may be introduced into the dietary regimen of a subject suffering of a trichological disorder such as hair loss, hair thickening or discoloration.

The inventors observed that the iminooxothiazolidine derivatives of formula (I) when administered through the topical or systemic route to a subject suffering from hair thinning or hair loss, give a progressive thickening of thinner areas of the scalp.

In accordance with certain aspects, a composition is provided containing the iminooxothiazolidine derivatives of formula (I) and a physiologically acceptable carrier for use in the treatment of hair growth disorders.

According to certain embodiments, the composition of the present invention is a pharmaceutical composition.

Typically, the composition of the invention contains a trichologically or pharmaceutically active amount of the iminooxothiazolidine derivatives of formula (I).

Certain aspects and advantages of the invention are further described with reference to the appended drawing.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows a 3 to 6 fold increase of the Lef1 gene expression obtained by its stimulation with two compounds of the invention identified as C1 and C5 in Example 4, in respect with the one obtained by the vehicle alone.

Figure 2 shows additional tests with the aim to show the hair growth promotion activity of the iminooxothiazolidine compounds of the invention, especially those reported in the following Table I using Hair cell line (derma papilla cells).

DETAILED DESCRIPTION OF THE INVENTION

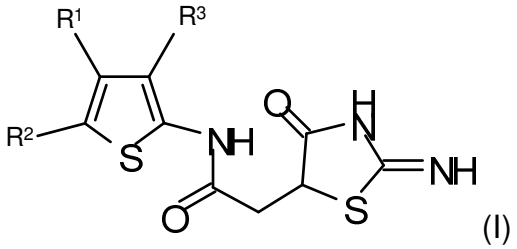
In accordance with certain aspects of the invention, the inventors found that a selected class of iminooxothiazolidine derivatives of formula (I) found application in the trichological field and are useful in the prevention or treatment of hair growth disorders.

The compounds of formula (I) find application in the prevention or treatment of trichological or hair growth disorders.

The Applicant discovered that compounds of formula (I) exert a trichological activity and stimulates the activity of follicle cells and of quiescent hair bulbs of

the scalp. These activities activate the life cycle of hair follicles promoting the hair growth.

In accordance with a first aspect the present invention thus provides the cosmetic/ non therapeutical use of iminooxothiazolidine compounds of formula (I)



(I)

wherein

R_1 is H, linear or branched C_1-C_6 alkyl preferably C_1-C_3 alkyl, (C_3-C_6)cycloalkyl, phenyl, –CH_2-NH-phenyl;

R_2 is H, linear or branched C_1-C_6 alkyl preferably C_1-C_3 alkyl, halogen, phenyl;

R_3 is COO(C_1-C_6) alkyl preferably COO(C_1-C_3)alkyl, $CONR_4R_5$ wherein R_4 and R_5 individually are H or C_1-C_6 alkyl preferably C_1-C_3 alkyl, or R_1 and R_2 together form a C_5-C_7 cycloalkyl fused with the thiophene ring

for promoting hair growth or preventing or slow down hair loss.

A preferred halogen is Br.

In accordance with certain embodiments R_1 is H or C_1-C_3 alkyl, preferably methyl; R_2 is H, halogen preferably Br or C_1-C_3 alkyl preferably CH_3 and R_3 is COO(C_1-C_3) alkyl preferably a methyl or ethyl ester.

In certain embodiments R_1 is H or C_1-C_3 alkyl,

R_2 is H or halogen, preferably Br,

R_3 is $COOCH_3$ or $COOCH_2CH_3$.

Preferred compounds of formula (I) have the following substituent groups:

R_1 is methyl, R_2 is Br and R_3 is COOethyl;

R_1 is methyl, R_2 is H and R_3 is COOethyl

R_1 is H, R_2 is H and R_3 is COOmethyl

R_1 is –CH_2-NH-phenyl, R_2 is Br and R_3 is COOethyl.

In accordance with certain embodiments the compounds of formula (I) reported in the following Table 1 are provided, preferably compounds C1, C5, C7, C17 which are more active, especially compounds C1 and C5.

subjects of male and female gender. The composition is also suitable for treating or preventing female pattern hair loss, baldness, or androgenetic alopecia.

Iminooxothiazolidine compounds and method for preparation thereof are disclosed in the International patent application WO 2006124887 to Wyeth Corp., USA.

In accordance with a third aspect, the present invention provides the cosmetic, non-therapeutic, use of a composition comprising an iminooxothiazolidine compound of formula (I) according to any one of the embodiments described hereinabove, and a physiologically acceptable carrier for stimulating the physiological growth of hair. The composition of the invention is effective in preventing and/or treating forms of baldness or hair thinning as described above.

The composition for cosmetic use is either for the local or systemic administration.

In accordance with some embodiments, the composition for oral administration is a functional food, a nutraceutical composition, a dietary product, a food supplement or a nutritional product or a medical device.

Functional food means any modified food or food ingredient which may provide a benefit or protection against a drawback or a physiological condition, besides the traditional nutrients it contains.

Nutraceutical product means a product isolated or purified from edible substances. A nutraceutical product is such when it shows to have a physiological advantage or to provide protection against a drawback or physiological disorder.

Independently from the routes of administration, the cosmetic composition increases the viability of follicle cells during the anagen phase and activates the quiescent cells of the scalp by stimulating the metabolism of follicles and the growth of new hair.

In accordance with a fourth aspect, a composition is provided containing an iminooxothiazolidine compound of formula (I) according to any one of the embodiments described hereinabove, and a pharmaceutically acceptable carrier for use in the treatment of hair growth disorders.

Example of hair growth disorders treated by these compositions include alopecia and telogen effluvium. Typically, these compositions are formulated as pharmaceutical compositions.

The amount of active compounds of formula (I) in compositions for cosmetic/ pharmaceutical/nutritional uses is such that a prophylactic or therapeutically effective dosage is obtained.

In accordance with certain embodiments the composition for both cosmetic and medical uses may contain an iminooxothiazolidine compound of formula (I) in an amount of 0.0001 to 15% by weight, 0.001 to 10% by weight or 0.1 to 3% by weight.

In certain embodiments, the composition of the invention may further comprise one or more additional ingredients having a trichological activity.

The amount administered and the frequency of administration of the composition will depend on the type and severity of the trichological condition to be treated.

In certain embodiments the compositions for both cosmetic and therapeutical uses further includes additional ingredients such as vitamins, for example vitamin A, vitamin C, vitamins of the B group, niacin.

In accordance with some embodiments the composition of the invention further contains an amino acid, in particular a sulfur-containing amino acid, such as L-cysteine or L- Methionine, especially for formulations for the oral administration.

In accordance with some embodiments the composition of the invention also includes one or more micronutrients and/or minerals such as Mg, Zn, Ca, Fe, Cr, Se, and others, especially for formulations for the oral administration.

In certain embodiments the composition may further contain additional substances such as folic acid, calcium pantothenate, mucopolysaccharides such as hyaluronic acid, or soy derivatives such as soy isoflavones.

In some embodiments the composition of the invention for both cosmetic and medical uses comprises a physiologically and/or pharmaceutically acceptable carrier, diluent or excipient.

Typically, the physiologically acceptable carrier of the composition of the invention is an excipient, carrier or diluent suitable for topical application and/or systemic administration. Physiologically and pharmaceutically carriers may be the same carriers.

Typically, the active compounds of formula (I) of the pharmaceutical compositions are formulated in dosage units. The dosage unit may contain from 0.1 to 1000 mg of active ingredient per dosage unit for daily administration.

In some embodiments, the amounts effective for formulation will depend on the severity of the disorder or condition to be treated. In some embodiments, the dose is in the range from 0.001% by weight to about 60% by weight of the formulation.

In accordance with certain aspects the invention relates to compositions for topical use which are useful in the stimulation of hair follicles, with a resulting benefit of treating hair growth disorders, such as alopecia, in females in particular. Methods of treatment of relevant populations are also a feature of the invention.

According to another aspect of the invention, there is provided a method of cosmetic treatment which comprises the application to the scalp of an effective amount of a composition of the type described previously.

For example, for local uses, a cosmetically or pharmaceutically active amount of the composition of the invention is applied directly to the scalp one or more times daily, conveniently for cycles having a duration of 2-3 months alternating with periods of rest.

The following examples are provided merely for illustrating the present invention and are not to be intended as limiting the scope of protection as results from the appended claims.

EXAMPLE 1

A tablet for oral administration containing a compound of formula (I) for stimulating hair growth

Ingredient	q.ty (mg)
Microcrystalline cellulose	60 – 120
Hydroxypropylmethylcellulose K100	20 – 60
2-(2-(2-Imino-4-oxo-thiazolidin-5-yl)-acetylamino)-4,5-dimethyl-thiophene-3-carboxylic acid ethyl ester	5 - 30
Magnesium stearate	5 - 10
Silicon dioxide	10 – 20
Polyvinylpyrrolidone K30	5 – 10
Hyaluronic acid	0.5 – 2.0

Polyethylene glycol	5 – 10
Methacrylic Acid - Methyl Methacrylate Copolymer	10 - 20
Triethyl Citrate	1.5 - 3
Polysorbate 80	0.5 – 1.0
Titanium dioxide	1.5 - 2.5
Talc	3 - 6

EXAMPLE 2

A lotion for topical application containing a compound of formula (I) for stimulating hair growth

Ingredient	q.ty (mg)
Aqua	1350 - 1850
Hydroxypropyltrimonium hyaluronate	1.3 - 3.8
Ethanol	1900 - 2600
2-(2-(2-Imino-4-oxo-thiazolidin-5-yl)-acetylamino)-4,5-dimethyl-thiophene-3-carboxylic acid methyl ester	0.01 – 1.00
Lactic acid 80%	7 – 10
PEG-40 Hydrogenated castor oil	35 – 45
Octadecyl di-t-butyl-4-hydroxyhydrocinnamate	1.5 - 2.0
Lecithin	1.5 - 2.0
Fomblin HC/PU-CAT5	0.6 - 0.8

5

EXAMPLE 3

A composition for topical application containing a compound of formula (I) for treating alopecia

Ingredient	q.ty (mg)
Aqua	940 – 2800
Ethanol	900-1800
Hydroxypropyltrimonium hyaluronate	3 - 4
Acetyl L-carnitine hydrochloride	17 – 23

Caffein	2 – 3
L-Arginine hydrochloride	2 – 3
Calcium Pantothenate	6.5 - 8.5
2-(2-(2-Imino-4-oxo-thiazolidin-5-yl)-acetylamino)-4,5-dimethyl-thiophene-3-carboxylic acid methyl ester	0.01 – 1.00
Lactic acid 80%	2 – 3
Biotin	0.5 -0.8
PEG-40 Hydrogenated castor oil	700 – 1000
Lecithin	17 – 23
Parfum	2.0 – 2.5
Ajuga Reptans dry extract	8.5 - 11.5
Fomblin HC/PU-CAT5	0.7 - 1.1

EXAMPLE 4

Experimental evidence.

Evidence of the activity of compounds of the invention is provided by the expression of Lymphoid enhancer binding factor-1 (Lef1), which plays an important role in bulge stem cells cell growth and differentiation (International Journal of Medical Sciences 2013; 10(6):738-746).

Test compounds

Compounds tested were:

C1: 2-(2-(2-Imino-4-oxo-thiazolidin-5-yl)-acetylamino)-4,5-dimethyl-thiophene-3-carboxylic acid methyl ester

C5: 2-(2-(2-Imino-4-oxo-thiazolidin-5-yl)-acetylamino)-4,5-dimethyl-thiophene-3-carboxylic acid ethyl ester.

These products were reconstituted in DMSO (stock solution) and intermediate or working solutions were prepared diluting the stock or the intermediate solution in William's E medium.

Hair follicle (HF) organ culture

Microdissected human anagen VI scalp HFs (were cultured at 37°C with 5% CO_2 in a minimal media of William's E media (Gibco, Life Technologies) supplemented with 2mM of L-glutamine (Gibco), 10ng/ml hydrocortisone (Sigma-Aldrich), 10µg/ml insulin (Sigma-Aldrich) and 1% penicillin/streptomycin

mix (Gibco) to make Williams Complete Media (WCM) as previously described (Philpott et al., 1990; Kloepper et al., 2010 ; Langan et al., 2015). After 24 hrs, the medium was replaced with fresh medium containing either WCM containing a final concentration of 0.1% of DMSO (vehicle) or test compounds at final concentrations of 15μM for C1 & C5. For qRT-PCT, RNA was extracted from hair follicles treated only for 6 hours.

qRT-PCR analysis

RNA was extracted from hair follicles with RNeasy kit (Qiagen, USA), and reverse transcribed with Tetro cDNA synthesis kit (Bioline) according to manufacturer's instructions. qPCR was performed in QuantStudio3 (Thermo Fisher) with Taqman probe for Axin2 (Hs00610344_m1, Thermo Fisher), Lef1 (Hs01547250_m1, Thermo Fisher), b-actin (Hs01060665_g1, Life Technologies) and Gapdh (Hs02758991_g1, Thermo Fisher). Expression levels were calculated with the delta-delta CT method.

Results

Lef-1 (genic expression)

The following table reports the genic expression of Lef1 (mRNA) in follicles microsectioned treated with the vehicle, C1 and C5.

The Lef1 expression is normalized on the average of housekeeping (GAPDH) gene. N=8 HFs/group from 1 donor.

	Vehicle	C1	C5
mean	1	3.425	6.134
SEM	0.01	0.251	0.326

The expression with compounds C1 and C5 shows a 3 to 6 fold increase in respect with the one obtained by the vehicle alone.

The results are illustrated in Figure 1.

EXAMPLE 5 (FIGURE 2)

Additional tests with the aim to assess the hair growth promotion activity of the iminooxothiazolidine compounds of the invention, especially those reported in the following Table I.

The cell line was cultured in Follicle Dermal Papilla Cell medium in 25 cm^2 culture flasks at 37°C, 5% CO_2. Every two days confluent cultures were splitted 1:3 – 1:6, after washing with PBS 1X (without Ca^{2+} and Mg^{2+}), using Trypsin/EDTA and seed at 2-5·10^4cell/cm^2, 37°C, 5% CO_2.

Freezing medium: Cryo-SFM. Cell quantification: trypan blue assay

Controls

Positive control: Way316606

Negative control: Untreated cells in Follicle Dermal Papilla Cell under the same sample cultural condition.

Methods

The expression of LEF1 was investigated by RT-PCR. The analysis was performed according to the reported method (Gibson et al, 1996; Heid et al, 1996) using TaqMan Gene Expression Assay supplied by Applied Biosystems. Briefly, within a gene-specific PCR oligonucleotide primer pair, an oligonucleotide probe labelled with a reporter fluorescent dye (FAM) at the 50-end and a quencher fluorescent dye (TAMURA) at the 30-end was designed. When the probe was intact, the reporter dye emission was quenched. During the extension phase of the PCR cycle, the nucleolytic activity of the DNA polymerase cleaved the hybridization probe and released the reporter dye from the probe. Fluorescence intensity produced during PCR amplifications was monitored by a sequence detector directly in the reaction tube (“real time”). A computer algorithm compared the amount of reporter dye emission with the quenching dye emission and calculated the threshold cycle number (CT) when signals reached ten times the standard deviation of the baseline, from which the levels of the mRNAs transcribed from the various genes tested were obtained (Gibson et al, 1996).

Gene expression analysis involves four following step:

1. Treatments of cells with active compounds for 24h;
2. RNA extraction;
3. cDNA retro transcription;
4. Quantitative RT-PCR;

Day 1: Seed cells

When the cells (Human Follicle Dermal Papilla Cells) reached approximately 80% confluence, they were harvested with trypsin/EDTA and seeded at a density of 1×10^6 cells/ml into 12-well plates and then incubated at 37°C, 5% CO_2 (24h).

Day 2-3: Chemical exposure 24 h

When the cells reached approximately 80% confluence, complete medium was removed; cells were washed once with PBS 1X and the incubated in Human Follicle Dermal Papilla Cells at 37°C, 5% CO_2 for at least 24h;

Compounds to be tested (WAY316606, 1, 5, 7, 17, 18, 20, 21, 24) were dissolved in DMSO and diluted at final concentration in Human Follicle Dermal Papilla Cells.

Controls (medium only) were also included.

Cells were then incubated at 37°C, 5% CO_2 for different 24h.

RNA extraction

Total RNAs were extracted from the cells using Tri Reagent (Thermo Fisher), that is a single, homogenous solution for the isolation of total RNA. This procedure is an improvement of the single-step method reported by (Chomczynski and Mackey, 1995) for total RNA isolation. Tri Reagent, a mixture of guanidine thiocyanate and phenol in a monophase solution, effectively dissolves DNA, RNA, and protein on homogenization or lysis of tissue sample and contains a unique combination of denaturants and RNase inhibitors and is used in a convenient, single-step disruption/separation procedure.

The purity of total RNA extracted was assessed by reading absorbance at 260nm, the λ at which the nucleic acid has the highest absorbance. It was also read the absorbance at 280 nm, to assess contamination by proteins or phenol. Whereas measuring the absorbance at 230 nm and 330 nm provides information about the purity of both samples and cuvette. The RNA is considered of good quality if the R=A260/A280 ratio is $>$1.6.

After have determining the total RNA concentration and purity each sample of RNA was diluted in DEPC water to a final concentration of 2 µg/ml. This is the required concentration by the retro transcription kit (in the follow phase)

It was also performed an electrophoresis gel running to verify the integrity of the total RNA extracted.

Reverse transcription PCR

Total RNA, extracted and quantified, was amplified using the "PRIME SCRIPT RT reagent kit (Perfect Real time) (Takara).

Random primers were used in order to ensure an efficient synthesis of the first strand of the mRNA.

For amplification we used the Real time PCR System Mx3000P (Stratagene) and each total RNA was amplified in duplicate.

AMPLIFICATION CONDITIONS	**Step 1:**	**Step 2:**	**Step 3:**
Temperature	37° C	85°C	25° C
Time	15 min	5 sec	hold

After amplification samples were diluted with 30 µl of DEPC water and stored at -20 °C until use.

Real Time PCR

The RT-PCR analysis was set, using TaqMan linear probes (Applied Biosystems). Those probes are the most widely used and published detection chemistry for qPCR applications.

Inventoried probes and primers were chosen according to previously specific bibliographic studies.

We have used the relative quantification method in which the relative concentration of the gene of interest (target) in unknown samples is compared to a calibrator, or control sample (untreated cells). Here, the calibrator is a baseline for the expression of a given target gene. Using this approach, differences in Ct value

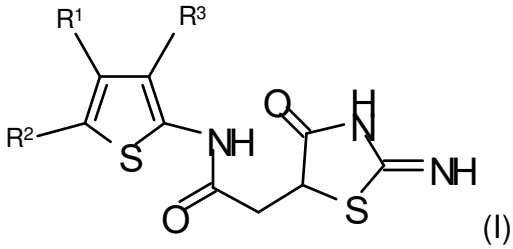
	Lef1	
	fold of change mRNA expression	SEM
Vehicle	1	0.668
WAY-316606	2.18	0.097
C1	8.43	0.314
C5	6.05	0.226
C7	2.46	0.559
C17	2.52	0.229
C18	1.56	0.592
C20	1.39	0.621
C21	1.79	0.299
C24	1.88	0.314

The major induction of Lef1 was obtained with compounds 1 and 5.

After 24h of all tested compounds were able to inhibit Lef1 mRNA expression, as illustrated in (Figure 2). Also WAY316606, used as positive control, exerted such inhibitory action.

CLAIMS

1. Cosmetic use of an iminooxothiazolidine compound of formula (I)



(I)

wherein

R_1 is H, C_1-C_6 alkyl, cycloalkyl, phenyl, $–CH_2$-NH-phenyl;

R_2 is H, C_1-C_6 alkyl, halogen, phenyl;

R_3 is $COO(C_1$-$C_6)$ alkyl, $CONR_4R_5$ wherein R_4 and R_5 individually are H or C_1-C_6 alkyl, or R_1 and R_2 together form a C_5-C_7 cycloalkyl fused with the thiophene ring for promoting hair growth and/or preventing and/or reducing hair loss.

2. Use according to claim 1 wherein

R_1 is H or C_1-C_3 alkyl, preferably methyl,

R_2 is H, halogen preferably Br or C_1-C_3 alkyl, preferably CH_3 and

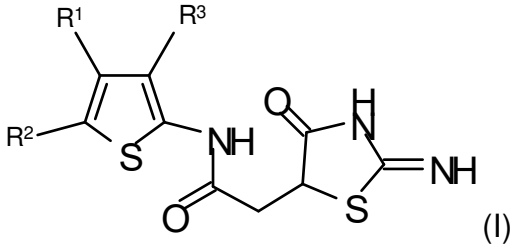
R_3 is $COO(C_1$-$C_3)$ alkyl, preferably a methyl or ethyl ester.

3. Use according to claim 1 or 2 wherein the compounds are 2-(2-(2-Imino-4-oxo-thiazolidin-5-yl)-acetylamino)-4,5-dimethyl-thiophene-3-carboxylic acid methyl ester or 2-(2-(2-Imino-4-oxo-thiazolidin-5-yl)-acetylamino)-4,5-dimethyl-thiophene-3-carboxylic acid ethyl ester.

4. Cosmetic use of a composition comprising an iminooxothiazolidine compound of formula (I) according to anyone of claims 1-3 and a physiologically acceptable carrier for promoting hair growth and/or preventing and/or reducing hair loss.

5. Use according to claim 4 wherein the composition is for local or topical application or for oral administration.

6. An iminooxothiazolidine compound of formula (I)



(I)

wherein

R_1 is H, C_1-C_6 alkyl, cycloalkyl, phenyl, $–CH_2$-NH-phenyl;

R_2 is H, C_1-C_6 alkyl, halogen, phenyl;

R_3 is $COO(C_1$-$C_6)$ alkyl, $CONR_4R_5$ wherein R_4 and R_5 individually are H or C_1-C_6 alkyl, or R_1 and R_2 together form a C_5-C_7 cycloalkyl fused with the thiophene ring for use in the prevention or treatment of an hair growth disorder.

7. An iminooxothiazolidine compound of formula (I) for use according to claim 5 wherein

R_1 is H or C_1-C_3 alkyl, preferably methyl;

R_2 is H, halogen preferably Br or C_1-C_3 alkyl preferably CH_3 and

R_3 is $COO(C_1$-$C_3)$ alkyl preferably a methyl or ethyl ester.

8. An iminooxothiazolidine compound of formula (I) for use according to claim 1 or 2 wherein the compounds are 2-(2-(2-Imino-4-oxo-thiazolidin-5-yl)-acetylamino)-4,5-dimethyl-thiophene-3-carboxylic acid methyl ester or 2-(2-(2-Imino-4-oxo-thiazolidin-5-yl)-acetylamino)-4,5-dimethyl-thiophene-3-carboxylic acid ethyl ester.

9. A pharmaceutical composition comprising an iminooxothiazolidine compound of formula (I) for use according to anyone of claims 6-8 and a pharmaceutically acceptable carrier.

10. A pharmaceutical composition for use according to claim 9 wherein the composition is for local/topical application or for systemic administration.