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**(54) Title:** SKIN LIGHTENING COMPOSITION

**(57) Abstract:** Desired skin colour is a major unmet consumer need in Asia. Consumers particularly desire even skin colour, absence of age spots (solar lentigines) and lighter overall skin tone. One solution is to use biological actives that reduce the activity of melanocytes in skin. These cells, present in the basal layer of the epidermis, make the darkly coloured pigment melanin and export it in small export vesicles called melanosomes to the neighbouring keratinocytes. It is well described in the literature that compounds which reduce melanin synthesis when topically applied to the skin will reduce skin darkness over time. This invention relates to a composition comprising climbazole for use in skin lightening. This invention is based on the fact that climbazole has been found to inhibit pigment production in B16 monolayer and MelanoDerm™ cultures. Thus climbazole, when applied topically over an appropriate length of time in-vivo, would be expected to cause skin lightening, or to reduce blemishes and/or hyperpigmented spots and/or solar lentigines leading to an improvement in skin tone and evenness.

# SKIN LIGHTENING COMPOSITION

Desired skin colour is a major unmet consumer need in Asia. Consumers particularly desire even skin colour, absence of age spots (solar lentigines) and lighter overall skin tone. One solution is to use biological actives that reduce the activity of melanocytes in skin. These cells, present in the basal layer of the epidermis, make the darkly coloured pigment melanin and export it in small export vesicles called melanosomes to the neighbouring keratinocytes. It is well described in the literature that compounds which reduce melanin synthesis when topically applied to the skin will reduce skin darkness over time.

This invention relates to a composition comprising climbazole for use in skin lightening. This invention is based on the fact that climbazole has been found to inhibit pigment production in B16 monolayer and MelanoDerm™ cultures. Thus climbazole, when applied topically over an appropriate length of time in-vivo, would be expected to cause skin lightening, or to reduce blemishes and/or hyperpigmented spots and/or solar lentigines leading to an improvement in skin tone and evenness.

Climbazole is a topical antifungal agent commonly used in the treatment of human fungal skin infections such as dandruff and eczema. Climbazole has shown a high in-vitro and in-vivo efficacy against Pityrosporum ovale that appears to play an important role in the pathogenesis of dandruff. Its chemical structure and properties are similar to other fungicides such as ketoconazole and miconazole.

In Mun et al (Biol. Pharm. Bull., 27(6), 806-809 (2004)), the inhibitory effect of miconazole is described on melanogenesis in B16 melanoma cells. Tyrosinase activity and melanin content were dose dependency decreased by the azole as compared with untreated cells, however this is accompanied by decreased cellular viability.

WO 95/34280 discloses methods of lightening hyper-pigmented regions in mammalian skin by administering to the skin a composition comprising an effective amount of a sulfhydryl compound. Preferred sulfhydryl compounds include lipoic acid.

WO 2010/121919 discloses a hair growth composition comprising lipoic acid as a microcirculation promoter and climbazole as an anti-dandruff agent.

**Summary of the invention**

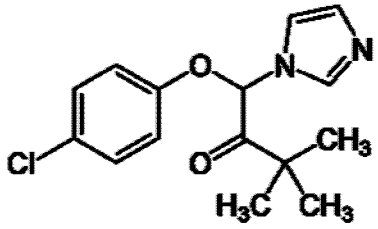
In a first aspect of the invention, a skin lightening composition is provided, the composition comprising:

a. Climbazole;

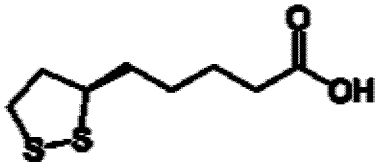
b. Lipoic acid; and

c. An ingredient selected from the group consisting of an organic sunscreen, an inorganic sunscreen, an antiperspirant active, a microbiocide, and mixtures thereof.

Climbazole has the following structure:




and lipoic acid has the following structure:




By skin is included under-arm skin.

In a second aspect of the invention, a composition according to the first aspect is provided for use in skin lightening. By skin lightening is preferably a skin benefit selected from the group consisting of treating freckles, treating age spots, treating uneven skin tone, treating underarm hyperpigmentation, reducing blemishes and treating hyperpigmented spots.

In a third aspect of the invention, an effective amount of climbazole is provided for use in skin lightening.

**Detailed description of the invention**

This invention relates to a composition comprising climbazole for use in skin lightening. This invention is based on the fact that climbazole has been found to inhibit pigment production in B16 monolayer and MelanoDerm™ cultures. Thus climbazole, when applied

topically over an appropriate length of time in-vivo, would be expected to cause skin lightening, or to reduce blemishes and/or hyperpigmented spots and/or solar lentigines leading to an improvement in skin tone and evenness. Thus the invention provides an effective amount of climbazole for use in skin lightening. Alternatively, use of an effective amount of climbazole is provided in the manufacture of a medicament for lightening skin. In a further alternative, a method of lightening the skin of a human is provided, the method comprising the step of applying an effective amount of climbazole to skin of a person in need thereof.

A skin lightening composition is also provided, the composition comprising:

a. Climbazole;
b. Lipoic acid; and
c. An ingredient selected from the group consisting of an organic sunscreen, an inorganic sunscreen, an antiperspirant active, a microbiocide, and mixtures thereof.

The composition may comprise 0.001 to 2, preferably 0.005 to 0.5 % w/w climbazole and/or 0.001 to 2, preferably 0.002 to 0.2 % w/w lipoic acid. These concentration ranges will provide about 10 μM of each of climbazole and lipoic acid in the basal epidermis. The lipoic acid is preferably in the form of the R-(+)-isomer, preferably exclusively in the form of the R-(+)-isomer.

Sunscreens include those materials commonly employed to block ultraviolet light. Illustrative organic compounds are the derivatives of PABA, cinnamate and salicylate. For example, avobenzophenone (Parsol 1789®) octyl methoxy cinnamate and 2-hydroxy-4-methoxy benzophenone (also known as oxybenzone) can be used. Octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone are commercially available under the trademarks, Parsol MCX and Benzophenone-3, respectively. Inorganic sunscreens include oxides like titanium dioxide and zinc oxide which reflect or scatter the suns rays. The exact amount of sunscreen employed in the compositions can vary depending upon the degree of protection desired from the sun's UV radiation. However levels can be in the range 0.01 to 15, preferably 0.1 to 10, most preferably 0.5 to 7.5 % by weight of the composition.

The composition preferably contains an antiperspirant active. Antiperspirant actives are preferably incorporated in an amount of from 0.5 to 50, particularly from 5 to 30 and

especially from 10 to 26 % by weight of the composition. It is often considered that the main benefit from incorporating of up to 5 % by weight of the composition of an antiperspirant active in a stick composition is manifest in reducing body odour, and that as the proportion of antiperspirant active increases, so the efficacy of that composition at controlling perspiration increases.

Antiperspirant actives for use herein are often selected from astringent active salts, including in particular aluminium, zirconium and mixed aluminium/zirconium salts, including both inorganic salts, salts with organic anions and complexes. Preferred astringent salts include aluminium, zirconium and aluminium/zirconium halides and halohydrate salts, such as chlorohydrates.

Aluminium halohydrates are usually defined by the general formula $Al_2(OH)xQy.wH_20$ in which Q represents chlorine, bromine or iodine, x is variable from 2 to 5 and x + y = 6 while $wH_20$ represents a variable amount of hydration. Especially effective aluminium halohydrate salts, known as activated aluminium chlorohydrates, are described in EP 6739 A (Unilever NV et al), the contents of which specification is incorporated herein by reference. Such activated aluminium chlorohydrates are made by a method in which the weight concentration of aluminium compounds in the solution is controlled within specified limits and simultaneously the temperature of that solution is controlled within a specified elevated temperature range whilst polymeric aluminium species are formed, and drying conditions are strictly controlled as described in the said EP 6739 A. Some activated salts do not retain their enhanced activity in the presence of water but are useful in substantially anhydrous formulations, i.e. formulations that do not contain a distinct aqueous phase.

Zirconium actives can usually be represented by the empirical general formula: $ZrO(OH)2n-nzBz.wH_20$ in which z is a variable in the range of from 0.9 to 2.0 so that the value 2n-nz is zero or positive, n is the valency of B, and B is selected from the group consisting of chloride, other halide, sulphamate, sulphate and mixtures thereof. Possible hydration to a variable extent is represented by $wH_20$. Preferable is that B represents chloride and the variable z lies in the range from 1.5 to 1.87. In practice, such zirconium salts are usually not employed by themselves, but as a component of a combined aluminium and zirconium-based antiperspirant.

The above aluminium and zirconium salts may have co-ordinated and/or bound water in various quantities and/or may be present as polymeric species, mixtures or complexes. In particular, zirconium hydroxy salts often represent a range of salts having various amounts of the hydroxy group. Zirconium aluminium chlorohydrate may be particularly preferred.

Antiperspirant complexes based on the above-mentioned astringent aluminium and/or zirconium salts can be employed. The complex often employs a compound with a carboxylate group, and advantageously this is an amino acid. Examples of suitable amino acids include dl-tryptophan, dl-P-phenylalanine, dl-valine, dl-methionine and β-alanine, and preferably glycine which has the formula $CH_2(NH_2)COOH$.

It is highly desirable to employ complexes of a combination of aluminium halohydrates and zirconium chlorohydrates together with amino acids such as glycine, which are disclosed in U S 3 792 068 (Luedders et al). Certain of those Al/Zr complexes are commonly called ZAG in the literature. ZAG actives generally contain aluminium, zirconium and chloride with an Al/Zr ratio in a range from 2 to 10, especially 2 to 6, an Al/Cl ratio from 2.1 to 0.9 and a variable amount of glycine. Actives of this preferred type are available from Westwood, from Summit and from Reheis, though with differing particle size distributions.

Many aluminium and/or zirconium-containing astringent antiperspirant salts employed herein have metal: chloride mole ratio in the range of 1.3:1 to 1.5:1. Others having a lower metal: chloride mole ratio, such as from 1:1 to 1.25:1 tend to generate lower pHs when applied to skin and thus tend to be more irritating.

The proportion of solid antiperspirant salt in a suspension composition normally includes the weight of any water of hydration and any complexing agent that may also be present in the solid active.

Many particulate antiperspirants employed in the instant invention have a refractive index (RI) of at least 1.49 and not higher than 1.57. Actives which are free from zirconium tend to have an RI of from 1.49 to 1.54, depending on their formula and at least partly on their residual water content. Likewise, actives which contain zirconium tend to have an RI of from 1.52 to 1.57.

The selection of the antiperspirant active material desirably takes into account the type of applicator from which it is dispensed. Thus, in many particularly preferred embodiments in which the composition is dispensed from a contact applicator, for example using a stick or cream (soft solid dispenser, the antiperspirant active comprises an aluminium-zirconium active, such as AZAG. However, in some other preferred embodiments, the antiperspirant active is highly desirably an aluminium chlorohydrate (ACH) and in others an activated aluminium chlorohydrate (AACH).

For incorporation of compositions according to the present invention, desirably at least 90, preferably at least 95 and especially at least 99 % by weight of the particles have a diameter in the range of from 0.1 up to 100 μπι, and usually have an average particle diameter of at least 1 μηι and especially below 20 μιη. In some highly desirable contact compositions the particles by weight have a weight average particle size of at least 2 μιη and particularly below 10 μπι, such as in the range of from 3 to 8 μιη.

Suitable deodorant actives can comprise deodorant effective concentrations of antiperspirant metal salts, deoperfumes, and/or microbiocides, including particularly bactericides, such as chlorinated aromatics, including biguanide derivatives, of which triclosan (e.g. Irgasan DP300 or Triclorban), and chlorhexidine warrant specific mention. Another class of effective deodorants comprises polyaminopropyl biguanide salts such as are available under the trade mark Cosmosil. Such materials commonly act as bactericides. A still further class of materials that can inhibit malodour formation comprise chelators that can sequester iron, and thereby retard bacterial growth, including aminopolycarboxylates such as ethylenediamine tetraacetic acid (EDTA) or higher homologues such as diethylenetriamine pentaacetic acid (DTPA). Deodorant actives other than astringent metal antiperspirant salts are commonly employed at a concentration of from 0.1 to 5, and particularly 0.1 to 2 % by weight.

Commercially acceptable and conventional vehicles may be used in the compositions of the invention, acting as diluents, dispersants and/or carriers for the skin lightening agents described herein and for any other optional but often preferred ingredients. Therefore, cosmetically acceptable vehicle suitable for use in this invention may be aqueous-based, anhydrous or an emulsion whereby a water-in-oil or oil-in-water emulsion is generally preferred. If the use of water is desired, water typically makes up the balance of the

composition, and preferably makes up from 5 to 99 %, and most preferably from 40 to 80 % by weight of the topical composition, including all ranges subsumed therein.

In addition to water, organic solvents may be optionally included to act as carriers or to assist carriers within the compositions of the present invention. Illustrative and non-limiting examples of the types of organic solvents suitable for use in the present invention include alkanols like ethyl and isopropyl alcohol, mixtures thereof or the like.

Other optional additives suitable for use include ester oils like isopropyl myristate, cetyl myristate, 2-octyldodecyl myristate, avocado oil, almond oil, olive oil, neopentylglycol dicaprate, mixtures thereof or the like. Typically, such ester oils assist in emulsifying the composition of this invention, and an effective amount is often used to yield a stable, and most preferably, water-in-oil emulsion.

Emollients may also be used, if desired, as carriers within the composition of the present invention. Alcohols like 1-hexadecanol (i.e. cetyl alcohol) are often desired as are the emollients generally classified as silicone oils and synthetic esters. Silicone oils suitable for use include cyclic or linear polydimethylsiloxanes containing from 3 to 9, preferably from 4 to 5, silicon atoms. Non-volatile silicone oils useful as an emollient material in the inventive composition described herein include polyalkyl siloxanes, polyalkylaryl siloxanes and polyether siloxane copolymers. The essentially non-volatile polyalkyl siloxanes useful herein include, for example, polydimethylsiloxanes.

The ester emollients that may optionally be used are:

(1) Alkenyl or alkyl esters of fatty acids having 10 to 20 carbon atoms. Examples thereof include isoarachidyl neopentanoate, isononyl isonanonoate, oleyl myristate, oleyl stearate, and oleyl oleate.

(2) Ether-esters such as fatty acid esters of ethoxylated fatty alcohols.

(3) Polyhydric alcohol esters. Ethylene glycol mono and di-fatty acid esters, diethylene glycol mono- and di-fatty acid esters, polyethylene glycol (200-6000) mono- and di-fatty acid esters, propylene glycol mono- and di-fatty acid esters, polypropylene glycol 2000 monooleate, polypropylene glycol 2000 monostearate, ethoxylated propylene glycol monostearate, glyceryl mono- and di-fatty acid esters, polyglycerol

poly-fatty esters, ethoxylated glyceryl mono-stearate, 1,3-butylene glycol monostearate, 1,3-butylene glycol distearate, polyoxyethylene polyol fatty acid ester, sorbitan fatty acid esters, and polyoxyethylene sorbitan fatty acid esters are satisfactory polyhydric alcohol esters.

(4) Wax esters such as beeswax, spermaceti, stearyl stearate and arachidyl behenate.

(5) Sterols esters, of which cholesterol fatty acid esters are examples.

Emollients when used, typically make up from 0.1 to 50 % by weight of the composition, including all ranges subsumed therein.

Fatty acids having from 10 to 30 carbon atoms may also be included as acceptable carriers within the composition of the present invention. Illustrative examples of such fatty acids include pelargonic, lauric, myristic, palmitic, stearic, isostearic, oleic, linoleic, arachidic, behenic or erucic acid, and mixtures thereof. Compounds that are believed to enhance skin penetration, like dimethyl sulfoxide, may also be used as an optional carrier.

Humectants of the polyhydric alcohol type may also be employed in the compositions of this invention. The humectant often aids in increasing the effectiveness of the emollient, reduces scaling, stimulates removal of built-up scale and improves skin feel. Typical polyhydric alcohols include glycerol, polyalkylene glycols and more preferably alkylene polyols and their derivatives, including propylene glycol, dipropylene glycol, polypropylene glycol, polyethylene glycol and derivatives thereof, sorbitol, hydroxypropyl sorbitol, hexylene glycol, 1,3-butylene glycol, 1,2,6-hexanetriol, ethoxylated glycerol, propoxylated glycerol and mixtures thereof. For best results the humectant is preferably propylene glycol or sodium hyaluronate. The amount of humectant may range anywhere from 0.2 to 25%, and preferably, from 0.5 to 15 % by weight of the composition, based on total weight of the composition and including all ranges subsumed therein.

Thickeners may also be utilized as part of the acceptable carrier in the compositions of the present invention. Typical thickeners include cross-linked acrylates (e.g. Carbopol 982), hydrophobically-modified acrylates (e.g. Carbopol 1382), cellulosic derivatives and natural gums. Among useful cellulosic derivatives are sodium carboxymethylcellulose, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, ethyl cellulose and hydroxymethyl cellulose. Natural gums suitable for the present invention

include guar, xanthan, sclerotium, carrageenan, pectin and combinations of these gums. Amounts of the thickener may range from 0.0 to 5, usually from 0.001 to 1, optimally from 0.01 to 0.5 % by weight of the composition.

Collectively the water, solvents, silicones, esters, fatty acids, humectants and/or thickeners will constitute the acceptable carrier in amounts from 1 to 99.9, preferably from 80 to 99 % by weight of the composition.

Surfactants may also be present in compositions of the present invention. Total concentration of the surfactant will range from about 0 to about 40%, and preferably from about 0 to about 20%, optimally from about 0 to about 5% by weight of the composition. The surfactant may be selected from the group consisting of anionic, nonionic, cationic and amphoteric actives. Particularly preferred nonionic surfactants are those with a C10-C20 fatty alcohol or acid hydrophobe condensed with from 2 to 100 moles of ethylene oxide or propylene oxide per mole of hydrophobe; mono- and di- fatty acid esters of ethylene glycol; fatty acid monoglyceride; sorbitan, mono- and di- C8-C20 fatty acids; block copolymers (ethylene oxide/propylene oxide); and polyoxyethylene sorbitan as well as combinations thereof. Alkyl polyglycosides and saccharide fatty amides (e.g. methyl gluconamides) are also suitable nonionic surfactants.

Preferred anionic surfactants include soap, alkyl ether sulfate and sulfonates, alkyl sulfates and sulfonates, alkylbenzene sulfonates, alkyl and dialkyl sulfosuccinates, C8-C20 acyl isethionates, acyl glutamates, C8-C20 alkyl ether phosphates and combinations thereof.

Perfumes may be used in the composition of this invention. Illustrative non-limiting examples of the types of perfumes that may be used include those comprising terpenes and terpene derivatives like those described in Bauer, K., et al, Common Fragrance and Flavor Materials, VCH Publishers (1990).

Illustrative yet non-limiting examples of the types of fragrances that may be used in this invention include myrcene, dihydromyrenol, citral, tagetone, cis-geranic acid, citronellic acid, mixtures thereof or the like.

Preferably, the amount of fragrance employed in the composition of this invention is in the range from 0.0 to 10, more preferably 0.00001 to 5, most preferably 0.0001 to 2 % by weight of the compound.

Various types of optional additional active ingredients may be used in the compositions of the present invention. Actives are defined as skin benefit agents other than emollients and other than ingredients that merely improve the physical characteristics of the composition. Although not limited to this category, general examples include talcs and silicas, as well as alpha-hydroxy acids, beta-hydroxy acids and zinc salts.

Beta-hydroxy acids include salicylic acid, for example. Zinc pyrithione is an example of the zinc salts useful in the composition of the present invention.

Many compositions, especially those containing water, should be protected against the growth of potentially harmful microorganisms. Anti-microbial compounds, such as triclosan, and preservatives are, therefore, typically necessary. Suitable preservatives include alkyl esters of p-hydroxybenzoic acid, hydantoin derivatives, propionate salts, and a variety of quaternary ammonium compounds. Particularly preferred preservatives of this invention are methyl paraben, propyl paraben, phenoxyethanol and benzyl alcohol. Preservatives will usually be employed in amounts ranging from 0.1 to 2 % by weight of the composition.

Still other optional ingredients that may be used with the composition of this invention include dioic acids (e.g. malonic acid and sebacic acid), antioxidants like vitamin E, retinoids, including retinoic acid, retinal, retinol and retinyl esters, conjugated linoleic acid, petroselinic acid and mixtures thereof, as well as any other conventional ingredients well known for wrinkle-reducing, anti-acne effects and reducing the impact of sebum.

When making the composition of the present invention, the desired ingredients are mixed in no particular order and usually at temperatures from 70 to 80 °C and under atmospheric pressure.

The packaging for the composition of this invention can be a patch, bottle, tube, roll-ball applicator, propellant driven aerosol device, squeeze container or lidded jar.

The composition according to the invention may be used as a medicament, more specifically for skin lightening. Alternatively, use of climbazole and lipoic acid in the manufacture of a composition of the invention is provided for lightening skin. In a further alternative, use of a composition according to the invention is provided for lightening skin. In another alternative, a method of lightening the skin of a human is provided, the method comprising the step of applying the composition of the invention to skin of a person in need thereof.

**Examples**

1.0 In-vitro studies of the skin lightening effect of climbazole

1.1 Materials

1.1.1 MelanoDerm™ cultures

MatTek MelanoDerm™ cultures (a pigmented 3D-Living Skin Equivalent (LSE) model) purchased from the MatTek Corporation. Dark cultures (MEL-300-B), for evaluation of skin lightening potential, were prepared in a long life maintenance medium (EPI-IOO-LLMM available from the MatTek Corporation) for good pigment production whilst preserving acceptable histology.

On arrival samples were replaced into growth medium and left at 37 °C for 2 hours to recover prior to the administration of test items. Test actives were applied to the samples by addition to the growth medium. Fresh growth medium containing dimethyl sulphoxide (DMSO) alone or test actives was replaced every 2-3 days and the cultures were grown in this way for 14 days prior to harvesting and analysis. Prior to extraction or fixation cultures were assayed using the WST-1 cell proliferation assay.

1.1.2 B16 monolayer cultures

B16 mouse melanoma cells were cultured in Eagle's minimal essential medium (EMEM) supplemented with 10% foetal calf serum (FCS) and 2mM L-glutamine at 37 °C, 5 % C02 in T175 tissue culture flasks and were sub-cultured twice weekly using trypsin in EDTA. For assay, 25 000 cells per well in 500 µï volume were seeded to a 48 well plate and adhered overnight. The following morning standard culture media was replaced by EMEM, 10% FCS, 4mM L-glutamine (500 µï) and test actives added. Cells were then incubated for 72 hours. At 72 after which the supernatants were removed and analysed for melanin content by spectrophometry at 450 nm against a reference standard curve. The B16 cell monolayer was dissolved in Triton™/PBS at 4 °C for 20 minutes, centrifuged at 13 000 rpm for 5 minutes at

4 °C and analysed for total protein content using a standard bicinchoninic acid assay (BCA) assay against a reference standard curve. Melanin results were normalised to total protein. The results are summarised in table 1.

The results in table 2 were obtained using B16 F10 cells (mouse melanoma cell line) obtained from ATCC grown in Lonza Biowhittaker BE12-662F media. Trypsin in ethylenediaminetetraacetic acid (EDTA) (Sigma T3924) and Dulbecco's Phosphate Buffered Saline (DPBS) (Sigma D8537) were used to split the cells. Plates were set up at a concentration of 2.5 x $10^4$ cells per well in 48 well plates in Phenol Red free media (Sigma D 1145).

After overnight incubation, media was removed and media with climbazole in DMSO solution were added. 500 µï of the media and solutions were added to 4 wells of a 48 well plate and the plates incubated at 37 °C for a further 3 days. Thereafter the melanin concentration and cell numbers were measured.

Melanin concentration was measured by making up standards each day and pipetting 100 µï of each standard in duplicate onto Greiner plates. For the blank plate Phenol Red free media was used. 100 µï from each well was also pipetted in duplicate onto Greiner plates and the A450 nm was measured.

100 µï of RIPA buffer (Sigma R0278) with protease inhibitor was added to each well. Pierce BCA Protein Assay (Thermo Scientific # 23225) was used as per manufacturer's instructions with IOµI of sample in replicate.

1.1.3 Azoles

Climbazole, fluconazole, imidazole, oxazole and thiazole were obtained from Sigma Aldrich Company Limited.

1.2 Melanin quantitation

Melanin content of the cultures was determined following the recommended by MatTek (solvable extraction protocol) using a proprietary solvent known as Solvable™ (available from Perkin-Elmer) as the extraction solvent. Protein was extracted as described previously and quantified using the BCA assay.

1.3 Results

1.3.1 Melanogenesis in B16 monolayer cultures

Each test active was applied in DMSO to n = 3 or 4 cultures (as stated in tables 1 and 2). Data are shown as mean values and range. The results are summarised in tables 1 and 2.

Table 1: Normalised melanin (µg per mg protein) from B16 monolayer cultures for a number of azoles (the first DMSO control relates to the climbazole results only and the second DMSO control relates to the remaining results).

| Treatment | Normalised melanin ^g/mg protein) | |
|---|---|---|
| | Mean (n = 3) | Range |
| DMSO control | 186 | 41 |
| 5 µM climbazole | 185 | 44 |
| 20 µM climbazole | 162 | 11 |
| 50 µM climbazole | 78 | 6 |
| DMSO control | 182 | 16 |
| 20 µM fluconazole | 225 | 18 |
| 20 µM imidazole | 217 | 6 |
| 20 µM oxazole | 204 | 32 |
| 20 µM thiazole | 240 | 27 |

Table 2: Normalised melanin (µg per mg protein) from B16 monolayer cultures for climbazole.

| Treatment | Normalised melanin (µg/mg protein) | | % of control mean | % pigment reduction |
|---|---|---|---|---|
| | Mean (n=4) | range | | |
| 50 µM climbazole | 196 | 60 | 57 | 43 |
| 25 µM climbazole | 304 | 124 | 88 | 12 |
| 10 µM climbazole | 337 | 94 | 98 | 2 |
| 5 µM climbazole | 393 | 172 | 114 | none |
| 2 µM climbazole | 343 | 98 | 99 | 1 |
| DMSO | 345 | 149 | 100 | none |

1.3.2 Melanogenesis in MelanoDerm™ cultures

Climbazole was applied in DMSO to n = 2 cultures. Two cultures from each treatment group were extracted for melanin and protein quantisation. The results for normalised melanin (per mg protein) are summarised in table 3.

Table 3: Normalised melanin (per mg protein) for climbazole from MelanoDerm™ cultures.

| Treatment | Normalised melanin ^g/mg protein) |
|---|---|
| DMSO control | 0.099477 |
| | 0.078425 |
| 50 μM climbazole | 0.040708 |
| | 0.042679 |

The total extracted protein concentration was relatively consistent for all treatments (data not shown) suggesting that there were not any substantial cytotoxic effects on the cultures.

1.4 Conclusions

Climbazole very effectively inhibits melanin production in B16 monolayer cultures and MelanoDerm™ cultures at concentrations of 20 and 50 μM. Furthermore there is no detectable effect on the viability of epidermal cultures at this dose. Surprisingly other azoles and in particular fluconazole, imidazole, oxazole and thiazole did not effectively inhibit melanin production in B16 monolayer cultures at 20 μM.

2.0 Combination of climbazole with lipoic acid

2.1 Method

B16 F10 cells (mouse melanoma cell line) were obtained from ATCC and grown in Lonza Biowhittaker BE12-662F media. Trypsin in ethylenediaminetetraacetic acid (EDTA) (Sigma T3924) and Dulbecco's Phosphate Buffered Saline (DPBS) (Sigma D8537) were used to split the cells. Plates were set up at a concentration of 2.5 x $10^4$ cells per well in 48 well plates in Phenol Red free media (Sigma Dl 145).

After overnight incubation, media was removed and media with lipoic acid (Lipoec™ from Cognis which is 99 % lipoic acid itself comprises a racemic mixture of R- and S- lipoic acids) in ethanol and climbazole in DMSO solutions were added. 500 μï of the media and solutions were added to 4 wells of a 48 well plate and the plates incubated at 37 °C for a further 3 days. Thereafter the melanin concentration and cell numbers were measured.

Melanin concentration was measured by making up standards each day and pipetting 100 μï of each standard in duplicate onto Greiner plates. For the blank plate Phenol Red free media

was used. 100 µï from each well was also pipetted in duplicate onto Greiner plates and the A450 nm was measured.

100 µï of RIPA buffer (Sigma R0278) with protease inhibitor was added to each well. Pierce BCA Protein Assay (Thermo Scientific # 23225) was used as per manufacturer's instructions with IOµI of sample in replicate.

2.2 Results

Each test active was applied in DMSO to n = 4 cultures. Data are shown as mean values and range

The results are summarised in table 4.

Table 4: Melanin ^g) per mg protein from B16 monolayer cultures for the combination of climbazole and lipoic acid.

| | Normalised melanin µg/ mg protein | | | |
|---|---|---|---|---|
| | Mean **(n=4)** | Range | % of control mean | % pigment reduction |
| 10µM climbazole + ethanol | 275 | 34 | 95.9 | 1.09 |
| Ethanol + DMSO | 287 | 97 | 100 | 0 |
| 10µM lipoic acid + DMSO | 285 | 80 | 99.2 | 0.769 |
| 10µM lipoic acid + 10µM climbazole | 0 | 0 | 0 | 100 |
| 10µM climbazole + ethanol | 267 | 57 | 83.4 | 16.5 |
| Ethanol + DMSO | 319 | 122 | 100 | 0 |
| 10µM lipoic acid + DMSO | 293 | 131 | 91.6 | 8.42 |
| 10µM lipoic acid + 10µM climbazole | 192 | 52 | 60. 1 | 39.8 |
| 10µM climbazole + ethanol | 310 | 44 | 84. 1 | 15.9 |
| Ethanol + DMSO | 368 | 37 | 100 | 0 |
| 10µM lipoic acid + DMSO | 288 | 75 | 78.2 | 21.8 |
| 10µM lipoic acid + 10µM climbazole | 122 | 36 | 33. 1 | 66.9 |

2.3 Conclusion

Significant synergy in reducing melanin content is exhibited in this in-vitro assay by combining lipoic acid with climbazole.

**Claims**

1. A topical skin lightening composition comprising:
   a. Climbazole;
   b. Lipoic acid; and
   c. An ingredient selected from the group consisting of an organic sunscreen, an inorganic sunscreen, an antiperspirant active, a microbiocide, and mixtures thereof.

2. A composition according to claim 1 comprising 0.001 to 2, preferably 0.005 to 0.5 % w/w climbazole.

3. A composition according to claim 1 or claim 2 comprising 0.001 to 2, preferably 0.002 to 0.2 % w/w lipoic acid.

4. A composition according to any one of the preceding claims, wherein lipoic acid is in the form of the **R**-(+)-isomer, preferably exclusively in the form of the **R**-(+)-isomer.

5. A composition according to any one of the preceding claims, wherein the antiperspirant active is selected from the group consisting of an aluminium salt, a zirconium salt, a mixed aluminium/zirconium salt, and mixtures thereof.

6. A composition according to any one of the preceding claims, wherein the microbiocide is selected from the group consisting of triclosan, a polyaminopropyl biguanide salt, and mixtures thereof.

7. A composition according to any one of the preceding claims for use as a medicament.

8. A composition according to any one of claims 1 to 6 for use in skin lightening.

9. Use of climbazole and lipoic acid in the manufacture of a composition according to any one of claims 1 to 6 for lightening skin.

10. Use of the composition according to any one of claims 1 to 6 for lightening skin.

11. A method of lightening the skin of a human, the method comprising the step of applying the composition of any one of claims 1 to 6 to skin of a person in need thereof.

12. An effective amount of climbazole for use in skin lightening.

13. Use of an effective amount of climbazole in the manufacture of a medicament for lightening skin.

14. A method of lightening the skin of a human, the method comprising the step of applying an effective amount of climbazole to skin of a person in need thereof.