Natural skin-whitening compounds for the treatment of melanogenesis (Review)

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Abstract. Melanogenesis is the process for the production of melanin, which is the primary cause of human skin pigmentation. Skin-whitening agents are commercially available for those who wish to have a lighter skin complexions. To date, although numerous natural compounds have been proposed to alleviate hyperpigmentation, insufficient attention has been focused on potential natural skin-whitening agents and their mechanism of action from the perspective of compound classification. In the present article, the synthetic process of melanogenesis and associated core signaling pathways are summarized. An overview of the list of natural skin-lightening agents, along with their compound classifications, is also presented, where their efficacy based on their respective mechanisms of action on melanogenesis is discussed.

Contents

1. Introduction

Lighter skin tone has long been associated with youth and beauty among a variety of Asian cultures. Investment in skin-whitening agents, boosted by markets in Asian countries, especially those in China, India and Japan, is increasing annually (1). Skin color is influenced by a number of intrinsic factors, including skin types and genetic background, and extrinsic factors, including the degree of sunlight exposure and environmental pollution (2-4). Skin color is determined by the quantity of melanosomes and their extent of dispersion in the skin (5). Under physiological conditions, pigmentation can protect the skin against harmful UV injury. However, excessive generation of melanin can result in extensive aesthetic problems, including melasma, pigmentation of ephelides and post-inflammatory hyperpigmentation (1,6). Traditional pharmacological agents, including corticosteroids, hydroquinone and aminomercuric chloride, lighten skin tone through the inhibition of either melanocyte maturation or interference with the process of melanogenesis. However, most if not all of the aforementioned agents are closely associated with adverse effects including prickling sensation, contact dermatitis, irritation, high toxicity and sensitivity (7-10). Therefore, recent research by cosmetic companies and research institutions has been focusing on the development of novel whitening agents that selectively suppress the activity of tyrosinase (TYR) to reduce hyperpigmentation whilst avoiding cytotoxicity to normal, healthy melanocytes. As a result, natural skin whitening compounds are currently garnering significant attention in the cosmetic and medical industry (11,12).

The present review summarizes the biosynthetic process of melanogenesis and the associated core regulatory signaling pathways. It also reviews natural skin-whitening agents in terms of their compound classification and discusses their efficacy based on their mechanism of action on melanogenesis. In addition, an overview of the current research methodology applied for the evaluation of compound bioactivity is provided. The aim of the present review is to provide informative guidance for the development of safe and effective depigmenting agents for use in the cosmetic industry.

2. Melanogenesis

Melanin is mainly produced by melanocytes that are localized in the epidermis, the outermost layer of the skin; it is also this layer that determines skin color in humans (4). Melanin is primarily synthesized in melanosomes, which function as specialized organelles in melanocytes. Melanogenesis

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is a complex process that involves a series of enzymatic and chemical reactions inside the melanosomes, resulting in the production of two types of melanin: Eumelanin and pheomelanin. Eumelanin is an insoluble polymer that is dark brown-black in color, whereas pheomelanin is a soluble polymer light red-yellow in color that also contain sulfur (13). Both eumelanin and pheomelanin are formed by the conjugation of cysteine or glutathione (14-16). To gain an understanding of the mechanism of whitening agents, a summary of the signaling pathways associated with skin melanogenesis is presented in Fig. 1. The pigmentation process starts with the oxidation of L-tyrosine to L-dopaquinone (DQ) in the presence of the rate-limiting enzyme TYR. Following DQ formation, the resulting quinone undergoes intramolecular cyclization and oxidation, where it serves as a substrate for the synthesis of eumelanin and pheomelanin (17,18). During the process of melanogenesis, hydroxylation of L-tyrosine to form L-3,4-dihydroxyphenylalanine (L-DOPA) is the rate-limiting step of the whole process, which is catalyzed by TYR.

3. Core signaling pathways in the regulation of melanogenesis

Melanogenesis is a complex process that is modulated by a network of pivotal signaling cascades and transcription factors, which is controlled at different levels. In particular, modulation of TYR activity is the most commonly applied strategy for the clinical intervention of pigmentation disorders. Since naturally occurring inhibitors of melanogenesis usually garner more attention compared with chemically synthesized compounds due to the cosmetic demands of consumers, the present review focuses on natural compounds that have been documented to exhibit skin-whitening effects through the inhibition of TYR activity. The three core signal pathways involved in the regulation of melanogenesis are: i) melanocortin-1 receptor (MC1R) signaling; ii) the Wnt/β-catenin signaling pathway; and iii) the tyrosine kinase receptor KIT/stem cell factor (SCF) pathway, all of which converge downstream to activate the master regulator-microphthalmia-associated transcription factor (MITF) (Fig. 2) (19). The following sections will describe the genetic and molecular modulators that are involved in the control of melanogenesis by these three key pathways.

α-melanocyte-stimulating hormone (α-MSH)-MC1R signaling pathway. α-MSH is a precursor polypeptide derived from pro-opiomelanocortin that can modulate pigmentation through paracrine action, whilst MC1R is a member of the G-protein-coupled receptor family (20). α-MSH binding to MC1R results in the activation of adenylyl cyclase, increasing the intracellular levels of cAMP and subsequently upregulating TYR, tyrosinase related protein-1 (TRP-1) and tyrosinase related protein-2 (TRP-2) expression. The biological effects downstream of cAMP elevation have been previously demonstrated to be predominately mediated by cAMP-dependent protein kinase (PKA), which phosphorylates cAMP-response element (CRE) binding protein (CREB) (21). However, it has also been suggested that neither TRP-1 nor TRP-2 have cAMP response elements in their respective promoter regions. Evidence has indicated that regulating the gene expression of TRP-1 and TRP-2 by cAMP is directly associated with MITF, which binds to the M-box sequence (AGTCATGTG CT) located in the tyrosinase distal elements (TDEs) after its activation (22). Since the promoter region of MITF contains the consensus CRE sequence, the expression of MITF can also be increased by α-MSH stimulation in a cAMP-dependent manner (23). This demonstrated that the α-MSH-MC1R signaling pathway induces melanin production predominantly by elevating intracellular cAMP levels, and the inhibition of which can exert inhibitory effects on melanogenesis.

Wnt signaling pathway. The Wnt signaling pathway has been previously reported to serve an important role in melanogenesis (24,25). Wnt ligands bind to Frizzled receptors on the cell surface, resulting in the increased stability of cytoplasmic β-catenin, and its subsequent translocation into the nucleus, where it activates the transcription of MITF by interplay with lymphoid enhancer-binding factor 1 (LEF1)/T-cell factor (LEF1/TCF) (26). Previous studies on melanocytes suggest that β-catenin and LEF1 synergistically regulate the M promoter activity of MITF via LEF1 binding sites, which upregulate MITF expression in melanoma (27,28). By regulating MITF transcription, the Wnt/β-catenin signaling pathway can control the expression of TYR and other pigmentation enzymes.

SCF-KIT signaling pathway. Recent studies have verified the important roles of the SCF-KIT signaling pathway in melanocyte proliferation and differentiation, and the process of melanogenesis (29,30). SCF is a paracrine factor that is secreted by fibroblasts, whereas c-KIT, its receptor, is expressed on melanocytes (31). When SCF binding to its receptor-c-KIT, it stimulates tyrosine kinase activity, resulting in receptor auto-phosphorylation to initiate signal transduction (32,33). c-KIT phosphorylation directly activates p38 mitogen-activated protein kinase (MAPK), a member of the MAP kinase family, which in turn phosphorylates CREB and subsequently activates MITF to promote TYR transcription (34). c-KIT can also activate ERK. c-KIT-mediated ERK signaling pathway can induces CREB phosphorylation to activate melanin synthesis on one hand, and on the other hand, the activation of ERK signaling has been demonstrated to phosphorylate MITF at the serine 73 residue, which leads to the ubiquitination and degradation of MITF, this is the feedback mechanism of the ERK pathway to regulate melanin production (3,35). In addition to p38 MAPK and ERK, c-KIT activation is associated with the phosphoinositide 3-kinase (PI3K) signaling pathway, which not only regulates cell survival but also causes pigmentation by activating the serine/threonine-specific protein kinase AKT. Downstream, PI3K activation leads to the phosphorylation of glycogen synthase kinase 3β (GSK-3β) to increase MITF activity (36). Therefore, inhibitors of the SCF-KIT signaling pathway can potentially exhibit anti-melanogenesis activity.

MITF. MITF serves as the central hub of the regulatory network of melanin synthesis that is comprised of numerous transcription factors and signaling pathways that modulate the survival, proliferation and differentiation of melanoblasts and melanocytes (37). The MITF gene contains multiple promoters, the M promoter is one of such promoters, which is located adjacent to the common downstream exons and is targeted by several transcriptional factors, including CREB, paired box gene 3

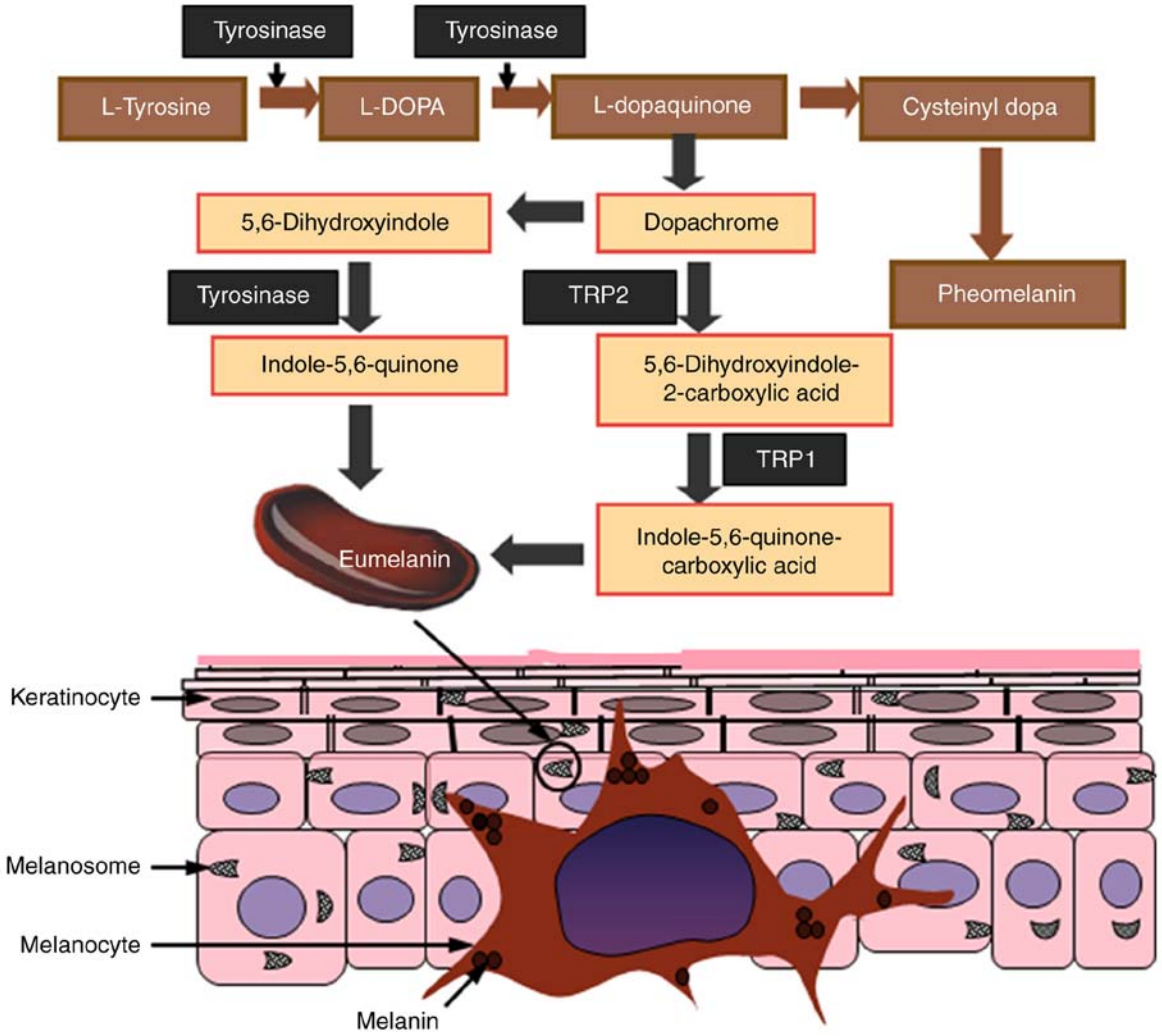

Figure 1. Melanogenesis and transport. Melanocytes are located on the basal layer of the skin, which synthesizes melanin. Eumelanin and pheomelanin are produced in melanosomes, a specialized organelle within melanocytes, through a series of reactions that are catalyzed by melanogenic enzymes. The synthetic pathways are divided into two branches: Eumelanogenesis and pheomelanogenesis. Melanocytes transport the melanin pigments produced by melanosomes through their elongated dendrites into neighboring keratinocytes in the epidermis layer. TRP1, tyrosinase related protein-1; TRP2, tyrosinase related protein-2; L-DOPA, L-3,4-dihydroxyphenylalanine.

(PAX3), LEF1/TCF, SRY-related HMG-box 10 (SOX10), SOX9 and MITF itself (38). In melanocytes, these transcription factors bind to the promoter of MITF-M to regulate MITF expression whilst controlling the transcription of several important genes. These genes are not only related to the production of melanin, including TYR, TRP-1 and TRP-2, but are also linked to the regulation of melanocyte differentiation, proliferation and cell cycle progression. Cyclin-dependent kinase 2 (CDK2), B-cell lymphoma-2 (BCL-2) and Hypoxia-inducible factor 1-alpha (HIF-1α) are such genes that are regulated by MITF. In addition, MAPK, ribosomal S6 kinase (RSK), glycogen synthase kinase-3β (GSK-3β) and p38 can all phosphorylate MITF and simultaneously modulate its transcriptional activity in response to specific environmental cues (39-43).

4. Naturally occurring skin-whitening agents for inhibiting melanogenesis

Naturally occurring skin-whitening agents exert their effects by regulating melanin production through a number of mechanisms, including inhibiting the expression and activity of TYR and suppressing the uptake and distribution of melanosomes. In the cosmetics industry, since skin-whitening compounds from natural sources are usually more appealing to consumers, a greater demand exists for inhibitors of melanogenesis derived from herbal plants that prevent hyperpigmentary disorders. Naturally occurring bioactive compounds, including flavonoids, terpenoids, polysaccharides and coumarin derivatives, all of which have been previously demonstrated to exhibit antioxidant and anti-inflammatory properties, are now becoming increasing recognized to possess anti-melanogenesis functions (44,45). Therefore, this section focuses on the natural active skin-whitening agents that are currently known based on their compound classification along with their mechanism of action on melanogenesis.

Transcriptional control of TYR expression via MITF. MITF serves an indispensable role in melanogenesis as it controls the transcription of TYR and other pigmentation-associated enzymes (46-48). Naturally occurring bioactive compounds

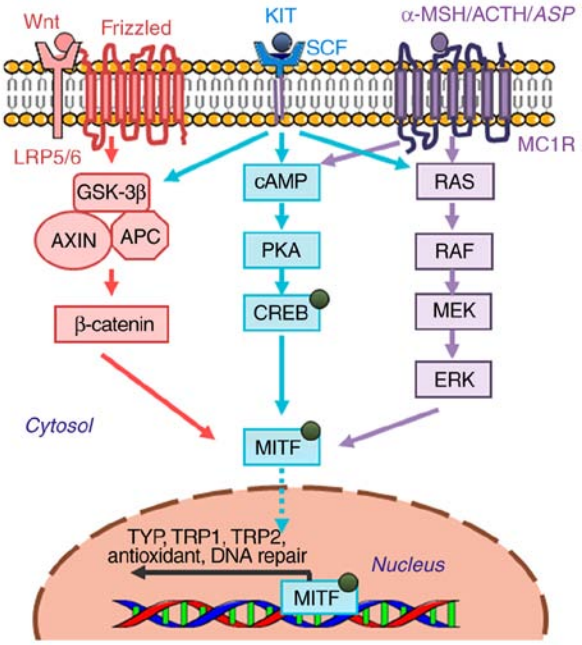

Figure 2. Core molecular pathways associated with the regulation of melanin production in melanocytes. Genes encoding specific melanogenic enzymes, including TYP, TRP1 and TRP2, are regulated by the MITF transcription factor, which is in turn regulated by a number of important signaling pathways, including α-MSH/MC1F (purple), KIT/SCF (blue) and Wnt/frizzled (red). Signal transduction is mediated by cAMP/PKA, RAS/MEK/ERK and β-catenin pathways. cAMP, cyclic AMP; MEK, MAPK/ERK kinase; Wnt, wingless-related integration site; LRP5/6, low-density lipoprotein receptor-related protein 5/6; GSK-3β, glycogen synthase kinase-3β; AXIN, axis inhibitor; APC, adenomatous polyposis coli; SCF, stem cell factor; MC1R, melanocyte-specific melanocortin-1 receptor; α-MSH, α-melanocyte-stimulating hormone; ACTH, adrenocorticotropic hormone; ASP, agonist stimulating protein; TRP1, tyrosinase related protein-1; TRP2, tyrosinase related protein-2; PKA, protein kinase A; MITF, microphthalmia-associated transcription factor; CREB, cAMP response element binding protein.

have now been reported to exert an anti-melanogenesis function by interfering with signaling pathways to downregulate MITF expression. Among them, phenolic compounds, including [6]-Shogaol (49), derived from *Heracleum moellendorffii* Hance extracts (50), the ethyl acetate fraction of *Oroxylum indicum* Vent. seeds (51) and 2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (35) from *Juglans mandshurica* plants, inhibit melanogenesis by mediating the degradation of MITF in a manner that is associated with ERK signaling. By contrast, other phenolic compounds (52-55) exert anti-melanogenic properties by downregulating the cAMP/CREB signaling pathway and/or activating related caspases to trigger apoptosis of melanocyte cells (Table I). Flavonoids, including isoorientin, catechin, coumaric acid and kaempferol-7-O-D-glucuronide, derived from *Gentiana* (56), *Phyllostachys nigra* (57), *Cryptotaenia japonica* (57) and dried pomegranate concentrate powder (58) exhibit skin-whitening effects by downregulating PKA/CREB-mediated MITF expression. A list of other bioactive compounds, including terpenoids, polysaccharides and lignanoids, and their respective molecular mechanism of action on the melanogenesis pathway is provided in Table I (49,59-72). It can be observed that bioactive compounds are able to suppress MITF or TYR activity by either binding to transcription factors directly or by inhibiting melanogenic pathways upstream, including that of cAMP/PKA, ERK, Wnt/β-catenin and MAPK. Therefore, these aforementioned compounds represent promising skin-whitening agents, but those targeting TYR gene expression are not recommended for clinical use mainly for their non-specific effects through intracellular signaling cascades (73).

TYR modulation. TYR is a popular target for the development of skin-whitening agents due to its position at the rate-limiting step of the melanogenesis pathway. Additionally, TYR inhibitors have highly specificity for targeting melanogenesis, reducing the risk of side effects. Therefore, TYR inhibitors remain as the most successful and commonly applied skin-whitening agents. The majority of the naturally occurring compounds currently applied are botanical inhibitors of TYR, where their mechanism of action mainly entails two processes.

Inhibition of TYR catalytic activity. A number of studies have reported TYR inhibitors from natural sources, most of which are originate from Asia. Table II provides a summary of studies that have previously applied such types of TYR inhibitors. In a substantial number of these studies, mushroom TYR has been used as the protein model, and the IC_{50} values of the prospective TYR inhibitor were compared with those of other established inhibitors, including kojic acid and arbutin. TYR is a multi-functional type-3 copper-containing glycoprotein that is located on the membrane of the melanosome (1,74). Structurally, the active site of TYR consists two copper ions surrounded by three histidine residues (75). Anthraquinones, flavonoids and phenylpropanoids can serve as competitive inhibitors of TYR due chemical structures similar to those of L-tyrosine or L-DOPA (76,77).

Within the quinone family of compounds, the most frequently applied skin-whitening agents are hydroquinone (HQ) (78,79) and arbutin (80,81). Although HQ can function as an alternative substrate for TYR, the subsequent enzymatic reaction results in the production of reactive oxygen species (ROS), which is thought to be responsible for its skin-lightening properties, with possible associated side effects including leukoderma and exogenous ochronosis (82,83). Therefore, HQ has been banned in the EU, USA and a number of African and Asian countries (5). By contrast, arbutin is an effective agent for treating for hyperpigmentation in the cosmetics industry, which is also commonly applied as a positive control for melanogenesis studies.

Flavonoid compounds, including epigallocatechin gallate (84), quercetin (85), aloesin (86,87), hydroxystilbene derivates and licorice extracts are used for hyperpigmentation due to the ability to remove ROS and to chelate metals ions at the active sites of metalloenzymes (88). The hydroxystilbene family of compounds, which includes resveratrol as a well-known example, is the most efficient at alleviating hyperpigmentation compared with other families of flavonoid compounds. Resveratrol, which is found in a wide variety of plants such as grapes, exhibits potent inhibitory effects towards TYR (89,90). Regarding other flavonoid compounds, licorice-glabiridin (91), the main component found in the hydrophobic fraction of licorice extracts (92), has previously been demonstrated to exhibit inhibitory activity against TYR

Table I. Bioactive, naturally occurring compounds and their respective mechanism of action on tyrosinase and MITF expression.

Structure type	Source	Mechanism of action	(Refs.)
Phenolic compounds			
2-[4-(3-Hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol	*Juglans mandshurica*	ERK-associated pathway resulting in the downregulation of MITF	(34)
Protocatechuic acid	Pear fruits	Downregulation of the cAMP/CREB signaling pathway	(49)
Ethyl acetate fraction of *Oroxylum indicum* Vent. seeds	*Oroxylum indicum*	Activation p38, ERK1/2 and JNK phosphorylation and suppression of MITF expression	(47)
Hispolon	*Phellinus linteus*	Downregulation of MITF and activation of caspase-3, -8 and -9	(51)
Heracleum moellendorffii Hance extract	*Heracleum moellendorffii*	Activation of ERK1/2 and subsequent degradation of MITF	(46)
[6]-Shogaol	Ginger rhizome	Acceleration of ERK and PI3K/Akt-mediated MITF degradation	(45)
Sesamol	Sesame	Regulation of melanin-related signal transduction	(48)
Phenolic extracts	Rape bee pollen	Inhibition of the cAMP/MITF/TYR pathway	(50)
Flavonoids			
Isoorientin	*Gentiana veitchiorum* Hemsl. flowers	Suppression of MITF through CREB	(52)
Hesperidin	Rutaceae citrus species	Activation of ERK1/2 and downregulation of MITF	(56)
Gallic acid	Gallnut, lacquer tree, tea	Inhibition of PI3K/AKT, MEK/ERK and Wnt/β-Catenin signaling to downregulate MITF	(58)
Ethyl acetate fraction of bamboo stems	*Phyllostachys nigra* f. *henosis*	PKA/CREB-mediated MITF downregulation	(53)
Kaempferol-7-O-D-glucuronide (K7G) and tilianin	*Cryptotaenia japonica*	CREB- and MAPK-associated signaling pathways	(54)
Flavonoids and polyphenolic compounds			
Pomegranate concentrate powder	Pomegranates	Inactivation of p38 and PKA signaling pathways to reduce the phosphorylation of CREB and MITF	(55)
Sorghum ethanolic extract	Sorghum	Suppression of PAX3-mediated MITF gene promoter activity	(59)
Terpenoids			
Zerumbone	*Zingiber officinale*	Increased phosphorylation of ERK1/2 to downregulate MITF	(69)
Ganodermanondiol	*Ganoderma lucidum*	Inhibition of the MAPK cascade and cAMP-dependent signal pathway	(68)
N-hexane fraction rich in methyl linoleate and methyl linolenate	*Sageretia thea*	Suppression of the AKT/GSK3β signaling pathway	(61)
Hinokitiol	Cupressaceous plants	Inhibition of the AKT/mTOR signaling pathway	(60)
Ginsenoside Rg3	*Panax ginseng*	Activation of ERK to downregulate MITF	(62)
Polysaccharides			
Ganoderma lucidum polysaccharide	*Ganoderma lucidum*	Inhibition of cAMP/PKA and ROS/MAPK signaling pathways	(65)
S. japonicus extracts	*Stichopus japonicus*	Inhibition of ERK activation and reduction of the expression of MITF	(66)
Alkaloids			
Betaine	Crustaceans, beetroot	Inhibition of cAMP/PKA/CREB signaling and activation of AKT-GSK3β signaling, leading to the degradation of MITF	(63)

Table I. Continued.

Structure type	Source	Mechanism of action	(Refs.)
Lignanoids			
Gomisin N	*Schisandra chinensis*	Inactivation of the PI3K/AKT and MAPK/ERK signaling pathways	(57)
Quinolines			
3,8-Dihydroxyquinoline (jineol)	*Scolopendra subspinipes mutilans*	Activation of ERK1/2 and p38 MAPK signaling, leading to the proteolytic degradation of MITF	(64)
Fatty acids			
Linoleic acid and oleic acid	Spent coffee grounds	Downregulation of cAMP/PKA, PI3K/AKT and MAPK signaling pathways	(67)

cAMP, cyclic AMP; GSK-3β, glycogen synthase kinase-3β; PKA, protein kinase A; MITF, microphthalmia-associated transcription factor; CREB, cAMP response element binding protein; GSK-3β, glycogen synthase kinase-3β.

in B16 murine melanoma cells (93,94). A list of other recently discovered flavonoids that have been reported to inhibit the activity of TYR is shown in Table II (95-104).

Phenylpropanoids and olefinic unsaturated compounds, which include ferulic acid, benzaldehyde (105), astaxanthin, curcumin and cinnamic acid esters (106), have been revealed to exert inhibitory effects on TYR. According to a study by Park *et al* (107), ferulic acid, one of the main phenolic components found in *Tetragonia tetragonioides*, suppressed melanin synthesis by reducing the expression of TYR and MITF in B16-F10 cells at concentrations of between 5 and 20 μM. Additionally, Rao *et al* (108), Niwano *et al* (109) and Tu *et al* (110) demonstrated that astaxanthin and curcumin exhibit suppressive properties on melanin synthesis and cellular TYR activity. Other typical agents with reported inhibitory activities on TYR include kojic acid (111,112), methyl gentisate (113,114), ganodermanondiol (71,115), 10-hydroxy-2-decenoic acid (116), *Stichopus japonicus* extracts (69) and bis (4-hydroxybenzyl)sulphide (117). Information on their specific respective mechanisms of action are shown in Table II.

Post-translational regulation of TYR. Substances that can regulate melanin synthesis by affecting protein levels of the melanogenic enzymes without any changes in mRNA levels likely regulate the activity of melanogenic enzymes at post-translational levels. Post-translational modification of components in this pathway primarily lead to the inhibition of melanin synthesis. Currently, two main pathways are known for the degradation of TYR, namely proteasomal and lysosomal degradation (118,119). Unsaturated fatty acids, including oleic acid (C18:1), linoleic acid (C18:2) and α-linolenic acid (C18:3), have been demonstrated to accelerate the protein degradation of TYR by activating one of these two pathways, leading to anti-melanogenesis activity (120). These agents downregulate intracellular TYR protein levels by promoting ubiquitin-dependent degradation, inhibiting melanin synthesis and suppressing hyperpigmentation. According to previous studies by Park *et al* (121) and Lee *et al* (122), terrein, a novel fungal metabolite reduces TYR expression by downregulating MITF in a manner that is dependent on ERK activation, with its inhibitory effects on melanin synthesis prolonged by ubiquitin-mediated proteasomal degradation. By contrast, lysosomes can also target TYR for degradation. Geoditin A, an isomalabaricane triterpene compound derived from the South China Sea Sponge *Geodia japonica*, has been previously found to suppress melanogenesis by post-translational regulation in the endoplasmic reticulum and the degradation of TYR in the lysosome (123). Resveratrol, a promising pigment-lightening flavonoid found in red wine, was recently found to suppress TYR expression not via the inhibition of MITF expression, but by directly inhibiting TYR activity by a post-translational modification that reduces the levels of fully mature TYR protein (119). Retention of misfolded TYR proteins in the endoplasmic reticulum results in the loss of pigmentation, which has also been proposed to be one of the major post-translational mechanisms responsible for the effects of resveratrol.

Inhibition of melanin dispersion. Following melanin synthesis, one key step of melanogenesis in the skin is the translocation

Table II. Tyrosinase inhibitors from natural sources and characterization of the active compounds.

Structure type	Source	Mode of action		(Refs.)
		Mechanism	IC_{50}	
Anthraquinones				
Hydroquinone	Coffee, cranberries and blueberries.	Alternative substrate	75 μM^a	(74,75)
Arbutin	Cranberries, blueberries, wheat and pears	Alternative substrate	0.17 μM^a; 4.0 μM^b	(76,77)
Flavonoids				
Epigallocatechin gallate	Tea	Copper chelation	-	(80)
Quercetin	*Sophora japonica*	Competitive inhibition	30.8 μM^a	(81)
Aloesin	Aloe vera	Competitive inhibition, Dopa oxidation	0.17 mM^a	(82,83)
Glabridin, semilicoisoflavone B, allolicoisoflavone B	*Glycyrrhiza*	****	0.43 μM^a; 0.25 μM^a; 0.80 μM^a	(87,88)
Anthocyanosides	Black soya bean, red grapes	Scavenging free radicals	-	(96)
Flavonoid compounds and ferulic acid	*Spiranthes sinensis*	Copper chelation	0.21 mg/ml^a	(95)
2,4,2',4'-Hydroxycalcone	*Morus australis*	Competitive inhibition	0.21 μM^a	(93)
Fleminchalones A-C	*Flemingia philippinensis*	Competitive inhibition	1.28 μM^a	(92)
Baicalin	*Scutellaria baicalensis*	Inhibition of tyrosinase expression and activity	-	(97)
Soybean extracts	Soybean	Inhibition of tyrosinase expression and activity	-	(91)
Carthamus yellow	*Carthamus tinctorius*	Competitive inhibition	1.01 mg/ml^a	(94,100)
Coumestrol	*Medicago sativa*	Inhibition of tyrosinase expression	-	(98)
Resveratrol analogs				
Resveratrol	Grape, *Polygonum cuspidatum*	Alternative substrate	54 μM^a; 250 μM^b	(85,86)
Phenylpropanoids				
Ferulic acid	*Tetragonia tetragonioides,* Angelicae Gigantis Radix	Inhibition of tyrosinase expression		(103)
Cinnamic acid esters	*Origanum vulgare*	-	16.13 μM^a	(102)
Benzaldehyde	Cinnamon oil, bitter almond oil	Partial noncompetitive inhibition	31.0 $m\mu M^a$	(101)
Olefinic unsaturated compounds				
Astaxanthin, astaxanthin esters	Green alga *Haematococcus pluvialis*	Inhibition of tyrosinase expression	-	(104,105)
Curcumin	*Curcuma aromatica Salisb.*	Inhibition of tyrosinase expression	0.18 mM^a	(106)
Others				
Kojic acid	*Aspergillus* solid culture	Copper chelation	6.2 μM^a; 250 μM^b	(107,108)
Bis(4-hydroxybenzyl)sulfide	Rhizome of *Gastrodia elata*	Copper chelation	0.53 μM^a	(114)
Methyl gentisate	Radix gentianae	Copper chelation	11.2 μM^a; 30.9 μM^b	(109,110)
Ganodermanondiol	*Ganoderma lucidum*	Inhibition of tyrosinase expression	-	(111,112)

Table II. Continued.

Structure type	Source	Mode of action		(Refs.)
		Mechanism	IC_{50}	
10-Hydroxy-2-decenoic acid	Royal jelly	Inhibition of tyrosinase expression	-	(113)
S. japonicus extracts	*Stichopus japonicus*	Inhibition of tyrosinase expression	-	(66)

[a]Kinetics study on mushroom tyrosinase; [b]kinetics study on melanocyte or melanoma cell cultures.

of mature melanosomes into keratinocytes, which are then transported up to the epidermidis where the melanin is dispersed. Therefore, agents that can inhibit the transfer of melanosomes and/or accelerate epidermal turnover can result in the whitening of the skin.

Inhibition of melanosome transfer. A number of studies have previously proposed regulatory mechanisms of melanosome movement in dendrites and interplay between keratinocytes and melanocytes during the transfer process (124,125). In this regard, early skin-whitening compounds, including niacinamide and soybean extracts, are reported to interfere with this process. Niacinamide is has been shown to reduce pigmentation by inhibiting melanosome transfer using a skin-co-culture model (126), whilst soymilk and soybean extracts have been previously suggested to inhibit protease-activated receptor 2 activation in the skin, which may enhance pigment transfer that results in skin whitening (127,128). In addition, it was recently reported that ginsenoside F1 exhibited skin lightening effects by disrupting melanin transfer from the basal layer of melanocytes to the upper layer of keratinocytes (129). Further microscopic research revealed that melanosome transport between cells requires a number of steps, including bidirectional long-range transfer to the apical surface on microtubules, transfer to actin filaments, irreversible short-range transfer by actin dynamics followed by binding to the cell membrane (130). A number of important molecules, including Rab27A, melanophilin (MLPH) /SLP homolog lacking C2 domains-A, synaptotagmin-like protein (SLP) 2A/synaptotagmin 2 and myosin Va are involved in the regulation of melanosome transport (130,131). Kudo *et al* (132) reported that O-methylated flavones extracted from *Scutellaria baicalensis* Georgi, such as wogonin, can inhibit the transport of intracellular melanosomes by degrading melanophilin (MLPH), a carrier protein associated with melanosome transport on actin filaments. Additionally, gagunin D, a highly oxygenated diterpenoid from the marine sponge *Phorbas* sp., was also found to exhibit anti-melanogenic properties by downregulating the expression of proteins associated with melanosome transfer, including Rab27A, MLPH and myosin Va (133). Therefore, these observations suggest that downregulating the expression and activity of the aforementioned proteins associated with melanosome transport may be useful for reversing the process of skin hyperpigmentation.

Inhibition of melanin dispersion and acceleration of epidermal turnover. A number of compounds have been documented to possess the capacity to inhibit the dispersion of melanin granules and accelerate skin turnover, which can result in a lighter skin tone. Topical application of these compounds to the skin has been demonstrated to effectively reduce the visibility of skin spots without affecting their size or quantity, which can be used for treating melasma. Examples of these compounds include α-hydroxy acids, salicylic acid, linoleic acid and retinoic acids, which can promote cellular renewal and facilitate the elimination of melanized keratinocytes, leading to the loss of melanin pigmentation (134,135). However, the application of those acids is associated with side effects including erythema, scaling and increased risk of sunburn (136-138). Therefore, current research efforts are mainly focused on the

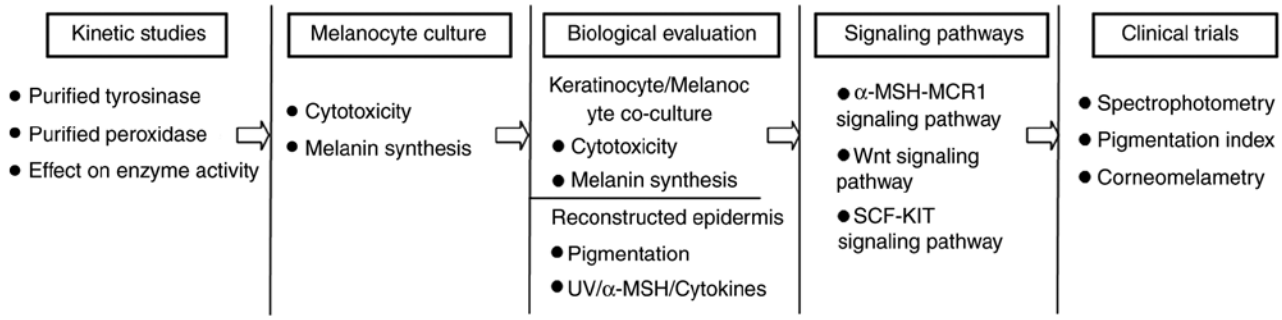

Figure 3. Recommended multi-step research strategy for the evaluation of prospective skin-whitening agents.

discovery of novel components of natural compounds with minimal off-target effects. Liquiritin, a flavonoid glycoside of liquorice, has previously been shown to significantly reduce hyperpigmentation in 20 women with a clinical diagnosis of melasma. The mechanism was proposed to be associated with melanin dispersion mediated by the pyran ring of the flavonoid chemical structure and the acceleration of epidermal turnover (139). This suggest flavonoids to be promising candidates for the development of safe and effective interventions for hyperpigmentation.

5. Conclusions

Over the past few years, significant progress has been made on the understanding of melanocyte biology and its underlying mechanism, opening up new research avenues for the discovery of novel melanogenesis inhibitors. In addition to the direct suppression of TYR catalytic activity, other methods for the disruption of melanogenesis include the post-transcriptional control of TYR, regulation of melanosome transfer and the suppression TYR transcription by suppressing upstream signaling pathways. These mainly involve the inhibition of the master regulator MITF, resulting from the reduction of intracellular cAMP levels, increases in cytoplasmic β-catenin and/or the activation of ERK signaling. Therefore, a large number of inhibitors acting through these aforementioned alternative mechanisms have been successfully identified (73).

Among these inhibitors, a mild, stable, safe and effective compound is sought from natural extracts as a raw material for the development of skin-whitening and skin care products. For such prospective melanogenesis inhibitors, further *in vitro*/*in vivo* studies and clinical trials are required to evaluate efficacy and safety. To accelerate the process of drug discovery, a variety of models and methodologies should be applied to assess their potential hypopigmentation activity. From a methodological perspective (140,141), a multi-step process should be adopted for these investigations (Fig. 3). Initial evaluation of whitening properties *in vitro* should be conducted on purified TYR and/or other melanogenic proteins, followed by the use of melanocyte cultures to examine potential cytotoxic and melanin synthetic effects. For further biological evaluation, co-culture systems and reconstructed skin models should be adopted to screen for the ability of the novel compounds to interfere with the melanogenesis process, especially following stimuli including UV irradiation, exposure to a-MSH or proinflammatory cytokines. In addition, investigation into the regulatory mechanism involved in melanogenesis should be performed. Finally, the *in vivo* activity of the prospective agents should be evaluated using non-invasive techniques such as UV light photography or spectrophotometry to obtain comparable results (12,142). It is expected that the aforementioned research methodology can provide better opportunities for the development of novel lightening agents that are effective and safe for use in the clinical and cosmetic industries.

Although promising, the use of skin-lightening compounds requires further research due to diverse modes of action or off-target effects (77). Kojic acid and arbutin remain the classic compounds that can be topically used as skin-lightening agents in a clinical setting due to proven efficacy. Additional natural skin-lightening compounds, including mulberry, licorice and lemon extract, are regularly supplemented into skin care products, to strengthen the effect of arbutin or kojic acid (143,144). The ideal skin-lightening cosmetic product should include a formulation that comprises compounds acting on different pathways during the melanogenesis process. This future combination should contain multiple targets and layers, including the control of TYR expression on transcription and protein levels, inhibition of enzymatic activity in the melanogenesis pathway, suppression of melanocyte proliferation and the transport of melanosomes on a cellular level. Although the mechanisms of these inhibitors have been well characterized *in vitro*, they have not been topically applied in cosmetics and cosmeceuticals. Therefore, further evaluation of their skin-whitening activity *in vivo* or in parallel human clinical trials are required. In summary, from a clinical point of view, additional mechanistic investigations of the novel natural modulators on melanogenesis are urgently required.

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Authors' contributions

GG and HS designed the theme of the review. WL, YC and AT retrieved the relevant literature. WQ wrote and reviewed the article. DZ helped to revise the manuscript and provided important intellectual revision suggestions.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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