Technical Report

Product

CHROMABRIGHT®

synthetic molecule

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Lightening as an ancient request

The desire for a lighter skin tone is not new. In ancient civilisations, it was a common wish as a pale complexion was associated with aristocratic lineage and wealth. Pearl powder, natural pure hydroquinone, lead and mercury are some examples of compounds that were applied on the face, neck and chest many decades ago to make skin tone lighter.

Even if the first whiteners were highly toxic and certainly damaging, the concern for getting a lighter skin remained until today. Presenting a **light and homogeneous skin tone** is still in trend, as it is normally **related to health and youth** even in completely different cultures.

In Asia for example, a **fair skin tone** is considered a **symbol of beauty and femininity** inherited from ancient times. Thus, getting an overall lighter tone is a widespread objective in Asian skin care.

In Western countries, increasing cutaneous **evenness** is a common goal to improve skin appearance and beauty, as well as **reducing undesired pigmented areas** and protecting from the appearance of new ones [1].

Generally speaking, presenting spots, freckles (irregular grouping of pigment-containing cells), melasma (local hyperpigmentation), post-inflammatory hyperpigmentation and/or lentigines (dark spots appearing when getting older and in sun-exposed skin) is not gladly accepted. Some of these pigmented alterations are clear aging signs (lentigines for example) while others are just not aesthetic.

Summarising, it can be said that there is a **shared wish** for **homogenising the skin tone**, getting a **brighter skin without undesired pigmented areas**.

There is a market demand and need for skin whitening agents, with proven efficacy and improved safety profiles.

Melanin as the key to pigmentation

The **color of the skin** and hair is mainly determined by the amount, distribution and type of a specific natural pigment, known as **melanin**. Melanins are pigmented biopolymers synthesised by the dendritic melanocytes in the dermo-epidermal junction that terminate in the superficial layer of the skin, known as Stratum Corneum (SC).

Melanin synthesis starts within **melanocyte** cells with the amino acid tyrosine as the raw material. Actually, melanin pigments are assembled in specialised membrane-bound organelles called **melanosomes**, whose multi-step maturation process is under the responsibility of melanocytes at the basal layer of the epidermis [2].

Melanosomes start as spherical vesicles and their early maturation process involves melanocyte stimulating hormone and its receptor (MC1R) among others. In the later steps of maturation, crucial enzymes are processed and transported into the organelle, including Tyrosinase (TYR) [3-4]. All these enzymes work together to produce two distinct types of pigments: **pheomelanin** (red/yellow) and **eumelanin** (black/brown, more photoprotective), collectively known as **melanin pigments**. While melanosomes are maturing, melanocytes develop finger-like projections (**dendrites**) in order to prepare for the later **pigment transfer**.

The mature organelles migrate towards the extremities of the melanocyte dendrites where melanins are transferred to the neighboring keratinocytes of the SC, and in hair bulbs to the hair shaft, where the final distribution patterns of the pigment are determined [5].

The **balance between pheomelanin and eumelanin** (MC1R controls the switch between these two pigments) **determines skin and hair color.** However, the resulting skin pigmentation is cleared by the degradation that such pigments suffer during their way to the SC [6-8]. The remaining melanin pigments are posteriorly shed with desquamation.

On this basis, it can be said that the first and **rate-limiting step of melanogenesis** is mediated by **tyrosinase**. This enzyme catalyses the hydroxylation of tyrosine into 3,4-Dihydroxyphenylalanine (DOPA) and the subsequent oxidation from DOPA to DOPAquinone, which is the precursor of melanin [5].

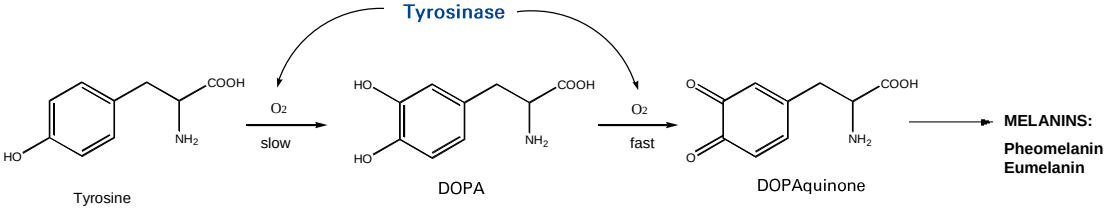

Fig. 1. Melanin synthesis summary.

Skin color results from a multi-step process where melanin is synthesised and transferred to the SC, tyrosinase playing a key role.

Desired features for brightening molecules

Presenting an **even and bright skin is a global objective**, so the potential brighteners need to accomplish several features to be successful among customers. Obviously, their **first one would be brightening and unifying the skin tone** meeting the market demand but the key is how to achieve it.

Intrinsic aging and photoaging (induced by UV exposure) imply the abnormal accumulation of melanin, which results in visible **hyperpigmentation areas** (melasma, freckles, spots…) that normally causes distress [9]. Acute or persistent UV exposure can exacerbate the accumulation of the already present melanin pigments, leading to additional hyperpigmentation [6].

Tyrosinase inhibition is the most common approach to **skin hypopigmentation** as this key enzyme catalises the rate-limiting step of pigmentation. It is agreed that the *in vitro* mushroom tyrosinase inhibition assay is a basic step to assess the direct effect of a potential skin lightener on tyrosinase activity [3]. However, the use of mammalian-derived tyrosinase is thought to be more appropriate as DOPA oxidation activities of human and mushroom tyrosinases show very different inhibitory effects depending upon the nature of the lightening agents [1]. Additionally, the optimum pH and temperature, IC_{50} values, and kinetic parameters are dissimilar depending on the tyrosinase type. Therefore, it is important to use **human tyrosinase** for the screening and the evaluation of skin brightening agents [9].

Despite the large number of molecules showing lightening properties *in vitro*, only some of them are able to induce an effective result measurable in clinical trials. This gap between *in vitro* and *in vivo* studies suggests that a different approach is needed to discover other depigmenting agents and validate the efficacy. In addition, many inhibitors in cell-free enzymatic assays are likely to have **toxicity or delivery problems** in cell-based assays [3]. Actually, some current brighteners from the cosmetic market show adverse effects such as high irritant and sensitising potential, cytotoxicity and/or instability when formulated.

That is why depigmenting agents should present specific features that answer the market demand. Such list of characteristics would include an **inhibitory effect of mammalian tyrosinase**, **lack of toxicologic** and mutagenic potential, **clinical efficacy**, **formulation stability** and novelty and patent protection of the agent and/or formulation [6].

Depigmentation products need to be effective in reducing melanin accumulation without presenting toxicity.

CROMABRIGHT® *synthetic molecule*, at the cutting-edge for a radiant skin

CHROMABRIGHT® *synthetic molecule* is a patented ingredient designed for skin brightening applications that fulfills all of the desired characteristics for a safe lightening agent.

In vitro, it demonstrated to noticeably **inhibit mushroom and human tyrosinase**, key enzyme for melanin production. In addition, its *in vitro* efficacy in **reducing melanogenesis** proved to be dose-dependent and noticeably higher than other well-known lightening agents like arbutin, kojic acid and magnesium ascorbyl phosphate. Besides, its brightening activity showed to be similar to hydroquinone at the same concentration, but presenting no cytotoxicity.

Furthermore, it provided a **photoprotective** effect on human epidermal keratinocytes, helping to prevent the UV-induced skin damage, like melanin naturally does.

Low concentrations of this ingredient demonstrated to significantly **brighten** the skin in a panel of volunteers. In another *in vivo* study, it **reduced** the **undesired hyperpigmentation** (melasma and lentigines) after 30 days, with even more visible results after 60 days.

Unlike other brightening agents that may induce skin irritation and alterations, this synthetic molecule presents a **complete safety profile** studied in melanocytes, keratinocytes and fibroblasts. It also proved to be stable in different types of formulations and detectable at very low concentrations.

CHROMABRIGHT® *synthetic molecule* is a completely safe skin brightening active for cosmetic applications.

In vitro efficacy

MUSHROOM TYROSINASE INHIBITION

The aim of this study was to evaluate the inhibitory effect of CHROMABRIGHT® *synthetic molecule* on mushroom tyrosinase activity, as a direct assessment of potential whiteners.

Kojic acid (0.1 mM, positive control) or CHROMABRIGHT® *synthetic molecule* (1 mM) were pre-incubated with L-DOPA (10 mM, acting as a substrate). Afterwards, synthetic mushroom tyrosinase was added and the samples were incubated protected from light. Reaction was quenched by cooling at 4 °C and absorption was measured at 470 nm.

The controls included non-treated wells (without any test item) and the wells treated with kojic acid.

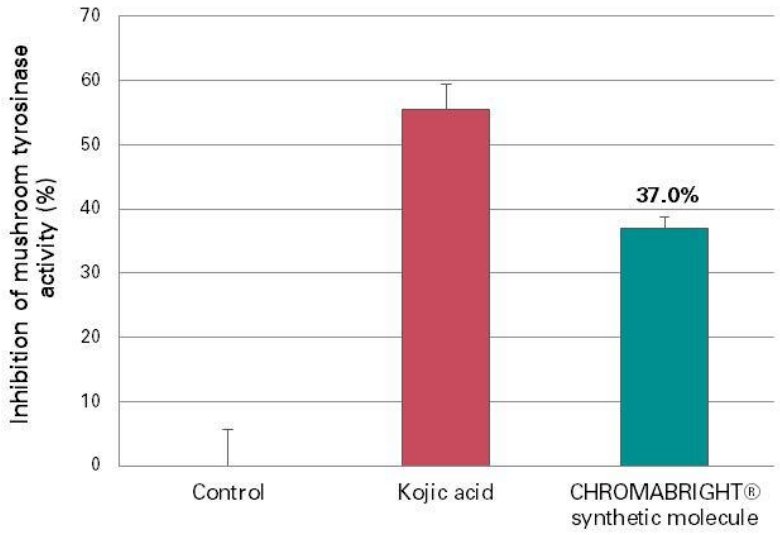

Fig. 2. Reduction of mushroom tyrosinase activity.

Results showed that the tested molecule **reduced mushroom tyrosinase activity** by **37.0%** at the tested concentration, which is linked to a melanin decrease.

CHROMABRIGHT® *synthetic molecule* decreases mushroom tyrosinase activity.

HUMAN TYROSINASE INHIBITION

The inhibitory effect of CHROMABRIGHT® *synthetic molecule* on human tyrosinase activity was evaluated, as it is related with melanin formation and pigmentation.

A human tyrosinase kit was used to measure the conversion of L-Tyrosine into a dopachrome complex. First, the reaction mix was incorporated to the wells and, then, kojic acid (1.12 mM, positive control) or CHROMABRIGHT® *synthetic molecule* (0.1 mM or 0.25 mM) were added. After loading the tyrosinase enzyme, the absorbance was read at 490 nm in a microtiter plate reader.

Wells without the enzyme were used as negative control.

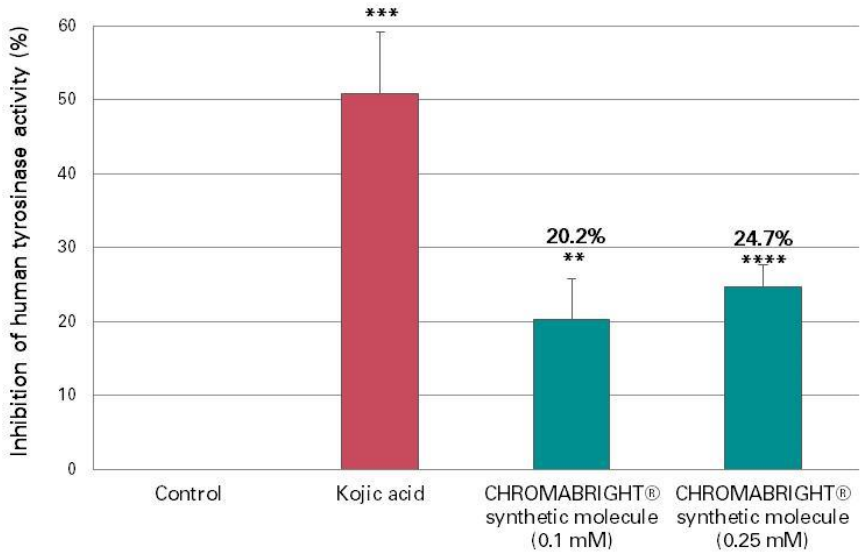

Fig. 3. Diminution of human tyrosinase activity (**p<0.01, ***p<0.001, ****p<0.0001).

The synthetic molecule **diminished human tyrosinase** activity, in a statistically significant manner, up to **24.7%**, which implies a melanin decrease.

CHROMABRIGHT® *synthetic molecule* reduces human tyrosinase activity.

MELANOGENESIS REDUCTION IN MELANOCYTES

Primary human melanocytes were used to compare the efficacy of CHROMABRIGHT® *synthetic molecule* in inhibiting melanogenesis with several recognised whiteners.

Cells were seeded and, then, the culture medium was changed by the medium with the selected test item (day zero). The treatment was repeated on days 3, 6, 8, 10, 13, 15 and 17. The test products were hydroquinone, arbutin, Magnesium Ascorbyl Phosphate (MAP), kojic acid (10 µM all) and CHROMABRIGHT® *synthetic molecule* (5, 10, 100, 150 or 200 µM). The medium without any test item was used as control.

After 3 days from the last treatment, the absorbance was measured at 450 nm in a plate reader and melanin concentration was determined from a standard curve plotted with synthetic melanin at known concentrations.

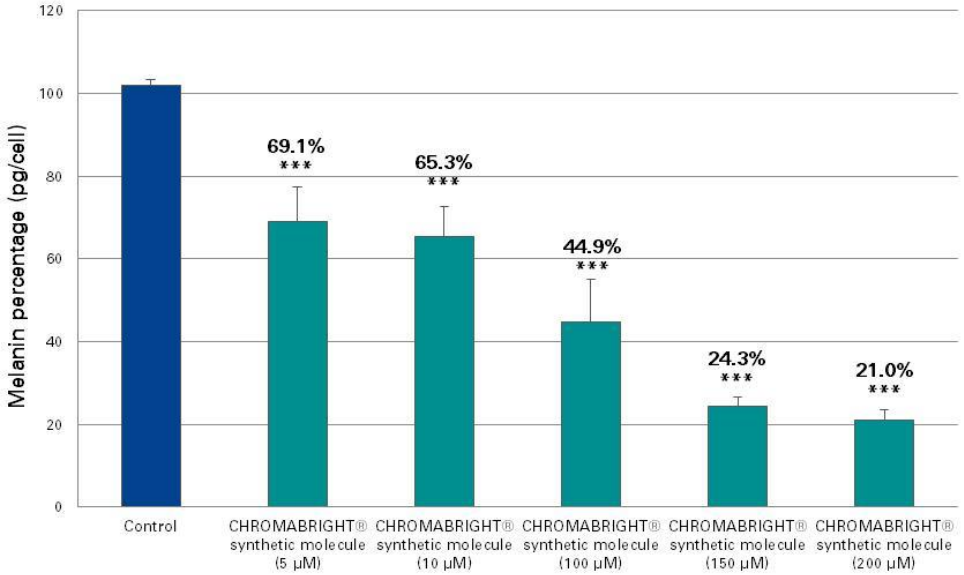

Fig. 4. Percentage of melanin after the different treatments (***p<0.01).

As the image shows, the active molecule **decreased** melanin content in a **dose dependent-manner**. All results were statistically significant.

CHROMABRIGHT® *synthetic molecule* diminishes melanin content.

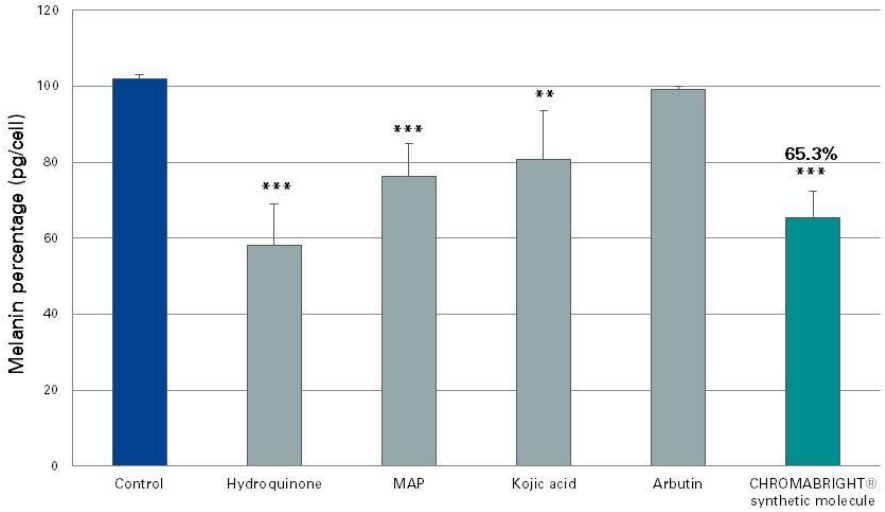

Fig. 5. Percentage of melanin after the different treatments (**p<0.05, ***p<0.01).

The results demonstrated that the tested synthetic molecule was **similar to hydroquinone** in **reducing melanin**, while the other products were less effective at the same concentration (10 µM).

CHROMABRIGHT® *synthetic molecule* offered similar efficacy to hydroquinone.

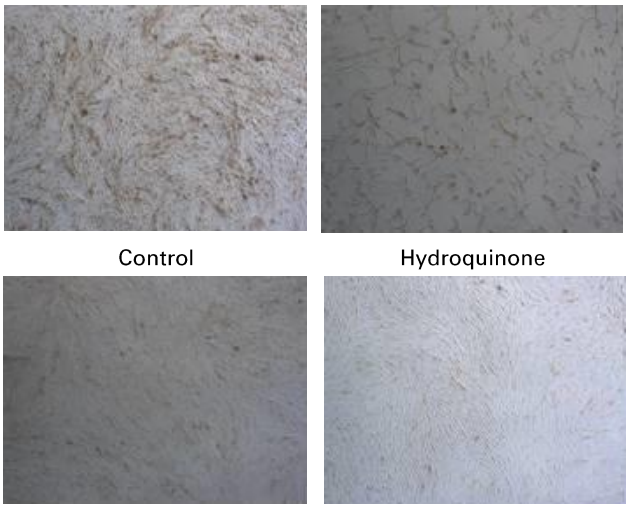

Fig. 6. Human melanocytes at the end of the different treatments.

The microscopic images confirmed the depigmenting efficacy of the synthetic molecule (**less pigment**) and that it **did not affect cellular growth**.

CHROMABRIGHT® *synthetic molecule* presented lower toxicity compared to hydroquinone.

PHOTOPROTECTION ON KERATINOCYTES

This assay was developed to determine the photoprotective effect of CHROMABRIGHT® *synthetic molecule* on Human Epidermal Keratinocytes from adult (HEKa) exposed to a cytotoxic dose of simulated solar light.

HEKa were pre-incubated with PBS (control) or CHROMABRIGHT® *synthetic molecule* (47 or 150 µg/mL) in the dark. Afterwards, cells were exposed to the irradiation dose (37 J/cm^2) during 150 min. A different plate was kept in the dark during the same exposure period.

Then, the treatment medium was replaced by culture medium. After 24 h of incubation, cell viability was determined by the Neutral Red (NR) uptake method. NR is a dye that accumulates inside the cells but alterations on cell surface decrease its uptake. Optical density was measured at 540 nm in a spectrophotometer.

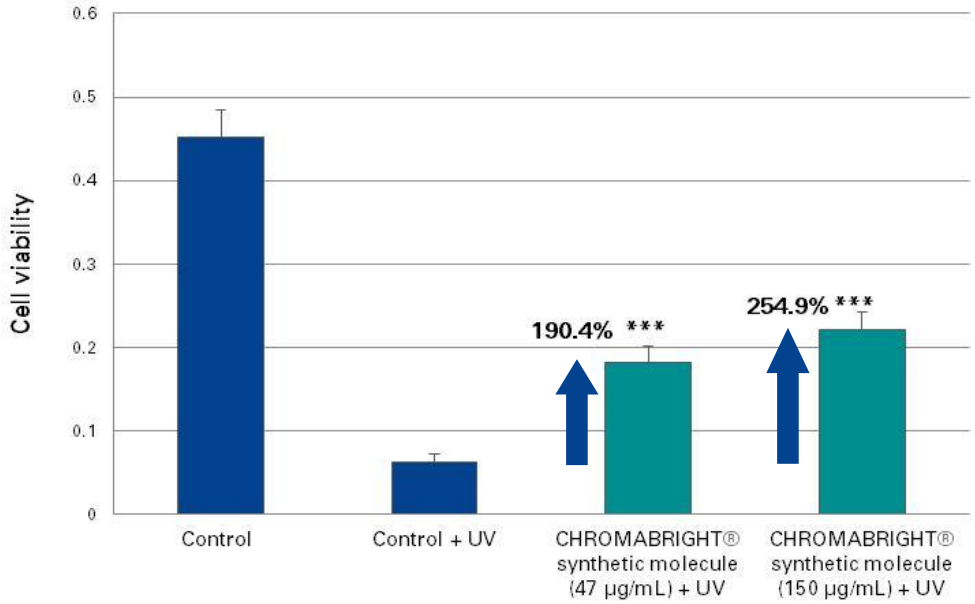

Fig. 7. Cell viability of HEKa after different treatments (***p<0.01).

Results showed that the molecule highly **increased viability** of cells exposed to toxic UV (up to 254.9%). Both values were statistically significant.

CHROMABRIGHT® *synthetic molecule* raises cellular survival after UV exposure.

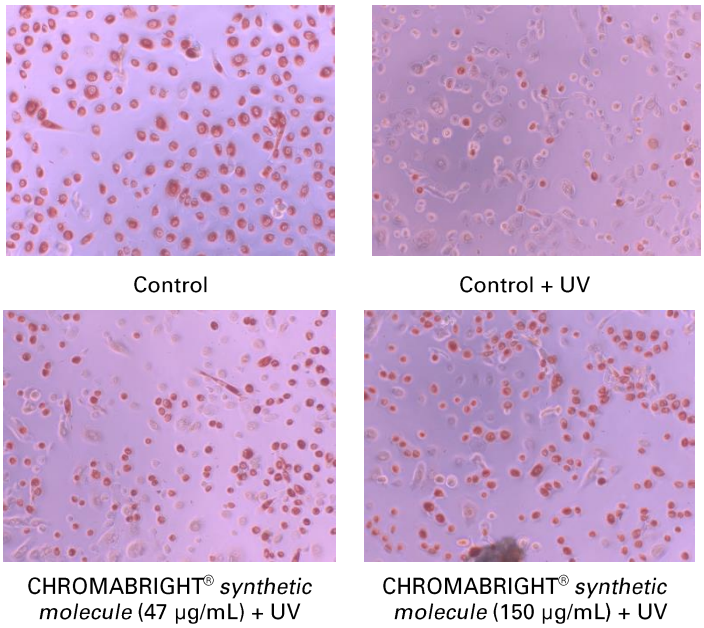

Fig. 8. Images of HEKa cultures after different treatments.

As the pictures indicated, the active molecule **reduced cell damage** (membrane) compared to control, allowing higher NR accumulation (more colorful dots).

CHROMABRIGHT® *synthetic molecule* protects from UV-induced damage.

In vivo efficacy

BRIGHTENING EFFECT

The main goal of this study was to evaluate the *in vivo* brightening efficacy of CHROMABRIGHT® *synthetic molecule* using chromametry (measurement of the color of the skin with a chromameter).

Twenty Asian female volunteers between 18-46 years old applied a placebo cream on half of the face and a cream with 0.1% CHROMABRIGHT® *synthetic molecule* on the other half, twice a day for 60 days.

The instrumental evaluation of skin colorimetry was carried out at the initial time and after 30 and 60 days on the right and left cheekbones (sites defined to be reproducible each time). The following parameters were used to evaluate the brightening effect: L* which is luminance (represents the relative brightness from total darkness, L*=0, to absolute white, L*=100), a* (red-green color axis), and b* (yellow-blue color axis). The best description of a brightening effect is given by combining the L* and b* parameters, in the Individual Typological Angle (ITA°). The higher the L* and ITA° parameters are, the lighter the skin will be.

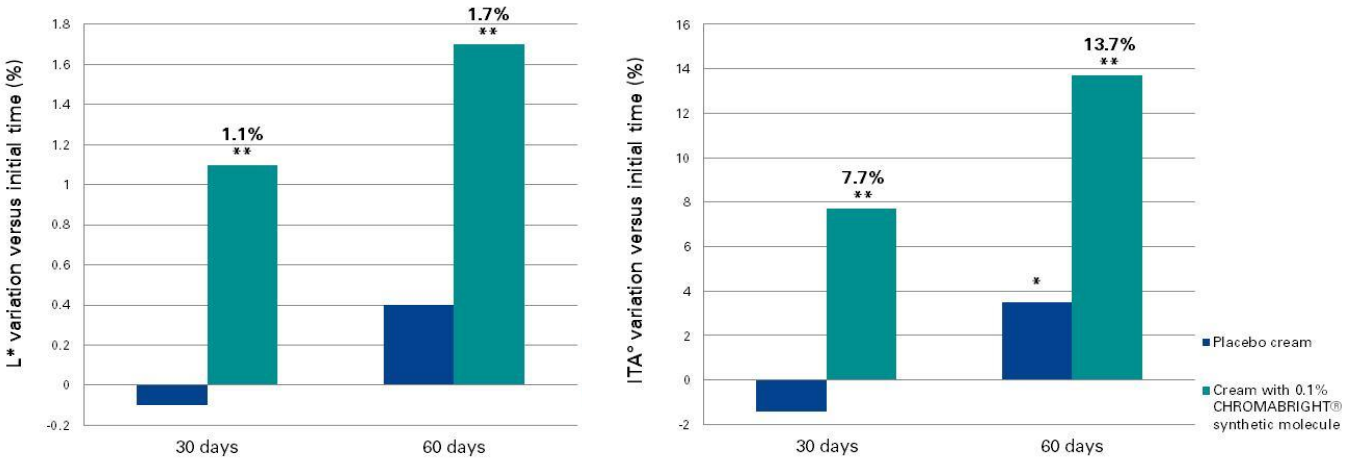

Fig. 9. Evolution of L* and ITA° parameters versus the initial time (*p<0.05, **p<0.01).

The active ingredient **increased L* and ITA° parameters after 30 and 60 days**, which implied a skin **brightening effect**. Apart from being highly superior to the placebo, all the effects of the active molecule were statistically significant.

CHROMABRIGHT® *synthetic molecule* induces a skin brightening effect.

DEPIGMENTATION EFFICACY

The aim of this assay was to evaluate the *in vivo* efficacy of CHROMABRIGHT® *synthetic molecule* in volunteers presenting hyperpigmentation in concrete zones.

Ten volunteers (between 23-70 years old) presenting melasma and/or actinic lentigines were chosen to apply a cream with 0.5% CHROMABRIGHT® *synthetic molecule* to their face and/or hands twice a day for 60 days. Five volunteers presented melasma and nine presented actinic lentigines.

Clinical and iconographic controls were carried out at the beginning of the study and after 30 and 60 days. A dermatologist scored the percentage of improvement of melasma and/or lentigines according to a scale going from score zero (0% improvement) to score 4 (76-100% improvement).

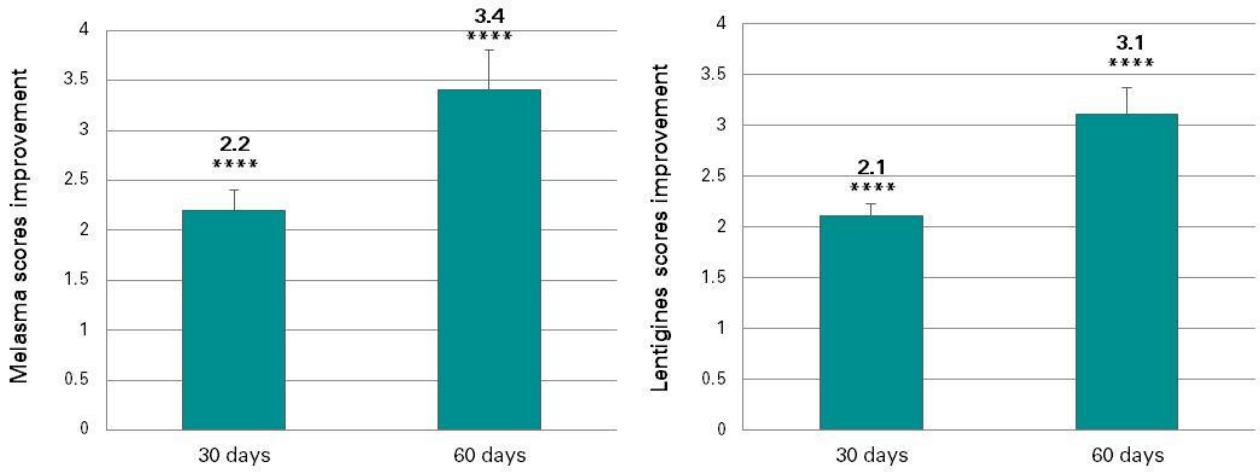

Fig. 10. Improvements after 30 and 60 days applying a cream with the active molecule (****p<0.0001).

The treatment **ameliorated** the appearance of **melasma and lentigines after 30 and 60 days**, reducing both by **26-50%** after 30 days and by **51-75%** after 60 days.

CHROMABRIGHT® *synthetic molecule* evens skin tone.

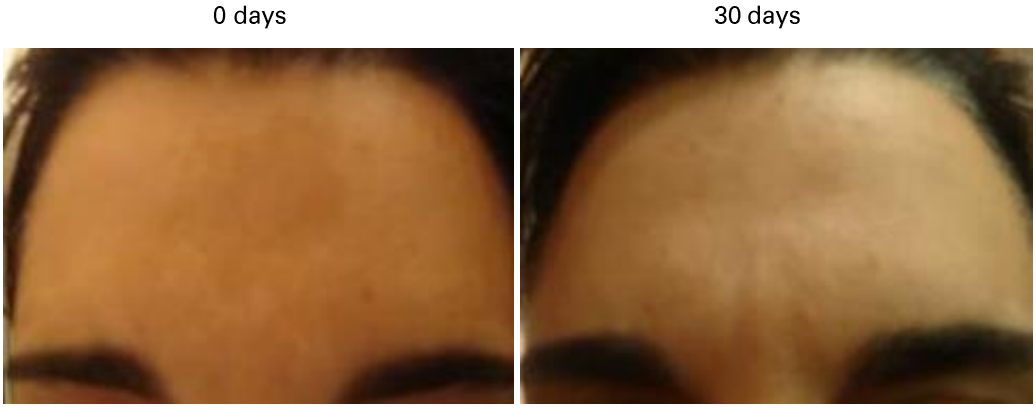

Fig. 11. Real images of a volunteer before and after 30 days.

Fig. 12. Real images of another volunteer before and after the whole treatment.

The pictures showed the **observable improvement induced** by the active treatment in volunteers with melasma and lentigines after **30 and 60 days**.

CHROMABRIGHT® *synthetic molecule* visibly decreases melasma and lentigines.

Cosmetic properties

CHROMABRIGHT® *synthetic molecule:*

- patented ingredient to lighten and even the skin tone, and reduce undesired hyperpigmentation.
- **decreased mushroom tyrosinase activity** by **37.0%**, known enzyme linked to melanin formation.
- **inhibits the activity of human tyrosinase**, directly related to melanin synthesis. It decreased it up to **24.7%** (statistically significant).
- **diminished melanin content** in a **dose-dependent manner** (up to **21.0%** in melanocytes), being statistically significant at all tested concentrations. Compared to other well-known brighteners, this active molecule provided, more **safely**, the **same** level of **efficacy** as **hydroquinone**.
- offered a statistically significant **photoprotection**, **increasing cellular viability** up to 254.9% after UV exposure, and therefore offering protection from its induced damage.
- **increased** the **L* and ITA°** parameters *in vivo*, which are associated with a **brightening effect**. Apart from being superior to the placebo, its effects in Asian volunteers after 30 and 60 days were statistically significant (0.1% *synthetic molecule)*.
- **visibly improves** the appearance of **melasma and lentigines.** It has been proven to reduce both of these conditions by 26-50% after 30 days and by 51-75% after 60 days (0.5% *synthetic molecule)*.

Cosmetic applications

CHROMABRIGHT® *synthetic molecule* is an ideal ingredient to incorporate into facial, body and hands formulations designed to **brighten and even the skin tone** due to its demonstrated effects on reducing undesired spots.

It can be used in anti-aging products and/or treatments to diminish hyperpigmentation, as well as in daily care products to increase photoprotection and avoid the appearance of undesired pigmented areas.

Technical data

INCI NAME OF THE ACTIVE INGREDIENT

Active ingredient	INCI name
CHROMABRIGHT® *synthetic molecule*	Dimethylmethoxy Chromanyl Palmitate

PRESENTATION AND PRESERVATIVE

100% Powder.

Code	Product presentation	Preservative
ES291	CHROMABRIGHT® *synthetic molecule*	Preservative free

Application data

PROCESSING

CHROMABRIGHT® *synthetic molecule* can be incorporated into emulsions, oily sera and formulations containing oil (2% minimum) or silicon phases, in the oily phase. In case of emulsions, it is recommended to add it at 70 ºC in the oily phase, in which it may remain stable for 2 h at 100 ºC. Please ask for more details if needed.

Recommended pH range between 3.8 and 8.4 for CHROMABRIGHT® *synthetic molecule*.

INCOMPATIBILITIES

Not expected.

SOLUBILITY

Insoluble in water and glycols. Soluble in oils and certain solvents (see the table below, although these solubility values may change when other ingredients or solvents are added into the formulation).

INCI name of the solvents	Solubility at room temperature
Ethylhexyl Cocoate	26.89%
C-12-15 Alkyl Benzoate	34.61%
Soybean (Glycine Soja) Oil	19.80%
Caprylic/Capric Trigliceride	24.35%
Ethylhexyl Methoxycinnamate	35.88%
Mineral (Paraffinum Liquidum) Oil	13.52%
Isohexadecane	15.66%
Peg-7 Glyceryl Cocoate	9.27%
Cyclopentasiloxane	1.27%
Ethanol	6.39%

DOSAGE

A dosage of 0.1-0.5% of CHROMABRIGHT® *synthetic molecule* is recommended in final cosmetic formulations.

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