; 530/300, 350; 536/23.5

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)

U.S. : 435/320.1,325, 530/00, 350, 536/235

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)

WEST, MEDLINE, HCAPLUS, PIR, GENBANK: SEQ ID NO: 2, IAP, ligand, BIR3, caspase, apoptosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	DATABASE **PIR 73, Accession No. T10375**. Ahrens CA, et al. 16 July 1999 (see search report var13pir.res, pages 11 and 12).	1, 5, 23, 27, 88, and 90 ---------- 19-22 and 89
X	**LIU Z,** et al. Structural basis for binding of Smac/DIABLO to the XIAP BIR3 domain. Nature. 28 December 2000, Vol. 408, No. 21, pages 1004-1008.	1-4, 19-26, and 88-90
X,P	**SRINIVASULA SM, et al.** A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. Nature. 01 March 2001, Vol. 410, pages 112-116.	1-4, 19-26, and 88-90
X,T --- Y,T	**KIPP RA, et al.** Molecular targeting of inhibitor of apoptosis proteins based on small molecule mimics of natural binding partners. Biochem. 2002, vol. 41, pages 7344-7349.	1-4, 23-26, 88, and 90 ---------- 19-22 and 89
X,P	**WRIGHT CW, et al.** Sequence requirements of Hid binding and apoptosis regulation in the Baculovirus Inhibitor of Apoptosis OP-IAP. J Biol Chem. 25 January 2002, Vol. 277, No. 4, pages 2454-2462.	1, 2, 19-22, 23, 24, amd 88-90

☐ Further documents are listed in the continuation of Box C. ☐ 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

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

"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)

"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
12 March 2003 (12.03.2003)	14 APR 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Stephen L. Rawlings, Ph.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/03553

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

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

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 49-65,75 and 92-94
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:
Claims 49-65, 75, and 92-94 are unsearchable under Article 17 (2)(b) because of defects under Article 17 (2)(a).

3. ☐ Claim 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:
Please See Continuation Sheet

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.: Please See Continuation Sheet

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

INTERNATIONAL SEARCH REPORT

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Group I, claim(s) 1-5, 10-12, 19-27, 32-34, and 88-90, insofar as the claims are drawn to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of SEQ ID NO: 13 or composition thereof, wherein said polypeptide binds an Inhibitor of Apoptosis Protein (IAP), an expression vector comprising said nucleic acid molecule or composition thereof, a host cell containing said vector, a polypeptide comprising an amino acid sequence of SEQ ID NO: 13 or composition thereof, wherein said polypeptide binds an Inhibitor of Apoptosis Protein (IAP).

Group II, claim(s) 6, 19-22, 28, and 88-90. insofar as the claims are drawn to a nucleic acid molecule consisting essentially of polynucleotide sequence encoding at least an N-terminus sequence of SEQ ID NO: 11 or a composition thereof, an expression vector comprising said nucleic acid molecule or composition thereof, a host cell containing said vector, and a polypeptide or composition thereof, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 11 or a variant thereof and binds IAP.

Group III, claim(s) 7, 19-22, 29, and 88-90, insofar as the claims are drawn to a nucleic acid molecule consisting essentially of polynucleotide sequence encoding at least an N-terminus sequence of SEQ ID NO: 15 or a composition thereof, an expression vector comprising said nucleic acid molecule or composition thereof, a host cell containing said vector, and a polypeptide or composition thereof, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 15 or a variant thereof and binds IAP.

Group IV, claim(s) 8, 19-22, 30, and 88-90, insofar as the claims are drawn to a nucleic acid molecule consisting essentially of polynucleotide sequence encoding at least an N-terminus sequence of SEQ ID NO: 12 or a composition thereof, an expression vector comprising said nucleic acid molecule or composition thereof, a host cell containing said vector, and a polypeptide or composition thereof, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 12 or a variant thereof and binds IAP.

Group V, claim(s) 9, 19-22, 31, and 88-90, insofar as the claims are drawn to a nucleic acid molecule having a polynucleotide sequence encoding a polypeptide or a composition thereof, an expression vector comprising said nucleic acid molecule or composition thereof, a host cell containing said vector, and a polypeptide or composition thereof, wherein said polypeptide comprises a first portion of a procaspase-9 that specifically binds IAP and a second portion of a procaspase-9 containing a mutated active site and lacking cysteine protease activity.

Group VI, claim(s) 13-22, 35-39, and 88-90, insofar as the claims are drawn to a nucleic acid molecule having a polynucleotide sequence encoding a polypeptide comprising a portion of a mutated procaspase-9 or a composition thereof, wherein said portion fails to undergo normal processing and possesses wild-type caspase-9 activity, an expression vector comprising said nucleic acid molecule or composition thereof, a host cell containing said vector, and a polypeptide or composition thereof, wherein said polypeptide comprises a portion of a mutated procaspase-9, wherein said portion fails to undergo normal processing and possesses wild-type caspase-9 activity.

Group VII, claim(s) 40, 41, 44-47, and 91, insofar as the claims are drawn to an antibody that specifically binds a polypeptide set forth in SEQ ID NO: 13 or a composition thereof, wherein said polypeptide binds an IAP.

Group VIII, claim(s) 42-48 and 91, insofar as the claims are drawn to an antibody that specifically binds an epitope of the N-terminus of a caspase-9-p12 or a composition thereof.

Group IX, claim(s) 66, drawn to a method for identifying an inhibitor or enhancer of apoptosis comprising contacting a cell containing a vector expressing a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13 that is capable of specifically binding an IAP with a candidate inhibitor or enhancer and detecting cell viability.

Group X, claim(s) 67, drawn to a method for identifying an inhibitor or enhancer of apoptosis comprising contacting a cell containing a vector expressing a polypeptide comprising a portion of a mutated procaspase-9, wherein said portion fails to undergo normal processing and possesses wild-type caspase-9 activity, and detecting cell viability.

Group XI, claim(s) 68 and 69, drawn to a method for identifying an inhibitor or enhancer of apoptosis comprising contacting a cell containing a vector expressing a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13 that is capable of specifically binding an IAP with a candidate inhibitor or enhancer and detecting the presence of large and small caspase subunits.

Group XII, claim(s) 70 and 71, drawn to a method for identifying an inhibitor or enhancer of apoptosis comprising contacting a cell containing a vector expressing a polypeptide comprising a portion of a mutated procaspase-9, wherein said portion fails to undergo normal processing and possesses wild-type caspase-9 activity, and detecting the presence of large and small caspase subunits.

Group XIII, claim(s) 72-74, drawn to a method for identifying an inhibitor or enhancer of apoptosis comprising contacting a cell containing a vector expressing a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13 that is capable of specifically binding an IAP with a candidate inhibitor or enhancer and detecting caspase activity.

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Group XIV, claim(s) 76-79, drawn to a method for identifying an inhibitor or enhancer of apoptosis comprising contacting a cell containing a vector expressing a polypeptide comprising a portion of a mutated procaspase-9, wherein said portion fails to undergo normal processing and possesses wild-type caspase-9 activity, and detecting caspase activity.

Group XV, claim(s) 80-82, drawn to a method for identifying a compound that inhibits a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13, wherein said polypeptide specifically binds an IAP, wherein said method comprises contacting a candidate compound with said polypeptide in the presence of an IAP and detecting displacement of said IAP from said polypeptide.

Group XVI, claim(s) 83-87, drawn to a method for identifying a compound that inhibits a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13, wherein said polypeptide specifically binds an IAP, wherein said method comprises contacting a candidate compound with said polypeptide in the presence of an IAP and performing a functional assay.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Claim 1 is generic to a plurality of inventions, wherein said nucleic acid molecules encodes a polypeptide comprising an amino acid sequence selected from the group consisting of the first four amino acids of (a) SEQ ID NO: 2, (b) SEQ ID NO: 3, (c) SEQ ID NO: 4, (d) SEQ ID NO: 5, (e) SEQ ID NO: 6, (f) SEQ ID NO: 7, (g) SEQ ID NO: 8, (h) SEQ ID NO: 9, and (i) SEQ ID NO: 10, according to claim 5.

Claim 10 is generic to a plurality of inventions, wherein said nucleic acid molecule encodes a polypeptide selected from the group consisting of (a) a polypeptide consisting essentially of a caspase-3 in which the amino acid residues corresponding to the amino terminal two residues of p12 are substituted with alanine-valine and (b) a polypeptide consisting essentially of a caspase-3 in which the amino acid residues corresponding to the amino terminal four residues of p12 are substituted with residues set forth in SEQ ID NO: 13, as set forth in claims 11 and 12, respectively.

Claim 13 is generic to a plurality of inventions, wherein said nucleic acid molecule encodes a polypeptide comprising a portion of a mutated procapase-9 selected from the group consisting of (a) a portion corresponding to SEQ ID NO: 1 with amino acid residue 315 substituted by alanine, (b) a portion corresponding to SEQ ID NO: 1 with amino acid residues 315 and 330 substituted by alanine, (c) a portion corresponding to SEQ ID NO: 1 with amino acid residues 306, 315, and 330 substituted by alanine, and (d) a portion corresponding to SEQ ID NO: 1 with amino acid residues 316-330 deleted, as set forth in claims 15, 16, 17, and 18, respectively.

Claim 23 is generic to a plurality of inventions, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of the first four amino acids of (a) SEQ ID NO: 2, (b) SEQ ID NO: 3, (c) SEQ ID NO: 4, (d) SEQ ID NO: 5, (e) SEQ ID NO: 6, (f) SEQ ID NO: 7, (f) SEQ ID NO: 8, (g) SEQ ID NO: 9, and (h) SEQ ID NO: 10, according to claim 27.

Claim 32 is generic to a plurality of inventions, wherein said polypeptide is selected from the group consisting of (a) a polypeptide comprising a caspase-3 or variant thereof in which the amino acid residues corresponding to the amino terminal two residues of p12 are substituted with alanine-valine and (b) a polypeptide consisting essentially of a caspase-3 in which the amino acid residues corresponding to the amino terminal four residues of p12 are substituted with residues set forth in SEQ ID NO: 13, as set forth in claims 33 and 34, respectively.

Claim 35 is generic to a plurality of inventions, wherein said polypeptide comprises a portion of a mutated procapase-9 or variant thereof selected from the group consisting of (a) a portion corresponding to SEQ ID NO: 1 with amino acid residue 315 substituted by alanine, (b) a portion corresponding to SEQ ID NO: 1 with amino acid residues 315 and 330 substituted by alanine, (c) a portion corresponding to SEQ ID NO: 1 with amino acid residues 306, 315, and 330 substituted by alanine, and (d) a portion corresponding to SEQ ID NO: 1 with amino acid residues 316-330 deleted, as set forth in claims 36, 37, 38, and 39, respectively.

Claims 40 and 42 are generic to a plurality of inventions, wherein said antibody inhibits binding of the protein to which the antibody binds to a portion of an IAP selected from the group consisting of (a) BIR1, (b) BIR2, and (c) BIR3, as set forth in claims 45, 46, and 47, respectively.

Claims 68 and 70 are generic to a plurality of inventions, wherein said method comprises detecting the small and large subunits of a caspase selected from the group consisting of (a) caspase-3, (b) caspase-7, and (c) caspase-9, according to claim 69 or 71.

Claims 72 and 76 are generic to a plurality of inventions, wherein said method comprises detecting the enzymatic activity of a caspase selected from the group consisting of (a) caspase-3, (b) caspase-7, and (c) caspase-9, according to claim 73 or 77.

Claim 83 is generic to a plurality of inventions, wherein said method comprises functionally assaying a caspase selected from the group consisting of (a) caspase-3, (b) caspase-7, and (c) caspase-9, according to claim 85.

The inventions listed as Groups I-XVI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

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The special technical feature of Group I is a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of SEQ ID NO: 13 and inducing or stimulating apoptosis in a cell by a process comprising contacting the cell with said nucleic acid molecule.

The special technical feature of Group II is a nucleic acid molecule consisting essentially of polynucleotide sequence encoding at least an N-terminus sequence of SEQ ID NO: 11.

The special technical feature of Group III is a nucleic acid molecule consisting essentially of polynucleotide sequence encoding at least an N-terminus sequence of SEQ ID NO: 15.

The special technical feature of Group IV is a nucleic acid molecule consisting essentially of polynucleotide sequence encoding at least an N-terminus sequence of SEQ ID NO: 12.

The special technical feature of Group V is a nucleic acid molecule having a polynucleotide sequence encoding a polypeptide comprising a first portion of a procaspase-9 that specifically binds IAP and a second portion of a procaspase-9 containing a mutated active site and lacking cysteine protease activity.

The special technical feature of Group VI is a nucleic acid molecule having a polynucleotide sequence encoding a polypeptide comprising a portion of a mutated procaspase-9, wherein said portion fails to undergo normal processing and possesses wild-type caspase-9 activity.

The special technical feature of Group VII is an antibody that specifically binds a polypeptide set forth in SEQ ID NO: 13 or a composition thereof, wherein said polypeptide binds an IAP.

The special technical feature of Group VIII is an antibody that specifically binds an epitope of the N-terminus of a caspase-9-p12 or a composition thereof.

The special technical feature of Group IX is identifying an inhibitor or enhancer of apoptosis by a process comprising contacting a cell containing a vector expressing a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13 that is capable of specifically binding an IAP with a candidate inhibitor or enhancer and detecting cell viability.

The special technical feature of Group X is identifying an inhibitor or enhancer of apoptosis by a process comprising containing a vector expressing a polypeptide comprising a portion of a mutated procaspase-9, wherein said portion fails to undergo normal processing and possesses wild-type caspase-9 activity, and detecting cell viability.

The special technical feature of Group XI is identifying an inhibitor or enhancer of apoptosis by a process comprising contacting a cell containing a vector expressing a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13 that is capable of specifically binding an IAP with a candidate inhibitor or enhancer and detecting the presence of large and small caspase subunits.

The special technical feature of Group XII is identifying an inhibitor or enhancer of apoptosis by a process comprising contacting a cell containing a vector expressing a polypeptide comprising a portion of a mutated procaspase-9, wherein said portion fails to undergo normal processing and possesses wild-type caspase-9 activity, and detecting the presence of large and small caspase subunits.

The special technical feature of Group XIII is identifying an inhibitor or enhancer of apoptosis by a process comprising contacting a cell containing a vector expressing a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13 that is capable of specifically binding an IAP with a candidate inhibitor or enhancer and detecting caspase activity.

The special technical feature of Group XIV is identifying an inhibitor or enhancer of apoptosis by a process comprising contacting a cell containing a vector expressing a polypeptide comprising a portion of a mutated procaspase-9, wherein said portion fails to undergo normal processing and possesses wild-type caspase-9 activity, and detecting caspase activity.

The special technical feature of Group XV is identifying a compound that inhibits a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13, wherein said polypeptide specifically binds an IAP by a process comprising contacting a candidate compound with said polypeptide in the presence of an IAP and detecting displacement of said IAP from said polypeptide.

The special technical feature of Group XVI is identifying a compound that inhibits a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13, wherein said polypeptide specifically binds an IAP by a process comprising contacting a candidate compound with said polypeptide in the presence of an IAP and performing a functional assay.

Accordingly, groups I-XVI do not share the same or a corresponding special technical feature and therefore do not form a single general inventive concept. Moreover, PCT Rules 13.1 and 13.2 only provide for the unification of claims drawn to the first claimed product and claims drawn to the first claimed methods for making and using said product.

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The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

The special technical features of the species of invention encompassed by claim 1 are nucleic acid molecules that encode a polypeptide comprising an amino acid sequence selected from the group consisting of the first four amino acids of (a) SEQ ID NO: 2, (b) SEQ ID NO: 3, (c) SEQ ID NO: 4, (d) SEQ ID NO: 5, (e) SEQ ID NO: 6, (f) SEQ ID NO: 7, (f) SEQ ID NO: 8, (g) SEQ ID NO: 9, and (h) SEQ ID NO: 10.

The special technical features of the species of invention encompassed by claim 10 are nucleic acid molecules that encode a polypeptide selected from the group consisting of (a) a polypeptide consisting essentially of a caspase-3 in which the amino acid residues corresponding to the amino terminal two residues of p12 are substituted with alanine-valine and (b) a polypeptide consisting essentially of a caspase-3 in which the amino acid residues corresponding to the amino terminal four residues of p12 are substituted with residues set forth in SEQ ID NO: 13.

The special technical features of the species of invention encompassed by claim 13 are nucleic acid molecules that encode a polypeptide comprising a portion of a mutated procapase-9 selected from the group consisting of (a) a portion corresponding to SEQ ID NO: 1 with amino acid residue 315 substituted by alanine, (b) a portion corresponding to SEQ ID NO: 1 with amino acid residues 315 and 330 substituted by alanine, (c) a portion corresponding to SEQ ID NO: 1 with amino acid residues 306, 315, and 330 substituted by alanine, and (d) a portion corresponding to SEQ ID NO: 1 with amino acid residues 316-330 deleted.

The special technical features of the species of invention encompassed by claim 23 are polypeptides comprising an amino acid sequence selected from the group consisting of the first four amino acids of (a) SEQ ID NO: 2, (b) SEQ ID NO: 3, (c) SEQ ID NO: 4, (d) SEQ ID NO: 5, (e) SEQ ID NO: 6, (f) SEQ ID NO: 7, (f) SEQ ID NO: 8, (g) SEQ ID NO: 9, and (h) SEQ ID NO: 10.

The special technical features of the species of invention encompassed by claim 32 are (a) a polypeptide comprising a caspase-3 or variant thereof in which the amino acid residues corresponding to the amino terminal two residues of p12 are substituted with alanine-valine and (b) a polypeptide consisting essentially of a caspase-3 in which the amino acid residues corresponding to the amino terminal four residues of p12 are substituted with residues set forth in SEQ ID NO: 13.

The special technical features of the species of invention encompassed by claim 35 are polypeptides comprising a portion of a mutated procapase-9 or variant thereof selected from the group consisting of (a) a portion corresponding to SEQ ID NO: 1 with amino acid residue 315 substituted by alanine, (b) a portion corresponding to SEQ ID NO: 1 with amino acid residues 315 and 330 substituted by alanine, (c) a portion corresponding to SEQ ID NO: 1 with amino acid residues 306, 315, and 330 substituted by alanine, and (d) a portion corresponding to SEQ ID NO: 1 with amino acid residues 316-330 deleted.

The special technical features of the species of invention encompassed by claim 40 are an antibody that inhibits binding of the protein to which the antibody binds to (a) BIR1, (b) BIR2, or (c) BIR3.

The special technical features of the species of invention encompassed by claim 42 are an antibody that inhibits binding of the protein to which the antibody binds (a) BIR1, (b) BIR2, or (c) BIR3.

The special technical features of the species of invention encompassed by claim 68 are detecting the small and large subunits of a caspase selected from the group consisting of (a) caspase-3, (b) caspase-7, and (c) caspase-9.

The special technical features of the species of invention encompassed by claim 70 are detecting the small and large subunits of a caspase selected from the group consisting of (a) caspase-3, (b) caspase-7, and (c) caspase-9.

The special technical features of the species of invention encompassed by claim 72 are detecting the enzymatic activity of a caspase selected from the group consisting of (a) caspase-3, (b) caspase-7, and (c) caspase-9.

The special technical features of the species of invention encompassed by claim 76 are detecting the enzymatic activity of a caspase selected from the group consisting of (a) caspase-3, (b) caspase-7, and (c) caspase-9.

The special technical features of the species of invention encompassed by claim 83 are functionally assaying a caspase selected from the group consisting of (a) caspase-3, (b) caspase-7, and (c) caspase-9.

Accordingly, each species of invention lacks the same or corresponding special technical feature as any other, and therefore do not related to a single general inventive concept.

Continuation of Box II Item 4:
1-5, 10-12, 19-27, 32-34, and 88-90 insofar as the claims are drawn to the first named species of invention