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(54) **USE OF PEPTIDE DERIVATIVES FOR INDUCING CELL DIFFERENTIATION**

VERWENDUNG VON PEPTIDDERIVATEN ZUM INDUZIEREN DER ZELLDIFFERENZIERUNG

UTILISATION DE DERIVES PEPTIDIQUES POUR L'INDUCTION DE LA DIFFERENTIATION
CELLULAIRE

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GB-A- 2 143 732   RU-A- 96 110 217
RU-C1- 2 141 483**

• **KULESZ-MARTIN M.F. ET AL.: 'Differentiation
and tumor response to retinobenzoic acid re-80
in a malignant conversion model' CANCER
DETECT PREV. vol. 19, no. 4, 1995, pages 355 -
366, XP002995423**
• **DATABASE BIOSIS [Online] BIOSCIENCES
INFORMATION SERVICE, PHILADELPHIA, PA,
US Database accession no. prev19850034327**

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- DATABASE WPI Section Ch, Week 198912, Derwent Publications Ltd., London, GB; Class B03, AN 1989-090814 NAGAI KANESHIRO ET AL: 'Side-effect remover for chemotherapeutic agent' & JP 1 042 430 A (UNIV NIPPON) 14 February 1989

- DATABASE WPI Section Ch, Week 200011, Derwent Publications Ltd., London, GB; Class B02, AN 1997-319428 KAUROV O.A. ET AL: 'Immunostimulating agent and a preparation based on thereof' & RU 2 120 298 A (TSITOKIN CO LTD, VERTA CO LTD, WEI E T) 20 October 1998

Description

[0001] The invention relates to medicine and in particular, to treating cancer diseases and it is useful in treating tumors of different genesis as specified in the claims.

[0002] The invention relates to the induction of cells differentiation using peptides derivatives as an agent inducing cells differentiation and in particular, to their use in antitumor noncytotoxic therapy as specified in the claims.

[0003] Absence of the ability to differentiate in most tumor cells is known to result in an uncontrollable tumor growth.

[0004] Search for agents of both specific and non-specific induction of cells differentiation is therefore one of novel approaches to anti-tumor noncytotoxic therapy.

[0005] Under "induction of cells differentiation" is averaget the capability of the different substances to restore (or to drive) the following functions lost or lowered because of the various reasons: passing a normal cellular cycle by a cell, synthesis of biologically active vitally important substances therein etc.

[0006] Substances or compounds the action mechanism of which is not associated with one particular cell function and those can cause its differentiation by several parameters, can be attributed to non-specific differentiation inductors.

[0007] Methods for inducing tumor cells differentiation by administering retinoids or α-2-interferon are known [Cancer Res.,40, 2245-3350, 1980].

[0008] The cells differentiation inductor polytransretinoic acid (PTRA) is used as an agent to prolong remission following induction or post-remission therapy of acute promyelocytic leukemia. Cells differentiation as affected by retinoic acid derivatives leads to stabilization of tumor cells growth [Abelev G.I. Differentiation and tumor phenotype in cells of leukoses and lymphomas/ In: The Clinical Oncohematology (edited by M.A. Volkova). Moscow, the Meditsina publishers, 2001, Chapter 11, pages 116-123].

[0009] The use of α-interferon preparations as immunotherapy agents in treating melanoma is also associated with induction of tumor cells differentiation in which adhesion capability is enhanced and antigenic profile changes. Therapy with interferon results in reduced progression of tumor growth as well as prevents the development and rate of metastasis [Atzpodien J., Kirchner H. Cancer, Cytokines, and cytotoxic cells: interleukin-2 in the immunotherapy of human neoplasms. Klin. Wochenschr, 1990, v.68, pp.1-7].

[0010] Preparations that cause hemopoietic cells differentiation damaged owing to cytotoxic chemotherapy have been introduced into clinical practice recently. These preparations are different cytokines prepared from bone marrow such as hematohormones: granulocytic colony-stimulating factor, granulocytic-macrophagal colony-stimulating factor and others. Their use in treating different human tumors results in accelerated maturation of the bone marrow cells and prevents hematologic cytotoxic effect of the chemotherapy preparations [Crawford j., Ozer H., Stoller R. et al. Phase II of clinical investigation of GM-CSF by the patients of SCLC with the dose-intensive chemotherapy. The New England Journal of Medicine. 1991, v.325, No.3, pp.164-170].

[0011] Thus, induction of tumor cells differentiation is one of the leading mechanisms of neoplasm growth stabilization, increased immunotherapy effect and correcting hematologic toxicity of the chemotherapy preparations.

[0012] DE-A-3424781 relates to the use of L-carnosine, and salts thereof, in the treatment of tumours. JP-A-1042430 relates to L-camosine in the context of alleviating side effects of chemotherapeutic agents.

[0013] GB-A-2143732 discloses a combination of homocarnosine with OK-432, which is said to be an immunopotentiator.

[0014] The present inventors have discovered that the peptide derivatives of general formula (I):

$$R_1 \text{---}(CH_2)_n \text{---} CONH \text{---} \underset{\underset{R_2}{|}}{CH} \text{---}(CH_2)_m \text{---} R_3 \qquad (I)$$
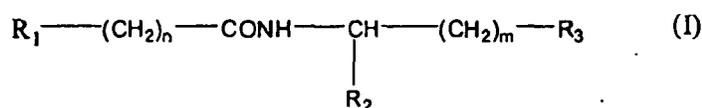
are potent inductors of cell differentiation and are useful as agents for non-cytotoxic therapy of cancer diseases in particular melanoma and hemoblastomas as well as hematocorregating agents.

[0015] The compounds of formula (I)

$$R_1 \text{---}(CH_2)_n \text{---} CONH \text{---} \underset{\underset{R_2}{|}}{CH} \text{---}(CH_2)_m \text{---} R_3 \qquad (I)$$
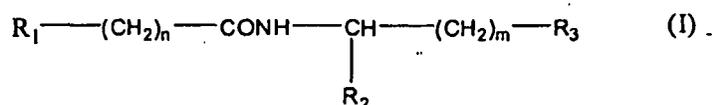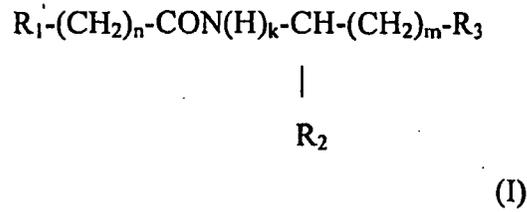
are disclosed in the International application PCT/RU98/00215 as possessing antioxidant, antiasthmatic, antihypoxic,

anti-inflammatory, antiviral, antibacterial, lipidregulating, anti-metastatic as well as other kinds of therapeutic effect. The compounds of cyclic aspartyl-histamine and acetyl-aspartyl-histamine structure are disclosed in the work Kvamme, E.; Reichelt, K.L.; Edminson, P.D.; et al. N-substituted peptides in brain. Fed. Eur. Biochem. Society Meet., {Proc.], 1975, 41, 127-136.

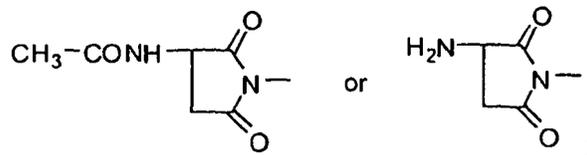**[0016]** The present invention provides a peptide derivative of general formula (I)

$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3$$
$$|$$
$$R_2$$

(I)

wherein:

- n=0-4, m=1-4, k=0-1;
- R_1 is a C_1-C_3 hydrocarbon radical substituted by a functional group selected from amino, C_1-C_5 amido- or carboxylic groups, the carboxylic group being optionally esterified; or
- R_1 is a C_1-C_3 hydrocarbon radical simultaneously substituted by (a) an amino group which is optionally substituted by an acyl substituent and (b) a carboxylic group; or
- R_1 is a C_1-C_3 hydrocarbon radical substituted by a 5-6-membered unsaturated heterocyclic group, wherein the hydrocarbon radical can simultaneously comprise an amino group optionally substituted by an acyl substituent; or
- R_1 is a saturated heterocyclic group;
- R_2 is hydrogen atom or a functional group selected from carboxyl, that can be esterified;
- R_3 is 5-6-membered saturated or unsaturated cyclic or heterocyclic group, or an amino- or carboxyl group, the carboxyl group being optionally esterified;
  or a pharmaceutically acceptable salt thereof, for use in the treatment of hemoblastosis or melanoma.
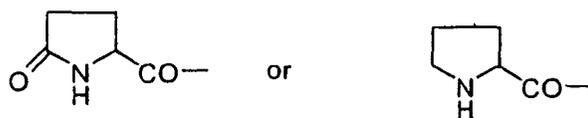 In a preferred embodiment, the present invention relates to a peptide derivative of general formula
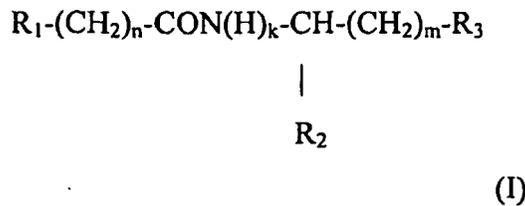
$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3$$
$$|$$
$$R_2$$

(I)

wherein:
- n is 0, k is 0 and R_1-CON- is
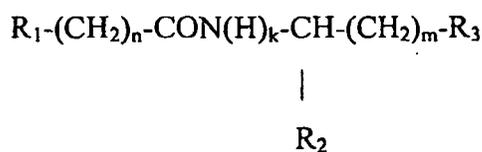

or
- n is 0, k is 1 and R_1-CO- is

or

- R$_2$ is H, COOH or COOCH$_3$;
- R$_3$ is
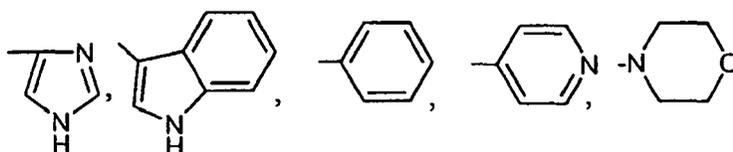
NH$_2$, -COOH, or -COOCH$_3$; and
- m is 1-4,
  or a pharmaceutically acceptable salt thereof, for use in the treatment of hemoblastosis or melanoma.

[0017]   Also provided is a peptide derivative of general formula (I)

$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3$$

$$R_2$$

wherein R$_1$, n, k, R$_2$, m and R$_3$ are as defined above, or a pharmaceutically acceptable salt thereof, for use in reducing neutropenia caused by myelosuppresive therapy.

[0018]   Preferred compounds of general formula (I) used in the present invention are compounds of general formula (I) shown below:

Number of compound	R$_1$	n	k	R$_2$	m	R$_3$
1	NH$_2$-CH-COOH	2	1	H	1	(-4-Im)
2	HOOC-CH$_2$-	2	1	H	1	-4-Im
3	HOOC-CH$_2$-	1	1	H	1	-4-Im
4	NH$_2$-CH-COOH	2	1	-COOH	1	-4-Im
5	NH$_2$-CH-CH$_2$-CH$_2$-COOH	0	1	H	1	-4-Im
6	HOOC-CH$_2$-	2	1	-COOCH$_3$	1	-4-Im
7	HOOC-CH$_2$-	1	1	-COOH	1	-4-Im
8	NH$_2$-CH$_2$-	2	1	-COOH	1	-4-Im
9	NH$_2$-CH$_2$-	2	1	-COOCH$_3$	1	-4-Im
10	NH$_2$-CH$_2$-	1	1	-COOH	1	-4-Im

(continued)

Number of compound	R_1	n	k	R_2	m	R_3
11	$NH_2\text{-}CH_2\text{-}$	1	1	H	1	-4-Im
12	$NH_2\text{-}CH_2\text{-}$	2	1	H	1	-4-Im
13	$HOOC\text{-}CH_2\text{-}$	2	1	-COOH	1	-4-Im
14	$CH_3\text{-}CONH\text{-}CH\text{-} / CH_2\text{-}COOH$	0	1	H	1	-4-Im
15	$R_1CON\text{-} = CH_3CONH\text{-}CH\text{-}C(=O)\text{-}N\text{-}/CH_2\text{-}C(=O)$	0	0	H	1	-4-Im
16	$R_1CON\text{-} = NH_2\text{-}CH\text{-}C(=O)\text{-}N\text{-}/CH_2\text{-}C(=O)$	0	0	HH	11	-4-Im
17	$CH_3CONH\text{-}CH_2\text{-}$	2	1	H	1	-4-Im
18	$NH_2\text{-}CH\text{-} / COOH$	1	1	H	1	-4-Im
19	$CH_3OCO\text{-}CH_2\text{-}$	2	1	H	1	-4-Im
20	$NH_2\text{-}CH\text{-} / COOH$	2	1	COOH	1	(-3-Ind)
21	$NH_2\text{-}CH\text{-} / CH_2\text{-}CH_2\text{-}COOH$	0	1	H	1	-3-Ind
22	$HOOC\text{-}CH_2\text{-}$	2	1	-COOH	1	-3-Ind
23	$NH_2\text{-}CH_2\text{-}$	2	1	-COOH	1	-3-Ind
24	$R_1CO\text{-} =$ (pyrrolidinone ring, CO-)	0	1	H	1	-3-Ind
25	$COOH\text{-}CH_2\text{-}$	2	1	H	1	-3-Ind
26	$NH_2\text{-}CH\text{-} / COOH$	2	1	H	1	-3-Ind
27	$NH_2\text{-}CH_2\text{-}$	2	1	H	1	$-C_6H_5$
28	$NH_2\text{-}CH_2\text{-}$	2	1	H	1	(-Py)
29	$NH_2\text{-}CH\text{-} / COOH$	2	1	H	1	-Py
30	$HOOC\text{-}CH_2\text{-}$	2	1	H	1	-Py

(continued)

Number of compound	R_1	n	k	R_2	m	R_3
31	$HOOC-CH_2-$	2	1	-COOH	4	$-NH_2$
32	NH_2-CH- $\quad CH_2-4Im$	0	1	H	1	-COOH
33	$R_1CO- =$	0	1	H	1	-4-Im
34	$HOOC-CH_2-$	2	1	H	1	
35	NH_2-CH- $\quad CH_2-4Im$	0	1	H	1	$-COOCH_3$
36	$CH_3CO-NH-CH-$ $\qquad COOH$	2	1	H	1	-4-Im
37	$CH_3CONH-CH_2-$	1	1	-COOH	1	-4-Im
38	NH_2-CH_2-	4	1	H	1	-4-Im

[0019]   Also provided are compounds 1 to 9, and 11 to 38 above, and pharmaceutically acceptable salts thereof, for use in reducing neutropenia caused by myelosuppressive chemotherapy.

[0020]   The present invention also provides compounds 1 to 7, 9 and 11 to 38 above, and pharmaceutically acceptable salts thereof, for use in the treatment of hemablastosis or melanoma, in combination with interferon, for enhancing the efficacy of the immunotherapy.

[0021]   The most preferred compound used in the present invention is the compound of the formula
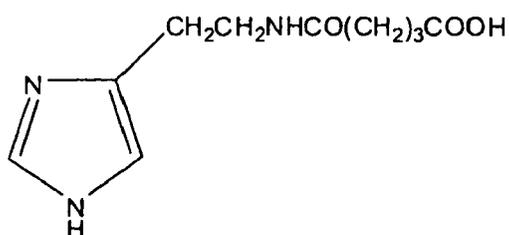
(Dicarbamine®)

[0022]   In a further embodiment, the present invention provides a peptide derivative of formula

$$CH_2CH_2NHCO(CH_2)_3COOH$$
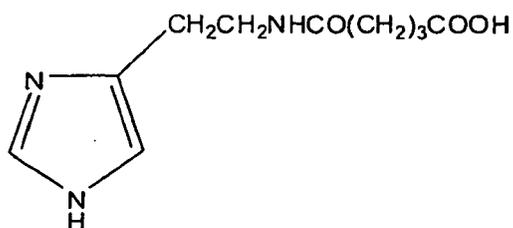
(Dicarbamine®)

or a pharmaceutically acceptable salt thereof, for use in reducing neutropenia and thrombocytopenia caused by mye-losuppressive therapy. Also provided is said peptide derivative and pharmaceutically acceptable salt thereof, for use in immunotherapy against malignant tumours, in combination with interferon, for improving the efficacy of immunotherapy.

[0023] In a preferred embodiment of the invention peptides derivatives of general formula (I) are for administration for a long period of time at a single dose 0.5-5.0 mg/kg body weight.

[0024] In a preferred embodiment of the invention the peptide derivatives of the invention are for use in induction of cell differentiation wherein in order to stabilize malignant tumor growth, in particular melanoma or hemoblastosis, peptides derivatives of general formula (I) are to be administered at the single dose 0.5-5.0 mg/kg body weight for at least 15 days when the capabilities of the chemotherapy has been exhausted.

[0025] Administering peptides derivatives of general formula (I) in combination with the immunotherapy agent interferon results in enhancement of its efficacy in respect to malignant tumor cells in particular melanoma.

[0026] In another preferred embodiment of the present invention, the peptide derivatives of the present invention are for use in induction of cell differentiation wherein in order to enhance efficacy of melanoma immunotherapy, peptide derivatives of general formula (I) are to be administered at dose 0.5-5.0 mg/kg body weight for not less than 15 days together with administering interferon.

[0027] In a further preferred embodiment of the present invention the peptide derivatives of the invention are for use in invention of cell differentiation wherein in order to lower hematological toxicity, peptide derivatives of general formula (I) are to be administered daily at the single dose 0.5-5.0 mg/kg body weight 5 days before the chemotherapy course starting, during the chemotherapy and within the period between the chemotherapy courses until next course of cytotoxic therapy.

[0028] Below, the examples illustrating preferred embodiments of the instant invention are presented.

Example 1. Activity of peptides derivatives of general formula (I) to melanoma M-6 cells differentiation

[0029] The study was conducted on 10-12 week old thymus free (nude) female Balb/C mice weighing 20-22 grams (breeding of the Russian Cancer Research Center (RCRC) named after N.N.Blokhin of the Russian Academy of Medical Sciences (RAMS)). A human melanoma strain earlier obtained from primary clinical material was taken from the bank of tumor strains of the RCRC of the RAMS for transplantation to thymus free "nude" mice. Tumor was disintegrated by Versen solution with vital Tripan blue staining and inoculated subcutaneously to mice in the amount of 1.6 million cells per a mouse.

[0030] Dicarbamine was administered to mice intragastrically using a metal probe daily at dose 1.0 mg/kg beginning 4 days before the tumor inoculation and thereafter for 10-11 days (administration course up to 15 days). The mice were sacrificed with ether anesthesia in 12, 24 and 48 hours after the last administration.

[0031] 4 groups of mice were used in the experiment:

Group 1 - the control, no Dicarbamine is administered. Mice are sacrificed at the same terms as those of the groups received Dicarbamine.
Group 2 - Dicarbamine is administered and mice are sacrificed in 12 hours after termination of the administration.
Group 3 - Dicarbamine is administered and mice are sacrificed in 24 hours after termination of the administration.
Group 4 - Dicarbamine is administered and mice are sacrificed in 48 hours after termination of its administration.

[0032] Four morphologic parameters such as the number of cells with pigment and the number of cells with apoptosis signs (capability to differentiate), the number of mitoses (proliferation activity) and necrosis area were determined to monitor differentiation and proliferation degree of M-6 melanoma in the groups of control animals and the Dicarbamine groups. These parameters were determined dynamically and correlated with general morphologic picture of a tumor growth as an integral sign. For this purpose the tumor was removed in mice, placed into formaline and histologically processed for light microscopy. The data obtained are shown in Table 1.

Table 1. Morphologic parameters of M-6 melanoma (light microscopy)

Parameters (in %)		Time passed since Dicarbamine withdrawal		
		12 hours	24 hours	48 hours
Necrosis area	Control	1-2	2-3	3-5
	Following Dicarbamine administration	6-7	7-9	8-10
Mitoses	Control	3-5	3-5	3-5
	Following Dicarbamine administration	3-5	3-5	3-5
Apoptosis	Control	0.1-0.2	0.1-0.2	0.1-0.2
	Following Dicarbamine administration	0.1-0.2	0.2-0.3	0.2-0.3
Cells with pigment	Control	1-2	1-2	2-3
	Following Dicarbamine administration	2-3	2-4	3-5

[0033]   The conducted study allowed the inventors to establish that human melanoma inoculated to nude mice at day 9 forms the tumor consisting of polymorph cells that grow by continuous fields with insignificant stroma development. Small necrosis sites are ennumberered in the tumor, which sites slightly increase by 48 hours (up to 3-5 % of slice area) as compared to sacrificing terms 12 and 24 hours (1-2 % and 2-3 % , respectively). 3-5 % of imitoses are observed in the tumor during all growth periods. Apoptosis is slightly expressed. Cells containing pigment are rarely found and their number during the first day does not exceed 1-2 % and only in 48 hours of growth it increases up to 2-3 %. Thus intensity of melanin genesis during this period is insignificant. The obtained characteristic allows to conclude that melanoma is a rapidly growing tumor practically lost capability of differentiating on the basis of both apoptosis degree and first of all, on the basis of the basal functional capability of melanin genesis.

[0034]   The effect of Dicarbamine on melanoma cells differentiation was assessed on the basis of the intensity of melanin genesis by numbering the number of cells with melanin in the tumor slices. With this object, the tumor was excised in mice, placed into glutaraldehyde and
histologically processed for electronic microscopy was done. The Melanin Genesis Intensity Index (MGII) that reflects the degree of cells differentiation was calculated in the prepared slices according to the following equation:

$$MGII = NCM \times NM,$$
wherein: NCM is the number of cells containing melanosomes;
NM is average number of melanosomes per a cell.

[0035]   The analysis of melanin genesis intensity conducted by this index is shown in Table 2.

Table 2. Comparative melanin genesis intensity in melanoma cells following administration of Dicarbamine (electronic microscopy)

Parameters (absolute values)		Time passed since Dicarbamine withdrawal		
		12 hours	24 hours	48 hours
Number of cells with melanosomes (per 500 cells)	Control	135.0	144.0	159.0
	Following Dicarbamine administration	175.0	210.0	227.0
Average amount of melanosomes per a cell	Control	19.0	21.0	26.0
	Following Dicarbamine administration	28.0	35.0	42.0

(continued)

Parameters (absolute values)		Time passed since Dicarbamine withdrawal		
		12 hours	24 hours	48 hours
MGII	Control	5.1	6.0	8.2
	Following Dicarbamine administration	9.8	14.7	19.0
*beginning from day 9 following the tumor inoculation				

[0036] Electronic microscopy test shows that as compared to the control, the number of tumor cells comprising melanosomes and the number of melanosomes per one cell are increased as due to Dicarbamine effect. The MGII index increases for the observes terms as follows: in 12 hours - 1.9-fold, in 24 hours - 2.4-fold and in 48 hours - 2.3-fold.

[0037] Thus following a 15 day administration course of Dicarbamine, average increase of M-6 melanoma tumor cells differentiation degree is 2.2-fold that is supported by melanin genesis intensity (the MGII index), increase in the number of cells comprising melanosomes (1.3-fold) and increase in the number of melanosomes (1.3-fold).

[0038] Example 2. The effect of Dicarbamine on melanin synthesizing function of inoculated human melanoma cells

[0039] Mice with subcutaneously inoculated human melanoma as described in Example 1, were daily p.o. dosed with Dicarbamine at a higher single dose 4.5 mg/kg for 3 weeks from the moment of the tumor transplantation.

[0040] Animals were sacrificed in 3 weeks since the tumor transplantation. By sacrifice the tumors volume was in average 150 mm^3. Following sacrifice, the tumor wsa excised in mice and disintegrated with Versen solution and the cell fraction was isolated in which the number of cells with pigment was calculated in the Goryaev's chamber during light microscopy.

[0041] The conducted studies show that in the control average number of cells with melanin was 39.14 ± 8.72, and in the test it was 108.42 ± 11.91, i.e. the number of cells synthesizing melanin significantly ($p<0.01$) 3-fold increased.

[0042] Thus in the conducted series of tests using Dicarbamine at the different doses, a statistically significant effect of pronounced induction of human melanoma cells differentiation was obtained as evidenced on the basis of the melanin genesis intensity.

[0043] The data are presented in Table 3.

Table 3. Melanin genesis intensity in human melanoma cells induced by Dicarbamine

Tumor number	Control	Tumor number	Test
	Number of cells with melanin		Number of cells with melanin
1	32	1	95
2	35	2	111
3	29	3	95
4	42	4	110
5	46	5	130
6	36	6	106
7	54	7	111
Average	39.14	Average	108.42*
Standard deviation	8.72	Standard deviation	11.91
*p<0.01			

Example 3. The effect of Dicarbamine on inoculated human melanoma Mel-6growth dynamics

[0044] The study was conducted on 10-12 week old thymus free "nude" female Balb/C mice weighing 20-22 grams (breeding of the RCRC named after N.N.Blokhin of the RAMS). A human melanoma strain Mel-6 earlier obtained from primary clinical material was taken from the bank of tumor strains of the RCRC of the RAMS for transplantation to thymus free mice.

[0045]   Dicarbamine at the single doses 1.5 mg/kg and 4.5 mg/kg was p.o. administered daily to two mice groups for 3 weeks from the moment of the tumor development (from day 15 to day 36 from the tumor transplantation).

[0046]   Measurement of the tumor was done at days 18, 25, 33, 39, 46 and 53 from the transplantation. Dicarbamine effect was assessed on the basis of the tumor growth dynamics for 8 weeks in multiple measurements of tumor volumes "V" according to the formula:

$$V = \pi * L * s * h\left(mm^3\right)$$

wherein L is length in mm, s is width in mm; and h is height in mm.

[0047]   Ratio between volumes of tumors V_t/V_{t-1} that was expressed in percent was then calculated and statistically processed according to the Student's method to calculate statistically significant difference. The data obtained are shown in Table 4.

[0048]   The data obtained showed a 7-day delay in maximum tumor mass gain in comparison with the control. As compared with the control group, statistically significant differences in tumor growth rate were found at day 25 from the transplantation in the mice group received Dicarbamine at the single doses 4.5 mg/kg that corresponds to a 10-day course of Dicarbamine dosing at a course dose 45 mg/kg. In this group average tumor volume increased by 166.0±93.0%, whereas in the control this parameter was 329.0±88.9% (p<0.015)

Example 4. The Dicarbamine effect in combination with the chemotherapy on the growth of inoculated human M-6 melanoma transplanted to thymus free mice.

[0049]   The study was conducted according to the technique described in Example 3. Dicarbamine was administered daily p.o. at the single dose 4.5 mg/kg for 3 weeks from the moment of tumor appearance (from day 1 to day 36). In the groups of combined treatment Dicarbamine was also administered daily at the single dose 4.5 mg/kg for 3 weeks (days 15-36) in combination with a single administration of anti-tumor cytostatic agents Cysplatin at dose 6 mg/kg i.v. (day 25) and Aranoza at dose 40 mg/kg i.p. (day 27). Cytostatic therapy with was commenced at average tumors volume being 200±62 mm3. At days 18, 25, 33, 39, 46 and 53 from the transplantation tumor volumes were measured and the value V_t/V_{t-1} was calculated which value was expressed in percent. The data obtained are shown in Table 5.

Table 5. The effect of combined the chemotherapy with anti-tumor cytostatic agents and Dicarbamine on human M-6 melanoma growth dynamics

Mice group	Scheme of therapy		M±m (%) at days after tumor inoculation				
	Dose (mg/kg) single/course	Days of dosing	25 Day	33 Day	39 Day	46 Day	53 Day
Control	Physiological saline p.o.	15-36	329.0+88.9	132.9+57.3	21.9+12.6	10.5+8.1	3.8+10.3
Cysplatin Aranoza	6mg/kgi.v.	25	413.0+276.0	177.0+46.0	62.04+30.1	21.2+18,7	18.2+12.7
	40mg/kgi.p.	27					
Dicarbamine	4.5/94.5 mg/kg po.	15-36	166.0+93.0**	276.0+104.0	39.8+27.3	19.6+17.5	4.2+22.5
Dicarbamine Cysplatin Aranoza	4.5/94.5 mg/kgp.o*	15-36	182.0+60.0**	191.0+71.0	24.5+17.4	28.7+9.8	8.0+30.6
	6 mg/kgi.v.	25					
	40 mg/kgi.p.	27					
* - daily; ** - p<0.05							

[0050]   It follows from the data presented that Dicarbamine in the used dosing routdelays the tumor growth at initial stagesthat can be shown by decrease in tumor mass gain at day 25 166.0±93.0% as compared to the control where the gain was 329.0±88.9%. Thus the results of Dicarbamine effect on melanoma growth were reproduced (see Example 3). Combined the chemotherapy with Aranoza and Cysplatin in the indicated regimes appeared to be inefficient, i.e. gain of the tumor at this term was higher than the control value (413.0±276.0%). This proves the absence of sensitivity

of the used Mel-6 human melanoma strain to the given the chemotherapy scheme. Introduction of Dicarbamine into the inefficient the chemotherapy scheme resulted in a statistically significant ($p<0.05$) decrease in the tumor mass gain at day 25 by $182.0\pm60.0\%$ that proves its efficacy in case of the absence of the chemotherapy effect.

Example 5. The effect of peptides derivartives of general formula (I) on proliferation capability of melanoma cells when interferon administration.

[0051]   The effect of Dicarbamine on proliferation capability of melanoma cells along with α-interferon (Introne®, IN) administration was studied. It should be noted that Dicarbamine itself is capable of slowing proliferation activity of melanoma cells without changing their survival.

[0052]   The study was conducted on two continuous cell cultures growing in the form of a monolayer in a tissue culture on murine B-16 melanoma cells and human M-5 melanoma cells. IN was administered at concentrations 70-700 IU/ml. Dicarbamine (D) was transferred into stock solution (1.000 μM), sterilized through filters with 0.22 μm pore diameter and then diluted to concentrations 0.01 and 1.0 μM.

[0053]   The effect of preparations on cells was assessed on the basis of initial rate of cell proliferation (IRCP). This index (IRCP) that is usually called colony rate growth, was determined by numbering the number of cells in micro colonies during the first days following affection in "test" (with preparations) and "control" (without preparations) dishes, by analyzing 50 colonies in each of them. Each "point" included not less than three Petri dishes with growing cell colonies in adding specific concentrations of preparations under study. Growth rate of colonies (in %) was calculated according to the formula:

$$\frac{Number\ of\ cells\ /\ colony\ (average\ value)\ in\ the\ test\ dishes\ -\ 1}{Number\ of\ cells\ /\ colony\ in\ the\ control\ dishes\ -\ 1} \times 100\%$$

[0054]   Cell numbers in micro colonies were calculated for every "point". Toxicity of preparations in the selected range of concentrations was judged by cells survival that was determined by the ratio between numbers of the grown colonies in "test" and "control" dishes. Test results are shown in Table 6.

Table 6. The effect of Dicarbamine and α-interferon on proliferation activity of murine B-16 melanoma and human M-5 melanoma cells

Preparation	IN concentration in IU/ml	Initial rate of cell proliferation (%cells/colony relative to the control) at a term following contact with preparations								
		48 hours			72 hours			96 hours		
		Without D	D,0.01 μM	D, 1.0μM	Without D	D,0.01 μM	D, 1.0 μM	Without D	D,0.01 μM	D, 1.0 μM
Control of M-5		100.0	84.2	69.0	100.0	73.6	50.0	100.0	70.2	49.1
IN	7.0	111.3	79.1	54.7	94.8	49.1	36.9	73.0	46.9	33.3
	70.0	53.7	40.5	30.7	51.9	34.9	24.5	48.8	31.7	23.9
Control of B-16		100.0	52.9	44.6	100.0	61.0	43.6	-	-	-
IN	70.0	-	-	-	50.2	-	26.1	-	-	-
	700.0	38.0	24.9	21.5	29.8	22.0	16.0			

[0055]   One can see from the table that in the control with M-5 melanoma the IRCP index was preserved at the 100% level for 96 hours.

[0056]   In the samples with M-5 cells when α-interferon was added at the concentration 7.0 IU/ml in 48 hours the IRCP index increased up to 111.3% and slowed down to 94.8 and 73.0% respectively only in 76 and 96 hours. When α-interferon was added at concentration 70 IU/ml in 48 hours the IRCP index slowed down to 53.7%, in 72 hours - down to 51.9% and in 96 hours - down to 48.8%. I.e. the maximum inhibiting effect of α-interferon at concentration 70 IU/ml achieves 50% IRCP.

[0057]   When Dicarbamine at the concentration 0.01 μM was added, the IRCP index in 48 hours slowed down to 82.4%, in 72 hours - down to 73.6% and in 96 hours - down to 70.2% and when Dicarbamine at the concentration 1 μM

was added, the IRCP index in 48 hours slowed down to 69.0% and in 72 hours - down to 50.0%.

[0058] Thus the maximum inhibiting effect of Dicarbamine also achieves 50% of the IRCP index and was obtained at the preparation concentration 1.0 μM.

[0059] In the tests on B-16 melanoma when α-interferon was added at the concentration 70 IU/ml, the IRCP index in 72 hours slowed down to 50.0%, and when Dicarbamine was added at two indicated concentrations, the IRCP index in 48 hours slowed down to 52.9 and 44.6% respectively and in 72 hours to 61.0 and 44.6% respectively. Significant reducing the IRCP index down to 38.0 and 29.8% was obtained only when α-interferon was added at concentration 700 IU/ml.

[0060] Thus the conducted tests show that α-interferon and Dicarbamine inhibit M-5 melanoma and B-16 melanoma cells growth at the level of 40.0-50.0% that is characteristic of differentiation inductors. A more pronounced effect on the IRCP index can be obtained only in case of a 100-fold increase in α-interferon concentration.

[0061] Combined addition of α-interferon at concentration 70.0 IU/ml and Dicarbamine to M-5 cells showes that in all cases the IRCP index lowered down to 30.7-24.0-31.0% respectively to recording terms. The most pronounced effect was obtained on B-16 melanoma when α-interferon at concentration 700 IU/ml and Dicarbamine at the both concentrations were used in combination: the IRCP index lowered down to 24.9 and 29.8% in 48 hours and down to 22.0 and 16.0% in 72 hours, respectively.

[0062] Thus Dicarbamine similarly to α-interferon slows down murine B-16 melanoma and human M-5 melanoma cells proliferation and does not show toxicity (according to the survival index). As it was shown by the given examples, the effect of Dicarbamine is characteristic of differentiation inductors and has an additive character in combination with the known differentiation inductor α-interferon on melanoma cells. This effect results in enhancement of tumor growth inhibition and it is an indication for raising immunotherapy efficacy of melanomas.

5.2. The effect of peptide derivatives on melanoma cells proliferation capability .

[0063] The study was conducted on a continuous cell culture of murine B-16 melanoma growing in the form of a monolayer in a tissue culture. α-Interferon selected as a preparation of comparison, was administered at concentration 70 IU/ml.

[0064] The tested compounds were transferred into stock solution (1,000 μM), sterilized through filters with 0.22 μm pore diameter and then diluted down to concentration 100 μM.

[0065] The effect of compounds on cells was assessed on the basis of initial rate of cell proliferation (IRCP). This index was determined by numbering the number of cells in micro colonies during the first days following affection in "test" (with preparations) and "control" (without preparations) dishes, by analyzing 50 colonies in each of them.

[0066] Growth rate of colonies (in %) was calculated according to the formula:

$$\frac{\text{Number of cells/colony (average value) in the test dishes -1}}{\text{Number of cells/colony in the control dishes -1}} \times 100\%$$

[0067] Calculations of cell numbers in micro colonies were done for every "point". Toxicity was judged by B-16 melanoma cells survival that was determined by the ratio between numbers of the grown colonies in "test" and "control" dishes. Test results are shown in Table 7.

Table 7. The effect of peptide derivatives at concentration 100 μM and α-interferon at concentration 70 IU/ml on murine B-16 melanoma cells proliferative activity

Compound		Initial rate of cell proliferation (%cells/colony relative to the control) at a term following contact with preparations	
		48 hours	72 hours
control		2.52=100%	3.49=100%
interferon		29.6+2.3	27.4$\pm$2.1
dicarbamine		30.8$\pm$2.8	28.2$\pm$2.2
	1	26.6$\pm$2.7	26.6$\pm$2.8
	2	25.5$\pm$1.9	25.5$\pm$1.7
	3	35.6$\pm$2.9	35.6$\pm$2.9

(continued)

Compound	Initial rate of cell proliferation (%cells/colony relative to the control) at a term following contact with preparations	
4	38.3±3.5	38.3+3.5
5	32.4±2.6	32.4±2.3
6	29.3±2.4	29.3+2.2
7	38.8+2.7	38.8±2.8
8	21.4±1.5	16.9±0.9
9	27.1±1.7	17.2±1.3
10	35.9±3.6	23.1±1.6
11	21.5±1.9	20.7±1.8
12	28.7+2.1	20.3±1.9
13	44.9±4.0	18.9±1.4
14	33.8±3.5	19.9±1.8
15	39.7+2.5	29.8+2.3
16	41.3±4.0	28.9+2.5
17	39.7±2.1	26.6±2.1
18	42±3	39±3
19	21±1	41±5
20	44+3	42+4
21	42±4	28±2
Differences with the control are significant (p<0.01)		

[0068]   Data presented in Table 7 show that peptide derivatives inhibit B-16 melanoma cell colonies growth at the level 50.0-70.0% that is characteristic of differentiation inductors.

Example 6. Distribution of tumor cells by cell cycle phases at different terms following Dicarbamine administration.

[0069]   Tests were done on inoculated B-16 melanoma. The effect of Dicarbamine on the distribution of tumor cells was studied on the basis of DNA content at different terms following administration of the preparation. From day 6 after the tumor inoculation mice for 10 days were daily intragastrically given with 0.5 mg/kg Dicarbamine. Animals were sacrificed with subsequent investigation of tumor material at days 10, 12, 16 and 18 after inoculation, i.e. days 5 and 7 respectively after Dicarbamine administration as well as in 2 and 4 days after termination of Dicarbamine 10-day dosing.
[0070]   Testal results shows that Dicarbamine caused a significant increase of the portion of inter-phase tumor cells (IIG[1]) ($\approx$25%). In a constant portion of proliferating cells ($\approx$30%) increase in the portion of IIG[2] cells (12-14%) is noted. Accordingly the portion of normal stromal cells (IG[1]) in the samples compensatory dicrease. Said changes are most clearly pronounced after 5-10 administrations of Dicarbamine.
[0071]   Course dosing of Dicarbamine causes kinetic rearrangement of tumor cell population. Inhibition of cells in the synthetic cycle phase (S-phase) is noted with compensatory decrease in the portion of cells that are ready for proliferation or proliferating cells (G[2] phase). Accumulation of tumor cells in the stationary phase G[1] simultaneously occurs.
[0072]   Lowering the level of proliferative activity Dicarbamine promotes accumulation of cells in stationary (non-pro-liferating) cell cycle phase. It can slow down tumor growth and promote transition of cells to a more differentiated state.

Example 7. Efficacy of Dicarbamine in respect to hematological toxicity of Cyclophosphamide and its combinations with Cysplatin and Carboplatin.

[0073]   Hematocorregating effect of Dicarbamine was studied on the first generation of male mice hybrids F_1 (CBA x C_{57}Bl).

[0074] 7.1. 4 groups of animals were used to study the effect of Dicarbamine on hematotoxicity of Cyclophosphamide (CPH):

Group 1 - Dicarbamine, 0.5 mg/kg daily beginning 5 days before CPH administration and for 5 days after the single administration of CPH at dose 200 mg/kg;
Group 2 - the single administration of 200 mg/kg CPH;
Group 3 - intact control;
Group 4 - Dicarbamine 0.5 mg/kg daily for 10 days.

[0075] The data obtained are shown in Table 8.

Table 8. Total leukocyte number in peripheral mice blood under Cyclophosphamide effect and Cyclophosphamide with Dicarbamine

Group	Total leukocyte number (in thousand in mm^3) at days post Cyclophosphamide dosing						
	3	5	7	10	13	17	21
1	2.80±0.22	7,96±1.10	13.38±1.54	11.88±1.92	13.30±1.48	12.40±1.76	12.90±2.60
2	1.06±0.44	4.38±0.77	10.50±3.02	6.44±0.60	12.20±3.02	12.20±1.80	11.86±1.32
3	16.50±8.20	16.10±3.20	14.80±3.30	15.80±1.90	14.90±2.70	16.90±4.70	14.70±2.80
4	15.70±4.30	15.30±7.80	17,30±5.10	15.70±3.80	12.50±3.52	17.80±4.70	16.30±3.90

[0076] The data obtained show that use of Dicarbamine in combination with CPH allows reducing hematotoxic effect of the latter and speeding up recovering blood parameters.

[0077] 7.2. When studying the effect of Dicarbamine on hematotoxic action of CPH combinations with platinum derivatives, Dicarbamine was intragastrically daily administered to mice for 20 days daily at a single dose 0.5 mg/kg. Cytostatic preparations were once administered intraperitoneally at day five from starting Dicarbamine administration course. Doses of cytostatic preparations are shown in Tables 10 and 11.

[0078] The results of studying the effect of Dicarbamine on leukocytes number in mice peripheral blood when combined dosing of CPOH with Cysplatin or Carboplatin are shown in Tables 9 and 10 respectively.

Table 9. The effect of Dicarbamine on hematotoxicity of Cyclophosphamide in combination with Cysplatin

Cytostatic preparation	Cdtistatic preparation dose (mg/kg)	Total leukocyte number (in thousand in mm^3) at days after cytostatic preparations dosing					Terms of death (days)
		0	3	5	7	21	
Dicarbamine CPH Cysplatin	200 8	11.30± 2.30	2.32± 0.49	6.60± 0.90	10.4± 1.54	12.30± 1.56	8; 16
CPH Cysplatin	200 8	11.30± 2.30	1.20± 0.33	4.32± 0.77	6.24±1.15	10.80±1.02	3;4;7
Dicarbamine CPH Cysplatin	100 4	11.30± 230	4.14± 0.60	11.40± 1.10	14.90± 132	11.80± 1.32	no
CPH Cysplatin	100 4	11.3± 2.3	2.65± 0.66	4.74± 0.66	8.05± 0.88	12.0± 1.4	no
Dicarbamine CPH Cysplatin	50 2	11.30± 2.30	6,70± 1.15	17.00± 5.17	14.50± 2.00	12.40± 0.99	no
CPH Cysplatin	50 2	11.30± 2.30	4.04±0.77	7.62± 0.99	8.72± 1.15	13.10± 1.54	no

[0079] The presented data show that already by day 5 in the mice group received cytostatic preparations at maximal doses along with Dicarbamine, leukocyte number achieved lower border of physiological norm and by day 7 it practically

restored up to the initial level. Without Dicarbamine restoration was observed only by day 21 of the test. In mice received cytostatic preparations at maximum doses without Dicarbamine death of animals was noted at days 3, 4 and 7 of the test. In animals that were given cytostatic preparations at maximum doses along with Dicarbamine, only delayed death at days 8 and 16 was noted.

Table 10. The effect of Dicarbamine on hematotoxicity of Cyclophosphamide in combination with Carboplatin

Cytostatic preparation	Cytostatic preparation dose (mg/kg)	Total leukocyte number (in thousand in mm^3) at days after cytostatic preparations dosing					Terms of death
		0	3	5	7	21	
Dicarbamine CPH Cysplatin	200 30	11.50± 2.80	3.10± 0.70	12.80± 137	15.30± 126	1230± 0.89	10
CPH Cysplatin	200 30	11.30± 2.30	1.18± 0.49	4.60± 0.60	7.54± 0.77	12.60± 1.28	3
Dicarbamine CPH Cysplatin	100 15	11.50± 2.80	4.04± 0.44	10.40± 1.59	14.80± 1.76	11.80± 134	no
CPH Cysplatin	100 15	11.30± 2.30	2.74± 0.49	6.48± 0.60	10.50± 1.38	13.20± 1.50	no
Dicarbamine CPH Cysplatin	50 7.5	11.30± 2.30	6.60± 0.77	10.90± 1.21	11.20± 1.20	10.90± 1.28	no
CPH Cysplatin	50 2	11.50± 2.80	3.94± 1.04	8.72± 1.98	10.80± 2.40	11.20± 0.99	no

[0080] The data presented show (Table 10) that in case of using Dicarbamine together with Carboplatin and Cyclophosphane at the lethal doses, leukocyte number in peripheral blood and terms of animals death are similar to the data presented in Table 9.

[0081] Thus Dicarbamine inhibits the development of leukopenia in all the courses studied, it speeds up recovering total leukocyte number and delays term of mice death when using cytostatic preparations at lethal doses.

[0082] 7.3. When studying the effect of peptide derivatives of general formula (I) on hematotoxic action of CPH combinations with Carboplatin, the compounds were intragastrically administered to mice daily at the dose 0.5 mg/kg for 10 days. At day five after starting administration of the tested compounds mice were intraperitoneally injected CPH at dose 200 mg/kg and Carboplatin at a single dose 15 mg/kg. Thereafter administration of the tested compounds was continued for 5 more days.

[0083] Before starting dosing the tested compounds, blood was drawn from mice tail to calculate total leukocyte number. At days 3, 5 and 7 after administration of Cyclophosphamide with Carboplatin blood also was drawn from mice tail to calculate total leukocyte number. Each group included 15 animals.

[0084] As a control a mice group that received only cytostatic preparations was used.

[0085] The data presented in Table 11 show that peptide derivatives of general formula (I) inhibit the development of leukocytopenia and speed up recovering total leukocyte number.

Table 11. The effect of peptide derivatives of general formula (I) on hematotoxic action of Cyclophosphamide combination with Carboplatin

No. of compound	Total leukocyte number in peripheral blood (in thousand in mm^3) at days after dosing of cytostatic preparations			
	0	3	5	7
1	13,3±3.5	3.3±1.0*	10.0±1.3*	11.6±2.5*
2	13.8±3.5	3.18±0.82*	9.1±0.5*	11.9±2.5*
3	15.5±3.2	4.18±2.0	19.2±1.9	17.5±1.7*

(continued)

No. of compound	Total leukocyte number in peripheral blood (in thousand in mm^3) at days after dosing of cytostatic preparations			
	0	3	5	7
4	15.3±2.7	3.02±0.83	9.62±3.84	16.1±0.15
5	12.1±1.4	2.1±1.04	10.5±2.08	15.26±1.23
6	14.2±1.1	3.04±1.61	14.56±2.65	25.68±3.1*
7	13.7±1.1	3.14±0.62	13.7±0.57	16.58±2.9
8	12.9±2.5	3.98±0.78	10.8±0.57	16.16±0.85
9	13.2±3.0	5.04±0.20*	8.64±1.97	19.38+1.8
10	12.9±1.9	5.18±1.97*	19.76±3.22*	21.82±3.74*
11	14.8±2.3	3.32±1.3	10.28±1.35	17.56±2.6
12	12.8±0.8	3.56±0.12	20.66±3.7*	17.4±2.8
13	14,9±0,6	2,66±0.21	25.7±4.1*	32.1±4.87*
14	13.8±0.5	2.66±0.23	16.24+2.3	28.9±3.65*
15	12.7±0.7	3.76±0.14	26.4±5.8*	27.6±4.12*
16	12.6±0.6	3.9±0.23	15.44±1.3	24.9±4.31*
17	13.4±0.8	3.36±0.27	17.6±3.1	26.1±3.97*
CPH+C	16.1±3.5	1.14±0.55	4.31±1.3	8.3±0.58
*significant at p ≤0.05				

[0086] 7.4. The effect of Dicarbamine on cells differentiation is supported by the study of mice peripheral blood differential number under Cyclophosphamide effect in combination with Dicarbamine in comparison with dosing Cyclophosphamide alone.

[0087] Two groups of mice are used. Group one is administered Dicarbamine at dose 0.5 mg/kg 5 days prior to and 5 days after CPH administration at dose 200 mg/kg. Group two of mice is administered CPH alone at dose 200 mg/kg. Results of the studies are shown in Tables 12 and 13.

[0088] Data presented in Tables 12 and 13 show that recovering peripheral blood occurs due to burst of mature forms that confirms the differentiation effect of Dicarbamine. This is especially seen at days 3 and 5 by peripheral blood number and cellularity of bone marrow (Tables 12 and 13). In the group with Dicarbamine myelocytes and band neutrophils are absent in peripheral blood and in group without Dicarbamine these form elements are present (Table 12).

Example 8. Decrease in the rate of and dimensions of subcutaneously inoculated Friend erythroblastosis (FEB) in mice as effected by peptide derivatives.

[0089] The studies were conducted on male mice hybrid 100 BDF$_1$ that were divided into groups containing 10 mice each. Lineal DBA$_2$ mice were used for passaging FEB in vivo.

[0090] A strain of Friend erythroblastosis was obtained from the bank of tumor strains of the GU RCRC named after N.N.Blokhin of the RAMS, it was twice passaged intraperitoneally using generations 3-8 in subcutaneous inoculation. The inoculation was done using cellular suspension at amount 1x10^6 in 0.3 ml 1999 medium.

[0091] Solutions of the tested compounds were intragastricaally daily administered to mice using a probe from day 3 to day 7 after tumors inoculation.

[0092] Therapy efficacy was assessed on the basis of tumor growth inhibition (TGI, %) and an average life span (ALS). Increase in life span was determined by the commonly accepted criterion T/C (%) that was calculated as ratio between ALS in the test and control groups. Tumor growth rate V_t/V_1 was calculated on the basis of change dynamics in average tumor volumes.

[0093] Data of the studies on the effect of peptide derivatives on tumor size and on tumor growth rate are shown in Tables 14 and 15, respectively.

[0094] The results obtained show that peptides derivartives cause growth inhibition of subcutaneously inoculated FEB

for 19 days after termination of therapy. Said effect started to be recorded immediately after termination of administering compounds at a single dose 1.5 mg/kg and was preserved at a significant level ($p<0.05$) up to day 13. Tumor growth rate got stabilized during one week following withdrawal of the compounds.

[0095]   It was established resulting from the conducted studies that the compounds of general formula (I) possess inhibiting effect on the development of FEB subcutaneous node. The data obtained allow to consider that the test compounds are useful for therapy of human hemoblastoses.

Example 9. The effect of Dicarbamine and 2α-interferon (Reaferon) on Friend erythroblastosis tumor cells.

[0096]   Friend erythroblastosis was investigated which was inoculated subcutaneously to DBA_2 female mice via spleen cells.

[0097]   4 groups of tests were carried out.

Group 1 - control animals without therapy, a physiological saline was administered;
group 2 - Reaferon at dose 100 thousand IU/kg was administered daily s.c. from day 3 to day 7 after inoculation;
group 3 - Dicarbamine at a single dose 4.5 mg/kg was administered p.o. from day 3 to day 7 after inoculation;
group 4 - Dicarbamine and Reaferon were administered simultaneously according to a similar scheme.

[0098]   Material for light microscopy was taken in sacrificed animals at days 3, 7 and 14 after termination of therapy or administration of a physiological saline, material for electronic microscopy was taken at days 7 and 14.

[0099]   For histological examination tumor pieces were fixed in 10% neutral formaline and imbedded into paraffin; slices obtained were stained with hematoxylin-eosin and examined for glycogen (polysaccharides) content using periodic acid Schiff reaction, for RNA content according to Brachet, for lipids and iron. Slices were looked through and photographed in the Polivar light microscope (Austria).

[0100]   For electronic microscopy tumor pieces were fixed in 2.5% glutaraldehyde solution and 1% osmium quadrioxide, embedded into EPON-812. Semi-thick and ultra-sick slices were prepared on the LKB-III ultratome (Sweden). The obtained semi-sick slices were stained with Toluidine blue and looked through in the light microscope. Ultra-sick slices were additionally stained with uranyl acetate and lead citrate; the slices were looked through and photographed in the JEOL 1200 EX-II electronic microscope (Japan).

[0101]   Percentage of cells with different types of differentiation (blast cells, lymphocytes and granular leukocytes) was calculated during electronic microscopy for quantitative assessment.

[0102]   Percentage of mitoses and cells with apoptosis as well as areas of necroses were assessed during histological examination.

Histological examination

Group 1. Control animals without therapy.

[0103]   It was found at histological examination that tumor cells are large, polymorphous, their nuclei are light, cytoplasm is moderately developed. Cellular size sometimes fluctuates and individual smaller cells are ennumberered but large cells represent the main mass of cells.

[0104]   Tumor cells form continuous outgrowths. In individual tumors necrosis sites surrounding preserved fields of tumor cells are ennumberered. Area of necroses did not exceed 10-15% of the slice surface.

[0105]   In a majority of tumor cells Brachet reaction for RNA is strongly pronounced, less often it is weak or absent (in individual small cells).

[0106]   Periodic acid Schiff reaction had diffuse character, reaction to iron was positive only in individual cells.

[0107]   In the tumor among large cells mitoses (up to 1-1.5%) and cells with apoptosis signs (up to 0.5%) were ennumberered.

[0108]   As the tumor grew, the area of necroses was increasing up to 20-30% of the slice surface, the number of metoses increased (up to 1.5-2%), apoptosis activity did not change. The number of large polymorphous cells significantly prevailed at all terms.

Group II. Administration of Reaferon

[0109]   Tumors have usual histological structure. As in the control, among large polymorphous cells smaller cells with hyperchromatic nucleus are found.

[0110]   By day 14 the area of necroses is 40-50% of the slice surface, mitotic activity is 0.5-1%, by day 7 apoptosis increased up to 1-2%, but by day 14 it lowered down to 1-1.5%.

Group III. Administration of Dicarbamine

[0111] Increase in the quantity of small tumor cells with hyperchromatic nuclei is noted. Quantity of large polymorphous cells significantly prevails. The area of necroses did not significantly change as compared to the picture in group I. Mitotic activity also remained within the limits of the control figures. At days 3 and 7 the rate of apoptosis slightly lowered (down to 0.1 =0.5% at day 7).

Group IV. Simultaneous administration of Dicarbamine and Reaferon

[0112] The area of necroses and mitotic activity did not show significant shifts as compared to the changes in group II. At day 3 apoptosis lowered down to 0.2-0.5%, at days 7 and 14 it is 0.5% (as in the control).
[0113] Large polymorphous cells of blast type significantly prevail in the tumor.

Electronic microscopy

Group I. Control animals without treatment

[0114] Large polymorphous low-differentiated cells of blast type are mainly found in the tumor during electronic microscopy. Nuclei in these cells have rounded or slightly irregular shape occasionally with uneven surface. Diffuse distribution of chromatin is usually seen in them, only in some of them formation of marginally located heterochromatin is noted. Nuclei usually occupy a major portion of cytoplasm wherein ribosomes, single mitochondria, occasionally the structures of slightly rough endoplasmic reticulum prevail. Blast cells amount 90-95% of all the tumor population.
[0115] In addition to blast cells, lymphocytes of different maturity degree are ennumberered, i.e. lymphoblasts, lymphocytes (large, medium, small). Nuclei in these cells are rounded, oval, often with uneven surface, they comprise heterochromatin in the form of large accumulations, nucleoli are ennumberered. Cytoplasm is moderately developed, it comprises a lot of ribosomes; there are little other organelles, dense granules are occasionally ennumberered.
[0116] Granular leukocytes are small, granules characteristic of neutrophils, less often eosinophils are visible in cytoplasm. Nuclei in cells are segmented or have deep concavities. Cells having granules in cytoplasm, irregular nucleus and protruding plasmatic membrane in the form of processes can be occasionally seen (monocytes). Freely lying red blood cells were ennumberered in the tumor.
[0117] Large blast cells mainly prevail in the tumor (up to 90-95%). Lymphoid cells are ennumberered within the range 4-8%, granular cells amount 1-2%.
[0118] No significant shifts in the ratio between different cell types were noted as the tumor grew following engrafting.

Group II. Administration of Reaferon

[0119] General ultrastructure of tumor cells of different type is preserved.
[0120] Quantity of large blast cells does not lower, lymphoid cells amount up to 4-8%, granular leukocytes amount up to 1-2%. Individual red blood cells are present in the tumor.

Group III. Administration of Dicarbamine

[0121] Ultrastructure of tumor cells of different type remains previous. Their quantitative ratio changes and differentiation level somewhat raises. The quantity of large blast type cells lowers down to 70-80%, the quantity of lymphocytes and granulocytes increases up to 18-25% and 2-5% respectively. Individual red blood cells are present in the tumor.
[0122] The most constantly found changes are ennumberered at day 7 post treatment termination.

Group IV. Administration of Reaferon and Dicarbamine

[0123] Ultrastructure of tumor cells of different type practically corresponds to what is described above (see Group I).
[0124] The quantity of large blast type cells fluctuates within the range of 70-80%. The number of lymphocytes achieves 18-25%, the quantity of leukocytes remains at the level of 2-5%. Red blood cells lying among the other cells are ennumberered.
[0125] As in the previous groups, the changes found are most pronounced at day 7.
[0126] Thus Dicarbamine orally administered to mice with Friend erythroblastosis at dose 4.5 mg/kg daily for 5 days was established to cause differentiation of immature tumor cells mainly in the direction of forming granulocytes as well as cells of erythroid lineage.
[0127] As compared to tumors of the control animals, when Dicarbamine was used, the quantity of immature tumor

cells lowered down from 90-95% to 70-80%, i.e. by 15-20% and the quantity of lymphocytes increased from 4-8% up to 18-25%, i.e. 4-fold.

[0128] The quantity of granulocyte lineage cells increased less significantly (from 1-2% to about 2-5%).

[0129] It should be noted that most frequently changes were found at day 7 after termination of treatment. At day 14 after termination of treatment these changes got stabilized.

[0130] Reaferon in subcutaneous administration for 5 days at dose 100 thousand IU/kg caused increase in the area of necroses in the tumor (from 15-20% in the control to 40-50% in the test at day 7 after termination of treatment and from 20-30% to 40-50% at day 14). The rate of mitoses somewhat decreased (from 1.5-2% to 0.5-1%) and the quantity of cells with apoptosis signs increased (from 0.5% to 1-2% at day 7 after termination of treatment). Differentiation of tumor cells did not practically change.

[0131] In simultaneous administration of Dicarbamine and Reaferon at the same doses and at the same terms summation of the effect of each preparation was found. Enhancement of differentiation of blast immature cells characteristic of the effect of Dicarbamine alone was observed as well as growing area of necroses and decrease in the number of mitoses were found that was seen in administering Reaferon alone.

[0132] Thus it has been established that Dicarbamine is capable of enhancing differentiation of immature tumor hemopoietic cells of Friend erythroblastosis in different directions in particular with formation of lymphoid and myeloid lineage tumor cells.

[0133] The effect of Dicarbamine on cells differentiation represents its general property as it was earlier observed in the example of melanoma study.

Example 10. Electronic microscopy of Dicarbamine protective effect in respect to hemopoietic cells of the bone marrow and peripheral blood in patients with ovarian cancers during the chemotherapy

[0134] In the previous studies devoted to studying action mechanism of Dicarbamine on the bone marrow, the given preparation was found to protect the bone marrow in animals under testal conditions against adverse cytotoxic effect of Cyclophosphamide by reducing apopotosis in normal hemopoietic cells.

[0135] Similar data were obtained on the bone marrow puncture biopsies and peripheral blood of 10 patients with stage III-IV ovarian cancer.

[0136] The patients were divided into two equal groups: group I - patients who received the chemotherapy alone and group II - patients who received the chemotherapy along with Dicarbamine administration.

[0137] Patients in groups I and II received 600 mg/m^2 Cyclophosphane and 400 mg/m^2 Carboplatin during the first day of therapy; courses were repeated with 3-4 week interval. Average the chemotherapy course of one patient included 6 courses without Dicarbamine and 5.7 courses with Dicarbamine.

[0138] In group II patients received the chemotherapy along with Dicarbamine dosing at a single dose 100 mg beginning 5 days before the first course and then until the beginning of next course at the same dose. Duration of Dicarbamine use between two courses averaged 24.5 days. Average total dose was 2.5 grams.

[0139] Puncture biopsy of the bone marrow and peripheral blood for electronic microscopy were taken in patients prior to the chemotherapy starting and at the end of treatment course with Dicarbamine or without it.

[0140] Fresh bone marrow puncture biopsies were placed on slide plate and many times stirred with stirring rod until small dense fragments were obtained. The latter were fixed in 2.5% glutaraldehyde solution additionally fixed in 1% osmium quadrioxide solution; following washing with phosphate buffer at pH 7.4 they were dehydrated in alcohols of increasing concentration and embedded into the mixture of epoxy resins EPON-812. Semi-thick and ultra-sick slices were prepared on the LKB-III ultratome (Sweden). The semi-sick slices were stained with Methylene or Toluidine blue, ultra-sick slices were contrasted with aqueous solution of uranyl acetate and lead citrate.

[0141] Peripheral blood comprising heparin was centrifuged for 1 hour at 3,000 rpm. Then 2.5% glutaraldehyde solution was poured on the surface of the film formed for 10-15 minutes the film was removed and then treatment was proceeded as described above.

[0142] Thin slices were jbserved in the light microscope Polivar (Austria) and semi-thin slices were observed in the electronic microscope JEOL-1200-CX-11 (Japan).

1. Control studies prior to starting of the chemotherapy and Dicarbamine administration. Patients of groups I and II.

[0143] Hemopoietic cells of different maturity degree and differentiation direction are found in puncture biopsies of the bone marrow a portion of cell being with the signs of vacuolization and dystrophy.

[0144] There are blast non-differentiated cells of large size with narrow cytoplasm rim comprising mainly ribosomes. In these cells a nucleus of rounded-oval shape with diffuse chromatin and individual nucleoli occupy the main portion of cytoplasm.

[0145] A portion of the cells differentiates in the direction of granulocytic lineage of leukocytes of different type and

differentiation degree.

[0146] Promyelocytes and myelocytes with rounded-oval nuclei, diffuse chromatin comprising in cytoplasm different amount of specific granules are seen. Red blood cells and more mature granulocytes are often situated around these cells.

[0147] Accumulations of more differentiated granulocytes, i.e. band neutrophils and segmented neutrophils are often seen. Specific granules of different type characteristic of neutrophils, eosinophils and basophils are present in their cytoplasm.

[0148] Lymphoid cells of different differentiation degree (small, medium, large - lymphoblastic) are disposed among granulocytes.

[0149] Many mature red blood cells, often having different shape as well as normoblasts comprising nuclei and platelets are ennumberered.

2. Bone marrow following the chemotherapy with Cyclophosphane and Carboplatin - group I.

[0150] In the preserved hemopoietic cells of different type (granulocytes, lymphocytes, normocytes, red blood cells, platelets) the signs of dystrophy and low degree maturity are ennumberered in the bone marrow puncture biopsies taken after the chemotherapy course.

[0151] In blast cells cytoplasm contains ribosomes and it is often vacuolated. Nuclei are large with diffuse chromatin or accumulations of heterochromatin, occasionally of irregular shape and with sites drawn inside.

[0152] The quantity of specific granules in promyelocytes and myelocytes is insignificant and cytoplasm often has pronounced dystrophic changes.

[0153] In the preserved granulocytes of band and segmented type dystrophic changes and insignificant amount of specific granules are also observed. The present granules are also often dystrophically modified and vacuolated.

[0154] The preserved normoblasts are often of irregular shape with processes and projections of cytoplasm.

[0155] It should be noted that in puncture bone marrow biopsies especially among granulocytes cells with apoptosis signs were ennumberered. In such cells margination of heterochromatin, the signs of nucleus and cytoplasm fragmentation and formation of apoptotic bodies were noted.

3. Bone marrow after the chemotherapy with Cyclophosphane and Carboplatin along with Dicarbamine administration - group II.

[0156] In puncture bone marrow biopsies of patients who underwent the chemotherapy along with Dicarbamine administration hemopoietic cells of different degree and type of differentiation (granulocytes, lymphocytes, platelets, normoblasts) are ennumberered.

[0157] The cells of blast type are large, they contain rounded nuclei with diffuse chromatin and individual nucleoli, their cytoplasm is narrow and therein ribosomes, individual mitochondria and occasionally single primary dense granules are seen.

[0158] There are many promyelocytes and myelocytes comprising rounded or oval nuclei with diffuse or condensed chromatin; their cytoplasm comprises a rather big amount of specific granules both primary ones (dark) and less mature ones (more mature).

[0159] Band and segmented leukocytes are also frequently ennumberered. The have a concave (bean-like) or segmented nucleus, abundance of specific granules of predominantly neutrophile type in their cytoplasm, less often of eosinophile type with crystalloid structures.

[0160] Lymphocytes of different differentiation degree comprise in cytoplasm mitochondria, structures of rough endoplasmic reticulum, occasionally single inclusions in the form of single granules.

[0161] Cells of granulocytic type, lymphocytes often form compact accumulations.

[0162] Along with red blood cells, normoblasts of different differentiation degree and relatively usual shape are ennumberered.

[0163] Cells with apoptosis signs are rarely ennumberered.

[0164] The same regularities of composition that were earlier described for bone marrow elements were found in studying hemopoietic cells of peripheral blood.

[0165] The conducted comparative electronic microscopy of bone marrow and peripheral blood hemopoietic cells in patients with ovarian cancer before and after combined the chemotherapy (Cyclophosphamide + Carboplatin) and during the chemotherapy along with Dicarbamine dosing allowed establishing mechanisms of its protective effect from cytotoxic influence of the used preparations.

[0166] The study showed that the the chemotherapy preparations used in the present work exert a pronounced cytotoxic effect on different types of hemopoietic cells of granulocytic, lymphoid and erythroid lineage.

[0167] This cytotoxic effect is expressed in the form of dystrophic changes in cytoplasm and death of specific granules that develop in bone marrow cells (and respectively peripheral blood).

[0168] Said disorders especially concern granulocytic and to a less extent lymphoid cells at early stages of their differentiation, i.e. formation of blast cells, promyelocytes, myelocytes, lymphoblasts, and they involve erythroid lineage as well that results in insufficient accumulation of differentiated functionally competent forms of hemopoietic cells.

[0169] Further as it was found in the elements of granulocytic lineage the genetically programmed cell death, i.e. apoptosis is enhanced.

[0170] Dystrophic changes and apoptosis generally result in the development of leukopenia, neutropenia, thrombocytopenia and other disorders of hemopoiesis state and limit capabilities of the chemotherapy.

[0171] Based on the conducted study, Dicarbamine was established to protect hemopoietic cells of the bone marrow (and respectively peripheral blood) from cytotoxic effect of the used the chemotherapy preparations, to promote differentiation of young forms to mature cellular elements and to reduce the events of apoptosis.

[0172] As a result of the found effect of Dicarbamine, in the bone marrow of patients during the chemotherapy accumulation of young (blast) forms of hemopoietic cells occurs and what is especially important, their differentiation to functionally competent forms is enhanced.

[0173] Thus under the conditions of the chemotherapy stimulation of the bone marrow hemopoietic cells differentiation especially of granulocytic lineage cells, and preventing the growth of apoptosis are those mechanisms that underlie the protective effect of Dicarbamine.

Example 11. The efficacy of Dicarbamine in respect to reducing hematological toxicity of the chemotherapy in ovarian cancer

[0174] The effect of Dicarbamine was studies in 13 patients with stage III-IV ovarian cancer who underwent 77 courses of the chemotherapy according to the scheme: 400 mg/m^2 Carboplatin i.v. drop-wise, once + 600 mg/m^2 Cyclophosphan i.v. drop-wise, once; the courses were repeated in 28 days. Dicarbamine was prescribed daily at dose 100 mg orally after meals beginning 5 days prior to the first course and then for three weeks. Dosing duration was 26 days, course dose being 2600 mg. Dicarbamine was given again 5 days prior to second the chemotherapy course and dosing was continued for 21 days. Total duration of Dicarbamine intake during two the chemotherapy courses was 52 days.

[0175] Hematological toxicity (leukopenia, neutropenia, thrombocytopenia) was assessed in 13 patients who received 77 courses of the chemotherapy with Dicarbamine as compared with the group of 7 patients who received 25-27 courses of the chemotherapy without Dicarbamibne (control).

[0176] Hemopoiesis parameters were assessed dynamically many times before and after conducted the chemotherapy (control) as well as dynamically before and after Dicarbamine dosing in the test group. Below hemopoiesis parameters in individual patients who received the chemotherapy according to the indicated scheme with Dicarbamine or without it are presented.

8.1. Patients who received the chemotherapy without Dicarbamine

[0177] 51 year old female, diagnosis: stage III ovarian cancer; she received the first course of the chemotherapy according to therapy scheme as follows: 600 mg/m^2 Cyclophosphan and 400 mg/m^2 Carboplatin once. Complete blood analysis , course 1 of the chemotherapy

Parameter, units of measurement	Before course 1 the chemotherapy starting	5 days after course 1 the chemotherapy	2 weeks after course 1 the chemotherapy	3 weeks after course 1 the chemotherapy
Leukocytes 10^9/l	4.5	3.8	2.2	2.0
Neutrophils 10^9/l	2.9	2.4	0.9	0.8
Platelets	168	160	154	150

[0178] The second course of therapy was delayed by 7 days because of neutropenia.

[0179] The second course of the chemotherapy according to therapy scheme was as follows: 600 mg/m^2 Cyclophosphan + 400 mg/m^2 Carboplatin once without Dicarbamine.

[0180] Complete blood analysis, course 2 of the chemotherapy

Parameter, units of measurement	Before course 2 the chemotherapy starting	5 days after course 2 the chemotherapy	2 weeks after course 2 the chemotherapy	3 weeks after course 2 the chemotherapy
Leukocytes 10^9/l	3.5	3.3	2.0	2.1
Neutrophils 10^9/l	2.2	2.0	0.8	0.9
Platelets	178	170	154	150

[0181]   The third course was delayed because of neutropenia.

[0182]   63 year old female, diagnosis: stage IV ovarian cancer, matastatic involvement of right groin lymph node, ascites; she received the first course of the chemotherapy according to therapy scheme as follows: 600 mg/m^2 Cyclo-phosphan + 400 mg/m^2 Carboplatin once, without Dicarbamine.

[0183]   Complete blood analysis, course 1 of the chemotherapy

Parameter, units of measurement	Before course 1 of CT starting	5 days after course 1 of CT	2 weeks after course 1 of CT	3 weeks after course 1 of CT
Leukocytes 10^9/l	5.0	3.9	2.1	2.0
Neutrophils 10^9 /l	3.2	1.7	0.9	1.0
Platelets	160	150	151	152

[0184]   The second course of therapy was delayed for 4 days because of leuko- and neutropenia.

[0185]   The second course of the chemotherapy was conducted according to therapy scheme was as follows: 600 mg/m^2 Cyclophosphan + 400 mg/m^2 Carboplatin once without Dicarbamine.

[0186]   Complete blood analysis, course 2 of the chemotherapy

Parameter, units of measurement	Before course 2 of CT starting	5 days after course 2 of CT	2 weeks after course 2 of CT	3 weeks after course 2 of CT
Leukocytes 10^9/l	3.7	2.9	2.0	2.2
Neutrophils 10^9/l	2.2	1.8	0.9	0.9
Platelets	166	160	140	155

[0187]   The third course was delayed because of neutropenia.

8.2. Patients who received the chemotherapy together with Dicarbamine

[0188]   51 year old female, diagnosis: stage III ovarian cancer; she received the first course of the chemotherapy according to therapy scheme as follows: 600 mg/m^2 Cyclophosphan and 400 mg/m^2 Carboplatin at day 1 of therapy. Dicarbamine was prescribed daily at dose 100 mg beginning 5 days prior to course 1 of the chemotherapy and then for 21 days. Period of therapy with Dicarbamine was 26 days before course 2.

[0189]   Complete blood analysis, course 1 of the chemotherapy with Dicarbamine

Parameter, units of measurement	Before starting Dicarbamine administration	Before starting course 1 of CT	After termination of Dicarbamine intake	After course 2 of CT
	Day "0"	Day 5	Day 21	Day 33
Leukocytes 10^9/l	5.9	5.5	4.7	4.0
Neutrophils 10^9/l	4.2	4.0	3.3	2.9
Platelets	170	164	160	158

[0190] Course 2 of the chemotherapy was conducted in time according to the scheme of therapy as follows: 600 mg/m^2 Cyclophosphan and 400 mg/m^2 Carboplatin once at day 28 after first course of the chemotherapy was conducted + Dicarbamine. Dicarbamine was administered at dose 100 mg 5 days prior to course 2 and then daily for 21 days. Total duration of Dicarbamine intake (2 courses of the chemotherapy) was 52 days.

[0191] Complete blood analysis, course 2 of the chemotherapy

Parameter, units of measurement	Before starting Dicarbamine administration	Before starting course 1 of CT	After termination of Dicarbamine intake	Before course 3 of CT
	Day 28 after course 1 of CT	Day 33	Day 54	Day 61
Leukocytes 10^9/l	4.9	5.0	4.2	4.2
Neutrophils 10^9/l	3.2	3.3	3.1	3.0
Platelets	180	170	160	160

[0192] Third course of CT was given in time.

[0193] 75 year old female, diagnosis: stage III ovarian cancer, ascites; she received the chemotherapy with Dicarbamine according to therapy scheme as follows: 600 mg/m^2 Cyclophosphan and 400 mg/m^2 Carboplatin at day 1 of therapy. Dicarbamine was prescribed at dose 100 mg daily beginning 5 days prior to course 1 of CT and then for 21 days. Period of therapy with Dicarbamine was 26 days before course 2.

[0194] Complete blood analysis, course 1 of the chemotherapy with Dicarbamine

Parameter, units of measurement	Before starting Dicarbamine administration	Before starting course 1 of CT	After termination of Dicarbamine intake	Before course 2 of CT
	Day "0"	Day 5	Day 21	Day 33
Leukocytes 10^9/l	7.4	7.2	6.6	5.2
Neutrophils 10^9/l	5.7	5.0	5.2	3.8
Platelets	174	165	162	167

[0195] Course 2 of the chemotherapy was conducted in time according to the scheme of therapy as follows: 600 mg/m^2 Cyclophosphan and 400 mg/m^2 Carboplatin once at day 28 after the first course of the chemotherapy was conducted + Dicarbamine. Dicarbamine was administered at dose 100 mg 5 days prior to course 2 and then daily for 21 days. Total duration of Dicarbamine intake (2 courses of the chemotherapy) was 52 days.

[0196] Complete blood analysis, course 2 of the chemotherapy

Parameter, units of measurement	Before starting Dicarbamine administration	Before starting course 1 of CT	After termination of Dicarbamine intake	Before course 3 of CT
	Day 28 after course 1 of CT	Day 33	Day 54	Day 61
Leukocytes 10^9/l	7.8	8.2	7.6	7.2
Neutrophils 10^9/l	5.2	6.0	6.2	5.8
Platelets	165	160	162	157

[0197] The third course of CT was given at term.

[0198] 65 year old female, diagnosis: stage IV ovarian cancer, ascites, metastatic involvement of the umbilical region; she received the chemotherapy with Dicarbamine according to therapy scheme as follows: 600 mg/m^2 Cyclophosphan and 400 mg/m^2 Carboplatin at day 1 of therapy. Dicarbamine was prescribed at dose 100 mg daily beginning 5 days prior to course 1 of CT and then for 21 days. Period of therapy with Dicarbamine was 26 days before course 2.

[0199] Complete blood analysis, course 1 of the chemotherapy with Dicarbamine

Parameter, units of measurement	Before starting Dicarbamine administration	Before starting course 1 of CT	After termination of Dicarbamine intake	Before course 2 of CT
	Day "0"	Day 5	Day 21	Day 33
Leukocytes 10^9/l	6.6	5.9	5.5	5.0
Neutrophils 10^9/l	5.0	4.2	4.4	3.4
Platelets	170	172	166	164

[0200] Course 2 of the chemotherapy was conducted in time according to the scheme of therapy as follows: 600 mg/m^2 Cyclophosphan and 400 mg/m^2 Carboplatin once at day 28 after the first course of the chemotherapy was conducted + Dicarbamine. Dicarbamine was administered at dose 100 mg 5 days prior to course 2 and then daily for 21 days. Total duration of Dicarbamine intake (2 courses of the chemotherapy) was 52 days.

[0201] Complete blood analysis, course 2 of the chemotherapy

Parameter, units of measurement	Before starting Dicarbamine administration	Before starting course 1 of CT	After termination of Dicarbamine intake	Before course 3 of CT
	Day 28 after course 1 of CT	Day 33	Day 54	Day 61
Leukocytes 10^9/l	5.6	5.8	5.7	5.5
Neutrophils 10^9/l	3.0	3.2	3.4	3.2
Platelets	170	170	176	165

[0202] The third course of CT was given at term.

8.3. Comparative data on hematological toxicity in patients who received the chemotherapy and those who received or not received Dicarbamine are shown in Tables 16 and 17.

[0203]

Table 16. Number (%) of patients with hematological toxicity who received the chemotherapy without Dicarbamine

Type of toxicity	Number of CT courses	Degree of hematological toxicity according to WHO					
		0	I	II	III	IV	III+IV
Leukopenia	26	3 11.5%	5 19.2%	12 46.1%	5 19.2%	1 3.8%	6 23.07%
Neutropenia	26	7 26.9%	0	8 30.7%	6 23.07%	5 19.2%	11 42.3%
Thrombocytopenia	25	10 40.0%	3 12.0%	7 28.0%	4 16.0%	1 4.0%	5 20.0%

Table 17. Number (%) of patients with hematological toxicity who received the chemotherapy with Dicarbamine

Type of toxicity	Number of CT courses	Degree of hematological toxicity according to WHO					
		0	I	II	III	IV	III+IV
Leukopenia	77 100%	6 7.7%	18 23.3%	43 55.8%	10 12.9%	0	10 12.9%
Neutropenia	67 100%	21 31.3%	12 17.9%	23 34.3%	5 7.4%	6 8.9%	11 16.4%

(continued)

Type of toxicity	Number of CT courses	Degree of hematological toxicity according to WHO					
		0	I	II	III	IV	III+IV
Thrombocytopenia	76 100%	27 35.5%	32 42.1%	10 13.1%	6 7.8%	1 1.3%	7 9.1%

[0204] The data obtained show that the limiting hematological toxicity of stage III-IV without use of Dicarbamine (table 16) achieves on the basis of leukopenia over 23.0%, on the basis of neutropenia 42.3% and by thrombocytopenia 20.0%.

[0205] In the group of patients who received Dicarbamine the occurrence rate of leuko-, neutro- and thrombocytopenia was significantly lower (Table 17). Hematological toxicity on the basis of leukopenia lowered down to 12.9%, i.e. 1.8-fold, on the basis of neutropenia 2.6-fold and on the basis of thrombocytopenia 2.2-fold. Thus the use Dicarbamine resulted in reducing all the listed kinds of hematological toxicity.

[0206] Below the data are presented supporting the fact that administration of Dicarbamine does not lower therapy efficacy with cytostatic agents but on the contrary enhances to some extent the effect achieved.

[0207] Efficacy of therapy was assessed in groups of patients following two courses of the chemotherapy with or without Dicarbamine according to the above described scheme. Efficacy was assessed according to the generally accepted parameters: CR - complete remission, PR - partial remission, SB - stabilization; and Progr. - progression.

[0208] The data obtained are presented in Table 18.

Table 18. Efficacy of treating patients according to scheme Cyclophosphane + Carboplatin with Dicarbamine

Groups of patients	Number of patients	CR	PR	SB	Progr.
The chemotherapy	6 100.0%	2 33.3%	1 16.6%	2 33.5%	1 16.6%
The chemotherapy + Dicarbamine	15 100.0%	4 26.6%	7 46.6%	2 13.5%	2 13.3%

[0209] The data presented show that in the group of patients who received the chemotherapy without Dicarbamine, tumor growth control (CR+PR) amounts to 49.9%. In the group of patients who received the chemotherapy with Dicarbamine efficacy of therapy is 73.2%.

[0210] Thus the use of Dicarbamine in treating patients who receive the chemotherapy results in reducing main kinds of hematological toxicity without decrease in the efficacy of therapy.

[0211] The testal and clinical data presented above evidently prove the efficacy of peptide derivatives of general formula (I) as non-specific inductors of differentiation that when using peptide derivatives together with myelosuppressive the chemotherapy is showed by reducing the degree and number of neutropenias and in use alone results in growth stabilization of murine hemoblastosis, of differentiating murine and human melanoma including the case of absent efficacy of the chemotherapy.

[0212] The effect of peptide derivatives of general formula (I) on tumor growth was shown to be associated with delay of proliferation activity of tumor cells and raised degree of differentiation in particular melanin synthesizing capability of melanoma cells and differentiation induction of Friend erythroblastosis precursor cells.

[0213] Clinical investigations revealed the properties of peptide derivatives of general formula (I) to significantly lower hematological toxicity in treating cancer patients using different schemes of combined the chemotherapy. Thus when treating patients suffering from ovarian cancer with platinum preparations (Cyclophosphane) along with peptide derivatives, the degree

of limiting neutropenia and thrombocytopenia lowered 2-3-fold. At the same time efficacy of therapy did not lower.

Table 4. The effect of Dicarbamine on M-6 human melanoma growth in nude mice

Day after inoculation	Control n=7		Dicarbamine 1.5 mg/kg n=10			Dicarbamine 4.5 mg/kg n=10		
	M+m*		M+m			M+m		
	V	%	V	%	p**	V	%	P
18 day	66.2+2.8	100	21.8+12.8	100		91.9+54.4	100	
25 day	266.0+69.4	329.0+88.9	77.5+46.4	302.0+186.0	0.82	266.0+198.0	166.0+93.0	0.015
33 day	582.0+127.4	132.9+57.3	342.0+142.0	428.0+313.1	0.11	852.0+495.0	276.0+104.0	0.011
39 day	701.0+123.5	21.9+12.6	435.0+187.0	23.2+22.1	0.92	1129.0+600.0	39.8+27.3	0.169
46 day	778.0+148.4	10.5+8.1	662.0+417.0	23.9+31.0	0.45	1354.0+735.0	19.6+17.5	0.276
53 day	821.0+221.8	3.8+10.3	783.0+423.0	18.6+54.0	0.43	1550.0+780.0	4.2+22.5	0.538

* - average value with standard deviation,
** - significance calculation was done only for the data of percent change in tumor volume

Table 12. Dynamics of mice peripheral blood leukocytes formula under Cyclophosphamide effect in combination with Dicarbamine

Formed elements	Group 1						
	Days post Cyclophosphamide administration						
	3	5	7	10	13	17	21
Myelocytes	0	0	0,4/54±0,4	0	0	0	0
Young	0	0	1.4/188±148	0.4/48±3	0	0	0
Band	3.2/89±16	4.6/370±13	4.8/660±223	5.7/620±260	2.2/293±146	1.6/198±150	2.7/286±214
Segmented	10.4/290±210	17.6/1400±	31.4/4200±960	27/3200±450	24/3190+440	19.6/2430±545	18.8/2444±585
Eosinophils	1.2/34±15	1.2/95.5±44	0	0	1.2/290±73	2.6/322±93	1.4/182±72
Monocytes	6.2/172±62	7.2/570±17	5.4/724±74	5.2/690±130	4.6/612±219	5.4/60±150	4.0/520±143
Lymphocytes	75.6/2120±310	69.4/5520±	56.2/753 0±1250	65.4/7780±450	67.8/9017±580	70-8/8780±545	73.8/9594±585
Group 2							
Myelocytes	0	0.6/26±5	0	0	0	0	0
Young	0.414±1	1.6/70±24	0.6/63±12	0.4/26±5	0	0	0
Band	1.2/13±3	4.2/184±48	3.6/378±41	3.2/206±71	2.2/2681134	1.6/195±67	1.4/167±67
Segmented	5.2/55±0.6	16/700±240	35.2/3700±705	23.7/1494±390	18.2/2220±470	20.2/2460±37	19.6/2330±714
Eosinophils	0.4/4±1	0.4/17±5	0	1.0/64±35	1.4/170±134	1.4/170±79	1.6/190±130
Monocytes	4.649±17.5	6.4/280±48	4.8/504±115	5.4/350±160	3.8/464±134	4.4/537±134	4.6/547±65
Lymphocytes	88.2/935±23	71/3110±217	55.8/6860±750	65.8/4240±708	75.0/9150±402	71.6/8740±604	71.6/8520±785

Table presents %/absolute amount in mm^3

Table 13. Cellularity* of murine bone marrow under effect of Cyclophosphamide and Cyclophosphamide in combination with Dicarbamine

Group No	Days after Cyclophosphamide administration						
	3	5	7	10	13	17	21
1	14.5±3.43	21.55±1.92	32.35 ±3.57	33.8±3.85	32.6±5.22	28.25±3.02	30.4±2.75
2	8.2±1.65	13.05±2.75	25.22±2.75	27.9±2.75	30.15±6.6	25.0±3.16	26.55±4.53
* - number of cells in millions							

Table 14. The effect of peptide derivatives on tumor size in mice with Friend erythroblastosis

Compound	Single dose (mg/kg) in daily dosing for 5 days	Average tumor volumes at days after termination of therapy			Tumor growth inhibition, %		
		8	13	19			
Control	-	342[139÷545]	706[457÷961]	777[199÷1355]			
1	1.5	157[73÷241]	284[197÷371]	318[136÷500]	54	60*	63
2	1.5	130[68÷192]	367[105÷629]	367[105÷629]	62	48	57
Control**	-	249[150÷348]	678[373÷983]	645[385÷905]	-	-	-
3	1.5**	77[52÷102]	219[104÷334]	368[193÷543]	69*	68*	43
Dicarbamine	1.5	96[37÷155]	150[87÷213]	290[103÷477]	61	78*	55
	4.5**	129[67÷191]	300[130÷470]	485[1354÷835]	62	58	38
Notes: * - difference from the control is significant at p<0.05 ** - the second test							

Table 15. The effect of peptide derivatives on tumor growth rate in mice with Friend erythroblastosis

Compound	Single dose (mg/kg), daily dosing for 5 days	Relative tumor volumes at days after tumor inoculation along with daily Dicarbamine intake V_t/V1			Tumor growth inhibition, %		
		8	13	19			
Control	-	1,0	2,0	2.3			
1	1.5	1.0	1.8	2.0	54	60**	63
2	1.5	1.0	2.8	2.8	62	48	57
Control***	1.5	1.0	2.72	2.59	-	-	-
3	1.5***	1.0	2.8	4.78	69**	68**	43
Dicarbamine	1.5	1.0	1.56	3.0	61	78**	55
	45***	1.0	2.3	3.76	62	58	38
Notes: * - p<0.05 ** - the second test							

Claims

1. A peptide derivative of general formula

$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3$$
$$|$$
$$R_2$$

(I)

wherein:

- n=0-4, m=1-4, k=0-1;
- R_1 is a C_1-C_3 hydrocarbon radical substituted by a functional group selected from amino, C_1-C_5 amido- or carboxylic groups, the carboxylic group being optionally esterified; or
- R_1 is a C_1-C_3 hydrocarbon radical simultaneously substituted by (a) an amino group which is optionally substituted by an acyl substituent and (b) a carboxylic group; or
- R_1 is a C_1-C_3 hydrocarbon radical substituted by a 5-6-membered unsaturated heterocyclic group, wherein the hydrocarbon radical can simultaneously comprise an amino group optionally substituted by an acyl substituent; or
- R_1 is a saturated heterocyclic group;
- R_2 is hydrogen atom or a functional group selected from carboxyl, that can be esterified;
- R_3 is 5-6-membered saturated or unsaturated cyclic or heterocyclic group, or an amino- or carboxyl group, the carboxyl group being optionally esterified;
or a pharmaceutically acceptable salt thereof, for use in the treatment of hemoblastosis or melanoma.

2. A peptide derivative of general formula
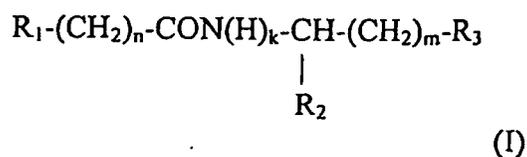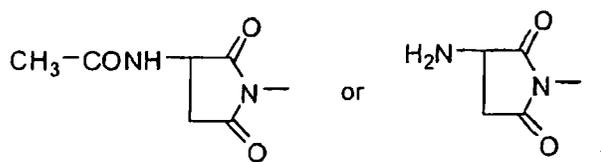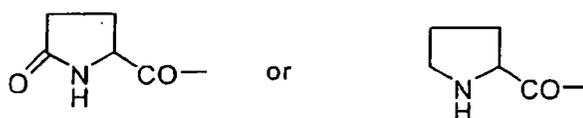
$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3$$
$$|$$
$$R_2$$

(I)

wherein:

- n is 0, k is 0 and R_1-CON- is

or
- n is 0, k is 1 and R_1-CO- is

- R_2 is H, COOH or $COOCH_3$;
- R_3 is
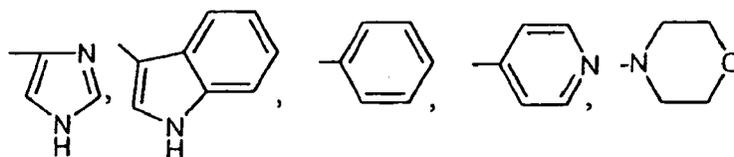
NH_2, -COOH, or -COOCH$_3$; and
- m is 1-4,
or a pharmaceutically acceptable salt thereof, for use in the treatment of hemoblastosis or melanoma.

3. A peptide derivative of general formula (I)

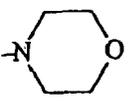$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3$$
$$|$$
$$R_2$$
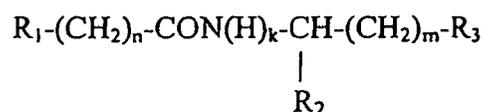
wherein R$_1$, n, k, R$_2$, m and R$_3$ are as defined in claim 1 or 2, or a pharmaceutically acceptable salt thereof, for use in reducing neutropenia caused by myelosuppresive therapy.

4. A compound for use according to any one of claims 1 to 3, wherein n = 0-4,
k = 0- 1,
R$_1$ = NH$_2$CH$_2$-, HOOC-CH$_2$-, CH$_3$CONH-CH$_2$-, CH$_3$OCO-CH$_2$-,
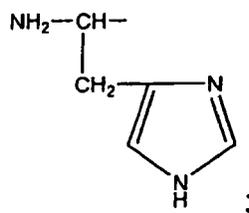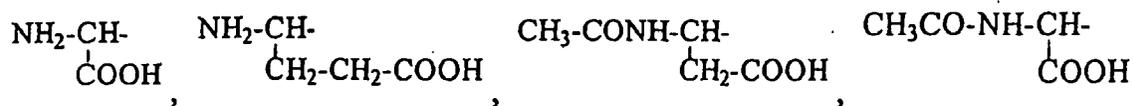
or, when n is 0 and k is 0,
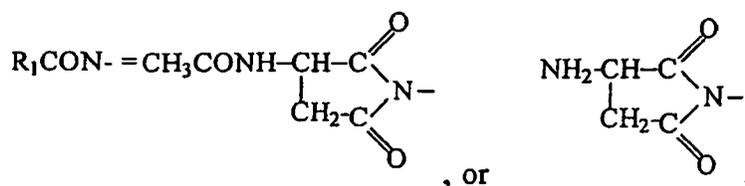
or, when n is 0 and k is 1,

$R_1CO- =$

$R_1CO- =$

, or ,

$R_2 = H, COOH, COOCH_3;$

$R_3 =$

, , , , ,
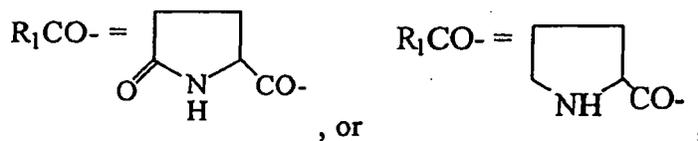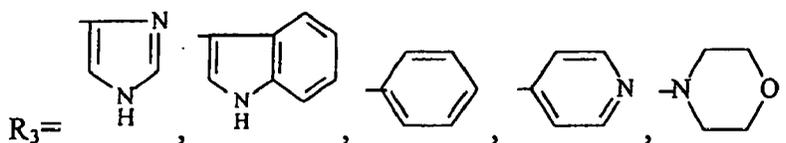
$NH_2, COOH,$
$-COOCH_3,$
and m=1-4.

5. A compound for use according to any one of the preceeding claims, wherein the peptide derivative is a compound of the formula

$CH_2CH_2NHCO(CH_2)_3COOH$
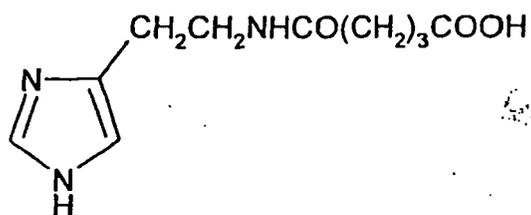
or a pharmaceutically acceptable salt thereof.

6. A peptide derivative according to claim 3, which has the formula
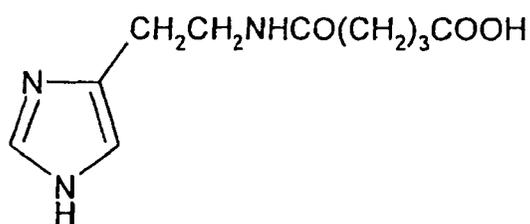
$CH_2CH_2NHCO(CH_2)_3COOH$

or a pharmaceutically acceptable salt thereof, for use in reducing neutropenia and thrombocytopenia caused by chemotherapy.

7. A peptide derivative of general formula (I),
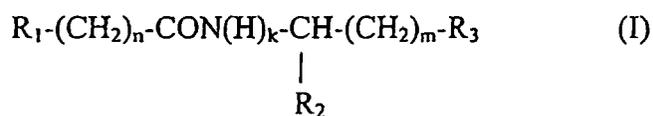
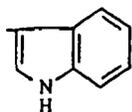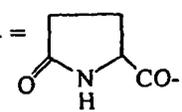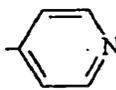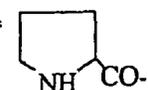$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \qquad (I)$$
$$|$$
$$R_2$$

wherein R_1, n, k, R_2, m and R_3 are as defined below, or a pharmaceutically acceptable salt thereof, for use in reducing neutropenia caused by myelosuppressive chemotherapy,

Compound Number	R_1	n	k	R_2	m	R_3
1	NH_2-CH- 　　　COOH	2	1	H	1	 (-4-Im)
2	HOOC-CH_2-	2	1	H	1	-4-Im
3	HOOC-CH_2-	1	1	H	1	-4-Im
4	NH_2-CH- 　　　COOH	2	1	-COOH	1	-4-Im
5	NH_2-CH- 　　　CH_2-CH_2-COOH	0	1	H	1	-4-Im
6	HOOC-CH_2-	2	1	-$COOCH_3$	1	-4-Im
7	HOOC-CH_2-	1	1	-COOH	1	-4-Im
8	NH_2-CH_2-	2	1	-COOH	1	-4-Im
9	NH_2-CH_2-	2	1	-$COOCH_3$	1	-4-Im
11	NH_2-CH_2-	1	1	H	1	-4-Im
12	NH_2-CH_2-	2	1	H	1	-4-Im
13	HOOC-CH_2-	2	1	-COOH	1	-4-Im
14	CH_3-CONH-CH- 　　　　　　CH_2-COOH	0	1	H	1	-4-Im
15	R_1CON- = CH_3CONH-CH-C(=O)(N-)-CH_2-C(=O)	0	0	H	1	-4-Im
16	R_1CON- = NH_2-CH-C(=O)(N-)-CH_2-C(=O)	0	0	H	1	-4-Im
17	CH_3CONH-CH_2-	2	1	H	1	-4-Im
18	NH_2-CH- 　　　COOH	1	1	H	1	-4-Im
19	CH_3OCO-CH_2-	2	1	H	1	-4-Im

(continued)

Compound Number	R$_1$	n	k	R$_2$	m	R$_3$
20	NH$_2$-CH- COOH	2	1	COOH	1	(-3-Ind)
21	NH$_2$-CH- CH$_2$-CH$_2$-COOH	0	1	H	1	-3-Ind
22	HOOC-CH$_2$-	2	1	-COOH	1	-3-Ind
23	NH$_2$-CH$_2$-	2	1	-COOH	1	-3-Ind
24	R$_1$CO- =	0	1	H	1	-3-Ind
25	COOH-CH$_2$-	2	1	H	1	-3-Ind
26	NH$_2$-CH- COOH	2	1	H	1	-3-Ind
27	NH$_2$-CH$_2$-	2	1	H	1	-C$_6$H$_5$
28	NH$_2$-CH$_2$-	2	1	H	1	(-Py)
29	NH$_2$-CH- COOH	2	1	H	1	-Py
30	HOOC-CH$_2$-	2	1	H	1	-Py
31	HOOC-CH$_2$-	2	1	-COOH	4	-NH$_2$
32	NH$_2$-CH- CH$_2$-4Im	0	1	H	1	-COOH
33	R$_1$CO- =	0	1	H	1	-4-Im
34	HOOC-CH$_2$-	2	1	H	1	
35	NH$_2$-CH- CH$_2$-4Im	0	1	H	1	-COOCH$_3$
36	CH$_3$CO-NH-CH- COOH	2	1	H	1	-4-Im
37	CH$_3$CONH-CH$_2$-	1	1	-COOH	1	-4-Im
38	NH$_2$-CH$_2$-	4	1	H	1	-4-Im

8. A peptide derivative for use according to claim 1 or 2, which has the general formula (I),
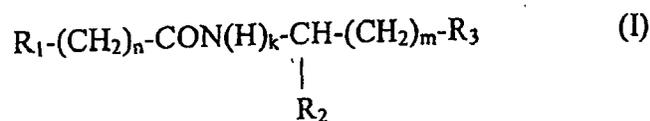
$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \qquad (I)$$
$$\underset{R_2}{|}$$

wherein R_1, n, k, R_2, m and R_3 are as defined below, or a pharmaceutically acceptable salt thereof, for use in the treatment of hemablastosis or melanoma, in combination with interferon, for enhancing the efficacy of the immuno-therapy,

Compound number	R_1	n	k	R_2	m	R_3
1	NH_2-CH- COOH	2	1	H	1	(-4-Im)
2	$HOOC\text{-}CH_2$-	2	1	H	1	-4-Im
3	$HOOC\text{-}CH_2$-	1	1	H	1	-4-Im
4	NH_2-CH- COOH	2	1	-COOH	1	-4-Im
5	NH_2-CH- CH_2-CH_2-COOH	0	1	H	1	-4-Im
6	$HOOC\text{-}CH_2$-	2	1	$-COOCH_3$	1	-4-Im
7	$HOOC\text{-}CH_2$-	1	1	-COOH	1	-4-Im
9	$NH_2\text{-}CH_2$-	2	1	$-COOCH_3$	1	-4-Im
11	$NH_2\text{-}CH_2$-	1	1	H	1	-4-Im
12	$NH_2\text{-}CH_2$-	2	1	H	1	-4-Im
13	$HOOC\text{-}CH_2$-	2	1	-COOH	1	-4-Im
14	CH_3-CONH-CH- CH_2-COOH	0	1	H	1	-4-Im
15		0	0	H	1	-4-Im
16		0	0	H	1	-4-Im
17	$CH_3CONH\text{-}CH_2$-	2	1	H	1	-4-Im

(continued)

Compound number	R₁	n	k	R₂	m	R₃
18	NH_2-CH- $COOH$	1	1	H	1	-4-Im
19	$CH_3OCO-CH_2-$	2	1	H	1	-4-Im
20	NH_2-CH- $COOH$	2	1	COOH	1	(-3-Ind)
21	NH_2-CH- CH_2-CH_2-COOH	0	1	H	1	-3-Ind
22	$HOOC-CH_2-$	2	1	-COOH	1	-3-Ind
23	NH_2-CH_2-	2	1	-COOH	1	-3-Ind
24	$R_1CO- =$	0	1	H	1	-3-Ind
25	$COOH-CH_2-$	2	1	H	1	-3-Ind
26	NH_2-CH- $COOH$	2	1	H	1	-3-Ind
27	NH_2-CH_2-	2	1	H	1	$-C_6H_5$
28	NH_2-CH_2-	2	1	H	1	(-Py)
29	NH_2-CH- $COOH$	2	1	H	1	-Py
30	$HOOC-CH_2-$	2	1	H	1	-Py
31	$HOOC-CH_2-$	2	1	-COOH	4	$-NH_2$
32	NH_2-CH- CH_2-4Im	0	1	H	1	-COOH
33	$R_1CO- =$	0	1	H	1	-4-Im
34	$HOOC-CH_2-$	2	1	H	1	
35	NH_2-CH- CH_2-4Im	0	1	H	1	$-COOCH_3$
36	$CH_3CO-NH-CH-$ $COOH$	2	1	H	1	-4-Im

36

(continued)

| Compound number	R₁	n	k	R₂	m	R₃
37	CH_3CONH-CH_2-	1	1	-COOH	1	-4-Im
38	NH_2-CH_2-	4	1	H	1	-4-Im

9. A peptide derivative of formula
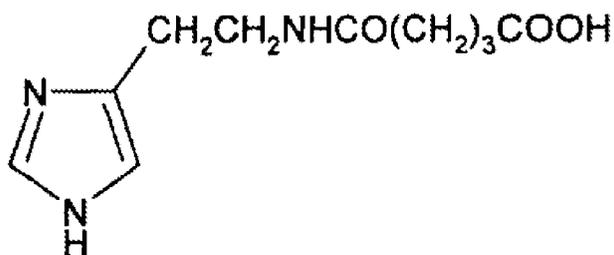
or a pharmaceutically acceptable salt thereof, for use in immunotherapy against malignant tumours, in combination with interferon, for improving the efficacy of the immunotherapy.

Patentansprüche

1. Peptidderivat der allgemeinen Formel

$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \ (I),$$
$$|$$
$$R_2$$
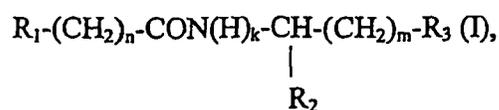
worin
n= 0-4, m=1-4, k=0-1;
R₁ ein C₁-C₃ _ Kohlenwasserstoffrest ist, welcher durch eine funktionelle Gruppe, ausgewaehlt aus Amino-, C₁-C₅-Amido- oder Carboxylgruppen substituiert ist, wobei die Carboxylgruppe verestert werden kann; oder
R₁ ein C₁-C₃-Kohlenwasserstoffrest ist, gleichzeitig substituiert durch (a) eine Aminogruppe, wobei die Aminogruppe durch einen Acylsubstituenten substituiert werden kann, und durch (b) eine Carboxylgruppe, oder
R₁ ein C₁-C₃-Kohlenwasserstoffrest ist, welcher durch eine 5 bis 6-gliedrige ungesaettigte heterocyclische Gruppe substituiert ist, wobei der Kohlenwasserstoffrest gleichzeitig eine Aminogruppe enthalten kann, welche durch einen Acylsubstituenten nicht obligatorisch substituiert ist; oder
R₁ eine gesaettigte heterocyclische Gruppe ist;
R₂ ein Wasserstoffatom oder eine funktionelle Gruppe ausgewaehlt aus Carboxyl, welches verestert werden kann, ist;
R₃ eine 5 bis 6-gliedrige gesaettigte oder ungesaettigte cyclische oder heterocyclische Gruppe, oder eine Amino- oder Carboxylgruppe ist, wobei die Carboxylgruppe verestert werden kann; oder sein pharmazeutisch annehmbares Salz fuer die Verwendung zur Behandlung eines Melanoms oder Hemoblastosen.
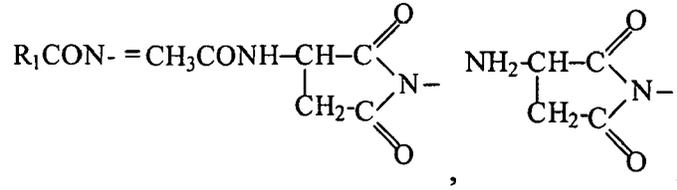
2. Peptidderivat der allgemeinen Formel:

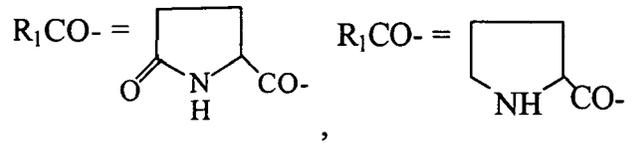$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \text{ (I)},$$

$$R_2$$

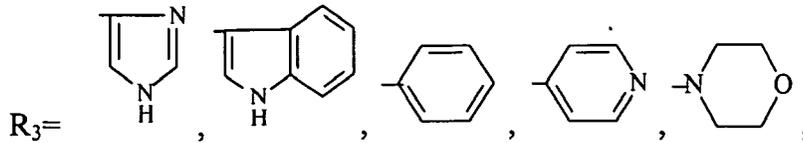worin n=0, k=0 und

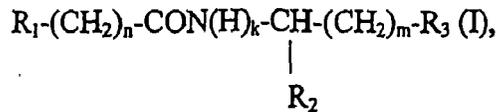oder n=0, k=1 und

R_2=H, COOH, COOCH$_3$;

R_3=
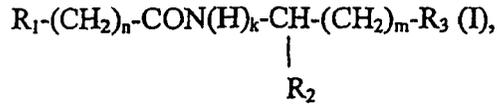
NH$_2$, COOH, -COOCH$_3$, und m=1-4 oder dessen pharmazeutisch annehmbares Salz fuer die Verwendung zur Behandlung eines Melanoms und Hemoblastosen.
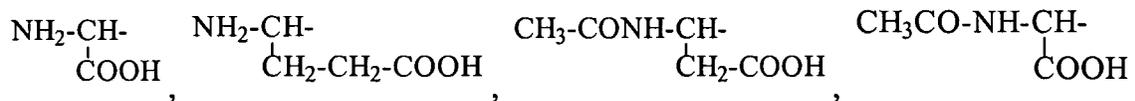
3. Peptidderivat der allgemeinen Formel (I),

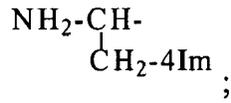$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \text{ (I)},$$

$$R_2$$

worin R$_1$, n, k, R$_2$, m und R$_3$ die im Anspruch 1 oder 2 definierten Bedeutungen annehmen oder dessen pharmazeutisch annehmbares Salz zur Linderung der Neutropenie, welche durch eine Myelomsuppressivtherapie verursacht ist.

4. Verbindung nach jedem derAnsprueche 1 bis 3 fuer die Verwendung, worin n=0-4; k=0-1; R$_1$=NH$_2$-CH$_2$-, HOOC-CH$_2$-, CH$_3$CONH-CH$_2$-, CH$_3$OCO-CH$_2$-,

$$NH_2\text{-}CH\text{-}$$
$$CH_2\text{-}4Im \quad ;$$

bei n=0 und k=0

$$R_1CON\text{-} = CH_3CONH\text{-}CH\text{-}C \overset{O}{\underset{CH_2\text{-}C \overset{}{\underset{O}{\diagdown}}}{\diagdown}} N\text{-} \quad , \quad NH_2\text{-}CH\text{-}C \overset{O}{\underset{CH_2\text{-}C \overset{}{\underset{O}{\diagdown}}}{\diagdown}} N\text{-} \quad ;$$

oder, wenn n=0 und k=1,

$$R_1CO\text{-} = \quad , \quad R_1CO\text{-} = \quad ,$$

R_2=H, COOH, COOCH$_3$;

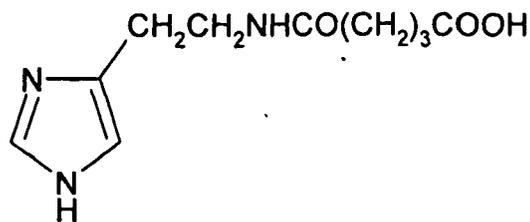$$R_3 = \quad , \quad , \quad , \quad , \quad ,$$
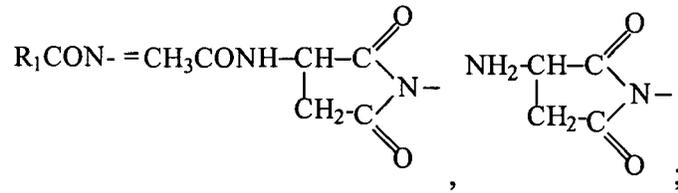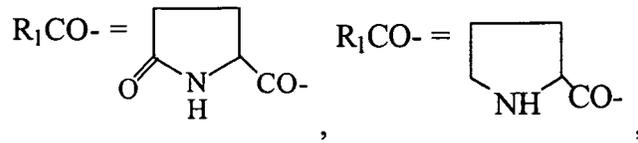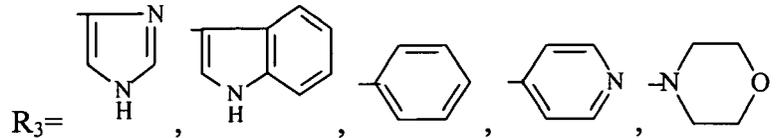
NH$_2$,COOH, -COOCH$_3$, und m=1-4.

5. Verbindung nach einem der vorhergehenden Ansprueche fuer die Verwendung, worin das Peptidderivat eine Verbindung der Formel
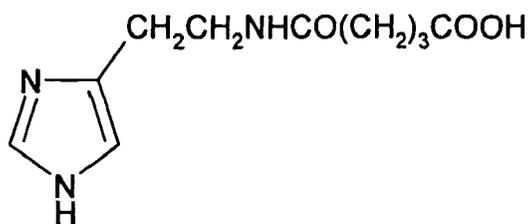
$$CH_2CH_2NHCO(CH_2)_3COOH$$

darstellt, oder deren pharmazeutisch annehmbares Salz.
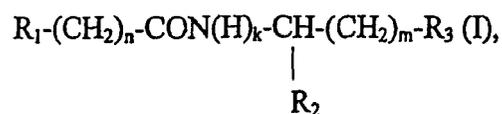
6. Peptidderivat nach Anspruch 3, welches die Formel

hat,

oder dessen pharmazeutisch annehmbares Salz fuer die Verwendung zur Linderung der Neutropenie und Throm-
bozytpenie, welche durch die Chemotherapie verursacht sind.

7. Peptidderivat der allgemeinen Formel (I),

$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \ (I),$$
$$|$$
$$R_2$$

worin R_1, n, k, R_2 und R_3 die unten definierten Bedeutungen annehmen, oder dessen pharmazeutisch annehmbares
Salz zur Linderung der Neuropenie, welche durch die Chemotherapie verursacht ist

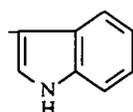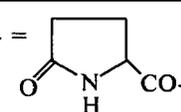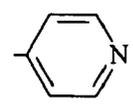| Nummer der Verbindung	R_1	n	k	R_2	m	R_3
1	$NH_2\text{-}CH\text{-}$ $COOH$	2	1	H	1	(-4-Im)
2	$HOOC\text{-}CH_2\text{-}$	2	1	H	1	-4-Im
3	$HOOC\text{-}CH_2\text{-}$	1	1	H	1	-4-Im
4	$NH_2\text{-}CH\text{-}$ $COOH$	2	1	-COOH	1	-4-Im
5	$NH_2\text{-}CH\text{-}$ $CH_2\text{-}CH_2\text{-}COOH$	0	1	H	1	-4-Im
6	$HOOC\text{-}CH_2\text{-}$	2	1	$-COOCH_3$	1	-4-Im
7	$HOOC\text{-}CH_2\text{-}$	1	1	-COOH	1	-4-Im
8	$NH_2\text{-}CH_2\text{-}$	2	1	-COOH	1	-4-Im
9	$NH_2\text{-}CH_2\text{-}$	2	1	$-COOCH_3$	1	-4-Im
11	$NH_2\text{-}CH_2\text{-}$	1	1	H	1	-4-Im
12	$NH_2\text{-}CH_2\text{-}$	2	1	H	1	-4-Im
13	$HOOC\text{-}CH_2\text{-}$	2	1	-COOH	1	-4-Im
14	$CH_3\text{-}CONH\text{-}CH\text{-}$ $CH_2\text{-}COOH$	0	1	H	1	-4-Im

(fortgesetzt)

Nummer der Verbindung	R_1	n	k	R_2	m	R_3
15	$R_1CON- =CH_3CONH-CH-C$ (mit Ringstruktur CH_2-C, $N-$, O)	0	0	H	1	-4-Im
16	$R_1CON- =NH_2-CH-C$ (mit Ringstruktur CH_2-C, $N-$, O)	0	0	H	1	-4-Im
17	$CH_3CONH-CH_2-$	2	1	H	1	-4-Im
18	NH_2-CH- / $COOH$	1	1	H	1	-4-Im
19	$CH_3OCO-CH_2-$	2	1	H	1	-4-Im
20	NH_2-CH- / $COOH$	2	1	COOH	1	(-3-Ind)
21	NH_2-CH- / CH_2-CH_2-COOH	0	1	H	1	-3-Ind
22	$HOOC-CH_2-$	2	1	-COOH	1	-3-Ind
23	NH_2-CH_2-	2	1	-COOH	1	-3-Ind
24	$R_1CO- =$ (Pyrrolidinon-Ring, $O=$, $N-H$, $CO-$)	0	1	H	1	-3-Ind
25	$COOH-CH_2-$	2	1	H	1	-3-Ind
26	NH_2-CH- / $COOH$	2	1	H	1	-3-Ind
27	NH_2-CH_2-	2	1	H	1	$-C_6H_5$
28	NH_2-CH_2-	2	1	H	1	(-Py)
29	NH_2-CH- / $COOH$	2	1	H	1	-Py
30	$HOOC-CH_2-$	2	1	H	1	-Py
31	$HOOC-CH_2-$	2	1	-COOH	4	$-NH_2$
32		0	1	H	1	-COOH

(fortgesetzt)

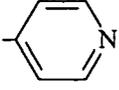| Nummer der Verbindung	R$_1$	n	k	R$_2$	m	R$_3$
33	R$_1$CO- = (Pyrrolidin: NH–CO-)	0	1	H	1	-4-Im
34	HOOC-CH$_2$-	2	1	H	1	(Morpholin: –N O)
35	NH$_2$-CH- / CH$_2$-4Im	0	1	H	1	-COOCH$_3$
36	CH$_3$CO-NH-CH- / COOH	2	1	H	1	-4-Im
37	CH$_3$CONH-CH$_2$-	1	1	-COOH	1	-4-Im
38	NH$_2$-CH$_2$-	4	1	H	1	-4-Im

8. Peptidderivat nach Anspruch 1 oder 2 fuer die Verwendung, welches eine allgemeine Formel (1) hat,

$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \ (I),$$
$$|$$
$$R_2$$
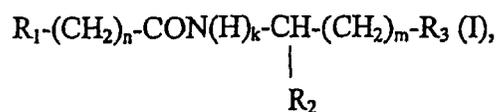
worin R$_1$, n, k, R$_2$, m und R$_3$ die unten angegebenen Bedeutungen annehmen oder dessen pharmazeutisch an-nehmbares Salz fuer den Einsatz zur Behandlung von Hemoblastosen oder eines Melanoms in Kombination mit Interferon zur Erhoehung der Effektivitaet der Immunotherapie

Nummer der Verbindung	R$_1$	n	k	R$_2$	m	R$_3$
1	NH$_2$-CH- / COOH	2	1	H	1	(Imidazol) (-4-Im)
2	HOOC-CH$_2$-	2	1	H	1	-4-Im
3	HOOC-CH$_2$-	1	1	H	1	-4-Im
4	NH$_2$-CH- / COOH	2	1	-COOH	1	-4-Im
5	NH$_2$-CH- / CH$_2$-CH$_2$-COOH	0	1	H	1	-4-Im
6	HOOC-CH$_2$-	2	1	-COOCH$_3$	1	-4-Im
7	HOOC-CH$_2$-	1	1	-COOH	1	-4-Im
8	NH$_2$-CH$_2$-	2	1	-COOH	1	-4-Im
9	NH$_2$-CH$_2$-	2	1	-COOCH$_3$	1	-4-Im

(fortgesetzt)

Nummer der Verbindung	R_1	n	k	R_2	m	R_3
11	NH_2-CH_2-	1	1	H	1	-4-Im
12	NH_2-CH_2-	2	1	H	1	-4-Im
13	$HOOC-CH_2-$	2	1	-COOH	1	-4-Im
14	$CH_3-CONH-CH-$ (mit CH_2-COOH)	0	1	H	1	-4-Im
15	$R_1CON-\ =CH_3CONH-CH-C(=O)-N-$ Ring mit $CH_2-C(=O)$	0	0	H	1	-4-Im
16	$R_1CON-\ =NH_2-CH-C(=O)-N-$ Ring mit $CH_2-C(=O)$	0	0	H	1	-4-Im
17	$CH_3CONH-CH_2-$	2	1	H	1	-4-Im
18	NH_2-CH- (mit $COOH$)	1	1	H	1	-4-Im
19	$CH_3OCO-CH_2-$	2	1	H	1	-4-Im
20	NH_2-CH- (mit $COOH$)	2	1	COOH	1	Indol (-3-Ind)
21	NH_2-CH- (mit CH_2-CH_2-COOH)	0	1	H	1	-3-Ind
22	$HOOC-CH_2-$	2	1	-COOH	1	-3-Ind
23	NH_2-CH_2-	2	1	-COOH	1	-3-Ind
24	$R_1CO-\ =$ (Pyrrolidindion-Ring mit $O=$, $N-H$, $CO-$)	0	1	H	1	-3-Ind
25	$COOH-CH_2-$	2	1	H	1	-3-Ind
26	NH_2-CH- (mit $COOH$)	2	1	H	1	-3-Ind
27	NH_2-CH_2-	2	1	H	1	$-C_6H_5$

(fortgesetzt)

| Nummer der Verbindung	R_1	n	k	R_2	m	R_3
28	NH_2-CH_2-	2	1	H	1	(-Py)
29	NH_2-CH- $\overset{|}{COOH}$	2	1	H	1	-Py
30	$HOOC-CH_2-$	2	1	H	1	-Py
31	$HOOC-CH_2-$	2	1	-COOH	4	$-NH_2$
32	NH_2-CH- $\overset{|}{CH_2-4Im}$	0	1	H	1	-COOH
33	$R_1CO- =$	0	1	H	1	-4-Im
34	$HOOC-CH_2-$	2	1	H	1	
35	NH_2-CH- $\overset{|}{CH_2-4Im}$	0	1	H	1	$-COOCH_3$
36	$CH_3CO-NH-CH-$ $\overset{|}{COOH}$	2	1	H	1	-4-Im
37	$CH_3CONH-CH_2-$	1	1	-COOH	1	-4-Im
38	NH_2-CH_2-	4	1	H	1	-4-Im

9. Peptidderivat der Formel

$$CH_2CH_2NHCO(CH_2)_3COOH$$
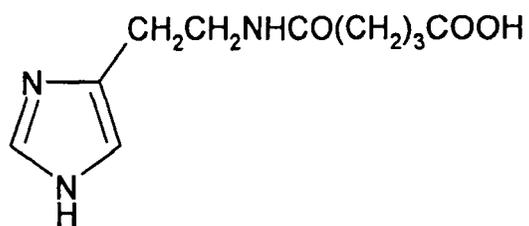
oder dessen pharmazeutisch annehmbares Salz fuer die Verwendung in der Immunotherapie von boesartigen Geschwulsten in Kombination mit Interferon zur Erhoehung der Effektivitaet der Immunotherapie.

Revendications

**1.** Dérivé d'un peptide de formule générale

$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \text{ (I)},$$
$$|$$
$$R_2$$
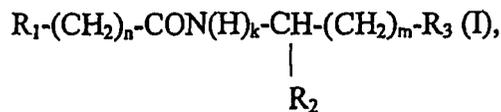
dans laquelle n= de 0 à 4, m= de 1 à 4, k= 0 à 1;

R_1 représente un radical hydrocarboné en C_1-C_3 substitué par un groupe choisi parmi un groupe aminé, amide en C_1-C_5 ou carboxylique, le groupe carboxylique étant éventuellement estérifié; soit

R_1 représente un radical hydrocarboné en C_1-C_3 substitué à la fois (a) par un groupe aminé, le groupe aminé, étant éventuellement substitué par un substituant acylique et (b) par un groupe carboxylique, soit

R_1 représente un radical hydrocarboné en C_1-C_3 substitué par un groupe hétérocyclique insaturé comprenant de 5 à 6 chaînons, le radical hydrocarboné comprenant éventuellement à la fois un groupe aminé éventuellement substitute par un substituant acylique; soit

R_1 représente un groupe hétérocyclique saturé;

R_2 représente un atome d'hydrogène ou un groupe choisi parmi un carboxyle éventuellement estérifié;

R_3 représente un groupe cyclique ou hétérocyclique saturé ou insaturé comprenant de 5 à 6 chaînons, ou un groupe aminé ou carboxylique, le groupe carboxylique étant éventuellement estérifié; ou son sel pharmaceutiquement acceptable pour une utilisation dans le traitement du mélanome ou des hémoblastoses.

2. Dérivé d'un peptide de formule générale

$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \text{ (I)},$$
$$|$$
$$R_2$$
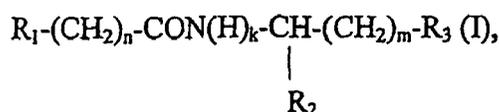
dans laquelle n=0, k=0 et
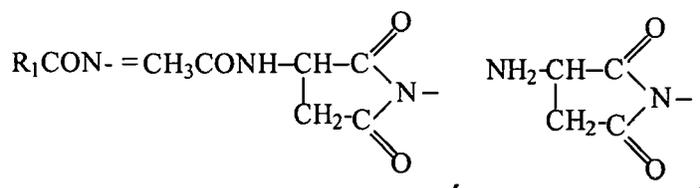
ou n=0, k=1 et

R_2=H, COOH, COOCH$_3$;

NH$_2$, COOH, -COOCH$_3$, et m=de 1 à 4 ou son sel pharmaceutiquement acceptable pour une utilisation dans le traitement du mélanome ou des hémoblastoses.

3. Dérivé d'un peptide de formule générale (I)
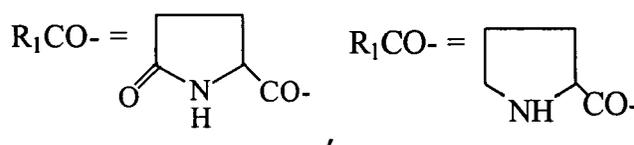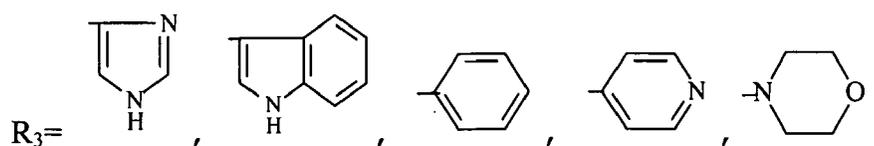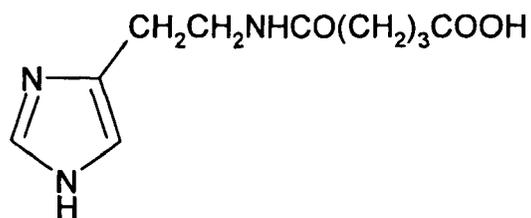
$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \; (I),$$
$$|$$
$$R_2$$

dans laquelle R_1, n, k, R_2, m et R_3 sont tels que définis dans les revendications 1 ou 2 ou son sel pharmaceutiquement acceptable pour atténuer la neutropénie provoquée par le traitement myélosupresseur.

4. Composé pour une utilisation selon l'une quelconque des revendications 1 à 3, où n= de 0 à 4, k= de 0 à 1; R_1=NH_2-CH_2-, HOOC-CH_2-, CH_3CONH-CH_2-, CH_3OCO-CH_2-,

$$NH_2\text{-}CH\text{-} \atop COOH \quad , \qquad NH_2\text{-}CH\text{-} \atop CH_2\text{-}CH_2\text{-}COOH \quad , \qquad CH_3\text{-}CONH\text{-}CH\text{-} \atop CH_2\text{-}COOH \quad , \qquad CH_3CO\text{-}NH\text{-}CH\text{-} \atop COOH$$

$$NH_2\text{-}CH\text{-} \atop CH_2\text{-}4Im \quad ;$$

n=0 et k=0

ou lorsque n=0 et k=1,

R_2=H, COOH, $COOCH_3$;

NH_2, COOH, -$COOCH_3$, et m= de 1 à 4.

5. Composé pour une utilisation selon l'une quelconque des revendications précédentes où le dérivé d'un peptide représente un composé de formule

$$CH_2CH_2NHCO(CH_2)_3COOH$$

ou son sel pharmaceutiquement acceptable.

6. Dérivé d'un peptide selon la revendication 3 qui répond à une formule
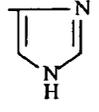
$$CH_2CH_2NHCO(CH_2)_3COOH$$
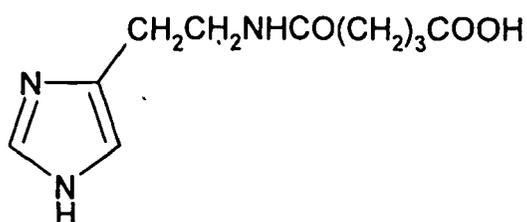
ou son sel pharmaceutiquement acceptable pour atténuer la neutropénie et la thrombocytopénie provoquées par la chimiothérapie.

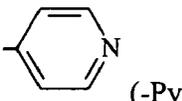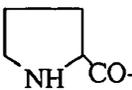**7.** Dérivé d'un peptide de formule générale (I)
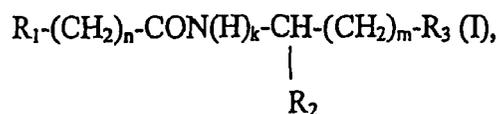
$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \text{ (I)},$$
$$|$$
$$R_2$$

dans laquelle R_1, n, k, R_2, m et R_3 sont tels que définis ci-dessous ou son sel pharmaceutiquement acceptable pour atténuer la neutropénie provoquée par la chimiothérapie.

No composé	R_1	n	k	R_2	m	R_3
1	$NH_2\text{-}CH\text{-}$ $\quad COOH$	2	1	H	1	(-4-Im)
2	$HOOC\text{-}CH_2\text{-}$	2	1	H	1	-4-Im
3	$HOOC\text{-}CH_2\text{-}$	1	1	H	1	-4-Im
4	$NH_2\text{-}CH\text{-}$ $\quad COOH$	2	1	-COOH	1	-4-Im
5	$NH_2\text{-}CH\text{-}$ $\quad CH_2\text{-}CH_2\text{-}COOH$	0	1	H	1	-4-Im
6	$HOOC\text{-}CH_2\text{-}$	2	1	$COOCH_3$	1	-4-Im
7	$HOOC\text{-}CH_2\text{-}$	1	1	-COOH	1	-4-Im

(suite)

No composé	R_1	n	k	R_2	m	R_3
8	NH_2-CH_2-	2	1	-COOH	1	-4-Im
9	NH_2-CH_2-	2	1	$COOCH_3$	1	-4-Im
11	NH_2-CH_2-	1	1	H	1	-4-Im
12	NH_2-CH_2-	2	1	H	1	-4-Im
13	$HOOC-CH_2-$	2	1	-COOH	1	-4-Im
14	$CH_3-CONH-CH-$ CH_2-COOH	0	1	H	1	-4-Im
15	$R_1CON- = CH_3CONH-CH-C$ (cyclic imide structure with CH_2-C, $N-$, two $=O$)	0	0	H	1	-4-Im
16	$R_1CON- = NH_2-CH-C$ (cyclic imide structure with CH_2-C, $N-$, two $=O$)	0	0	H	1	-4-Im
17	$CH_3CONH-CH_2-$	2	1	H	1	-4-Im
18	NH_2-CH- $COOH$	1	1	H	1	-4-Im
19	$CH_3OCO-CH_2-$	2	1	H	1	-4-Im
20	NH_2-CH- $COOH$	2	1	-COOH	1	(indole structure) (-3-Ind)
21	NH_2-CH- CH_2-CH_2-COOH	0	1	H	1	-3-Ind
22	$HOOC-CH_2-$	2	1	-COOH	1	-3-Ind
23	NH_2-CH_2-	2	1	-COOH	1	-3-Ind
24	$R_1CO- =$ (pyrrolidinone ring with $O=$, $N-H$, $CO-$)	0	1	H	1	-3-Ind
25	$COOH-CH_2-$	2	1	H	1	-3-Ind
26	NH_2-CH- $COOH$	2	1	H	1	-3-Ind
27	NH_2-CH_2-	2	1	H	1	$-C_6H_5$

(suite)

No composé	R$_1$	n	k	R$_2$	m	R$_3$
28	NH$_2$-CH$_2$-	2	1	H	1	(-Py)
29	NH$_2$-CH- | COOH	2	1	H	1	-Py
30	HOOC-CH$_2$-	2	1	H	1	-Py
31	HOOC-CH$_2$-	2	1	-COOH	4	-NH$_2$
32	NH$_2$-CH- | CH$_2$-4Im	0	1	H	1	-COOH
33	R$_1$CO- = NH CO-	0	1	H	1	-4-Im
34	HOOC-CH$_2$-	2	1	H	1	
35	NH$_2$-CH- | CH$_2$-4Im	0	1	H	1	-COOCH$_3$
36	CH$_3$CO-NH-CH- | COOH	2	1	H	1	-4-Im
37	CH$_3$CONH-CH$_2$-	1	1	-COOH	1	-4-Im
38	NH$_2$-CH$_2$-	4	1	H	1	-4-Im

8. Dérivé d'un peptide pour une utilisation selon la revendication 1 ou 2 qui répond à la formule générale (I).
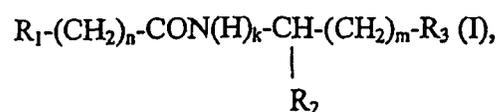
$$R_1\text{-}(CH_2)_n\text{-}CON(H)_k\text{-}CH\text{-}(CH_2)_m\text{-}R_3 \text{ (I)},$$
$$\underset{\displaystyle R_2}{|}$$

dans laquelle R$_1$, n, k, R$_2$, m et R$_3$ sont tels que définis ci-dessous ou son sel pharmaceutiquement acceptable pour une utilisation dans le traitement des hémoblastoses ou du mélanome en combinaison avec l'interféron pour rendre l'immunothérapie plus efficace
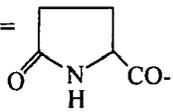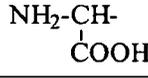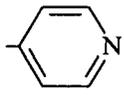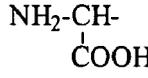
No com posé	R$_1$	n	k	R$_2$	m	R$_3$
1	NH$_2$-CH- | COOH	2	1	H	1	(-4-Im)
2	HOOC-CH$_2$-	2	1	H	1	-4-Im

(suite)

No composé	R$_1$	n	k	R$_2$	m	R$_3$
3	HOOC-CH$_2$-	1	1	H	1	-4-Im
4	NH$_2$-CH- / COOH	2	1	-COOH	1	-4-Im
5	NH$_2$-CH- / CH$_2$-CH$_2$-COOH	0	1	H	1	-4-Im
6	HOOC-CH$_2$-	2	1	COOCH$_3$	1	-4-Im
7	HOOC-CH$_2$-	1	1	-COOH	1	-4-Im
8	NH$_2$-CH$_2$-	2	1	-COOH	1	-4-Im
9	NH$_2$-CH$_2$-	2	1	-COOCH$_3$	1	-4-Im
11	NH$_2$-CH$_2$-	1	1	H	1	-4-Im
12	NH$_2$-CH$_2$-	2	1	H	1	-4-Im
13	HOOC-CH$_2$-	2	1	-COOH	1	-4-Im
14	CH$_3$-CONH-CH- / CH$_2$-COOH	0	1	H	1	-4-Im
15	R$_1$CON- = CH$_3$CONH–CH–C(=O)–N–(...)–CH$_2$-C(=O)	0	0	H	1	-4-Im
16	R$_1$CON- = NH$_2$–CH–C(=O)–N–(...)–CH$_2$-C(=O)	0	0	H	1	-4-Im
17	CH$_3$CONH-CH$_2$-	2	1	H	1	-4-Im
18	NH$_2$-CH- / COOH	1	1	H	1	-4-Im
19	CH$_3$OCO-CH$_2$-	2	1	H	1	-4-Im
20	NH$_2$-CH- / COOH	2	1	-COOH	1	(-3-Ind)
21	NH$_2$-CH- / CH$_2$-CH$_2$-COOH	0	1	H	1	-3-Ind
22	HOOC-CH$_2$-	2	1	-COOH	1	-3-Ind
23	NH$_2$-CH$_2$-	2	1	-COOH	1	-3-Ind

(suite)

No com posé	R$_1$	n	k	R$_2$	m	R$_3$
24	R$_1$CO- = (pyroglutamyl, O=C–N(H)–CH–CO-)	0	1	H	1	-3-Ind
25	COOH-CH$_2$-	2	1	H	1	-3-Ind
26	NH$_2$-CH- / COOH	2	1	H	1	-3-Ind
27	NH$_2$-CH$_2$-	2	1	H	1	-C$_6$H$_5$
28	NH$_2$-CH$_2$-	2	1	H	1	pyridine (-Py)
29	NH$_2$-CH- / COOH	2	1	H	1	-Py
30	HOOC-CH$_2$-	2	1	H	1	-Py
31	HOOC-CH$_2$-	2	1	-COOH	4	-NH$_2$
32	NH$_2$-CH- / CH$_2$-4Im	0	1	H	1	-COOH
33	R$_1$CO- = (proline, NH–CH–CO-)	0	1	H	1	-4-Im
34	HOOC-CH$_2$-	2	1	H	1	morpholine
35	NH$_2$-CH- / CH$_2$-4Im	0	1	H	1	-COOCH$_3$
36	CH$_3$CO-NH-CH- / COOH	2	1	H	1	-4-Im
37	CH$_3$CONH-CH$_2$-	1	1	-COOH	1	-4-Im
38	NH$_2$-CH$_2$-	4	1	H	1	-4-Im

9. Dérivé d'un peptide de formule

$$CH_2CH_2NHCO(CH_2)_3COOH$$

(imidazole ring, N / N–H)
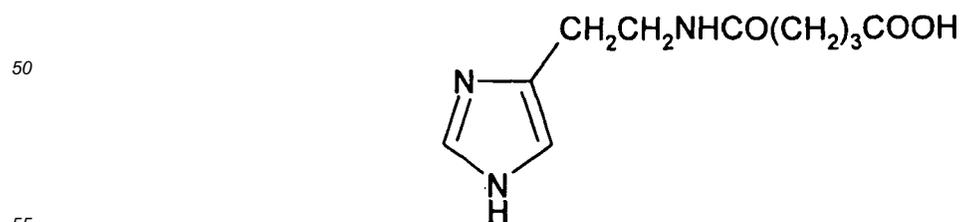
ou son sel pharmaceutiquement acceptable pour une utilisation dans l'immunothérapie des tumeurs malignes en combinaison avec l'interféron pour rendre l'immunothérapie plus efficace.

REFERENCES CITED IN THE DESCRIPTION

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