METHOD FOR IDENTIFYING LIGANDS OF THE AhR RECEPTOR HAVING THERAPEUTIC SEBOSUPPRESSIVE ACTIVITY AND SAID LIGANDS

Field of the invention.

The present invention relates to a sorting method of substances for the purpose of determining their capacity for sebosuppressive activity in topical skin treatment. The invention also concerns substances identifiable by such sorting.

The present invention relates more particularly to a pharmaceutical composition for topical use, intended for the treatment and/or prevention of hyperseborrhea and thereby skin diseases that are associated, such as acne, seborrheic dermatitis and rosacea.

Description of the prior or art.

Numerous substances are able to interact as ligands with the AhR receptor (Aryl hydrocarbon Receptor) which is notably expressed by the epithelial and mesenchymal skin cells. Documents WO 2004/041758, WO 2007/060256 and WO 2007/128725 describe *in vitro* tests to determine the antagonist or agonist nature of such ligands and propose the use of *antagonist* ligands in dermatological treatments. The *in vitro* tests of this type which are most frequently used in the state of the art are the CALUX and EROD tests., Document WO 2009/093207 also discloses such an in vitro test .

Compounds which interact as agonist ligands with the AhR receptor, the prototype of which is 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD), are mostly xenotoxic compounds inducing various types of tissue lesions. For this reason, the therapeutic and/or preventive use of such *agonist*

compounds as active agents that modulate skin functions appears, in the state of the art to be excluded.

The applicant, on the contrary, has shown in document WO 2009/093207, the content of which is hereby incorporated by reference, that the application to the skin of certain *agonist* ligands of the AhR receptor may favourably modulate some skin functions such as the sebaceous gland function, anti-infection defence, wound healing, skin atrophy involving dermatoporosis and oestrogen deprivation. In that document, the Applicant showed that this property can be used to treat ill-functioning of these skin functions and how these ligands should be chosen so that they do not cause one of the main complications of excessive AhR activation by xeno-toxic ligands such as dioxin i.e. proliferative cystic lesions. These cystic lesions were formerly called “chloracne” and henceforth MADISH (J.H.Saurat *et al The cutaneous lesions of dioxin exposure: Lessons from the poisoning of V. Yushchenko,* Toxicological Sciences 2012; 125; 310-317). To prevent the application of therapeutic AhR ligands from leading to MADISH, agonists must be selected to have a half-life in the body of hours/days so that they are rapidly metabolised, contrary to prototype agonist, dioxin, whose half-life in human skin can be counted in years.

The ligands, according to document WO 2009/093207 are preferably chosen such that they meet four criteria:

1. An ability to activate the AhR receptor.
2. An ability to modulate a specific gene regulated by AhR.
3. A short half-life in the human organism, preferably of between 2 hours and 96 h, and more specifically between 6 and 24 hours.
4. A measurable positive effect on a recognized criterion of ill-functioning of these skin functions.

The ligands having agonist characteristics are numerous., Document WO 2009/093207 lists several such agonist ligands. Some of

them belong to so-called « natural ligand » AhR classes called NAhRAs (Natural AhR Agonists).

It is also unknown how to really identify the best candidates from these ligands that are likely to be endowed with a sebosuppressive effect that is therapeutically efficient in man. Current approaches entail atrophy-inducing tests of the differentiated regions of the sebaceous glands, in suitable animal species. These tests involve complex interpretation and to be sensitive they may require the prolonged application of the ligands under consideration over multiple weeks.

It is therefore the objective of the invention to propose a sorting method such as defined in the foregoing that is sensitive, rapid and offers high correlation between the sorting result and the sebosuppressive properties of the selected substances on human skin.

SUBJECT OF THE INVENTION

For this purpose, the invention proposes a method for sorting substances with a view to determining their capacity for sebosuppressive activity in topical skin treatments, comprising an *in vivo* test, the said *in vivo* test comprising the following steps:

- choosing a substance from among the ligands of the AhR receptor;
- choosing a mammal in which the CYP1A1 gene can be induced in the skin;
- treating part of the skin of the said mammal chosen in relation to the sebaceous glands thereof, via topical route, with a composition containing the said substance, following a dose/response/ response time protocol;
- examining, by immunohistological staining, the expression of CYP1A1 in the sebaceous glands of the said part of the skin of the said mammal;

- selecting the said substance in relation to the sequence of onset of immunohistochemical labelling in several different types (compartments) of cells of the said sebaceous glands.

It is possible in particular to choose the skin of the ears which are known to comprise sebaceous glands lending themselves well to analysis and in which the CYP1A1 gene is most likely to be induced.

The said substance is selected if the said *in vivo* test exhibits staining in a plurality of cell types within a determined time period;

The said substance to be tested is preferably chosen from among the ligands of the AhR receptor having an agonist nature on the basis of at least one *in vitro* test.

The choice of the best natural candidate from among the NAhRAs therefore initially entails the demonstration of sufficient AhR agonist effect on the basis of an *in vitro* test, for example using CALUX and EROD tests, and the demonstration of a short *in vivo* half-life.

According to one embodiment of the method of the invention, the said mammal is a mouse. It is possible to choose the C57/B6 mouse strain.

According to this embodiment, it is more particularly the ears of the said mice that are treated via topical route, then sampled, and the expression of CYP1A1 is examined by immunohistochemical analysis using an antibody; in particular, but not limited thereto, the antibody used may by the rabbit anti-rat CYP1A1 polyclonal antibody (Millipore AB1247).

In this embodiment, the examination of the expression of CYP1A1 in the sebaceous glands may comprise:

- examination of the isthmus region, in particular examination of the progenitor cells;
- examination of the peripheral region of the gland, in particular examination of the undifferentiated cells;
- examination of the intermediate region of the gland, in particular examination of the differentiated cells;

- examination of the central region of the gland, in particular examination of the mature cells.

After examining these four cell types, the said substance can be selected if the expression of CYP1A1 is labelled in at least two cell types after one week's treatment.

After examining these four cell types, the said substance is preferably selected if the expression of CYP1A1 is labelled in the 4 cell types after one week's treatment.

Therefore, according to a further aspect of the present invention, an object of the invention is a composition for treating and/or preventing skin diseases of a human being, in particular acne, seborrheic dermatitis and rosacea, wherein the composition is configured to treat and/or prevent hyperseborrhea by means of topical application of said composition on a skin, said composition comprising an active substance selected from the group consisting of AhR agonist ligands having:

-an ability to activate the AhR receptor;

-an ability to modulate a gene regulated by AhR;

-a short half-life in the human organism of between 2 hours and 96 hours;

-a measurable positive effect on a recognized criterion of hyperseborrhea, and

wherein said active substance is positively selected by an in vivo test as defined above..

The invention proposes in particular 5,6 benzoflavone (5,6 BZF) also called beta-naphthoflavone as a preferred active substance in a sebosuppressive composition for topical use.

The invention also proposes rutecarpine as active substance in a sebosuppressive composition for topical use.

According to a still further aspect, an object of the invention is a treatment method for treating and/or preventing skin diseases of a human being , such as acne, seborrheic dermatitis and rosacea, comprising providing a composition that is configured to treat and/or prevent hyperseborrhea by means of topical application of said composition on a skin, said composition comprising an active substance selected from the group consisting of AhR agonist ligands having:

-an ability to activate the AhR receptor;

-an ability to modulate a gene regulated by AhR;

-a short half-life in the human organism of between 2 hours and 96 hours;

-a measurable positive effect on a recognized criterion of hyperseborrhea, wherein said active substance is a substance positively selected by an in vivo test as defined above,

and administering topically said composition to said human being.

A particular object of the invention is thus a process for treating and/or preventing hyperseborrhea induced skin conditions in a human being, comprising providing a composition configured for topical application of said composition on a skin, wherein said composition contains 5,6 benzoflavone (5,6 BZF) as active substance, and administering topically said composition to said human being.

Persons skilled in the art, through the detailed description below of particular embodiments of the invention given with reference to the accompanying Figures, will understand how it was possible to determine a novel method for identifying ligands of the AhR receptor having therapeutic, topical sebosuppressive activity.

BRIEF DESCRIPTION OF THE FIGURES

- Figure 1 comprises parts 1A, 2A, 3A, 4A which are a schematic illustration of the location inside a sebaceous gland of four types of cells able to express the CYP1A1 gene, namely:
  - progenitor cells (1)
  - undifferentiated cells (2);
  - differentiated cells (3);
  - mature cells (4).
- Figure 2 comprises parts 1B, 2B, 3B, 4B which are microphotographic observations corresponding to drawings 1A, 2A, 3A, 4A; 1C shows a different viewing angle and magnification of region 1B i.e. of the progenitor cells;
- Figure 3 illustrates the onset of CYP1A1 activity as a function of time in all the sub-populations of sebaceous cells in Figures 1 and 2, after topical application of several ligands of the AhR receptor;
- Figure 4 is a table showing the correlations between *in vitro* tests, *in vivo* tests according to the invention, and clinical examination of the sebosuppressive activity in man of the ligands in Figure 3;
- Figure 5 is a table showing the correlations between *in vitro* tests, *in vivo* tests according to the invention, and clinical examination of the sebosuppressive activity in man of three flavones;
- Figure 6 is a comparative table of the inhibition of the transcription of genes of sebogenic enzymes by three flavones;
- Figure 7 is a comparative table of the relative surface occupied by the sebaceous glands in a dermis, subsequent to treatment with 5,6 BZF at three different concentrations,;
- Figure 8 is a comparative table of the differentiation index in the sebaceous glands subsequent to treatment with 5,6 BZF at the same concentrations as in Figure 7;
- Figure 9 is a comparative table of the number of differentiated and mature sebocytes subsequent to treatment with 5,6 BZF at the same concentrations as in Figure 7;

- Figure 10 is a comparative table of the number of active sebaceous glands subsequent to treatment with 5,6 BZF at the same concentrations as in Figure 7.

Description of an embodiment of the sorting method.

The method according to the invention for sorting ligand molecules of AhR, intended for sebosuppressive therapy in man, is based on the chance, unexpected observation made at the time of topical and systemic administering of several AhR ligands listed in WO 2009/093207.

So that persons skilled in the art may appreciate the most surprising nature of the present invention, the following elements of the state of the art must be recalled relating to the distribution of biological phenomena in the skin triggered by the application of a ligand for a nuclear receptor such as AhR, or, as a further example, the retinoic acid receptors (RAR), the vitamin D receptors (VDR).

According to the state of the art, the biological activity triggered by the topical or systemic administering of the ligand should be uniform:

- in all the body parts which express the receptor; and
- the ligand being diffused in these parts in sufficient quantitative manner and in non-metabolised active form.

In this case, the application of an AhR ligand to the skin should activate the pathway of this receptor uniformly first in the surface layers of the epidermis, then progressively diffusing as per the entry gradient of the ligand into the deeper layers of the epidermis, and then possibly the dermis and annexes, hairs and sebaceous glands. It is effectively known, and this has been confirmed by the applicant, that the AhR receptor is uniformly expressed in all these compartments of the skin.

Yet, the applicant has found in most surprising manner that the application to the skin of an AhR ligand activates the pathway of this receptor in focal manner on the sebaceous glands starting with the

progenitor cells i.e. the sebaceous stem cells located in the isthmic region of the pilosebaceous follicle, followed in sequence by the non-differentiated sebaceous cells, the differentiated sebaceous cells, and finally the mature cells. These observations are illustrated in Figures 1 and 2.

Further surprisingly, the ligands endowed with strong sebosuppressive activity are those which, without necessarily being the most active during *in vitro* activation tests of the AhR receptors, are those which follow this sequence of activation *in situ* at an early stage, rapidly and completely as shown by the Table in Figure 4 and by Figure 3.

These observations are effectively surprising since they differ from those of Rowe MJ *et al*. (*J. Invest. Derm. 2008:128; 1866-68*). These authors had shown that in a transgenic mouse (CYP1A1-GFP promoter) the biological activity induced by an AhR ligand administered via systemic route could be localized to the sebaceous glands, but in uniform manner within the latter:

- first, in the applicant's observations, the administration route is transcutaneous, and the priority distribution is fully unexpected on account of the reasons of transepidermal diffusion explained above. In the work conducted by Rowe MJ *et al.*), the ligand, 3-methyl cholantrene, is administered via systemic route which entails access to the skin via the blood route, which means that the distribution of a lipophilic ligand in the sebaceous gland is not surprising;

- secondly, and more especially, the activation sequence: progenitor > undifferentiated > differentiated > mature cells at the base of the present invention was not observed by Rowe MJ *et al*. (*J. Invest. Derm. 2008:128;1866-68*) who demonstrate diffuse biological activity in the region of the sebaceous glands. This aspect shown by Rowe *et al*. would not have allowed either the determination or the suggestion of the method for sorting sebosuppressive ligands subject of this invention.

Demonstration of the sequential focal regions of AhR-receptor activation in the sebaceous glands

The embodiment described here entails treating the ears of C57/B6 mice, via topical route following dose/response/response time protocols, with ligands of the AhR receptor previously characterised for their *in vitro* activation properties of the receptor e.g. using the CALUX and EROD methods which are widely used in this field (see Table 1).

The ears are sampled and CYP1A1 expression is examined by immunohistochemical analysis using a specific antibody. Figure 1 shows the different types of labelling observed. The positivity of immunohistochemistry i.e. the cells stained brown in Figure 2, indicates that the region expresses the CYP1A1 protein.

1. In the basal state the protein is not detectable.
2. In every case in which the AhR ligands used led to positivity of CYP1A1 immunohistochemistry, the first region in time to be stained, indicating an increase in the CYP1A1 protein induced by activation of the AhR receptor, is the region of the isthmus where the «progenitor» cells of the sebaceous glands are located (Fig. 1A and 1B): Stage 1. This corresponds to multipotent clonogenic cells, hence in general to the «sebaceous stem cells». In addition to the particular topography of the isthmus region, these cells are characterized by the expression of keratin 15 and L-RIG1 a marker of isthmic multipotent clonogenic cells. Using double CYP1A1 and L-RIG1 staining it was possible to assert that the cells activated by the AhR ligand, of topical origin, effectively correspond to this population at this isthmic region.
3. The following stage is the extension of staining to undifferentiated cells which do not contain lipids and in general are located on the periphery of the sebaceous gland (Fig. 2A and 2B): Stage 2.

4. The following stage is extension of staining to differentiated cells which contain lipids and are in general located at the intermediate part of the sebaceous gland (Fig. 3A and 3B): Stage 3.
5. The following stage is the extension of staining to mature cells which contain lipids and in general are located at the central part of the sebaceous gland (Fig. 4A and 4B): Stage 4.

Correspondence between the sequential activation stage and the sebosuppressive properties of the ligand.

The table in Fig. 4 indicates the correspondence between the stages of focal activation expression and the index of sebum inhibition, and the effect on human skin. The sebum inhibition index is calculated by counting the number of mature and differentiated cells in relation to the total number of cells in the sebaceous glands. A decrease in mature and differentiated cells indicates blocking of sebogenesis. The effect on human skin is determined by sebumetric examination using what is known as the « casual level » (*J. Cosmet Dermatol.* 2007 June;6(2):113-8).

In Figure 3 and in the Table 1 in Figure 4, the abbreviations used have the following meanings:

NSA1: rutecarpine
NSA2: beta naphtoflavone
NSA3: TCDD
NSA4: FICZ
NSA5: Hispidine
NSA6: beta carboline
NSA7: esomeprazole

A comparison between Figure 3 and Figure 4 (Table 1) shows that the two ligands, namely beta naphthoflavone, also called 5,6-benzoflavone or 5,6 BZF and TCDD, which activate the AhR pathway in the four cell sub-populations within a period of less than one week, are also those which display the strongest sebosuppressive activity in man. Rutecarpine which

activates the AhR pathway in two cell sub-populations has potentially useful sebosuppressive activity although weaker than that of 5,6 BZF. On the contrary, other ligands which only activate the AhR receptor in one cell sub-population and even if this activation reaches a high level, only exert poor sebosuppressive activity in man.

Description of the sebosuppressive properties of a 5,6 BZF and structural analogues

The sebosuppressive activity of 5,6 BZF was compared with that of 7,8 BZF (7,8-benzoflavone) and flavone under the same conditions, namely *in vitro* tests, *in vivo* tests according to the invention and clinical examination in man, as those described above. The results are given in Figure 5. It can be seen that 5,6 benzoflavone, and not 7,8 BZF or flavone, distributes its agonist activity in the sebaceous glands in similar manner to the most active AhR agonist (TCDD) whilst flavone and 7,8 benzoflavone do not have these effects. Without wishing to be bound by any theory, it would appear that this capacity for tissue distribution is not fully linked to the affinity of the molecule for the AhR receptor, since molecules having stronger affinity such as FICZ are not distributed in the sebaceous glands like 5,6 BZF. In combination with its other properties set forth below, this property of targeting the sebaceous glands is able to explain the sought-after therapeutic effect.

Figure 6 gives the results of experiments during which the ears of C57BL/6 mice were treated with the three above different flavones at 0.5 % for 5 weeks. The reference activity substance was TCDD at a concentration 1000 times smaller.

After RNA extraction, RTqPCR reaction was performed for three major enzymes in the production of sebaceous lipids. The results were compared with control mice treated with the vehicle: Figure 6 shows that among the 3 flavones solely 5,6 benzoflavone induces major inhibition of the expression of the mRNA encoding the three enzymes, whereas 7,8

benzoflavone rather more has a slightly stimulating effect and flavone has no significant effect. 5,6 benzoflavone and not 7,8 benzoflavone strongly inhibits the expression of the genes of key enzymes in the production of lipids characteristic of sebum such as AWAT 1, ELOVL3 and FADS2 in mice, which partly accounts for its sebosuppressive effect. These targets had not been disclosed in the prior art.

These differences in molecular structures lead to changes relating firstly to the receptor activating property and secondly to solubility properties. These latter properties can be determinant for targeting of the sebaceous glands as described in the foregoing.

RELATIONSHIP BETWEEN 5,6 BZF DOSE AND SEBOSUPPRESSIVE EFFECT

The doses of 5,6 benzoflavone to obtain significant suppression of sebogenesis must be defined in particular to ensure good tolerance and prevent the onset of cysts of MADISH type such as mentioned above, which could be dose-related and related to pharmacokinetics which themselves partly depend on dose within this context.

This essential step was approached in several phases.

1. Dose effect in mice

Preliminary studies showed good tolerance of concentrations higher than 0.05 %, and activity in the sequential *in vivo* activation test described above. For these dose-effects tests, C57BL/6 mice were treated for 3 to 5 weeks, 5 days per week on the ears and in three concentrations, namely 0.1 , 0.5 and 1 % of 5,6 BZF.

a. The sebosuppressive effect was analysed at the 3rd week although expression thereof starts after one week. Figures 7-10 show very marked effects which few substances other than toxics such as TCDD are able to induce in this model both regarding the relative surfaces occupied by the sebaceous glands and their activity, and regarding the ratios between non-differentiated, differentiated and mature sebocytes.

In particular, a reduction in the differentiated compartment of the gland was observed, which tallies well with the suppressive effect of the genes of sebaceous lipogenesis enzymes since the differentiation of the sebocyte is defined by cytoplasmic lipid overload.

b. Tolerance, in the absence of MADISH-type cyst production, was assessed after 5 weeks' treatment. No cystic lesion was observed at these high concentrations. The blood assay of 5,6 BZF by HPLC in these mice did not detect any measureable level (5nM sensitivity) which confirms the teaching of WO 2009/093207 concerning the use of agonists having a short life in the body.

2. Tolerance and effect in man:

a. A stable 0.5 % formulation of 5,6 BZF was defined.

i. Formulation: 5,6 BZF 0.5 g/100 ml in ethanol / PEG 400 (1:1).
Solvents: Ethanol EMSURE® Merck catalog number 1.00983, batch K42754183.
Polyethylene 400, Fluka catalog number 81170, batch 260154 286 or PEG 400, Aldrich catalog number 202398.

ii. Stability: no degradation products observed 6 months after preparation.

b. Use in man. The formulation was applied once per day to the face in eleven patients suffering from intense seborrhea and not eligible for oral treatment with Isotretinoin, six with acne, four with rosacea and one with seborrheic dermatitis.

i. No side effect was noted and in particular no clinical sign suggesting the onset of microcysts. This gives confirmation in man of observations made in animals. In this field of activation of the AhR receptor the sensitivity of species is of essential importance; even if the mouse strain chosen for the tests was the most sensitive to the topical effects of TCDD and 5,6 BZF. This tolerance in man therefore amounts to original data of primary importance. It

confirms the accuracy of the teaching in WO 2009/093207 concerning the use of short-life agonists in the body and gives an illustration of a precise application thereof.

ii. Seborrhea was markedly reduced with distinct appearance of the effect after 2- 3 weeks of application. Symptoms of acne, rosacea and seborrheic dermatitis were either significantly improved or cleared. Patients who had previously taken oral Isotretinoin at the intermediate dose of 0.4-0.5 mg/bw/day considered that they obtained a similar effect with the topical 5,6 BZF thus formulated with less irritation and discomfort.

iii. The use of the Sebutape® (CUDerm) patch test, to determine the amount of sebum produced in 6 individuals after treatment, indicated a casual level corresponding to normal seborrhea range.

The topical pharmaceutical composition of the invention may have a concentration of 5,6 benzoflavone (5,6 BZF) of between 0.005 % and 1 % by weight and more particularly between 0.1 % and 1 % by weight of the said composition.

Conclusion: In addition to novel information on the physiopathology of the sebaceous gland that is involved, the invention allows rapid sorting of candidate sebosuppressive molecules for therapeutic use.

Persons skilled in the art will easily appreciate that the method of the invention could be implemented, without departing from the scope of the invention, in another laboratory mammal other than the C57/B6 mouse strain, provided the described activation sequence is reproduced.

The discrepancy between the *in vitro* data which represent receptor activation power and the *in vivo* effect probably reflects intra-tissular kinetic elements particular to each molecule. The method subject of this invention

is therefore the first ever described which allows investigation of the specific targeting of the sebaceous cells within skin tissue.

The ligands able to induce a significant sebosuppressive effect at clinical level are those which, as early as the first week of treatment, exceeded Stage 2. The most active reached Stage 4 as early as the first week of treatment.

During the above-described tests, the 5,6 benzoflavone selected after the *in vivo* test according to the invention confirmed its capacity for use in sebosuppressive treatment as a topical application in the human being

CLAIMS

1. A method for sorting substances for the purpose of determining their capacity for sebosuppressive activity in topical skin treatment, characterized in that the said method comprises an *in vivo* test, the said *in vivo* test comprising the following steps:

- choosing a substance from among the ligands of the AhR receptor;
- choosing a mammal expressing the CYP1A1 gene;
- treating via topical route part of the skin of the said mammal comprising sebaceous glands, with a composition containing the said substance following a dose/response/response time protocol;
- examining by immunohistochemical staining the expression of CYP1A1 in sebaceous glands of the said part of the skin of the said mammal;
- selecting the said substance as a function of the sequence of onset of immunohistochemical staining in several different types of cells of the said sebaceous glands.

2. The method according to claim 1, characterized in that the said substance is selected if the said *in vivo* test exhibits staining in a plurality of cell types within a determined time interval.

3. The method according to claim 1 or 2, characterized in that the said substance is chosen from among the ligands of the AhR receptor having an agonist nature on the basis of at least one *in vitro* test.

4. The method according to claim 3, characterized in that the said *in vitro* test is chosen from among the CALUX, EROD tests and combinations thereof.

5. The method according to one of the preceding claims, characterized in that the said mammal is a mouse strain chosen so that the sebaceous glands allow reproduction of the activation sequence:

- progenitor cells
- undifferentiated cells
- differentiated cells
- mature cells.

6. The method according to one of the preceding claims, characterized in that the said mammal is a C57/B6 mouse strain;

7. The method according to claim 5 or 6, characterized in that the ears of said mice are treated via topical route then sampled and CYP1A1 expression is examined by immunohistochemical examination using an antibody.

8. The method according to one of the preceding claims, characterized in that the examination of CYP1A1 expression in the sebaceous glands comprises:

- examination of the isthmus region, in particular examination of the progenitor cells;
- examination of the peripheral region of the gland, in particular examination of the undifferentiated cells;
- examination of the intermediate region of the gland, in particular examination of the differentiated cells;
- examination of central region of the gland, in particular examination of the mature cells.

9. The method according to claim 7, characterized in that the said substance is selected if the expression of CYP1A1 is labelled in more than two types of cells after one week's treatment.

10. The method according to claim 7, characterized in that the said substance is selected if the expression of CYP1A1 is labelled in 4 types of cells after one week's treatment.

11. A composition for treating and/or preventing skin diseases of a human being, in particular acne, seborrheic dermatitis and rosacea, wherein the composition is configured to treat and/or prevent hyperseborrhea by means of topical application of said composition on a skin, said composition comprising an active substance selected from the group consisting of AhR agonist ligands having:

-an ability to activate the AhR receptor;

-an ability to modulate a gene regulated by AhR;

-a short half-life in the human organism of between 2 hours and 96 hours;

-a measurable positive effect on a recognized criterion of hyperseborrhea, and

wherein said active substance is positively selected by an in vivo test as claimed in any one of claims 1-10.

12. A pharmaceutical composition for topical use intended to reduce sebogenesis, characterized in that it contains 5,6 benzoflavone (5,6 BZF) as active substance.

13. A pharmaceutical composition for treating and/or preventing human skin diseases, in particular acne, seborrheic dermatitis and rosacea, wherein the composition is configured to treat and/or prevent hyperseborrhea by means of topical application of said composition on a skin, said composition comprising 5,6 benzoflavone (5,6 BZF) as active substance.

14. The pharmaceutical composition according to claim 12 or 13, characterized in that the concentration of 5,6 benzoflavone (5,6 BZF) is

between 0.005 % and 1 % by weight, and more particularly between 0.1 % and 1 % by weight of the said composition.

15. 5,6 benzoflavone (5,6 BZF) as active substance in a sebosuppressive composition for topical use.

16. Rutecarpine as active substance in a sebosuppressive composition for topical use.

17. A treatment method for treating and/or preventing skin diseases of a human being , such as acne, seborrheic dermatitis and rosacea, comprising providing a composition that is configured to treat and/or prevent hyperseborrhea by means of topical application of said composition on a skin, said composition comprising an active substance selected from the group consisting of AhR agonist ligands having:
-an ability to activate the AhR receptor;
-an ability to modulate a gene regulated by AhR;
-a short half-life in the human organism of between 2 hours and 96 hours;
-a measurable positive effect on a recognized criterion of hyperseborrhea,
wherein said active substance is a substance positively selected by an in vivo test as claimed in any one of claims 1-10,
and administering topically said composition to said human being.

18. A process for treating and/or preventing hyperseborrhea in a human being, comprising providing a composition configured for topical application of said composition on a skin, wherein said composition contains 5,6 benzoflavone (5,6 BZF) as active substance, and administering topically said composition to said human being.

19. A process as claimed in claim 18, wherein the concentration of 5,6 benzoflavone (5,6 BZF) is between 0.005 % and 1 % by weight, and more particularly between 0.1 % and 1 % by weight of the said composition.

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