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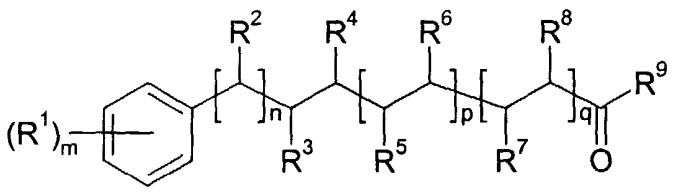
(54) Title: COMPOUNDS FOR USE IN THE TREATMENT OF SKIN CONDITIONS



(I)

(57) Abstract: The present invention provides compounds of formula (I) and analogues or derivatives thereof for the treatment of skin conditions, such as Vitiligo, which are treatable by the stimulation of melanocyte proliferation and also for treating skin cancer. The compounds may also be used to cosmetically enhance the natural coloration of the skin.

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COMPOUNDS FOR USE IN THE TREATMENT OF SKIN CONDITIONS

Field of the invention

This invention relates to the treatment of skin conditions, comprising those conditions requiring stimulation of melanocyte proliferation and to the inhibition of melanomas. The invention is of especial application to the treatment of vitiligo and skin cancer.

Vitiligo is a common skin pigment disorder characterised by the development of patchy de-pigmented lesions. Current treatments which include the use of photosensitisers (eg psoralens) with UVA radiation (PUVA), corticosteroids or skin grafting have low success rates and are generally accompanied by unpleasant side effects. Vitiligo has a highly detrimental impact on the emotional well-being of the sufferer, the disfiguring effects of the disease being compounded by the absence of a suitable treatment. Although vitiligo patches are not believed to contain melanocytes (pigment producing cells), a reservoir exists in hair follicles in vitiliginous skin. Thus activation of hair follicular melanocytes is a crucial process in the repigmentation of vitiliginous skin.

Certain plant remedies, usually administered as mixtures of herbs or extracts, particularly those used in traditional Chinese medicine and Indian Ayurvedic medicine, have been employed for the treatment of vitiligo for a long time and in many cases have given positive results in small scale studies. Herbs such as *Psoralea corylifolia L.* and *Vernonia anthelmintica Willd.* (=*Centratherum anthelminticum Kuntze*) are well known for their use in this disease. Psoralens, which are employed in the modern PUVA and khellin in KUVA therapy were originally derived from plant sources (*Psoralea corylifolia L* and *Ammi visnaga* respectively) used in traditional remedies for vitiligo. However these therapies rely on the use of UV irradiation for their efficacy, which is associated with the aetiology of skin cancer.

The fruit of black pepper (*Piper nigrum L.*) and long pepper (*Piper longum L.*) are both important medicinal herbs in Ayurvedic and Unani (traditional Indian) medicine systems, in which remedies generally consist of mixtures of herbs. A wide range of the medicinal uses of black pepper have been documented by Kirtikar and Basu (Indian Medicinal Plants, 2nd Edition, Vol. 3, (1935) pages 2128 - 2135), including its use in the treatment of leucoderma. Black pepper has also been implicated as a possible adjunct to *Vernonia anthelmintica* in the treatment of leucoderma (Indian Medicinal Journal, Vol. 1, 3rd Edition, (1982) 1267 - 1270).

These two herbs are employed as a constituent in many traditional herbal preparations for a variety of uses, including gastro-intestinal and skin ailments. Compositions comprising black pepper, ginger and pipali have been used in the treatment of vitiligo (Ancient Science of Life, Vol. IX, No. 4 (1990) 202 - 206); however, the specific therapeutic action of black pepper in this orally administered composition has not been established.

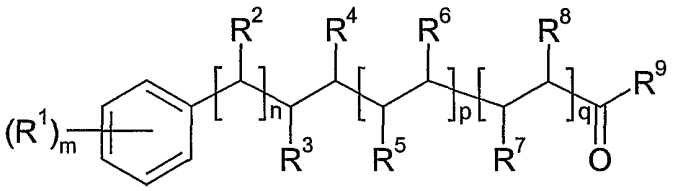
It has been found (WO 001/02544) that, piperine, which is present in the fruit of *Piper nigrum*, stimulates the replication of melanocytes. The action of piperine is to increase the number of cells which confer pigmentation. Piperine is the compound (E,E)-1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine and should not be confused with piperidine.

Pharmaceutical compositions containing piperine have been used in the treatment of tuberculosis and leprosy (EP 0 650 728). It has also been suggested that piperine is able to enhance the bioavailability of the other constituents of a pharmaceutical composition (WO 96/25939).

There is, therefore, a need for further compounds and compositions, which are able to stimulate the proliferation of melanocytes.

Summary of the invention

The invention provides a compound or formula (1) for use in the treatment of a skin condition requiring stimulation of melanocyte proliferation and melanomas, in which



(I)

m is 2

n is 0 or 1

p is 0 or 1

q is 0 or 1

the two R^1 groups together represent a 3′,4′-methylenedioxy group

R^2 is hydrogen

R^3 and R^4 represent hydrogen atoms or together represent a carbon to carbon double bond;

R^5 and R^6 represent hydrogen atoms or together represent a carbon to carbon double bond;

R^7 and R^8 represent hydrogen atoms or together represent a carbon to carbon double bond; and

R^9 represents piperidino, morpholino, cyclohexylamino, methylamino, ethylamino and isopropylamino in any of its E, Z geometrically isomeric forms or an active analogue or derivative thereof, as hereinafter defined or optionally when n is 1 R^2 and R^3 together represent a carbon to carbon double bond and one or more of R^4 and R^5 together, R^5 and R^6 together, R^6 and R^7 together or R^7 and R^8 together represent a carbon to carbon double bond the other of R^4 to R^8 representing hydrogen with the proviso that the compound is not piperine, 3,4-dihydropiperine; 1,2,3,4–tetrahydropyridine, Ilepeimide or piperettine.

The invention also provides the use of a compound of formula (I) in the preparation of a medicament for use in the treatment of a skin condition requiring stimulation of melanocyte proliferation and melanomas. Pharmaceutical compositions comprising a compound of formula (I) and a pharmaceutically acceptable carrier are also provided.

The active ingredient may be used on its own, but is more suitably used in combination with a carrier or excipient and optionally one or more further active ingredients.

Stimulation of melanocyte proliferation greatly facilitates the re-pigmentation of de-pigmented skin, e.g. post traumatised de-pigmented skin. The term "post traumatised de-pigmented skin" means the skin formed during the healing process that occurs after a skin trauma. De-pigmentation may arise, for example, from scar tissue formed as a result of a skin trauma such as burn or other skin lesion or may be due to vitiligo. The present invention can be used to treat any of these skin disorders in a patient.

Generally in this invention, the compounds of formula (I) or active derivatives or analogues thereof may be administered by oral, topical, intravenous or subcutaneous (intra-muscular) routes but are preferably applied topically (to the area of the skin where treatment is desired). Indeed, the twice-daily topical application of compounds of formula (I) has been

found to induce significant pigmentation in mice. Skin coloration in the mouse population under study was first observed at approximately four weeks after the treatment was started. This coloration was enhanced further as a result of subsequent topical applications.

The active ingredient may be formulated as a solid powder; a paste, ointment or cream; a tablet or capsule or a solution.

The compounds of formula (I) may also be used to treat a person having a skin condition which would benefit from coloration, e.g. to enhance or promote the natural colouring of the skin. The treatment may be used for prophylactic, therapeutic or cosmetic purposes.

The compounds of formula (I) and their analogues or derivatives as hereinafter defined inhibit the proliferation of melanoma cells. Thus, they may also be used in the treatment of skin cancer. Another aspect of the invention therefore provides a method of treating skin cancer in a human or animal patient comprising the administration to said patient of a therapeutically effective amount of a compound of formula (I) or an active analogue or derivative thereof, as hereinafter defined.

The compounds of formula (I) or active analogues or derivatives thereof may be administered by oral or topical routes. Suitable dosage forms may be any of those discussed above.

Certain of the active analogues or derivatives of the compound of formula (1) are new. The present invention therefore includes such compounds, and pharmaceutical compositions containing them together with a carrier or excipient.

Description of the preferred embodiments

Preferred compounds of formula (I) are those in which

(a) n is 0, one of p or q is other than 0, R^3 and R^4 together form the second bond of a carbon to carbon double bond and either R^5 and R^6 together or R^7 and R^8 together form the second bond of a carbon to carbon double bond; and

(b) n is 0, one of p or q is other than 0, R^3 and R^4 are each hydrogen and either R^5 and R^6 or R^7 and R^8 are also hydrogen and R^9 is cyclohexylamino or piperidino.

Particularly preferred compounds are those in which

(a) n is 0, one of p or q is other than 0, R^3 and R^4 together form the second bond of a carbon to carbon double bond and either R^5 and R^6 together or R^7 and R^8 together form the second bond of a carbon to carbon double bond and R^9 is selected from morpholino, cyclohexylamino, methylamino, ethylamino and isopropylamino;

(b) n is 0, p is 0, q is 1, R^3, R^4, R^7 and R^8 represent hydrogen and R^9 is cyclohexylamino; and

(c) n is 0, p is 1, q is 1, R^3, R^4, R^5, R^6, R^7 and R^8 represent hydrogen and R^9 is piperidino.

The compounds of formula (1) can be prepared from the appropriate acid with the appropriate connecting chain between the carboxylic acid function and the benzene ring and having the appropriate stereochemistry. Where necessary, this may be preceded or followed by reduction to reduce the double bond or bonds in the connecting chain. Methods of preparing amides and esters from these acids are illustrated by the Examples below. They may also be adapted from the references cited herein, the disclosure of which is herein incorporated by reference.

The active compounds may be formulated for topical use in the form of creams, soft paraffin or lotions. Aqueous cream BP or Yellow Soft Paraffin BP may suitably contain the active at 0.03-3.0 mg % w/w or an equivalent amount of plant extract. A suitable lotion is typically prepared from 20% glycerol and 80% ethanol in purified water and contains 0.03-3.0 mg % w/w of the active material. These topical formulations may also contain penetration enhancers such as oleic acid, propylene glycol, ethanol, urea, lauric diethanolamide or azone, dimethyl sulphoxide, decylmethyl sulphoxide, or pyrrolidone derivatives. Liposomal delivery systems may also be used.

Compositions for oral formulation include tablets or capsules containing 1.5-150 mg active for daily administration.

The invention will now be described with reference to the following non-limiting examples, with reference to the accompanying tables and drawings.

Examples

Introduction

Cell culture experiments

Microplate culture and sulforhodamine B (SRB) assay

Cells of mouse melan-a cell line (passage number 18-24), a first known line of non-tumorigenic pigmented mouse melanocytes were maintained in a flask (Costar, Cambridge, MA, USA) using RPMI 1640 (ICN, Costa, Mesa, CA, USA) as a basic medium. For microplate proliferation assays, subconfluent melan-a cultures were trypsinized (0.25% trypsin at 37°C for 5-10 min) and inoculated with a repeater-pipettor (Finn pipette, Labsystems, Finland) into 96-well microtiter plates (Costar, Cambridge, MA, USA) at a seeding concentration of 6 x 10 cells per well. A supplemental growth medium of 10% foetal bovine serum (FBS) was added to the 36-well microtiter plates. The plates were incubated at 37°C in a 10% CO_2, 90% air humidified atmosphere for 4 days. At the end of the incubation, an SRB assay was performed. Briefly, cells attached to the bottom of the plate were fixed by addition of cold trichloroacetic acid (TCA, 4°C, Aldrich, Dorset, UK) on the top of the growth medium (final TCA 20% w/v). The plate was placed at 4°C for 1 hour before being gently washed five times with tap water. It was allowed to dry in air, or aided with a hair dryer to speed up the drying process, then 50 µl of 4% w/v SRB dissolved in 1% acetic acid in water was added to each well for 30 min. At the end of the staining period, unbound SRB was removed by washing 4 times with 1% acetic acid. The plate was air dried again, and 150 µl of 10 mM aqueous Tris base (Sigma-Aldrich Co. Ltd, Irvine, UK) was added into each well to solubilize the cell-bound dye. The plate was shaken for 15 min on a gyratory shaker followed by reading the optical density (OD) at 550 nm in a microplate spectrophotometer (Anthos Labtec HT3, version 1.06). A control assay was carried out on cells incubated without test compound. There were 2 or 3 series of experiments, each of which consisted of six replicate experiments. The results are tabulated below.

Example 1

Compounds of formula (1)

1.0 Introduction

Vitiligo is defined as a circumscribed, acquired, idiopathic, progressive hypomelanotic skin disorder which is characterised by the development of patchy depigmented macules due to progressive loss of melanocytes which is often familial with lack of established aetiology.

Various compounds of formula (1) were synthesised and tested for melanocyte (mouse melan-a) proliferant activity *in-vitro*. Cells were incubated with the test compound for 4 days, as described above.

1.1 Percentage cell growth (A)

Percentage cell growth was obtained with a given compound calculated as **(optical density in the presence of the compound/ control optical density) x 100.**

1.2 Relative activity to piperine

Melan-a cell proliferant activity for tested compounds was compared with that obtained with piperine. Percentage stimulant activity is (A-100) where A stands for piperine or a test compound's percentage cell growth (see 1.1). All figures are given with Standard Error of the Mean.

Relative activity to piperine was calculated as **(A-100) compound / (A-100) piperine).**

Interpretation of the relative active value is as follows

< 0	-	Inhibition of cell growth
0	-	No effect (equal to control)
0-1	-	Stimulant but weaker effect than piperine
1	-	Equal stimulant effect to piperine
> 1	-	Stimulant and stronger effect than piperine

1.3 Dendricity

Effect on dendricity of melan-a cells by the test compounds was by observation under microscope. Dendricity is relevant to vitiligo since normal skin melanocytes have dendrites, but in vitiligo the melanocytes seem to lose these before they disappear from the patches.

1.4 Synthesis of Compounds of formula (1)

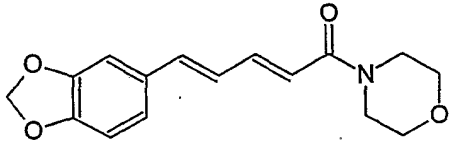
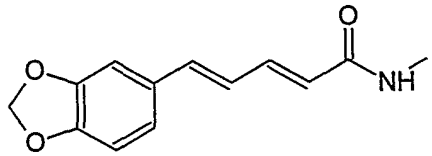
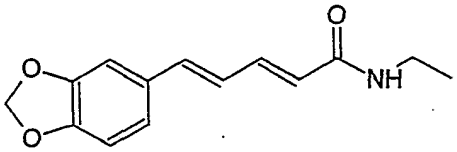
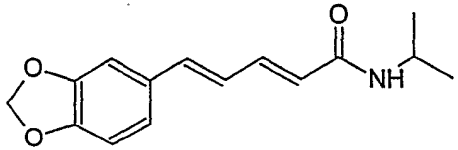
The compounds of formula (1) were synthesised using methods described in the literature, adapted from the literature or devised in the inventors' laboratory. Structures of

compounds were verified using NMR, MS, IR spectroscopy and melting point. Unless a synthetic method is given, reagents and reactants were purchased from Sigma Aldrich.

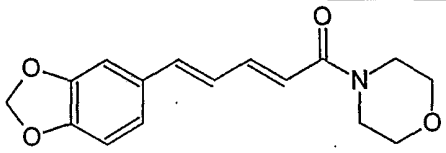
1.5 Results

The activity of compounds of formula (I) at a single concentration of test compound (10μM) is shown in Table 1. This is followed by data showing results at other concentrations. Many compounds showed a "cross-over" effect in which the test compound was less active than piperine at 10μM but more active at 50μM.

Table 1

Variation on Nitrogen Substituent of Piperine		Effect on melan-a cells at µM concentration				
Code N°	Structure	Percentage cell growth (Repeated experiments) Test cpd.	Piperine	Stimulant activity	Relative activity to piperine	Dendricity
RV-A02		156±58 187±40** 153±19**	210±65** 170±22 155±19**	Positive	0.5 1.02 0.9	+++
RV-A07		170±24*	216±33*	Positive	0.6	++
RV-A08		200±14	236±17	Positive	0.73	+++
RV-A09		224±19	263±16**	Positive	0.76	+++

RV-A10		308±29**	302±17**	Positive	1.02	+++
*P<0.05, **P<0.01 compared to vehicle treatment (Dunnett's t test) +++ highly dendritic, ++ moderately dendritic, + weakly dendritic, - no effect						

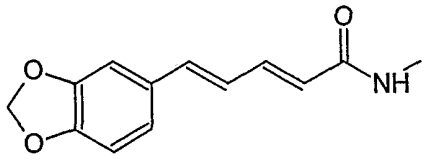
Code N°	Structure
RV-A02	

Compounds Tested	1μM	10μM	25μM	50μM
Piperine	147±11**♦	192±13**♦	167±19**	142±15**
RV-A02	125±10	167±17**	171±8**	168±12**□

** P<0.01 Compared to vehicle treatment (Dunnet's t test)

♦ Piperine is significantly more active than test compound P<0.05

□ Test compound is significantly more active than Piperine P<0.05

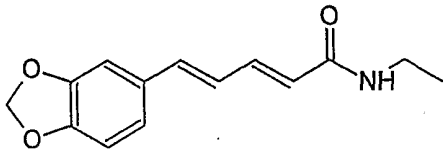
Code N°	Structure
RV-A07	

Compounds Tested	1μM	10μM	50μM	100μM
Piperine	211±16**♦	216±33**	52±15	16±3
RV-A07	140±12**	170±24**	71±5	46±2
Dentricity of RV-A07	++	++	+	+

** P<0.01 Compared to vehicle treatment (Dunnet's t test)

♦ Piperine is significantly more active than test compound P<0.05

++ moderately dendritic, + weakly dendritic

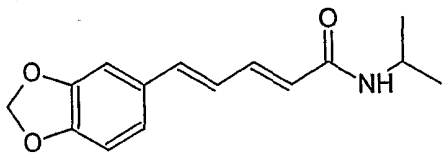
Code N°	Structure
RV-A08	

Compounds Tested	1μM	10μM	50μM	100μM
Piperine	216±14**♦	236±17**	61±11	32±5
RV-A08	139±27**	200±14**	81±12	62±13
Dendricity of RV-A08	++	+++	+	+

** P<0.01 Compared to vehicle treatment (Dunnet's t test)

♦ Piperine is significantly more active than test compound P<0.05

+++ highly dendritic, ++ moderately dendritic, + weakly dendritic

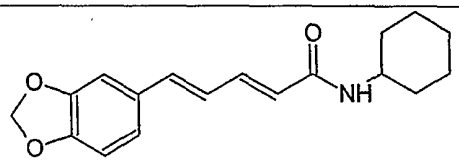
Code N°	Structure
RV-A09	

Compounds Tested	1μM	10μM	50μM	100μM
Piperine	221±17**♦	263±16**	77±12	24±2
RV-A09	187±15**	224±19**	85±5	42±6
Dendricity of RV-A09	+++	+++	+	+

** P<0.01 Compared to vehicle treatment (Dunnet's t test)

♦ Piperine is significantly more active than test compound P<0.05

+++ highly dendritic, + weakly dendritic

Code N°	Structure
RV-A10	

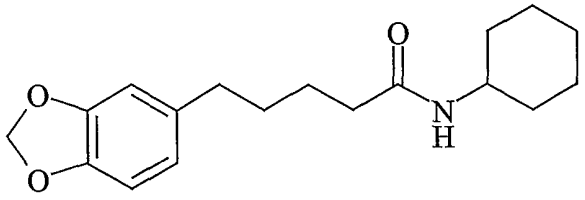
Compounds Tested	1μM	10μM	50μM	100μM
Piperine	236±30**	302±17**	78±11	21±4
RV-A10	301±20**□	308±29**	155±22**□	100±13
Dendricity of RV-A10	+++	+++	++	+

** P<0.01 Compared to vehicle treatment (Dunnet's t test)

□ Compound is significantly more active than piperine P<0.05

+++ highly dendritic, ++ moderately dendritic, + weakly dendritic

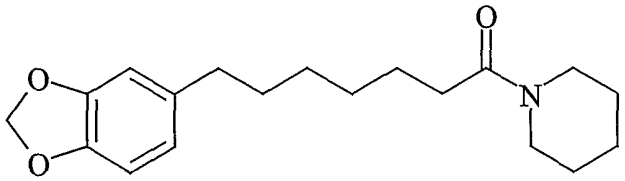
RV-C04

Code No	Structure
RV-C04	

Compound	1μM	10μM	50μM	100μM
Piperine	191±12**♦	216±18**	184±6**	96±6
RV-C04	129±6**	192±6**	192±10**	191±12**
Dendricity of RV-C04	+	+++	+++	+++

5

RV-C05

Code No	Structure
RV-C05	

Compound	1μM	10μM	50μM	100μM
Piperine	161±13**	192±2** ♦	189±15**	87±13
RV-C05	118±1	160±5** ♦	158±19**	113±15
Dendricity of RV-C05	+	++	++	+

2. Synthesis of amide derivatives of piperinic acid

2.1 Preparation of piperinic acid (RV-A00)

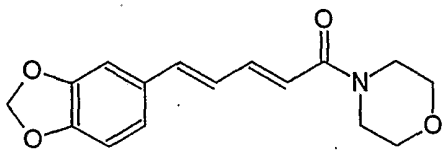
To piperine (1) (2g, 0.7mmol, 1eq), 20% of methanolic KOH (100ml) was added and refluxed for 2days. After completion of the hydrolysis, methanol was removed under reduced pressure and a yellow coloured oily solid was obtained. This residue was dissolved in water (50ml) and acidified with 6N HCl to pH <1 yielding a yellowish precipitate of piperinic acid. Recrystallization from methanol gave yellow needles (0.9g, 60% yield). m.p. 206^{0}C-208^{0}C (Lit m.p. 217^{0}C-218^{0}C)[1]

Piperine (1) Piperinic acid

2.2 Synthesis of amide derivatives of piperinic acid

The following amines were reacted with piperinic acid in accordance with the following procedure: morpholine; methylamine; ethylamine; isopropylamine and cyclohexylamine. A mixture of piperinic acid (1eq) and triethylamine (2eq) in dichloromethane (50ml) was stirred for 15min at 0^{0}C. To this mixture methanesulfonyl chloride (1.5eq) was added and stirred for further 30 min at 0^{0}C. The amine (1.5eq) was added to the mixture and stirred for 1h at 0^{0}C and 2h at room temperature. Dichloromethane (50ml) was added to the mixture which was then washed with 5% HCl (3x100ml), saturated aqueous $NaHCO_3$ (3x100ml) and water (3x100ml). The organic fraction was dried over anhydrous sodium sulphate, filtered and rotary evaporated to yield a yellowish solid residue. Recrystallisation from ethylacetate/petroleum spirit yielded colourless needles of piperlonguminine (120mg, 32% yield)[2]. The reaction is presumed to proceed through a mesylate ester intermediate.

5-E,E-piperinoyl morpholine (RV-A02)



^{1}H-NMR ($CDCl_3$) δ: 6.37 (d, 1H, J=14.6, CH=CH-CH=**CH**), 7.45 (d,d, 1H, J=10.2, 14.6, CH=CH-**CH**=CH), 6.72 (d,d, 1H, J=15.5, 10.2, CH=**CH**-CH=CH), 6.79 (d, 1H, J=15.5 **CH**=CH-CH=CH), 6.98 (d,1H J=1.5, Ar-**7-H**), 6.80 (d,1H J=8.0, Ar-**10-H**), 6.89 (d, d, 1H J=1.5, 8.0 Ar-**11-H**), 5.98 (s, 2H, **O-CH_2-O**), 3.70 (t, 2H, J=4.0 **CH_2**-N- **CH_2** (morpholine)) 3.60 (t, 2H, J=4.0 **CH_2**-O- **CH_2** (morpholine))

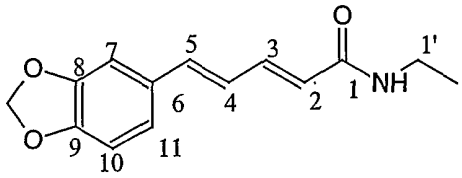
^{13}C-NMR ($CDCl_3$) : 42.3 (**CH_2**), 46.1(**CH_2**), 66(**CH_2**), 66(**CH_2**), 101.3 (**CH_2**), 106.5 (**CH**), 108.5 (**CH**), 118.7 (**CH**), 122.7 (**CH**), 124.9 (**CH**), 130.8 (**C**), 139.1 (**CH**), 143.4 (**CH**), 148.2 (**C**), 148.3 (**C**), 165.6 (**C**)

MS m/z (%) : 287 (M^+ 57), 201 (100), 173 (25), 171 (10) 143 (10), 115 (30)

IR (KBr): ν_{max} (carbonyl group) 1641

m.p. 161.8^0-162.5^{0}C (Lit m.p. 167-168^{0}C)[3], yield 44.1%

5-E,E-piperinoylmethylamine (RV-A07)



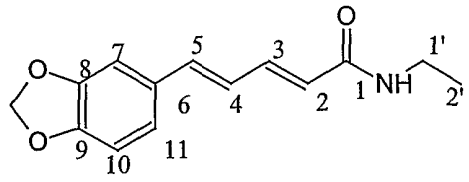
^{1}H-NMR ($CDCl_3$) δ: 5.91 (d, 1H, J=14.8, CH=CH-CH=**CH**), 7.36 (d,d, 1H, J=10.7, 14.8, CH=CH-**CH**=CH), 6.66 (d,d, 1H, J=15.4, 10.6, CH=**CH**-CH=CH), 6.77 (d, 1H, J=15.4 **CH**=CH-CH=CH), 6.97 (d,1H J=1.5, Ar-**7H**), 6.77 (d,1H J=8.0, Ar-**10H**), 6.88 (d, d, 1H J=1.6, 8.0 Ar-**11H**), 5.97 (s, 2H, **O-CH_2-O**), 2.91(t, 3H, **CH_3**), 5.61 (br, **NH**)

^{13}C-NMR ($CDCl_3$) : 26.9 (**CH_3**), 101.7 (**CH_2**), 106.1 (**CH**), 108.9 (**CH**), 123.0 (**CH**), 123.3 (**CH**), 125.0 (**CH**), 131.2 (**C**), 139.2 (**CH**), 141.4 (**CH**), 148.6 (**C**), 148.6 (**C**), 167.2 (**C**)

MS m/z (%) : 231(M^+89), 201 (42), 173 (67), 172 (32), 171 (17), 143 (27), 116 (21) 115 (100), 89 (12)

m.p. 181.1^0-182.4^{0}C (Lit m.p. 186^{0}C)[5], yield 48.2%

5-E,E-piperinoylethylamine (RV-A08)



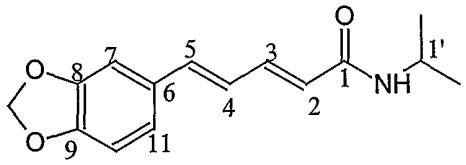
^{1}H-NMR (CD_3OD) δ: 6.14 (d, 1H, J=15.0, CH=CH-CH=**CH**), 7.37 (d,d, 1H, J=10.2, 15.0, CH=CH-**CH**=CH), 6.93 (d,d, 1H, J=15.7, 10.6, CH=**CH**-CH=CH), 6.87 (d, 1H, J=15.7 **CH**=CH-CH=CH), 6.97 (d,1H J=1.5, Ar-**7H**), 6.77 (d,1H J=8.0, Ar-**10H**), 6.88 (d, d, 1H J=1.6, 8.0 Ar-**11H**), 5.97 (s, 2H, **O-CH_2-O**), 3.39 (m, 2H, J= 6.2, **CH_2**),1.22(t, 3H, J= 6.1, **CH_3**),

^{13}C-NMR ($CDCl_3$) : 14.7 (**CH_3**), 36.9 (**CH_2**), 103.2 (**CH_2**), 107.2 (**CH**), 109.8 (**CH**), 121.2 (**CH**), 124.9 (**CH**), 125.9 (**CH**), 132.4 (**C**), 142.9 (**CH**), 145.2 (**CH**), 150.2 (**C**), 150.6 (**C**),170 (**C**)

MS m/z (%) : 245(M^+78), 218 (34), 201 (71), 200 (49), 174 (64), 173 (80), 172 (76), 171 (65), 143 (75), 116 (68),115 (100)

m.p. 158.5^0-159.9^{0}C (Lit m.p. 162^0-164^{0}C)[4], yield 45.6%

5-E,E-piperinoylisopropylamine (RV-A09)



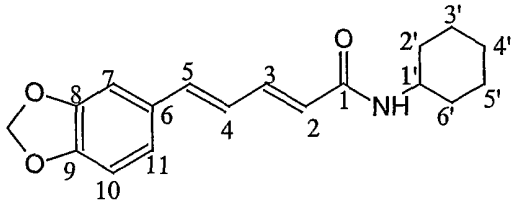
^{1}H-NMR ($CDCl_3$) δ: 5.87 (d, 1H, J=14.8, CH=CH-CH=**CH**), 7.36 (d,d, 1H, J=10.7, 14.8, CH=CH-**CH**=CH), 6.66 (d,d, 1H, J=15.4, 10.6, CH=**CH**-CH=CH), 6.76 (d, 1H, J=15.2 **CH**=CH-CH=CH), 6.97 (d,1H J=1.6, Ar-**7H**), 6.77 (d,1H J=8.0, Ar-**10H**), 6.88 (d,d, 1H J=1.6, 8.0 Ar-**11H**), 5.97 (s, 2H, **O-CH_2-O**), 4.15(m, 1H, J=6.6, **CH**), 5.36 (d, 1H, J=7.3 **NH**), 1.19 (d, 6H, J=6.6, **$(CH_3)_2$**)

^{13}C-NMR ($CDCl_3$) : 23.2 (**CH_3**) $_2$, 41.9 (**CH**), 101.9 (**CH_2**), 106.4 (**CH**), 108.9 (**CH**), 123.0 (**CH**), 123.8 (**CH**), 124.1 (**CH**), 131.3 (**C**), 140.2 (**CH**), 141.2 (**CH**), 148.8 (**C**), 148.6 (**C**) 165.6 (**C**)

MS m/z (%) : 259(M^+80), 201 (62), 174 (34), 173 (74), 172 (31), 171 (15), 143 (30), 116 (16), 115 (100)

m.p. 169^0-169.4^{0}C (Lit m.p. 171^0-173^{0}C)[4], yield 52%

5-E,E-piperinoyl cyclohexylamine (RV-A10)



^{1}H-NMR ($CDCl_3$) δ: 5.93 (d, 1H, J=14.8, CH=CH-CH=**CH**), 7.35 (d,d, 1H, J=10.6, 14.8, CH=CH-**CH**=CH), 6.66 (d,d, 1H, J=15.3, 10.6, CH=**CH**-CH=CH), 6.76 (d, 1H, J=15.4 **CH**=CH-CH=CH), 6.96 (d,1H J=1.6, Ar-**7H**), 6.76 (d,1H J=8.0, Ar-**10H**), 6.87 (d, d, 1H J=1.6, 8.0 Ar-**11H**), 5.97 (s, 2H, **O-CH_2-O**), 3.87 (m, 1H, **CH** (cyclohexyl)) 1.99 (m, 2H, **CH_2**(cyclohexyl)) 1.65 (m, 4H, **CH_2-CH_2**(cyclohexyl) 1.39 (m, 2H, **CH_2**(cyclohexyl)) 1.18 (m, 2H, **CH_2**(cyclohexyl)) 5.48 (d,J=8.0 **NH**)

^{13}C-NMR ($CDCl_3$) : 25.3 (($\mathbf{CH_2}$)$_2$), 25.9 ($\mathbf{CH_2}$), 33.6 (($\mathbf{CH_2}$)$_2$), 48.6 (**CH**), 101.3 ($\mathbf{CH_2}$), 101.7 (**CH**), 106.1 (**CH**), 108.9 (**CH**), 123.0 (**CH**), 124.0 (**CH**), 125.1 (**CH**),131.3 (**C**), 139.0 (**CH**), 141.2 (**CH**) 148.5 (**C**), 148.5 (**C**), 165.5 (**C**)

MS m/z (%) : 299(M^+56), 259 (48) 216 (33), 201 (60),174 (33), 173 (61), 172 (18), 171 (16) 143 (17), 115 (100)

m.p. 196.4^0-197.3^{0}C (Lit m.p. 199^0-200^{0}C)[4], yield 57.4%

References:

[1]Chatterjee, A., and Dutta, C.P. (1967). Alkaloids of *Piper longum* Linn-I Structure and synthesis of piperlongumine and piperlonguminine, Tetrahedron, **23**,1769-1781.

[2]Nokio Nakumara, Fumiyuki Kiuchi, and Yoshisuke Tsuda (1988). Infrared spectra of conjugated amides: Reassignment of the C=O and C=C absorptions: Chemical and Pharmaceutical Bulletin, **36**, 2647-2651.

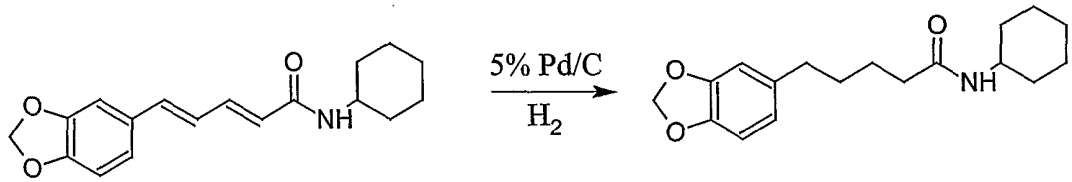
[3]H.Oediger and A.Schulze (Bayer AG), (1979), Deutsche Auslegeschrift 2757 483

[4]Paula, Vanderlucia F. de; A Barbosa, Luiz C. de; Demuner, Antonio J.; Pilo-Veloso, Dorila; Picanco, Marcelo C. (2000) Pest Management Science **56**, 2, 168 – 174.

[5]Gokale et al., (1948) Journal of University Bombay Science **16**/5A 32-35

4. Preparation of 5-(3,4-methylenedioxy phenyl)-pentanoic acid cyclohexylamide (RV-C04)

To 5-(3,4-methylenedioxy phenyl)-2E,4E-pentadienoic acid cyclohexyl amide (300mg) was added 5% Pd/C (30mg) and hydrogenated the contents at 30 psi for 1hr. The solution was filtered and rotary evaporated to yield a white solid. Recrystallisation from ethylacetate and petroleum spirit yielded pure white crystals (255mg, yield 84%). m.p. 145.4^{0}C-146.3^0 C.

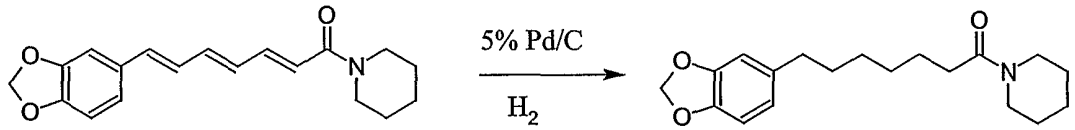


5-(3,4-methylenedioxy phenyl)-2E,4E-pentadienoic acid cyclohexyl amide

5-(3,4-methylenedioxy phenyl)-2,4-pentanoic acid cyclohexyl amide

5. Preparation of 7-(3,4-methylenedioxy phenyl)-heptanoic acid piperidineamide (RV-C05)

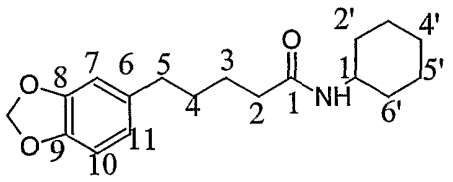
To 7-(3,4-methylenedioxy phenyl)-2E,4E,6E-heptatrienoic acid piperidine amide (150mg, 0.06mmole) was added 5% Pd/C (15mg) and hydrogenated the contents at 30 psi for 30min to give 7-(3,4-methylenedioxy phenyl)-heptanoic acid piperidine amide as an oil.



7-(3,4-methylenedioxy phenyl)-2E,4E,6E-heptatrienoic acid piperidine amide

7-(3,4-methylenedioxy phenyl)-2,4,6-heptanoic acid piperidine amide

RV-C04

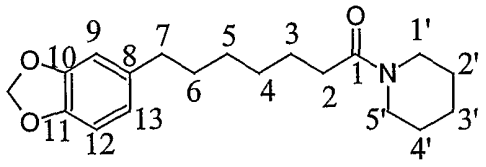


^{1}H-NMR ($CDCl_3$) δ: 6.65 (d,1H J=1.6, Ar-**7-H**), 6.71 (d,1H J=7.8, Ar-**10-H**), 6.60 (d, d, 1H J=1.6, 8.0 Ar-**11-H**), 5.90 (s, 2H, **O-CH_2-O**), 5.43 (s, 1H, **NH**), 2.53 (t, 2H, J=7.7 (**CH_2**-CH_2-CH_2CH_2)) 2.14 (t, 2H, J=7.7 ((CH_2-CH_2-CH_2-**CH_2**)) 1.62-1.91 (m, 10H, CH_2-**CH_2**-**CH_2**-CH_2, **CH_2**-**CH_2**-**CH_2** (cyclohexyl amide) 1.07-1.30 (m, 4H, **CH_2**-CH-**CH_2** (cyclohexylamide))

^{13}C-NMR ($CDCl_3$) : 25.3 ((**CH_2**)$_2$), 25.7 (**CH_2**),25.9 (**CH_2**), 31.3 (**CH_2**), 31.7 (**CH_2**), 33.6 (**CH_2**), 35.8 (**CH_2**), 37.3 (**CH_2**), 48.4 (**CH**), 101.1 (**CH_2**), 108.4 (**CH**), 109.2 (**CH**), 121.4 (**CH**), 136.4 (**C**), 145.8 (**C**), 147.8 (**C**), 172.2 (**C**),

MS m/z (%) : 303 (M^+ 98), 204 (72), 176 (13), 168(16), 162 (12) 161 (14), 154 (27), 148 (66), 141 (61) 135 (100) 74 (24) 60 (60)

RV-C05



^{1}H-NMR ($CDCl_3$) δ: 6.66 (d,1H J=1.5, Ar-**7-H**), 6.71 (d,1H J=7.8, Ar-**10-H**), 6.60 (d, d, 1H J=1.6, 8.0 Ar-**11-H**), 5.90 (s, 2H, **O-CH_2-O**), 3.53 (t, 2H, J=5.4 CH_2-N-**CH_2**) 3.37 (t, 2H, J=5.7, (**CH_2**-N- CH_2) 2.51 (t, 2H, J=7.7 (**CH_2**-CH_2-CH_2-CH_2-CH_2-CH_2)) 2.33(t, 2H, J=7.7 ((CH_2-CH_2-CH_2-CH_2-CH_2-**CH_2**)) 1.52-1.65 (m, 10H, hydrocarbon CH_2, CH_2, **CH_2-CH_2-CH_2** (Piperidine)) 1.34 (m, 4H, CH_2 CH_2)

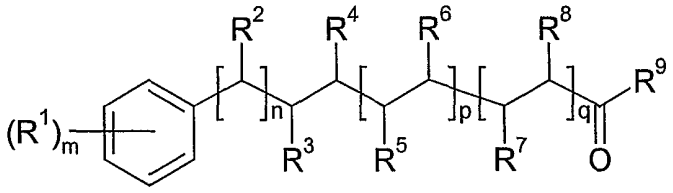
^{13}C-NMR ($CDCl_3$) : 24.9 (**CH_2**), 25.8 (**CH_2**),25.9 (**CH_2**), 26.9 (**CH_2**), 29.3(**CH_2**), 29.7 (**CH_2**), 31.3 (**CH_2**), 31.9 (**CH_2**), 33.8 (**CH_2**), 42.9 (**CH_2**), 47.1 (**CH_2**), 101.8 (**CH_2**), 108.4 (**CH**), 109.2 (**CH**), 121.4 (**CH**), 137.0 (**C**), 145.7 (**C**), 147.8 (**C**), 171.8 (**C**),

MS m/z (%) : 317 (M^+ 78), 232 (11), 204 (10), 183 (30), 182 (15), 154 (21) 148 (43), 141 (41), 127 (100), 112 (43), 85 (49)

Yield 51.2%

CLAIMS

1. A compound of formula (1) for use in the treatment of a skin condition



in which

m is 2

n is 0 or 1

p is 0 or 1

q is 0 or 1

the two R^1 groups together represent a 3',4'-methylene methylenedioxy group,

R^2 is hydrogen;

R^3 and R^4 represent hydrogen atoms or together represent a carbon to carbon double bond;

R^5 and R^6 represent hydrogen atoms or together represent a carbon to carbon double bond;

R^7 and R^8 represent hydrogen atoms or together represent a carbon to carbon double bond; and

R^9 represents piperidino, morpholino, cyclohexylamino, methylamino, ethylamino, isopropylamino, isopropoxy, propoxy and butoxy in any of its E,Z geometrically isomeric forms or an active analogue or derivative thereof or optionally when n is 1 R^2 and R^3 together represent a carbon to carbon double bond and one or more of R^4 and R^5 together, R^5 and R^6 together, R^6 and R^7 together or R^7 and R^8 together represent a carbon to carbon double bond the other of R^4 to R^8 representing hydrogen with the proviso that the compound is not piperine, 3,4-dihydropiperine; 1,2,3,4–tetrahydropyridine, Ilepeimide or piperettine.

2. A compound according to claim 1 or claim 2, wherein n is 0.

3. A compound according to claim 2, wherein p is 1, q is 0 and R^3 and R^4 together and R^5 and R^6 together represent a carbon double bond.

4. A compound according to claim 1, selected from the group 5-E,E piperinoyl morphaline, 5-E,E-piperinoylmethylamine, 5-E,E,-piperinoylethylamine, 5-E,E-piperinoylisopropylamine and 5-E,E-piperinoylcyclohexylamine.

5. A compound according to claim 1, wherein each of R^2 to R^8 are hydrogen.

6. A compound according to claim 1, being 5-(3,4-methylenedioxyphenyl)-2E,4E-pentanoic acid cyclohexylamide or 7-(3,4-methylenedioxyphenyl)-2E,4E,6E-heptanoic acid cyclohexylamide.

7. A compound according to any one of the preceding claims in which the skin condition is treatable by stimulation of melanocyte proliferation.

8. A compound according to any one of claims 1 to 7 in which the skin condition is a melanoma.

9. Use of a compound of formula (I) as defined in any one of the preceding claims in the preparation of a medicament for use in the treatment of a skin condition selected from the group of conditions consisting of (a) those treatable by stimulation of melanocyte proliferation and (b) a melanoma.

10. Use according to claim 9, in which the skin condition is vitiligo.

11. A pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (I) as defined in any one of claims 1 to 8 and a pharmaceutically acceptable carrier.

12. A pharmaceutical composition according to claim 11, in which the pharmaceutically acceptable carrier includes glycerol.

13. A pharmaceutical composition according to claim 11 or claim 12, in which the pharmaceutically acceptable carrier includes ethanol.

14. A pharmaceutical composition according to any one of claims 11 to 13, which includes a penetration enhancer.

15. A pharmaceutical composition according to any one of claims 11 to 14 in the form of a lotion.

16. A pharmaceutical composition according to claim 15, comprising 0.03 to 3.0 mg % w/w of the compound of formula (I).

17. A compound of formula (I) as defined in claim 1 being 5-(3,4-methylenedioxyphenyl)-2E,4E-pentanoic acid cyclohexylamide

INTERNATIONAL SEARCH REPORT

PCT/GB 02/00158

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D413/10 C07D317/60 A61P17/02 A61K31/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CA 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; GAIND, K. N. ET AL: "Preservatives. IX" retrieved from STN Database accession no. 83:72580 XP002198540 abstract & INDIAN J. PHARM. (1974), 36(6), 149-50 , ---	3-17
X	DE PAULA, VANDERLUCIA F. ET AL: "Synthesis and insecticidal activity of new amide derivatives of piperine" PEST MANAGEMENT SCIENCE (2000), 56(2), 168-174 , XP001070610 tables 1,,PAGE,170 --- -/--	3-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 May 2002	28/05/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Wolf, C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 02544 A (BTG INT LTD ;RAMAN AMALA (GB); LIN ZHIXIU (GB)) 20 January 2000 (2000-01-20) compounds listed on page 5	3-17
A	LIN Z ET AL: "STIMULATION OF MOUSE MELANOCYTE PROLIFERATION BY PIPER NIGRUM FRUIT EXTRACT AND ITS MAIN ALKALOID, PIPERINE" PLANTA MEDICA, THIEME, STUTTGART, DE, vol. 65, October 1999 (1999-10), pages 600-603, XP000879055 ISSN: 0032-0943 page 602	3-17

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1,2

Present claims 1 and 2 relate to an extremely large number of possible compounds. In fact, the claims contain so many options that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely claims 3-17. Compounds and pharmaceutical compositions have been searched e.g those compounds and compositions recited in the examples and closely related homologous compounds thereof.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

PCT/GB 02/00158

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0002544 A	20-01-2000	AU 4920999 A	01-02-2000
		CN 1330545 T	09-01-2002
		EP 1094813 A2	02-05-2001
		WO 0002544 A2	20-01-2000
		US 6346539 B1	12-02-2002