NEW DYE FOR FLUORESCENT DEPIGMENTING OF STRATUM CORNEUM OF EPIDERMIS

Product description

New fluorescent dye from the *Safirinium-Q* family is an organic chemical compound for visualization of biological samples, in particular the stratum corneum of the epidermis, dedicated to the biomedical, pharmaceutical, and cosmetic industries.
The compound is useful in fluorescent and confocal microscopy, providing unique information related, inter alia, to:

- ✓ identification of building structures of the stratum corneum of epidermis i.e. corneocytes and lipid matrix surrounding them;
- ✓ evaluation of the capacity of stratum corneum of epidermis' penetration by xenobiotics, including therapeutic substances and their penetration into the deeper layers of the skin (*epidermis and dermis*);
- ✓ identification of ways of transport of substances to the skin, including through transfollicular through hair follicle, sebaceous gland and sweat gland and transepidermal, perpendicular to the corneocyte cells and the lipid matrix, as well as through the lipid matrix and canyons surrounding the corneocytes groups (so called clusters);
- ✓ knowing the mechanisms of loss of skin barrier features under the action of different substances;
- ✓ the study of interaction between the substances with the building components of the skin;
- ✓ assessment of the qualitative condition of hair (dyeing of keratin protein);
- ✓ diagnostics of skin diseases;
- ✓ comparative analysis of disorder or improvement of the structure of stratum corneum of epidermis under the influence of medicinal or beauty formulations applied on the skin.

The new dye is characterized by the amphiphilic properties with a predominance of lipophilic properties. The nature of the compound, causes the dye to have a high affinity on the one hand to the lipophilic and hydrophobic and hydrophilic structures, and on the other, to lyofophobic and hydrophilic ones, thus it is characterized by a wider range of applications in the examination of biological samples in comparison to fluorescent dyes available currently on the market.

Fluorescent dyes from the *Safirinium-P* and *Safirinium-Q* families have been already applied particularly in the biomedical industry for marking of bacterial spores, and in studies of protein hydrolysates. The technology of their production was disclosed in the patent description PL223740 B and it is the subject of technological offer of the Medical University of Gdańsk. A modification targeted within the structure of *Safirinium* chromophore and optimization of chemical synthesis have led to developing the innovative and economical manufacturing method of fluorescent dye based on the fluorogenic reaction, by the use of the isoxazole-structured substrates.

Key words

fluorescent dyes, derivatives of *Safirinium-Q*, chemical synthesis, visualization of cell structures, stratum corneum of the epidermis, fluorescence microscopy, fluorescent confocal microscopy

Legal status of product

European Patent Office:
European patent EP 3 578 559 A1 – entity solely entitled to the invention – Medical University of Gdańsk

Subject of the offer

The subject of the offer is a new, universal fluorescent dye from *Safirinium-Q* family and dye production technology dedicated to applying in fluorescence microscopy and confocal microscopy to visualize stratum corneum of the epidermis, evaluate structural disorders of this layer, identify ways of penetration of lipophilic and hydrophilic substances into the skin.

Previous funding of research on the product

The project was financed partially with funds from the grant received in the framework of the programme of the Ministry of Science and Higher education under the supervision of the „Iuventus Plus" IP 2012 055472.

Analysis of market competition

Fluorescent dyes' market grows continuously with a tempo of several percentage points on a year-by-year basis, and in the years 2014-2016 it reached the value of more than 30% higher than in the previous years. The interest in this group of compounds increases due to the growing use of the newest techniques of identification, imaging and visualization of cell structures, useful in, for example, diagnosis of diseases or verification of the ability to overcome barriers of human skin by xenobiotics, including therapeutic or toxic substances. Currently, an increased interest in the synthesis of innovative fluorescent dyes with more specific affinity to cell structures is reported, which will allow for the realization of various research purposes. In case of *stratum corneum* imaging, the most frequently applied commercial fluorescent dyes include fluorescein (FLU), sulforhodamine B (SRB), rhodamine hexyl ester (RBHE). However, these dyes show a specific affinity for specific skin construction structures of stratum corneum of epidermis, which results in either colouration of the matrix lipids, as in the case of RBHE, or the proteins of corneocytes, as in the case of the FLU or SRB. However, the new proposal for a fluorescent dye has the ability of dye primary lipids, but also corneocytes' proteins, and in addition protein – hair keratin.
From the groups of *Safirinium-P* and *Safirinium-Q* there, fluorescent dyes used for fluorescent marking of bacterial spores and in proteomics as ionization markers in studies of protein hydrolysates are already known. However, these dyes do not indicate a specific affinity to the stratum corneum layers of the epidermis, or in the case of interaction with the stratum corneum components they are characterized by a very short duration of fluorescence.

Advantages of the proposed product

Production technology of a new class of fluorescent dye in the *Safirinium-Q* family is simple, fast, efficient and uses a new type of fluorogenic reaction by the use of isoxazole derivatives. The innovative dye which is formed as a result of this synthesis is characterized by the amphiphilic properties, it is water

soluble, chemically stable, showing high quantum performance of fluorescence, a large Stoke's shift and fotooptic durability.
It also stands out for its optimal for diffusion to the skin size of molecule below 500 Da and lipophilicity, which allows to penetrate stratum corneum of the epidermis. All these features make, it more universal than commercial fluorescent dyes, as well as being able to be used in studies for a wider range of compounds applied to the skin, and this significantly broadens the spectrum of research on skin barrier features. It shows an affinity to both lipid canyons, surrounding corneocytes clusters, lipid matrix, foremost ceramides, surrounding a single corneocyte, as well as keratin being the building component of corneocytes, enabling their simultaneous observation. A unique feature of the received dye is the ability to undergo colourization in the stratum corneum of the so-called canyons, or lipid space between corneocytes clusters, important for the skin barrier features. By colourizing the canyons one may observe the penetration of substances to the skin, including active substances, from different formulation of medicinal products or cosmetics, as well as carriers, including liposomes applied on the skin.

An additional advantage of the use of innovative dye is not only the ability to make an assessment of the impact of skin structures on dermal preparations' components (medicinal and cosmetic) or xenobiotics originating from the environment but also to test regenerative action or action which destroys formulations on the skin through visualization of the shape of corneocytes. Fluorescent staining technique with the use of the innovative dye allows for repeatable obtaining of high-quality microscopic images. The fact which seems important to be highlighted is the ability of the new fluorescent dye to create a mixture with dyes of hydrophilic properties. In view of the fact that the new dye has amphiphilic properties and is of blue colouration, it can be mixed with hydrophilic orange dye SRB goal in order to contrast colouring of the specific structures of the stratum corneum. The mixture has the ability to colourize both corneocytes, the lipid matrix, and canyons. This feature can be a chance for new quality of examining the penetration of active substances and excipient substances with diverse properties present in preparations for the skin, both for medicinal agents as well as skin care products.