Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2923388 C 2022/07/19

(11)(21) **2 923 388**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) Date de dépôt PCT/PCT Filing Date: 2014/09/03
(87) Date publication PCT/PCT Publication Date: 2015/03/12
(45) Date de délivrance/Issue Date: 2022/07/19
(85) Entrée phase nationale/National Entry: 2016/03/04
(86) N° demande PCT/PCT Application No.: IB 2014/064223
(87) N° publication PCT/PCT Publication No.: 2015/033276
(30) Priorité/Priority: 2013/09/06 (IT MI2013A001475)

(51) Cl.Int./Int.Cl. *A61K 8/36* (2006.01), *A61K 31/202* (2006.01), *A61K 31/232* (2006.01), *A61K 8/37* (2006.01), *A61P 17/16* (2006.01), *A61P 35/00* (2006.01)

(72) Inventeurs/Inventors:
GIULIANI, GIAMMARIA, IT;
BENEDUSI, ANNA, IT;
MARZANI, BARBARA, IT;
BARONI, SERGIO, IT;
PICARDO, MAURO, IT

(73) Propriétaire/Owner:
GIULIANI S.P.A., IT

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : COMPOSITIONS UTILES DANS LE TRAITEMENT PROPHYLACTIQUE OU THERAPEUTIQUE DU CANCER DE LA PEAU
(54) Title: COMPOSITIONS USEFUL IN THE PREVENTION OR TREATMENT OF SKIN CANCER

(57) Abrégé/Abstract:
The invention relates to compounds of general formula (I): CH_3-$(CH=CH)_n$-R (I) wherein n = 3, 5, 7; R is selected from -CO-OR', -CO-$O^{(-)}$, or -CH_2-O-CO-R', R' being selected from H, C_1-C_{22} alkyl or alkenyl.aryl or aralkyl, or sugars; with the proviso that when R is -CH_2-O-CO-R' and R' alkyl, said alkyl being selected from C_1 to C_{11}; and pharmaceutically acceptable salts thereof, preferably such as sodium, potassium, lysine salts, each compound of general formula (I) being used as such or in admixture with one or more of the other ones, for use in the prevention or treatment of skin cancer resulting from DNA damage produced by UV radiation.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(10) International Publication Number
WO 2015/033276 A1

(43) International Publication Date
12 March 2015 (12.03.2015)

(51) International Patent Classification:
A61K 8/36 (2006.01) *A61K 31/232* (2006.01)
A61K 8/37 (2006.01) *A61P 17/16* (2006.01)
A61K 31/202 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number: PCT/IB2014/064223

(22) International Filing Date: 3 September 2014 (03.09.2014)

(25) Filing Language: Italian

(26) Publication Language: English

(30) Priority Data:
MI2013A001475 6 September 2013 (06.09.2013) IT

(71) Applicant: **GIULIANI S.P.A.** [IT/IT]; Via Palagi 2, I-20129 Milano (IT).

(72) Inventors: **GIULIANI, Giammaria**; Via Mascheroni 2, I-20123 Milano (IT). **BENEDUSI, Anna**; Viale Nazario Sauro 9, I-20124 Milano (IT). **MARZANI, Barbara**; Via G. Marconi, 13, I-27020 Carbonara Al Ticino (IT). **BARONI, Sergio**; Via Piazzolo 3, I-24030 Villa d'Adda (IT). **PICARDO, Mauro**; Via Capodistria 15, I-00198 Roma (IT).

(74) Agents: **APPOLONI, Romano** et al.; Notarbartolo & Gervasi S.p.a., Corso di Porta Vittoria 9, I-20122 Milano (IT).

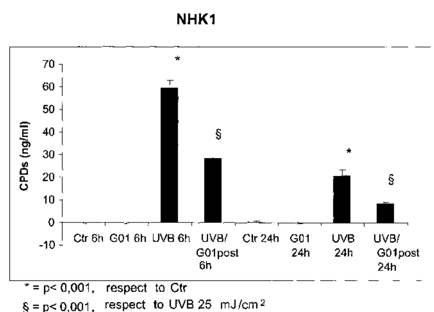
(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,

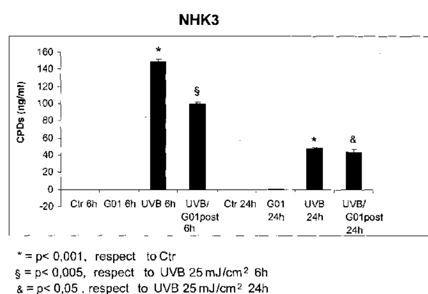
[Continued on next page]

(54) Title: COMPOSITIONS USEFUL IN THE PREVENTION OR TREATMENT OF SKIN CANCER

(57) **Abstract**: The invention relates to compounds of general formula (I): CH_3-$(CH=CH)_n$-R (I) wherein n = 3, 5, 7; R is selected from -CO-OR', -CO-$O^{(-)}$, or -CH_2-O-CO-R', R' being selected from H, C_1-C_{22} alkyl or alkenyl.aryl or aralkyl, or sugars; with the proviso that when R is -CH_2-O-CO-R' and R' alkyl, said alkyl being selected from C_1 to C_{11}; and pharmaceutically acceptable salts thereof, preferably such as sodium, potassium, lysine salts, each compound of general formula (I) being used as such or in admixture with one or more of the other ones, for use in the prevention or treatment of skin cancer resulting from DNA damage produced by UV radiation.



* = p< 0,001, respect to Ctr
§ = p< 0,001, respect to UVB 25 mJ/cm²



* = p< 0,001, respect to Ctr
§ = p< 0,005, respect to UVB 25mJ/cm² 6h
& = p< 0,05 , respect to UVB 25mJ/cm² 24h

Fig. 7

WO 2015/033276 A1

WO 2015/033276 A1

DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

COMPOSITIONS USEFUL IN THE PREVENTION OR TREATMENT OF SKIN CANCER

Field of the Invention

The object of the present invention is a novel anticancer use of known
5 compounds, and compositions thereof which contain said compounds as active ingredient.

Background of the Invention

In each cell, the DNA is the repository of the genetic information; its stability and integrity are essential to life. The DNA is subject to environmental insults and any
10 consequent damage, if not repaired, leads to a mutation and, possibly, to the onset of a disease state.

Skin cancer from damage caused by excessive exposure to ultraviolet radiation is an example of the link between environmental damage induced to theDNA and the onset of a disease.

15 Another example is the DNA damage caused by tobacco smoke, which may thus lead to mutations in the lung cells and subsequent lung cancer.

In addition to the attack of environmental agents, the DNA is also subject to damage caused by metabolism by-products.

Whatever the origin of the harmful attack, it is estimated that a single cell can
20 suffer up to a million DNA changes in a day. In addition to the damage caused by environmental insults or metabolites, the process of DNA replication during cell divisionis subject to errorsitself. The speed at which the DNA polymerase adds not correctnucleotides during the DNA replication is an important factor in determining the rate of spontaneous mutation in an organism.

25 Although the cell is equipped with enzymatic systems that normally recognize and correct many of these errors, some mutations remain. For a given gene,estimates of the frequency with which the human DNA undergoes errors that are not corrected are roughly between 1 x 10^{-4} to 1 x 10^{-6} mutations per gamete. A rate of 1 x 10^{6} expresses the possibility of finding a mutation in a specific locus for a
30 million gametes.

The DNA repair processes are present in both prokaryote and eukaryote organisms. Many proteins involved in these processes are highly conserved during

evolution because of the importance of this repair function. The cells have developed several mechanisms to detect and correct the various types of damage that can occur to DNA, regardless of the source of damage. Because the DNA is a molecule that plays an active and critical role in cell division, the DNA repair is closely linked to the regulation of the cell cycle.

During the cell cycle, in fact, control mechanisms (checkpoints) ensure that the DNA of a cell is intact before allowing that the DNA replication and the cell division happen. A defect in the checkpoints may lead to an accumulation of damages, which in turn lead to mutations.

Among the harmful environmental factors, for example, it is particular well-known that the ultraviolet radiation (UVA and UVB) is the main etiologic agent in the development of many skin cancers. It causes DNA damage through the formation of pyrimidine cyclobutane dimers (CPD) and pyrimidine(6-4)pyrimidone adducts, or 6-4 photoproducts (6-4 PP). Both compounds are able to modify the DNA structure, thus preventing transcription and replication.

The phosphorylated form of histone γH2AX, the p53 protein and the protein GADD45a (growth arrest and DNA damage response gene),are also cited as known markers of DNA damage induced by UV irradiation.

In addition, 8-oxo-2'-deoxyguanosine (8-oxo-dG or 8-OHdG), an oxidized derivative of deoxyguanosine, is one of the major products of the DNA oxidation and, therefore, another typical marker of damage against it, particularly under exposure to UVA ultraviolet radiation.

The DNA repair is a physiological process that continuously operates in cells to protect the genome from damage and harmful mutations. The damage from CPD dimers and 6-4 PP photoproducts is counteracted through a process known as nucleotide excision repair (NER). In eukaryotes this complex process may repair a damage that involves up to 30 nucleotides.

Defects to some of these genes, if not repaired, can lead for example to genodermatoses, such as xeroderma pigmentosum, as well as other serious pre-cancerous dermatological disorders, *i.e.* characterized by a very high risk of developing skin cancer (about one thousand times greater than normal).

With reference to other technical problems and, therefore, in view of different uses, the same Applicant has in the past studied a class of dienes characterized by a general formula $CH_3(-CH=CH)_n-R$ (wherein n=2-7) and, in this respect, is the holder of various publications.

5 WO2010/052328 suggests for these compounds, investigated with reference to retinol as a positive control, a pharmaceutical or cosmetic use to improve the human epidermis cell repair activity by means of the production of keratin mediated by cytokeratins cK-19. The transcriptional study on the expression of cK-19 showed an increase (up-regulation) of cK-19 in the final differentiation of the

10 human epidermis reconstructed *in vitro*.

WO2010/052329 describes for these compounds, through the definition of an inhibitory action of the enzyme 5α-reductase, a cosmetic use directed to promote the integrity of the skin through the effect of increasing the strength of the collagen and elastin.

15 WO2011/132177 suggests the use of some of these compounds to counteract the oxidizing action of the free radicals ROS and preserve the physiological conditions of the human epidermis.

WO2012/007572 describes an activity of such compounds in the induction of melanogenesis through a non-receptor route. In particular, experiments performed

20 on primary melanocytes cultures showed the ability to induce the expression and activity of tyrosinase, the main melanogenic enzyme, and to increase the intracellular content of melanin.

None of these prior art documents relates to the behavior of these diene compounds with reference to the problem of human DNA damage caused by

25 environmental agents, such as those caused by excessive UV radiation may be. In fact, none of the experiments on which the evidence of activity in the uses as described in these prior art is based, contemplate, for example, UV irradiation, and therefore none of them measuresthe effects of UV radiation as a damage factor to human DNA, and thus as a carcinogen in case of skin cancers that develop

30 through an initial stage of DNA damage caused by UV radiation.

Summary of the Invention

Experimental studies, reported in the following paragraphs of the present description, have now surprisingly shown that for some of said compounds it is possible to obtain a significant activity of inhibition of the DNA damage produced by UV radiation, thereby envisaging a use for them as anticancer, both as
5 prevention and therapy, in the case of skin cancer induced by this kind of DNA damage.

Object of the invention are therefore compounds of general formula (I):

$$CH_3\text{-}(CH{=}CH)_n\text{-}R \qquad (I)$$

10 wherein n = 3, 5, 7; R is selected from -CO-OR', $-CO-O^{(-)}$, or $-CH_2-O-CO-R'$, R'being selected from H, C_1-C_{22} alkyl or alkenyl,aryl or aralkyl, or sugars;

with the proviso that when R is $-CH_2-O-CO-R'$ and R' alkyl, said alkyl being selected from C_1 to C_{11};

and pharmaceutically acceptable salts thereof, preferably such as sodium,
15 potassium, lysine salts,

each compound of general formula (I) being used as such or in admixture with one or more of the other ones,

for use in the prevention or treatment of skin cancer resulting from DNA damage produced by UV radiation.

20 Among the dermatological disorders to be treated according to such use, for example, xeroderma pigmentosum and photocarcinogenesis are cited as non-limiting examples.

A secondary use, dependent on the anticancer one, particularly in the case of prevention of DNA damage caused by UV radiation, is envisaged as active
25 ingredient in compositions directed to the photoprotection of the skin from UVA and UVB, specifically to prevent DNA damage.

Therefore, a further object of the present invention is such photoprotective use dependent on the above described use.

The present invention also relates to any composition for the uses described
30 above in both the therapeutic and the preventionfield, comprising as active ingredient a compound of general formula (I) as identified above, with any suitable excipient, particularly for topical application on the skin, including the scalp.

Detailed Description of the Invention

Some preferred compounds corresponding to the formula (I) for the use envisaged in the present invention are:

2,4,6 octatrienoic acid

5 2,4,6 octatrienoic acid sodium salt, or potassium salt

2,4,6-octatrienoic acid ethyl ester

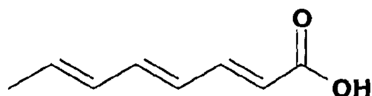
2,4,6-octatrienoic acid L-lysine salt

2,4,6-octatrien-1-ol acetate

The following examples illustrate the invention without limiting its scope in any

10 way.

The formula and the molecular weight for some of the compounds of general formula (I) are shown below.

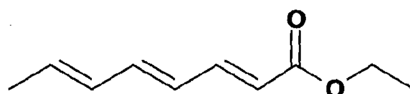


$C_8H_{10}O_2$M.W.138.17

15 2,4,6 octatrienoic acid

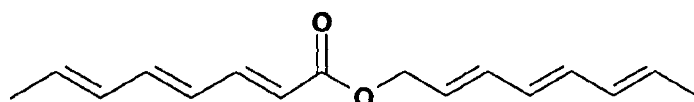
Sodium salt: $C_8H_9O_2Na$

Lysin salt: $C_8H_9O_2 . C_6H_{15}N_2O_2$,



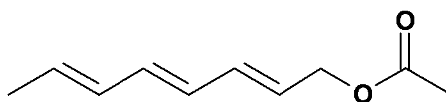
20 $C_{10}H_{14}O_2$ M.W. 166.22

2,4,6-octatrienoic acid ethyl ester



25 $C_{16}H_{20}O_2$ M.W. 244.34

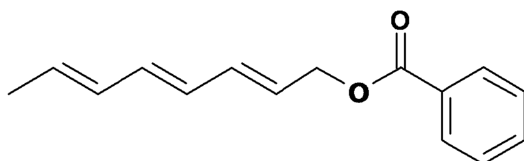
2,4,6-octatrienyl ester of 2,4,6-octatrienoic acid



$C_{10}H_{14}O_2$ M.W. 166.22

2,4,6-octatrien-1-ol acetate

5



$C_{15}H_{16}O_2$ M.W.228.29

2,4,6-octatrienyl ester of benzilic acid; 2,4,6-octatrien-1-ol, benzoate

In a composition according to the invention, said compound is present as active

10 ingredient in an amount preferably in the range from $10^{-6}$to $3x10^{-2}$mol/100g.

Description of the Drawings

Figs.1, 3, 6 show Western Blot immunofixation patterns, as described in more detail below.

15 Figs. 2, 4, 5, 7, 8, 9, 10, 11, 12 show diagrams relating to activity tests, as described in more detail below.

In the figures, the following meanings have to be understood:

Ctr = control

UVA or UVB = treatment with radiation only

20 G01 = treatment with compound of the invention only

G01/UVA = pre-treatment with compound of the invention, and subsequent UVA radiation

G01/UVB = pre-treatment with compound of the invention, and subsequent UVB radiation

25 UVA/G01post = UVA radiationand subsequenttreatment with compound of the invention

UVB/G01post = UVB radiationand subsequenttreatment with compound of the invention

Date Reçue/Date Received 2020-10-08

Non-limiting examples of compositions particularly suitable for the above-stated use are given below.

The quantities of the components are expressed as percentages by weight:

5 Example 1

ANTICANCER DERMATOLOGICAL PREPARATION

Component	Amount (% w/w)
2-Octyldodecanol	5-20
Cetostearyl alcohol	5-15
Cetyl esters wax	1-5
Sorbitan monostearate	1-5
Polisorbate 60	1-5
Benzilic alcohol	0.1-1
2,4,6-Octatrienoic acid	0.1-0.5
Purified water	q.s. to100 mL

Example 2

DERMATOLOGICAL PREPARATION HIGH PROTECTION AGAINST UV DAMAGE

Component	Amount (% w/w)
C_{20}-C_{22} alkyl phosphate	0.50-5.00
C_{20}-C_{22} alcohols	0.50-5.00
Cetearyl glucoside	0.10-2.00
Tromethamine	0.10-0.80
Dibutyl adipate	1.00-7.00
Ethylhexyl methoxycinnamate	1.00-10.00
Isostearyl isostearate	2.00-8.00
Ethylhexyl salicylate	1.00-5.00
Butylene glycol cocoate	1.00-5.00
Butyl methoxydibenzoylmethane	1.00-5.00
Diethylamino Hydroxybenzoyl Hexyl Benzoate	1.00-5.00
Octocrylene	1.00-5.00

Date Reçue/Date Received 2020-10-08

Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine 1.00-4.00
Diethylhexyl syringylidene malonate 0.10-1.00
2,4,6-Octatrienoic acid 0.05-0.50
Lysine Octatrienoate 0.05-0.50
5 Phenoxyethanol.. 0.80-1.00
Glycerin 1.00-5.00
Disodium EDTA... 0.01-0.09
Tocopheryl acetate.. 0.05-1.00
Potassium sorbate.. 0.005-0.10
10 Citric acid.. q.s. to pH 5.5
Purified water .... q.s. to100 g

Example 3

DERMATOLOGICAL PREPARATION FOR FACE PROTECTION

15 Component.. Amount (% w/w)
Xanthan gum 0.10-0.20
Sodium hyaluronate 0.05-1.00
Inositol 0.05-1.00
Xylitol... 0.05-1.00
20 Taurine 0.05-1.00
Betaine 0.05-1.00
C_{14}-C_{22} Alcohols 0.05-2.00
C_{12}-C_{20} Alkyl glucoside 0.05-1.00
Octyldodecanol.. 1.00-3.00
25 Ethylhexyl methoxycinnamate 0.50-3.00
Octocrylene 0.10-3.00
Butylene glycol 0.10-5.00
Glycerin 1.00-4.00
2,4,6-Octatrienoic acid 0.05-0.50
30 Phenoxyethanol.. 0.80-1.00
Sodium hydroxide.. 0.001-0.20
Citric acid.. 0.001-0.30

Date Reçue/Date Received 2020-10-08

Hydroxyethyl acrylate/Sodium acryloyldimethyl..................0.01-2.00
Taurate copolymer
Polyisobutene...0.01-1.50
PEG-7 Trimethylolpropane Coconut Ether 0.01-1.00
5 Purified water ....q.s. to100

Example 4

PROTECTIVE AND ANTICANCER DERMATOLOGICAL PREPARATION FOR THE SCALP

10 Component...Amount (% w/w)
Ethanol 10.00-30.00
Pentylene glycol 0.025-0.20
Potassium Octatrienoate 0.05-0.50
PEG-6 Caprylic/Capric Glycerides 0.10-1.00
15 Purified water ....q.s. to100.00

Example 5

DERMATOLOGICAL PREPARATION WITHSUNSCREEN FOR SKIN PROTECTION

20 Component...Amount (% w/w)
Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine...............1.00-4.00
Octyldodecanol...1.00-6.00
Denat. Alcohol Type D 1.00-15.00
C_{12}-C_{15}Alkyl benzoate 1.00-15.00
25 Diethylamino Hydroxybenzoyl Hexyl Benzoate 1.00-5.00
Caprylic/capric Triglyceride 1.00-60.00
Ethylhexyl methoxycinnamate...0.50-10.00
Octocrylene 0.10-5.00
Simmondsia Chinensis Seed Oil 0.10-10.00
30 2,4,6-Octatrienoic acid 0.01-1.00

Example 6

Date Reçue/Date Received 2020-10-08

DERMATOLOGICAL PREPARATION FOR THE TREATMENT OF THE FACE

Component	Amount (% w/w)
Propanediol	1.00-7.00
Xylitol	0.30-1.00
Cetearyl glucoside	0.10-2.00
Polyglyceryl-3 Rice Branate	0.10-3.00
Cetearyl alcohol	0.05-1.00
Disodium EDTA	0.01-0.10
2,4,6-Octatrienoic acidpotassium salt	0.05-0.50
C_{12}-C_{15} alkyl benzoate	1.00-5.00
Ethylhexyl methoxycinnamate	0.50-10.00
Octocrylene	0.50-5.00
Butyrospermum parkii butter	0.50-3.00
Citric acid	0.001-0.30
Simmondsia Chinensis seed oil	0.10-0.30
Hydrogenated Evening Primrose Oil	0.50-3.00
Octyldodecanol	0.50-3.00
Caprylic/Capric triglyceride	1.00-5.00
Isosterayl isostearate	0.10-5.00
Beta sitosterol	0.10-0.50
Delta tocoferol	0.05-0.20
Caprylyl glycol	0.05-0.50
1.2 Hexanediol	0.10-0.70
Sodium hydroxide	0.001-0.20
Phenoxyethanol	0.50-0.99
Perfume	0.05-0.50
Purified water	q.s. to 100.00

Example 7

DERMATOLOGICAL PREPARATION FOR SCALP SKIN

Component	Amount (% w/w)
Cyclopentasiloxane	1.00-50.00

Date Reçue/Date Received 2020-10-08

Denat. Alcohol Type C 1.00-15.00
C_{12}-C_{15} alkyl benzoate.. 1.00-10.00
Ethylhexyl methoxycinnamate 1.00-5.00
Octocrylene 0.10-0.50
5 Disiloxane.. 1.00-49.00
Perfume.. 0.05-0.30
Oleyl erucate 0.50-3.00
Dimethiconol.. 0.10-10.00
2,4,6-Octatrienoic acid 0.01-1.00
10 Octyldodecanol.. 0.01-1.00

Example 8

PROTECTIVE TREATMENT CREAM

Component.. Amount (% w/w)
15 Propylene glycol 0.50-7.00
Pentylene glycol 1.00-3.00
Steareth-21.. 0.10-3.00
Steareth-2.. 0.10-3.00
Caprylic/capric triglyceride.. 1.00-10.00
20 Cycolopentasiloxane 0.50-20.00
Cetearyl alcohol.. 0.01-2.00
Octyldodecanol.. 0.10-5.00
Disodium EDTA... 0.01-0.10
2,4,6-Octatrienoic acidL-lysine salt...................................... 0.05-1.00
25 Phenoxyethanol.. 0.80-1.00
Sodium hydroxide.. 0.001-0.20
Citric acid.. 0.001-0.30

An experimental study on cell cultures was conducted as described below, with reference to the figures in the attached drawings, to demonstrate the activity of the 30 compounds of the present invention.

EXPERIMENTAL STUDY

Date Reçue/Date Received 2020-10-08

The aim of the study is to evaluate the repair activity of UV induced DNA damage exercised by a compound according to said general formula (I), namely 2,4,6-trans-octatrienoic acid, identified in the description of the study hereinafterwith the initials G01.

5 The study is performed on primary cultures of human keratinocytes, NHKs.

This activity has been evaluated in several respects, as follows.

Cell viability after treatment with G01

The cells (three different cultures of keratinocytesNHKs) were plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the

10 plate. The cells were treated with eight different concentrations of the compound of the invention G01under evaluation, diluted in the culture medium, for 48 ± 2h. The values of cell viability for each concentration tested, resulting from the spectrophotometric reading, were compared with the value obtained from the control. The results obtained showed the absence of cellular toxicity in all eight

15 different concentrations.

1) Assessment of the activity in the pre-treatment with the compound of the invention

Cell viability after pre-treatment with G01 and subsequent UV irradiation

The cellswere plated in the specific culture medium and maintained in culture for

20 24 ± 2h to allow adhesion to the plate. The cells were treatedfor 24 ± 2h with the concentrations of the compound G01 under evaluation, selected based on the previous results. Subsequently, the cells in the plates identified as +UVA and +UVB were respectively exposed, in the irradiation medium, to dosesof UVA of10 J/cm^2andUVB of 25 mJ/cm^2, while the control plate was maintained in the dark

25 and at room temperature for a period of time equal to the irradiation time. The irradiation medium was then replaced with the culture medium. The cell viability was measured (incorporation of NR)24 ± 2h and 48 ± 2h after the irradiation, following examination of the morphology of the cells under a microscope, in the absence and the presence of treatment with G01 and/or UVA/UVB irradiation. The

30 cell viability values for each condition tested were compared with the value obtained from the control and plotted on a graph to highlight any differences between the various experimental conditions.

The results obtained showed a reduction in cell viability following irradiation with UVA and UVB, and a general protective effect of cell viability, particularly 48h after irradiation, if a pre-treatment with a compound of the invention G01 was present, particularly significant at the highest concentration tested (90μM).

5 Western Blot assessment of the ability to repair a DNA damage after pre-treatment with G01 and subsequent UV irradiation

The NHKs cellswere plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate. Then, the keratinocytes were treated for 24 ± 2h with the compound of the invention G01, at concentrations of
10 60 μMand 90μM.Subsequently, the plates identified as +UVA, +G01/UVA, +UVB,and +G01/UVB were respectively exposed, in the irradiation medium, to dosesof UVA of10 J/$cm^2$and UVB of 25 mJ/cm^2, while the control plates (Ctr) and those treated only with G01 were maintained in the dark and at room temperature for a period of time equal to the irradiation time. The irradiation medium was then
15 replaced with the culture medium. Based on the preliminary results obtained on a broader time range, a post-treatment time of 6h was selected, after which the plates were processed for Western Blot protein determination.

For each condition tested (treatment with G01 and/or UVA/UVB irradiation), the protein expression of the phosphorylated histone γH2AX, p53 and GADD45a
20 (growth arrest and DNA damage response gene), known markers of DNA damage induced by UV radiation, was compared with the expression in the control cells (Ctr).

The results obtained by averaging the responses of three different keratinocytes cultures have shown the protective activity of the compound G01 under
25 examination, especially at the highest dose, as the cells pre-treated with G01 induced the protein expression of p53 and Gadd45ato a lesser extent. In confirmation of these results, the pre-treated keratinocytes show, after UVB irradiation, a reduced fraction of phosphorylated histone γH2AX (fosfoγH2AX), whose expression is also associated with the amount of DNA damage.

30 Fig.1 shows the Western Blot patternsdrawn from experiments performed on one (NHK2) of the three different keratinocytes culturesdescribedabove, in which

GAPDH is the constitutively expressed housekeeping, used to normalize the values of the samples.

Flow cytometric assessment of UV induced apoptosis after a pre-treatment with G01 and subsequent UV irradiation

5 The NHKs cellswere plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate.Each experimental condition was plated in duplicate.The cells were treated for 24 ± 2h with G01,in a concentration of 90µM.Subsequently, the plates identified as +UVA, +G01/UVA, +UVB,and +G01/UVB were respectively exposed, in the irradiation medium, to dosesof UVA 10 of25 J/cm^{2}and UVB of80 mJ/cm^2, while the control plates (Ctr) and those treated only with the compound under examination (G01)were maintained in the dark and at room temperature for a period of time equal to the irradiation time. The irradiation medium was then replaced with the culture medium. Based on the preliminary results obtained on a broader time range, a post-treatment time of 24h 15 was selected,after which the plates were processedaccording to the procedure given in the user manual of the specific kit for annexin V flow cytometric analysis (Annexin V), a typical assay to detect the early stages of apoptosis.The positivity for annexin V in each condition tested (treatment with G01 and/or UVA/UVBirradiation) was compared with the positivity in the control cells.

20 The results obtained have shown the ability of G01to reduce the apoptosis induced by with UVA and UVB radiation.

Fig. 2 shows the corresponding diagrams drawn from the experiments performed on three different keratinocytes cultures, NHK1, NHK2 and NHK3.

Western Blot assessment of UV induced apoptosis after a pre-treatment with G01 25 and subsequent UV irradiation

The NHKs cellswere plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate.Each experimental condition was plated in duplicate. The cells were treated for 24 ± 2h with the compound G01,in a concentration of 90µM.Subsequently, the cells identified as +UVA, +G01/UVA, 30 +UVB,and +G01/UVB were respectively exposed, in the irradiation medium, to dosesof UVA of 25 J/cm^{2}andUVB of80 mJ/cm^2, while the control plates and those treated only with G01 were maintained in the dark and at room temperature for a

period of time equal to the irradiation time. The irradiation medium was then replaced with the culture medium. Based on the preliminary results obtained on a broader time range, a post-treatment time of 24h was selected, after which the plates were processedfor Western Blotprotein determination. The protein expression of non-cleaved caspase 3 in each condition tested (treatment with G01 and/or UVA/UVBirradiation) was compared with the expression in the control cells (Ctr).

The results obtained have shown the ability of G01 to reduce caspase 3 activation (corresponding to a reduction of the protein expression of the zymogen form of the protein), one of the key mediators of the apoptosis process induced by UVA and UVB.

Fig. 3 shows the Western Blot patternsdrawn from the experiments performed on three different keratinocytes cultures, NHK1, NHK2 and NHK3.

ELISA assessment of DNA damage after pre-treatment with G01 and subsequent UVB irradiation: CPDs determination

The NHKs cellswere plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate. Then, the keratinocytes were treated for 24 ± 2h with the compound G01,in a concentration of 90μM.Subsequently, the plates identified as +UVB and +G01/UVB were exposed, in the irradiation medium, to a UVB doseof 80 mJ/cm^2, while the control plates (Ctr) and those treated only with the compound under examination (G01) were maintained in the dark and at room temperature for a period of time equal to the irradiation time. The irradiation medium was then replaced with the culture medium. Based on the preliminary results obtained on a broader time range, post-treatment times of 6h and 24h were selected, after which the plates were processed according to the procedure described in the work instruction specific for the DNA extraction. The determination of the pyrimidine cyclobutane dimers, CPDs, was performed using a specific ELISA kit following the procedure described in the user manual.

The results obtained have shown the ability of the compound G01 to promote the removal of CPDs adducts, known signal of DNA damage induced by UVB.

Fig. 4 shows the corresponding diagrams drawn from the experiments performed on three different keratinocytes cultures, NHK1, NHK2 and NHK3.

2) Assessment of the activity in the post-treatment with the compound of the invention

5 The following experiments were performed in order to assess the efficacy of compound G01 applied to NHKs cell cultures after irradiation with UVA and UVB.

Cell viability after UV irradiation and subsequent treatment with G01

The cellswere plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate. The cells in the plates identified as +UVA 10 and +UVB were respectively exposed, in the irradiation medium, to dosesof UVA of 10 J/cm^{2}and UVB of 25 mJ/cm^2, while the control plate (Ctr) was maintained in the dark and at room temperature for a period of time equal to the irradiation time. The irradiation medium was then replaced with the culture medium, containing the compound G01 in concentrations selected based on the cell viability results 15 obtained after treatment with the same compound (see above). The cell viability was measured (incorporation of NR)at a time of 24 ± 2h and 48 ± 2h after the irradiation, in the absence and the presence of UVA/UVB irradiationand/ortreatment with the compound of the invention G01. The cell viability values for each condition tested were compared with the value obtained 20 from the control and plotted on a graph to highlight any differences between the various experimental conditions.

The results obtained showed a reduction in cell viability following irradiation with UVA and UVB, and a significant protective action, particularly 48h after irradiation, of the highest concentration tested (90μM) of the compound G01.

25 Flow cytometric assessment of apoptosis induced by UV irradiation and subsequent treatment with G01

The NHKs cells were plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate.Each experimental condition was plated in duplicate. Subsequently, the plates identified as +UVA, +G01/UVA, 30 +UVB,and +G01/UVB were respectively exposed, in the irradiation medium, to dosesof UVA of 25 J/cm^{2}and UVB of80 mJ/cm^2, while the control plates (Ctr) and those treated only with the compound under examination (G01) were maintained

in the dark and at room temperature for a period of time equal to the irradiation time. The irradiation medium was then replaced with the culture medium containing the compound G01in a concentration of 90μM. Based on the preliminary results obtained on a broader time range, a post-treatment time of 24h
5 was selected, after which the plates were processed according to the procedure given in the user manual of the specific kit for annexin V flow cytometric analysis. The positivity for annexin V in each condition tested (treatment with the compound of the invention under examination and/or UVA/UVBirradiation) was compared with the positivity in the control cells.
10 The results obtained have shown the ability of the compound G01 to reduce the apoptosis induced by irradiation with UVA and UVB.
Fig. 5 shows the corresponding diagrams drawn from the experiments performed on twodifferent keratinocytes cultures, NHK1and NHK2.
Western Blot assessment of apoptosis induced by UV irradiation and subsequent
15 treatment with G01
The NHKs cellswere plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate. Subsequently, the plates identified as +UVA, +G01/UVA, +UVB,and +G01/UVB were respectively exposed, in the irradiation medium, to dosesof UVA of 25 J/cm^{2}and UVB of80 mJ/cm^2, while
20 the control plates (Ctr) and those treated only with the compound under examination (G01) were maintained in the dark and at room temperature for a period of time equal to the irradiation time. The irradiation medium was then replaced with the culture medium containing the compound G01,in a concentration of 90μM. Based on the preliminary results obtained on a broader time range, a
25 post-treatment time of 24h was selected, after which the plates were processed for Western Blot protein determination. The protein expression of non-cleaved caspase 3 in each condition tested (treatment with the compound G01 under examinationand/or UVA/UVBirradiation) was compared with the expression in the control cells.
30 The results obtained have shown the ability of the compound of the invention to reduce caspase 3 activation (corresponding to a reduction of the protein

expression of the zymogen form of the protein), one of the key mediators of the apoptosis process induced by UVA and UVB.

Fig. 6 shows the Western Blot patterns drawn from the experiments performed on two different keratinocytes cultures, NHK1and NHK3.

5 <u>ELISA assessment of the damage induced by UVB irradiation and subsequent treatment with G01: CPDs determination</u>

The NHKs cellswere plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate.Then, the plates identified as +UVB and +G01/UVB were respectively exposed, in the irradiation medium, to
10 aUVB doseof 25 mJ/cm^2, while the control plates (Ctr) and those treated only with the compound under examination (G01) were maintained in the dark and at room temperature for a period of time equal to the irradiation time. The irradiation medium was then replaced with the culture medium containing the compound G01,in a concentration of 90μM. Based on the preliminary results obtained on a
15 broader time range, post-treatment times of 6h and 24h were selected, after which the plates were processed according to the procedure described in the work instruction specific for the DNA extraction. The CPDs determination was performed using a specific ELISA kit following the procedure described in the user manual.

20 The results obtained have shown the ability of the compound G01 under examination to promote the removal of CPDs products, known signal of DNA damage induced by UVB.

Fig. 7 shows the corresponding diagrams drawn from the experiments performed on two different keratinocytes cultures, NHK1and NHK3.

25 <u>ELISA assessment of the damage induced by UVA irradiation and subsequent treatment with G01: 8-OHdG determination</u>

The NHKs cellswere plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate. Then, the plates identified as +UVA and +UVA/G01postwere respectively exposed, in the irradiation medium, to
30 a UVA doseof 10 J/cm^2. The irradiation medium was then replaced with the culture medium containing the compound of the invention G01,in a concentration of 90μM. Based on preliminary results obtained on a broader time range, post-treatment

times of 6h and 24h were selected, after which the plates were processed according to the procedure described in the work instruction specific for the DNA extraction. The determination of 8-OHdG, marker of DNA oxidative damage induced by UVA, was performed using a specific ELISA kit following the procedure
5 described in the user manual.
The results obtained are summarized in in the graph of Fig. 8. They show, in the cells irradiated and post-treated with the compound of the invention, a significant reduction in the amount of 8-OHdG produced by UVA radiation at both post-treatment times compared to the cells only irradiated and not treated with the
10 compound.

Immunofluorescence assessment of the ability to repair a DNA damage following UVA or UVB irradiation and subsequent treatment with G01

The NHKs cellswere plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate. Subsequently, the plates
15 identified as +UVA, +UVA/G01post, UVB,and +UVB/G01postwere respectively exposed, in irradiation medium, to dosesof UVA of 10 J/cm^{2}or UVB of25 mJ/cm^2. The irradiation medium was then replaced with the culture medium containing the compound of the invention G01, in a concentration of 90μM. Based on preliminary results obtained on a broader time range, a post-treatment time of 24h was
20 selected, after which the plates were processed for the immunofluorescence determination of the protein expression of the phosphorylated histone γH2AX, whose expression is associated to the amount of DNA damage(double strand breaks). The protein expression of the phosphorylated histone γH2AX after treatment with the compound of the invention G01, or only after irradiation with
25 UVA/UVB, was compared with the expression in the control cells.
The results obtained are summarized in the graph of Fig. 9. They show, in the cells irradiated and post-treated with the compound of the invention, a significant reduction in the fraction of phosphorylated histone γH2AX compared to the cells only irradiated and not treated with the compound.

30 Western Blot assessment of the ability to repair a DNA damage following UVB irradiation and subsequent treatment with G01

The NHKs cellswere plated in the specific culture medium and maintained in culture for 24 ± 2h to allow adhesion to the plate. Subsequently, the cells identified as +UVB and +G01/UVBpostwere exposed, in the irradiation medium, to a UVB doseof25 mJ/cm^2.The irradiation medium was then replaced with the culture
5 medium containing the compound of the invention G01,in a concentration of 90μM. Based on preliminary results obtained on a broader time range, a post-treatment time of 24h was selected, after which the plates were processed for Western Blot protein determination. The protein expression of p53, phospho-p53, GADD45a, and phosphorylated histone γH2AX was measured in the condition of UVB
10 irradiation.

Western Blot analysis at pre-set post-treatment times "time course" of the expression levels of p53, phospho-p53 and GADD45a after UVB irradiation and subsequent treatment with G01

The NHKs cellswere plated in the specific culture medium and maintained in
15 culture for 24 ± 2h to allow adhesion to the plate. Subsequently, the plates identified as +UVB and +G01/UVBpostwere exposed, in the irradiation medium, to a UVB doseof25 mJ/cm^2. The irradiation medium was then replaced with the culture medium containing the compound of the invention G01,in a concentration of 90μM. Post-treatment times of 1-3-6-24h were selected, after which the plates
20 were processed for Western Blot protein determination. The protein expression of p53, phospho-p53, andGADD45a after UVB irradiation and post-treatment with the compound of the invention G01 was compared with the expression in the control cells (Ctr).

The results obtained are summarized in the diagrams of Figures 10 to 12, which
25 represent the densitometries of the Western Blot electrophoretic patterns of immunofixation obtained at the above-specified times. They show that the post-treatment with the compound of the invention of cells exposed to UVB causes an acceleration of the increase in the levels of p53, phospho-p53. and GADD45a in the shorter times, resulting in a rapid activation of the DNA damage repair
30 mechanisms. As final general result, this action causes a decrease in the expression of the markers of DNA damage at 24h after UVB irradiation and, thus, a cytoprotective effect.

Overall, the experimental results obtained in the post-treatment of UV irradiation show, for the compound of the invention, an effective activity against DNA damage, similar to that demonstrated above in the pre-treatment before UV irradiation, particularly for what concerns key parameters such as preservation of
5 cell viability, positivity to annexin V, inhibition of caspase 3 protein expression,CPDs and 8-OHdG inhibition, markers of DNA damage.
Moreover, in cells exposed to UVB irradiation, the post-treatment with compounds of the invention results in a timely activation of the DNA damage repair mechanisms. This results in a decrease of the expression of the markers of
10 damage at 24h after the irradiation, and a cytoprotective effect.
Taken together, the experimental data therefore envisage an anticanceruse of the compounds of general formula (I), both in the prevention of a cancer that may develop and in the treatment of an already developed cancer, as a result of a human DNA damaged induced by UV radiation.
15 As secondary use, dependent on the anticancer one, particularly in the case of prevention of DNA damage caused by UV radiation, a use as active ingredient is envisaged in compositions directed to the photoprotection of the skin, including the scalp skin, from UVA and UVB, to prevent DNA damage potentially developable in a cancer.
20 For this reason, a further object of the present invention is also such photoprotective use dependent on the one described above.

CLAIMS

1. Compound of general formula (I):

$CH_3-(CH=CH)_n-R$ (I)

wherein n = 3, 5 or 7; R is -CO-OR' or $-CO-O^{(-)}$, R' is H, C_1-C_{22} alkyl, alkenyl, aryl, aralkyl or sugars,

or pharmaceutically acceptable salts thereof,

for use in the treatment of skin cancer resulting from DNA damage produced by UV radiation, wherein the compound is for topical administration to the skin in an amount sufficient to produce at least one of: (i) preservation of cell viability, (ii) positivity to annexin V, (iii) apoptosis inhibition, (iv) inhibition of caspase-3 protein expression and (v) inhibition of pyrimidine cyclobutane dimers and 8-oxo-2'-deoxyguanosine in human keratinocytes.

2. The compound for use according to claim 1, wherein the compound is for further use for photoprotection of the scalp from DNA damage produced by UV radiation.

3. The compound for use according to claim 1 or 2, wherein the pharmaceutically acceptable salts are selected from sodium, potassium and lysine salts.

4. A composition comprising a compound of general formula (I):

$CH_3-(CH=CH)_n-R$ (I)

wherein n = 3, 5 or 7; R is -CO-OR', $-CO-O^{(-)}$ or -CH2-O-CO-R', R' is H, C_1-C_{22} alkyl, alkenyl, aryl, aralkyl or sugars,

or pharmaceutically acceptable salts thereof,

and an excipient,

for use in the treatment of skin cancer resulting from DNA damage produced by UV radiation, wherein the composition is for topical administration to the skin in an

Date Reçue/Date Received 2021-09-22

amount sufficient to produce at least one of: (i) preservation of cell viability, (ii) positivity to annexin V, (iii) apoptosis inhibition, (iv) inhibition of caspase-3 protein expression and (v) inhibition of pyrimidine cyclobutane dimers and 8-oxo-2'-deoxyguanosine in human keratinocytes.

5. The composition for use according to claim 4, wherein the composition is for further use for photoprotection of the scalp from DNA damage produced by UV radiation.

6. The composition for use according to claim 4 or 5, wherein the pharmaceutically acceptable salts are selected from sodium, potassium and lysine salts.

7. The composition for use according to any one of claims 4 to 6, wherein said compound is 2,4,6-octatrienoic acid.

8. The composition for use according to any one of claims 4 to 6, wherein said compound is a 2,4,6-octatrienoic acid salt.

9. The composition for use according to any one of claims 4 to 6, wherein said compound is 2,4,6-octatrienoic acid sodium salt.

10. The composition for use according to any one of claims 4 to 6, wherein said compound is 2,4,6-octatrienoic acid potassium salt.

11. The composition for use according to any one of claims 4 to 6, wherein said compound is 2,4,6-octatrienoic acid L-lysine salt.

12. The composition for use according to any one of claims 4 to 6, wherein said compound is 2,4,6-octatrienoic acid ethyl ester.

13. The composition for use according to any one of claims 4 to 6, wherein said compound is 2,4,6-octatrienyl ester of 2,4,6-octatrienoic acid.

Date Reçue/Date Received 2021-09-22

14. The composition for use according to any one of claims 4 to 6, wherein said compound is 2,4,6-octatrien-1-ol acetate.

15. The composition for use according to any one of claims 4 to 6, wherein said compound is 2,4,6-octatrien-1-ol benzoate.

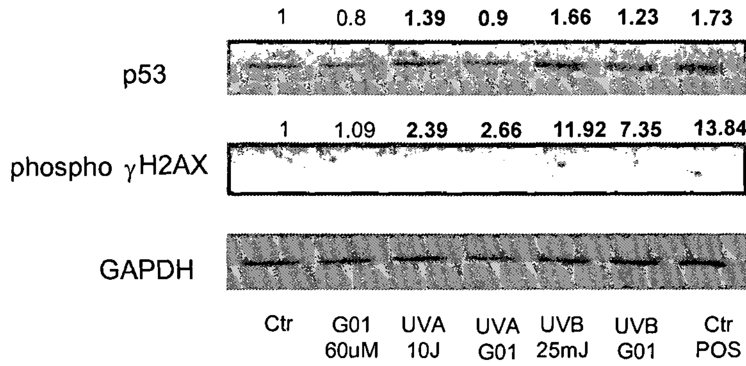
16. The composition for use according to any one of claims 4 to 6, comprising a mixture of two or more compounds of formula (I).

17. The composition for use according to any one of claims 4 to 15, comprising said compound in an amount from 10^{-6} to $3x10^{-2}$ mol/100g.

18. The composition for use according to any one of claims 4 to 15, comprising said compound in an amount of 90µM.

19. The composition for use according to any one of claims 4 to 18, wherein the excipient is formulated for topical administration on the human skin or scalp.

Date Reçue/Date Received 2021-09-22



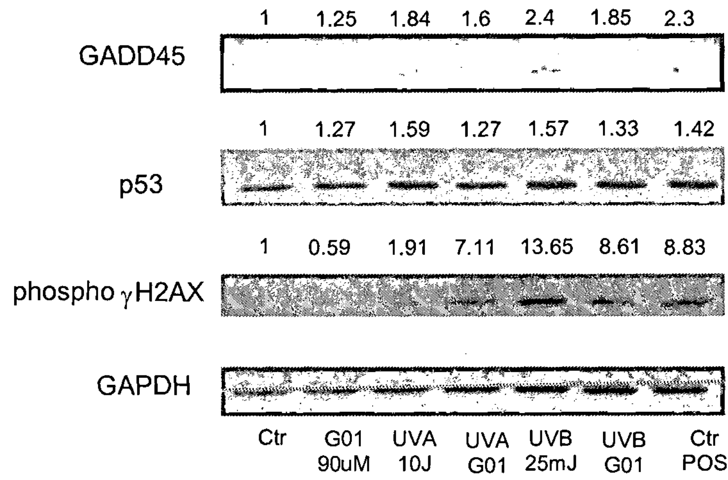
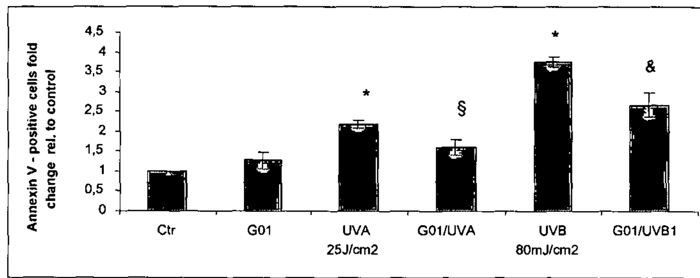


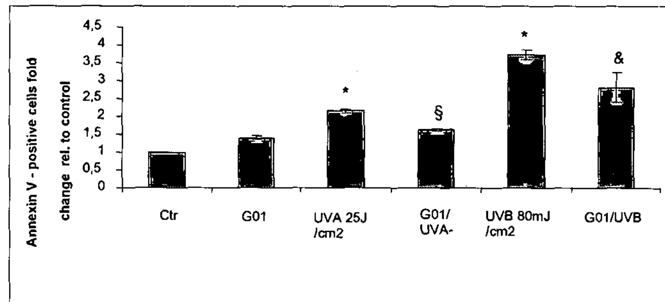
Fig. 1

NHK1



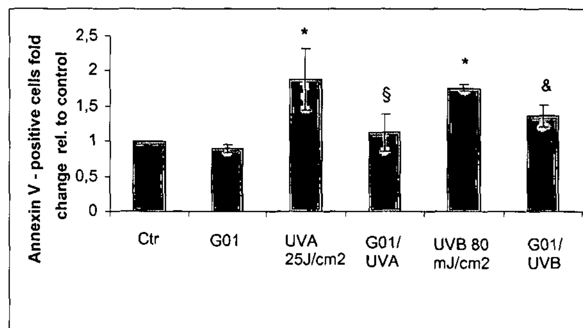
* = p< 0,01, respect to Ctr
§ = p< 0,05, respect to UVA 25 J/cm^2
& = p< 0,05, respect to UVB 80 mJ /cm^2

NHK2



* = p< 0,01, respect to Ctr
§ = p< 0,05, respect to UVA 25 J/cm^2
& = p< 0,05, respect to UVB 80 mJ/cm^2

NHK3



* = p< 0,01, respect to Ctr
§ = p< 0,05, respect to UVA 25 J/cm^2
& = p< 0,05, respect to UVB 80 mJ/cm^2

Fig.2

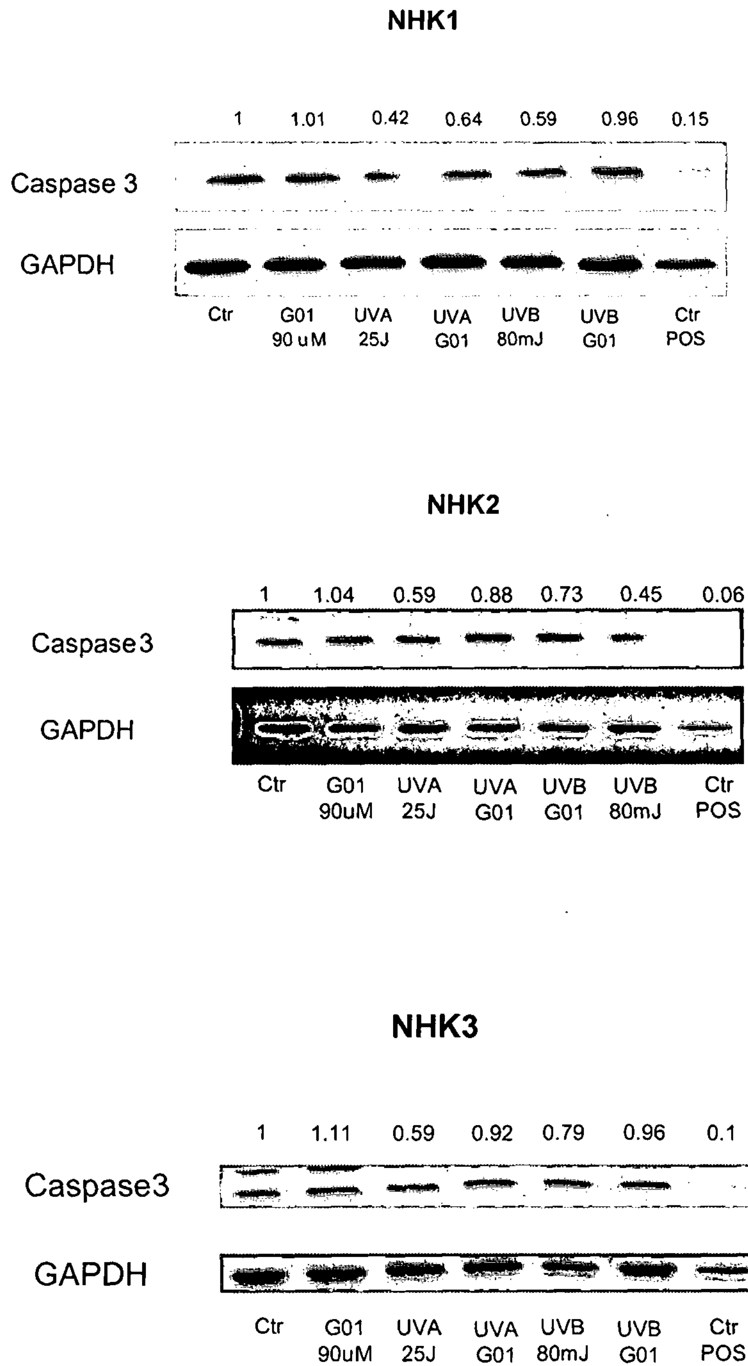
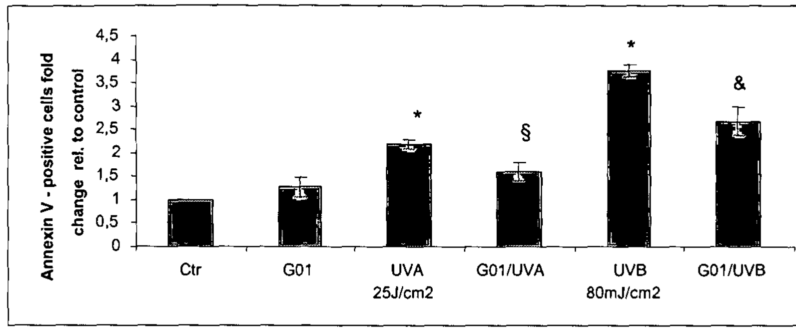


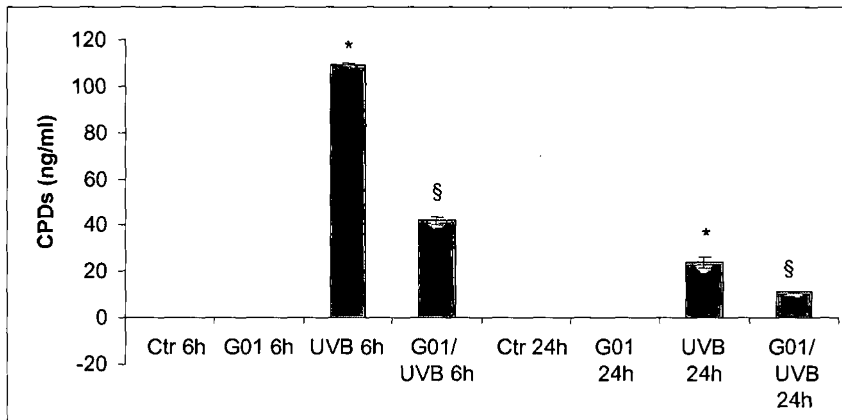
Fig. 3

NHK1



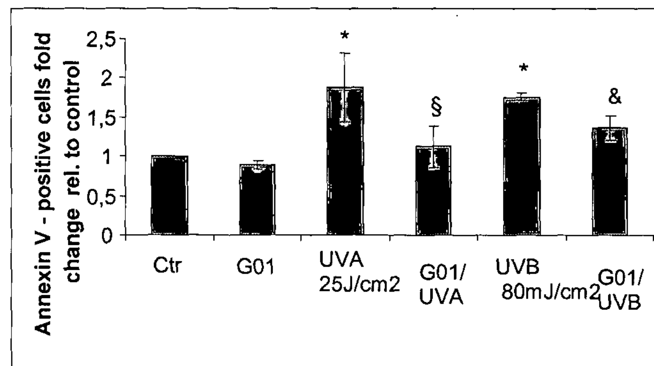
* = p< 0,01, respect to Ctr
§ = p< 0,05, respect to UVA 25 J/cm ^{2}
& = p< 0,05, respect to UVB 80 mJ /cm ^{2}

NHK2



* = p< 0,001, respect to Ctr
§ = p< 0,001, respect to UVB 25 mJ /cm ^{2}

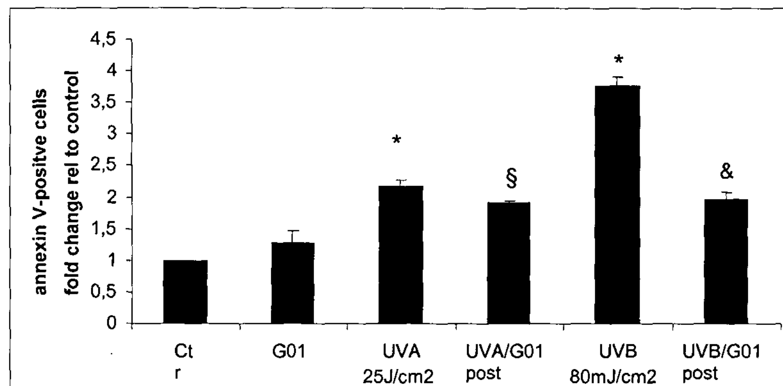
NHK3



* = p< 0,01, respect to Ctr
§ = p< 0,05, respect to UVA 25 J /cm ^{2}
& = p< 0,05, respect to UVB 80 mJ /cm ^{2}

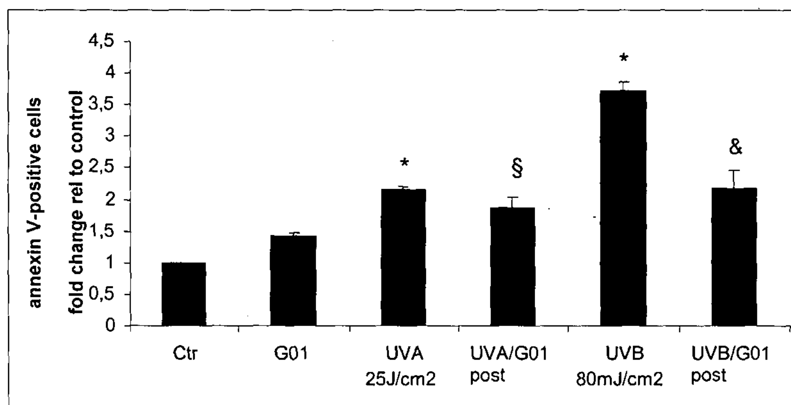
Fig. 4

NHK1



* = p< 0,01, respect to Ctr
§ = p< 0,05, respect to UVA 25 J/cm ^{2}
& = p< 0,01, respect to UVB 80 mJ/cm ^{2}

NHK2



* = p< 0,01, respect to Ctr
§ = p< 0,05, respect to UVA 25 J/cm ^{2}
& = p< 0,01, respect to UVB 80 mJ/cm ^{2}

Fig. 5

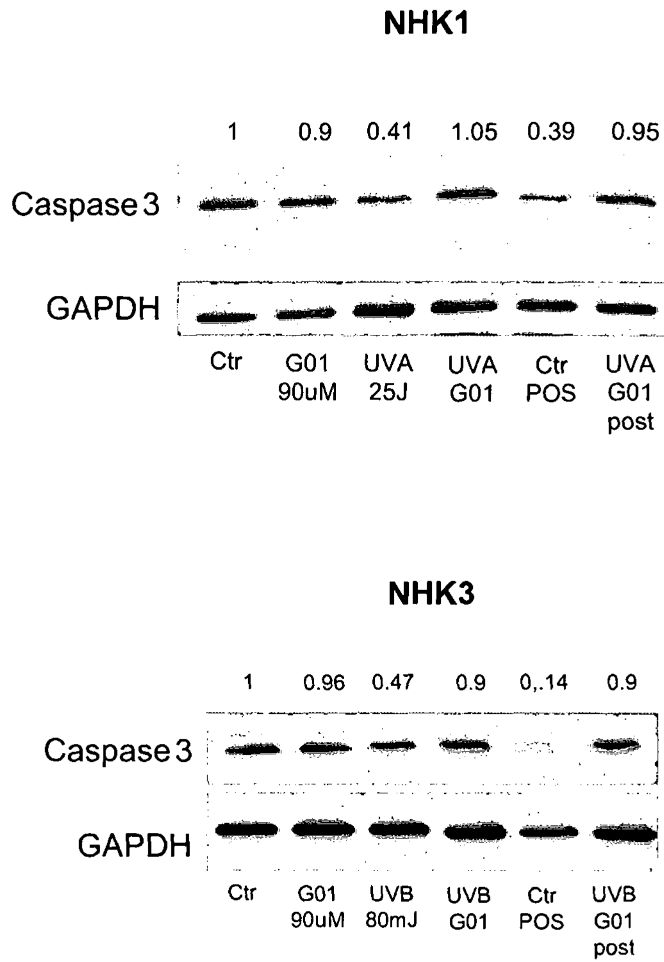
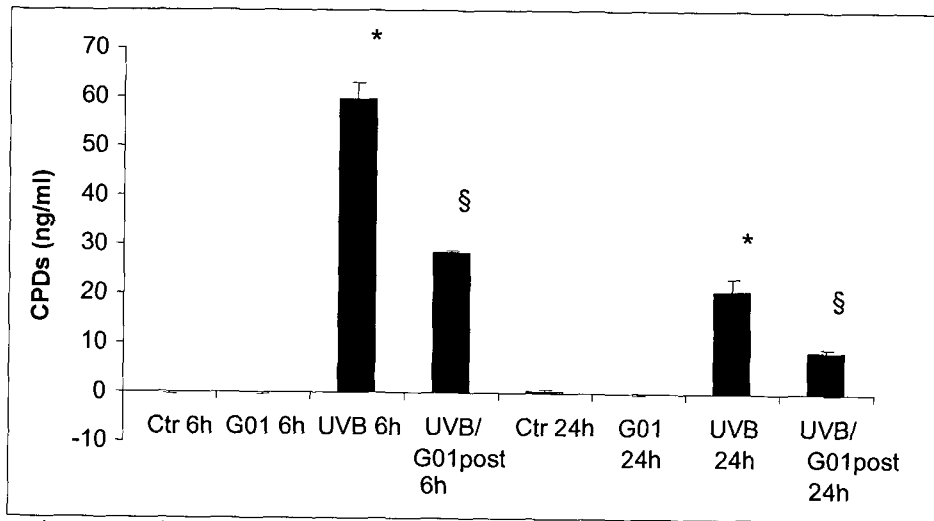


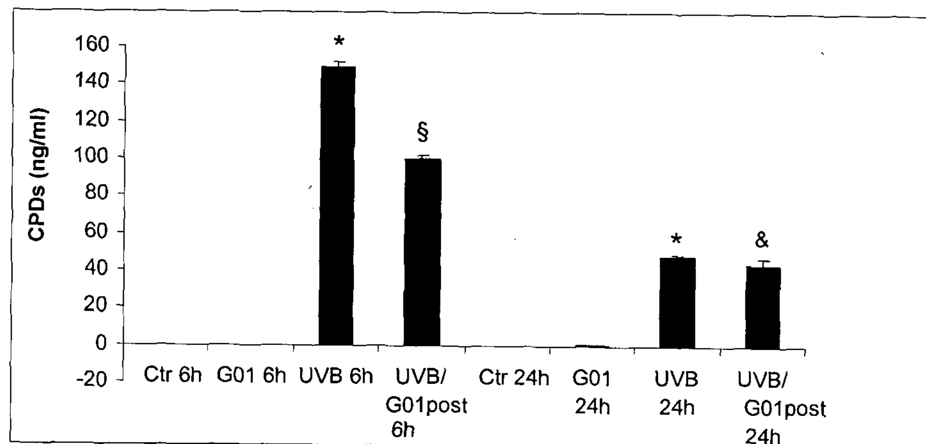
Fig. 6

NHK1



* = p< 0,001, respect to Ctr
§ = p< 0,001, respect to UVB 25 mJ/cm^2

NHK3



* = p< 0,001, respect to Ctr
§ = p< 0,005, respect to UVB 25 mJ/cm^2 6h
& = p< 0,05 , respect to UVB 25 mJ/cm^2 24h

Fig. 7

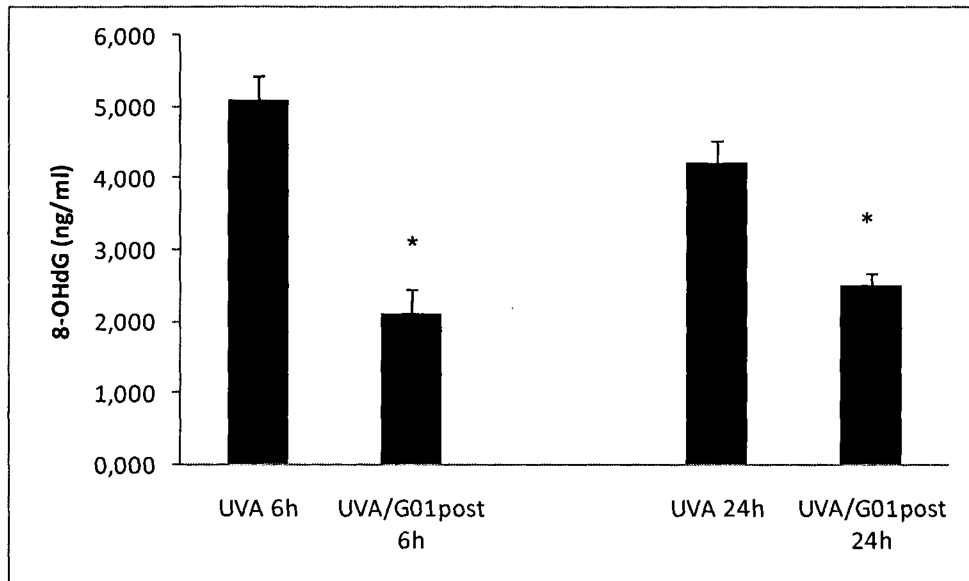


Fig. 8

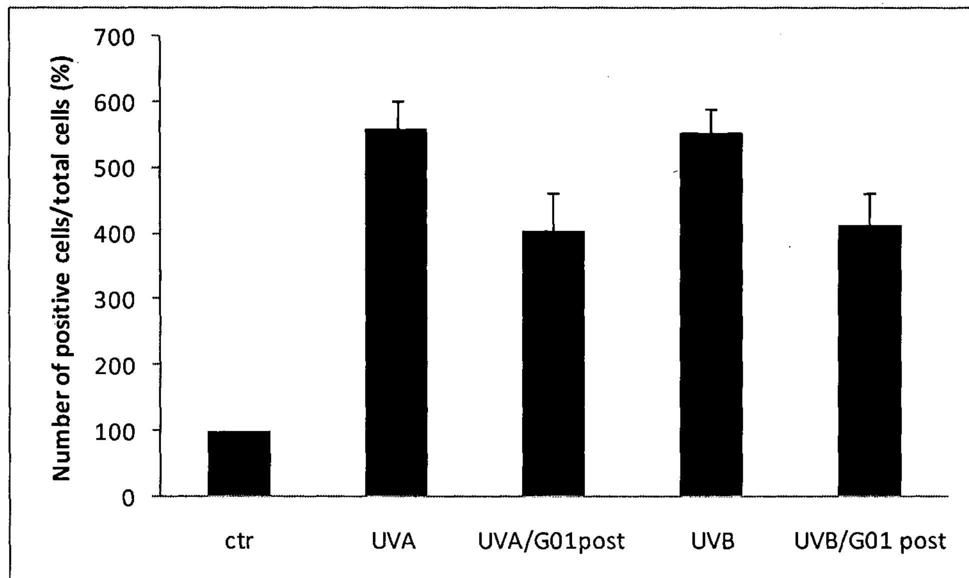


Fig. 9

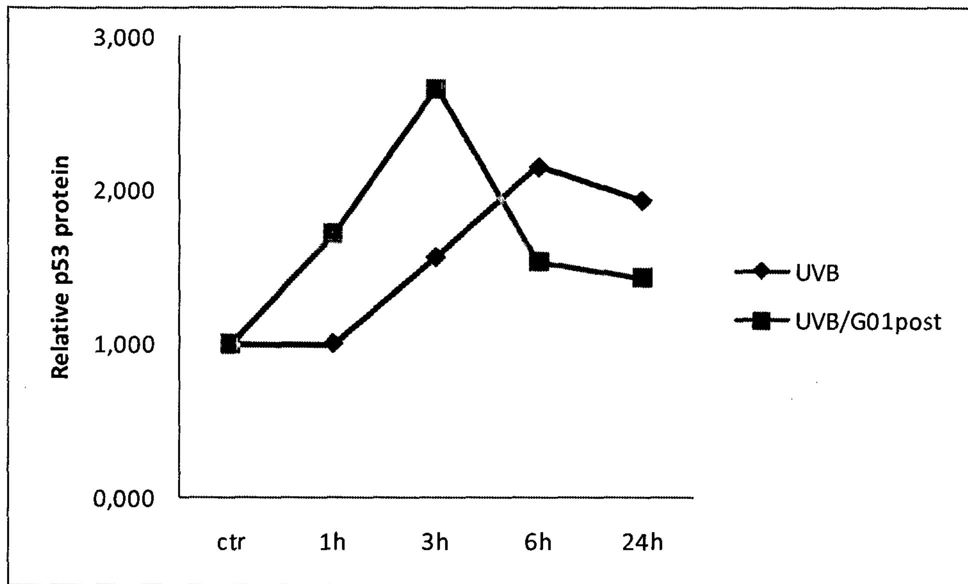


Fig. 10

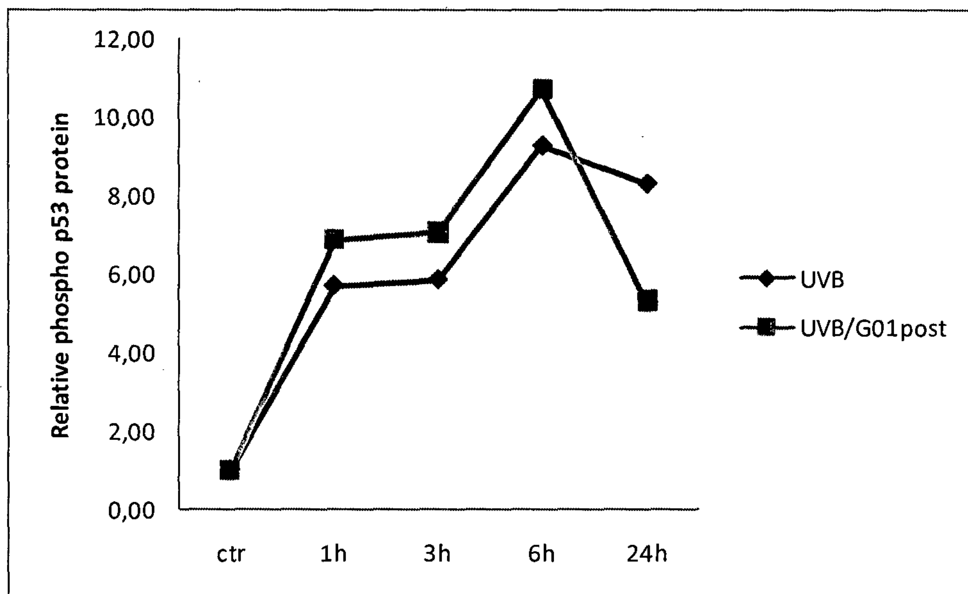


Fig. 11

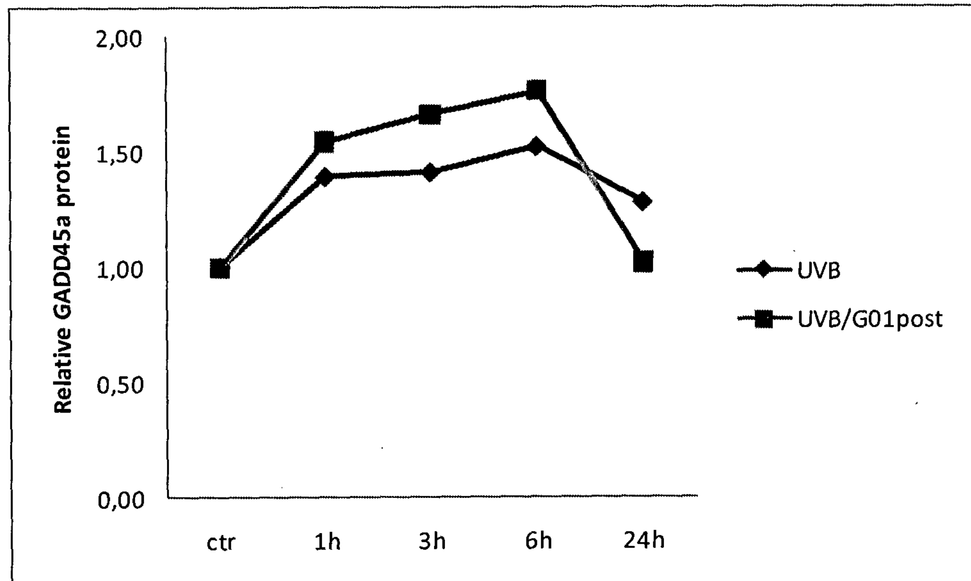


Fig. 12