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(54) Titre : UTILISATION COMBINEE D'INHIBITEURS ENZYMATIQUES ET DE PREPARATIONS PHARMACEUTIQUES
CONSTITUEES DE CES INHIBITEURS POUR LE TRAITEMENT ET LA PROPHYLAXIE DE L'ARTERIOSCLEROSE,
POUR LE TRAITEMENT ET LA PREVENTION DE REACTIONS ALLERGIQUES DE TYPE I SELON LA
CLASSIFICATION DE GELL ET COOMBS ET POUR LE TRAITEMENT ET LA PREVENTION
(54) Title: USE OF INHIBITORS OF DIPEPTIDYL PEPTIDASE IV (DP IV) IN COMBINATION WITH INHIBITORS OF
ALANYL-AMINOPEPTIDASE (APN)

(57) **Abrégé/Abstract:**

Combined Use of Enzyme Inhibitors and of Pharmaceutical Compositions thereof for a Therapy and a Prophylactic Treatment of Arteriosclerosis, for a Therapy and Prevention of Allergic Reactions of the Type I according to the Gell and Coombs Classification and for a Therapy and Prevention of Dermatological Diseases with Follicular and Epidermal Hyperkeratoses and an Enhanced Proliferation of Keratinocytes The present invention relates to the use of inhibitors of dipeptidyl peptidase IV (DP IV) and of enzymes having the same substrate specificity (DP IV-analogous enzymatic activity) in combination with inhibitors of alanyl aminopeptidase (aminopeptidase N, APN) and of enzymes having the same substrate specificity (APN-analogous enzymatic activity) for a more than additive to superadditive inhibition of the activation and proliferation (DNA synthesis) of human T lymphocytes and mononuclear cells and of the production of T_{H2} cytokines for a therapy and prevention of allergic reactions of the type I according to the Gell and Coombs classification and for a more than additive to superadditive inhibition of the activation, proliferation (DNA synthesis) of human epidermal and follicular keratinocytes and of keratinocytes of the transitional zone between the skin and the mucous membranes as well as for a therapy and prevention of dermatological diseases with follicular and epidermal hyperkeratoses and an enhanced proliferation of keratinocytes. The invention also relates to the use of inhibitors of dipeptidyl peptidase IV (DP IV) as well as of enzymes having the same substrate specificity (DP IV- analogous enzymatic activity) in combination with inhibitors of alanyl aminopeptidase (aminopeptidase N, APN) and of enzymes having the same substrate specificity (APN-analogous enzymatic activity), respectively, of X-Pro-aminopeptidase (aminopeptidase P, APP), of the "angiotensin-converting enzyme" (ACE) and/or of the prolyl oligopeptidase (POP, prolyl endopeptidase, PEP) for a more than additive to superadditive inhibition of activation, DNA synthesis and proliferation of human T lymphocytes and mononuclear cells for a therapy and prophylaxis of arteriosclerosis. The invention also relates to pharmaceutical preparations comprising more than one of said inhibitors of enzymes of the above group.



Abstract

Combined Use of Enzyme Inhibitors and of Pharmaceutical Compositions thereof for a Therapy and a Prophylactic Treatment of Arteriosclerosis, for a Therapy and Prevention of Allergic Reactions of the Type I according to the Gell and Coombs Classification and for a Therapy and Prevention of Dermatological Diseases with Follicular and Epidermal Hyperkeratoses and an Enhanced Proliferation of Keratinocytes

The present invention relates to the use of inhibitors of dipeptidyl peptidase IV (DP IV) and of enzymes having the same substrate specificity (DP IV-analogous enzymatic activity) in combination with inhibitors of alanyl aminopeptidase (aminopeptidase N, APN) and of enzymes having the same substrate specificity (APN-analogous enzymatic activity) for a more than additive to superadditive inhibition of the activation and proliferation (DNA synthesis) of human T lymphocytes and mononuclear cells and of the production of T_H2 cytokines for a therapy and prevention of allergic reactions of the type I according to the Gell and Coombs classification and for a more than additive to superadditive inhibition of the activation, proliferation (DNA synthesis) of human epidermal and follicular keratinocytes and of keratinocytes of the transitional zone between the skin and the mucous membranes as well as for a therapy and prevention of dermatological diseases with follicular and epidermal hyperkeratoses and an enhanced proliferation of keratinocytes. The invention also relates to the use of inhibitors of dipeptidyl peptidase IV (DP IV) as well as of enzymes having the same substrate specificity (DP IV-analogous enzymatic activity) in combination with inhibitors of alanyl aminopeptidase (aminopeptidase N, APN) and of enzymes having the same substrate specificity (APN-analogous enzymatic activity), respectively, of X-Pro-aminopeptidase (aminopeptidase P, APP), of the "angiotensin-converting enzyme" (ACE) and/or of the prolyl oligopeptidase (POP, prolyl endopeptidase, PEP) for a more than additive to superadditive inhibition of activation, DNA synthesis and proliferation of human T lymphocytes and mononuclear cells for a therapy and prophylaxis of arteriosclerosis. The invention also relates to pharmaceutical preparations comprising more than one of said inhibitors of enzymes of the above group.

**USE OF INHIBITORS OF DIPEPTIDYL PEPTIDASE IV (DP IV) IN COMBINATION WITH
INHIBITORS OF ALANYL-AMINOPEPTIDASE (APN)**

The present invention relates to the inhibition of the DNA synthesis and, thus, the proliferation of immune cells by the combined effect of inhibitors of aminopeptidase N (APN; E.C. 3.4.11.2; CD13), of dipeptidyl peptidase IV (DP IV; E.C. 3.4.14.5; CD26), of prolyl oligopeptidase (POP; prolyl endopeptidase; PEP; E.C. 3.4.21.26), of the membrane-located aminopeptidase P (X-Pro-Aminopeptidase; APP; XPNPEP2; E.C. 3.4.11.9) and of the angiotensin-converting enzyme (Angiotensin-konvertierendes Enzym; ACE; E.C. 3.4.15.1, CD143) or by the combined inhibition, respectively, of the activity of the above-mentioned enzymes as a result of the simultaneous application of respective specific inhibitors of said enzymes on the basis of amino acid derivatives, peptides or peptide derivatives, by which the activation, the DNA synthesis and, thus, the proliferation of immune cells is suppressed.

The invention also relates to the inhibition of the DNA synthesis essential for the proliferation as well as to the inhibition of the production of cytokines (interleukin-4, IL-4) by T_{H2} cells by a combined effect of inhibitors of aminopeptidase N (APN; E.C. 3.4.11.2; CD13) and of dipeptidyl peptidase IV (DP IV; E.C. 3.4.14.5; CD26) as a result of a simultaneous application of respective specific inhibitors of said enzymes on the basis of amino acid derivatives, peptides or peptide derivatives, by which the activation, the proliferation (DNA synthesis) and cytokine production (IL-4) by T_{H2} cells is suppressed.

The invention also relates to the inhibition of the DNA synthesis of keratinocytes essential for the proliferation by a combined effect of inhibitors of aminopeptidyl peptidase N (APN; E.C. 3.4.11.2; CD13) and of dipeptidyl peptidase IV (DP IV; E.C. 3.4.14.5; CD26) as a result of the simultaneous or immediately sequential application of the respective specific inhibitors of said enzymes or of enzymes having a similar effect on the basis of amino acid derivatives, peptides or peptide derivatives, by which the proliferation (DNA synthesis) of keratinocytes is suppressed.

It is applicable to all diseases with an autoimmune pathogenesis that the disease and its course of genesis and progress is based on, or consists of, an activation and proliferation of immune cells, particularly of autoreactive T cells. Similar mechanisms are effective for a number

1 of inflammatory diseases as, for example arteriosclerosis, where T lymphocytes play a central
2 role in the development and chronification of the disease.

3
4 It was shown that membrane-located peptidases as, for example, DP IV or APN play a
5 key role in the process of the activation and clonal expression of immune cells, particularly of T
6 lymphocytes [Fleischer B: CD26 a surface protease involved in T cell activation. Immunology
7 Today 1994; 15:180-184; Lendeckel U et al.: Role of alanyl aminopeptidase in growth and func-
8 tion of human T cells. International Journal of Molecular Medicine 1999; 4:17-27; Riemann D et
9 al.: CD13 - not just a marker in leukemia typing. Immunology Today 1999; 20:83-88]. Several
10 functions of mitogen-stimulated mononuclear cells (MNC) or accumulated T lymphocytes as, for
11 example, DNA synthesis production and secretion of immune-stimulating cytokines (IL-2, IL-6,
12 IL-12, IFN- γ) and helper functions of B cells (synthesis of IgG and IgM) may be inhibited in the
13 presence of specific inhibitors of DP IV and APN [Schön E et al.: The dipeptidyl peptidase IV, a
14 membrane enzyme involved in the proliferation of T lymphocytes. Biomed. Biochim. Acta 1985;
15 2: K9-K15; Schön E et al.: The role of dipeptidyl peptidase IV in human T lymphocyte activation.
16 Inhibitors and antibodies against dipeptidyl peptidase IV suppress lymphocyte proliferation and
17 immunoglobulin synthesis in vitro. Eur. J. Immunol. 1987; 17: 1821-1826; Reinhold D et al.: In-
18 hibitors of dipeptidyl peptidase IV induce secretion of transforming growth factor β 1 in PWM-
19 stimulated PBMNC and T cells. Immunology 1997; 91: 354-360; Lendeckel U et al. : Induction of
20 the membrane alanyl aminopeptidase gene and surface expression in human T cells by mito-
21 genic activation. Biochem. J. 1996; 319: 817-823; Kähne T et al.: Dipeptidyl peptidase IV: A cell
22 surface peptidase involved in regulating T cell growth (Review). Int. J. Mol. Med. 1999; 4: 3-15;
23 Lendeckel U et al.: Role of alanyl aminopeptidase in growth and function of human T cells (Re-
24 view). Int. J. Mol. Med. 1999; 4: 17-27].

25
26 On the other hand, scientific discoveries of the recent years characterized arteriosclero-
27 sis as an inflammatory disease, whereby T lymphocytes play a decisive role in the development
28 and progress of said disease [Ross R: Arteriosclerosis – an inflammatory disease. New England
29 J. Med. 1999; 340 (2): 115 – 126]. According to those discoveries, arteriosclerotic lesions are
30 understood as a series of specific cellular and molecular reactions which, when taken together,
31 are to be characterized as inflammations, unequivocally. Such lesions primarily occurring in
32 large and medium size elastic and muscular arteries result into ischemia (disturbed circulation)
33 of the heart, of the cerebrum and of the extremities up to infarcts of the above-mentioned or-

1 gans. Arteriosclerotic lesions are formed at defined arterial locations, where branches and
2 curves effect characteristic changes of the blood flow and of the sheer stresses as well as the
3 creation of turbulences [Gotlieb AI et al.: The role of rheology in atherosclerotic coronary artery
4 disease. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary athery disease. Vol.
5 1 Philadelphia: Lippincott-Raven, 1996: 595-606]. Vessel endothel cells generate specific mole-
6 cules at those locations, which molecules are responsible for the attraction, binding, accumula-
7 tion and activation of T lymphocytes and monocytes. T lymphocytes are essential inflammatory
8 cells in all phases of the arteriogenesis. T cells infiltrate from the peripheral blood into the arte-
9 riosclerotic plaques and multiply at the lesion location [Jonasson L et al.: Regional accumulation
10 of T cells, macrophages and smooth muscle cells in the human atherosclerotic plaque. Arterio-
11 sclerosis. 1986; 6: 131-138; van der Wal AC et al.: Atherosclerotic lesions in humans: in situ
12 immunophenotypic analysis suggesting an immune mediated response. Lab. Invest. 1989; 61:
13 166-170]. As a result of the accumulation, at the location of an arteriosclerotic lesion, of such
14 activated T lymphocytes which are characterized by a strong expression of alanyl aminopepti-
15 dase and of dipeptidyl peptidase IV, chemokines, cytokines, growth factors and proteases are
16 released, which compounds effect a further intensification of the disease conditions, as other
17 immune cells are recruited and activated [Libby P and Ross R. Cytokines and growth regulatory
18 molecules. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary athery disease.
19 Vol. 1, Philadelphia: Lippincott-Raven, 1996: 585-594].

20
21 In addition, monocytes localized in arteriosclerotic plaques are characterized by the con-
22 stitutive expression of, for example, alanyl aminopeptidase (APN) and are – as could be shown
23 by the present invention, capable of being suppressed effectively in their growth and function by
24 inhibitory substances of the above-mentioned enzymes. The same is true for endothelic cells
25 which express those ectopeptidases, too.

26
27 The angiotensin-converting enzyme plays a particular role in the pathogenesis of arterio-
28 sclerosis. Said enzyme effects the generation of angiotensin II (ang II) from ang I, the former
29 substance severely increasing the blood pressure. Hypertension is an important factor promot-
30 ing the risk of arteriosclerosis, and patients suffering therefrom often have increased ang II
31 blood levels. In addition, ang II is pro-arterogeneous by stimulating the growth of the smooth
32 muscles (vessels) [Chobanian AV et al. Renin angiotensin system and atherosclerotic vascular
33 disease. In: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary athery disease. Vol.

1 1, Philadelphia: Lippincott-Raven, 1996: 237-242; Gibbons GH et al. Vascular smooth muscle
2 cell hypertrophy vs. hyperplasia: autocrine TGF- β 1 expression determines growth response to
3 angiotensin II. J Clin. Invest. 1992; 90: 456-461]. Moreover, ang II also enhances the inflamma-
4 tory reaction by an increase of the lipoxygenase activity whereby inflammation-promoting me-
5 diators are released in increasing amounts.

6
7 The present invention is based on the surprising finding that the simultaneous effect of
8 inhibitors of the enzyme activity, or the simultaneous influence of the biologic activity, of (I)
9 dipeptidyl peptidase IV and amino peptidase N; (II) dipeptidyl peptidase IV and the "angiotensin-
10 converting enzyme"; (III) dipeptidyl peptidase IV and prolyl oligopeptidase; as well as (IV) dipep-
11 tidyl peptidase IV and X-Pro amino peptidase inhibits the DNA synthesis and, thus, the prolifera-
12 tion of mononuclear cells (MNC) and of T cells to an extent which cannot be achieved by an
13 application of a single one of those enzyme inhibitors, including the case of the administration of
14 an enhanced dose thereof. Although said inhibitors finally influence the same process, namely
15 the DNA synthesis and, thus, the proliferation of immune cells, this effect is substantially less
16 pronounced and is not long-lasting. Due to the functional overlap of the enzymatic activity of the
17 above-mentioned enzymes, there results a more than additive or super-additive inhibitory effect
18 on the synthesis of DNA and the proliferation from the simultaneous inhibition of two or more of
19 those enzymes, as the data of the present invention show.

20
21 The invention shows that the simultaneous application of inhibitory substances of the
22 above-mentioned enzymes or the simultaneous application of corresponding preparations and
23 administration forms, respectively, is definitely suitable for a therapy of inflammatory diseases
24 as, for example, of arteriosclerosis, for the development of which the proliferation and activation
25 of T lymphocytes plays a central and important role.

26
27 In detail, the invention is based on the finding that the DNA synthesis of mononuclear
28 cells (MNC) and T cells is inhibited, in a more than additive to super-additive manner, by a si-
29 multaneous administration of substances inhibiting the enzymatic activity of
30

- 31 (I) dipeptidyl peptidase IV and amino peptidase N;
32 (II) dipeptidyl peptidase IV and the "angiotensin-converting enzyme";
33 (III) dipeptidyl peptidase IV and prolyl oligopeptidase;

1 (IV) dipeptidyl peptidase IV and X-Pro amino peptidase.

2
3 The application of enzyme inhibitors is a novel method and a supplementary form of
4 therapy for the above-mentioned diseases.

5
6 The inhibitors of dipeptidyl peptidase IV, of amino peptidase N, of prolyl oligopeptidase,
7 of the "angiotensin-converting enzyme" and of X-Pro amino peptidase applied in accordance
8 with the present invention may be applied in the form of pharmaceutically applicable formulation
9 complexes as inhibitors, substrates, pseudo-substrates, peptides having inhibitory effect and
10 peptide derivatives as well as antibodies for this enzyme. Preferred effectors for DP IV are, for
11 example, Xaa-Pro dipeptides corresponding derivatives, preferably dipeptide phosphonic acid
12 diaryl esters, dipeptide boronic acids (e. g. Pro-boro-Pro) and their salts, Xaa-Xaa-(Trp)-Pro-
13 (Xaa)_n peptides (n = 0 to 10), corresponding derivatives and their salts, or amino acid-(Xaa)
14 amides, corresponding derivatives and their salts, wherein Xaa is an α -amino acid or -imino
15 acid or an α -amino acid derivative or -imino acid derivative, respectively, preferably N^ε-4-
16 nitrobenzyloxycarbonyl-L-lysine, L-proline, L-tryptophane, L-isoleucine, L-valine, and cyclic
17 amines as, for example pyrrolidine, piperidine, thiazolidine, and their derivatives serve as the
18 amide structure. Such compounds and their preparation were described in an earlier patent (K.
19 Neubert et al., DD 296 075 A5).

20
21 The inhibitors are administered simultaneously with known carrier substances. The ad-
22 ministration may occur, on the one hand, in the form of a topical application by means of
23 crèmes, ointments, pastes, gels, solutions, sprays, liposomes, shaken mixtures, hydrocolloid
24 dressings and other dermatologic bases/vehicles including instillative application and, on the
25 other hand, in the form of a systemic application for an oral, transdermal, intravenous, subcuta-
26 neous, intracutaneous or intramuscular application in suitable formulations or in a suitable
27 galenic form.

28
29 It was found for allergic reactions of the type I as, for example asthma bronchiale or hay
30 fever that the diseases are based on an activation, proliferation, and the production of cytokines
31 (in particular IL-4) by immune cells, particularly by T_{H2} cells [D.D. Corry et al., Induction and
32 regulation of the IgG response. Nature 1999; 402: B18 to B23].

1 It was shown that membrane-located peptidases as, for example, DP IV or APN play a
2 key role in the process of the activation and clonal expression of immune cells, particularly of T
3 lymphocytes [Fleischer B: CD26 a surface protease involved in T cell activation. Immunology
4 Today 1994; 15:180-184; Lendeckel U et al.: Role of alanyl amino-peptidase in growth and func-
5 tion of human T cells. International Journal of Molecular Medicine 1999; 4:17-27; Riemann D et
6 al.: CD13 - not just a marker in leukemia typing. Immunology Today 1999; 20:83-88]. Several
7 functions of mitogen-stimulated mononuclear cells (MNC) or accumulated T lymphocytes as, for
8 example, DNA synthesis production and secretion of immune-stimulating cytokines (IL-2, IL-6,
9 IL-12, IFN- γ) and helper functions of B cells (synthesis of IgG and IgM) may be inhibited in the
10 presence of specific inhibitors of DP IV and APN [Schön E et al.: The dipeptidyl peptidase IV, a
11 membrane enzyme involved in the proliferation of T lymphocytes. Biomed. Biochim. Acta 1985;
12 2: K9-K15; Schön E et al.: The role of dipeptidyl peptidase IV in human T lymphocyte activation.
13 Inhibitors and antibodies against dipeptidyl peptidase IV suppress lymphocyte proliferation and
14 immunoglobulin synthesis in vitro. Eur. J. Immunol. 1987; 17: 1821-1826; Reinhold D et al.: In-
15 hibitors of dipeptidyl peptidase IV induce secretion of transforming growth factor β 1 in PWM-
16 stimulated PBMNC and T cells. Immunology 1997; 91: 354-360; Lendeckel U et al. : Induction of
17 the membrane alanyl aminopeptidase gene and surface expression in human T cells by mito-
18 genic activation. Biochem. J. 1996; 319: 817-823; Kähne T et al.: Dipeptidyl peptidase IV: A cell
19 surface peptidase involved in regulating T cell growth (Review). Int. J. Mol. Med. 1999; 4: 3-15;
20 Lendeckel U et al.: Role of alanyl aminopeptidase in growth and function of human T cells (Re-
21 view). Int. J. Mol. Med. 1999; 4: 17-27].

22
23 On the other hand, scientific discoveries of the recent years characterized an allergic
24 reaction of the type I as an inflammatory disease, whereby T_{H2} lymphocytes play a decisive
25 role in the development and chronification of said disease [D.D. Corry et al.: Induction and
26 regulation of the IgE response. Nature 1999; 402: B18-B23. P.J. Barnes: Therapeutic strategies
27 for allergic diseases. Nature 1999; 402: B31-B38].

28
29 IL4 is a helper cytokine for the B cell proliferation, stimulates the generation of IgE and
30 the expression of Fc-IgE receptors of low affinity. Moreover, IL-4 enhances the induction of T_{H2}
31 cells themselves and controls the proliferation and activity of eosinophilic cells and mast cells.
32 That is why it plays a central role in allergic reactions of the type I [D.P. Stites, A.I. Terr, T.G.
33 Parslow: Medical Immunology. Appelton & Lange, Stamford, CT, 1997].

1
2 The present invention is also based on the surprising finding that the simultaneous effect
3 of inhibitors of dipeptidyl peptidase IV and amino peptidase N inhibits the proliferation (DNA
4 synthesis) of and the production of IL-4 by mitogene-stimulated mononuclear cells (MNC) to an
5 extent which cannot be achieved by an application of a single one of those enzyme inhibitors,
6 including the case of an enhanced dose thereof. Although said inhibitors finally influence the
7 same process, namely the DNA synthesis and, thus, the proliferation of and the IL-4 production
8 by T_{H2} cells, this effect is substantially less pronounced, when the inhibitors are applied singly,
9 and is not long-lasting. Due to the functional overlap of the enzymatic activity of the above-
10 mentioned enzymes, there results a more than additive or super-additive inhibitory effect on the
11 synthesis of DNA and the proliferation from the simultaneous inhibition of the two enzymes, as
12 the data of the present invention show.

13
14 The invention shows that the simultaneous application of inhibitory substances of the
15 enzymes DP IV and APN or the simultaneous application of corresponding preparations and
16 administration forms, respectively, is definitely suitable for a therapy of allergic diseases of the
17 type I, for the development of which the proliferation and activation of T lymphocytes plays a
18 central and important role.

19
20 In detail, the invention is based on the finding that the DNA synthesis of, and the IL-4
21 production by, mononuclear cells (MNC) is inhibited, in a more than additive to super-additive
22 manner, by a simultaneous administration of substances inhibiting dipeptidyl peptidase IV and
23 amino peptidase N.

24
25 The application of enzyme inhibitors is a novel method and a supplementary form of
26 therapy for the above-mentioned diseases.

27
28 The inhibitors of dipeptidyl peptidase IV and of amino peptidase N applied in accordance
29 with the present invention may be applied in the form of pharmaceutically applicable formulation
30 complexes as inhibitors, substrates, pseudo-substrates, peptides having inhibitory effect and
31 peptide derivatives as well as antibodies for this enzyme. Preferred effectors for DP IV are, for
32 example, Xaa-Pro dipeptides corresponding derivatives, preferably dipeptide phosphonic acid
33 diaryl esters, dipeptide boronic acids (e. g. Pro-boro-Pro) and their salts, Xaa-Xaa-(Trp)-Pro-

1 (Xaa)_n peptides (n = 0 to 10), corresponding derivatives and their salts, or amino acid-(Xaa)
2 amides, corresponding derivatives and their salts, wherein Xaa is an α-amino acid or –imino
3 acid or an α-amino acid derivative or –imino acid derivative, respectively, preferably N^ε-4-
4 nitrobenzyloxycarb-onyl-L-lysine, L-proline, L-tryptophane, L-isoleucine, L-valine, and cyclic
5 amines as, for example pyrrolidine, piperidine, thiazolidine, and their derivatives serve as the
6 amide structure. Such compounds and their preparation were described in an earlier patent (K.
7 Neubert at al., DD 296 075 A5).

8
9 Preferred inhibitors of alanyl aminopeptidase are bestatin (Ubenimex), actinonin,
10 probestin, phebestin, RB3014 or leuhistin.

11
12 The inhibitors are administered simultaneously with known carrier substances. The ad-
13 ministration may occur, on the one hand, in the form of a topical application by means of
14 crèmes, ointments, pastes, gels, solutions, sprays, liposomes, shaken mixtures, hydrocolloid
15 dressings and other dermatologic bases/vehicles including instillative application and, on the
16 other hand, in the form of a systemic application for an oral, transdermal, intravenous, subcuta-
17 neous, intracutaneous or intramuscular application in suitable formulations or in a suitable
18 galenic form.

19
20 A number of dermatologic diseases are accompanied by follicular and epidermal hy-
21 perkeratoses and an increased proliferation of keratinocytes. Such diseases cover inflammatory
22 and non-inflammatory epidermal hyperproliferation conditions (e. g. congenital ichthyoses and
23 psoriasis), benign and malignant epidermal clonal expansions (e. g. warts, condylomes, actinic
24 keratoses/precanceroses), benign and malignant follicular hyperproliferation conditions (e. g. kera-
25 tosis follicularis) as well as benign and malignant epithelial adnex tumors and primary and reactive
26 nail cell hyperproliferations. Details are set out in Table 1.

27
28 Peptidases as, for example, dipeptidyl peptidase IV and amino peptidase N or similarly
29 acting enzymes are of particular importance for the regulation and modulation, respectively, of
30 interactions between cells, since they are, inter alia, located, as ectoenzymes, in the plasma
31 membrane of the cells, interact with other extracellular structures, activate or inactivate pep-
32 tidergous messenger substances by an enzyme-catalysed hydrolysis and, thus, are important
33 for the intercellular communication [Yaron A, et al.: Proline-dependent structural and biological

1 properties of peptides and proteins. Crit Rev Biochem Mol Biol 1993;28:31-81; Vanhoof G, et
2 al.: Proline motifs in peptides and their biological processing. FASEB J 1995;9:736-744].

3
4 It was shown that membrane-located peptidases as, for example, DP IV or APN play a
5 key role in the process of the activation and clonal expression of immune cells, particularly of T
6 lymphocytes [Fleischer B: CD26 a surface protease involved in T cell activation. Immunology
7 Today 1994; 15:180-184; Lendeckel U et al.: Role of alanyl amino-peptidase in growth and func-
8 tion of human T cells. International Journal of Molecular Medicine 1999; 4:17-27; Riemann D et
9 al.: CD13 - not just a marker in leukemia typing. Immunology Today 1999; 20:83-88]. Several
10 functions of mitogen-stimulated mononuclear cells (MNC) or accumulated T lymphocytes as, for
11 example, DNA synthesis production and secretion of immune-stimulating cytokines (IL-2, IL-6,
12 IL-12, IFN- γ) and helper functions of B cells (synthesis of IgG and IgM) may be inhibited in the
13 presence of specific inhibitors of DP IV and APN [Schön E et al.: The dipeptidyl peptidase IV, a
14 membrane enzyme involved in the proliferation of T lymphocytes. Biomed. Biochim. Acta 1985;
15 2: K9-K15; Schön E et al.: The role of dipeptidyl peptidase IV in human T lymphocyte activation.
16 Inhibitors and antibodies against dipeptidyl peptidase IV suppress lymphocyte proliferation and
17 immunoglobulin synthesis in vitro. Eur. J. Immunol. 1987; 17: 1821-1826; Reinhold D et al.: In-
18 hibitors of dipeptidyl peptidase IV induce secretion of transforming growth factor β 1 in PWM-
19 stimulated PBMNC and T cells. Immunology 1997; 91: 354-360; Lendeckel U et al. : Induction of
20 the membrane alanyl aminopeptidase gene and surface expression in human T cells by mito-
21 genic activation. Biochem. J. 1996; 319: 817-823; Kähne T et al.: Dipeptidyl peptidase IV: A cell
22 surface peptidase involved in regulating T cell growth (Review). Int. J. Mol. Med. 1999; 4: 3-15;
23 Lendeckel U et al.: Role of alanyl aminopeptidase in growth and function of human T cells (Re-
24 view). Int. J. Mol. Med. 1999; 4: 17-27]. It is already known that a treatment of autoimmune dis-
25 eases and transplant rejection is possible by an inhibition of dipeptidyl peptidase IV located on
26 immune cells by means of synthetic inhibitors. (e. g. EP 0 764 151 A1; WO 95/29691; EP 0 731
27 789 A1; EP 0 528 858 A1).

28
29 The present invention is also based on the surprising finding that the simultaneous effect
30 of inhibitors of dipeptidyl peptidase IV/CD26 expressed in or on keratinocytes and of amino pep-
31 tidase N/CD13 or similar enzymes inhibits the proliferation (DNA synthesis) of those cells to an
32 extent which cannot be achieved by an application of a single one of those enzyme inhibitors,
33 administered at a given dose. Although said inhibitors finally influence the same process,

1 namely the DNA synthesis and, thus, the proliferation of the keratinocytes, this effect is substan-
2 tially less pronounced, when the inhibitors are applied singly, and is not long-lasting. Due to the
3 functional overlap of the enzymatic activity of the above-mentioned enzymes, there results an
4 additive inhibitory effect and, at lower concentrations, a more than additive or super-additive
5 inhibitory effect on the synthesis of DNA and the proliferation from the simultaneous inhibition of
6 the two enzymes, as the data of the present invention show.

7
8 The invention shows that the simultaneous application of inhibitory substances of the
9 enzymes DP IV and APN or of similar enzymes or the simultaneous application of correspond-
10 ing preparations and administration forms, respectively, is definitely suitable for a therapy or
11 prevention of inflammatory and non-inflammatory epidermal hyperproliferation conditions (e. g.
12 congenital ichthyoses and psoriasis), benign and malign epidermal clonal expansions (e. g.
13 warts, condylomes, actinic keratoses/precanceroses), benign and malign follicular hyperprolif-
14 eration conditions (e. g. keratosis follicularis) as well as benign and malign epithelial adnex tu-
15 mors and primary and reactive nail cell hyperproliferations, for the development of which the
16 proliferation and activation of epidermal and follicular keratinocytes as well as of keratinocytes
17 of the transitional mucous membrane zone is of central importance.

18
19 In addition to keratinocytes, T lymphocytes, too, play a central role in inflammatory dis-
20 eases of the skin, in particular in autoimmune diseases like psoriasis. As keratinocytes, T cells
21 express the above-mentioned peptidases DP IV and APN. As a consequence, the therapeutic
22 effect claimed or protected, respectively, for keratinocytes is even enhanced by influencing T
23 cells. This is also subject of the German Patent Application No. 100 25 464.0 entitled "Com-
24 bined Use of Enzyme Inhibitors and of Pharmaceutical Preparations Thereof for a Therapy of
25 Autoimmune Diseases as, for example, Rheumatoid Arthritis, Lupus Erythematoses, Multiple
26 Sclerosis, Insuline-Dependent Diabetes Mellitus (IDDM), Crohn's Disease, Colitis Ulcerosa,
27 Psoriasis, Neurodermitis, Glomerulonephritis, interstitial Nephritis, Vasculitis, Autoimmune Thy-
28 roid Gland Diseases or Autoimmune Hemolytic Anemia as well as transplantations and tumor
29 diseases".

30
31 In detail, the invention is based on the finding that the DNA synthesis of HaCaT kerati-
32 nocytes is inhibited, in an additive manner and, at lower concentrations in a super-additive

1 manner, by a simultaneous administration of inhibitors of dipeptidyl peptidase IV and amino
2 peptidase N.

3
4 Up to now, the above-mentioned diseases were treated topically by administering anti-
5 proliferative and differentiating substances (salicylic acid, urea, endogeneous and synthetic reti-
6 noids, Vitamin D3 derivatives, corticosteroids) as well as systemically by administering partially
7 immunosuppressive and antiproliferative preparations (e. g. Cyclosporin A, corticosteroids, reti-
8 noids). Particularly when administering substances systemically, often undesired side effects
9 were observed. The combined administration of inhibitors of DP IV and APN is a novel, expect-
10 edly very effective, possibly cheap therapeutical method and a valuable alternative constituent
11 element of the existing therapy concepts.

12
13 The inhibitors of dipeptidyl peptidase IV and of amino peptidase N or of similar enzymes
14 applied in accordance with the present invention may be applied in the form of pharmaceutically
15 applicable formulation complexes as inhibitors, substrates, pseudo-substrates, peptides having
16 inhibitory effect and peptide derivatives as well as antibodies for this enzyme. Preferred effec-
17 tors for DP IV are, for example, Xaa-Pro dipeptides corresponding derivatives, preferably dipep-
18 tide phosphonic acid diaryl esters, dipeptide boronic acids (e. g. Pro-boro-Pro) and their salts,
19 Xaa-Xaa-(Trp)-Pro-(Xaa)_n peptides (n = 0 to 10), corresponding derivatives and their salts, or
20 amino acid-(Xaa) amides, corresponding derivatives and their salts, wherein Xaa is an α-amino
21 acid or -imino acid or an α-amino acid derivative or -imino acid derivative, respectively, pref-
22 erably N^ε-4-nitrobenzyloxycarbonyl-L-lysine, L-isoleucine, L-valine, L-tryptophane, L-proline,
23 and cyclic amines as, for example pyrrolidine, piperidine, thiazolidine, and their derivatives
24 serve as the amide structure. Such compounds and their preparation were described in an ear-
25 lier patent (K. Neubert et al., DD 296 075 A5).

26
27 Preferred inhibitors of alanyl aminopeptidase are bestatin (Ubenimex), actinonin,
28 probestin, phebestin, RB3014 or leuhistin.

29
30 The inhibitors are administered simultaneously with known carrier substances. The ad-
31 ministration may occur, on the one hand, in the form of a topical application by means of
32 crèmes, ointments, pastes, gels, solutions, sprays, liposomes, shaken mixtures, hydrocolloid
33 dressings, plasters and similar new carriers substrates, jet injections and other dermatologic

1 bases/vehicles, respectively, including instillative application and, on the other hand, in the form
2 of a systemic application for an oral, transdermal, intravenous, subcutaneous, intracutaneous or
3 intramuscular application in suitable formulations or in a suitable galenic form.

4
5 In one embodiment, the present invention provides a use as described above wherein
6 the inhibitors of DP IV are XaaPro-dipeptides (Xaa = α -amino acid and side chain protected
7 derivative, respectively), corresponding derivatives, preferably dipeptide phosphonic acid diaryl
8 esters, dipeptide boronic acids (e. g. Pro-boro-Pro) and their salts, Xaa-Xaa(Trp)-Pro (Xaa)_n
9 peptides (Xaa = α -amino acid, n = 0 to 10), corresponding derivatives and their salts and amino
10 acid-(Xaa)-amides, respectively, corresponding derivatives and their salts, wherein Xaa is an α -
11 amino acid or a side chain protected derivative, respectively, preferably N^ε-4-
12 nitrobenzyloxycarbonyl-L-lysine, L-proline, Ltryptophane, L-isoleucine, L-valine, and cyclic
13 amines as, for example, pyrrolidine, piperidine, thiazolidine, and their derivatives serve as the
14 amide structure.

15
16 In another embodiment, the invention provides a use wherein amino acid amides, e. g.
17 N^ε-4-nitrobenzyloxycarbonyl-L-lysine thiazolidide, pyrrolidide and piperidide as well as the
18 corresponding 2-cyanothiazolidide, 2-cyanopyrrolidide and 2cyanopiperidide derivative are used
19 as DP IV inhibitors.

20
21 In a further embodiment, the invention provides a use wherein the following are used as
22 the inhibitors of APN: actinoin, leuhistin, phebestin, amastatin, bestatin, probestin, β -minothiols,
23 α -aminophosphinic acids, α -aminophosphinic acid derivatives, preferably D-Phe- ψ -[PO(OH)-
24 CH₂]-Phe-Phe and their salts.

25
26 In a further embodiment, the invention provides a use wherein, as the inhibitors of APP,
27 there are used apstatine, (2S,3R)-HAMH-L-proline, (2S,3R)-HAPB-L-proline, the corresponding
28 L-proline methyl ester, (2S,3R)-HAMH/(2S,3R)-HAPB-pyrrolidides, thiazolidides (HAMH = 3-
29 amino-2-hydroxy-5-methyl-hexanoyl, HAPB = 3-amino-2-hydroxy-4-phenylbutanoyl) and their
30 salts.

31
32 In a further embodiment, the invention provides a use wherein, as the inhibitors of ACE,
33 captopril, enalapril, lisinopril, cilazopril and their salts are used.

1
2 In a further embodiment, the invention provides a use wherein, as the inhibitors of POP
3 (PEP), there are used postatin, eurystatin A or B, N^a-protected peptide aldehydes, preferably
4 benzyloxycarbonyl-L-prolyl-L-prolinal and benzyloxycarbonyl-L-thioproyl-L-thioprolinal, N^a-
5 protected amino acid-(Xaa) pyrrolidides or thiazolidides (Xaa = α -amino acid, preferably L-
6 alanine, L-valine, L-isoleucine) as well as the corresponding 2-cyanopyrrolidide and 2-
7 cyanothiazolidide derivatives, substrate analogous N^a-protected peptide phosphonic acid diaryl
8 esters and peptide diazomethyl ketones and peptide ammonium methyl ketones, respectively,
9 and their salts.

10
11 In a further embodiment, the invention provides pharmaceutical preparations comprising,
12 as inhibitors of DP IV, preferably Xaa-Pro dipeptides (Xaa = α -amino acid and side chain
13 protected derivative, respectively), corresponding derivatives, preferably dipeptide phosphonic
14 acid diaryl esters, dipeptide boronic acids (e.g. Pro-boro-Pro) and their salts, Xaa-Xaa-(Trp)-
15 Pro-(Xaa)_n peptides (Xaa = α -amino acid, n = 0 to 10), corresponding derivatives and their salts
16 and amino acid-(Xaa) amides, corresponding derivatives and their salts, respectively, wherein
17 Xaa is an α -amino acid or a side chain protected derivative respectively, preferably N^ε-4-
18 nitrobenzyloxycarbonyl-L-lysine, L-proline, L-tryptophane, L-isoleucine, L-valine, and cyclic
19 amines as, for example, pyrrolidine, piperidine, thiazolidine, and their derivatives serve as the
20 amide structure.

21
22 In a further embodiment, the invention provides pharmaceutical preparations comprising
23 as inhibitors of APN, actinoin, leuhistin, phebestin, amastatin, bestatin, probestin, β -aminothiols,
24 α -aminophosphinic acids, . α -aminophosphinic acid derivatives, preferably D-Phe- ψ -[PO(OH)-
25 CH₂]-Phe-Phe and their salts.

26
27 In a further embodiment, the invention provides pharmaceutical preparations comprising,
28 as inhibitors of APP, apstatine, (2S,3R)-HAMH-L-proline, (2S,3R)-HAPB-L-proline, the
29 corresponding L-proline methyl ester, (2S,3R)-HAMH-/(2S,3R)-HAPB-pyrrolidides, thiazolidides
30 (HAMH = 3-amino-2-hydroxy-5-methyl-hexanoyl, HAPB = 3-amino-2-hydroxy-4-phenyl-butanoyl)
31 and their salts.

1 In a further embodiment, the invention provides pharmaceutical preparations comprising
2 as inhibitors of ACE captopril, enalapril, lisinopril, cilazopril and their salts.

3
4 In a further embodiment, the invention provides pharmaceutical preparations comprising
5 as inhibitors of POP (PEP) preferably postatin, eurystatin A or B, N^a-protected peptidaldehydes,
6 preferably benzyloxycarbonyl-L-prolyl-L-prolinal and benzyloxycarbonyl-L-thioproyl-L-
7 thioprolinal, N^a-protected amino acid-(Xaa) pyrrolidides or thiazolidides (Xaa = α -amino acid,
8 preferably L-alanine, L-valine, L-isoleucine) as well as the corresponding 2-cyanopyrrolidide and
9 2-cyanothiazolidide derivatives, substrate-analogous N^a-protected peptide phosphonic acid
10 diaryl esters and peptide diazomethyl ketones and peptide ammonium methyl ketones,
11 respectively, and their salts.

12
13 In a further embodiment, the invention provides pharmaceutical preparations comprising
14 two or more inhibitors of DP IV or of enzymes of DP IV-analogous enzymatic activity of APN or
15 of enzymes having APN-analogous enzymatic activity, of ACE, of POP (PEP) and of XPNPEP2
16 in a compartmentally separate formulation in combination with per se known carrier, auxiliary
17 and/or additive substances for a simultaneous or immediately timely consecutive administration
18 with the aim of a combined effect.

19
20 In a further embodiment, the invention provides pharmaceutical preparations for the
21 systemic application for an oral, transdermal, intravenous, subcutaneous, intracutaneous,
22 intramuscular, rectal, vaginal, sublingual administration together with per se known carrier,
23 auxiliary and/or additive substances.

Table 1

Epidermal hyperproliferation conditions

e.g. non-inflammatory

e. g. inflammatory

Congenital Ichthyoses

Psoriasis and sub-types including
nails and hair

Acquired ichthyoses (paraneoplast.)

Lichen ruber and subtypes

Palmoplantar keratoses

Parapsoriasis group

congenital

Keratosis lichenoides

acquired/paraneoplast.

Lichen simplex chronicus + reactive
lichenoid hyperproliferations (e. g.
atopic dermatitis)

M. Darier

Lichenoid reactions at GvHD

Epidermal Naevi

ILVEN-Naevus

Cutis rhomboidalis nuchae

Lupus Erythematoses chron.disc.
/SCLE/SLE

Acanthosis nigricans

Pityriasis rubra pilaris

Pachydermia

M. Grover

Vitiligo

Erythrodermia accompanied by a
hyperproliferation of keratinocytesEpidermal clonal expansion

benign

malign

HPV associated (warts, condylomes)

HPV associated tumors

Seborrheic keratoses

Actinic keratoses/precanceroses

Hidroacanthomes/poromes

M. Bowen + Bowen-CA

Epidermal cysts

M. Paget + Paget-CA

Milias

plate epithel-CA

M. Gottron

Merkel cell-CA

Follicular hyperproliferation conditions

benign

malign

Keratosis follicularis

Hair follicular cell tumors

Follicular hyperkeratoses

Proliferating trichilemmal cysts

Ulerythema ophryogenes

Mixed tumors

Hypertrichoses

Trichilemmal cysts

Epithelial adnex tumors

benign

malign

Poroms

eccrine/apocrine CA's + Subtypes

Syringoductal tumors

Hidradenomas

Spiradenomas

Cylindromas

Primary and reactive nail cell hyperproliferation

Congenital (e. g. pachyonychia)

Non-infectious

Acquired

Infectious with mycoses

The invention is further explained by means of the following working examples, but is not limited to these preferred embodiments.

Example 1

Inhibition of the DNA synthesis of human T lymphocytes by an incubation with synthetic inhibitors of DP IV and of APN

The searches according to the invention show that the DNA synthesis of human peripheral T lymphocytes is inhibited, in a more than additive to superadditive manner, by a simultaneous administration of inhibitors of DP IV (Lys[Z(NO₂)]-thiazolidide = I49) and of APN (Actinonin). The T cells were incubated for 72 h in the presence of said inhibitors, and the DNA synthesis was determined subsequently by a measurement of the ³[H]-thymidine incorporation, as described by Reinhold et al. (Reinhold D. et al.: Inhibitors of dipeptidyl peptidase IV induce secretion of transforming growth factor β 1 in PWM-stimulated PBMNC and T cells; Immunology 1997, 91: 354 – 360). Figure 1 (page 1/14) shows the dose-dependent, more than additive to superadditive inhibition of the DNA synthesis.

Example 2

Inhibition of the DNA synthesis of human peripheral mononuclear cells by an incubation with synthetic inhibitors of DP IV and of APN

1
2 The searches according to the invention show that the DNA synthesis of human pe-
3 ripheral mononuclear cells (MNC) is inhibited, in a more than additive to superadditive manner,
4 by a simultaneous administration of inhibitors of DP IV (Lys[Z(NO₂)]-thiazolidide = I49) and of
5 APN (Actinonin). The MNC were incubated for 72 h in the presence of said inhibitors, and the
6 DNA synthesis was determined subsequently by a measurement of the ³[H]-thymidine incorpo-
7 ration, as described by Reinhold et al. (Reinhold D. et al.: Inhibitors of dipeptidyl peptidase IV
8 induce secretion of transforming growth factor β1 in PWM-stimulated PBMNC and T cells; Im-
9 munology 1997, 91: 354 – 360). Figure 2 (page 2/14) shows the dose-dependent, more than
10 additive to superadditive inhibition of the DNA synthesis.

11 12 13 **Example 3**

14 **Inhibition of the DNA synthesis of human T lymphocytes by an incubation with synthetic** 15 **inhibitors of DP IV and of POP**

16
17 The searches according to the invention show that the DNA synthesis of human T lym-
18 phocytes is inhibited, in a more than additive to superadditive manner, by a simultaneous ad-
19 ministration of inhibitors of DP IV (Lys[Z(NO₂)]-thiazolidide = I49) and of prolyl oligopeptidase
20 (Boc-Ala thiazolidide). The T cells were incubated for 72 h in the presence of said inhibitors, and
21 the DNA synthesis was determined subsequently by a measurement of the ³[H]-thymidine in-
22 corporation, as described by Reinhold et al. (Reinhold D. et al.: Inhibitors of dipeptidyl peptidase
23 IV induce secretion of transforming growth factor β1 in PWM-stimulated PBMNC and T cells;
24 Immunology 1997, 91: 354 – 360). Figure 3 (page 3/14) shows the dose-dependent, more than
25 additive to superadditive inhibition of the DNA synthesis.

26 27 **Example 4**

28 **Inhibition of the DNA synthesis of human peripheral mononuclear cells by an incubation** 29 **with synthetic inhibitors of DP IV and of POP**

30
31 The searches according to the invention show that the DNA synthesis of human pe-
32 ripheral mononuclear cells (MNC) is inhibited, in an increased manner, by a simultaneous ad-
33 ministration of inhibitors of DP IV (Lys[Z(NO₂)]-thiazolidide = I49) and of prolyl oligopeptidase
34 (Boc-Ala thiazolidide). The MNC were incubated for 72 h in the presence of said inhibitors, and

1 the DNA synthesis was determined subsequently by a measurement of the $^3\text{[H]}$ -thymidine in-
2 corporation, as described by Reinhold et al. (Reinhold D. et al.: Inhibitors of dipeptidyl peptidase
3 IV induce secretion of transforming growth factor $\beta 1$ in PWM-stimulated PBMNC and T cells;
4 Immunology 1997, 91: 354 – 360). Figure 4 (page 4/14) shows the dose-dependent, increased
5 inhibition of the DNA synthesis.

6 7 **Example 5**

8 **Inhibition of the DNA synthesis of human T lymphocytes by an incubation with synthetic** 9 **inhibitors of DP IV and of ACE**

10
11 The searches according to the invention show that the DNA synthesis of human T lym-
12 phocytes is inhibited, in a more than additive to superadditive manner, by a simultaneous ad-
13 ministration of inhibitors of DP IV (Lys[Z(NO₂)]-thiazolidide = I49) and of the angiotensin-
14 converting enzyme (Captopril). The T cells were incubated for 72 h in the presence of said in-
15 hibitors, and the DNA synthesis was determined subsequently by a measurement of the $^3\text{[H]}$ -
16 thymidine incorporation, as described by Reinhold et al. (Reinhold D. et al.: Inhibitors of dipepti-
17 dyl peptidase IV induce secretion of transforming growth factor $\beta 1$ in PWM-stimulated PBMNC
18 and T cells; Immunology 1997, 91: 354 – 360). Figure 5 (page 5/14) shows the dose-dependent,
19 more than additive to superadditive inhibition of the DNA synthesis.

20 21 **Example 6**

22 **Inhibition of the DNA synthesis of human peripheral mononuclear cells by an incubation** 23 **with synthetic inhibitors of DP IV and of ACE**

24
25 The searches according to the invention show that the DNA synthesis of human pe-
26 ripheral mononuclear cells (MNC) is inhibited, in a more than additive to superadditive manner,
27 by a simultaneous administration of inhibitors of DP IV (Lys[Z(NO₂)]-thiazolidide = I49) and of
28 the angiotensin-converting enzyme (Captopril). The MNC were incubated for 72 h in the pres-
29 ence of said inhibitors, and the DNA synthesis was determined subsequently by a measurement
30 of the $^3\text{[H]}$ -thymidine incorporation, as described by Reinhold et al. (Reinhold D. et al.: Inhibitors
31 of dipeptidyl peptidase IV induce secretion of transforming growth factor $\beta 1$ in PWM-stimulated
32 PBMNC and T cells; Immunology 1997, 91: 354 – 360). Figure 6 (page 6/14) shows the dose-
33 dependent, more than additive to superadditive inhibition of the DNA synthesis.

Example 7

Inhibition of the proliferation of human peripheral mononuclear cells (MNC) by a single and simultaneous administration of inhibitors of DP IV (I49 = Lys[Z(NO₂)]-thiazolidide) and APN (Actinonin). (Figure 7: page 7/14)

Example 8

Inhibition of the proliferation of the human T cell line KARPAS-299 by a single and a simultaneous administration of inhibitors of DP IV (I49 = Lys[Z(NO₂)]-thiazolidide) and APN (Actinonin and Probestin). (Figure 8: page 8/14)

Example 9

Inhibition of the proliferation of activated human peripheral T cells by a single and simultaneous administration of inhibitors of DP IV (I49 = Lys[Z(NO₂)]-thiazolidide) and of APN (Actinonin and Probestin). (Figure 9: page 9/14)

Example 10

Inhibition of the proliferation of PHA-activated human nuclear cells (MNC) by a single and simultaneous administration of inhibitors of DP IV (I49 = Lys[Z(NO₂)]-thiazolidide) and of X-Pro-Aminopeptidase (APP) (Apstatin). (Figure 10: page 10/14)

Example 11

Inhibition of the DNA synthesis of pokeweed-mitogene (PWM) stimulated human mononuclear cells (MNC) of the peripheral blood by an incubation with synthetic inhibitors of DP IV and APN.

The searches according to the invention show that the DNA synthesis of pokeweed-mitogene stimulated human MNC of the peripheral blood is inhibited, in a more than additive manner, by a simultaneous administration of inhibitors of DP IV (Lys[Z(NO₂)]-thiazolidide = I49) and APN (Bestatine). The MNC were incubated for 72 hours in the presence of pokeweed-mitogene and of said inhibitors, and the DNA synthesis was determined subsequently by a measurement of the ³[H]-thymidine-incorporation, is described by Reinhold et al. (Reinhold D et al.: Inhibitors of dipeptidyl peptidase IV induce secretion of transforming growth factor β1 in

1 PWM-stimulated PBMNC and T cells. Immunology 1997; 91; 354-360). Figure 11 (page 11/14)
2 shows the dose-dependent more than additive inhibition of DNA synthesis.

4 **Example 12**

5 **Inhibition of the IL-4-production by pokeweed-mitogene stimulated human mononuclear** 6 **cells of the peripheral blood by an incubation with synthetic inhibitors of DP IV and of** 7 **APN**

8
9 The searches according to the invention show the interesting result that the production of
10 the (T_{H2}-cell characteristic) cytokine IL-4 by pokeweed-mitogene stimulated human mononu-
11 clear cells (MNC) of the peripheral blood is inhibited, in a more than additive to superadditive
12 manner, by a simultaneous administration of inhibitors of DP IV (Lys[Z(NO₂)]-thiazolidide = I49)
13 and APN (Bestatine). The MNC were incubated for 48 hours in the presence of pokeweed-
14 mitogene and of said inhibitors, and the concentration of IL-4 was subsequently determined in
15 the respective culture supernatants by means of commercially available IL-4 analysis kits
16 (ELISA) as described by Reinhold et al. (Reinhold D et al.: Inhibitors of dipeptidyl peptidase IV
17 induce secretion of transforming growth factor β 1 in PWM-stimulated PBMNC and T cells. Im-
18 munology 1997; 91; 354-360). Figure 12 (page 12/14) shows the dose-dependant superadditive
19 inhibition of the IL-4-production.

21 **Example 13**

22 **Inhibition of the DNA synthesis of human keratinocytes (HaCaT cell line) by an incuba-** 23 **tion with synthetic inhibitors of DP IV and of APN.**

24
25 The searches according to the invention show that the DNA synthesis of human HaCaT
26 keratinocytes is inhibited, in a more than additive and, at lower concentrations, also in a super-
27 additive manner, by a simultaneous administration of inhibitors of DP IV (Lys[Z(NO₂)]-
28 thiazolidide = I49) and of APN (actinonin).

29
30 The human keratinocyte cell line HaCaT which is an accepted cell model for psoriasis
31 expresses DP IV and APN. The enzymatic activity of DP IV in vital cells is 30.2 ± 5 pkat/10⁶
32 cells, and the enzymatic activity of APN is 90 ± 4 pkat/10⁶ cells. In a corresponding manner, the
33 mRNA of APN and DP IV is detectable on those cells (Fig. 13, page 13/14).

1

2 HaCaT cells were incubated for 48 hours in presence of the above-mentioned inhibitors,
3 and the DNA synthesis was subsequently determined by a measurement of ³[H]-thymidine-
4 incorporation, as described by Reinhold et al. (Reinhold D et al.: Inhibitors of dipeptidyl pepti-
5 dase IV induce secretion of transforming growth factor β 1 in PWM-stimulated PBMNC and T
6 cells. Immunology 1997; 91; 354-360). Figure 14 (page 14/14) shows the dose-dependant inhi-
7 bition of the DNA synthesis.

Description of the Figures

Fig. 1: Synergistic and dose-dependent effect of inhibitors of DP IV (I49) and of aminopeptidase N (actinonin) on the DNA synthesis of human T lymphocytes. Human peripheral T cells were incubated for three days with the above-indicated concentrations of the inhibitors. Subsequently, ^3H -methyl thymidine was added to the culture medium, and after further 6 hours, the ^3H -thymidine amount incorporated into the DNA was measured.

Fig. 2: Synergistic and dose-dependent effect of inhibitors of DP IV (I49) and of APN (actinonin) on the DNA synthesis of human mononuclear cells (MNC). Human MNC were incubated for three days with the above-indicated concentrations of the inhibitors. Subsequently, ^3H -methyl thymidine was added to the culture medium, and after further 6 hours, the ^3H -thymidine amount incorporated into the DNA was measured.

Fig. 3: Synergistic and dose-dependent effect of inhibitors of DP IV (I49) and of prolyl oligopeptidase (Boc-Ala-Thia) on the DNA synthesis of human peripheral T lymphocytes. Human T cells were incubated for three days with the above-indicated concentrations of the inhibitors. Subsequently, ^3H -methyl thymidine was added to the culture medium, and after further 6 hours, the ^3H -thymidine amount incorporated into the DNA was measured.

Fig. 4: Enhanced and dose-dependent effect of inhibitors of DP IV (I49) and of prolyl oligopeptidase (Boc-Ala-Thia) on the DNA synthesis of human mononuclear cells (MNC). Human MNC were incubated for three days with the above-indicated concentrations of the inhibitors. Subsequently, ^3H -methyl thymidine was added to the culture medium, and after further 6 hours, the ^3H -thymidine amount incorporated into the DNA was measured.

Fig. 5: Synergistic and dose-dependent effect of inhibitors of DP IV (I49) and of angiotensin-converting enzyme (captopril) on the DNA synthesis of human peripheral T lymphocytes. Human T cells were incubated for three days with the above-indicated concentrations of the inhibitors. Subsequently, ^3H -methyl thymidine was added to the culture medium, and after further 6 hours, the ^3H -thymidine amount incorporated into the DNA was measured.

1 Fig. 6: Synergistic and dose-dependent effect of inhibitors of DP IV (I49) and of angio-
2 tensin-converting enzyme (captopril) on the DNA synthesis of human mononuclear cells (MNC).
3 Human MNC were incubated for three days with the above-indicated concentrations of the in-
4 hibitors. Subsequently, ^3H -methyl thymidine was added to the culture medium, and after further
5 6 hours, the ^3H -thymidine amount incorporated into the DNA was measured.

7 Fig. 7: The MNC were incubated for the time of 72 hours without (control), with mitogenic
8 lectin phytohemagglutinine (PHA) or with PHA plus the inhibitors indicated. Subsequently, there
9 was carried out a determination of the number of metabolically active cells by using the com-
10 mercially available WST-1 Cell-Proliferation Assay (Takara Inc.) in accordance with the propos-
11 als of the manufacturer.

12
13 Fig. 8: The KARPAS-299-cells were incubated for a time period of 72 hours without (con-
14 trol) and with the above-indicated inhibitors singly or in combination. Subsequently, there was
15 carried out a determination of the number of metabolically active cells by using the commercially
16 available WST-1 Cell-Proliferation Assay (Takara Inc.) in accordance with the proposals of the
17 manufacturer.

18
19 Fig. 9: The T cells with the exception of the untreated control, were activated by an addi-
20 tion of phytohemagglutinine and phorbol-12-myristate-13-acetate to the culture medium and
21 were incubated for a time of 72 hours in the presence of the above inhibitors singly or in combi-
22 nation. Subsequently, there was carried out a determination of the number of metabolically ac-
23 tive cells by using the commercially available WST-1 Cell-Proliferation Assay (Takara Inc.) in
24 accordance with the proposals of the manufacturer.

25
26 Fig. 10: The mononuclear cells (MNC) were incubated for a time of 72 hours in the pres-
27 ence of the above-indicated inhibitors singly or in combination. Subsequently, there was carried
28 out a determination of the number of metabolically active cells by using the commercially avail-
29 able WST-1 Cell-Proliferation Assay (Takara Inc.) in accordance with the proposals of the
30 manufacturer.

31
32 Fig. 11: Synergistic and dose-dependent effect of inhibitors of DP IV (I49) and of amin-
33 opeptidase N (bestatine) on the DNA synthesis of human PWM-stimulated MNC. Human pe-

1 peripheral MNC were incubated for three days with PWM (2 µg/ml) and the above-indicated con-
2 centration of inhibitors. Subsequently, ³[H]-methyl thymidine was added to the culture medium,
3 and the amount of ³[H]-thymidine incorporated into the DNA was measured after further 6 hours.
4

5 Fig. 12: Synergistic and dose-dependent effect of inhibitors of DP IV (I49) and of amin-
6 opeptidase N (bestatine) on the IL-4-production by human, PWM-stimulated MNC. Human pe-
7 ripheral MNC were incubated for 48 hours with PWM (2 µg/ml) and with the above-indicated
8 concentrations of the inhibitors. Subsequently, the concentration of IL-4 in the respective culture
9 supernatants were measured by means of IL-4-ELISA.
10

11 Fig. 13: Detection of the mRNA expression of DP IV and APN on HaCaT keratinocytes
12 by means of RT-PCR.
13

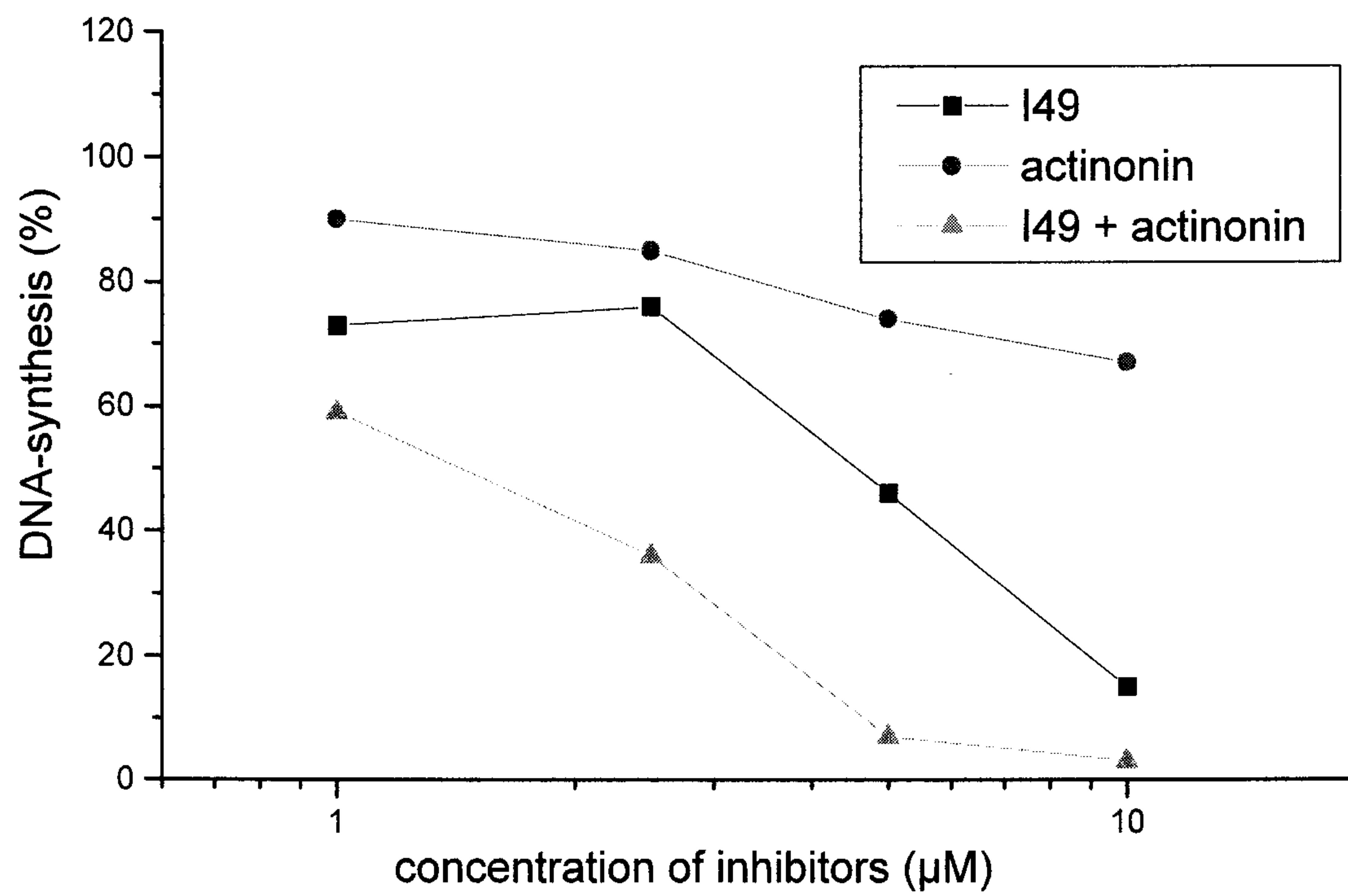
14 Fig. 14: Synergistic and dose-dependant effect of inhibitors of DP IV (I49) and of amin-
15 opeptidase N (actinonin) on the DNA synthesis of human HaCaT keratinocytes. The cells were
16 incubated for 48 hours with the above-indicated concentrations of inhibitors. Subsequently, ³[H]-
17 methyl thymidine was added to the culture medium, and after further 6 hours, the amount of
18 ³[H]-thymidine incorporated into the DNA was measured.
19

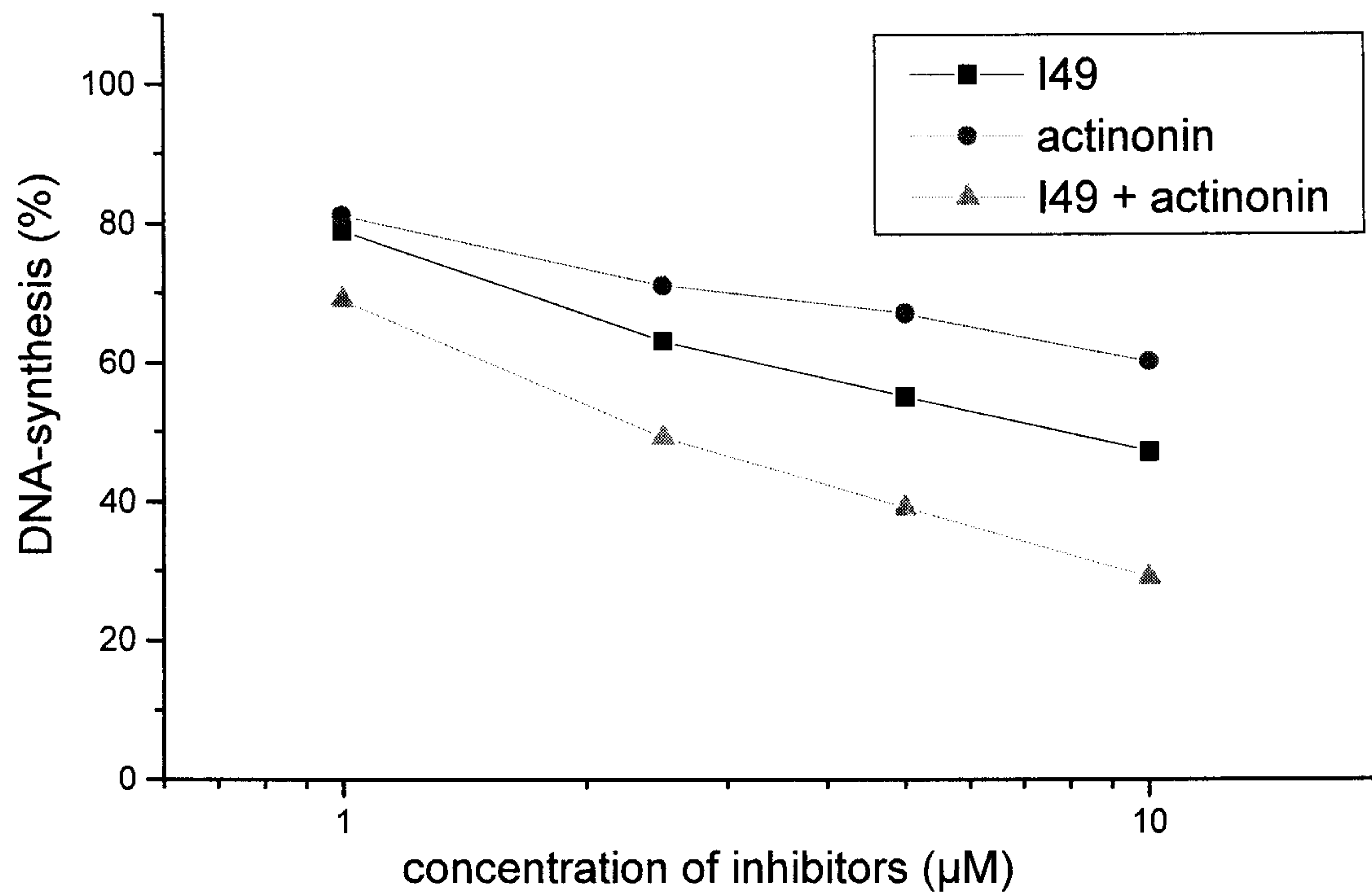
WE CLAIM:

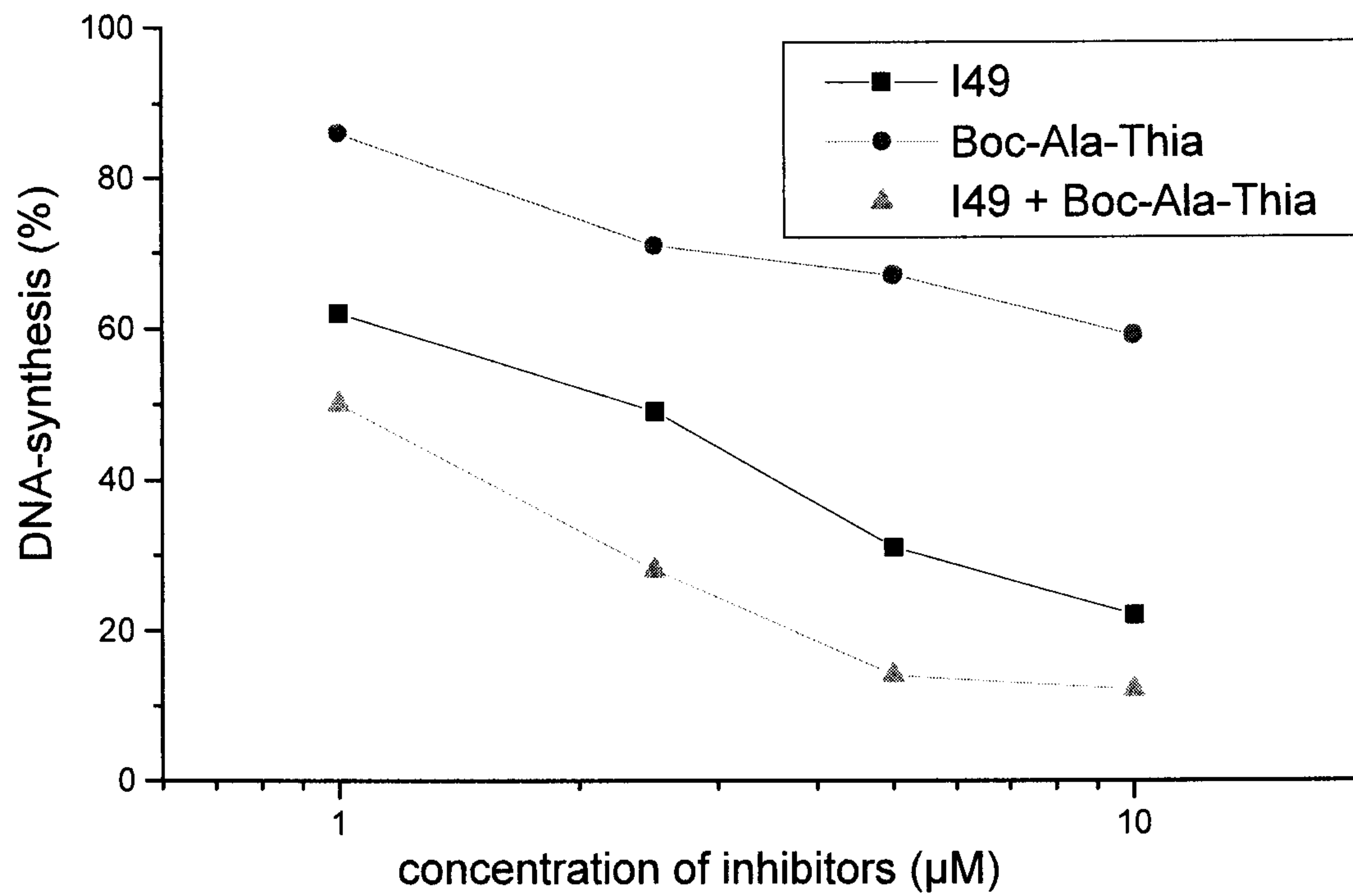
1. Use of an inhibitor combination comprising a first inhibitor and a second inhibitor, wherein:
 - (1) the first inhibitor is an inhibitor of dipeptidyl peptidase IV (DP IV) or an inhibitor of an enzyme having DP IV-analogous enzymatic activity; and,
 - (2) the second inhibitor is an inhibitor of alanyl-aminopeptidase (aminopeptidase N, APN) or an inhibitor of an enzyme having APN-analogous enzymatic activity;for a superadditive inhibition of the activation, DNA-synthesis and proliferation of human epidermal and follicular keratinocytes as well as keratinocytes of the transition zone from the skin to mucous membranes.
2. The use according to claim 1, wherein the first inhibitor is selected from the group consisting of:
 - Xaa-Pro-dipeptides or derivatives thereof, wherein Xaa is an α -amino acid or a side chain protected derivative thereof;
 - Xaa-Xaa-(Trp)-Pro-(Xaa)_n peptides or salts thereof, wherein Xaa is an α -amino acid and n is an integer from 0 to 10; and
 - amino acid (Xaa)-amides or salts thereof, wherein Xaa is an α -amino acid or a side chain protected derivative thereof and wherein the amide is derived from a cyclic amine or derivatives thereof.
3. The use according to claim 2, wherein the Xaa-Pro-dipeptide is a dipeptide phosphonic acid diaryl ester, or a dipeptide boronic acid or salts thereof.
4. The use according to claim 3, wherein the dipeptide boronic acid is Pro-Boro-Pro.
5. The use according to claim 2, wherein the Xaa of the amino acid (Xaa)-amide is N^ε-4-nitrobenzyloxycarbonyl-L-lysine, L-proline, L-tryptophane, L-isoleucine or L-valine.
6. The use according to claim 2, wherein the cyclic amine is a pyrrolidine, a piperidine or a thiazolidine.

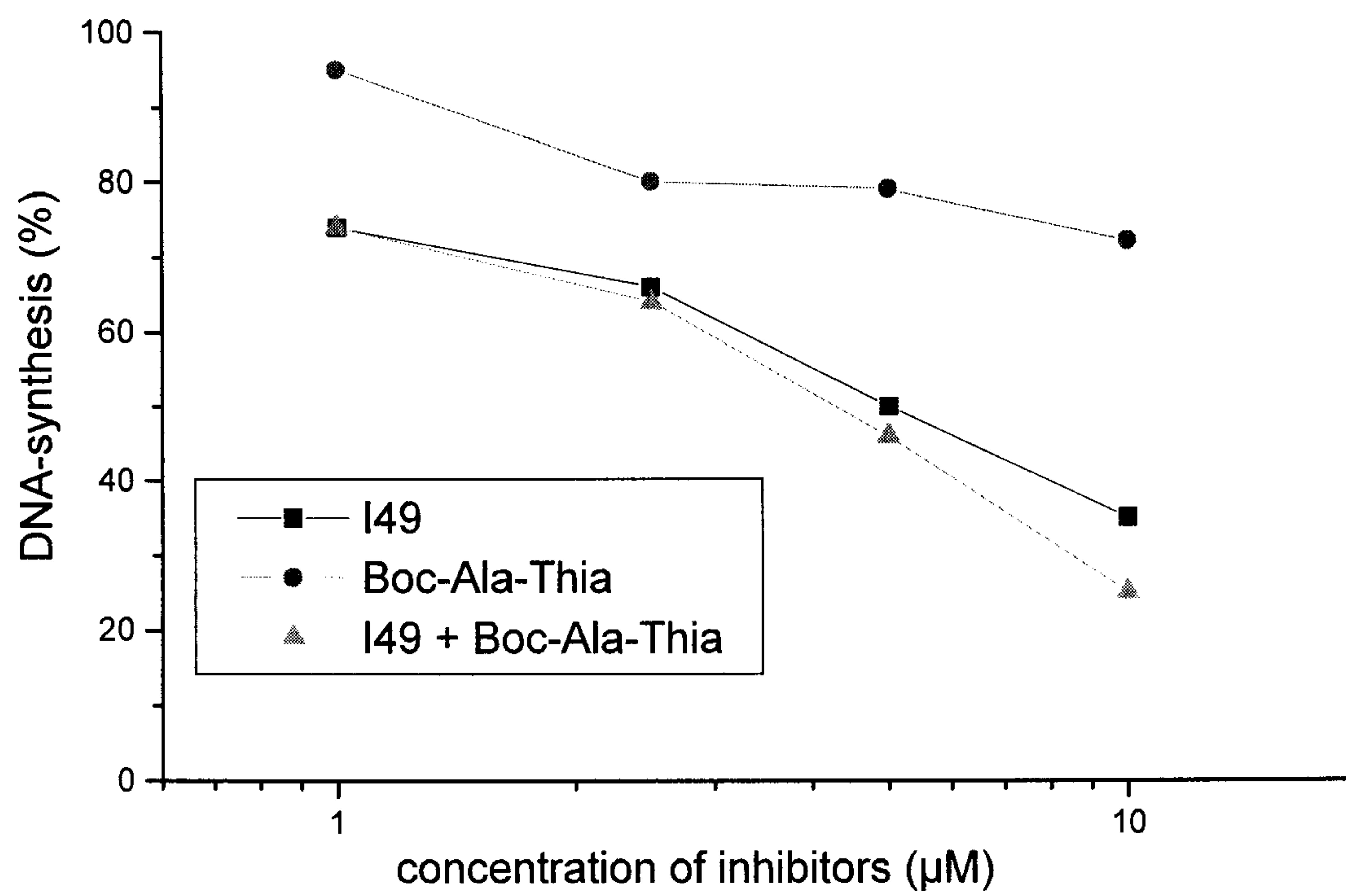
7. The use according to any one of claims 2 to 6, wherein the amino acid (Xaa)-amide is selected from the group consisting of N^ε-4-nitrobenzyloxycarbonyl-L-lysine thiazolidide, N^ε-4-nitrobenzyloxycarbonyl-L-lysine pyrrolidide, N^ε-4-nitrobenzyloxycarbonyl-L-lysine piperidide, N^ε-4-nitrobenzyloxycarbonyl-L-lysine 2-cyanothiazolidide, N^ε-4-nitrobenzyloxycarbonyl-L-lysine 2-cyanopyrrolidide and N^ε-4-nitrobenzyloxycarbonyl-L-lysine 2-cyano-piperidide.
8. The use according to any one of claims 1 to 7, wherein the second inhibitor is selected from the group consisting of actinonin, leuhistin, phebestin, amastatin, bestatin, probestin, RB3014, β-aminothiols, α-aminophosphinic acids, α-aminophosphinic acid derivatives and salts thereof.
9. The use according to claim 8, wherein the α-aminophosphinic acid derivative is D-Phe-ψ [PO(OH)-CH₂]-Phe-Phe and salts thereof.
10. Use of inhibitor combinations of any one of claims 1 to 9 for the prevention and therapy of inflammatory and non-inflammatory epidermal hyperproliferation conditions, benign and malign epidermal clonal expansions, benign and malign hyperproliferation conditions, benign and malignant epithelial adnex tumors or primary and reactive nail cell hyperproliferations.
11. The use according to claim 10, wherein the inflammatory and non-inflammatory epidermal hyperproliferation conditions are selected from the group consisting of congenital ichthyoses and psoriasis.
12. The use according to claim 10, wherein the benign and malignant epidermal clonal expansions are selected from the group consisting of warts, condylomes and actinic keratoses or precanceroses.
13. The use according to claim 10, wherein the benign and malign hyperproliferation condition is keratosis follicularis.
14. Use of inhibitor combinations of any one of claims 1 to 13 in a systemic application form selected from the group consisting of oral, transdermal, intravenous, subcutaneous, intracutaneous, intramuscular, rectal, vaginal and sublingual administration.

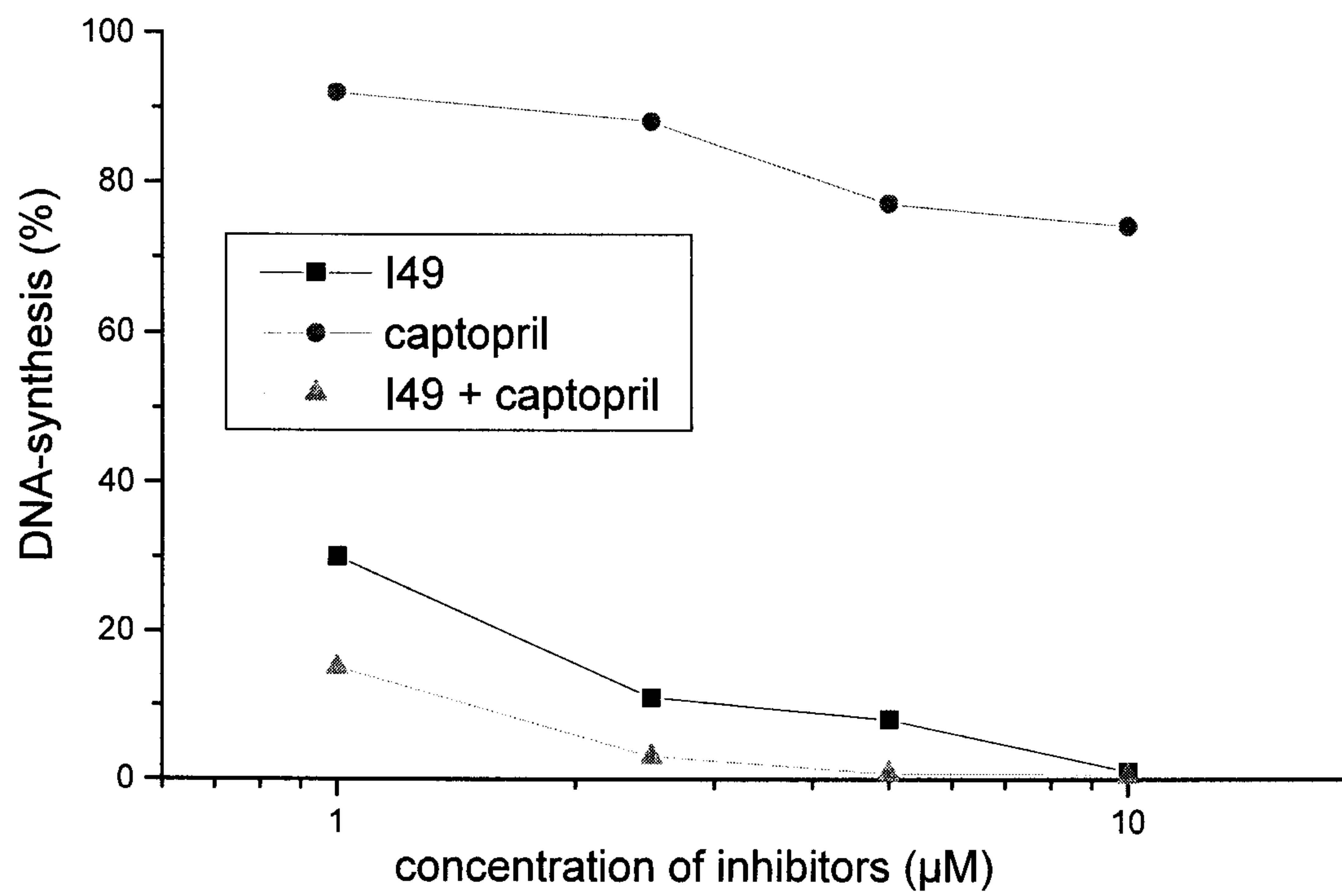
15. Use of the inhibitor combinations according to any one of claims 1 to 13 in a topical application form selected from the group consisting of creams, ointments, pastes, gels, solutions, sprays, liposomes, hydro-colloid dressings and other dermatological bases or vehicles and instillative applications.
16. Use of the inhibitor combination of any one of claims 1 to 15, wherein the first inhibitor and the second inhibitor are in a compartmentally separate formulation in combination with an adjuvant selected from the group consisting of a carrier, an auxiliary and an inert additive, for simultaneous administration with the aim of a combined effect.
17. Use of the inhibitor combination of any one of claims 1 to 15, wherein the first inhibitor and the second inhibitor are in a compartmentally separate formulation in combination with an adjuvant selected from the group consisting of a carrier, an auxiliary and an inert additive, for consecutive administration with the aim of a combined effect.

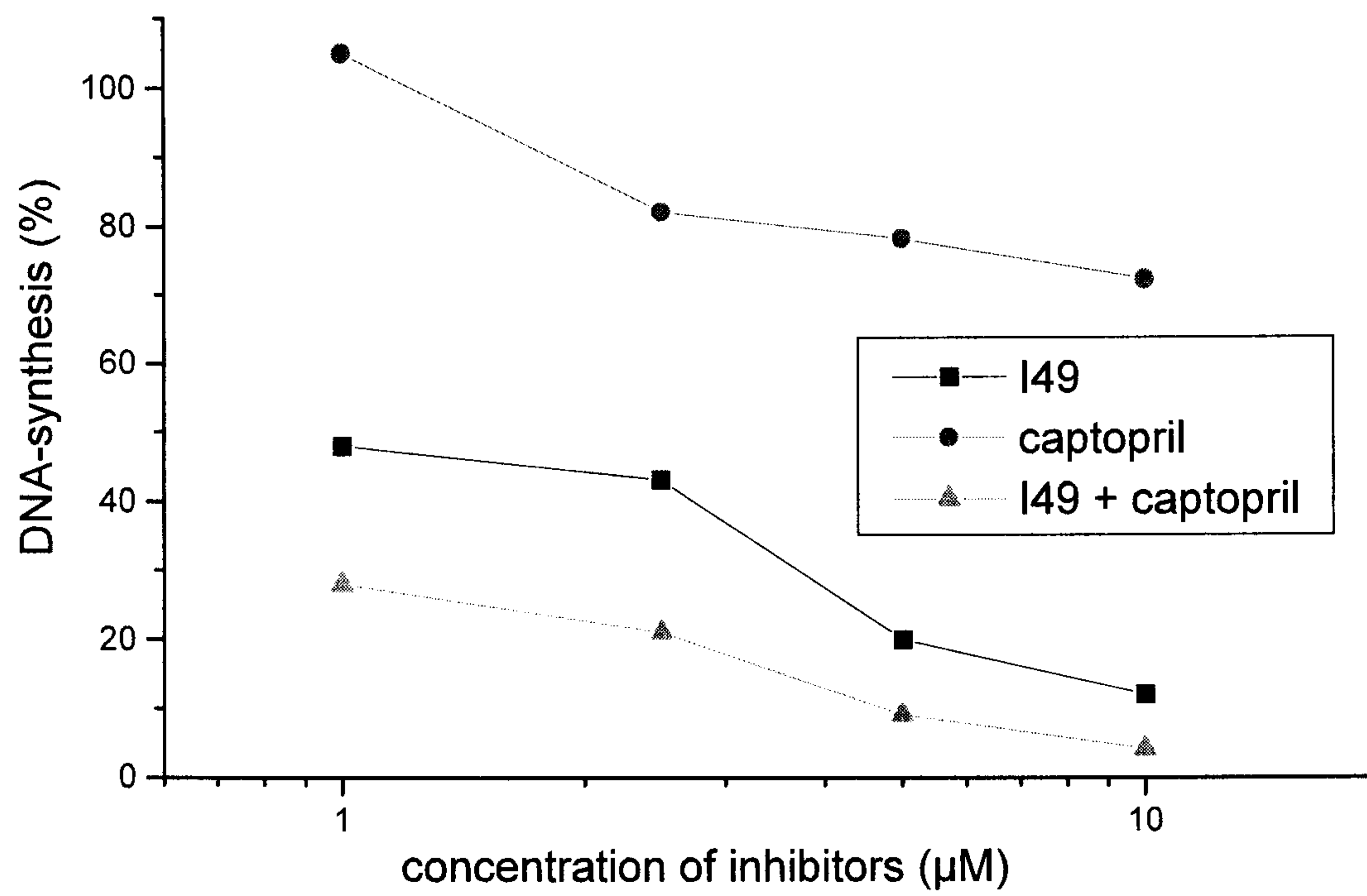
**Figure 1**

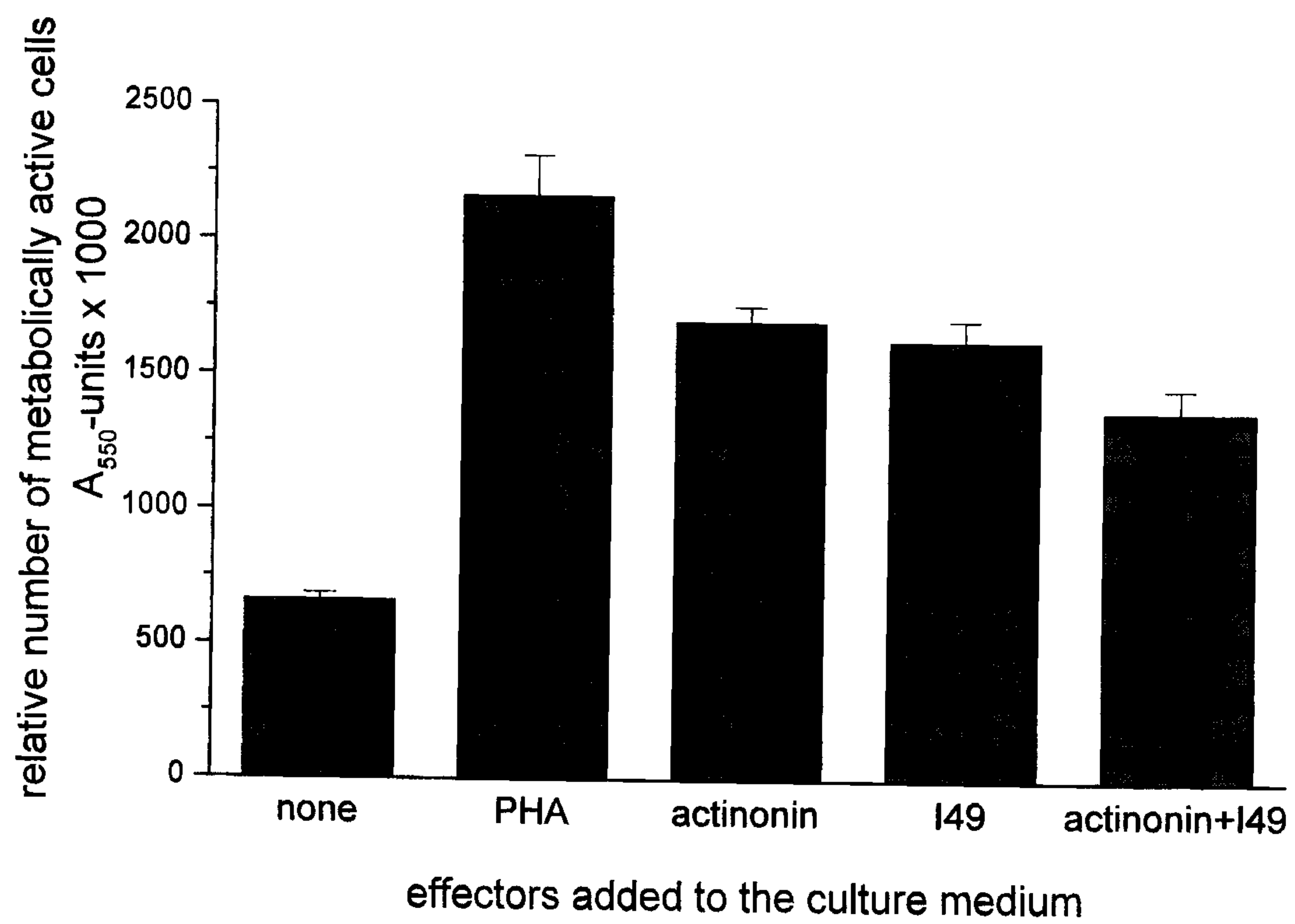
**Figure 2**

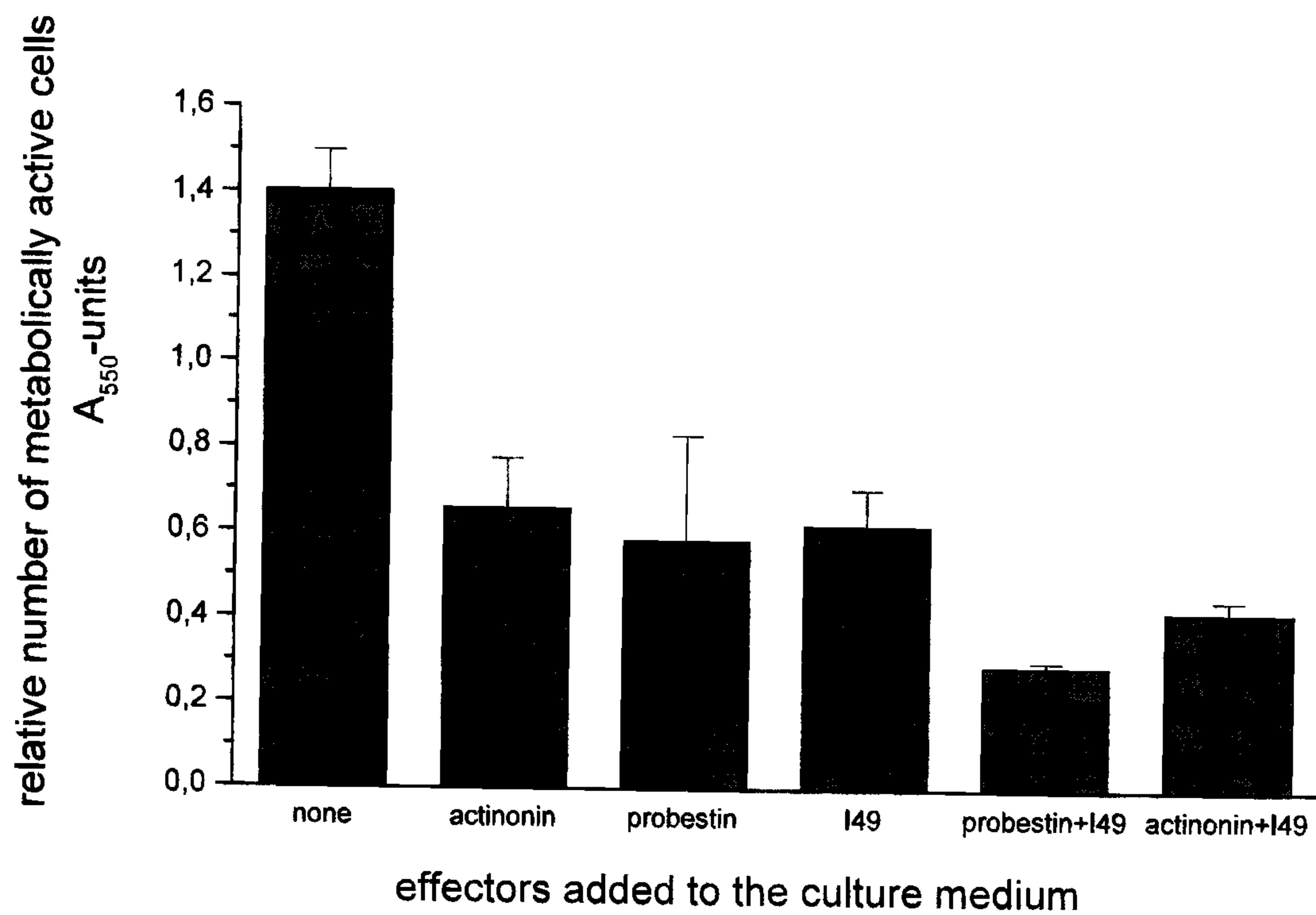
**Figure 3**

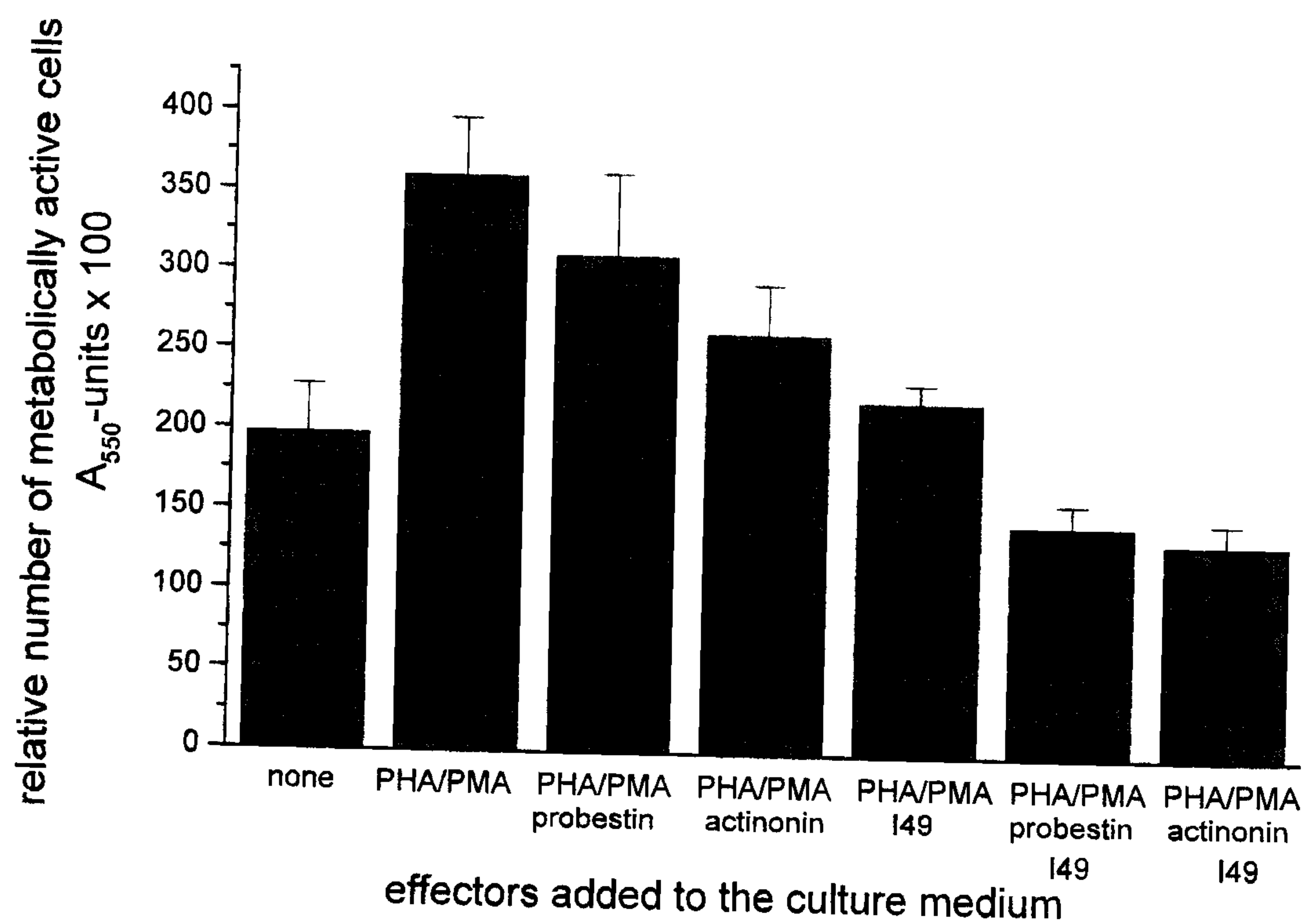
**Figure 4**

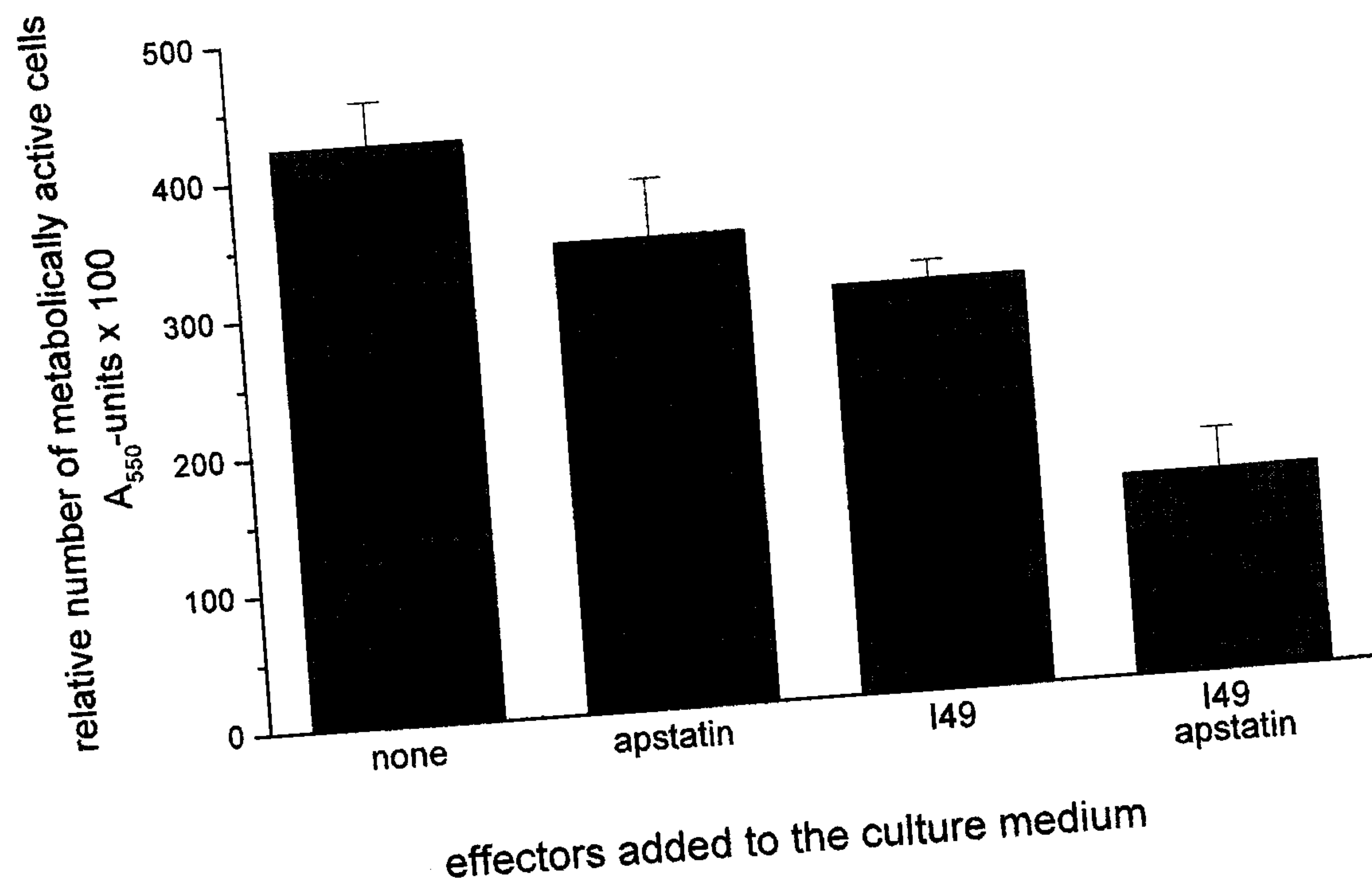
**Figure 5**

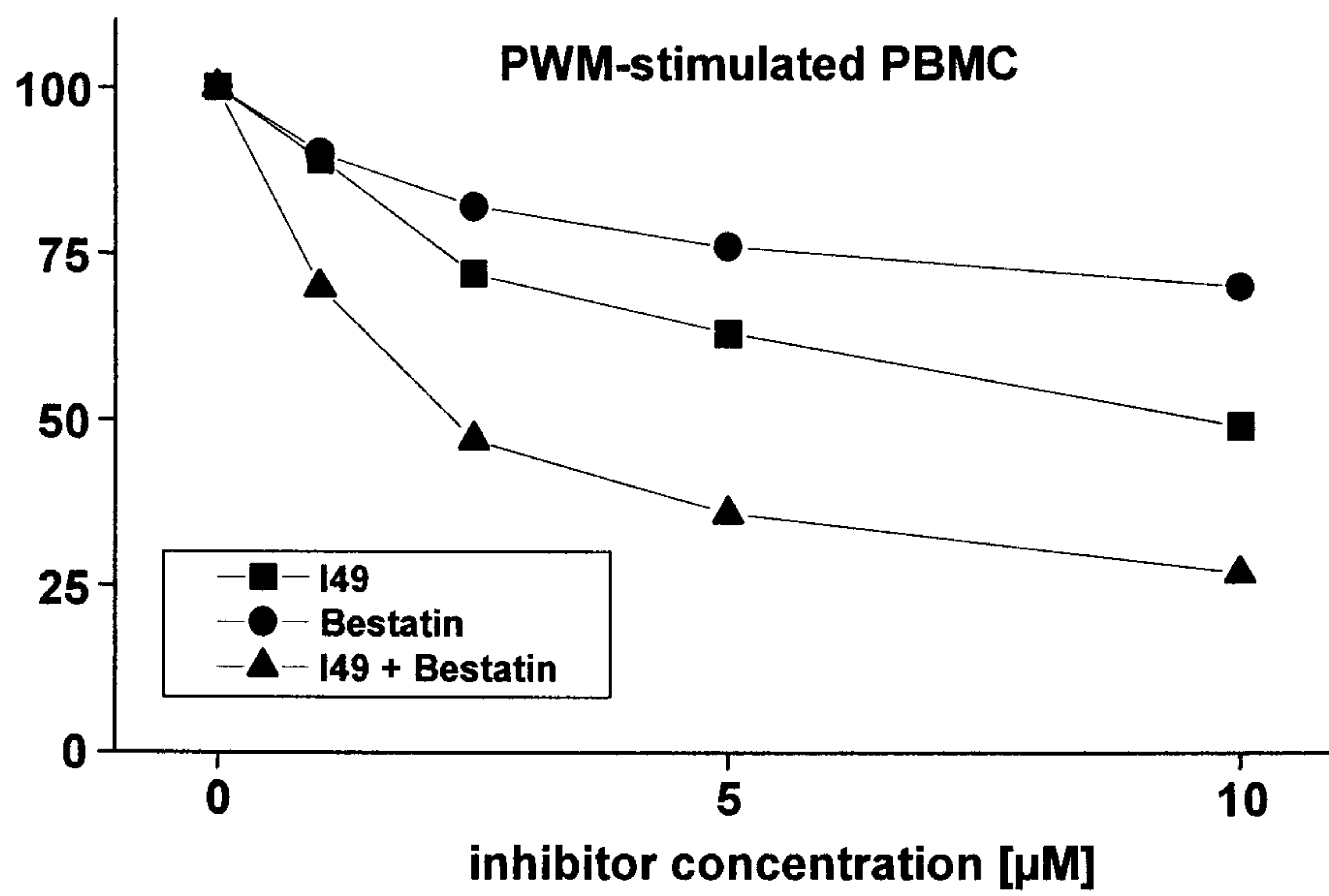
**Figure 6**

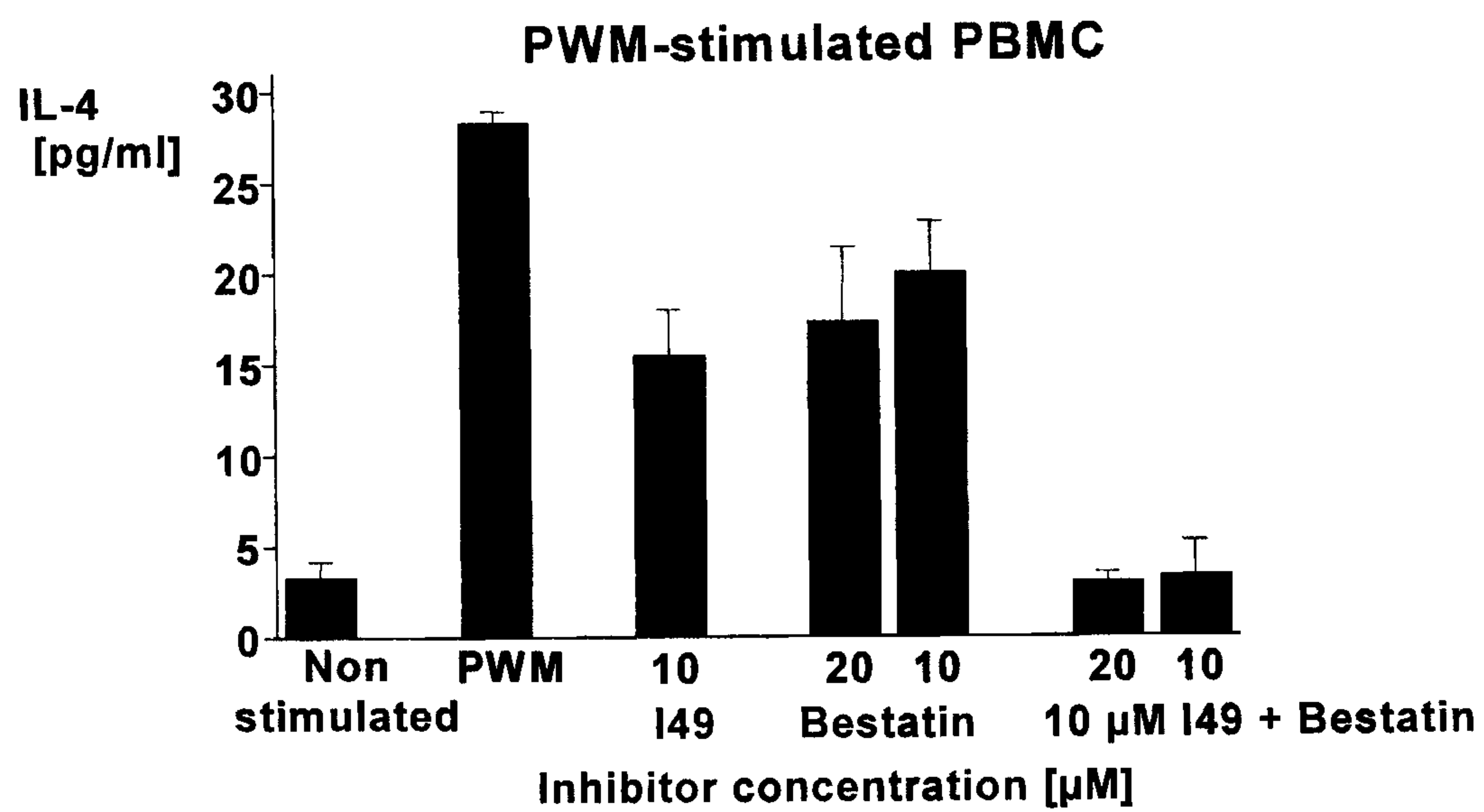
**Figure 7**

**Figure 8**

**Figure 9**

**Figure 10**

% DNA synthesis**Figure 11**

**Figure 12**

Application number/numéro de demande: 2,436,724

Figures: 13

Pages: _____

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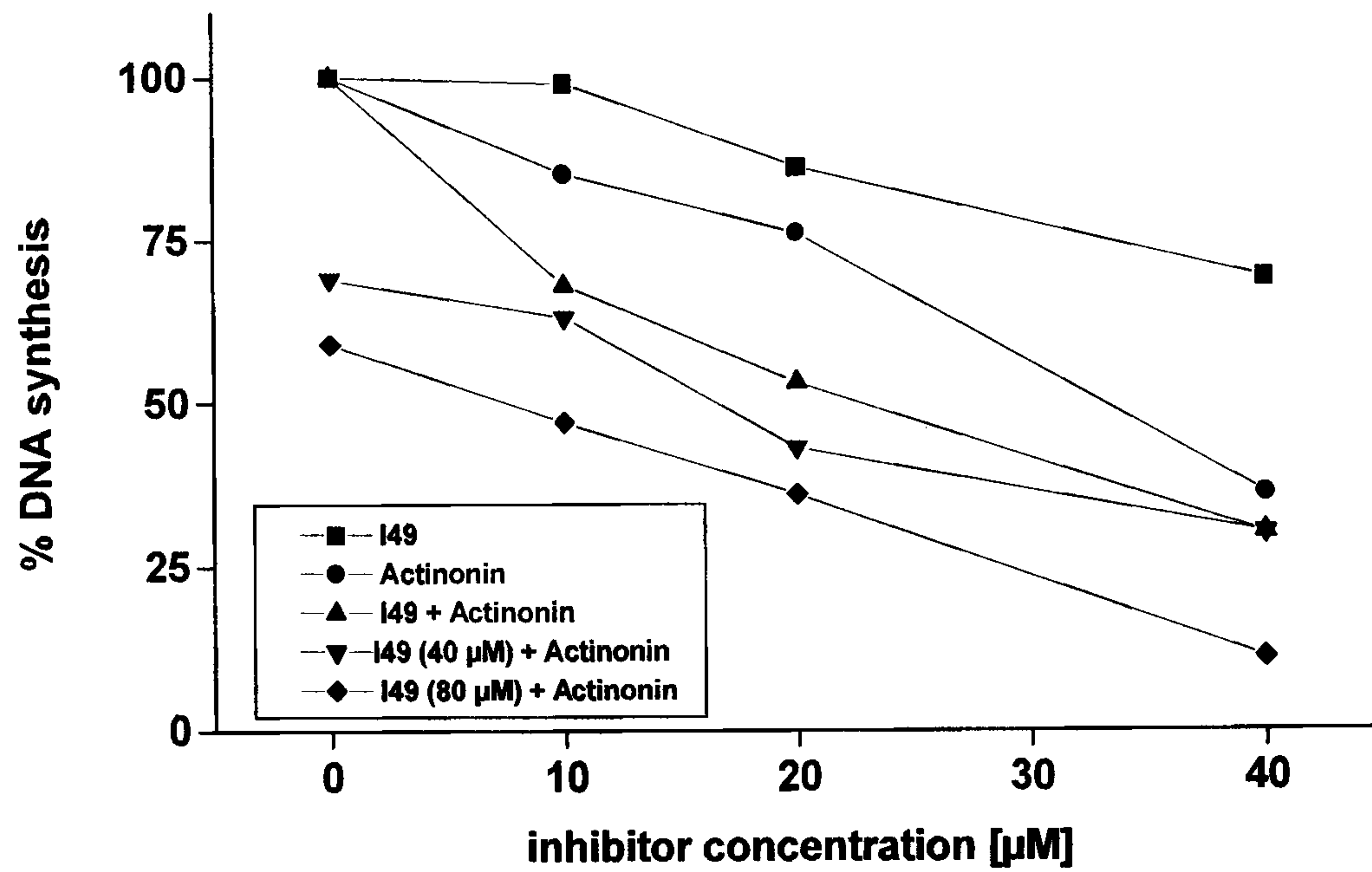
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**Figure 14**