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(54) Title: USE OF ANTAGONISTS OF EGF OR TGF-ALPHA FOR THE TREATMENT AND PROPHYLAXIS OF ACNE

(57) Abstract

Compositions comprising one or more antagonists of EGF, TGF-α or EGF-receptor function for use in treating acne, spots and pimples are described.

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USE OF ANTAGONISTS OF EGF OR TGF-ALPHA FOR THE TREATMENT AND PROPHYLAXIS OF ACNE

The present invention relates to compositions for topical application to the skin and to their cosmetic and pharmaceutical use. In particular the invention relates to compositions suitable for use in the treatment of acne and cosmetic conditions associated with acne such as spots and pimples.

BACKGROUND TO THE INVENTION

Acne vulgaris is a disease of the human sebaceous unit. The sebaceous pilosebaceous duct has been implicated in the aetiology of acne, with hyperproliferation, hyperkeratinisation and abnormal desquamation of the duct cells leading to open or closed comedone formation which are the primary symptom of acne.

The isolation and maintenance of the human pilosebaceous duct in-vitro has previously been described by Guy et al., British Journal of Dermatology (1993) 128 242-248. This paper further reported that the known anti-acne treatment, 13-cis retinoic acid, acts directly at the level of the duct.

The present inventors have significantly improved upon the isolated human sebaceous duct model reported previously. By maintaining the sebaceous duct in "keratinocyte serum-free medium" supplied commercially, supplemented with bovine pituitary extract and a high concentration of calcium chloride (ca 2mM), in place of supplemented William's E medium as previously reported, retention of duct architecture over a period of seven days with no fall in the rate of cell division is obtained. Furthermore, the rates of cell division in ducts maintained in keratinocyte

medium have been found to be significantly higher than in ducts maintained in William's E medium after twenty four hours and seven days.

The present inventors have further surprisingly found that the addition of epidermal growth factor (hereinafter EGF) or transforming growth factor α (hereinafter TGF-α) to the keratinocyte maintenance medium causes disruption of the duct architecture but without an accompanying decrease in the rate of cell division or protein synthesis. This closely mimics the rupturing of the duct observed in acne vulgaris leading to inflammatory skin reactions.

Treatments directed to antagonising EGF function are therefore useful in reducing or limiting this tissue disruption and consequent inflammatory reactions which are the main symptoms of acne.

DEFINITION OF THE INVENTION

The invention provides a topical composition comprising one or more antagonists of EGF, TGF-α or EGF receptor function; and, optionally a cosmetically or physiologically acceptable vehicle.

In a second aspect, the invention provides a method of treating acne comprising applying to the skin a composition comprising one or more antagonists of EGF, TGF-α or EGF receptor function and optionally a cosmetically or physiologically acceptable vehicle.

The invention further provides the use of one or more antagonists of EGF, TGF-α or EGF receptor function in the treatment of acne.

In an alternative aspect the invention provides the use of one or more antagonists of EGF, TGF-α or EGF receptor

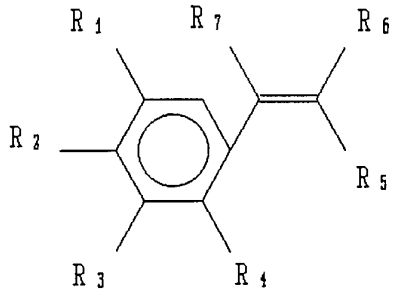
function for the manufacture of a medicament for the treatment of acne.

In a related aspect, the invention provides a method for screening or testing candidate substances to identify substances suitable for treating acne comprising contacting the test substance with an isolated human sebaceous duct maintained in keratinocyte serum-free medium (or a medium having a similar beneficial effect on duct maintenance) in the presence of EGF or TGF-α and assessing the response of the duct to the test substance. Substances which antagonise EGF, TGF-α or EGF receptor function and are therefore of use in the treatment of acne will reduce or prevent ductal rupture.

DISCLOSURE OF THE INVENTION

As used herein, the term "antagonist of EGF function" means any agent which is capable of interfering with the stimulatory effect of EGF or TGF on cell growth. In particular, it means any agent which has the effect of reducing or eliminating any changes in the properties of the isolated human pilosebaceous duct preparation as herein described caused by administration of EGF alone. Antagonists of EGF function include agents that interfere with the activity of EGF, TGF-α and the function of, or pathways stimulated by, the EGF receptor.

One suitable class of compounds which antagonise EGF function and may be used according to the present invention are the protein tyrosine kinase inhibitors of formula (I), commonly known as tyrphostins.



(I)

where R^1, R^2, R^3 and R^4 are the same of different, and are chosen from

-H, -OH, $-C_nH_{2n+1}$, $-NO_2$, -Cl, -Br, -F

and $-\overset{\overset{O}{\|}}{C}H$;

and where R^5 and R^6 are the same or different, and are chosen from:

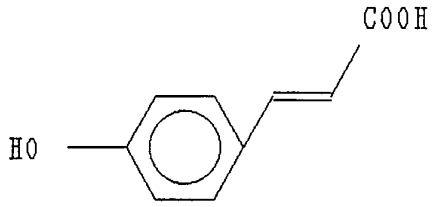
-H, -CN, $-\overset{\overset{O}{\|}}{C}OH$, $-\overset{\overset{O}{\|}}{C}NH_2$ and $-\overset{\overset{S}{\|}}{C}NH_2$;

and where R^7 is chosen from -H and -OH
and where n is an integer of from 1 to 8.

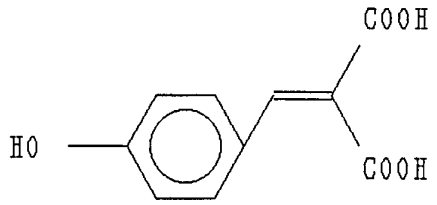
The composition according to the invention can also comprise mixtures of said inhibitors.

Examples of the inhibitors are:

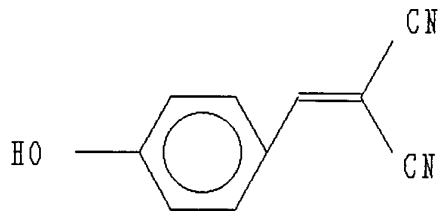
1-carboxy-2-(4-hydroxyphenyl)ethylene having the structure:



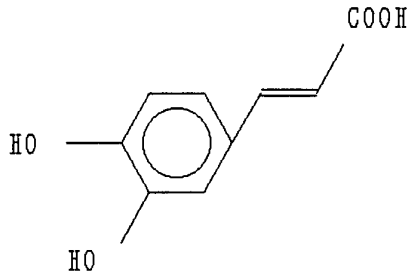
1,1-dicarboxy-2-(4-hydroxyphenyl)ethylene having the structure:



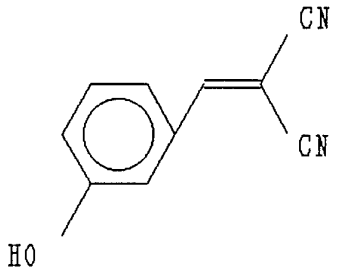
1,1-dicyano-2-(4-hydroxyphenyl)ethylene having the structure:



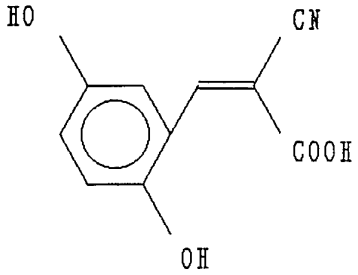
1-carboxy-2-(3,4-dihydroxyphenyl)ethylene having the structure:



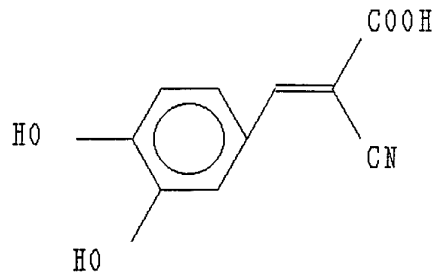
1,1-dicyano-2-(3-hydroxyphenyl)ethylene having the structure:



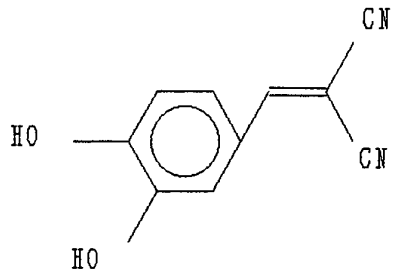
1-cyano-1-carboxy-2-(2,5-dihydroxyphenyl)ethylene having the structure:



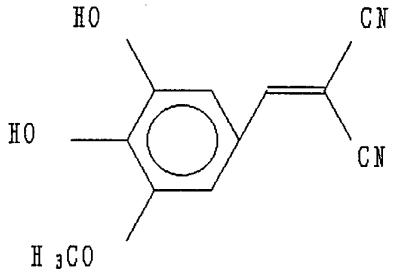
1-carboxy-1-cyano-2-(3,4-dihydroxyphenyl)ethylene having the structure:



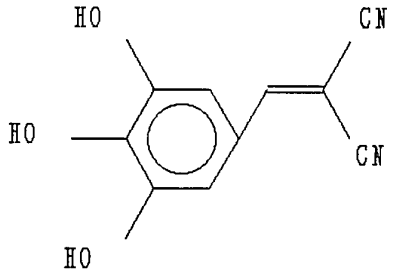
1,1-dicyano-2-(3,4-dihydroxyphenyl)ethylene having the structure:



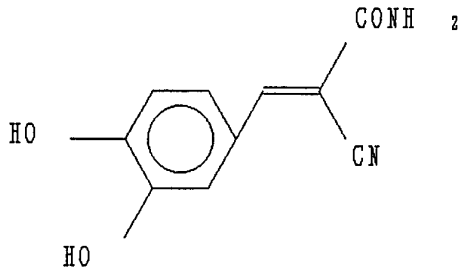
1,1-dicyano-2-(3-methoxy-4,5-dihydroxyphenyl)ethylene having the structure:



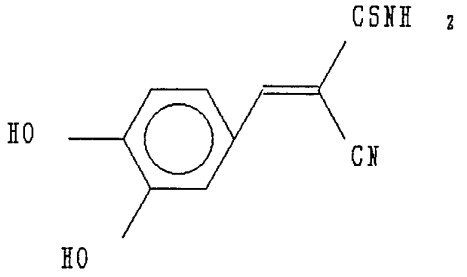
1,1-dicyano-2-(3,4,5-trihydroxyphenyl)ethylene having the structure:



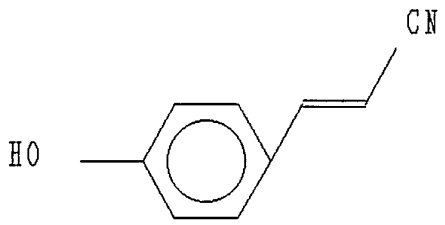
1-amido-1-cyano-2-(3,4-dihydroxyphenyl)ethylene having the structure:



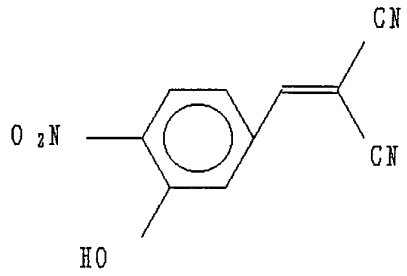
1-thioamido-1-cyano-2-(3,4-dihydroxyphenyl)ethylene having the structure:



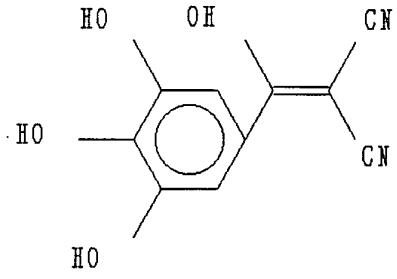
1-cyano-2-(4-hydroxyphenyl)ethylene
having the structure:



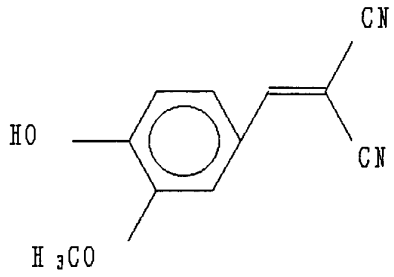
1,1-dicyano-2-(3-hydroxy-4-nitrophenyl)ethylene
having the structure:



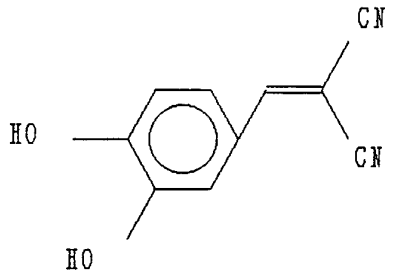
1,1-dicyano-2-hydroxy-2-(4-hydroxyphenyl)ethylene
having the structure:



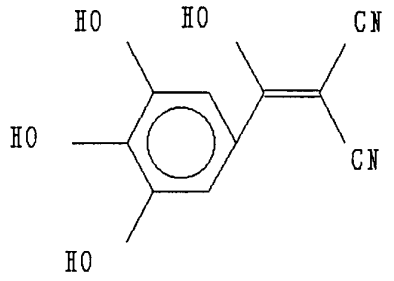
1,1-dicyano-2-(3-methoxy-4-hydroxyphenyl)ethylene having the structure:



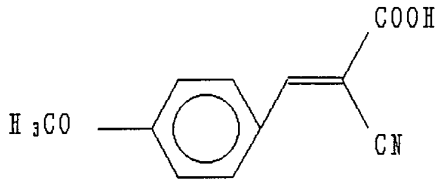
1,1-dicyano-2-(3,5-dihydroxyphenyl)ethylene having the structure:



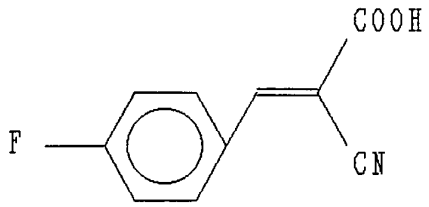
1,1-dicyano-2-hydroxy-2-(3,4,5-trihydroxyphenyl)ethylene having the structure:



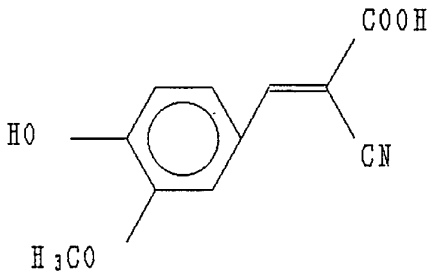
1-carboxy-1-cyano-2-(4-methoxyphenyl)ethylene having the structure:



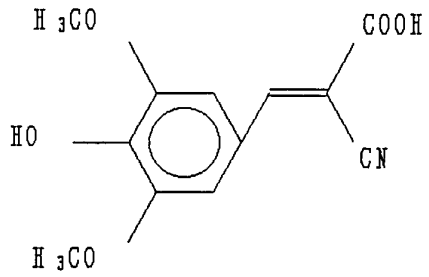
1-carboxy-1-cyano-2-(4-fluorophenyl)ethylene having the structure:



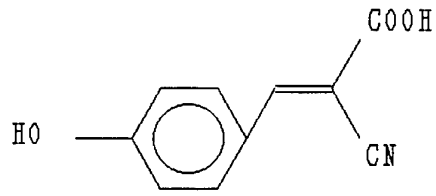
1-carboxy-1-cyano-2-(3-methoxy-4-hydroxyphenyl)ethylene having the structure:



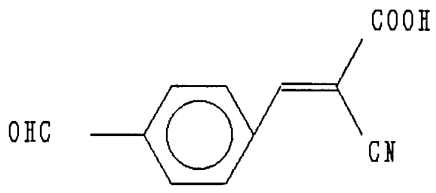
1-carboxy-1-cyano-2-(3,5-dimethoxy-4-hydroxyphenyl)ethylene having the structure:



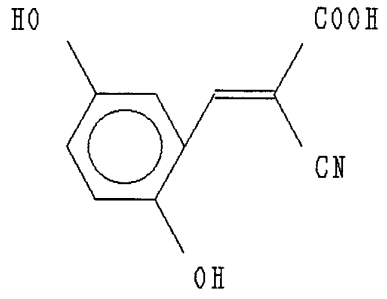
1-carboxy-1-cyano-2-(4-hydroxyphenyl)ethylene having the structure:



1-carboxy-1-cyano-2-(4-phenylcarboxyaldehyde)ethylene having the structure:



1-cyano-1-carboxy-2-(2,5-dihydroxyphenyl)ethylene having the structure:



Compounds of formula (I) are known from European Patent Application EP-A-0403238. They are described as suitable for inducing, maintaining or increasing hair growth.

An alternative possibility is to use an antibody to EGF or TGF-α or the EGF receptor to antagonise their function.

Such an antibody, which may be monoclonal or polyclonal or an antibody fragment, may be generated by techniques conventional in the art, for example by using recombinant DNA techniques. Specific binding subunits or antibody fragments may also be used. These may similarly be generated by conventional techniques such as enzymic digestion of intact antibody molecules, for example using papain or pepsin, or using recombinant DNA techniques. Antibody fragments may also be generated by conventional molecular biology techniques.

Conveniently, the compositions according to the invention comprises one or more antagonists of EGF function in an amount of from 0.000001 to 10% by weight of the composition, preferably from 0.00001 to 10% by weight of the composition.

The compositions according to the invention preferably also comprises a vehicle to act as a diluent, disperser or

carrier for the antagonist of EGF function in the composition so as to facilitate its distribution when the composition is applied to the skin. Preferably the vehicle is cosmetically and/or physiologically acceptable. The vehicle must be acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Vehicles can include water or substances such as liquid or solid emollients, solvents, humectants, thickeners and powders.

Examples of each of these types of vehicle, which can be used singly or as mixtures of one or more vehicles, are as follows:

Emollients, such as stearyl alcohol, glyceryl monoricinoleate, glyceryl monostearate, mink oil, cetyl alcohol, isopropyl isostearate, stearic acid, isobutyl palmitate, isocetyl stearate, oleyl alcohol, isopropyl laurate, hexyl laurate, decyl oleate, octadecan-2-ol, isocetyl alcohol, eicosanyl alcohol, behenyl alcohol, cetyl palmitate, silicone oils such as dimethylpolysiloxane, di-n-butyl sebacate, isopropyl myristate, isopropyl palmitate, isopropyl stearate, butyl stearate, polyethylene glycol, triethylene glycol, lanolin, cocoa butter, corn oil, cotton seed oil, tallow, lard, olive oil, palm kernel oil, rapeseed oil, safflower seed oil, evening primrose oil, soybean oil, sunflower seed oil, avocado oil, olive oil, sesame seed oil, coconut oil, arachis oil, castor oil, acetylated lanolin alcohols, petroleum jelly, mineral oil, butyl myristate, isostearic acid, palmitatic acid, isopropyl linoleate, lauryl lactate, myristyl lactate, decyl oleate, myristyl myristate;

Propellants, such as air, propane, butane, isobutane, dimethyl ether, carbon dioxide, nitrous oxide;

Solvents such as ethyl alcohol, methylene chloride, isopropanol, acetone, ethylene glycol monoethyl ether, diethylene glycol monobutyl ether, diethylene glycol monoethyl ether, dimethyl sulphoxide, dimethyl formamide, tetrahydrofuran;

Powders, such as chalk, talc, fullers earth, kaolin, starch, gums, colloidal silica sodium polyacrylate, tetra alkyl and/or trialkyl aryl ammonium smectites, chemically modified magnesium aluminium silicate, organically modified montmorillonite clay, hydrated aluminium silicate, fumed silica, carboxyvinyl polymer, sodium carboxymethyl cellulose, ethylene glycol monostearate.

The vehicle will usually form from 10 to 99.9%, preferably from 50 to 99% by weight of the emulsion, and can, in the absence of other adjuncts, form the balance of the composition.

The composition according to the invention can also comprise other materials which are conventionally useful in cosmetic or therapeutic products for topical application to the skin, such as surfactants, for example anionic, nonionic and amphoteric surfactants, preservatives, perfumes, moisturisers and antioxidants.

USE OF THE COMPOSITION

The composition according to the invention is intended primarily as a product for topical application to human skin, for treating acne, spots and pimples. References herein to treatment extend to prophylaxis as well as the treatment of established conditions.

It will be appreciated that the amount of the composition and the frequency of application to the skin will depend on the condition of the patient and the particular antagonist

of EGF function used. In general, topical application of from 0.1mg to 10mg daily of a selected antagonist is proposed.

In use, a small quantity of the composition, for example from 1ml is applied to areas of the skin from a suitable container or applicator and, if necessary, it is then spread over and/or rubbed into the skin using the hand or fingers or a suitable device.

The topical skin treatment composition of the invention may conveniently be formulated as a lotion having a viscosity of from 4,000 to 10,000 mPas, a fluid cream having a viscosity of from 10,000 to 20,000 mPas or a cream having a viscosity of from 20,000 to 100,000 mPas, or above. The composition can be packaged in a suitable container to suit its viscosity and intended use by the consumer. For example, a lotion or fluid cream can be packaged in a bottle or a roll-ball applicator or a propellant-driven aerosol device or a container fitted with a pump suitable for finger operation. When the composition is a cream, it can simply be stored in a non-deformable bottle or squeeze container, such as a tube or a lidded jar.

The invention accordingly also provides a closed container containing a cosmetically acceptable composition as herein defined.

In order that the invention may be well understood the following examples are given by way of illustration only.

Example 1

A lotion has the following formulation:

	% w/w
3,4 dihydroxy-d-cyanocinnamamide	0.001
Ethanol	10
Perfume	qs
Butylated hydroxytoluene	0.01
Water	to 100

Example 2

The effectiveness of compositions comprising an antagonist of EGF function was studied as follows:

Isolation of sebaceous ducts

Redundant human female facial skin was obtained from cosmetic surgical procedures. Thereafter, layers of skin were removed by means of a keratome. Initially 0.1mm of the skin surface was taken, to remove the epidermal layer. A second layer of 0.2mm of dermis was then taken and placed in phosphate-buffered saline. This layer contains the pilosebaceous ducts. The ducts were easily identified using a dissecting microscope, as they are much larger than the ducts of the vellus follicle, and lack the prominent hair of the terminal follicle. In addition, the ducts were seen to contain large quantities of sebum which appeared dark on transillumination. The ducts were removed by gentle microdissection.

Maintenance of Isolated Pilosebaceous Ducts

Ducts were maintained in keratinocyte serum free basal medium (supplied by Gibco) supplemented by 50μg/ml bovine pituitary extract, and where appropriate 5ng/ml EGF or

5ng/ml TGF-α at 37°C in an humidified atmosphere of 5%CO_2/95% air. Where appropriate the 'antagonist of EGF function' was added at the same time as the EGF or TGF-α. The antagonists used were of the tyrphostin family:

tyrphostin 1(4-methoxybenzylidene)alononitrite at 1000μM as a negative for tyrphostin toxicity, and
tyrphostin 46(3,4, dihydroxy-α-cyanocinnamamide) at 100μM or tyrphostin 47(3,4, dihydroxy-α-cyanothiocinnamamide) 24μM

as antagonists of EGF function.

Optionally antibodies and antifungal agents may be added to the culture medium to prevent bacterial and fungal contamination.

Morphology of Ducts and Behaviour in Culture

Ducts maintained in vitro in the absence of EGF or TGF-α maintained normal morphology for at least 7 days. The duct shows an organised stratified keratinised squamous epithelium similar to that seen in tissue sections both on isolation and at the end of the culture period. (For an example of normal duct morphology see Figure 3 of Guy et al. British Journal of Dermatol. 1993 128, 242-248). However on the addition of either EGF or TGF-α (typically at 5ng/ml) to the culture medium, the normal duct morphology was lost within 4 days. The duct ruptured in a process resembling that which occurs in acne when the pilosebaceous duct ruptures. (See Figure 4 of Guy et al for an example of a ruptured duct.) Addition of an antagonist of EGF function (tyrphostin 46 or tyrphostin 47) prevented the rupture of the duct in the presence of EGF. Addition of the negative control (tyrphostin 1) did not prevent duct rupture and had no effect on cell toxicity. Furthermore addition of a neutralising antibody to EGF

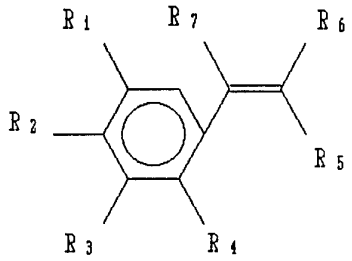
(e.g. at 50 micrograms/ml) in the presence of EGF also prevented the rupture of the duct. These data show that antagonists of EGF function, including antagonists that can inhibit receptor function or antagonists that can prevent ligand function, can prevent duct rupture in vitro in response to EGF.

The maintenance of the duct over 7 days described here is vastly superior to that reported by Guy et al (as above) using the different medium Williams E.

CLAIMS

1. Use of one or more antagonists of EGF, TGF-α or EGF-receptor function in the manufacture of a composition for the treatment of acne, spots and pimples.

2. Use according to claim 1 wherein the antagonist of EGF function comprises a compound of formula (I)



(I)

Where R_1, R_2, R_3 and R_4 are the same or different and are chosen from -H, -OH, $-C_nH_{2n+1}$, $-NO_2$, -Cl, -Br, -F and -CHO;
and R_5 and R_6 are the same or different and are chosen from -H, -CN, $-CO_2H$, $CONH_2$ and $CSNH_2$;
and where R_7 is chosen from -H and -OH;
and n is an integer from 1 to 8;
and mixtures thereof.

3. Use according to claim 1 wherein the antagonist of EGF, TGF-α or EGF-receptor function comprises an antibody or antibody fragment to EGF, TGF-α or the EGF receptor.

4. Use according to any one of claims 1 to 3 wherein the antagonist of EGF, TGF-α or EGF receptor function is present in an amount of from 0.0000001 to 10% by weight of the composition.

5. Use according to any one of claims 1 to 4 wherein the composition further comprises a cosmetically or physiologically acceptable vehicle.

6. A composition for topical application comprising an antibody to EGF, TGF-α or the EGF receptor.

7. A method of testing a substance for its ability to treat acne comprising the steps of:

(i) contacting the test substance with an isolated human sebaceous duct maintained in a keratinocyte serum-free medium, in the presence of EGF or TGF-α; and

(ii) assessing the response of the duct to the test substance.