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ELABORATO DI LAUREA

Methods for chemical inhibition of melanin synthesis

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Abstract

Melanin is a heteropolymeric pigment essential for protecting human skin from UV-visible radiation through broad-spectrum absorption. It is produced within melanocytes and stored in melanosomes. Melanogenesis begins with the activity of tyrosinase, a membrane-bound enzyme that converts L-tyrosine into DOPAquinone. Dysregulated tyrosinase activity contributes to several skin disorders, including hyperpigmentation, oxidative stress and an increased risk of malignant melanoma (Xinhua Ni, 2025).

Over the years, extensive research has focused on understanding how tyrosinase functions and how its activity can be modulated. Numerous tyrosinase inhibitors have been identified and are now widely used in dermatology, cosmetology and clinical treatments for pigmentation disorders. These compounds act through different mechanisms; some directly inhibit tyrosinase, while others interfere with various steps of the melanogenesis pathway.

This work aims to summarize the current knowledge on melanin synthesis and to examine key chemical inhibitors involved in its regulation, ranging from commonly used molecules such as glabridin and kojic acid to newer approaches including the cyclopeptide CHP-9 and salicylic acid. Several of these modulators have also shown potential in melanoma studies, highlighting their value not only in pigmentation control but also as possible therapeutic agents.

1. Introduction

1.1. Melanin

Melanin is a family of heteropolymeric pigments widely distributed in animals, plants and microorganisms. The word “melanin” derives from the Greek word “melanos”, which means black or dark. The name is usually attributed to the chemist Berzelius, who in 1840 used it to refer to the dark-brown pigment that was isolated from the eye membranes (P.A. Riley, 1997). Today we know that the term melanin refers to a wide number of pigments that range from black and brown colors to yellow and red. According to their features, the pigments can be classified into five categories: eumelanin, neuromelanin, pheomelanin, allomelanin, and pyomelanin (in humans, we can find eumelanin, pheomelanin, and neuromelanin). Depending on the organism from which the melanin is extracted, it can have different shapes, like round, granular, shallow, and empty, or round and slender, and have different exterior colors (Wen Song et al., 2023).

This work will concentrate on eumelanin and pheomelanin, which are responsible for dark and light color phenotype of human skin. Melanin is responsible for protection from UV light likely due to its structure. As already mentioned, melanin has a heterogeneous structure, thanks to the presence of different chemical species, and is usually represented by covalently linked indoles. There are different substitutes within the same eumelanin: this heterogeneous structure provides this polymer the property to absorb a broad range of wavelengths. Eumelanin (the darker family of melanin) contains a higher amount of indole quinones and has a strong absorbance in the red spectrum, thereby providing dark coloration. Pheomelanin is characterized by a red and yellow pigmentation, due to the lower amount of carbonyl groups in its structure.

Melanin pigment represents a physical barrier against UV irradiation. While UVC (200-280 nm) does not reach the earth surface thanks to the ozone layer, UVA (320-400 nm) and UVB (280-320 nm) are not absorbed by the ozone layer. Thereby, they cause a wide variety of effects on the human body, such as erythema, mutation and immunosuppression. UVB is more cytotoxic, being a direct cause of DNA mutation, but UVA can penetrate deeper into the skin and generate reactive oxygen species (ROS), causing indirect DNA damage. Thanks to the presence of melanin in the epidermis, absorption of ultraviolet radiation (UVR) by melanin can occur before it reaches the deeper parts of the skin and create damage to the cells.

Apart from photoprotection, melanin also has other important functions in the human body, such as chemoprotection from free radicals and toxic cations (P.A. Riley, 1997).

1.2. Melanogenesis

Melanin synthesis takes place inside epidermal cells called melanocytes. When it is fully synthesized, it is stored in a specific organelle named a melanosome. Melanocytes can be found in the basal stratum of the epidermis, the epithelial tissue of skin, and they mature from the neural crest during the development of the embryo. The precursors of melanocytes, called melanoblasts, develop from the neural crest and then migrate to their final location (skin, hair bulbs, eyes, leptomeninges), subsequently developing into melanocytes. In the skin, melanoblasts can be found in the dermis, where they mature into melanocytes, and then migrate to the epidermis. Melanocytes transfer melanosomes to other differentiated cells of the epidermis, keratinocytes.

The major enzyme responsible for melanin synthesis is tyrosinase, which biogenesis involves microphthalmia-associated transcription factor (MITF), an important transcription factor that regulates the expression of melanosomal proteins.

Melanosomal proteins can be structural proteins, such as premelanosome protein (PMEL), and enzymatic proteins like tyrosinase (TYR), tyrosinase-related protein 1 (TRP-1) and tyrosinase-related protein 2 (TRP-2). After MITF gene enhancement, tyrosinase (TYR) is co-translated in the endoplasmic reticulum (ER). Here, TYR maturation requires two different classes of molecular chaperones: Binding Immunoglobulin Protein (BiP), a HSP70 family member (Heat Shock Protein 70 kilodalton) that recognises unfolded regions; lectin chaperones such as calnexin and calreticulin.

Their role is to prolong ER retention and facilitate copper incorporation in the active site and conformational folding. A correct folding requires a suitable retention time; one of the most common mutations of TYR from improper folding causes depigmentation and is associated with human Oculocutaneous Albinism type 1 (OCA1) (Hidenori Watabe et al., 2004). Other studies demonstrate TRP-1 also being an important chaperone in the ER for TYR maturation (Toyofuku K., et al., 2001): proper folding and maturation of TYR in the ER is essential for its activity. While being co-translated, tyrosinase is being translocated into the ER lumen by the Sec61 translocon and glycosylated by the oligosaccharide transferase (OST). Chaperones complete the maturation by helping the folding and forming homodimers. The tyrosinase is then packed into Coat protein complex II (COPII) vesicles and goes through the secretory pathway by entering the *cis*-Golgi network. In the *trans*-Golgi, the N-linked glycans are further modified and complex sugars are added; coppers are inserted in the active site. TYR and tyrosinase-related proteins leave from *trans*-Golgi in vesicles and develop into melanosomes. Inside these vesicles, tyrosinase starts to form an ordered pattern, and melanin biosynthesis takes place.

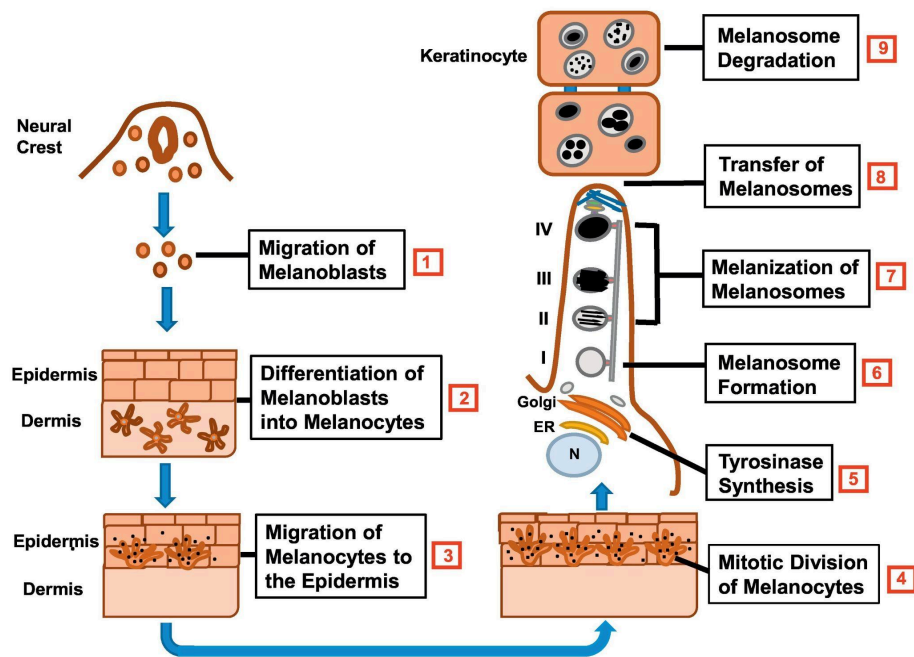


Figure 1 (Muriel W. Lambert, 2019): stages of melanocyte development, the formation of melanosome and melanization.

Melanosomes pass through 4 maturation stages (Figure 1): in stage I, the melanosome is a spherical vesicle that contains tyrosinase and filamentous proteins. In stage II, it gets into an ellipsoidal shape with membranous filaments of PMEL (premelanosome protein), organized into parallel fibrils. Between stage II and stage III, melanogenesis starts: the amino acid L-tyrosine is oxidized to L-dopa (L-dihydroxyphenylalanine) by the enzyme tyrosinase, which also catalyzes the conversion of L-dopa to L-dopaquinone. Dopaquinone can undergo different chemical reactions, to produce both pheomelanin and eumelanin. To form the latter, dopaquinone spontaneously cyclizes in dopachrome (indolene-2-carboxylic acid-5,6-quinone), from which two pathways depart: tautomerization of dopachrome is catalysed by TRP-2 (tyrosinase-related protein-2) in DHICA (5,6-dihydroxyindole-2-carboxylic acid) and then in DHI (5,6-dihydroxyindole), probably by TRP-1 (the function is still not clear). In stage IV, melanosome synthesis is completed, and the internal structure of the organelle is electron opaque, appearing as a black organelle via electron microscopy.

When the organelle is complete, it is transported through microtubules due to kinesin's bonds to the tips of the melanocyte dendrite structures. Here, motor proteins along microtubules to the melanocyte dendrites translocate the melanosome to the neighboring keratinocytes (1 melanosome surrounded by 36 keratinocytes).

Because the mechanisms are not yet completely understood, there are different theoretical models of the transfer (Xufeng Wu, 2014): cytophagocytosis, membrane fusion, shedding-phagocytosis, and exocytosis-endocytosis.

1.3. Intracellular pathways that induce melanin synthesis

α -MSH/cAMP-dependent signaling pathway

Several signaling pathways regulate the expression of melanogenic enzymes. One of these pathways involves a seven-transmembrane G protein-coupled receptor residing in the membrane of melanocytes: the melanocortin 1 receptor (MC1R). MC1R is activated by melanocortins, a family of hormones that regulates different subcellular pathways such as melanin synthesis, inflammatory response and fibrotic scarring; α -MSH (alpha-melanocyte stimulating hormone) is the hormone responsible for the stimulation of melanogenesis. Some genetic studies have been made in *Mus musculus*, and a loss-of-function mutation on the MC1R locus showed that when the locus is recessive, *yellow* for the mutation, the mice assume a yellow coat color (Robbins LC et al., 1993).

When MC1R is activated, an intracellular pathway mediated by the second messenger cAMP (thereby known as “cAMP pathway”) is triggered: MC1R activates its coupled $G_{\alpha s}$ (G_s protein alpha subunit), leading to adenylyl cyclase (AC) activation, which promotes the synthesis of cAMP and activation of the kinase PKA (protein kinase a). PKA then translocates to the nucleus, where it activates transcription factors such as CREB (cAMP response element-binding protein), which in turn enhance the transcription of the MITF gene (microphthalmia transcription factor). MITF is important for melanogenesis as it upregulates the transcription of key melanogenic enzymes, such as tyrosinase, TRP-1, and TRP-2.

MAPK signaling pathway

MITF expression levels are controlled also via the MAPK (mitogen-activated protein kinase) signaling pathway. MAPKs are protein kinases activated by agents that stimulate cell division (mitogens), and include several members, among which ERK (extracellular signal-regulated kinases), JNK (c-Jun N-terminal kinases), and p38 MAPK. Depending on the MAPK activated, melanogenesis can be stimulated (p38 promotes MITF transcription) or inhibited (ERK activation reduces MITF transcription).

Wnt/ β -catenin signaling pathway

The Wnt signaling pathway is one of the key regulators of MITF; Wnt binds to the G protein-coupled receptor (called Frizzled), leading to the inhibition of GSK3 β and the subsequent accumulation of β -catenin. The accumulated β -catenin forms a complex with the transcription factor LEF-TCF and is transported into the nucleus, where it promotes MITF expression. Elevated levels of nuclear β -catenin thus enhance MITF activity, ultimately stimulating melanogenesis.

2. Chemical inhibitors of melanogenesis

2.1. Modulators targeting enzymes (tyrosinase)

Tyrosinase (TYR) is the critical enzyme that regulates melanogenesis by catalyzing the conversion of L-tyrosine to DOPAquinone, two oxidations that initiate the synthesis of melanin. Tyrosinase is a membrane-bound glycoprotein containing two copper atoms in its active site (CuA and CuB), each binding three histidine residues: CuA linked to His162, 184, and 193; CuB to His345, 349, and 371. Indeed, the copper-binding domains with these six residues of histidine are the only conserved domain between different species (Ning Wang, 2006).

Because tyrosinase controls this crucial initial phase of melanogenesis, it holds a primary role in determining the amount and rate of melanin formed in melanocytes. Dysregulated or excessive tyrosinase activity is responsible for a variety of skin-related and systemic problems, including hyperpigmentation disorders such as freckles, pigmented acne, chloasma, and age spots; It might also increase the risk of malignant melanoma (Xinhua Ni, 2025). For these reasons, tyrosinase has been the main subject of extensive research: scientists aim to learn how to modulate its activity, in order to develop effective strategies for preventing and treating pigmentation disorders.

One of the most common uses of tyrosinase inhibitors is for whitening treatment. In Asian countries, there is a wide production of skin-lightening products containing tyrosinase inhibitors, Asian cultures being the main reason: light skin is associated with beauty, youth and status. Numerous natural and synthetic compounds with tyrosinase-inhibiting properties have been identified, including hydroquinone, azelaic acid, arbutin, licorice extract, sodium ascorbyl phosphate (an active form of vitamin C), mandelic acid, glycine hydroxamate, mimosine, magnesium ascorbyl phosphate, various hydroxy acids, and kojic acid; all used to treat hyperpigmentation disorders.

The aim of this work is to describe the main chemical modulators of melanin synthesis and the subcellular mechanism that compounds use to block melanogenesis; their potential use in treating hyperpigmentation, melanoma and also their use in dermatology and cosmetology.

Kojic acid

Kojic acid (5-hydroxy-2 [hydroxymethyl]-gamma-pyrone; $C_6H_6O_4$) became widely recognized for its main role as a skin-lightening compound. It is commonly used in skincare formulations because it helps protect the skin from UV radiation and reduces hyperpigmentation by inhibiting tyrosinase activity, and its use is approved by the FDA (U.S. Food and Drug Administration).

It is an organic acid that functions as a chelant agent and it's produced by several types of fungus; it has an antibacterial effect produced by aerobic fermentation of glucose in *Aspergillus oryzae* and *Candida*. Kojic acid is not only used in cosmetology but also in the food industry thanks to its ability to sequester heavy

metal ions that speed up oxidation and to inhibit the enzyme that deteriorates food, preventing food browning.

Kojic acid derivatives have been widely developed to improve the original compound's activity and stability, as kojic acid easily oxidizes and therefore loses effectiveness. Numerous derivatives have been synthesized, many showing strong antibacterial, antifungal, anti-inflammatory, and skin-lightening properties. Some derivatives, such as resorcinol and cinnamamide, exhibit potent tyrosinase inhibition without cytotoxicity, while others, like kojic monooleate or phosphonate derivatives, display stronger depigmenting effects than kojic acid itself (Shifali Chib, 2023).

The inhibition of tyrosinase synthesis happens because kojic acid chelates copper ions at the active site of tyrosinase, which is essential for the enzyme's catalytic function. As a result, the conversion of L-tyrosine and L-DOPA into melanin intermediates is halted, effectively decreasing pigment synthesis. Overall, kojic acid and its derivatives are considered safe for human use and represent promising agents for medical and cosmetic applications.

Glabridin

Glabridin is an isoflavan discovered in 1976 from the root of *Glycyrrhiza glabra*. Beside the inhibition of melanogenesis, it has other useful properties and uses, such as anti-inflammatory and anti-cancer properties, neuroprotective effects and antioxidants (Jianmin Chen, 2016). Glabridin has shown strong tyrosinase inhibitory activity, even greater than that of kojic acid. It appears to be safe for human melanocytes, though its overall safety in living organisms is still uncertain. A major limitation is its tendency to oxidize easily, which reduces its stability and effectiveness. Because the exact molecular mechanism of action remains unclear, recent studies are undergoing to design more stable glabridin analogues and understand how its structure influences tyrosinase inhibition. Beside that, it is very widely used as an ingredient in cosmetics, and it's listed in the International Nomenclature of Cosmetic Ingredients (INCI) by the term "Glabridin-40". Glabridin strongly inhibits tyrosinase activity through a reversible, non-competitive mechanism. It doesn't interact with the enzyme's catalytic active site; instead, it binds to a separate region near the catalytic core, altering tyrosinase's function. Kinetic studies show that increasing glabridin concentrations reduce the enzyme's activity without affecting substrate binding, confirming non-competitive inhibition (Jianmin Chen, 2016).

In the same study, by Jianmin Chen, kojic acid was used as a positive control to study glabridin inhibition: 50% activity loss (IC_{50}) of mushroom tyrosinase was observed at a concentration of glabridin and kojic acid respectively at 0.43 and 75.74 $\mu\text{mol/L}$; this shows that glabridin, compared to kojic acid, has a much higher inhibitory activity. However, it does not inhibit melanin synthesis in zebrafish, suggesting species-specific differences or limited effect on vertebrate tyrosinase.

Arbutin

Arbutin is a naturally occurring hydroquinone glucoside widely distributed across the plant kingdom, having been identified in nearly 50 different plant families; since its discovery in *Arbutus unedo*, arbutin has become one of the most widely used skin-whitening agents due to its ability to inhibit tyrosinase. Beyond its dermatological applications, arbutin exhibits a broad spectrum of biological properties, including antioxidant, antimicrobial and anti-inflammatory effects. The most widely accepted mechanism is that arbutin acts as a competitive inhibitor of TYR by binding to the enzyme's active site due to its structural similarity to L-tyrosine. This prevents access of the natural monophenolic and diphenolic substrates, decreasing both activities. Arbutin interacts with tyrosinase in two distinct ways depending on the availability of L-DOPA. When L-DOPA is present, tyrosinase operates normally and arbutin can even be oxidized as an alternative substrate without disrupting the catalytic cycle. However, in the absence of L-DOPA or other diphenolic substrates, tyrosinase accumulates in its E_{met} state, a resting form that requires a diphenol to return to activity. The tyrosinase enzyme operates through a catalytic cycle involving three mutually converted forms: E_{met} , E_{deoxy} , and E_{oxy} (Yong Chool Boo, 2021). The E_{deoxy} form binds oxygen to create the E_{oxy} state, which can then capture a monophenol (M) or diphenol (D) substrate. When E_{oxy} reacts with a monophenol substrate, it eventually converts to E_{met} -D, releases a quinone product (Q), and generates E_{deoxy} . If E_{oxy} reacts with a diphenol substrate, E_{met} is formed as an intermediate. This E_{met} state is crucial because it can bind another diphenol substrate to complete the monophenolase cycle by regenerating E_{deoxy} . In conditions lacking diphenol substrates, E_{met} can bind to a monophenol inhibitor, such as arbutin and form a non-productive "dead-end" complex that traps the enzyme and prevents it from re-entering the catalytic cycle. As a result, arbutin does not merely compete with natural substrates but can actively inactivate tyrosinase when diphenols are lacking, providing an additional mechanism by which it suppresses melanogenesis. Arbutin also reduces melanogenesis by lowering intracellular oxidative stress; by shifting the redox balance, arbutin also favors pheomelanin production over eumelanin.

Azelaic acid

Azelaic acid (AZA) inhibits melanogenesis primarily through its direct action on tyrosinase. As a saturated dicarboxylic acid, AZA is structurally capable of interacting with the catalytic machinery of the enzyme, and to act as a competitive inhibitor of tyrosinase, blocking the conversion of tyrosine to L-DOPA and then to DOPA-quinone. AZA has a role in also interfering with the thioredoxin/thioredoxin-reductase system (Trx/TrxR), one of the cell's major regulation systems of redox, keeping the proteins in the correct oxidation state by transferring electrons. This system usually donates electrons to tyrosinase: by inhibiting thioredoxin reductase, AZA disrupts this regulatory loop, reducing the

catalytic efficiency of tyrosinase and contributing to its overall depigmenting action. Also, AZA acts more strongly on melanocytes that are overproducing melanin, such as those in melasma, lentiginos or post-inflammatory hyperpigmentation. These abnormal melanocytes have higher tyrosinase activity and higher oxidative stress; because AZA targets tyrosinase and redox-sensitive pathways, it affects these cells much more than normal melanocytes. In contrast, normal melanocytes, which have low basal tyrosinase activity and balanced redox conditions, aren't strongly inhibited by azelaic acid (Andrew Fitton, 2012). As a result, AZA can reduce excess pigmentation only when it's abnormal, without damaging the surrounding healthy skin.

Ellagic acid

Ellagic acid is a natural polyphenolic compound widely used as a depigmenting agent. Its precise mechanism of action within the melanogenesis pathway is more complex than a simple tyrosinase inhibitor; in fact, it acts as an alternative substrate of tyrosinase. The enzyme oxidises ellagic acid to an unstable o-quinone, demonstrating measurable monophenolase/diphenolase activity toward this molecule. In addition, ellagic acid has significant antioxidant effects, which plays a major role in its ability to reduce melanin synthesis. In fact, during melanogenesis, the oxidation of o-diphenols mediated by tyrosinase generates reactive intermediates such as o-quinones and semiquinones, as well as reactive oxygen species (ROS). Ellagic acid scavenges reactive intermediates far more effectively than ascorbic acid, shifting the redox equilibrium and limiting the formation of key melanogenic products. By reacting with o-quinones and semiquinones from substrates like L-DOPA, it reduces DOPAchrome production and slows the entire melanogenesis process.

However, the formation of reactive o-quinones from ellagic acid raises considerations regarding its potential cytotoxic effects and its capacity to perturb the cellular redox balance, which are important aspects for its cosmetic application.

CHP-9 cyclopeptide

In a study article by Huailong Chang, 2025, a novel cyclopeptide was discovered as a tyrosinase inhibitor, CHP-9, Cyclo(Gln-Gly-Pro-Gln-Gly-Pro).

The results from this experiment are very relevant for CHP-9's strong inhibition of tyrosinase activity, with a rate of 28.57% inhibition at 1% concentration, almost twice as potent as the positive control, glabridin (which showed about 14% inhibition). The inhibitor action translated directly to a reduction in pigmentation in human melanocytes. Treatment with CHP-9 (1%) alone significantly lowered cellular melanin content, reducing levels from 30.90 ± 1.13 to 23.51 ± 1.14 $\mu\text{g/mL}$. CHP-9 can also enhance the performance of other agents: the highest inhibition rate (34.52%) was observed when CHP-9 was combined with tranexamic acid and nonapeptide-1; using CHP-9 in multitarget approaches may lead to more effective overall suppression of TYR activity.

A final finding indicates that CHP-9, alone or combined with nonapeptide-1 and tranexamic acid, exhibits minimal cytotoxicity on human melanocytes, with cell viability remaining above 90% at effective concentrations (up to 2.5 mg/mL), demonstrating that CHP-9 does not compromise cell viability.

The results of the experiment are very clear, and what was just said is shown in *Figure 3* and *Figure 4*.

The cyclopeptide CHP-9 demonstrated strong tyrosinase inhibitory activity, significantly higher than the standard positive control glabridin. The most potent inhibition was observed when CHP-9 was combined with tranexamic acid and nonapeptide-1, suggesting that multitarget approaches can enhance tyrosinase suppression. These results highlight CHP-9's potential as a safe and effective agent for cosmetic applications, particularly in skin-lightening and hyperpigmentation treatments, and indicate that combining compounds may provide greater efficacy through complementary mechanisms of inhibition.

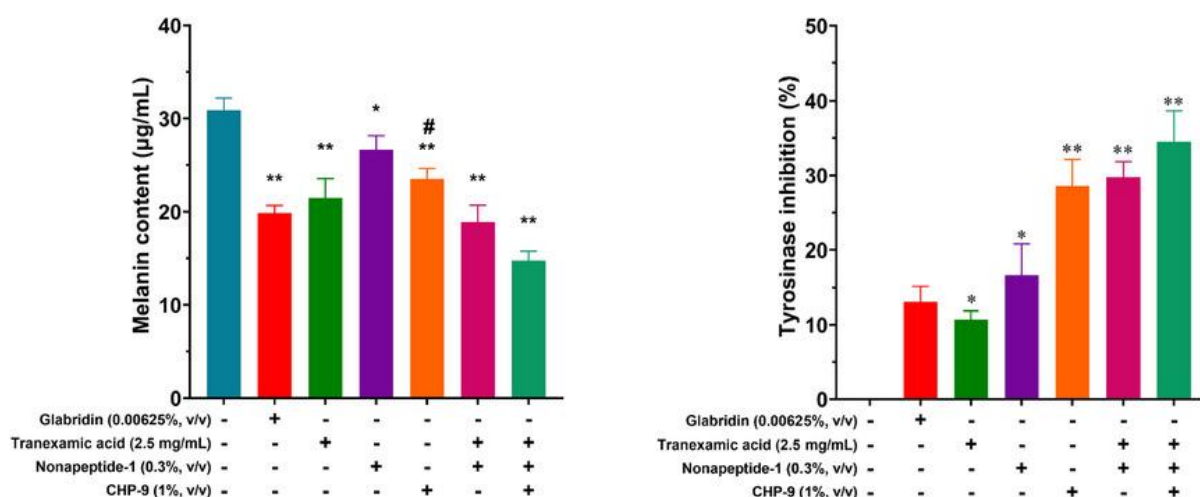


Figure 3 (Huailong Chang, 2025) (left): inhibition of melanin production by presence of cyclopeptides and other compounds.

Figure 4 (Huailong Chang, 2025) (right): tyrosinase inhibition by presence of cyclopeptides and other compounds. * = $p < 0.05$ ** = $p < 0.01$

2.2. Modulators that alter the biogenesis of melanosomes

Melanosomes are specialized organelles within melanocytes responsible for the synthesis, storage and transport of melanin. Their proper formation and maturation are essential for maintaining normal pigmentation and protecting the skin from ultraviolet (UV) damage. Certain compounds can influence these processes by affecting melanosome structure, maturation or intracellular trafficking. Unlike classical tyrosinase inhibitors, which act directly on the enzymatic synthesis of melanin, these modulators exert their effects at the

organelle level, altering the distribution, stability or functional capacity of melanosomes. Understanding these mechanisms provides insights into novel strategies for controlling pigmentation disorders while minimizing cytotoxicity to melanocytes.

Hydroquinone

Hydroquinone (HQ) is a diphenol, an aromatic organic compound widely used in dermatology as a skin-lightening agent. It is a chemical in some prescription creams and it is used to lighten dark spots on the skin. Because of safety concerns, hydroquinone is banned from use in cosmetics in most countries and in EU countries, but can be legally used in prescription creams for targeted dermatological treatments, under strict medical supervision. One of its most serious long-term side effects is exogenous ochronosis, a condition in which the skin develops dark, bluish-black patches: past hypotheses suggested that hydroquinone might inhibit homogentisate dioxygenase (HGD), an enzyme that catalyzes the conversion of homogentisic acid (HGA) into maleylacetoacetate in the tyrosine degradation pathway. Mutations in the HGD gene are typical in patients with alkaptonuria, in which homogentisic acid accumulates in the body.

All in all, the mechanisms behind exogenous ochronosis induced by hydroquinone are still not fully understood. A recent study (Shosuke Ito et al., 2025) has brought renewed attention to the topic, showing evidence that the inhibition of HGD is not involved in exogenous ochronosis. Instead, HQ inhibits the melanin biosynthetic pathway in the step from L-tyrosine to L-DOPAquinone, by acting as a ‘pseudo’ substrate of L-DOPA, thanks to their structural similarity. This leads to the production of potentially harmful metabolites that can promote the formation of ochronotic particles.

It has been shown that HGD is not expressed in human skin, by using gene expression datasets and both by attempting to measure its activity in fibroblasts (Human Protein Atlas): HGD is essentially absent in all skin samples, thereby the authors conclude that HQ-induced ochronosis can't be caused by HGD inhibition. Docking studies showed that hydroquinone and in general small phenols have very low affinity for HGD, and real HGD inhibitors are structurally very different. Instead, HQ could act as a pseudo substrate of tyrosinase, not just an inhibitor. It directly inserts in the melanin pathway: tyrosinase converts L-DOPA in DOPAquinone, which oxidizes hydroquinone forming p-benzoquinone. Cysteine strongly influences the pathways toward HQ-pheomelanin by reacting to p-benzoquinone, a toxic and reactive quinone. This explains why ochronosis occurs only in pigmented sun-exposed areas and not in vitiligo patches. Ochronotic particles form when HQ is converted into reactive quinones inside melanosomes. High-molecular-weight pigments remain in melanosomes and are transferred to keratinocytes, while low-molecular-weight derivatives diffuse into the dermis, binding to collagen and elastotic fibers. UV-induced collagen damage further promotes pigment formation, explaining the localization on sun-exposed

skin. These reactive quinones generated from HQ metabolism are highly cytotoxic and can induce oxidative stress within melanocytes. Their accumulation in melanosomes can disrupt normal melanosome structure and maturation, leading to dysfunctional or partially degraded melanosomes. Additionally, the formation of ROS and the covalent binding of quinones to melanosomal proteins may compromise melanosome integrity and reduce the efficiency of melanin synthesis. Low-molecular-weight HQ derivatives that diffuse into the dermis not only bind to extracellular matrix components but can also interfere with the normal turnover and biogenesis of melanosomes in nearby melanocytes. Prolonged exposure may lead to melanocyte damage or death, further impairing melanosome formation and contributing to the progression of exogenous ochronosis. These findings highlight that the depigmenting effects of hydroquinone are not solely due to direct enzymatic inhibition but also involve broader alterations in melanocyte physiology and pigment processing. This suggests that long-term use may have complex and potentially cumulative effects on skin homeostasis, emphasizing the need for safer alternatives that target hyperpigmentation without compromising melanocyte integrity.

Benzimidazole-2-butanol (BI2B)

Benzimidazole-2-butanol (BI2B) acts as a potent and selective modulator of melanogenesis by targeting a precise signaling pathway that governs melanin production (Song-Hee Kim et al., 2024).

In melanocytes, UVB exposure or α -MSH stimulation normally activates the MC1R-cAMP pathway, leading to phosphorylation of CREB, increased transcription of MITF and subsequent expression of melanogenic enzymes such as TYR, TRP-1 and TRP-2. BI2B disrupts this cascade at an early and highly specific regulatory point. BI2B inhibits the catalytic activity of mitogen-activated protein kinase (MAPK) kinase 3 (MKK3), the kinase responsible for phosphorylating and activating p38 MAPK. As a result, MSK1 (Mitogen- and stress-activated protein kinase-1) is inhibited, preventing the phosphorylation of CREB at Ser133, an essential step for MITF activation: this leads to a significant reduction in MITF levels and a broad down regulation of its target genes, including those required for melanin biosynthesis and for melanosome maturation transfer.

The modulatory action of BI2B is evident both in vitro and in vivo (as shown in the paper by Song-Hee Kim et al., 2024): in melanocyte cultures, BI2B significantly inhibits melanin accumulation induced by UV radiation, α -MSH, or cAMP analogs, without causing cytotoxicity.

In UVB irradiated hairless mice, topical BI2B treatment significantly reduces visual hyperpigmentation, decreases melanin granule deposition and suppresses the expression of MITF in pigmented skin, demonstrating that the compound effectively blocks melanogenesis.

Importantly, BI2B doesn't absorb UVB light, confirming that its anti-melanogenic function originates from biochemical pathway modulation rather than photoprotection.

Inulavosin

Inulavosin is an unreported melanogenesis inhibitor, isolated from *Inula nervosa*. A study by Hideaki Fujita demonstrated that inulavosin reduces melanin production in B16 melanoma cells by selectively destabilizing tyrosinase. Inulavosin disrupts only the late stages of melanosomes formation: melanosomes are the organelles where melanin is synthesized and stored, and mature through four stages. Electron microscopy images showed that control cells were characterized by numerous stage III and IV melanosomes (e.g. the stages in which organelles become loaded with melanin) but cells treated with inulavosin lack these mature structures. At variance, they still display normal stage I and II melanosomes, indicating that early organelle formation is unaffected (*Figure 5*). Immunofluorescence experiments revealed that inulavosin causes tyrosinase to disappear from melanosomes without altering the localization of other key melanosomal proteins, TRP-1, premelanosome protein (PMEL) and LGP85 (Lysosomal Glycoprotein 85). This indicates a selective effect on tyrosinase rather than a general disturbance of melanosome biology.

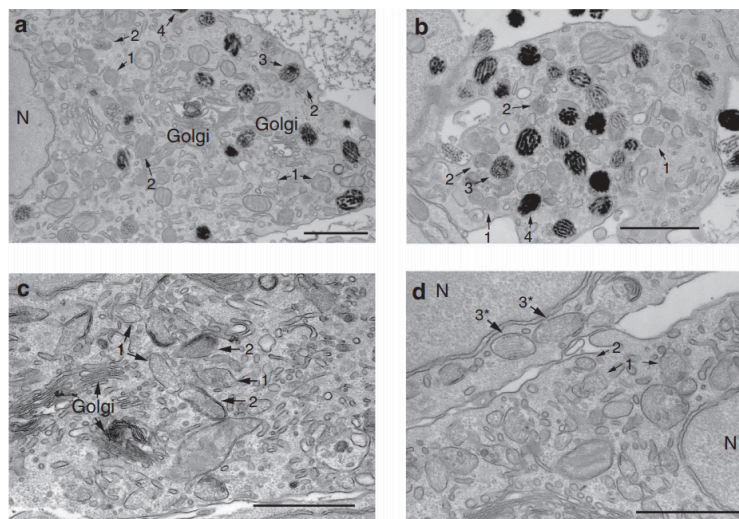


Figure 5 (Hideaki Fujita et al., 2008) : electron microscopic analyses of inulavosin on B16 melanoma cells. (a) and (b) as control cells; (c) and (d) cells are treated with 15 μ M inulavosin for 72 hours. N: nucleus. Stage 1, 2 and 3 melanosomes are shown.

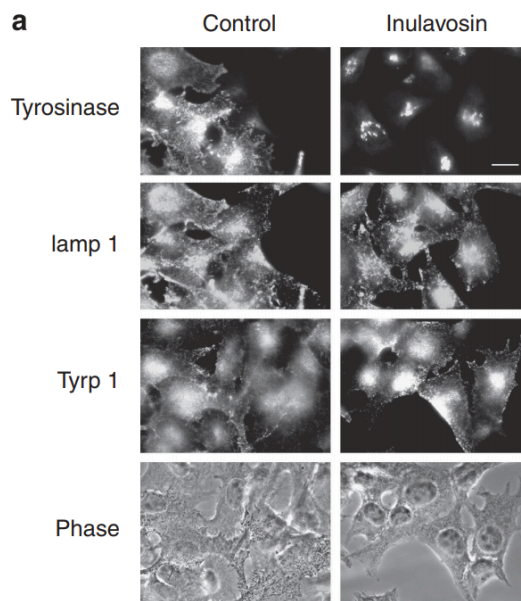


Figure 6 (Hideaki Fujita et al.,2008) (right): immunofluorescence of melanoma cells treated with DMSO (control) and 15 μ M for 72 hours (inulavosin). Fluorescence shows that while tyrosinase is not seen in inulavosin B16 melanoma cells, other key melanosomal proteins are unaltered (lamp 1 and trp-1).

Biochemical analysis further showed that inulavosin doesn't inhibit the enzymatic activities of tyrosinase, TRP-1 or TRP-2, nor it reduces the transcription of their genes. Instead it accelerates the degradation of newly synthesized tyrosinase via lysosomal degradation. Because other melanosomal components remain unaffected, the compound specifically alters tyrosinase trafficking, revealing an important role for lysosomal pathways in controlling pigmentation.

Oxyresveratrol

Oxyresveratrol is a natural stilbene derivative found in *Moraceae* plants, known for its antimelanogenic properties. It's associated with antioxidant, anti-inflammatory, anti-melanogenic and anticancer effects. In a study by Jianhua Zhang (202), the impact on melanin transfer was clarified. Oxyresveratrol decreases cAMP levels and downregulates MC1R (Melanocortin 1 Receptor), MITF (Microphthalmia-associated transcription factor) and its regulated genes, such as PMEL (Premelanosome protein), TYR, TRP-1 and TRP-2, demonstrating an inhibition of melanogenesis through the MC1R/cAMP/MITF signaling pathway. The researchers first tested how strongly oxyresveratrol inhibits human tyrosinase. They compared it to similar compounds (resveratrol, phenylethyl resorcinol and glabridin) and found that it was the most powerful inhibitor of the human enzyme (table 1).

They also noted that some compounds known to work well on mushroom tyrosinase perform poorly on the human form, highlighting the importance of using the human enzyme for reliable evaluations. When they treated melanoma cells (B16F10) with oxyresveratrol, they found that it safely reduced melanin levels without harming the cells, lowering cAMP levels and significantly reducing the expression of PMEL, TYR, TRP-1 and TRP-2. To understand whether oxyresveratrol also affects melanin transfer, the researchers used a co-culture of melanocytes and keratinocytes. They found that melanocytes treated with oxyresveratrol produced less melanin and also transferred less pigment to keratinocytes.

No.	Compounds	IC ₅₀ (µg/mL)	Max inhibition rate (%)
1	Oxyresveratrol	2.27	98.02
2	Resveratrol	13.06	93.78
3	Phenylethyl resorcinol	18.97	94.65
4	Glabridin	> 60	≥ 23.88

Table 1 (Jianhua Zhang, 2025): inhibitory effects of oxyresveratrol, resveratrol, phenylethyl resorcinol and glabridin to human tyrosinase activity. Oxyresveratrol showed an IC₅₀ of 2.27, indicating a potent inhibitory effect under the tested conditions.

Microscope images showed that the treated melanocytes had fewer dendrites, and the dendrites they did have were much shorter: because melanosomes must travel along dendrites to reach keratinocytes, this reduction directly limits melanin transfer. The molecules behind this process are small GTPases that control dendrite formation, cell shape and filopodia, whose levels are decreased upon treatment: they are CDC42 (cell division control protein 42), RAC1 (ras-related C3 botulinum toxin substrate 1), RAB17 (ras related protein rab 17), and RAB11B (ras related protein rab 11B); it also reduced the levels of proteins required for melanosome movement.

Oxyresveratrol can be used for treating hyperpigmentation, because it reduces melanin production; besides, it also blocks melanin transfer. These are two major targets in cosmetic and dermatological treatments, although more work is needed to confirm these last effects on human melanocytes.

Salicylic acid

An investigation on salicylic acid shows its properties in alleviating skin hyperpigmentation (Jianzeng Liu, 2021). Salicylic acid is an important phenolic acid found in ginseng roots that inhibits both melanogenesis and melanosome transport. In this study, salicylic acid showed no toxicity in murine B16F10 melanoma cells or normal human epidermal melanocytes. Even in the absence or

presence of α -MSH stimulation, salicylic acid reduced both melanin content and tyrosinase activity to levels comparable to the whitening agent arbutin. As the amount of salicylic acid increased, the α -MSH-induced rise in MITF, TYR, TRP-1, and TRP-2 was more strongly blocked by it, as shown by western blot analysis (Figure 7). This demonstrates that the compound lowers the production of these melanogenic enzymes and, as a result, decreases melanin formation.

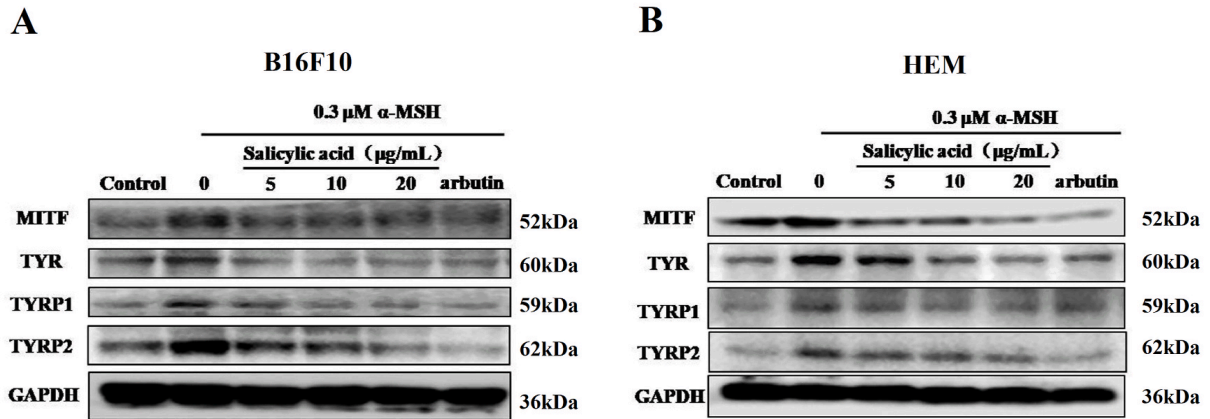


Figure 7 (Jianzeng Liu, 2021): western blot analysis of MITF, TYR, TRP-1 and TRP-2 levels in (A) α -MSH stimulated B16F10 cells treated with salicylic acid; (B) α -MSH stimulated HEM (human epidermal melanocytes) cells treated with salicylic acid. GAPDH is used as a loading control.

Melanosome transport was also examined: UVB irradiation normally increases the levels of proteins involved in the transport in melanocytes, such as melanophilin, Rab27a (ras associated binding 27a) and myosin Va. Salicylic acid blocked the UVB-induced increase in the expression levels of melanophilin and myosin Va both in B16F10 and human melanocytes, while Rab27a levels are unaffected. This indicates that salicylic acid inhibits actin-dependent melanosome transport by selectively reducing important motor and linker proteins. Studies on keratinocytes showed that while UVB increased keratinocytes melanosome uptake, salicylic acid reduced this increase and its involved signals.

The effects explain how salicylic acid alleviates hyperpigmentation, clarifying one of the molecular mechanisms behind the traditional use of ginseng to treat skin pigmentation disorders.

3. Discussion

Melanogenesis can be inhibited through multiple molecular strategies that act either directly on the enzymes responsible for melanin synthesis, or indirectly, on the biological processes that govern melanosome formation, maturation and transport. The compounds examined in this work revealed that the modulation of pigmentation extends far beyond the simple suppression of tyrosinase activity: a wide range of biochemical and cellular mechanisms can be used to regulate melanin production with varying efficacy and safety.

The first and most traditional strategy involves direct inhibition of melanogenic enzymes, especially tyrosinase, the enzyme responsible for converting L-tyrosine to L-DOPA and then DOPAquinone. We discussed several compounds that can modulate melanin synthesis: kojic acid reduces melanogenesis by chelating copper atoms in the tyrosinase active site, blocking the enzyme's catalytic cycles; glabridin is an even stronger inhibitor than kojic acid and is generally safe for human melanocytes, but it tends to oxidize easily, which makes it less stable. Arbutin interferes with tyrosinase by competing with natural substrates, and in conditions where diphenols are absent, it traps the enzyme in an inactive state (E_{met}) illustrating that inhibition can occur not only through competition but also through allosteric modulation of the enzyme's catalytic cycle. Azelaic acid, while being also a competitive inhibitor, extends its effect by targeting the redox machinery that supports tyrosinase activity, the thioredoxin/thioredoxin-reductase system (Trx/TrxR). This makes it especially effective in melanocytes with abnormally elevated oxidative states, such as those found in melasma or lentiginos.

Besides small molecules, newer approaches target the same enzymatic machinery with improved selectivity. The cyclopeptide CHP-9 shows remarkably high tyrosinase inhibition while preserving cell viability. Ellagic acid represents a more complex case, acting not only as a substrate for tyrosinase but also as a scavenger of reactive intermediates produced during melanogenesis. By reacting with quinones and semiquinones, it shifts the redox balance and slows the formation of melanin precursors. In contrast, hydroquinone shows the risks of interacting with melanogenic enzymes through unplanned chemical pathways: instead of inhibiting tyrosinase, it behaves as a pseudo substrate generating reactive quinones that disrupt melanocyte function and contribute to exogenous ochronosis. Together, these examples show that targeting enzymatic activity can be highly effective but also potentially harmful if the compounds introduce reactive species into the melanogenic pathway.

A second major strategy to inhibit pigmentation involves targeting melanosome biology, an area of growing interest due to the possibility of modulating melanin synthesis without directly interfering with catalytic enzymes. Inulavosin is a clear example of this approach: rather than altering gene expression or enzymatic activity, it destabilizes newly synthesized tyrosinase and redirects it toward lysosomal degradation. As a result, melanosomes fail to progress from early to

mature stages: selective manipulation of organelle trafficking can profoundly alter pigmentation outcomes.

Similarly, BI2B (Benzimidazole-2-butanol) modulates melanogenesis not by interacting with tyrosinase itself but by inhibiting MKK3, thereby preventing p38-mediated phosphorylation of transcription factors (such as CREB) and reducing MITF expression. This leads to decreased synthesis of tyrosinase and other melanosomal proteins, illustrating that altering signaling pathways upstream of melanosome formation is an effective way to reduce pigmentation.

Other compounds act on melanosome dynamics even more directly: for example oxyresveratrol inhibits melanogenesis by downregulating PMEL, TYR, TRP-1 and TRP-2; it also reduces dendrite formation and small GTPases involved in melanosome transport.

Salicylic acid exhibits similar effects, suppressing proteins required for melanosome trafficking (melanophilin and myosin Va), and interfering with keratinocyte uptake mechanisms.

In summary, we found that melanogenesis can be modulated at several interconnected levels: by blocking enzymatic reaction, lowering expression levels or enhancing degradation of melanosomal proteins, or preventing melanin transfer to keratinocytes.

Knowing these different methods for melanin synthesis inhibitions, we wonder which could be the best and safer to be used. The wide range of inhibitors described in this work makes it clear that no single method can be universally considered the best for suppressing melanogenesis. The optimal choice depends strongly on the biological context and the intended use, which could range from cosmetic skin-lightening purposes to treatment of hyperpigmentation disorders to potential therapeutic roles in melanoma modulation. Some inhibitors, such as kojic acid, arbutin and azelaic acid, are already incorporated into dermatological formulations aimed at reducing fleckles, melasma or post-inflammatory hyperpigmentation. Others, like CHP-9 or BI2B, represent more recent and selective findings that may be more suited for highly focused interventions.

4. Conclusions

Melanin production is a complex process that involves many steps: the activation of specific enzymes, the formation and growth of melanosomes, their movement inside the cell and finally the transfer of pigment to keratinocytes. Tyrosinase and related enzymes start the chemical reactions, while MITF and some signaling pathways control how much pigment the cell produces. At the same time, melanosomes change shape and structure as they mature, and their transport depends on proteins that guide them through the cell; all these elements work together to create skin pigmentation.

Because this process has many levels of control, there are also many ways to reduce or regulate melanin production. Some compounds block tyrosinase directly, others change the redox environment around the enzyme and some power MITF activity. Others slow melanosome maturation or reduce dendrite formation, preventing melanosomes from reaching keratinocytes.

It remains important to continue researching melanogenesis and its regulation because, despite the progress made, many aspects of this process are still not fully understood. Several steps, such as how tyrosinase is selectively degraded, how melanosomes mature and how signaling pathways precisely regulate MITF, remain only partially understood.

Continued research is essential to better understand the mechanisms behind these effects, discover new regulatory targets and develop better treatments.

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