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Remarks:

The complete document including Reference Table(s) and the Sequence Listing(s) can be downloaded from the EPO website

Description

Technical Field

[0001] The present invention relates to the field of antineoplastic compounds, in particular to anticancer peptides and anticancer compositions comprising said peptides. The invention also relates to the use of said peptides and said compositions for the prophylactic or therapeutic treatment of pancreatic cancer.

Background Art

[0002] The therapeutic use of proteins and peptides that act intracellularly holds much promise for the treatment of cancer and other diseases.

[0003] Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Cancer is a multifactorial disease, i.e. it is the result of the occurrence of multiple factors. Said factors usually converge in the generation of mutations in proto-oncogenes that cause cellular proliferation to increase. Mutations may also occur in tumor suppressor genes whose normal function is to regulate cellular proliferation. Mutations may also occur in DNA repair enzymes, impairing the ability of the cell to repair damage before proliferating therefore generating genomic instability.

[0004] Currently, there are few effective options for the treatment of many common cancer types. The course of treatment for a given individual depends on the diagnosis, the stage to which the disease has developed and factors such as age, sex and general health of the patient. The most conventional options of cancer treatment are surgery, radiation therapy and chemotherapy. Each of these therapies has varying degrees of efficacy and is accompanied with varying side effects. These side effects, together with the multidrug resistance already disclosed for traditional chemotherapy, have prompted urgent needs for novel anticancer drugs or therapeutic approaches.

[0005] One particularly deadly type of cancer is pancreatic cancer. This type of cancer is a malignant growth of the pancreas that mainly occurs in the cells of the pancreatic ducts. This disease is the ninth most common form of cancer, yet it is the fourth and fifth leading cause of cancer deaths in men and women, respectively. Cancer of the pancreas is almost always fatal, with a five-year survival rate that is less than 3%.

[0006] The most common symptoms of pancreatic cancer include jaundice, abdominal pain, and weight loss, which, together with other presenting factors, are nonspecific in nature. Thus, diagnosing pancreatic cancer at an early stage of tumor growth is often difficult and requires extensive diagnostic work-up, often times including exploratory surgery. Endoscopic ultrasonography and computed tomography are the best noninvasive means available today for diagnosis of pancreatic cancer. However, reliable detection of small tumors, as well as differentiation of pancreatic cancer from focal pancreatitis, is difficult. The vast majority of patients with pancreatic cancer are presently diagnosed at a late stage when the tumor has already extended outside of the capsule to invade surrounding organs and/or has metastasized extensively. Late detection of the disease is common, and early pancreatic cancer diagnosis is rare in the clinical setting.

[0007] Current treatment procedures available for pancreatic cancer have not led to a cure, nor to a substantially improved survival time. Surgical resection has been the only modality that offers a chance at survival. However, due to a large tumor load, only 10% to 25% of patients are candidates for "curative resection". For those patients undergoing a surgical treatment, the five-year survival rate is still poor, averaging only about 10%. Therefore, pancreatic cancer is one of the types of cancer where there is a higher need of development of efficient therapies.

[0008] One of the most promising therapeutic alternatives against cancer currently under development are anticancer peptides. These peptides have several important advantages over traditional anticancer agents such as high activity, specificity and affinity, and minimal drug-drug interaction. They can be used in combination with surgical resection. They also present several advantages with respect to therapies based on proteins or antibodies-they are small in size, easy to synthesize, they have the ability to penetrate the cell membranes, and have minimal biological and chemical diversity. An added benefit of using peptides as a treatment is that they do not accumulate in specific organs (e.g. kidney or liver), which can help to minimize their toxic side effects. They can also be rapidly synthesized and easily modified and are less immunogenic than recombinant antibodies or proteins. All these characteristics make peptide therapeutics a promising field for emerging anticancer agents. WO 2014/079943 discloses anti-cancer peptides that are useful for the treatment of pancreatic cancer.

[0009] However, therapeutic peptides do have some significant drawbacks such as low stability or resistance to proteases, which has hindered their development and arrival to the clinic.

[0010] Therefore, in spite of the efforts made, there continues to be a need in the clinical field of neoplastic diseases for therapeutic alternatives, such as effective anticancer peptides.

Summary of Invention

[0011] The present inventor has developed various peptides with the capacity to inhibit the growth of cancer cells. Surprisingly, the inventor has found that the presence of at least one cysteine residue in a terminal end confers the peptides of the invention a potent cancer inhibitory activity. Importantly, the peptides provided herein also display high solubility and high stability in frozen solutions, which makes them suitable for therapeutic compositions. All these characteristics make the peptides of the invention an important pharmacological alternative in the treatment of yet practically incurable tumors, like pancreatic tumors.

[0012] In a first aspect, the present invention provides a peptide of formula (I) or a pharmaceutically acceptable salt thereof,

$$C_aC_bC_cC_dC_eFEX_1SKYC_fC_gC_hC_iC_j \qquad (I)$$

wherein:

the N-terminal group of the peptide is a monoradical of formula $-NHR_1$,
the C-terminal group of the peptide is a monoradical of formula $-C(O)-R_2$;
R_1 is a monoradical selected from hydrogen and $-C(O)-(C_1-C_{20})$alkyl;
R_2 is a monoradical selected from -OH and $-NR_3R_4$ radical;
R_3 and R_4 are independently selected from hydrogen and (C_1-C_{10})alkyl;
"a" to "j" are integers from 0 to 1, provided that at least one of "a" to "j" is 1; and
X_1 represents any amino acid.

[0013] As it is shown below, the peptides of the invention are highly specific, being capable of specifically target cancer cells. That is, the peptides of the invention are able to "discriminate" between normal and cancer cells. This means a great advance in the field of cancer because one of the most widely known side-effects of current anti-cancer therapies is the side-effects due to their lack of specificity. This specificity towards cancer cells also explains the experimental data provided below, supporting the non-toxicity of said peptides when they are administered to various types of human primary cells.

[0014] These properties make the peptides of formula (I) of the invention suitable as cancer therapeutics.

[0015] A second aspect of the invention relates to a pharmaceutical composition comprising a therapeutically effective amount of the peptide of formula (I) or a pharmaceutically acceptable salt thereof of the first aspect with at least one pharmaceutically acceptable excipient, diluent or carrier.

[0016] A third aspect of the invention relates to the peptide or the pharmaceutical composition of the invention for use as a medicament.

[0017] And, finally, in a fourth aspect the present invention provides the peptide or pharmaceutical composition of the invention for use in the treatment or prevention of a neoplastic disease.

Brief Description of Drawings

[0018]

Fig. 1, related to Assay 1, are two bar diagrams showing the inhibitory effect of various peptides of the invention at two different concentrations on the growth of human pancreatic tumor cells (BXPC3) in comparison to mock treated cells. The y-axis represents the number of cells after 72 h of treatment as a percentage of the number of mock treated cells, which is accorded a 100% value. (A) Cells were treated with peptides at 20 μM concentration. The first column (CONTROL), corresponds to mock treated cells, the second column (P1) corresponds to the P1 peptide wherein the N-terminal end is acetylated and the C-terminal end is amidated; the third column (P1C), corresponds to a variant of the P1 peptide with a terminal cysteine wherein the N-terminal end is acetylated and the C-terminal end is amidated; the fourth column (P2) correspond to the P2 peptide wherein the N-terminal end is acetylated and the C-terminal end is amidated; the fifth column (P2C), corresponds to a variant of the P2 peptide with a terminal cysteine wherein the N-terminal end is acetylated and the C-terminal end is amidated; the sixth column (P1A) corresponds to a variant of the P1C peptide wherein the N-terminal end is free (not acetylated) and the C-terminal end is amidated; and the seventh column (P1B) corresponds to a variant of the P1C peptide wherein the N-terminal end is acetylated, the C-terminal end is amidated, and the isoleucine is substituted by a valine. (B) Cells were treated with peptides at 40 μM concentration. The first column (CONTROL), corresponds to mock treated cells, the second, third, fourth, and fifth columns correspond to cells treated with the P1, P1C, P1A, and P1B peptides, respectively. The sequences of the peptides are further detailed in Example 2.

Fig. 2, related to Assay 2, is a bar diagram showing the toxicity effect of the P1C peptide (Ac-CFEISKY-NH_2) on human umbilical cord primary endothelial cells (HUVEC) at various concentrations in comparison to mock treated cells. The y-axis represents the number of cells after 72 h of treatment as a percentage of the number of mock treated cells (control), which is accorded a 100% value. The first column (DMSO), corresponds to cells treated with the vehicle in which the peptides are dissolved. The second to fifth columns correspond to cells treated with the P1C peptide at 10, 20, 30, 40 μM concentrations, respectively.

Detailed description of the invention

[0019] All terms as used herein in this application, unless otherwise stated, shall be understood in their ordinary meaning as known in the art. Other more specific definitions for certain terms as used in the present application are as set forth below and are intended to apply uniformly through-out the specification and claims unless an otherwise expressly set out definition provides a broader definition.

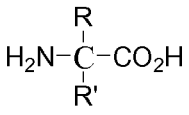
[0020] As above exposed, the inventors propose a set of peptides of formula (I) or pharmaceutically acceptable salts thereof with potent cancer inhibitory activity.

[0021] As used herein, the term "pharmaceutically acceptable salt", when referred to the peptide of the invention, refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and non-human animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, trifluoroacetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, and ammonium. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counter ions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate.

[0022] In the present invention, the term "amino acid" refers to a molecule containing both an amino group and a carboxyl group.

[0023] Suitable amino acids include, without limitation, alpha amino acids, such as the L-isomers of alpha-amino acids of the 20 common naturally occurring alpha-amino acids: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; natural beta-amino acids (e.g., beta-alanine); and unnatural amino acids.

[0024] The term "unnatural amino acid" comprises D-isomers of the 20 common naturally occurring alpha-amino acids or amino acids of formula (A)



(A)

wherein R and R' have the meaning provided in Table 1 below.

	Suitable amino acid side chains	
Exemplary unnatural alpha-amino acids	R	R'
D- Alanine	-H	$-CH_3$
D-Arginine	-H	$-CH_2CH_2CH_2$-NHC(=NH)NH_2
D-Asparagine	-H	$-CH_2C(=O)NH_2$

(continued)

Exemplary unnatural alpha-amino acids	Suitable amino acid side chains	
	R	R'
D-Aspartic acid	-H	$-CH_2CO_2H$
D-Cysteine	-H	$-CH_2SH$
D-Glutamic acid	-H	$-CH_2CH_2CO_2H$
D-Glutamine	-H	$-CH_2CH_2C(=O)NH_2$
D-Histidine	-H	$-CH_2$-2-(1H-imidazole)
D-Isoleucine	-H	-sec-butyl
D-Leucine	-H	-iso-butyl
D-Lysine	-H	$-CH_2CH_2CH_2CH_2NH_2$
D-Methionine	-H	$-CH_2CH_2SCH_3$
D-Phenylalanine	-H	$-CH_2Ph$
D-Proline	-H	-2-(pyrrolidine)
D-Serine	-H	$-CH_2OH$
D-Threonine	-H	$-CH_2CH(OH)(CH_3)$
D-Tryptophan	-H	$-CH_2$-3-(1H-indole)
D-Tyrosine	-H	$-CH_2$-(p-hydroxyphenyl)
D-Valine	-H	-isopropyl
Di-vinyl	$-CH=CH_2$	$-CH=CH_2$
Exemplary unnatural alpha-amino acids		R and R' are equal to:
α-methyl-Alanine (Aib)	$-CH_3$	$-CH_3$
α-methyl-Arginine	$-CH_3$	$-CH_2CH_2CH_2$-NHC(=NH)NH_2
α-methyl-Asparagine	$-CH_3$	$-CH_2C(=O)NH_2$
α-methyl-Aspartic acid	$-CH_3$	$-CH_2CO_2H$
α-methyl-Cysteine	$-CH_3$	$-CH_2SH$
α-methyl-Glutamic acid	$-CH_3$	$-CH_2CH_2CO_2H$
α-methyl-Glutamine	$-CH_3$	$-CH_2CH_2C(=O)NH_2$
α-methyl-Histidine	$-CH_3$	$-CH_2$-2-(1H-imidazole)
α-methyl-Isoleucine	$-CH_3$	-see-butyl
α-methyl-Leucine	$-CH_3$	-iso-butyl
α-methyl-Lysine	$-CH_3$	$-CH_2CH_2CH_2CH_2NH_2$

[0025] Further illustrative non-limitative examples of unnatural amino acids are summarized in Table 2:

Table 2

Three letter code	Amino acid
Aad	2-Aminoadipic acid
bAad	3-Aminoadipic acid
bAla	beta-Alanine, beta-Aminopropionic acid
Abu	2-Aminobutyric acid
4Abu	4-Aminobutyric acid, piperidinic acid
Acp	6-Aminocaproic acid
Ahe	2-Aminoheptanoic acid
Aib	2-Aminoisobutyric acid
bAib	3-Aminoisobutyric acid
Apm	2-Aminopimelic acid

(continued)

Three letter code	Amino acid
Dbu	2,4 Diaminobutyric acid
Des	Desmosine
Dpm	2,2'-Diaminopimelic acid
Dpr	2,3-Diaminopropionic acid
EtGly	N-Ethylglycine
EtAsn	N-Ethylasparagine
Hyl	Hydroxylysine
aHyl	allo-Hydroxylysine
3Hyp	3-Hydroxyproline
4Hyp	4-Hydroxyproline
Ide	Isodesmosine
alle	allo-Isoleucine
Nva	Norvaline
Nle	Norleucine
Orn	Ornithine

[0026] Each one of the amino acids forming the peptide of the invention can have, independently from the others, L- or D-configuration.

[0027] Amino acids used in the preparation of the peptides of the present invention may be prepared by organic synthesis, or obtained by other routes, such as, for example, degradation of or isolation from a natural source.

[0028] In one particular embodiment of the first aspect, optionally in combination with any embodiments provided above or below, X_1 is selected from the amino acids Ala, Ile, Leu, Phe, Val, Pro, and Gly. More particularly, X_1 is Ile or Val. Even more particularly, X_1 is Ile.

[0029] In another particular embodiment of the first aspect, optionally in combination with any embodiments provided above or below, R_1 is -C(O)(C_1-C_{10})alkyl. More particularly, R_1 is -C(O)(C_1-C_5)alkyl. Even more particularly, R_1 is -C(O)-CH_3.

[0030] In another particular embodiment of the first aspect, optionally in combination with any embodiments provided above or below, R_2 is -NR_3R_4. More particularly, R_3 and R_4 are the same or different and are selected from hydrogen and (C_1-C_5)alkyl.

[0031] In the present invention the term "alkyl" encompasses both lineal and branched hydrocarbon chains.

[0032] Illustrative non-limitative examples of "alkyl" are: methyl (C1), ethyl (C2), propyl (C3), isopropyl (C3), isobutyl (C4), sec-butyl (C4), tert-butyl (C4), pentyl (C5), hexyl, (C6), heptyl (C7), octyl (C9), nonyl (C9), and decyl (C10), among others.

[0033] Yet in another particular embodiment, optionally in combination with any embodiments above or below, one of "a" to "j" is 1 and the others are 0. In particular "a" is 1 and "b", "c", "d", "e", "f", "g", "h", "i" and "j" are 0.

[0034] Another particular embodiment of the first aspect comprises a peptide selected from the group consisting of sequences SEQ ID NO: 1 to SEQ ID NO: 3, which are summarized in Table 3.

Table 3

Peptide	SEQ ID	Sequence
P1A	SEQ ID NO: 1	CFEISKY-NH_2
P1B	SEQ ID NO: 2	CH_3-C(O)-CFEVSKY-NH_2
P1C	SEQ ID NO: 3	CH_3-C(O)-CFEISKY-NH_2

[0035] The peptides of the present invention can be prepared following routine protocols such as by solid phase

synthesis, wherein successive steps of (a) deprotecting the amino acid to be bound, and (b) protected-amino acid coupling cycles are performed.

[0036] The protecting group can be an N-protecting group, C-protecting group or a side-chain protecting group. There are commercially available protecting groups belonging to all three categories.

[0037] Illustrative non-limitative examples of amino acid protecting groups are the N-protecting groups t-Boc (or Boc) and Fmoc. When t-Boc or Fmoc is used in the synthesis of a peptide, the main four steps are: (a) protecting group is removed from the trailing amino acids (commercially available) in a deprotection reaction; (b) deprotection reagents are washed away to provide a clean coupling environment, (c) protected amino acids dissolved in a solvent such as dimethylformamide (DMF) combined with coupling reagents are pumped through the synthesis column, and (d) coupling reagents are washed away to provide clean deprotection environment. Depending on the particular N-protecting group, the deprotection reagent and the coupling reagent is one or another. The skilled person in the art, based on his general knowledge, and by routine methods, can optimize the particular conditions, if necessary.

[0038] Alternatively, the peptides of the invention can be obtained by means of recombinant DNA technology.

[0039] A second aspect of the invention relates to a pharmaceutical composition comprising a therapeutically effective amount of the peptide of FORMULA (I), a pharmaceutically acceptable salt thereof with at least one pharmaceutically acceptable excipient, diluent or carrier.

[0040] The expression "pharmaceutical composition" encompasses both compositions intended for human as well as for non-human animals.

[0041] The expression "therapeutically effective amount" as used herein, refers to the amount of the peptide when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the disease which is addressed. The particular dose of compound administered according to this invention will of course be determined by the particular circumstances surrounding the case, including the compound administered, the route of administration, the particular condition being treated, and the similar considerations.

[0042] The expression "pharmaceutically acceptable excipients or carriers" refers to pharmaceutically acceptable materials, compositions or vehicles. Each component must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the pharmaceutical composition. It must also be suitable for use in contact with the tissue or organ of humans and non-human animals without excessive toxicity, irritation, allergic response, immunogenicity or other problems or complications commensurate with a reasonable benefit/risk ratio.

[0043] Examples of suitable pharmaceutically acceptable excipients are solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

[0044] The relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered.

[0045] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Excipients such as colouring agents, coating agents, sweetening, and flavouring agents can be present in the composition, according to the judgment of the formulator.

[0046] The pharmaceutical compositions containing the peptide or the conjugate of the invention can be presented in any dosage form, for example, solid or liquid, and can be administered by any suitable route, for example, oral, parenteral, rectal, topical, intranasal or sublingual route, for which they will include the pharmaceutically acceptable excipients necessary for the formulation of the desired dosage form, for example, topical formulations (ointment, creams, lipogel, hydrogel, etc.), eye drops, aerosol sprays, injectable solutions, osmotic pumps, etc.

[0047] Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, corn-starch, powdered sugar, and combinations thereof.

[0048] Exemplary granulating and/or dispersing agents include, but are not limited to, potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked polyvinylpyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, and combinations thereof.

[0049] Exemplary binding agents include, but are not limited to, starch (e.g., corn-starch and starch paste); gelatin; sugars (e.g., sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol); natural and synthetic gums (e.g., acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, polyvinylpyrrolidone), magnesium aluminium silicate (Veegum), and larch arabogalactan); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; and combinations thereof.

[0050] Exemplary preservatives may include antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, ascorbyl palmitate, ascorbyl stearate, ascorbyl oleate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and sodium sulfite. Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and trisodium edetate.

[0051] Exemplary buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and combinations thereof.

[0052] Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behanate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, and combinations thereof.

[0053] As described above, another aspect of the invention relates to a peptide or a pharmaceutical composition of the invention for use as a medicament.

[0054] In a further aspect the present invention provides the peptide or the pharmaceutical composition of the invention is used in the treatment or prevention of a neoplastic disease, more particularly, in the treatment or prevention of pancreatic cancer. This aspect can also be formulated as the use of the peptide or the pharmaceutical composition of the invention for the manufacture of a medicament for the treatment or prevention of a neoplastic disease.

[0055] Illustrative non-limiting examples of neoplastic diseases which can be treated with the peptide and pharmaceutical composition of the invention include, although they are not limited to, papillomas, adenomas, lipomas, osteomas, myomas, angiomas, nevi, mature teratomas, carcinomas, sarcomas.immature teratomas, melanoma, myeloma, leukemia, Hodgkin's lymphoma, basalioma, spinalioma, breast cancer, ovarian cancer, uterine cancer, lung cancer, bronchial cancer, prostate cancer, colon cancer, pancreatic cancer, kidney cancer, esophageal cancer, hepatocarcinoma, head and neck cancer, etc. In a particular embodiment of the fifth aspect, the neoplastic disease is pancreatic cancer. From the data herein provided the peptide and pharmaceutical composition of the invention may also be useful in the treatment of other diseases such as metabolic, neurologic and inflammatory diseases.

[0056] Throughout the description and claims the word "comprise" and variations of the word, are not intended to exclude other technical features, additives, components, or steps.

[0057] Furthermore, the word "comprise" encompasses the case of "consisting of". Additional objects, advantages and features of the invention will become apparent to those skilled in the art upon examination of the description or may be learned by practice of the invention.

[0058] The following examples and drawings are provided by way of illustration, and they are not intended to be limiting of the present invention. Furthermore, the present invention covers all possible combinations of particular and preferred embodiments described herein.

Examples

Example 1: Synthesis of the peptides of the invention.

[0059] Peptides were synthesized by chemical synthesis using solid phase synthesis (SPPS) technology following a classical Fmoc/tBu strategy, either by an automatic peptide synthesizer or manually performed.

[0060] The Rink-amide resin was used for the synthesis and for the Fmoc-amino acid couplings N,N'-diisopropylcarbodiimide (DIPCDI) with 1-hydroxybenzotriazole (HOBt) as an additive in dimethylformamide (DMF). Deprotection was carried out with a solution of 20% piperidine in DMF.

[0061] In the case of the acetylated sequences, the acetylation was carried out in solid phase using a solution of acetic anhydride (Ac_2O) with diisopropylethylamine (DIEA) in DMF just before drying the resin. The dried peptidyl resins were treated with a trifluoroacetic acid (TFA) cocktail to cleave the peptide from the resin. The peptide crudes obtained were purified by preparative reverse phase HPLC using a H_2O/acetonitrile(can) purification system with TFA and the pure fractions were lyophilized. The results of the HPLC analysis are summarized in Table 4.

Table 4

Peptide	Identification MS spectrum	Peptide purity
P1	827.6 u.m.a.	90.54%
P1A	887.4 u.m.a.	97.54 %
P1B	915.5 u.m.a.	97.45 %
P1C	930.7 u.m.a.	97.00 %
P2	678.4 u.m.a.	94.14 %
P2C	781.5 u.m.a.	99.12%

Example 2: Cell culture and treatment of cells with the peptide of the invention

Cell cultures

[0062] The BXPC3 cell line from primary adenocarcinoma of human pancreas was provided by the Biomedical Research Institute (IRB) of Barcelona, and human umbilical cord primary endothelial cells (HUVEC) were obtained directly by the investigator and stored in liquid nitrogen in the laboratory. BXPC3 cells were maintained in RPMI-1640 culture medium (Gibco) supplemented with 10% fetal bovine serum and antibiotics. HUVEC cells were maintained in M199 culture medium (Gibco) supplemented with 20% fetal bovine serum, endothelial cell growth supplement (ECGS), Heparin (Hep) and antibiotics. Cultures were maintained in the cell incubator in a humid atmosphere at 37°C containing 5% CO_2.

[0063] The different peptides to be tested were easily dissolved in DMSO at a concentration of 50 mM and subsequently a 5 mM intermediate dilution was prepared in Dulbecco's PBS which was subjected to two short cycles of sonication, which retendered the peptides completely soluble. From this latter dilution, the different treatments at 10, 20, 30, and 40 uM concentrations were prepared in supplemented medium. The different treatments were prepared at a double concentration and 100 μl of them were added to the same volume of cell growth medium in the wells to reach the final concentrations above disclosed.

Cell treatments

[0064] Assays with the different peptides were performed following the protocol explained below. The cells were resuspended by trypsin/EDTA digestion with Trypsin 0,25%-EDTA in the case of BXPC3 cells, and Trypsin 0,25%-EDTA in the case of HUVEC cells. Once resuspended in culture medium, they were counted in Newbauer's chamber after a 1:1 dilution with trypan blue. This staining allows the number of living cells in the suspension to be known. From the counting, a suitable dilution of the cells (5000 cells/100 μl / well for BXPC3 and 10000 cells / 100 μl / well for HUVEC) was prepared. Cells were left 24 hours in culture within the cell incubator. After 24 hours of incubation, 100 μl / well of a double concentrated solution of the peptides prepared as explained above were added. Treatments were maintained for 72 hours by keeping the cells in the cell incubator. The sequences of the peptides used in the assay are shown in Table 5.

Table 5

Peptides of the invention	Sequence	SEQ ID NO:
P1A	CFEISKY-NH2	SEQ ID NO: 1
P1B	CH_3-C(O)-CFEVSKY-NH2	SEQ ID NO: 2
P1C	CH_3-C(O)-CFEISKY-NH2	SEQ ID NO: 3
P1	CH_3-C(O)-FEISKY-NH2	SEQ ID NO: 4
P2	CH_3-C(O)-VFSTAL-NH2	SEQ ID NO: 5

(continued)

Peptides of the invention	Sequence	SEQ ID NO:
P2C	CH_3-C(O)-CVFSTAL-NH2	SEQ ID NO: 6

[0065] After 72 hours, the culture media was removed by decantation, cells were washed twice with DPBS and then cells were fixed with 100 μl of 4% paraformaldehyde solution for 30 min. Two washes were then performed with 100 μl of mQ H_2O and immediately 50 μl of 0.25% Violet Crystal solution, prepared in distilled water, were added and maintained for 30 min at room temperature (RT). At the end of the staining time, several washes with distilled water were performed to completely remove the excess of Violet Crystal, the plates were then completely dried in the oven at 37°C.

[0066] The optical density values per well were obtained by a Biotek Synergy™2 multi-detection microplate reader, using a 590 nm filter and by scanning reading, obtaining the mean values per well.

RESULTS

In vitro proliferation assay to test the cancer growth-inhibitory activity of the peptides

[0067] In order to test the anticancer effect of the peptides of the invention, proliferation assays were conducted onto the human pancreatic tumor cell line BXPC3.

[0068] As can be observed in Fig. 1-A, when BXPC3 cells were treated with peptides at 20 μM final concentration, the P1C peptide (SEQ ID NO: 3) reduced cancer cell growth down to 40% in relation to the growth of mock treated cells, which was accorded a 100% value. A remarkable inhibitory capacity of the peptide was still maintained when either the carboxyterminal acetylation of the P1C peptide was lost-peptide P1A (SEQ ID NO: 1)-or the isoleucine residue was substituted by a valine residue-peptide P1B (SEQ ID NO: 2). When the peptides where applied at 40 μM final concentration (Fig. 1-B) their growth inhibitory effects were even higher.

[0069] No inhibition of cancer cell growth was observed for any of the other peptides tested.

In vitro proliferation assay to test the toxicity of the peptides of the invention

[0070] The administration of the peptide P1C-which presents the highest growth-inhibitory activity in cancer cells (see Assay 1)-did not affect the growth of normal non-transformed cells. The lack of toxicity was maintained independently of the dose of the peptide applied (ranging from 10 μM up to 40 μM final concentration, columns two to five of Fig. 2).

[0071] These results unambiguously demonstrate the high therapeutic potential of the peptides of the invention as anti-cancer agents given their low toxicity in non-transformed cells and their high growth-inhibitory activity in cancer cells.

Citation List

[0072]

WO 2014/079943 Copolovici D. M. et al., "Cell-Penetrating Peptides: Design, Synthesis, and Applications", ACS Nano, 2014, 8(3): 1972-1994;
Ford K.G. et al., "Protein transduction: an alternative to genetic intervention?" Gene Therapy, 2001; 8:1-4; and
Tandrup Schmidt S. et al., "Liposome-Based Adjuvants for Subunit Vaccines: Formulation Strategies for Subunit Antigens and Immunostimulators", Pharmaceutics, Mar. 2016 ; 8(1).

Claims

1. A peptide of formula (I), or a pharmaceutically acceptable salt thereof,

$$C_aC_bC_cC_dC_eFEX_1SKYC_fC_gC_hC_iC_j \qquad (I)$$

wherein:

the N-terminal group of the peptide is a monoradical of formula -NHR_1,
the C-terminal group of the peptide is a monoradical of formula -C(O)-R_2;
R_1 is a monoradical selected from hydrogen and -C(O)-(C_1-C_{20})alkyl;

R_2 is a monoradical selected from -OH and $-NR_3R_4$ radical;
R_3 and R_4 are independently selected from hydrogen and (C_1-C_{10})alkyl;
"a" to "j" are integers from 0 to 1, provided that at least one of "a" to "j" is 1; and
X_1 represents any amino acid.

2. The peptide according to claim 1 wherein X_1 is selected from Ala, Ile, Leu, Phe, Val, Pro, and Gly.

3. The peptide according to any of the claims 1-2, wherein X_1 is Ile or Val.

4. The peptide according to any of the claims 1-3, wherein R_1 is $-C(O)(C_1-C_{10})$alkyl.

5. The peptide according to any of the claims 1-4, wherein R_1 is $-C(O)-CH_3$.

6. The peptide according to any of the claims 1-5, wherein R_2 is $-NR_3R_4$.

7. The peptide according to any of the previous claims, wherein R_3 and R_4 are hydrogen.

8. The peptide according to any of the previous claims, wherein one of "a" to "j" is 1 and the others are 0.

9. The peptide according to any of the previous claims, wherein "a" is 1 and "b", "c", "d", "e", "f", "g", "h", "i" and "j" are 0.

10. The peptide according to any of the previous claims, which is selected from the group consisting of sequences SEQ ID NO: 1 to SEQ ID NO: 3

11. A pharmaceutical composition comprising a therapeutically effective amount of the peptide of formula (I) or a pharmaceutically acceptable salt thereof as defined in any of the claims 1-10, with at least one pharmaceutically acceptable excipient, diluent or carrier.

12. A peptide as defined in any of the claims 1-10, or a pharmaceutical composition as defined in claim 11, for use as a medicament.

13. A peptide as defined in any of the claims 1-10, or the pharmaceutical composition as defined in claim 11, for use in the treatment or prevention of a neoplastic disease.

14. The peptide, or the pharmaceutical composition for use according to claim 13, wherein the neoplastic disease is pancreatic cancer.

Patentansprüche

1. Ein Peptid der Formel (I), oder ein pharmazeutisch akzeptables Salz davon,

$$C_aC_bC_cC_dC_eFEX_1SKYC_fC_gC_hC_iC_j \qquad (I)$$

wobei:

die N-terminale Gruppe des Peptids ein Monoradikal der Formel $-NHR_1$ ist,
die C-terminale Gruppe des Peptids ein Monoradikal der Formel $-C(O)-R_2$ ist;
R_1 ein Monoradikal ausgewählt aus Wasserstoff und $-C(O)-(C_1-C_{20})$-Alkyl ist;
R_2 ein Monoradikal ausgewählt aus -OH und $-NR_3R_4$ ist;
R_3 und R_4 unabhängig voneinander aus Wasserstoff und (C_1-C_{10})-Alkyl ausgewählt sind;
"a" bis "j" ganze Zahlen von 0 bis 1 sind, unter der Voraussetzung, dass mindestens eines von "a" bis "j" 1 ist; und
X_1 für eine beliebige Aminosäure steht.

2. Das Peptid nach Anspruch 1, wobei X_1 ausgewählt aus Ala, Ile, Leu, Phe, Val, Pro und Gly ist.

3. Das Peptid nach einem der Ansprüche 1 bis 2, wobei X_1 Ile oder Val ist.

4. Das Peptid nach einem der Ansprüche 1 bis 3, wobei R_1 -C(O)(C_1-C_{10})-Alkyl ist.

5. Das Peptid nach einem der Ansprüche 1 bis 4, wobei R_1 -C(O)-CH_3 ist.

6. Das Peptid nach einem der Ansprüche 1 bis 5, wobei R_2 -NR_3R_4 ist.

7. Das Peptid nach einem der vorhergehenden Ansprüche, wobei R_3 und R_4 Wasserstoff sind.

8. Das Peptid nach einem der vorhergehenden Ansprüche, wobei eines von "a" bis "j" 1 ist und die anderen 0 sind.

9. Das Peptid nach einem der vorhergehenden Ansprüche, wobei "a" 1 ist und "b", "c", "d", "e", "f", "g", "h", "i" und "j" 0 sind.

10. Das Peptid nach einem der vorhergehenden Ansprüche, das ausgewählt ist aus der Gruppe bestehend aus den Sequenzen SEQ 1D NO: 1 bis SEQ 1D NO: 3.

11. Eine pharmazeutische Zusammensetzung umfassend eine therapeutisch wirksame Menge des Peptids der Formel (I) oder eines pharmazeutisch akzeptablen Salzes davon, wie in einem der Ansprüche 1 bis 10 definiert, mit mindestens einem pharmazeutisch akzeptablen Hilfsstoff, Verdünnungsmittel oder Trägersubstanz.

12. Ein Peptid wie in einem der Ansprüche 1 bis 10 definiert, oder eine pharmazeutische Zusammensetzung wie in Anspruch 11 definiert, zur Verwendung als Medikament.

13. Ein Peptid wie in einem der Ansprüche 1 bis 10 definiert, oder die pharmazeutische Zusammensetzung wie in Anspruch 11 definiert, zur Verwendung bei der Behandlung oder Prävention einer neoplastischen Erkrankung.

14. Das Peptid, oder die pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 13, wobei die neoplastische Erkrankung Bauchspeicheldrüsenkrebs ist.

Revendications

1. Un peptide de formule (I), ou un sel pharmaceutiquement acceptable de celui-ci,

$$C_aC_bC_cC_dC_eFEX_1SKYC_fC_gC_hC_iC_j \qquad (I)$$

où :

le groupe N-terminal du peptide est un monoradical de formule -NHR_1,
le groupe C-terminal du peptide est un monoradical de formule -C(O)-R_2 ;
R_1 est un monoradical choisi parmi l'hydrogène et -C(O)-alkyle en C_1 à C_{20} ;
R_2 est un monoradical choisi parmi -OH et un radical -NR_3R_4 ;
R_3 et R_4 sont indépendamment choisis parmi l'hydrogène et alkyle en C_1 à C_{10} ;
"a" à "j" sont des nombres entiers de 0 à 1, à condition qu'au moins l'un de "a" à "j" soit 1 ; et
X_1 représente un acide aminé quelconque.

2. Le peptide selon la revendication 1, dans lequel X_1 est choisi parmi Ala, Ile, Leu, Phe, Val, Pro et Gly.

3. Le peptide selon l'une quelconque des revendications 1 à 2, dans lequel X_1 est Ile ou Val.

4. Le peptide selon l'une quelconque des revendications 1 à 3, dans lequel R_1 est - C(O)alkyle en C_1 à C_{10}.

5. Le peptide selon l'une quelconque des revendications 1 à 4, dans lequel R_1 est - C(O)-CH_3.

6. Le peptide selon l'une quelconque des revendications 1 à 5, dans lequel R_2 est - NR_3R_4.

7. Le peptide selon l'une quelconque des revendications précédentes, dans lequel R_3 et R_4 sont l'hydrogène.

8. Le peptide selon l'une quelconque des revendications précédentes, dans lequel l'un de "a" à "j" vaut 1 et les autres valent 0.

9. Le peptide selon l'une quelconque des revendications précédentes, dans lequel "a" vaut 1 et "b", "c", "d", "e", "f", "g", "h", "i" et "j" valent 0.

10. Le peptide selon l'une quelconque des revendications précédentes, qui est choisi dans le groupe constitué par les séquences SEQ 1D NO : 1 à SEQ 1D NO : 3.

11. Une composition pharmaceutique comprenant une quantité thérapeutiquement efficace du peptide de formule (I) ou d'un sel pharmaceutiquement acceptable de celui-ci tel que défini dans l'une quelconque des revendications 1 à 10, avec au moins un excipient, diluant ou véhicule pharmaceutiquement acceptable.

12. Un peptide tel que défini dans l'une quelconque des revendications 1 à 10, ou une composition pharmaceutique telle que définie dans la revendication 11, pour une utilisation en tant que médicament.

13. Un peptide tel que défini dans l'une quelconque des revendications 1 à 10, ou la composition pharmaceutique telle que définie dans la revendication 11, destiné(e) à être utilisé(e) dans le traitement ou la prévention d'une maladie néoplasique.

14. Le peptide, ou la composition pharmaceutique pour l'utilisation selon la revendication 13, dans lequel la maladie néoplasique est le cancer du pancréas.

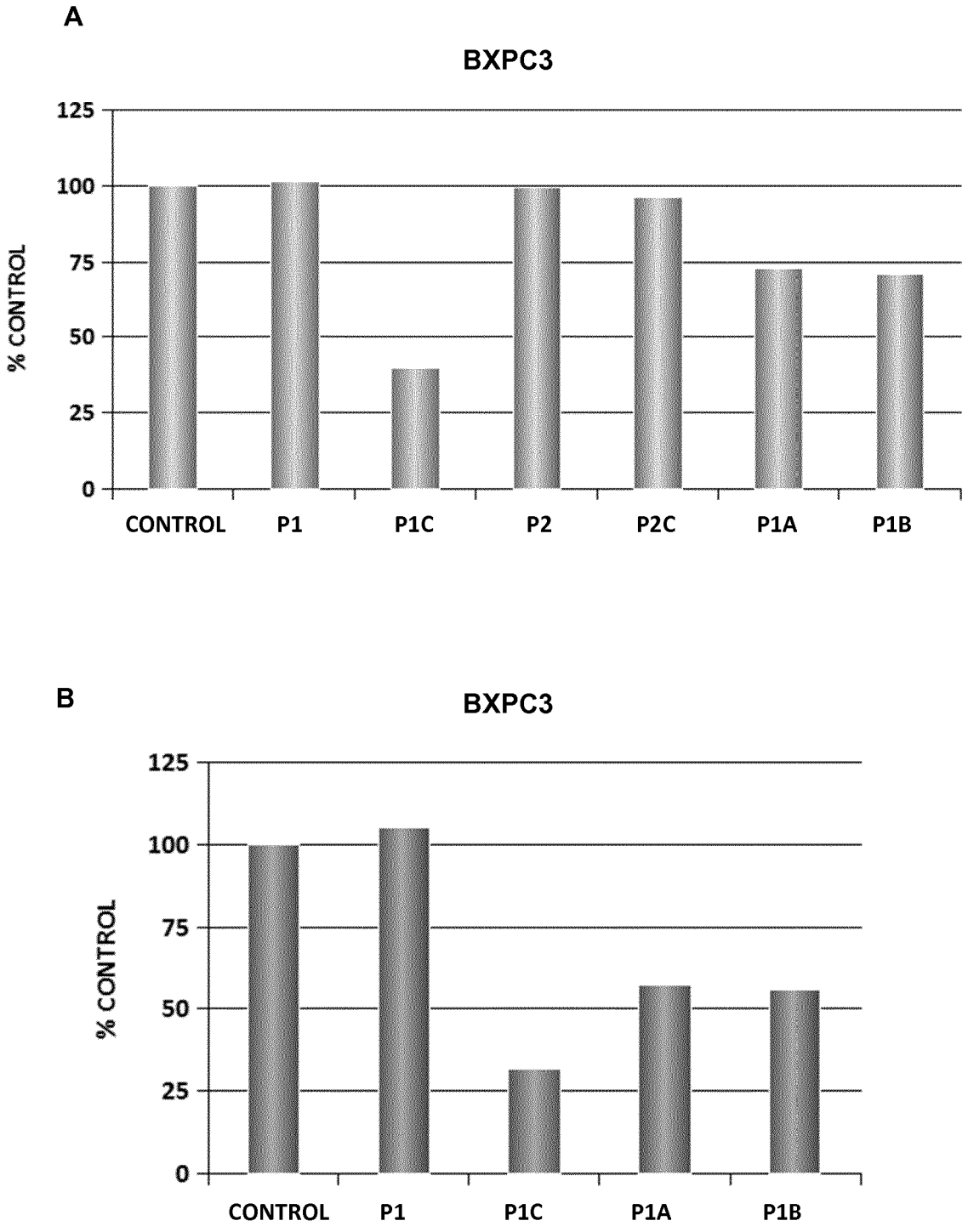

A
BXPC3
% CONTROL
125
100
75
50
25
0
CONTROL
P1
P1C
P2
P2C
P1A
P1B
B
BXPC3
% CONTROL
125
100
75
50
25
0
CONTROL
P1
P1C
P1A
P1B

Fig. 1

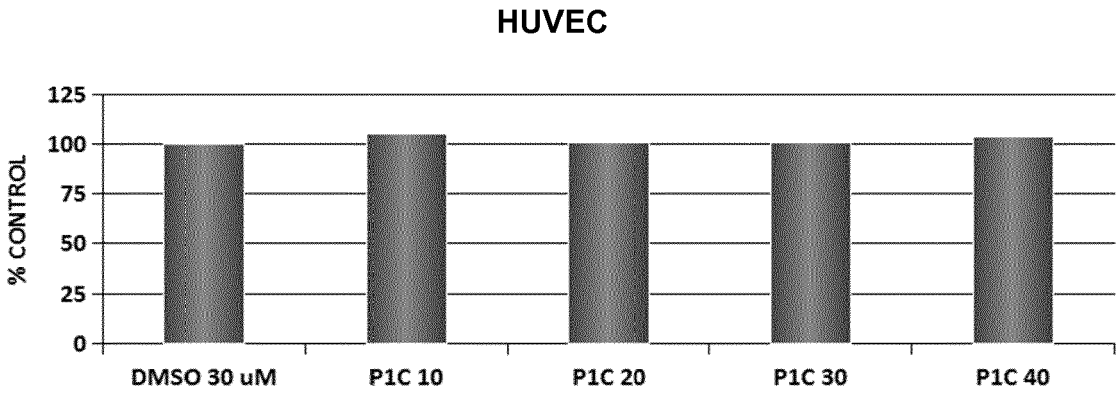

HUVEC
% CONTROL
125
100
75
50
25
0
DMSO 30 uM
P1C 10
P1C 20
P1C 30
P1C 40

Fig. 2

REFERENCES CITED IN THE DESCRIPTION

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- **TANDRUP SCHMIDT S. et al.** Liposome-Based Adjuvants for Subunit Vaccines: Formulation Strategies for Subunit Antigens and Immunostimulators. *Pharmaceutics,* March 2016, vol. 8 (1 **[0072]**