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REVIEW ARTICLE

Natural, semisynthetic and synthetic tyrosinase inhibitors

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Abstract

Tyrosinase plays a pivotal role in the synthesis of melanin pigment synthesis on skin utilizing tyrosine as a substrate. Melanin is responsible for the protection against harmful ultraviolet irradiation, which can cause significant pathological conditions, such as skin cancers. However, it can also create esthetic problems when accumulated as hyperpigmented spots. Various skin-whitening ingredients which inhibit tyrosinase activity have been identified. Some of them, especially ones with natural product origins, possess phenolic moiety and have been employed in cosmetic products. Semi-synthetic and synthetic inhibitors have also been developed under inspiration of the natural inhibitors yet some of which have no phenolic groups. In this review, tyrosinase inhibitors with natural, semi-synthetic and synthetic origins are listed up with their structures, activities and characteristics. Further, a recent report on the adverse effect of a natural melanin synthesis inhibitor which was included in skin-whitening cosmetics is also briefly discussed.

Keywords

Inhibitor, melanin, skin-whitening, tyrosinase

History

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Introduction

Skin pigmentation is an important human phenotypic trait whose regulation has not yet been fully understood despite recent advances[1–3]. The major constituent of pigment, melanin, is produced in melanosomes by the melanocyte through a complex process called melanogenesis. The melanocyte interacts with endocrine, immune, inflammatory and central nervous systems, and its activity is also regulated by extrinsic factors, such as ultraviolet radiation and chemicals[4]. Melanosomes are exported from melanocytes to adjacent keratinocytes, and variation of their number, size, distribution and composition can result in different pigmentations[5]. Melanin is the primary determinant of skin, hair and eye color, and performs important roles in human skin homeostasis, such as protecting from harmful ultraviolet radiation and scavenging toxic drugs and chemicals[6]. However, accumulation of abnormal melanin in specific parts of the skin as hyperpigmented spots causes esthetic problems.

There are two main types, red/yellow pheomelanin and brown/black eumelanin, of melanin, and they differ not only in color but also in the size, shape and granule packagings[7]. Enzymes those involved in melanogenesis are tyrosinase, tyrosine hydroxylase isoform I (TH-1), phenylalanine hydroxylase (PAH), tyrosinase-related protein-1 (TRP-1), tyrosinase-related protein-2 (TRP-2), etc. Melanin biosynthesis can be initiated from either L-tyrosine or L-dihydroxyphenylalanine (L-DOPA), and the oxidation of L-DOPA to dopaquinone, is common to both eu- and pheomelanogenic pathways. Following a series of oxidoreduction reactions produce intermediate dihydroxyindole (DHI) and DHI carboxylic acid (DHICA) that polymerize to form both melanins (Figure 1)[7].

In melanin biosynthetic pathway, tyrosinase catalyzes rate-limiting first two steps, thus inhibition of this enzyme might prevent the accumulation of the reactive intermediates as well[8]. The majority of known anti-melanogenesis agents from both natural and synthetic sources are tyrosinase inhibitors, and a number of research papers and reviews have been published and frequently updated in the aspect of tyrosinase inhibitors[9–13]. Tyrosinase was first isolated from the mushroom by Bourquelot and Bertrand in 1895[14]. After that, the enzyme seems to be universally distributed in animals, plants, fungi and bacteria. Tyrosinases contain a dinuclear copper center, which are able to insert oxygen in an *ortho* position to an existing hydroxyl group in an aromatic ring, followed by the oxidation of the diphenol to the corresponding quinone (Figure 2)[15]. Molecular oxygen is incorporated into dinuclear center to form an active oxy-tyrosinase. The structure of the active site of the enzyme, in which copper is bound by six or seven histidine residues and a single cysteine residue, is highly conserved. Due to the enzyme's biological importance and well established structural and functional motif, the inhibition of tyrosinase has been the primary target for hypopigmentation.

The anti-tyrosinase activity can be achieved by several ways: (i) by reducing the intermediate *o*-dopaquinone to dopa with suitable reducing agents, such as ascorbic acid; (ii) by introducing *o*-dopaquinone scavengers, such as alkyl thiols which can react with dopaquinone to form colorless products; (iii) by employing

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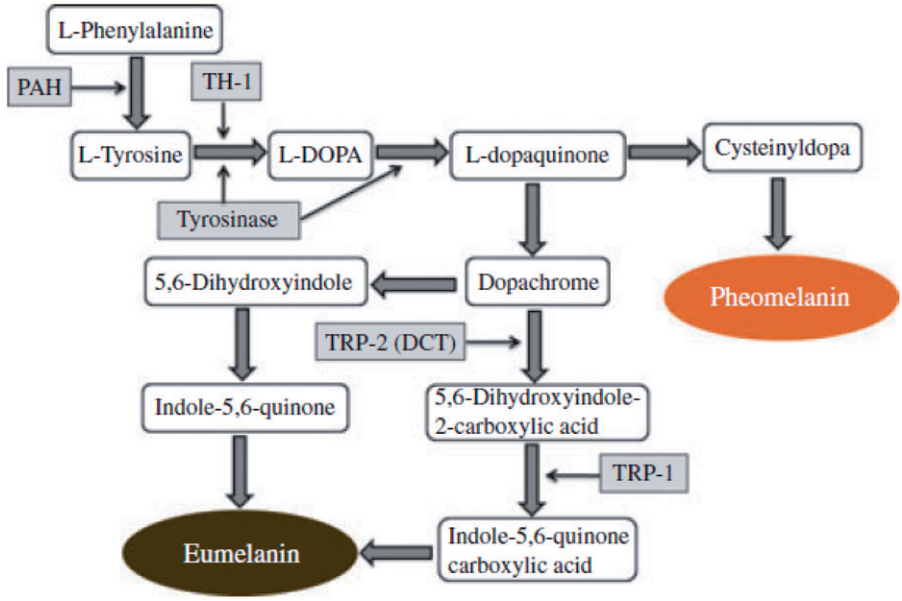

Figure 1. Synthesis of the two types of melanin and representation of the functions of the major enzymes involved (adapted from Gillbro and Olsson[5]).

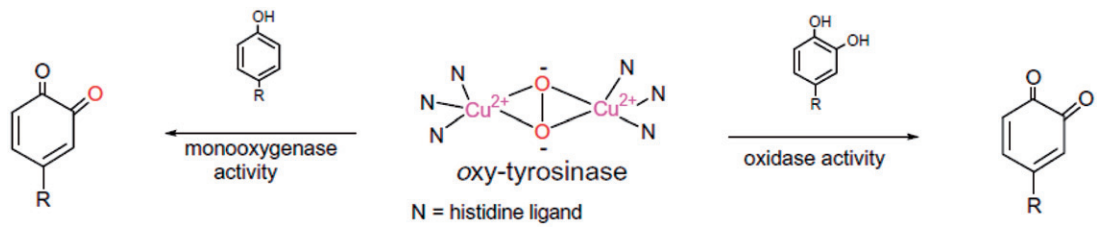

Figure 2. Two modes of oxidation by oxy-tyrosinase (adapted from Stratford et al.[8]).

alternative tyrosinase substrates, such as certain phenols whose enzymatic reaction products do not further undergo the next step; (iv) by denaturing the enzyme with non-specific enzyme inactivators, such as acids or bases; or by specific tyrosinase inactivators or inhibitors. Specific tyrosinase inactivators, called suicide substrates, are catalyzed by tyrosinase to result in the formation of a covalent bond with the enzyme thereby irreversibly inactivating the enzyme[12]. Specific tyrosinase inhibitors reversibly bind to the enzyme and reduce its catalytic capacity. They usually do not get involved in chemical modifications, such as a formation of covalent bond(s) with a part of the enzyme or electron transfer to enzyme's metal group, when inhibiting the enzyme activities. Both specific tyrosinase inactivators and inhibitors are called as ''true inhibitors'' because they actually bind to the enzyme and inhibit its activity. However, some of tyrosinase inhibitors display multifunctional roles such as reducing agents, dopaquinone scavengers and/or tyrosinase substrates, resulting in some ambiguity[16]. Recent advances in both natural and synthetic tyrosinase inhibitors are going to be thoroughly discussed in this review.

Naturally occurring tyrosinase inhibitors and their derivatives

The natural organisms have developed protective methods from harmful ultraviolet radiation, thus the nature is a myriad source of tyrosinase inhibitor. A number of researchers have been dedicated to identify inhibitors from plant, fungal metabolites and marine algae and to establish their structure-activity relationship (SAR). Tyrosinase inhibitors from natural sources usually attract more attention compared to chemically synthesized compounds due to the cosmetic demand. In the literature, the IC_{50} values for the tyrosinase inhibitors are incomparable due to the variations in assay conditions, including different substrate concentrations, varied incubation time, and different batches of commercial tyrosinase(s). Thus, most studies utilized a well-known tyrosinase inhibitor, such as kojic acid as a positive control in an effort to normalize the inhibitory activities of their inhibitors.

Flavonoids

Plant polyphenols are produced as the secondary metabolites by high plants and have many biological activities[13]. Flavonoids are one of the mostly explored polyphenol derivatives consisting of phenols and pyrane rings and distributed in the leaves, seeds, bark and flowers of plants. Over 4000 flavonoids have been identified to date, and they provide the protection against ultraviolet radiation, pathogens and herbivors[17]. Flavonoids occupy the largest portion in newly discovered natural tyrosinase inhibitors. They are divided into six major groups: flavanols, flavones, flavonols, flavanones, isoflavones and anthocyanidins (Figure 3). They differ in the conjugation of the A- and B-ring and in the arrangement of the substituents, such as hydroxy, methoxy, glycosides, etc. In principle, the structure of flavonoids is compatible with roles of both substrates and inhibitors of tyrosinase. Some flavonoids, such as kaempferol[18], quercetin[19] and morin[20], show the inhibitory activity against tyrosinase, while others such as catechin and rhamnetin behave as substrates and suppress tyrosinase activity by being a cofactor (catechin)[21] or acting as a free radical scavenger (rhamnetin)[22]. Recently, Zehng et al.[23] isolated a series of flavonoid derivatives from the twigs of *Cudrania tricuspidata*, and steppogenin, a flavanone derivative,

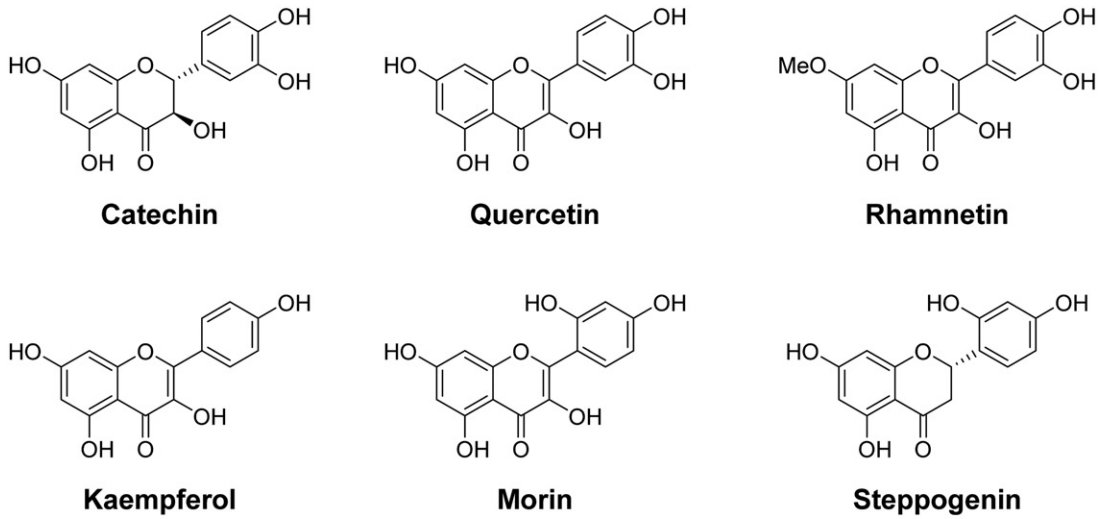

Figure 3. Representative anti-tyrosinase flavonoids.

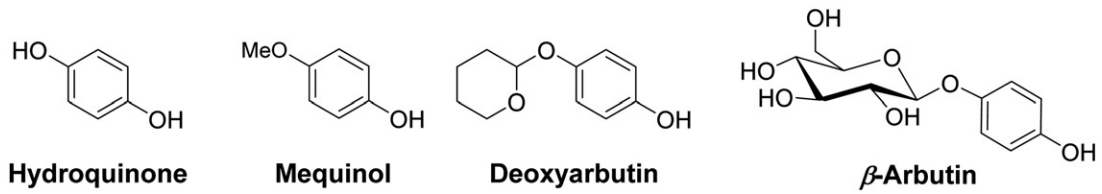

Figure 4. Hydroquinone and its representative derivatives.

showed the best tyrosinase inhibitory activity, whose activity was 10-fold higher than the known inhibitor, kojic acid.

Hydroquinone and its derivatives

1,4-Hydroquinone is widely distributed in tea, wheat, berries, beer and coffee, and had been widely used in the treatment of hyperpigmentation[24,25]. Hydroquinone competitively inhibits melanin synthesis by covalently binding to histone, interacting with copper at the active site, and acting as a poor substrate for tyrosinase[26]. Hydroquinone was considered as the standard treatment for the treatment of hyperpigmentation for a long period (Figure 4). However, it can cause permanent damage to melanosomes and melanocytes by the semiquinone-free radicals that produced during the enzymatic reaction[27]. In addition, it was recognized that this substance is transported rapidly from the epidermis to the vascular system and is detoxified inside the liver[28]. Due to the side effects, such as permanent depigmentation and exogenous ochronosis from long-term usage, hydroquinone has been forbidden in cosmetics by most countries.

Although the glorious days of hydroquinone in hyperpigment treatment is over, its derivatives, such as arbutin, deoxyarbutin and mequinol are still used in the cosmetic industry as skin-whitening agents[29]. β-arbutin is a β-D-glucopyranoside of hydroquinone found in the leaves of bearberry, cranberry, blueberry and pears[30]. β-Arbutin inhibits tyrosinase inhibition in a dose-dependent manner in cultured melanocytes. It is less cytotoxic to melanocytes than hydroquinone[31]. Controlled studies have showed that arbutin is comparably effective with kojic acid for hyperpigmentation[32]. The co-treatment of arbutin and aloesin showed the synergistic inhibitory effect on tyrosinase by acting through different mechanisms: arbutin inhibited competitively, and aloesin inhibited non-competitively[33]. Deoxyarbutin is a synthetic derivative of hydroquinone in which one of the two hydroxyls is protected as THP ether[34]. This derivative dose-dependently inhibits tyrosinase and is less cytotoxic than hydroquinone[35]. Mequinol, the monomethyl ether of hydroquinone, acts as a substrate for tyrosinase thereby inhibiting the formation of melanin precursors[36]. It is marketed in USA at a concentration of 2% in combination with 0.01% tretinoin[37]. This combination can cause side effects, such as erythema, burning, pruritus, desquamation, skin irritation and halo hypopigmentation, yet combination with sunscreens might mitigate the incidence of adverse effects[38].

Chalcones and their synthetic derivatives

Chalcones, 1,3-diaryl-2-propen-1-ones, exist in thermodynamically more stable *trans* isomeric form in nature. Their aryl rings are substituted with functionalities: hydroxy, methoxy, alkyl, etc, and these substituents influence the specific biological activities of chalcones[39]. Chalcones are considered as precursors of flavonoids and isoflavonoids and display a wide spectrum of biological activities[40]. A number of chalcones show tyrosinase inhibitory activity. Licochalcone A, isolated from the roots of the *Glycyrrhize* species, is 5-fold more active than kojic acid in the inhibition of the monophenolase activity of mushroom tyrosinase[41]. Kuraridin[42], kuraridinol[43] and 2,4,2′,4′-tetrahydroxy-3-(3-methyl-2-butenyl) chalcone (TMBC)[44] showed 34-, 18- and 26-fold higher tyrosinase inhibitory activities, respectively, than kojic acid (Figure 5). In an earlier study, Nerya et al.[45] reported that the position of the hydroxy groups attached to both aromatic rings is the major factor in their efficacy with a significant preference to a 4-substituted B ring rather than the substitution at A ring. Neither the number of hydroxyl groups nor the presence of a catechol moiety on ring B correlated with an increasing tyrosinase inhibition potency.

However, the results from their later study showed contradicted results that chalcones possessing resorcinol,

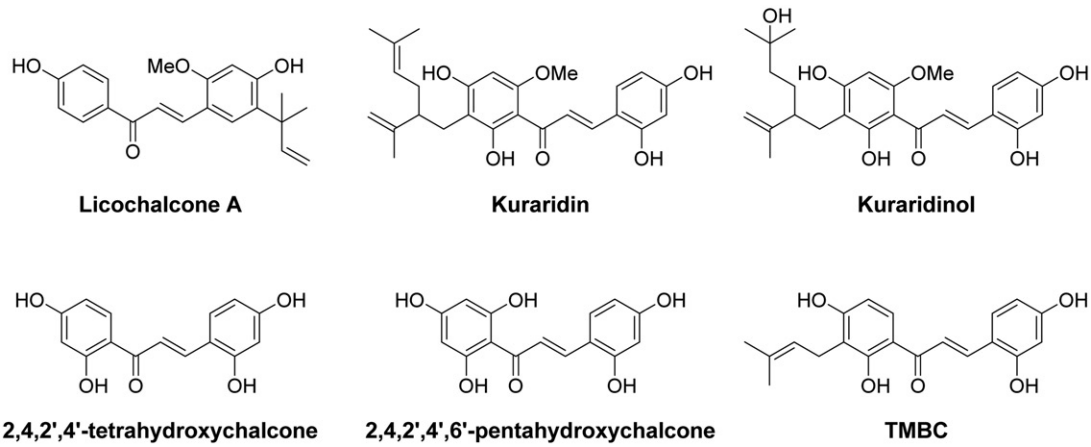

Figure 5. Representative tyrosinase inhibitory active chalcones.

2,4-dihydrophenyl, moiety showed the strong tyrosinase inhibitory activity, and resorcinol subunit on B ring contributed the most to the potency[46]. They also synthesized a series of chalcones and performed the SAR study. In short, the catechol on A ring acted as a metal chelator (in the presence of copper ions) and as a competitive inhibitor (in the presence of tyrosinase), and the catechol on B ring oxidized to *o*-quinone (in the presence of both copper ions and tyrosinase). The most active tyrosinase inhibitor was 2,4,2′,4′-tetrahydroxychalcone ($IC_{50} = 0.02\,\mu M$). Similarly, Jun et al.[47] prepared a series of chalcones and studied the tyrosinase inhibitory activity. The results were consistent with the previous work, and 2,4,2′,4′,6′-pentahydroxychalcone was found to be the most potent derivative such that its activity was 5-fold higher than that of 2,4,2′,4′-hydroxychalcone. Recently, Nguyen et al.[48] reported the isolation of a series of prenylated chalcones and flavones from the wood of *Artocarpus heterophyllus*. Several isolated compounds displayed good tyrosinase inhibitory activity, and the most active, morachalcone A that showed 3000-times higher activity ($IC_{50} = 13\,nM$) than kojic acid ($IC_{50} = 45\,\mu M$) was indeed TMBC.

Inspired by versatile bioactivity and unique structural motif of chalcones, a number of derivatives have been developed as effective tyrosinase inhibitor candidates. Cho et al.[49] modified the enone functionality in chalcone to a more stable amide linkage to prepare *N*-benzyl benzamide analogs as synthetic derivatives. The most potent compound was *N*-(2,4-dihydroxybenzyl)-3,5-dihydroxybenzamide, which was 8-times more potent than the positive control, kojic acid. Recently, Baek et al.[50] reported the preparation of another series of *N*-benzyl benzamide containing a bulky adamantly moiety as tyrosinase inhibitor. The lipophilic adamantly substituent conferred the enhancement of the potency without increasing in cytotoxicity. Among them, *N*-2,4-dihydroxybenzyl-2-hydroxy-4-methoxy-5-adamantyl benzamide **3b** showed 40-times higher tyrosinase inhibitory activity than kojic acid. Lately, Wu et al.[51] developed a series of phenylpropanoid amides for new synthetic analogs for chalcones.

Interestingly, the most active compound **1d**, which was 10-times more active in tyrosinase inhibition than positive control *tert*-butylhydroquinone, can be found in the skins of garlic. Thus, industrial waste garlic skins might be recycled as depigmentation agent. In another approach, Fang et al.[52] prepared a series of hydroxylated benzyl benzoates as the synthetic chalcone analogs. Several analogs were showed good activity, and the best one is 4-hydroxybenzyl 2,4-dihydroxybenzoate (**5**), which showed 5-times more potent tyrosinase inhibition than kojic acid. Recently, Bandgar et al.[53] reported the synthesis of a number of *N*-(3-(9-methyl-9*H*-carbazol-3-yl)acryloylphenyl)-amide derivatives for both xanthine oxidase and tyrosinase inhibitor. Several analogs displayed tyrosinase inhibitory activity comparable to kojic acid, and the structure of a representative compound **7a** is depicted in Figure 6.

Stilbenes and their derivatives

Stilbenes (diarylethenes) are biogenetically produced through the mixed shikimate-acetate pathway[54]. They can be classified according to the degree of oxygenation as di-, tri- and tetra-oxygenated stilbenes. A number of naturally occurring poly-oxygenated stilbenes such as resveratrol[55], oxyresveratrol[56], chlorophorin[57], andalasin A[58], and so forth, have been reported to possess tyrosinase inhibitory activity. Oxyresveratrol appears to be the most promising inhibitor, which exhibited 32-fold higher inhibitory activity than that of kojic acid. The structure of oxyresveratrol consists of 4-resorcinol moiety in the B ring and the 5-resorcinol moiety in the A ring. Resorcinol is converted to 3-hydroxy-ortho-quinone by monooxygenase that undergoes a reductive elimination to result in copper atoms from the active site[8]. Resveratrol, which lacks 4-resorcinol moiety, is 50-fold less active than oxyresveratrol, implying the importance of resorcinol functionality. Dihydroresveratrol turned out to be less active than resveratrol[59], however, the compound exhibited 8-fold stronger inhibitory effect on L-DOPA oxidase activity of mushroom tyrosinase than did oxyresveratrol, suggesting that not only numbers of phenols but also their arrangement in the space played an important role to the activity (Figure 7)[60].

There are many synthetic stillbene analogs, such as dihydrognetol[61], azostilbene[62], hydroxy-2-phenylnaphthalenes[63]. have been developed. Checking for the recent progresses, Bae et al.[64] synthesized several azo-stilbenes, and interestingly the most potent derivative was mono-tosylated analog **3**. Compound **3** was competitively inhibited tyrosinase and was 3-times more active than the positive control, kojic acid. They also reported the preparation of benzylidene-aniline derivatives for tyrosinase inhibitors[65]. In their work, 4-methoxy- or 4-hydroxy-anilino group exerted more potent inhibition against mushroom tyrosinase than those with a 2-substituted analogs. The most potent compound was a non-competitive inhibitor (*E*)-4-((4-hydroxyphenylimino)methyl)benzene-1,2-diol (**14**), whose inhibitory effect on mushroom tyrosinase was 3-fold higher than kojic acid. In addition, Song et al.[66] prepared a family of azo-resveratrols and azo-oxyresveratrols and compared their

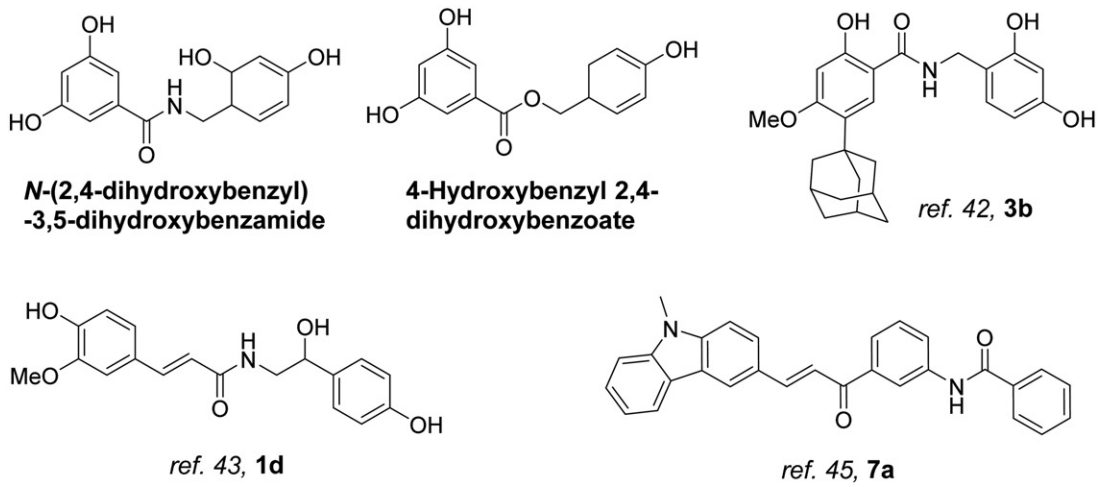

Figure 6. Representative synthetic chalcone derivatives.

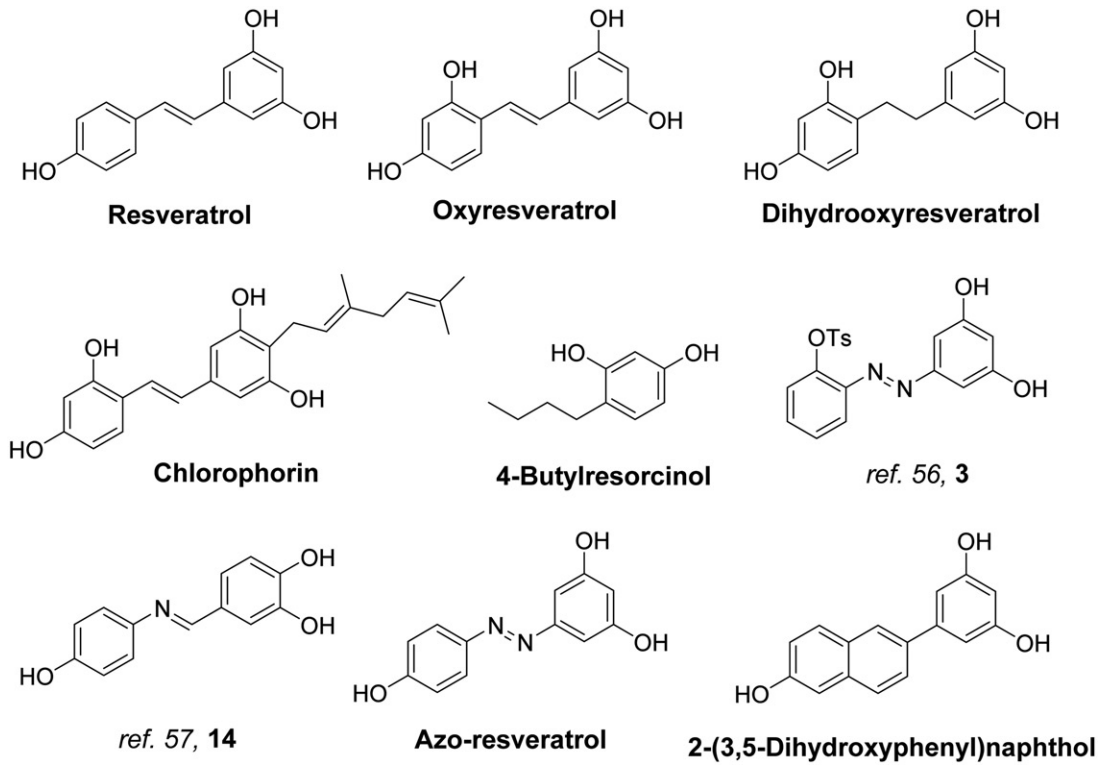

Figure 7. Representative reported natural stillbenes and synthetic derivatives.

tyrosinase inhibitory activity to those of resveratrols and oxyresveratrols. The introduction of an additional functionality to the 4-hydroxylphenyl moiety resulted in the decreasing mushroom tyrosinase inhibition. Azo-resveratrol showed the highest inhibitory activity, which is comparable to the parent compound, resveratrol. Additionally, Kolbe et al.[67] reported a known compound 4-*n*-butylresorcinol as a tyrosinase inhibitor. When 4-*n*-butylresorcinol was compared with known inhibitors, this alkylated resveratrol was 25- and 20-times more potent than kojic acid in the human tyrosinase and the artificial skin model melanin inhibition, respectively. Also, 4-butylresorcinol was more effective than longer alkyl chain analogs, 4-hexylresorcinol and 4-phenylethylresorcinol.

Kojic acid and its derivatives

Kojic acid, a fungal metabolite, a slow-binding tyrosinase inhibitor, is used as a skin-whitening cosmetic and as a food additive for preventing enzymatic browning[68,69]. Kojic acid acts as a bidentate chelator for transition metal ions such as Cu^{+2} and Fe^{+3} and shows the competitive inhibitory effect on the monophenolase activity and the mixed inhibitory effect on the diphenolase activity of mushroom tyrosinase[13,70]. However, its usage in cosmetics is limited due to its instability under the light and the aerobic environment. Thus, more stable an ester derivative, kojic dipalmitate is commonly used as an alternative in cosmetic formulations; however, there is no research showing

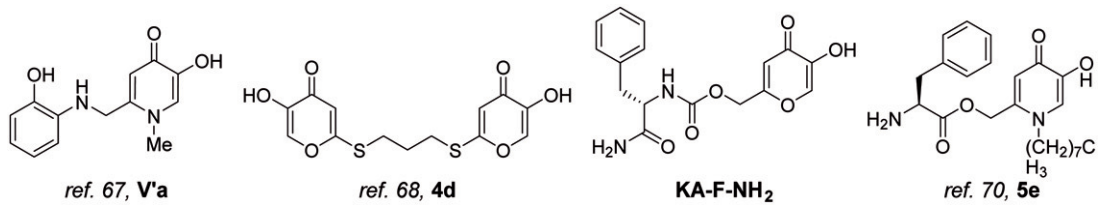

Figure 8. Recently developed kojic acid derivatives.

the ester to be as effective as kojic acid though. Kojic acid is frequently adapted in literature as a positive control to compare with the inhibitory activity of other inhibitors. There are many works for developing synthetic derivatives of kojic acid, such as ester[71], polymer[72], 4-pyridone[73], peptide-conjugate[74], were reported in order to improve the metal chelate ability or the stability.

For recent advances, Saghaie et al.[75] prepared a series of 3-hydroxy-4-pyridone derivatives and evaluated their tyrosinase inhibitory activity using the dopachrome method. All of synthesized derivatives showed the inhibitory effect, and pyridones containing two free hydroxyls were more potent than analogs having one hydroxyl. The most active compound, **V'a**, showed comparable inhibitory activity with the parent compound, kojic acid. In another work, Rho et al.[76] synthesized six dimeric analogs that two kojic acids were connected via an alkyl ester, an amide or a sulfide linkage. Compounds having flexible alkane dithioether linkage displayed the higher activity compare to others, and the dimer possessing propane 1,3-dithioether **4d** showed the highest activity that its tyrosinase inhibition activity was 25-times higher than that of kojic acid. In addition, Noh et al.[77] made 20 of mono and dipeptide conjugated kojic acid derivatives and screened their tyrosinase inhibitory activity. Amino acid moieties were connected to kojic acid via a carbamate linkage. Among them, kojic acid-phenylalanine amide (KA-F-NH_2) was found to be 6-times more potent than kojic acid and maintained its activity over three months. Recently, Li et al.[78] synthesized six of 4-pyridone-phenylalanine conjugates as tyrosinase inhibitors. The compounds are mixed-type inhibitors and might bind to both the free enzyme and the enzyme-substrate complexes. The most active compound **5e** was twice more active in the inhibition of tyrosinase than that of kojic acid (Figure 8).

Other natural inhibitors and their derivatives

One of the famous natural tyrosinase inhibitor is aloesin, a hydroxychromone glucoside isolated from *Aloe vera*. It does not show any cytotoxicity in cell-based assays, skin irritation in preliminary human studies and genotoxicity or mutagenicity in Ames assay[79]. Aloesin treatment might inhibit hyperpigmentation after UV radiation in a dose-dependent manner, and co-treatment with arbutin showed a synergistic effect, *vida supra*[80]. Aloesin showed more inhibitory activity toward murine tyrosinase than mushroom tyrosinase. Due to its multifunctional activity in skin care and natural origin, aloesin has been frequently used in topically applied cosmetics.

Coumarins (2*H*-1-benzopyran-2-one) are naturally occurring heterocyclic compounds, which present an aromatic ring fused to a condensed 6-member lactone ring. Coumarins and their derivatives have been found to exhibit a variety of biological and pharmacological activities and have raised substantial attention due to their potent beneficial effects[81]. Esuletin, 6,7-dihydroxylcoumarin that isolated from *Euphorbia lathyris*, acts as a substrate of mushroom tyrosinase and shows one-quarter of the tyrosinase inhibitory activity of kojic acid[82]. Other coumarin analogs, 9-hydroxy-4-methoxypsoralen[83], isolated from *Angelica dahurica*, and 8′-*epi*-cleomiscosin A[84], isolated from *Rhododendron collettianum*, exhibited 6- and 13-times more inhibitory activity toward mushroom tyrosinase than kojic acid, respectively.

p-Coumaric acid is a ubiquitous plant metabolite, structurally related to cinnamic acid, with antioxidant, anti-inflammatory and antiplatelet activy[85]. Recently, Lee et al.[86] prepared a series of small molecule-peptide conjugates and screened. When they first compared tyrosinase inhibitory activity of 17 natural compounds, *p*-coumaric acid and caffeic acid turned out to be highly effective, which were 10- and 3-fold more potent than kojic acid in mushroom tyrosinase assay. With the initial results, both *p*-coumaric acid and caffeic acid were incorporated into a series of oligomers by utilizing solid phase chemistry to obtain 28 analogs. Among them, a dipeptide conjugate, coumaric acid-RP-OH, showed the highest activity that exhibited 2-fold higher melanin inhibitory activity than *p*-coumaric acid in cultured B16F1 murine melanoma cell.

Naturally occurring benzaldehyde and benzoate derivatives, such as benzoic acid[87], benzaldehyde[88], anisic acid[89], anisaldehyde[90], cinnamic acid[91], methoxycinnamic acid[92], vanillic acid[93], are well known for their tyrosinase inhibitory activity. Whereas the tyrosinase inhibitory mechanism of benzaldehyde inhibitors comes from their ability to form a Schiff base with an primary amino group in the enzyme[94], benzoate inhibitors utilized the copper chelating ability that the interaction between the inhibitor and the copper in the active site of the enzyme provide the inhibition[95]. In terms of inhibitory strength, all the naturally occurring benzaldehyde and benzoate derivatives discovered so far showed only weak-to-moderate tyrosinase inhibitory activity that none of them are stronger than kojic acid.

Gallic acid from many plants displays tyrosinase inhibitory activity[87,96]. Gallic acid reduces dopaquinone back to L-DOPA through a redox cycling, similar to ascorbic acid. The acid also acts as a substrate and being slowly oxidized even without L-DOPA, but the addition of this cofactor significantly increases the oxidation rate. Several lipids and fatty acids, such as trilinolein[97], soyacerebroside I[98], *trans*-geranic acid[99], from the natural sources displayed weak tyrosinase inhibitory activity. The proposed inhibitory mechanism for lipids, based on their lack of copper chelating, might be free radical scavenging by unsaturated alkene or binding to the outside of catalytic domain. Steroids are biologically important structural scaffolds that perform imperative functions in human life. Many of them exhibited tyrosinase inhibitory activity, for instance, stigmast-5-ene-3β, 26-diol[100], from *Trifolium balansae*, 3β,21,22,23-tetrahydroxycycloart-24(31),25(26)-diene[101], from *Amberboa ramose*, and arjunilic acid[102], from *Rhododendron collettianum*, showed 12-, 13- and 17-fold more tyrosinase inhibitory activity than that of kojic acid. Hydrophobic steroid or long-chain lipid inhibitors have potential in the development as skin-whitening agents owing to their skin permeability. Still, they are less utilized as cosmetics because of the lack of cellular or clinical assays for determining their depigmentation activity. Anthraquinones display diverse

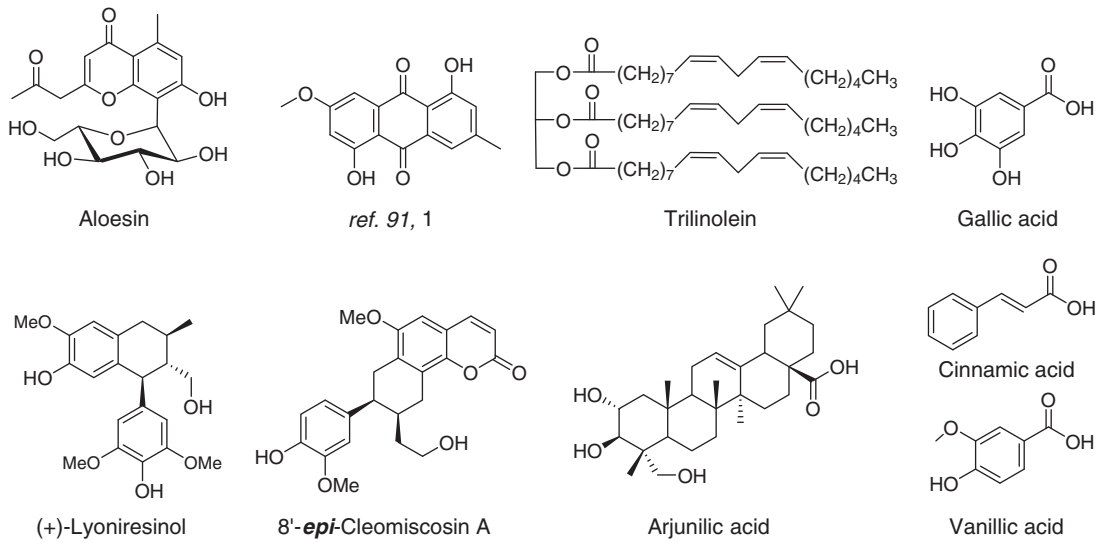

Figure 9. Selected natural tyrosinase from diverse sources.

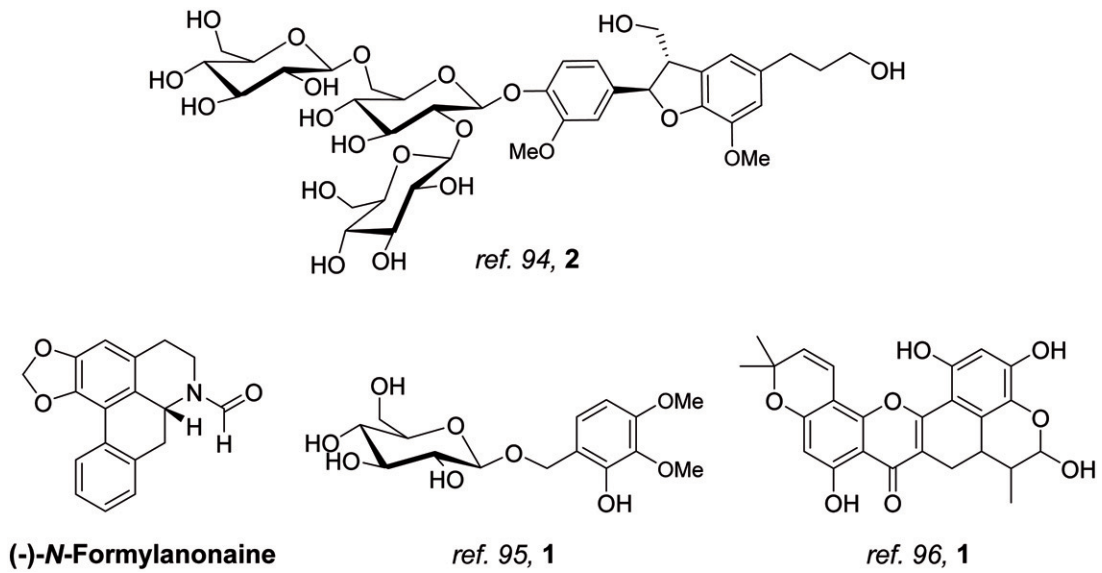

Figure 10. Selected recently discovered natural tyrosinase inhibitors.

pharmacological activities, such as antiinflammatory, wound healing, analgesic, antipyretic, antimicrobial, antitumor and anti-tyrosinase activities. Physcion, an anthraquinone, showed similar tyrosinase inhibitory activity comparable to that of kojic acid[103]. However, its analog, 1,5-dihydroxy-7-methoxy-3-methylanthraquinone (**1**), which possess hydroxyl and methoxyl groups at different positions comparing to physcion exhibited a 72-fold more inhibitory activity[104]. In addition, many lignans from *Vitex negundo* show higher tyrosinase inhibitory activity than kojic acid, and the most active one is (+)-lyoniresinol, whose activity was 5-fold higher than that of kojic acid (Figure 9)[105].

Recently discovered natural tyrosinase inhibitors

There is many new naturally occurring tyrosinase inhibitors that have isolated and updated due to the fact that the natural organisms can create countless methods to protect them from the sun light. In recent works, Wang et al.[106] reported the isolation of (-)-*N*-formylanonaine from magnoliaceous plants *Michelia alba* as a human tyrosinase inhibitor and antioxidant. In mushroom tyrosinase inhibition assay, (−)-*N*-formylanonaine showed the comparable activity to kojic acid, but the compound demonstrated higher inhibition against human tyrosinase and displayed much less cytotoxicity than that of kojic acid. On homology modeling, the compound binds the active site of tyrosinase by coordinating two Cu^{2+} ions (Figure 10). Additionally, Wu et al.[107] separated three new lignin glycosides from *Castanea ehnryi* along with two known lignins. All of them were subjected to mushroom tyrosinase inhibition assay, and among them, 2,3-dihydro-2-[4-(β-glucopyranosyl (1 → 2)-[β-glucopyranosyl(1 → 6)]-β-glucopyranosyloxy)-3-methoxyphenyl]-3-(hydroxymethyl)-7-methoxy-5-benzofuranpropanol (**2**) showed a comparable *o*-diphnolase inhibitory activity with kojic acid. In another progress, Akihisa et al.[108] isolated an aromatic glycoside, 3-*o*-demethylnikoenoside (**1**), and 11 known compounds from the stem bark of *Acer buergerianum*. All of the isolated compounds were evaluated for

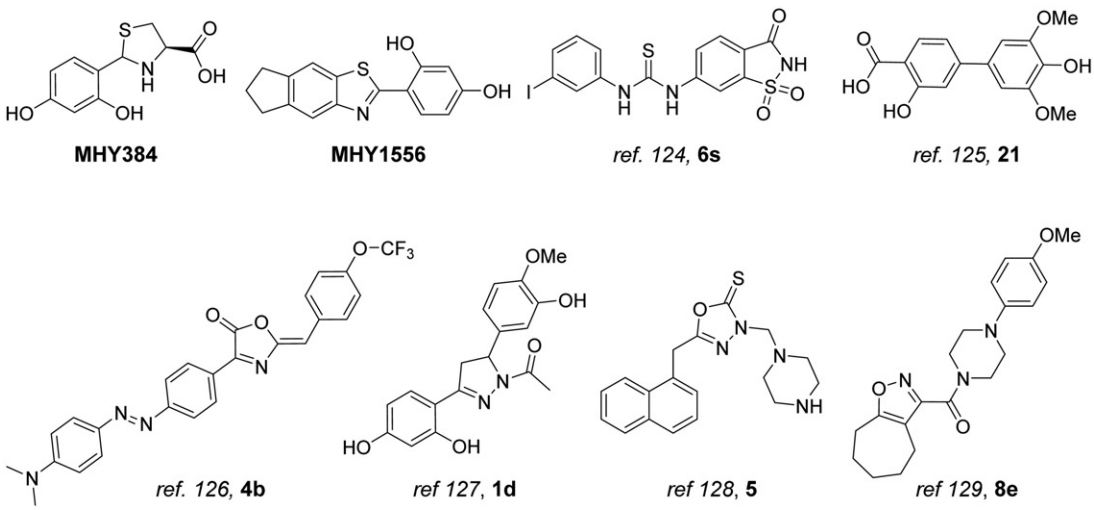

Figure 11. Recently developed synthetic tyrosinase inhibitors.

their melanogenesis-inhibitory activities in α-melanocyte stimulating hormone (α-MSH)-stimulated B16 melanoma cells, and 3-*o*-demethylnikoenoside displayed comparable activity with kojic acid. In addition, Mohd et al.[109] isolated three xanthone derivatives from *Artocarpus obtusus* F.M. Jarrett. Among them, pyranocycloartobiloxanthone A (**1**) inhibited mushroom tyrosinase in the comparable activity with kojic acid. The compound also exhibited a strong free radical scavenger towards DPPH free radicals with IC_{50} value of 2 μg/mL and displayed strong and moderate antimicrobial activity against MRSA and *Bacillus subtilis* (clinically isolated strain) with inhibition zones of 20 and 12 mm, respectively.

Synthetic tyrosinase inhibitors

Inspired by a diverse structural moiety of natural tyrosinase and the available methodology to rationally design efficient synthetic analogs, many synthetic tyrosinase inhibitors with the novel structural moiety together with derivatives of natural compounds have been prepared. The development of many synthetic tyrosinase inhibitors, such as *N*-hydroxy-*N*′-phenylthiourea[110], *N*-cyclopentyl-*N*-nitrosohydroxylamine[111], sildenafil methyl ether[112], 2,5-disubstituted-oxadiazoles[113], oxazolones[114], tetraketones[115], 4,4′-dihydroxybiphenyl[116], *S*-phenyl *N*-aryl *S*-alkylthiocarbamates[117], triazolothiadiazoles[118], 2-arylthiazolidine-4-carboxylic acids[119], benzaldehyde thiosemicarbazones[120], thiosemicarbazones[121–123], 4-hydroxybenzaldehyde derivatives[124], bis-salicylaldehydes[125], 3,4-dihydroxybenzoates[126], phenylurenyl chalcones[127], coumarin-resveratrol hybrid compounds[128], polyphenolic curcumin derivatives[129], curcumin diarylpentanoids[130], bis-benzyl glycosides[131], benzylidene hydantoins[132], rhodanine derivatives[133], 5-benzylidene barbiturates[134], have been reported, and the properties of those synthetic inhibitors were thoroughly reviewed[10,13]. Nevertheless, though a number of synthetic inhibitors displayed mushroom tyrosinase inhibitory activity, a few of them showed melanogenesis inhibition activity in cells or skin models.

Many researchers still have involved in the development of novel synthetic inhibitors. In analyzing continuous efforts for novel scaffolds of inhibitors, Han et al.[135] synthesized (4*R*)-2-(2,4-dihydroxyphenyl)thiazolidine-4-carboxylic acid (MHY384) as a tyrosinase inhibitor and investigated its inhibitory activity. In mushroom tyrosinase inhibition assay, MHY384 was 30-fold more potent than kojic acid, and the compound was also inhibited the tyrosinase activity in α-MSH-induced B16F10 melanoma cells in a dose-dependent manner (Figure 11). Similarly, Park et al.[136] designed 4-(6,7-dihydro-5*H*-indeno[5,6-*d*]thiazol-2-yl)benzene-1,3-diol (MHY1556) as an effective tyrosinase inhibitor. MHY1556 inhibited mushroom tyrosinase with 100-fold higher activity than kojic acid. 2,4-Dihydroxyl substitution on the phenyl ring plays a major role in binding to tyrosinase. In addition, MHY1556 showed a dose-dependent melanin content inhibitory effect together with no significant cytotoxicity in α-MSH-induced B16F10 melanoma cell line. In addition, Gencer et al.[137] prepared a series of saccharin derivatives as tyrosinase inhibitors and evaluated their activity on the diphenolase activity of banana tyrosinase. All the synthesized compounds inhibited the tyrosinase enzyme activity, 6-(3-iodophenylthiourenyl)saccharin (**6s**) was found to be the most active one. The inhibition constant, K_i value of **6s** was 130-fold lower than the positive control, ascorbic acid, and **6s** was found to be as a competitive inhibitor. SAR study showed that most of the 6-(phenylthiourenyl) derivatives exhibited higher inhibitory activity than 6-(phenylurenyl) derivatives. In another work, Bao et al.[138] extended the library of biphenyl tyrosinase inhibitors by adding new biphenyl compounds. SAR study revealed that 4-hydroxy-3,5-dimethoxyl substituent on the B ring is crucial for higher tyrosinase inhibitory activity in this class compounds, and the electron withdrawing groups on the A ring was advantageous. The most potent analog was **21**, which displayed 10-fold higher activity than positive control arbutin in mushroom tyrosinase inhibition assay. Kinetic study revealed that **21** act as a competitive inhibitor. Recently, Hamidian et al.[139] synthesized six new azo dyes containing 5(4*H*)-oxazolone ring. All synthesized compounds exhibited high tyrosinase inhibitory activity in mushroom tyrosinase inhibition assays. The results indicate that *N,N*′-dimethylaniline derivatives are more potent than 1-naphthol and 2-naphthol derivatives. The most active compound **4b** showed 12- and 2-fold more inhibitory activity than kojic acid in mushroom tyrosinase inhibition and in melanogenesis inhibition in α-MSH-induced B16F10 mouse melanoma cells, respectively. Additionally, Zhou et al.[140] provided 20 of polyhydroxyl 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives as tyrosinase inhibitors and examined their inhibitory activity on mushroom tyrosinase. The results showed that 1-(5-(3,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone **1d** to be the most potent inhibitor. Compound **1d** is a competitive inhibitor and 60-fold more potent than kojic acid in mushroom tyrosinase inhibition assay. Also, a series of 16 oxadiazole and triazolothiadiazole derivatives were prepared and

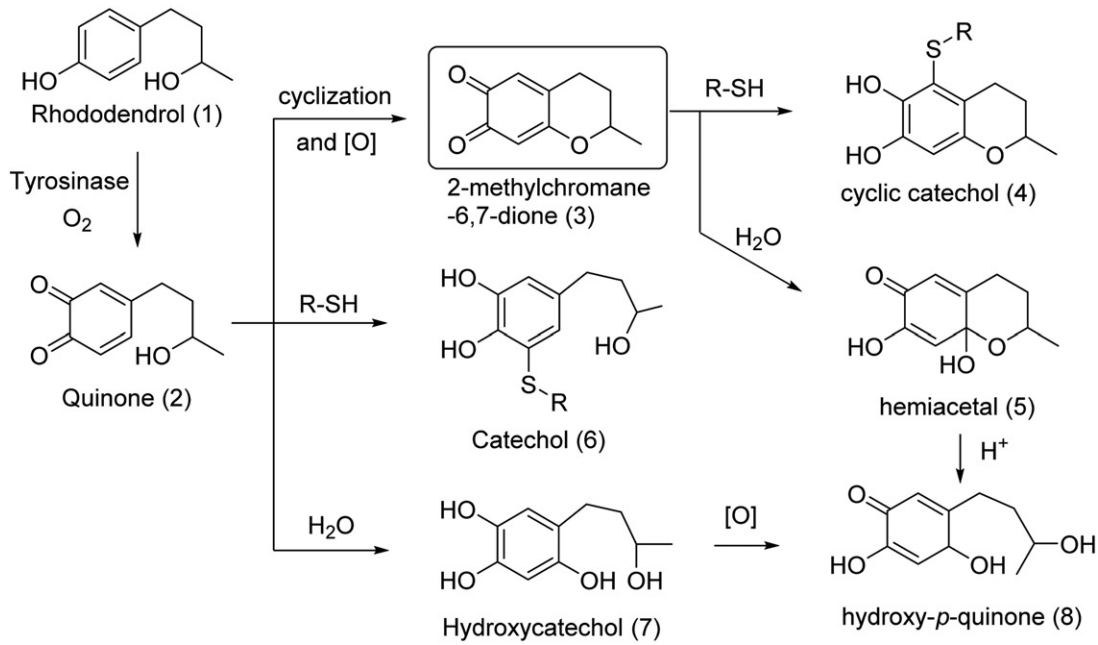

Figure 12. Mushroom tyrosinase-catalyzed transformation of rhododendrol (modified *Tajima et al.*[131]).

examined by Lam et al.[141] as tyrosinase inhibitors. Five oxadiazole thione compounds displayed high activity in mushroom tyrosinase inhibition, and 5-(naphthalen-1-ylmethyl)-3-(piperazin-1-ylmethyl)-1,3,4-oxadiazole-2(3*H*)-thione **5**, which was 11-fold more potent than kojic acid, showed the highest activity. In docking study, the thione group of **5** might have a metal-ligand interaction with dicopper nucleus of tyrosinase. Song et al.[142] added a new isoxazole scaffold to the arsenal of tyrosinase inhibitor. They synthesized a series of thirteen 5,6,7,8-tetrahydro-4*H*-cyclohepta[*d*]isoxazole derivatives and evaluated in a α-MSH-induced B16F10 mouse melanoma cell line. Compounds, **8e**, **8h** and **9b** not only show any detectable cytotoxicity but also exhibited high inhibitory activity that they were 126-, 84- and 85-fold more active than kojic acid, respectively. Additional investigation with compound **9b** revealed that its activity seemed to associate with an inhibition of tyrosinase expression without affecting the catalytic activity of tyrosinase.

Potential adverse effects

Not many cases regarding the life-threatening adverse effects of tyrosinase inhibitors have been reported mostly because the enzyme is responsible for the formation of melanin pigment mainly in skin. The most striking case recently reported would include 4-(4-hydroxyphenyl)-2-butanol, rhododendrol (Figure 12). It is a natural product isolated from *Acer nikoense*[143] and has been added as a skin-whitening ingredient in cosmetics since 2008. However, considerable number of consumers who used cosmetic products containing rhododendrol experienced leukoderma on their skins where the cosmetics were applied. In 2013, the products were finally recalled. Underlying mechanism of the leukoderma toxicity has not been clearly understood, but it was suggested that rhododendrol exerts cytotoxicity against melanocytes through production of a series of electrophilic *o*-quinone species which can deplete cytosolic GSH and then bind to crucial cellular proteins through sulfhydryl groups. The transformation into quinone species is catalyzed by tyrosinase itself. Of them, 2-methylchromane-6,7-dione (compound (**3**), Figure 12) was proposed to be the putative ultimate toxic metabolite of rhododendrol[144]. Rhododendrol (**1**) is rapidly oxidized by mushroom tyrosinase to 2-methylchromane-6,7-dione (compound (**3**) in Figure 12) through an intramolecular addition of the hydroxy group of quinone (**2**), which is the first oxidation product. In the presence of thiols (R-SH), quinones (**2**) and (**3**) further undergo an addition of thiols to afford catechol (**6**) and cyclic catechol (**4**), respectively. Water molecule can also be added to quinones (**2**) and (**3**) to produce hydroxycatechol (**7**) and hemiacetal (**5**), respectively. Quinone (**2**) and cyclic quinone (**3**) quantitatively bind to thiols, such as cysteine and GSH, and their covalent binding was confirmed as NAcCys adducts.

Quinone-type metabolites are generally considered as toxic because there are a lot of endogenous nucleophiles, which can attack the Michael acceptors. One good example is a cyclooxygenase inhibitor, acetaminophen, an active ingredient of Tylenol. Acetaminophen is oxidized to the toxic quinone by cytochrome P450[145], not by its target enzyme, cyclooxygenase, while rhododendrol is oxidized to the toxic quinones by its putative target enzyme. Therefore, the next generation tyrosinase inhibitors should be able to inhibit the activities of the enzyme without being its substrate or co-substrate.

Conclusion

The accumulation of an abnormal melanin in specific part of the skin causes an esthetic problem. Understanding of the melanocyte biology and the processes underlying melanin synthesis has made remarkable progress. Tyrosinase catalyzes rate-limiting first two steps in melanin biosynthetic pathway, and the majority of known anti-melanogenesis agents from both natural and synthetic sources are tyrosinase inhibitors. Along with treating some dermatological disorders from melanin hyperpigmentation, tyrosinase inhibitors have found a huge role in the cosmetic industry for their skin-whitening effect and depigmentation after sunburn. The nature is an unlimited source of tyrosinase inhibitor, and huge number of inhibitor from plant, fungal metabolites and marine algae have been isolated. Tyrosinase inhibitors from natural sources usually attract more attention than chemically synthesized compounds due to the cosmetic demand. However, only a few natural inhibitors have practically adapted as cosmetics due to

other parameters, such as cytotoxicity, solubility, effective cutaneous absorption, etc. In order to fulfil unmet need, many natural inhibitors were modified to semi-synthetic analogs, and many synthetic tyrosinase inhibitors with the novel structural scaffold have been designed. Although many synthetic inhibitors exhibited excellent mushroom tyrosinase inhibitory activity, only a few of them showed melanogenesis inhibition activity in cells or skin models. Thus, the primary goal for recently disclosed synthetic tyrosinase inhibitors is achieving suitable inhibitory activity in cell-based assay. Since a huge number of tyrosinase inhibitors have been developed, the necessity to clarify the viability of these inhibitors in terms of their skin-whitening efficiency has become an urgent task. Thus, thorough inspections of the identified inhibitors under human clinical point of view are required.

As shown in rhododendrol case, toxic metabolites can be formed by tyrosinase-catalyzed oxidation and can exert enormous adverse effects. The oxidation process can be catalyzed by both mushroom[144] and human[146] tyrosinase. Therefore, susceptibility to tyrosinase-catalyzed oxidation as a substrate or co-substrate should be checked for the successful next generation tyrosinase inhibitors. It is highly likely that many types of phenolic compounds can undergo such oxidation to afford quinone-type metabolites although not all quinone compounds are cytotoxic at the same level.

Declaration of interest

The authors report no declaration of interest.

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References

1. Gilchrest BA. Molecular aspects of tanning. J Invest Dermatol 2011; 131:E14–17.
2. Slominski A, Tobin DJ, Shibahara S, Wortsman J. Melanin pigmentation in mammalian skin and its hormonal regulation. Physiol Rev 2004;84:1155–228.
3. Slominski A, Zmijewski MA, Pawelek J. L-tyrosine and L-dihydroxyphenylalanine as hormone-like regulators of melanocyte functions. Pigment Cell Melanoma Res 2011;25:14–27.
4. Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. Nature 2007;445:843–50.
5. Gillbro JM, Olsson MJ. The melanogenesis and mechanisms of skin-lightening agents-existing and new approaches. Int J Cosmet Sci 2011;33:210–21.
6. Lee CH, Wu SB, Hong CH, et al. Molecular mechanisms of UV-induced apoptosis and its effects on skin residential cells: the implication in UV-based phototherapy. Int J Mol Sci 2013;14: 6414–35.
7. Videira IF, Moura DF, Magina S. Mechanisms regulating melanogenesis. An Bras Dermatol 2013;88:76–83.
8. Stratford MR, Ramsden CA, Riley PA. Mechanistic studies of the inactivation of tyrosinase by resorcinol. Bioorg Med Chem 2013;21: 1166–73.
9. Chang TS. Natural melanogenesis inhibitors acting through the down-regulation of tyrosinase activity. Materials 2012;5:1661–85.
10. Loizzo MR, Tundis R, Menichini F. Natural and synthetic tyrosinase inhibitors as antibrowning agents: an update. Compr Rev Food Sci Food Safety 2012;11:378–98.
11. Thomas NV, Kim SK. Beneficial effects of marine algal compounds in cosmeceuticals. Mar Drugs 2013;11:146–64.
12. Chang TS. An updated review of tyrosinase inhibitors. Int J Mol Sci 2009;10:2440–75.
13. Kim YJ, Uyama H. Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. Cell Mol Life Sci 2005;62:1707–23.
14. Bourquelot E, Bertrand A. Le bleuissement et le noircissement des champignons. Comp Rend Soc Biol 1895;2:582–4.
15. Ramsden CA, Riley PA. Tyrosinase: the four oxidation states of the active site and their relevance to enzymatic activation, oxidation and inactivation. Bioorg Med Chem 2014;22:2388–95.
16. Liang CP, Chang CH, Liang CC, et al. *In vitro* antioxidant activities, free radical scavenging capacity, and tyrosinase inhibitory of Flavonoid compounds and ferulic acid from *Spiranthes sinensis* (Pers.) Ames. Molecules 2014;19:4681–94.
17. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry 2000;55:481–504.
18. Kubo I, Kinst-Hori I, Chaudhuri SK, et al. Flavonols from *Heterotheca inuloides*: tyrosinase inhibitory activity and structural criteria. Bioorg Med Chem 2000;8:1749–55.
19. Kubo I, Kinst-Hori I, Ishiguro K, et al. Tyrosinase inhibitory flavonoids from *Heterotheca inuloides* and their structural functions. Bioorg Med Chem Lett 1994;4:1443–6.
20. Xie LP, Chen QX, Huang H, et al. Inhibitory effects of some flavonoids on the activity of mushroom tyrosinase. Biochemistry 2003;68:487–91.
21. No JK, Soung DY, Kim YJ, et al. Inhibition of tyrosinase by green tea components. Life Sci 1999;65:PL241–6.
22. Kim YJ. Rhamnetin attenuates melanogenesis by suppressing oxidative stress and pro-inflammatory mediators. Biol Pharm Bull 2013;36:1341–7.
23. Zehng ZP, Tan HY, Chen J, Wang M. Characterization of tyrosinase inhibitors in the twigs of *Cudrania tricuspidata* and their structure-activity relationship study. Fitoterapia 2013;84:242–7.
24. Denton CR, Lerner AB, Fitzpatrick TB. Inhibition of melanin formation by chemical agents. J Invest Dermatol 1952;18:119–35.
25. Amer M, Metwalli M. Topical hydroquinone in the treatment of some hyperpigmentary disorders. Int J Dermatol 1998;37:449–50.
26. Draelos ZD. Skin lightening preparations and the hydroquinone controversy. Dermatol Ther 2007;20:308–13.
27. Findlay GH. Ochronosis following skin bleaching with hydroquinone. J Am Acad Dermatol 1982;6:1092–3.
28. Wester RC, Melendres J, Hui X, et al. Human in vivo and in vitro hydroquinone topical bioavailability, metabolism, and disposition. J Toxicol Environ Health A 1998;54:301–17.
29. Sarkar R, Arora P, Garg KV. Cosmeceuticals for hyperpigmentation: what is available? J Cutan Aesthet Surg 2013;6:4–11.
30. Parejo I, Viladomat F, Bastida J, Codina C. A single extraction step in the quantitative analysis of arbutin in bearberry (*Arctostaphylos uva-ursi*) leaves by high-performance liquid chromatography. Phytochem Anal 2001;12:336–9.
31. Seo DH, Jung JH, Lee JE, et al. Biotechnological production of arbutins (α- and β-arbutins), skin-lightening agents, and their derivatives. Appl Microbiol Biotechnol 2012;95:1417–25.
32. Garcia A, Fulton JE. The combination of glycolic acid and hydroquinone or kojic acid for the treatment of melasma and related conditions. Dermatol Surg 1996;22:443–7.
33. Jin YH, Lee SJ, Chung MH, et al. Aloesin and arbutin inhibit tyrosinase activity in a synergistic manner via a different action mechanism. Arch Pharm Res 1999;22:232–6.
34. Boissy RE, Visscher M, DeLong MA. Deoxyarbutin: a novel reversible tyrosinase inhibitor with effective *in vivo* skin lightening potency. Exp Dermatol 2005;14:601–8.
35. Chawla S, deLong MA, Visscher MO, et al. Mechanism of tyrosinase inhibition by deoxyArbutin and its second-generation derivatives. Br J Dermatol 2008;159:1267–74.
36. Draelos ZD. Cosmetic therapy. In: Wolverton SE. ed. Comprehensive dermatologic drug therapy. 2nd ed. Philadelphia (PA): Saunders; 2007:P761–74.
37. Fleischer AB, Schwartzel EH, Colby SI, Altman DJ. The combination of 2% 4-hydroxyanisole (Mequinol) and 0.01% tretinoin is effective in improving the appearance of solar lentigines and related hyperpigmented lesions in two double-blind multicenter clinical studies. J Am Acad Dermatol 2000;42:459–67.
38. Colby SI, Schwartzel EH, Huber FJ, et al. A promising new treatment for solar lentigines. J Drugs Dermatol 2003;2:147–52.
39. Batovska DI, Todorova IT. Trends in utilization of the pharmacological potential of chalcones. Curr Clin Pharmacol 2010;5:1–29.
40. Sahu NK, Balbhadra SS, Choudhary J, Kohli DV. Exploring pharmacological significance of chalcone scaffold: a review. Curr Med Chem 2014;19:209–25.
41. Fu B, Li H, Wang X, et al. Isolation and identification of flavonoids in licorice and a study of their inhibitory effects on tyrosinase. J Agric Food Chem 2005;53:7408–14.

42. Kim SJ, Son KH, Chang HW, et al. Tyrosinase inhibitory prenylated flavonoids from *Sophora flavescens*. Biol Pharm Bull 2003;26: 1348–50.
43. Hyun SK, Lee WH, Jeong M, et al. Inhibitory effects of kurarinol, kuraridinol, and trifolirhizin from *Sophora flavescens* on tyrosinase and melanin synthesis. Biol Pharm Bull 2008;31:154–8.
44. Zhang X, Hu X, Hou A, Wang H. Inhibitory effect of 2,4,2′,4′-tetrahydroxy-3-(3-methyl-2-butenyl)-chalcone on tyrosinase activity and melanin biosynthesis. Biol Pharm Bull 2009;32:86–90.
45. Nerya O, Musa R, Khatib S, et al. Chalcones as potent tyrosinase inhibitors: the effect of hydroxyl positions and numbers. Phytochemistry 2004;65:1389–95.
46. Khatib S, Nerya O, Musa R, et al. Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol moiety. Bioorg Med Chem 2005;13:433–41.
47. Jun N, Hong G, Jun K. Synthesis and evaluation of 2′,4′,6′-trihydroxychalcones as a new class of tyrosinase inhibitors. Bioorg Med Chem 2007;15:2396–402.
48. Nguyen NT, Nguyen MH, Nguyen HX, et al. Tyrosinase inhibitors from the Wood of *Atrocarpus heterophyllus*. J Nat Prod 2012;75: 1951–5.
49. Cho SJ, Roh JS, Sun WS, et al. *N*-Benzylbenzamides: a new class of potent tyrosinase inhibitors. Bioorg Med Chem Lett 2006;16: 2682–4.
50. Baek HS, Hong YD, Lee CS, et al. Adamantyl *N*-benzylbenzamide: new series of depigmentation agents with tyrosinase inhibitory activity. Bioorg Med Chem Lett 2012;22:2110–13.
51. Wu Z, Zheng L, Li Y, et al. Synthesis and structure-activity relationships and effects of phenylpropanoid amides of octopamine and dopamine on tyrosinase inhibition and antioxidation. Food Chem 2012;134:1128–31.
52. Fang Y, Chen Y, Feng G, Ma L. Benzyl benzoates: new phlorizin analogs as mushroom tyrosinase inhibitors. Bioorg Med Chem 2011; 19:1167–71.
53. Bandgar BP, Adsul LK, Chavan HV, et al. Synthesis, biological evaluation, and molecular docking of *N*-{3-[3-(9-methyl-9*H*-carbazol-3-yl)-acryloyl]-phenyl}-benzamide/amide derivatives as xanthine oxidase and tyrosinase inhibitors. Bioorg Med Chem 2012; 20:5649–57.
54. Likhitwitayawuid K. Stilbenes with tyrosinase inhibitory activity. Curr Sci India 2008;94:44–53.
55. Aggarwal BB, Bhardwaj A, Aggarwal RS, et al. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Res 2004;24:2783–840.
56. Shin NH, Ryu SY, Choi EJ, et al. Oxyresveratrol as the potent inhibitor on dopa oxidase activity of mushroom tyrosinase. Biochem Biophys Res Commun 1998;243:801–3.
57. Shimizu K, Kondo R, Sakai K, et al. The inhibitory components from *Artocarpus incisus* on melanin biosynthesis. Planta Med 1998; 64:408–12.
58. Likhitwitayawuid K, Sritularak B. A new dimeric stilbene with tyrosinase inhibitory activity from *Artocarpus gomezianus*. J Nat Prod 2001;64:1457–9.
59. Ohguchi K, Tanaka T, Ito T, et al. Inhibitory effects of resveratrol derivatives from Dipterocarpaceae plants on tyrosinase activity. Biosci Biotechnol Biochem 2003;67:1587–9.
60. Likhitwitayawuid K, Sornsute A, Sritularak B, Ploypradith P. Chemical transformations of oxyresveratrol (*trans*-2,4,3′,5′-tetrahydroxystilbene) into a potent tyrosinase inhibitor and a strong cytotoxic agent. Bioorg Med Chem Lett 2006;16:5650–3.
61. Ohguchi K, Tanaka K, Kido T, et al. Effects of hydroxystilbene derivatives on tyrosinase activity. Biochem Biophys Res Commun 2003;307:861–3.
62. Choi SY, Kim S, Kim H, et al. (4-Methoxy-benzylidene)-(3-methoxy-phenyl)-amine, a nitrogen analog of stilbene as a potent inhibitor of melanin production. Chem Pharm Bull 2002;50:450–2.
63. Song S, Lee H, Jin Y, et al. Syntheses of hydroxy substituted 2-phenyl-naphthalenes as inhibitors of tyrosinase. Bioorg Med Chem Lett 2007;17:461–4.
64. Bae SJ, Ha YM, Kim JA, et al. A Novel Synthesized Tyrosinase Inhibitor: (*E*)-2-((2,4-Dihydroxyphenyl)diazenyl)phenyl 4-methylbenzenesulfonate as an azo-Resveratrol Analog. Biosci Biotechnol Biochem 2013;77:65–72.
65. Bae SJ, Ha YM, Park YJ, et al. Design, synthesis, and evaluation of (*E*)-*N*-substituted benzylidene-aniline derivatives as tyrosinase inhibitors. Eur J Med Chem 2012;57:383–90.
66. Song YM, Ha YM, Kim JA, et al. Synthesis of novel azo-resveratrol, azo-oxyresveratrol and their derivatives as potent tyrosinase inhibitors. Bioorg Med Chem Lett 2012;22:7451–5.
67. Kolbe L, Mann T, Gerwat W, et al. Stab F, 4-n-butylresorcinol, a highly effective tyrosinase inhibitor of the topical treatment of hyperpigmentation. JEADV 2013;27:19–23.
68. Burdock GA, Soni MG, Carabin IG. Evaluation of health aspects of kojic acid in food. Regul Toxicol Pharmacol 2001;33:80–101.
69. Bentley R. From *miso*, *sake* and *shoyu* to cosmetics: a century of science for kojic acid. Nat Prod Rep 2006;23:1046–62.
70. Kahn V, Ben-Shalom N, Zakin V. Effect of kojic acid on the oxidation of *N*-acetyldopamine by mushroom tyrosinase. J Agric Food Chem 1997;45:4460–5.
71. Lajis AF, Hamid M, Ariff AB. Depigmenting effect of kojic acid esters in hyperpigmented B16F1 melanoma cells. J Biomed Biotech 2012;2012:952452.
72. Tomita I, Mitsuhashi K, Endo T. Synthesis and radical polymerization of styrene derivative bearing Kojic acid moieties. J Polym Sci A1 1996;34:271–6.
73. Molenda JJ, Basinger MA, Hanusa TP, Jones MM. Synthesis and iron (III) binding properties of 3-hydroxypyrid-4-ones derived from Kojic acid. J Inorg Biochem 1994;55:131–46.
74. Kim H, Choi J, Cho JK, et al. Solid-phase synthesis of kojic acid-tripeptides and their tyrosinase inhibitory activity, storage stability, and toxicity. Bioorg Med Chem Lett 2004;14:2843–6.
75. Saghaie L, Pourfarzam M, Fassihi A, Sartippour B. Synthesis and tyrosinase inhibitory properties of some novel derivatives of kojic acid. Res Pharm Sci. 2013;8:233–42.
76. Rho HS, Baek HS, Ahn SM, et al. Synthesis of new anti-melanogenic compounds containing two molecules of kojic acid. Bull Kor Chem Soc 2008;29:1569–71.
77. Noh JM, Kwak SY, Seo HS, et al. Kojic acid-amino acid coujugates as tyrosinase inhibitors. Bioorg Med Chem Lett 2009;19:5586–9.
78. Li DF, Hu PP, Liu MS, et al. Design and synthesis of hydroxypyridinone-L-phenylalanine conjugates as potential tyrosinase inhibitors. J Agric Food Chem 2013;61:6597–603.
79. Jones K, Hughes J, Hong M, et al. Modulation of melanogenesis by aloesin: a competitive inhibitor of tyrosinase. Pigment Cell Res 2002;15:335–40.
80. Choi S, Lee SK, Kim JE, et al. Aloesin inhibits hyperpigmentation induced by UV radiation. Clin Exp Dermatol 2002;27:513–15.
81. Bubols GB, Vianna DR, Medina-Remon A, et al. The antioxidant activity of coumarins and flavonoids. Mini Rev Med Chem 2013;13: 318–34.
82. Masamoto Y, Ando H, Murata Y, et al. Mushroom tyrosinase inhibitory activity of esculetin isolated from seeds of *Euphorbia lathyris* L. Biosci Biotechnol Biochem 2003;67:631–4.
83. Piao XL, Baek SH, Park MK, Park JH. Tyrosinase-inhibitory furanocoumarin from *Angelica dahurica*. Biol Pharm Bull 2004;27: 1144–6.
84. Ahmad VU, Ullah F, Hussain J, et al. Tyrosinase inhibitors from *Rhododendron collettianum* and their structure-activity relationship (SAR) studies. Chem Pharm Bull 2004;52:1458–61.
85. Luceri C, Giannini L, Lodovici M, et al. *p*-Coumaric acid, a common dietary phenol, inhibits platelet activity *in vitro* and *in vivo*. Br J Nutr 2007;97:458–63.
86. Lee HS, Shin KH, Ryu GS, et al. Synthesis of small molecule-peptide conjugates as potential whitening agents. Bull Korean Chem Soc 2012;33:3004–8.
87. Duckworth HW, Coleman JE. Physicochemical and kinetic properties of mushroom tyrosinase. J Biol Chem 1970;245:1613–25.
88. Maghsoudi S, Adibi H, Hanzeh M, et al. Kinetic of mushroom tyrosinase inhibition by benzaldehyde derivarives. J Rep Pharm Sci 2013;2:156–64.
89. Kubo I, Chen QX, Nihei K, et al. Tyrosinase inhibition kinetics of anisic acid. Z Naturforsch C 2003;58:713–18.
90. Ha TJ, Tamura S, Kubo I. Effects of mushroom tyrosinase on anisaldehyde. J Agric Food Chem 2005;53:7024–8.
91. Shi Y, Chen QX, Wang Q, et al. Inhibitory effects of cinnamic acid and its derivatives on the diphenolase activity of mushroom (*Agaricus bisporus*) tyrosinase. Food Chem 2005;92:707–12.
92. Lee HS. Tyrosinase inhibitors of *Pulsatilla cernua* root-derived materials. J Agric Food Chem 2002;50:1400–3.
93. Miyazawa M, Oshima T, Koshino K, et al. Tyrosinase inhibitor from black rice bran. J Agric Food Chem 2003;51:6953–6.

94. Kubo I, Kinst-Hori I. Tyrosinase inhibitors from cumin. J Agric Food Chem 1998;46:5338–41.
95. Conrad JS, Dawso SR, Hubbard ER, et al. Inhibitor binding to the binuclear active site of tyrosinase: temperature, pH and solvent deuterium isotope effects. Biochemistry 1994;33:5739–44.
96. Kubo I, Kinst-Hori I, Kubo Y, et al. Molecular design of antibrowning agents. J Agric Food Chem 2000;48:1393–9.
97. Jeon HJ, Noda M, Maruyama M, et al. Identification and kinetic study of tyrosinase inhibitors found in sake lees. J Agric Food Chem 2006;54:9827–33.
98. Magid AA, Voutguenne-Nazabadioko L, Bontemps G, et al. Tyrosinase inhibitors and sesquiterpene diglycosides from *Guioa villosa*. Planta Med 2008;74:55–60.
99. Masuda T, Odaka Y, Ogawa N, et al. Identification of geranic acid, a tyrosinase inhibitor in lemongrass (*Cymbopogon citratus*). J Agric Food Chem 2008;56:597–601.
100. Sabudak T, Khan HTM, Choudhary MI, Oksuz S. Potent tyrosinase inhibitors from *Trifolium Balansae*. Nat Prod Res 2006;20:665–70.
101. Khan MT, Khan SB, Ather A. Tyrosinase inhibitory cycloartane type triterpenoids from the methanol extract of the whole plant of *Amberboa ramosa* Jafri and their structure-activity relationship. Bioorg Med Chem 2006;14:938–43.
102. Ullah F, Hussain H, Hussain J, et al. Tyrosinase inhibitory pentacyclic triterpenes and analgesic and spasmolytic activities of methanol extracts of *Rhododendron collettianum*. Phytother Res 2007;21:1076–81.
103. Leu YL, Hwang TL, Hu JW, Fang JY. Anthraquinones from *Polygonum cuspidatum* as tyrosinase inhibitors for dermal use. Phytother Res 2008;22:552–6.
104. Devkota KP, Khan MT, Ranjit R, et al. Tyrosinase inhibitory and antileishmanial constituents from the rhizomes of *Paris polyphylla*. Nat Prod Res 2007;21:321–7.
105. Azhar-Ul-Haq, Malik A, Khan MT, et al. Tyrosinase inhibitory lignans from the methanol extract of the roots of *Vitex negundo* Linn, and their structure-activity relationship. Phytomedicine 2006; 13:255–60.
106. Wang HM, Chen CY, Chen CY, et al. (−)-*N*-Formylanonaine from *Michelia alba* as a human tyrosinase inhibitor and antioxidant. Bioorg Med Chem 2010;18:5241–7.
107. Wu B, Zhang X, Wu X. New lignan glucosides with tyrosinase inhibitory activities from exocarp of *Castanea henryi*. Carbohydr Res. 2012;355:45–9.
108. Akihisa T, Orido M, Akazawa H, et al. Melanogenesis-inhibitory activity of aromatic glycosides from the stem bark of *Acer buergerianum*. Chem Biodivers 2013;10:167–75.
109. Hashim NM, Rahmani M, Ee GC, et al. Antioxidant, antimicrobial and tyrosinase inhibitory activities of xanthones isolated from *Artocarpus obtusus* F.M. Jarrett. Molecules 2012;17:6071–82.
110. Criton M, Le Mellay-Hamon V. Analogues of *N*-hydroxy-*N*′-phenylthiourea and *N*-hydroxy-*N*′-phenylurea as inhibitors of tyrosinase and melanin formation. Bioorg Med Chem Lett 2008; 18:3607–10.
111. Shiino M, Watanabe Y, Umezawa K. Synthesis of *N*-substituted *N*-nitrosohydroxylamines as inhibitors of mushroom tyrosinase. Bioorg Med Chem 2001;9:1233–40.
112. Khan KM, Maharvi GM, Perveen S, et al. Synthesis of methyl ether analogues of sildenafil (*Viagra*) possessing tyrosinase inhibitory potential. Chem Biodivers 2005;2:470–6.
113. Khan MT, Choudhary MI, Khan KM, et al. Structure-activity relationships of tyrosinase inhibitory combinatorial library of 2,5-disubstituted-1,3,4-oxadiazole analogues. Bioorg Med Chem 2005; 13:3385–95.
114. Khan KM, Mughal UR, Khan MT, et al. Oxazolones: new tyrosinase inhibitors; synthesis and their structure-activity relationships. Bioorg Med Chem 2006;14:6027–33.
115. Khan KM, Maharvi GM, Khan MT, et al. Tetraketones: a new class of tyrosinase inhibitors. Bioorg Med Chem 2006;14:344–51.
116. Kim YJ, No JK, Lee JH, Chung HY. 4,4′-Dihydroxybiphenyl as a new potent tyrosinase inhibitor. Biol Pharm Bull 2005;28:323–7.
117. Lee KH, Koketsu M, Choi SY, et al. Potent inhibitory effects of *N*-aryl *S*-alkylthiocarbamate derivatives on the dopa oxidase activity of mushroom tyrosinase. Chem Pharm Bull 2005;53: 747–9.
118. Lam KW, Syahida A, Ul-Haq Z, et al. Synthesis and biological activity of oxadiazole and triazolothiadiazole derivatives as tyrosinase inhibitors. Bioorg Med Chem Lett 2010;20:3755–9.
119. Ha YM, Park YJ, Lee JY, et al. Design, synthesis and biological evaluation of 2-(substituted phenyl)thiazolidine-4-carboxylic acid derivatives as novel tyrosinase inhibitors. Biochimie 2012;94: 533–40.
120. Chen LH, Hu YH, Song W, et al. Synthesis and antityrosinase mechanism of benzaldehyde thiosemicarbazones: novel tyrosinase inhibitors. J Agric Food Chem 2012;60:1542–7.
121. Lee KC, Thanigaimalai P, Sharma VK, et al. Structural characteristics of thiosemicarbazones as inhibitors of melanogenesis. Bioorg Med Chem Lett 2010;20:6794–6.
122. Yi W, Dubois C, Yahiaoui S, et al. Refinement of arylthiosemicarbazone pharmacophore in inhibition of mushroom tyrosinase. Eur J Med Chem 2011;46:4330–5.
123. Yi W, Cao R, Chen Z, et al. Rational design and synthesis of 4-*O*-substituted phenylmethylenethiosemicarbazones as novel tyrosinase inhibitors. Chem Pharm Bull 2010;58:752–4.
124. Yi W, Cao R, Peng W, et al. Synthesis and biological evaluation of novel 4-hydroxybenzaldehyde derivatives as tyrosinase inhibitors. Eur J Med Chem 2010;45:639–46.
125. Delogu G, Podda G, Corda M, et al. Synthesis and biological evaluation of a novel series of bis-salicylaldehydes as mushroom tyrosinase inhibitors. Bioorg Med Chem Lett 2010;20:6138–40.
126. Pan ZZ, Li HL, Yu XJ, et al. Synthesis and antityrosinase activities of alkyl 3,4-dihydroxybenzoates. J Agric Food Chem 2011;59: 6645–9.
127. Sonmez F, Sevmezler S, Atahan A, et al. Evaluation of new chalcone derivatives as polyphenol oxidase inhibitors. Bioorg Med Chem Lett 2011;21:7479–82.
128. Matos MJ, Santana L, Uriarte E, et al. New halogenated phenylcoumarins as tyrosinase inhibitors. Bioorg Med Chem Lett 2011;21:3342–5.
129. Du ZY, Jiang YF, Tang ZK, et al. Antioxidation and tyrosinase inhibition of polyphenolic curcumin analogs. Biosci Biotechnol Biochem 2011;75:2351–8.
130. Hosoya T, Nakata A, Yamasaki F, et al. Curcumin-like diarylpentanoid analogues as melanogenesis inhibitors. J Nat Med 2012;66: 166–76.
131. Tajima R, Oozeki H, Muraoka S, et al. Synthesis and evaluation of bibenzyl glycosides as potent tyrosinase inhibitors. Eur J Med Chem 2011;46:1374–81.
132. Ha YM, Kim JA, Park YJ, et al. Analogs of 5-(substituted benzylidene)hydantoin as inhibitors of tyrosinase and melanin formation. Biochim Biophys Acta 2011;1810:612–19.
133. Liu J, Wu F, Chen L, et al. Evaluation of dihydropyrimidin-(2*H*)-one analogues and rhodanine derivatives as tyrosinase inhibitors. Bioorg Med Chem Lett 2011;21:2376–9.
134. Yan Q, Cao R, Yi W, et al. Inhibitory effects of 5-benzylidene barbiturate derivatives on mushroom tyrosinase and their antibacterial activities. Eur J Med Chem 2009;44:4235–43.
135. Han YK, Park YJ, Ha YM, et al. Characterization of a novel tyrosinase inhibitor, (2*RS*,4*R*)-2-(2,4-dihydroxylphenyl) thiazolidine-4-carboxylic acid (MHY384). Biochim Biophys Acta 2012; 1820:542–9.
136. Park JW, Ha YM, Moon KM, Kim SR. De novo tyrosinase inhibitor: 4-(6,7-dihydro-5*H*-indeno[5,6-*d*]thiazol-2-yl)benzene-1,3-diol(MHY1556). Bioorg Med Chem Lett 2013;23:4172–6.
137. Gencer N, Demir D, Sonmez F, Kucukislamoglu M. New saccharin derivatives as tyrosinase inhibitors. Bioorg Med Chem 2012;20: 2811–21.
138. Bao K, Dai Y, Zhu ZB, et al. Design and synthesis of biphenyl derivatives as mushroom tyrosinase inhibitors. Bioorg Med Chem 2010;18:6708–14.
139. Hamidian H, Tagizadeh R, Fozooni S, et al. Synthesis of novel azo compounds containing 5(4*H*)-oxazolone ring as potent tyrosinase inhibitors. Bioorg Med Chem 2013;21:2088–92.
140. Zhou Z, Zhuo J, Yan S, Ma L. Design and synthesis of 3,5-diaryl-4,5-dihydro-1*H*-pyrazoles as new tyrosinase inhibitors. Bioorg Med Chem 2013;21:2156–62.
141. Lam KW, Syahida A, Ul-Haq Z, et al. Synthesis and biological activity of oxadiazole and triazolothiadiazole derivatives as tyrosinase inhibitor. Bioorg Med Chem Lett 2010;20:3755–9.
142. Song J, Lee HE, Kim YJ, et al. Discovery of small molecules that inhibit melanogenesis via regulation of tyrosinase expression. Bioorg Med Chem Lett 2012;22:6943–6.
143. Akazawa H, Akihisa T, Taguchi Y, et al. Melanogenesis inhibitory and free radical scavenging activities of diarylheptanoids and other

phenolic compounds from the bark of *Acer nikoense*. Biol Pharm Bull 2006;29:1970–2.
144. Ito S, Ojika M, Yamashita T, Wakamatsu K. Tyrosinase-catalyzed oxidation of rhododendrol produces 2-methylchromane-6,7-dione, the putative ultimate toxic metabolite: implications for melanocyte toxicity. Pigment Cell Melanoma Res 2014;27:744–53.
145. Dahlin DC, Miwa GT, Lu AY, Nelson SD. N-acetyl-p-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. Proc Natl Acad Sci USA 1984;81:1327–31.
146. Ito S, Gerwat W, Kolbe L, et al. Human tyrosinase is able to oxidize both enantiomers of rhododendrol. Pigment Cell Melanoma Res 2014;27:1149–53.