

Review

Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future

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Abstract. Tyrosinase is known to be a key enzyme in melanin biosynthesis, involved in determining the color of mammalian skin and hair. Various dermatological disorders, such as melasma, age spots and sites of actinic damage, arise from the accumulation of an excessive level of epidermal pigmentation. In addition, unfavorable enzymatic browning of plant-derived foods by tyrosinase

causes a decrease in nutritional quality and economic loss of food products. The inadequacy of current conventional techniques to prevent tyrosinase action encourages us to seek new potent tyrosinase inhibitors. This article overviews the various inhibitors obtained from natural and synthetic sources with their industrial importance.

Key words. Tyrosinase; melanogenesis; hyperpigmentation; food browning; inhibitor.

Introduction

Melanin is one of the most widely distributed pigments and is found in bacteria, fungi, plants and animals. It is a heterogeneous polyphenol-like biopolymer with a complex structure and color varying from yellow to black [1]. The color of mammalian skin and hair is determined by a number of factors, the most important of which is the degree and distribution of melanin pigmentation. Melanin is secreted by melanocyte cells distributed in the basal layer of the dermis [2]. The role of melanin is to protect the skin from ultraviolet (UV) damage by absorbing UV sunlight and removing reactive oxygen species (ROS). Various dermatological disorders result in the accumulation of an excessive level of epidermal pigmentation. These hyperpigmented lentigenes include melasma, age spots and sites of actinic damage [3]. Great interest has been shown in the involvement of melanins

in malignant melanoma, the most life-threatening skin tumors.

The type and amount of melanin synthesized by the melanocyte and its distribution in the surrounding keratinocytes determine the actual color of the skin. The characteristic skin patterns of zebra, giraffes and piebald animals in general are due to this uneven distribution of melanocytes. Melanin is formed through a series of oxidative reactions involving the amino acid tyrosine in the presence of tyrosinase.

Tyrosinase (EC 1.14.18.1) is a copper-containing enzyme that catalyzes two distinct reactions of melanin synthesis: the hydroxylation of tyrosine by monophenolase action and the oxidation of 3,4-dihydroxyphenylalanine (L-DOPA) to *o*-dopaquinone by diphenolase action. However, if L-DOPA is an active cofactor, its formation as an intermediate during *o*-dopaquinone production is still controversial. *o*-Dopaquinone is unstable in aqueous solution and rapidly suffers a non-enzymatic cyclization to leukodopachrome, which is further oxidized non-

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enzymatically by another molecule of *o*-dopaquinone to yield dopachrome and one molecule of regenerated L-DOPA [4–6].

Tyrosinase exists widely in plants and animals, and is involved in the formation of melanin pigments [7–9]. In the food industry, tyrosinase is a very important enzyme in controlling the quality and economics of fruits and vegetables [8–10]. Tyrosinase catalyzes the oxidation of phenolic compounds to the corresponding quinones and is responsible for the enzymatic browning of fruits and vegetables. In addition to the undesirable color and flavor, the quinone compounds produced in the browning reaction may irreversibly react with the amino and sulfhydryl groups of proteins. The quinone-protein reaction decreases the digestibility of the protein and the bioavailability of essential amino acids, including lysine and cysteine. Therefore, development of high-performance tyrosinase inhibitors is much needed in the agricultural and food fields.

Tyrosinase plays an important role in the developmental and defensive functions of insects. Tyrosinase is involved in melanogenesis, wound healing, parasite encapsulation and sclerotization in insects [11–13]. The development of tyrosinase inhibitors has become an active alternative approach in controlling insect pests. In addition, tyrosinase inhibitors have become increasingly important for medicinal and cosmetic products that may be used to prevent or treat pigmentation disorders [14, 15].

Biochemical characteristics and reaction mechanism of tyrosinase

In higher plants and fungi, tyrosinases exist in immature, mature latent and active isoforms [16, 17]. However, bio-

chemical characterization of the kinetics and relationship between the isoforms is not yet complete. The active site of tyrosinase consists of two copper atoms and three states. Structural models for the active site of these three forms of tyrosinase have been proposed [17–19].

Classification and properties of tyrosinase

By far the most well-studied multi-copper oxygenase is tyrosinase, which contains a coupled binuclear copper active site. Tyrosinase catalyzes both the *o*-hydroxylation of monophenols and the two-electron oxidation of *o*-diphenols to *o*-quinones. The latter is much more rapid than the former; thus, the hydroxylation of tyrosine to L-DOPA is considered to be the rate-determining step. Labeling studies demonstrated that the oxygen incorporated into the phenolic substrate derives from molecular O₂ [20]. The two electrons required to reduce the second oxygen atom to H₂O were supplied by the substrate.

The best-characterized tyrosinases are derived from *Streptomyces glaucescens*, the fungi *Neurospora crassa* and *Agaricus bisporus*, as shown in table 1. The first two are monomeric proteins, while the last is a tetramer with two different subunits, heavy and light. Tyrosinases have been isolated and at least partially purified from numerous plant and animal sources, but few of them have been well characterized. Unlike the fungal tyrosinase, human tyrosinase is a membrane-bound glycoprotein (13% carbohydrate) [21]. Many of these enzymes have been sequenced, including ones from *N. crassa* and humans [22–24]. A variety of mutations in the human tyrosinase gene have been correlated with the pigment deficiency of oculocutaneous albinism [25]. This nearly ubiquitous enzyme has been adapted to serve diverse physiological

Table 1. Properties of various tyrosinases [19].

Source	Number of subunits	Molecular weight of subunit (kDa)	Spectroscopy of oxy form	
			Absorption (nm)	CD (nm)
<i>Streptomyces glaucescens</i> (Eubacteria)	1	30.9	345 640	345 470 575 740
<i>Neurospora crassa</i> (Fungi)	1	46	345 425 600	345 520 600 750
<i>Agaricus bisporus</i> (Fungi; mushroom)	2	13.4 43	345 600	353
<i>Beta vulgaris</i> (Plant; spinach-beet)	1	40	345	— ^a
Human melanocyte (Animal)	1	66.7	— ^a	— ^a

^a No data available.

roles in different organisms [26, 27]. In fungi and vertebrates, tyrosinase catalyzes the initial step in the formation of the pigment melanin from tyrosine. In plants, the physiological substrates are a variety of phenolics. Tyrosinase oxidizes them in the browning pathway observed when tissues are injured; however, the function of this reaction is not clear. One possible role is protection of the wound from pathogens or insects. In the latter, tyrosinase is thought to be involved in wound healing and possibly sclerotization of the cuticle.

Structure of active center

Chemical and spectroscopic studies of tyrosinase have demonstrated that the geometric and electronic structures of the binuclear copper active site of this enzyme are extremely similar to those found in hemocyanins [28–30]. While it is unfortunate that there is no crystal structure presently available for any tyrosinases, much insight into their active site and contribution of the active site to reactivity can be obtained from correlations to hemocyanins, of which crystal structures exist for both the deoxy and oxy forms of the active sites [18, 31, 32].

In the formation of melanin pigments, three types of tyrosinase (met, oxy and deoxytyrosinases) with different binuclear copper structure of the active site are involved [33–35]. Mettyrosinase, the resting form of tyrosinase, contains two tetragonal Cu(II) ions antiferromagnetically coupled through an endogenous bridge, although hydroxide exogenous ligands other than peroxide are bound to the copper site. The antiferromagnetic coupling between the Cu(II) ions of mettyrosinase triggers the lack of an electron paramagnetic resonance (EPR) signal, which requires a superexchange pathway associated with a bridging ligand [36]. This species can be converted by addition of peroxide to oxytyrosinase, which in turn decays back to mettyrosinase when the peroxide is lost. The resting form of tyrosinase is found to be a mixture of 85% met and 15% oxy forms. In addition, a half-met derivative containing the two coppers in a mixed valence oxidation state [Cu(I)–Cu(II)] can be prepared, which is EPR detectable. Extensive spectroscopic studies on this form have showed electron delocalization between the coppers and exogenous ligands bridging at the binuclear copper active site [18, 37].

Oxytyrosinase also can be produced by the two-electron reduction of deoxytyrosinase, followed by the reversible binding of dioxygen [18], which reacts with monophenol as well as *o*-diphenol substrate. Thus, its geometric and electronic structures are key to understanding the hydroxylation chemistry of tyrosinase. Oxytyrosinase consists of two tetragonal Cu(II) atoms, each coordinated by two strong equatorial and one weaker axial N_{HIS} ligands. The exogenous oxygen molecule is bound as peroxide and bridges the two copper centers. Peroxide bound in

this mode confers a distinct $O_2^{2-} \rightarrow Cu(II)$ charge transfer spectrum which can be correlated to the optical features of the oxy form of tyrosinase and includes an extremely intense absorption band at 350 nm and CD band at 325 nm [34, 38].

Deoxytyrosinase, an analogue of deoxyhemocyanin, has a bicuprous structure [Cu(I)–Cu(I)]. From the crystal structure of deoxy *Panulirus interruptus* hemocyanin, no protein residue in the vicinity of the copper site can bridge; thus, the bridging ligand must be hydroxide from water, and a similar situation is likely the case for mettyrosinase. These three copper states in the active site of tyrosinase led to a structural model being proposed for the reaction mechanism involved in the *o*-hydroxylation of monophenols and oxidation of the resulting *o*-diphenols, which was based on an associative ligand substitution at the active site of tyrosinase.

Tyrosinase has three domains, of which the central domain contains two copper binding sites. Six histidine residues bind a pair of copper ions in the active site of tyrosinase, which interact with both molecular oxygen and its phenolic substrate [39]. The location of cysteine also plays an important role in the formation of disulfide linkages, which stabilize protein structure. The number of cysteine residues varies from one organism to another, as long the N-terminal and central part of the protein, human and mouse tyrosinases have 17 cysteine residues and plants have 11, whereas the C-terminal domain contains 1 cysteine residue. Interestingly, *N. crassa*, *A. bisporus* and prokaryotic tyrosinases contain 0 or 1 cysteine in mature protein. In the mushroom tyrosinase sequence only two cysteine residues are found in the C-terminal domain [40]. To determine the accessibility of various ligands toward the binuclear copper active site, a number of kinetic studies with several compounds were carried out, and it was found that large-size ligands have a higher affinity for the active site compared with smaller ones [18, 41].

Mechanism of tyrosinase action

The above considerations have led to the molecular mechanism for the monophenolase and diphenolase activity of tyrosinase. The mechanism for the monophenolase activity of tyrosinase has widely been studied [34, 42, 43] based on three forms of the enzyme. In the monophenolase cycle, the monophenol can react only with the oxy form and binds to the axial position of one of the coppers of this oxy form. Rearrangement through a trigonal bipyramidal intermediate leads to *o*-hydroxylation of monophenol by the bound peroxide (fig. 1). This generates a coordinated *o*-diphenol, which is oxidized to the *o*-quinone, resulting in a deoxy form ready for further dioxygen binding.

The *o*-diphenol can react with the met form present to give the coordinated *o*-diphenol in the monophenolase

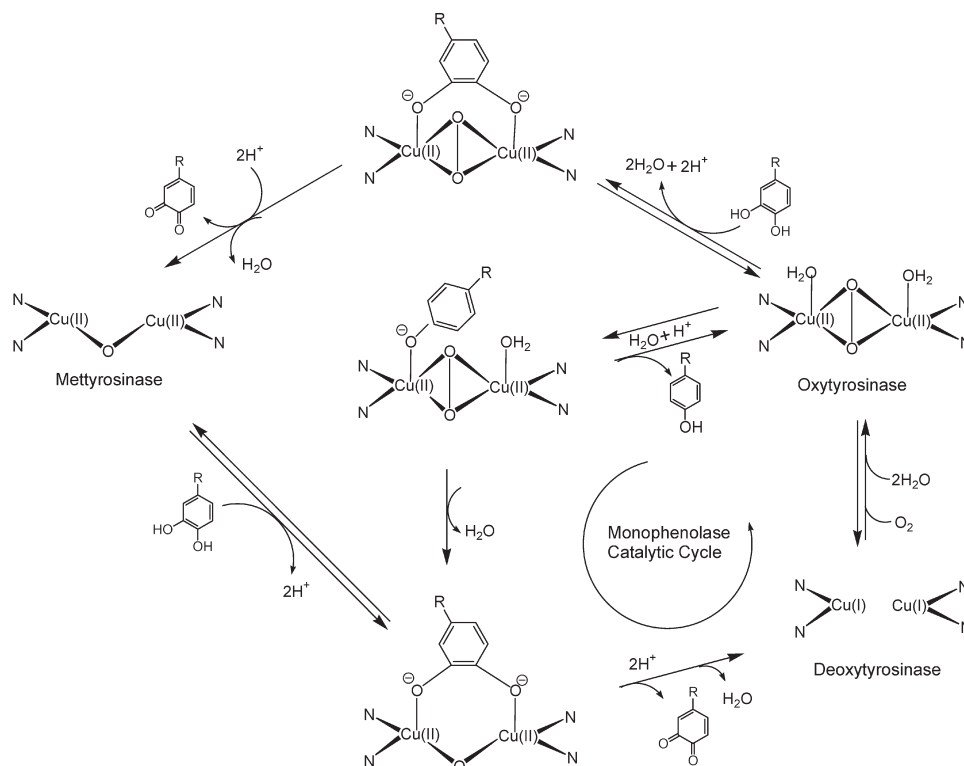


Figure 1. Catalytic cycles of the hydroxylation of monophenol and oxidation of *o*-diphenol to *o*-quinone by tyrosinase [4].

cycle. In the diphenolase cycle, both the oxy and met forms react with *o*-diphenol, oxidizing it to the *o*-quinone. However, monophenol can compete with *o*-diphenol for binding to the met form site, inhibiting its reduction. Comparing the kinetic constants for monophenolic versus *o*-diphenolic substrates, bulky substituents on the ring dramatically reduce monophenolase activity but not diphenolase activity [34]. This suggests that while monophenolic substrates require the axial to equatorial arrangement for *o*-hydroxylation, *o*-diphenolic substrates need not undergo rearrangement at the copper site for simple electron transfer.

Kinetic studies of the steady state of the pathway show the lower catalytic efficiency of tyrosinase on monophenols than on *o*-diphenols [35, 44, 45]. Monophenolase activity is typically characterized by a lag time [42, 43, 46, 47] which is dependent on factors such as substrate and enzyme concentrations, and presence of a hydrogen donor [6]. In the kinetic studies, lag time is the time required for the resting met form to be drawn into the active deoxy form by the reducing agent, arising via action of the small amounts of the oxy form that usually accompany the met form. In the presence of reducing agents known as cofactors, especially *o*-diphenol derivatives such as L-DOPA and (+)-catechin, tyrosinase was activated and the lag time was shortened or abolished as shown in figure 2 [38, 46, 47]. L-DOPA at a very low

concentration was the most effective reducing agent for eliminating lag time.

Pathway of melanin biosynthesis in mammalia

Mammalian melanocytes can produce two types of melanin: eumelanin is black or brown and pheomelanin is red or yellow in color [48, 49]. Switching between these two

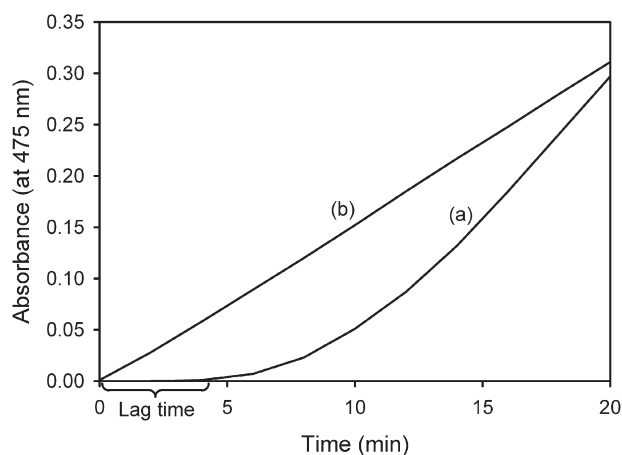


Figure 2. Hydroxylation of tyrosine by monophenolase action of mushroom tyrosinase in the absence (a) and presence (b) of (+)-catechin [38]. The increase of the absorbance at 475 nm is due to the formation of dopachrome by the hydroxylation of tyrosine.

types of melanins in follicular melanocytes elicits a temporary shift from eumelanogenesis to pheomelanogenesis, which is responsible for the wild-type agouti pigment of murine hair color [50]. For many decades, melanosomal proteins that regulate melanin biosynthesis have been studied and characterized, especially those required for eumelanogenesis [51].

The pathway of eumelanogenesis may be divided into two phases, one proximal and the other distal (fig. 3) [1, 52]. The proximal phase consists of the enzymatic oxidation of tyrosine or L-DOPA to its corresponding *o*-dopaquinone catalyzed by tyrosinase. This nascent *o*-dopaquinone can undergo two different types of reactions: intramolecular 1,4-addition to the benzene ring or a water addition reaction. The amino group of an *o*-dopaquinone side chain first undergoes an intramolecular 1,4-addition to the benzene ring, which causes its cyclization into leukodopachrome, as shown in figure 3. This intermediate is quickly oxidized to dopachrome by another *o*-dopaquinone, which is reduced back to L-DOPA [4–6].

The second reaction occurs with cyclizable and noncyclizable quinones and consists of a water addition to the benzene ring, which leads to the formation of a three-hydroxylated phenol, 2,4,5-trihydroxyphenylalanine (TOPA), which is chemically oxidized to *p*-topaquinone by another *o*-dopaquinone [4, 53]. This *p*-topaquinone evolves through a series of slow reactions to dopachrome, which is the final product of the proximal phase.

The distal phase is represented by chemical and enzymatic reactions which occur after dopachrome formation and lead to the synthesis of eumelanins (fig. 3). This

phase starts with the slow chemical decarboxylation of dopachrome to 5,6-dihydroxyindole (DHI) and its subsequent oxidation to indole-5,6-quinone. As an alternative to this chemical evolution in the distal phase, dopachrome may be enzymatically transformed into 5,6-dihydroxyindole-2-carboxylic acid (DHICA) by dopachrome tautomerase [54, 55]. DHICA is further oxidized by a redox reaction with *o*-dopaquinone to form indole-5,6-quinone carboxylic acid, which can exist in three tautomeric forms, including the quinone-imine and the corresponding highly reactive quinone-methide [56, 57]. Properties of DHI-derived and DHICA-derived melanins differ in each other; the former are black and flocculent, while the latter are yellowish-brown and finely dispersed [55].

During pheomelanogenesis, the thiol group of sulfhydryl compounds such as glutathione and cysteine nucleophilically attacks *o*-dopaquinone made enzymatically by tyrosinase to produce cysteinyl-dopa or glutathionyl-dopa. This thiol group can be added to different ring positions, although the 5-position is the favored position. Subsequent cyclization and polymerization of cysteinyl-dopa or glutathionyl-dopa in an uncharacterized series of reactions result in the production of pheomelanins and trichochromes [49, 51]. The interaction between the eumelanin and pheomelanin compounds gives rise to a heterogeneous pool of mixed-type melanins.

Enzymatic browning of plant-derived foods

The enzymatic browning of plant-derived foods and beverages takes place in the presence of oxygen when tyrosi-

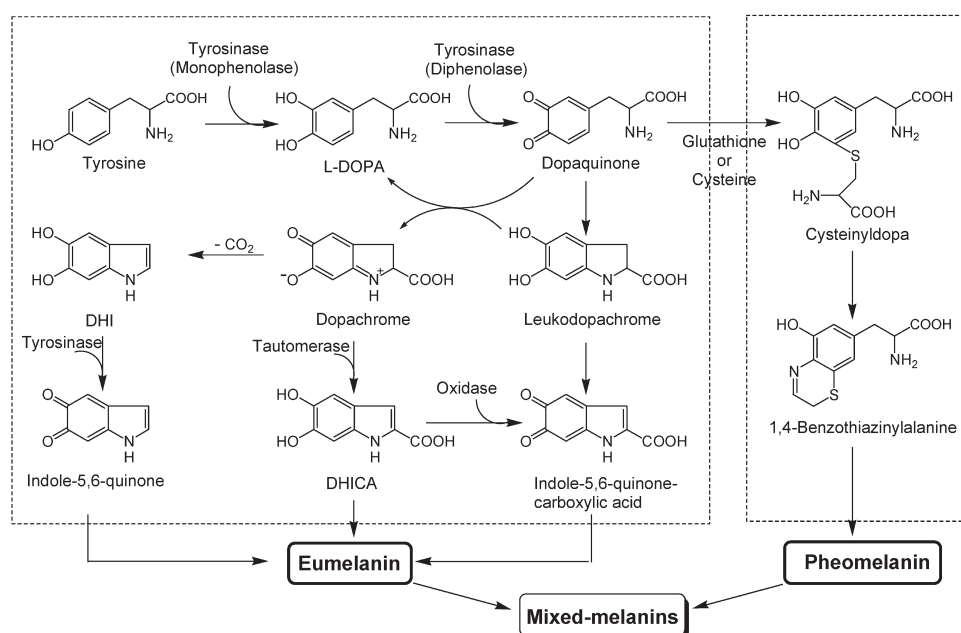


Figure 3. Biosynthetic pathway of melanin [1, 52].

nase and their polyphenolic substrates are mixed after brushing, peeling and crushing operations, which leads to rupture of cell structure [58]. The fundamental step in enzymatic browning is the transformation of an *o*-diphenol such as L-DOPA to the corresponding *o*-quinone, which can undergo further oxidation to brown melanin pigment [59, 60]. *o*-Quinones are powerful electrophiles which can suffer nucleophilic attack by water, other polyphenols, amino acids, peptides and proteins, leading to Michael-type addition products [61–63]. This enzymatic browning can be prevented by trapping the *o*-dopaquinone intermediate with cysteine or ascorbic acid (fig. 4) [10, 64]. Chlorogenic acid, the major phenolic compound of plant-derived foods, is also oxidized by tyrosinase to a highly reactive *o*-quinone intermediate which then could interact with NH_2 groups of lysine, SCH_3 groups of methionines and indole rings of tryptophan in nucleophilic addition and in polymerization reactions, the so-called browning and greening reactions. These transformations destroy essential amino acids, impair digestibility and nutritional quality, and may also result in the formation of toxic compounds [65, 66].

Tyrosinase inhibitors

Melanin plays a crucial protective role against skin photocarcinogenesis; however, the production of abnormal melanin pigmentation is a serious esthetic problem in human beings [67]. The cytotoxicity of L-DOPA has been attributed to its selective uptake by melanocytic cells and to the formation of reactive quinones and semiquinones formed in situ during metabolic activation of L-DOPA by tyrosinase [68–70]. The triphenolic amino acid TOPA also shows cytotoxic properties which seem to be due to the susceptibility of this substance to oxidation [71, 72]. It is possible that the cytotoxicity is mediated by oxygen radical or H_2O_2 formed on oxidation of TOPA [73]. An-

other explanation of the cytotoxicity of TOPA could be the reaction of the quinone formed by oxidation of TOPA with nucleophilic groups of cellular macromolecules [69, 71, 74, 75].

The oxidation products of L-DOPA and TOPA, quinones, are chemically reactive compounds that are potentially harmful, but in melanocytes the normal process of melanogenesis is not usually associated with significant toxicity due to the compartmentation of the reaction within membrane-limited organelles (melanosomes) and because of the rapid cyclization of the quinone intermediate. The discovery that certain substituted phenols have a depigmenting action due to their ability to act as substrates for tyrosinase, resulting in the generation of quinones, has led to the examination of this system as a possible targeted antimelanoma therapeutic strategy in the case of disseminated melanoma [76–79]. For such a chemotherapeutic agent to be useful, the prodrug must evade hepatic metabolism and other potentially toxic reactions [80, 81], reach the tumor tissue and enter the malignant melanocytes. Moreover, this prodrug has to avoid alternative cellular metabolism and enter melanosomes, leading to oxidation by tyrosinase to generate significant amounts of the quinone product, which requires the ability to initiate reactions damaging to the melanoma cell.

Melanin biosynthesis can be inhibited by avoiding UV exposure, the inhibition of tyrosinase, the inhibition of melanocyte metabolism and proliferation, or the removal of melanin with corneal ablation [82, 83]. Standard topical treatments for hyperpigmentation disorders such as melasma and postinflammatory hyperpigmentation include bleaching with hydroquinones, anti-inflammatory therapy by retinoids and use of tyrosinase inhibitors. A number of tyrosinase inhibitors from both natural and synthetic sources (figs. 5, 6) that inhibited monophenolase, diphenolase or both of these activities have been identified to date.

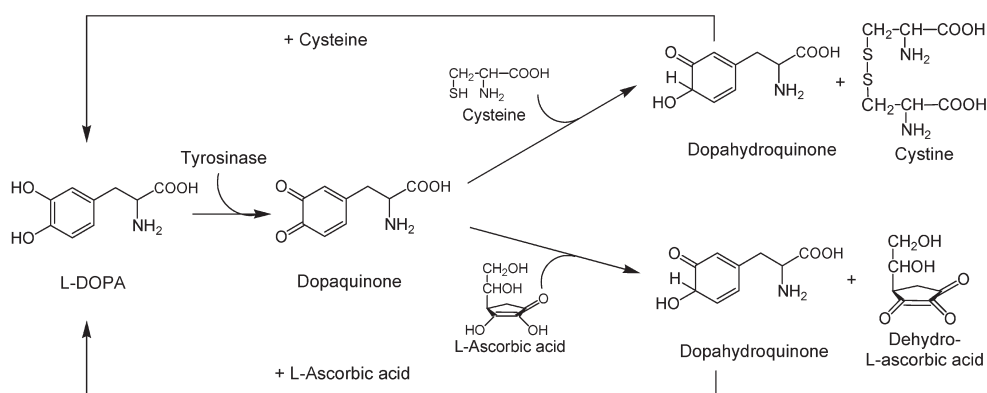


Figure 4. Inhibition of tyrosinase-catalyzed enzymatic browning by trapping the dopaquinone intermediate with cysteine or ascorbic acid [10, 64].

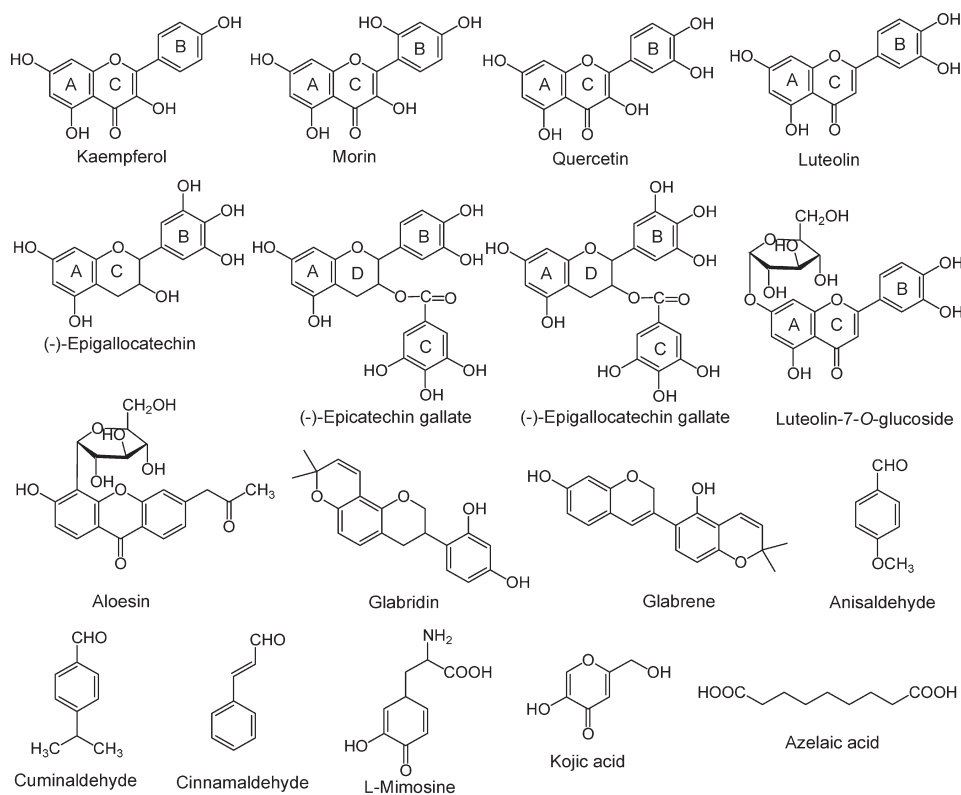


Figure 5. Structures of tyrosinase inhibitors from natural sources.

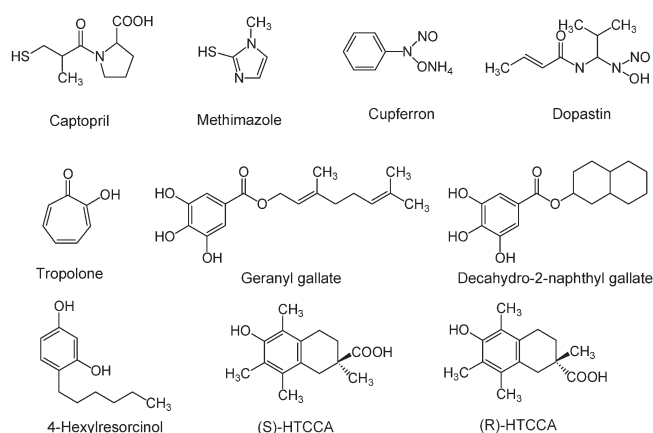


Figure 6. Structures of tyrosinase inhibitors from synthetic sources and derivatives of natural compounds.

Plant polyphenols as inhibitors

Plant polyphenols are usually referred to as a diverse group of compounds containing multiple phenolic functionalities [84]. These compounds are produced as secondary metabolites by most higher plants, in which they have numerous biological activities. Flavonoids are one of the most numerous and best-studied groups of plant polyphenols, that is benzo- γ -pyrone derivatives consist-

ing of phenolic and pyrane rings. Widely distributed in the leaves, seeds, bark and flowers of plants, over 4000 flavonoids have been identified to date. In plants, these compounds afford protection against UV radiation, pathogens and herbivores [85]. They are also responsible for the characteristic red and blue colors of berries, wines and certain vegetables [86–88].

Flavonoids may be subdivided into six major groups (flavanols, flavones, flavonols, flavanones, isoflavones and anthocyanidins), which differ in the arrangements of the hydroxyl, methoxy and glycosidic side groups, and in the conjugation between the A- and B-rings. Some flavonoids, such as kaempferol, quercetin and morin, show the inhibitory activity of tyrosinase, while others, e.g. catechin and rhamnetin, act as cofactors or substrates of tyrosinase [89–93]. A number of studies have been carried out to identify and characterize inhibitors from natural sources and to establish the relationship between their inhibitory activity and structure (table 2) [89, 91, 94]. It was found that flavonoids containing an α -keto group show potent tyrosinase inhibitory activity [94]. This inhibition ability may be explained in terms of similarity between the dihydroxyphenyl group in L-DOPA and the α -keto group in flavonoids.

Some flavonols possessing a 3-hydroxy-4-keto moiety, such as kaempferol and quercetin, competitively inhibit tyrosinase activity by their ability to chelate the copper in

Table 2. Summary of tyrosinase inhibitory activity of compounds from natural sources.

Compounds	Type of inhibition	IC ₅₀ (mM)	Reference
Flavanols			
(-)-Epigallocatechin	competitive ^a	0.035	90
(-)-Epicatechin gallate	competitive ^a	0.017	90
(-)-Epigallocatechin gallate	competitive ^a	0.034	90
Flavonols			
Quercetin	competitive ^b	0.070	91
Kaempferol	competitive ^b	0.230	91
Morin	competitive ^b	2.320	91
Flavones			
Luteolin	noncompetitive ^b	0.190	91
Luteolin 7- <i>O</i> -glucoside	noncompetitive ^b	0.500	91
Isoflavans			
Glabridin	noncompetitive ^b	0.004	98
Glabrene	mixed-type ^b	7.600	98
Isoliquiritigenin	mixed-type ^b	0.047	98
Other compounds			
Kojic acid	mixed-type ^b	0.014	119
Anisaldehyde	noncompetitive ^b	0.320	104
Cuminaldehyde	noncompetitive ^b	0.050	104
Cinnamaldehyde	noncompetitive ^b	0.980	106

^a Inhibition of monophenolase activity.^b Inhibition of diphenolase activity.IC₅₀, inhibitory concentration 50%.

the active site, leading to irreversible inactivation of tyrosinase [89, 91, 95]. After chelation to tyrosinase, kaempferol and quercetin in the resulting complex theoretically should lose their planar structure and should be somehow twisted. This chelation behavior was verified by UV-visible measurement; the characteristic bathochromic shifts in the spectra of kaempferol and quercetin were observed by adding excess copper ions. This copper chelation mechanism is further supported by observation of a noticeable spectral shift when kaempferol and quercetin are incubated with tyrosinase. Interestingly, quercetin and kaempferol can chelate copper in the met form of tyrosinase. This behavior differs from that of kojic acid, which chelates via the oxy form [96].

In contrast to kaempferol and quercetin, 3-*O*-glucoside derivatives (kaempferol 3-*O*-glucoside, kaempferol 3-*O*-sophoroside, quercetin 3-*O*-glucoside and quercetin 3-*O*-rutinoside) did not exhibit inhibitory activity at high concentration [89, 91]. This means that the free hydroxyl group at the C-3 position seems to play an important role in eliciting activity. As far as flavonols are concerned, it seems that aglycones exhibit tyrosinase inhibitory activity but not their 3-glycoside derivatives. However, although this hydroxyl group somehow relates to the activity, it may not be essential because several flavones, such as luteolin and luteolin 7-*O*-glucoside, which lack this 3-hy-

droxyl group still showed tyrosinase inhibitory activity [94]. Generally most competitive inhibitors closely resemble, at least in part if not all, the structure of the substrate. Based on this concern, the molecule of kaempferol or quercetin fits loosely into the active site of tyrosinase and prevents entry of L-DOPA. On the other hand, a bulky sugar moiety attached to the 3-hydroxyl group in the flavonols may hinder their approach to the active site of tyrosinase [91]. In contrast, the sugar moiety of flavone glycosides such as luteolin 7-*O*-glucoside and buddlenoids did not disturb their nearness to the active site for inhibition. The additional hydroxyl group at the 3'-position also somewhat affected the activity since quercetin exhibited slightly more potent activity than the more lipophilic kaempferol.

Structure-related activity study of flavonoids, stilbenes and related 4-substituted resorcinols, obtained from *Ar-tocarpus incisus* and other plants, suggested that compounds with the 4-substituted resorcinol skeleton have potent tyrosinase inhibitory activity [97]. However, the effective topical concentration of these compounds in disorders of hyperpigmentation is not yet known. The structural analysis of the extracts from licorice root, such as glabridin, glabrene, hispaglabridins and isoliquiritigenin (chalcone), revealed the relationship between the lipophilicity of compounds and their tyrosinase inhibitory activity [98]. Among the licorice extracts, glabridin, glabrene and isoliquiritigenin exhibited high inhibitory activity, and all effects were dose-dependent. Glabridin was the most active inhibitor of the licorice constituents with an isoflavan structure. Interestingly, all the licorice inhibitors were more effective against monophenolase than diphenolase activity, which underscores their role in the first step of oxidation, the rate-limiting reaction. Desirable skin-whitening agents should inhibit the synthesis of melanin in melanosomes by acting specifically to reduce the activity of tyrosinase, exhibit low cytotoxicity and be nonmutagenic. However, hispaglabidin A and hispaglabidin B were found to have no tyrosinase inhibitory activity. The general structures of these two isoflavans are similar to that of glabridin, except that hispaglabidin A has an isoprenyl side chain attached to 3'-position and hispaglabidin B has only one hydroxyl group at the 2'-position, both of which increase the lipophilic nature of the isoflavans and diminish the compound's activity as a tyrosinase inhibitor.

Gallates, gallic acid and its esters, are widely used as additives in food industries. Gallic acid, the parent compound of gallates, inhibits the oxidation of L-DOPA catalyzed by tyrosinase [99]. In addition, gallic acid itself seems to act as a substrate, being oxidized prior to L-DOPA, which accelerates the oxidation of gallic acid. The resulting *o*-quinones of gallic acid may condense with one another through a Michael-type addition, yielding a relatively stable quinol-quinone intermediate [100]. It

appears that *o*-dopaquinone could act as a redox cyler, oxidizing gallic acid to the corresponding *o*-quinone and being reduced back to L-DOPA. Various gallic acid derivatives have been isolated from green tea and *Galla rhois* [90, 101]. Some of them showed a potent inhibitory effect against tyrosinase, indicating that the flavan-3-ol skeleton with a galloyl moiety at the 3-position is an important structural requirement for optimum inhibition of tyrosinase activity. It has been reported that the tyrosinase inhibitory activity of aromatic carboxylic acids decreases through esterification, hydroxylation or methylation of the benzene ring [102, 103]. However, 1,2,3,4,6-penta-*O*-galloyl- β -D-glucose isolated from *G. rhois* exhibited high tyrosinase inhibitory activity [101].

Aldehydes and other compounds from higher plants

A large number of aldehydes and other compounds were also isolated and characterized as tyrosinase inhibitors, such as cinnaldehyde, (2E)-alkenals, 2-hydroxy-4-methoxybenzaldehyde, anisaldehyde, cuminaldehyde and cumic acid [104–108]. The aldehyde group is known to react with biologically important nucleophilic groups, e.g. sulfhydryl, amino and hydroxyl groups. Their tyrosinase inhibitory mechanism presumably comes from their ability to form a Schiff base with a primary amino group in the enzyme [105, 106]. Interestingly, the addition of an electron-donating group at the para position of benzaldehyde increases inhibitory activity, probably stabilizing the Schiff base. For example, the inhibitory activity of anisaldehyde and cuminaldehyde was about 2.5- and 16-fold more potent than that of benzaldehyde, respectively. In addition to stabilizing the binding site, the binding affinity of the hydrophobic electron-donating groups such as methoxy and isopropyl to the enzyme also may be related to the inhibitory effect.

Similar to aromatic aldehydes, it appears that aliphatic (2E)-alkenals, α,β -unsaturated aldehydes, form a Schiff base with a primary amino group in the enzyme rather than binding to the binuclear copper active center [104, 105]. This observation is supported by the fact that their corresponding saturated aldehydes (alkanals) did not show significant inhibitory activity. It should be noted that alkanals do not form a stable Schiff base but still show some inhibitory activity. This can be explained by their hydrophobic interaction with the enzyme, leading to disturbing the tertiary structure of enzyme [109].

Interestingly, (2E)-alkenals did not inhibit monophenolase activity but inhibited the oxidation of L-DOPA by tyrosinase as noncompetitive inhibitors, and the hydrophobic alkyl portion related to their inhibitory activity. (2E)-Alkenals with a longer alkyl group may better associate with the hydrophobic protein pocket close to the binuclear active site [34, 109]. However, a cyclic α,β -unsaturated aldehyde sesquiterpene, polygodial, did not inhibit

the oxidation of L-DOPA by tyrosinase. This polygodial is known to form a Schiff base, but its hydrophobic decaline moiety may not associate well with the protein pocket in the enzyme.

Fungal metabolites as inhibitors

Besides higher plants, some compounds from fungal sources have also been identified and reported for their inhibitory activity on tyrosinase [110–113]. Azelaic acid (1,7-heptanedicarboxylic acid) is a naturally occurring straight-chain, saturated dicarboxylic acid which is produced by lipoperoxidation of free and esterified *cis*-polyunsaturated fatty acids by yeast, *Pityrosporum ovale*. This dicarboxylic acid has a definite cytotoxic effect on malignant melanocytes of primary cutaneous melanoma, though normal melanocytes appeared not to be affected [111]. Azelaic acid acts as a rather weak competitive inhibitor of tyrosinase in vitro, which may be a major cause of its melanocytotoxicity.

Kojic acid [5-hydroxy-2-(hydroxymethyl)- γ -pyrone], a fungal metabolite produced by many species of *Aspergillus* and *Penicillium* [114], is a good chelator of transition metal ions and a good scavenger of free radicals [115, 116]. It inhibited the tyrosinases of various *Aspergillus* species, *N. crassa* and mushrooms, as well as those of some plants and crustaceans by chelating copper at the active site [117–120]. Recently, hemolymph serum tyrosinase from larvae of the noctuid moth *Spodoptera littoralis* was shown to be effectively inhibited by kojic acid when L-DOPA was used as the substrate [121]. Moreover, kojic acid effectively inhibited the formation of pigmented products and oxygen uptake when catecholamines such as DL-DOPA, norepinephrine and dopamine were oxidized by tyrosinase. This means that kojic acid is able to reduce *o*-quinone to *o*-diphenol to prevent the final pigment forming and be oxidized to a yellow product by chemical interaction with *o*-quinone [112].

The yeast metallothioneins are ubiquitous cytosolic proteins, usually characterized by selective binding of a large amount of heavy metal ions (Zn^{2+} , Cu^{2+} and Cd^{2+}) and high cysteine content [122, 123]. In addition, there are a number of reports indicating the detection of mammalian metallothioneins within the extracellular environment in vivo [124]. *Neurospora crassa* copper-metallothionein was reported as a metal donor for apotyrosinase [122]. Metallothionein from *Aspergillus niger* was also found to be an inhibitor for a commercially purified mushroom tyrosinase, and exhibited a higher inhibitory effect on the oxidation of catechin compared with that of chlorogenic acid [125]. Under conditions of high exposure, induced metallothioneins could provide highly effective demetalizing agents because of their strong avidity for the metal ion [126]. In addition, metallothioneins exhibit strong reduction activity [127]. Hence, metallothionein can be

used as an inhibitor for tyrosinase by a decrease in oxygen consumption and formation of colorless compounds. Other fungal extracts such as agaritine and inhibitors (Ia and Ib) from *Agaricus* species were also isolated, purified and characterized [128, 129]. Agaritine, β -N-(γ -L(+)-glutamyl)-4-hydroxymethylphenylhydrazine, showed a depigmenting effect that prevented melanin formation. The inhibition was uncompetitive when L-DOPA was used as the substrate and partially competitive when tyrosine was used as the substrate [129]. Taking into account that agaritine is very abundant in *Agaricus bisporus* mushrooms, it might be suggested that agaritine could play in vivo a role as endogenous regulator of mushroom tyrosinase activity and the extent of *o*-quinone concentration formed. Moreover, the inhibitory mechanism of two inhibitors, Ia and Ib, isolated from *Agaricus hortensis* was established [128]. Inhibitor Ia showed competitive inhibitory activity, whereas Ib showed noncompetitive inhibitory activity, as revealed by Lineweaver-Burk plots.

Derivatives of natural compounds

Recently, some natural compounds have been modified and examined as potential tyrosinase inhibitors [130, 131]. Gallic acid, often obtained by alkaline or acid hydrolysis of tannins, is used in the manufacturing of gallic acid alkyl esters (alkyl gallates) such as methyl gallate, propyl gallate and dodecyl gallate. Among them, short alkyl gallates such as methyl and octyl gallates were oxidized by tyrosinase, yielding yellowish oxidation products [130, 132]. However, long alkyl gallates such as dodecyl gallate inhibited the enzyme without producing the pigmented products, indicating that the carbon chain length is related to their tyrosinase inhibitory activity. Notably, the yield of yellowish oxidation products decreased with increasing the carbon number.

It is logical to conclude that the tyrosinase inhibitory activity of dodecyl gallate drives in part from hydrophobic interaction with the hydrophobic protein pocket surrounding the binuclear copper active site in the enzyme [133]. The hydrophobic dodecyl group, which is exposed on the outer side of the molecule, may disrupt tyrosinase's quaternary structure. The low conformational stabilities of native proteins make them easily susceptible to denaturation by altering the balance of the weak nonbonding forces that maintain the native conformation. This concept can be applied broadly for inhibitor design, that is the hydrophobic straight-chain dodecyl group should allow for conformational flexibility and interact with many types of hydrophobic domains.

The inhibitory effects of water-soluble derivatives of vitamin E, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acids (HTCCAs), on mushroom tyrosinase were examined and reported [131]. HTCCAs are stereoisomers with the R or S configuration on the C-2 and pos-

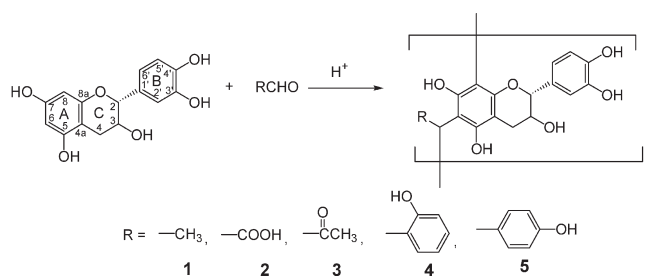


Figure 7. Schematic diagram of the regioselective synthesis of (+)-catechin-aldehyde polycondensates. Polycondensation of (+)-catechin and aldehydes was carried out in the presence of an acid catalyst [38].

sess the essential structural components observed in identified tyrosinase inhibitors, which are of competitive type. (R)-HTCCA had stronger tyrosinase affinity than the (S)-enantiomer, while the tyrosinase affinity of both HTCCA isomers were lower than that of L-DOPA, suggesting that tyrosinase may have stereoselectivity in binding substrates or inhibitors.

It was reported that some flavonoids inhibited tyrosinase activity by active site chelation and others acted as cofactors or substrates of tyrosinase [89–93]. For example, the lag time disappeared in the presence of catechin, in which catechin acted as cofactor and substrate for monophenolase action. However, (+)-catechin-aldehyde polycondensates showed strong inhibition toward both monophenolase and diphenolase activities (fig. 7) [38]. This amplified effect is probably caused by the high molecular weight of the polycondensates. Interestingly, the inhibition type of the polycondensate was competitive, and the polycondensate inhibited the action of the oxy form of tyrosinase. This result means the formation of polycondensate-tyrosinase complex, in which the ordered structure of tyrosinase is partially destroyed by chelation of polycondensate to the active site of the oxy form of tyrosinase.

Inhibitors from synthetic origins

A number of inhibitors from drugs and simple chemicals (e.g. captopril, tropolone and hydroxylamines) have been reported to date. Captopril [(2S)-N-(3-mercapto-2-methylpropionyl)-L-proline] is a drug widely used in the treatment of hypertension and heart failure through its inhibitory effect on the angiotensin-converting enzyme [134, 135]. This drug showed irreversible noncompetitive inhibition on the monophenolase activity of mushroom tyrosinase, while exhibiting irreversible competitive inhibition for the diphenolase activity of tyrosinase [136]. Captopril can also interact with the enzymatically generated *o*-quinone to give rise to a colorless conjugate. Inhibition of both monophenolase and diphenolase activities

of tyrosinase by captopril showed positive kinetic co-operativity which arose from the protection of both substrate and *o*-quinone against inhibition by captopril. Captopril is known as a copper chelator [137, 138]; therefore, it is reasonable to think that captopril mainly exerts its inhibitory effect by chelating copper ions at the active site of tyrosinase. In addition, the inhibitory process may involve a disulfide interchange reaction between captopril and cysteine-rich domains at the active site of the enzyme.

Some drugs also have an inhibitory effect on tyrosinase activity, as has been reported for penicillamine, which is used in therapy for Wilson's disease [139], and the antithyroid drug methimazole [140]. Methimazole (1-methyl-2-mercaptoimidazole) inhibited both the mono- and diphenolase activities of mushroom tyrosinase, which was a mixed-type inhibitor. Methimazole inhibited mushroom tyrosinase activity in two ways: conjugating with *o*-quinones, thereby causing apparent inhibition in pigmented product formation, and chelating copper at the active site of the enzyme.

Among all the inhibitors assayed to date, tropolone (2-hydroxy-2,4,6-cycloheptatriene) is one of the most potent tyrosinase inhibitors. It is structurally analogous to *o*-diphenolic substrates of tyrosinase, as well as an effective copper chelator [141]. Tropolone showed slow-binding inhibition on tyrosinase and could only bind the oxy form [142]. Slow-binding inhibition is characterized by the nonimmediate response of the enzymatic reaction to the presence of inhibitor, of which assumes the formation of an enzyme-inhibitor complex that undergoes slow and favorable isomerization [143]. The equilibria between enzyme, inhibitor and enzyme-inhibitor complexes occur slowly in the steady-state time scale.

Other slow-binding inhibition of tyrosinases from different sources (frog epidermis and mushroom) by several inhibitors (*m*-coumaric acid, L-mimosine and 4-substituted resorcinols) has also been reported [144–146]. 4-Substituted resorcinols, which are structurally related to phenolic substrates such as other slow-binding inhibitors, have recently been recognized as tyrosinase inhibitors. Although resorcinol is a poor tyrosinase inhibitor, substitution in the 4-position yields increased inhibitory activity. Among the 4-substituted resorcinols, the highest inhibition was obtained with hydrophobic substituents in the 4-position, such as 4-hexyl- and 4-dodecylresorcinol [147]. 4-Hexylresorcinol is claimed to be the most effective inhibitor for use in the food industry since it is water soluble, stable, nontoxic, nonmutagenic and noncarcinogenic, and it has been recognized as safe for use in the prevention of shrimp melanosis and for browning control of fresh and hot-air-dried apple slices as well as potatoes and avocados [148, 149]. In the kinetic study, progress curves of enzymatic reaction in the presence of 4-substituted resorcinols showed a progressive decrease in initial

velocity followed by a constant steady-state rate [146]. Both the initial and the constant rates decreased with increasing concentrations of the inhibitor.

A series of *N*-substituted *N*-nitrosohydroxylamines have been synthesized and examined for inhibition of mushroom tyrosinase [150]. Interestingly, activities of *N*-*n*-alkyl derivatives were not affected by the length of their alkyl chains, while inhibitory activity dramatically decreased with an increase in the length of the alkyl chain in compounds having the phenyl substituent at the α -carbon of the *N*-nitroso-*N*-hydroxylamino group. This means that the phenyl or alkyl substituent at the α -carbon of the *N*-nitroso-*N*-hydroxylamino group may cause steric hindrance for the approach of the inhibitors to the active site of the enzyme. Among these amines, *N*-cyclopentyl-*N*-nitrosohydroxylamine exhibited the most potent activity, as powerful as that of tropolone. As removal of nitroso or hydroxyl moiety, the enzyme inhibitory activity was completely diminished. Both *N*-nitroso and *N*-hydroxyl groups were suggested to be essential for the activity, probably by interacting with the copper ion at the active site of the enzyme.

Hydrogen peroxide is known as an inactivator of several copper-containing enzymes, such as dopamine β -monooxygenase and mushroom tyrosinase [151, 152]. Exposure of mushroom tyrosinase to high concentration of H_2O_2 resulted in loss of both monophenolase and diphenolase activities. H_2O_2 inactivated tyrosinase in a biphasic manner with the rate being faster in the first phase than in the second one. Additionally, the rate of inactivation of the enzyme was dependent on H_2O_2 concentration and was faster under anaerobic conditions than aerobic conditions. It is conceivable that in the presence of relatively high concentrations of H_2O_2 , tyrosinase is converted to an oxy-oxytyrosinase, which is a hypothetical intermediate analogous to the ternary complex formed between oxytyrosinase and *o*-dihydroxyphenol. Oxy-oxytyrosinase is probably a dead-end form of tyrosinase that cannot bind mono- and *o*-dihydroxyphenols and can therefore be considered to be an inactivated form of the enzyme. Moreover, hydroxyl radical produced from H_2O_2 binds to tyrosinase and causes the inactivation which is referred to as suicide-type inactivation [33]. In the case of *Neuspora* tyrosinase, the enzyme-bound hydroxyl radical attacks histidine-306 at the active site of the enzyme, leading to loss of activity. Other copper chelators (sodium azide and sodium thiosulfate) and substrate analogues (sodium benzoate and L-phenylalanine) are also known to be competitive inhibitors, which can inhibit diphenolase activity of tyrosinase [152].

Application and importance of inhibitors

Agricultural and food fields

Amino-carbonyl and related interactions of food constituents encompass those changes commonly termed browning reactions. Specifically, reactions of amines, amino acids, peptides and proteins with quinones (enzymatic browning) cause deterioration of food during storage and commercial or domestic processing. The loss of nutritional quality is attributed to the destruction of essential amino acids, decrease in digestibility, and inhibition of proteolytic and glycolytic enzymes. The production of antinutritional and toxic compounds may further reduce the nutritional value and possibly the safety of foods [153]. Thus, it is necessary to identify various methods to stop enzymatic browning caused by tyrosinase. Current conventional techniques to avoid browning include autoclave and blanching methods, whereby the food products were immersed in a liquid at 80–90 °C for 10–12 min or passed through a forced steam flow. These conventional processes are inherently linked to important weight and nutritional quality losses in the product [154]. One of the alternatives that have been proposed is microwave energy. The most restrictive factor for the application of microwave heating techniques to industrial blanching processes is the temperature gradient generated within the samples during microwave heating [155]. Hence, the enzyme inactivation effect was exhibited in overheated areas, while in colder areas the enzyme might not completely be inactivated. To overcome this restriction, combined conventional microwave heating techniques are envisaged, since surface enzymes may not be completely inactivated by microwave treatment alone. The application of combined microwave hot-water treatment has shown some improvement on final quality, weight loss and processing times [156]. Nevertheless, the rigorous control of microwave heating effects on enzymes requires homogenous heating of the sample and strict temperature control.

Compounds capable of inhibiting enzymatic browning in food products through the interference of tyrosinase-mediated reactions or through the reduction of *o*-quinones to *o*-diphenols have been identified [157–159]. However, the number of chemicals that can actually be used in food systems to inhibit enzymatic browning is limited due to off-flavors, off-odors, toxicity and economic feasibility [160]. Sulfiting agents have widely been used to prevent enzymatic browning in agricultural and seafood products. Due to health concerns, the use of sulfiting agents as food additives is being re-evaluated by the Food and Drug Administration (FDA) and, in some products, banned for use [161]. Most of the alternatives in the food industry are formulations of ascorbic and citric acids [158, 162]. But they are less effective than sulfiting agents, since ascorbic acid is quickly consumed in the process of reducing

quinones formed by tyrosinase. Recently the use of 4-hexylresorcinol is considered to be safe in the food industry and is quite effective in the prevention of shrimp melanosis and for browning control in fresh and dried fruit slices [147–149]. However, as safety is of prime concern for an inhibitor to be used in the food industry, there is a constant search for better inhibitors from natural sources as they are largely free of any harmful side effects.

Cosmetic and medicinal fields

Use of tyrosinase inhibitors is becoming increasingly important in the cosmetic and medicinal industries due to their preventive effect on pigmentation disorders. Tyrosinase inhibitors may result in a reduction in melanin biosynthesis and are used in cosmetic products for hyperpigmentation-related concerns, including the formation of freckles. Tyrosinase and its inhibitors may also be targets for developing medicines to treat hypopigmentation-related problems, such as albinism and piebaldism. A number of tyrosinase inhibitors have been reported from both natural and synthetic sources, but only a few of them are used as skin-whitening agents, primarily due to various safety concerns.

Among the skin-whitening agents, 1,4-dihydroquinone is one of the most widely prescribed [3, 163, 164]. It causes reversible inhibition of cellular metabolism by affecting both DNA and RNA synthesis. It is also a poor substrate for tyrosinase, thereby competing for tyrosinase oxidation in active melanocytes. Hence, 1,4-dihydroquinone can be considered to be a potent melanocyte cytotoxic agent and has also been reported to induce mutations. As a result of these and other side effects, such as chronosis in African nations [3], the use of 1,4-dihydroquinone has been forbidden in cosmetics by most countries, and there has been increasing pressure to find alternative herbal and pharmaceutical depigmentation agents.

Currently arbutin and aloesin are used in the cosmetic industry as skin-whitening agents because they show strong inhibition toward tyrosinase, which is responsible for pigmentation in human beings. β -Arbutin inhibited both tyrosinase activities from mushroom and mouse melanoma noncompetitively, while α -arbutin only inhibited tyrosinase from mouse melanoma by mixed-type inhibition [165]. On the other hand, the co-treatment of arbutin and aloesin exhibited the inhibitory effect on tyrosinase in a synergistic manner by acting through different mechanisms: arbutin inhibited competitively, whereas aloesin inhibited noncompetitively [166]. Taken together they inhibit melanin production synergistically by a combined mechanism of competitive and noncompetitive inhibition. This result indicates that it is beneficial to use arbutin and aloesin as a mixture in depigmentation because the co-treatment reduces the doses of these agents for the same inhibitory effect on tyrosinase activity and can di-

minish adverse side effects. In addition, methimazole has proven effective as a depigmenting agent both in vitro and in vivo, and is noncytotoxic and nonmutagenic. Therefore, methimazole could serve as a lead compound for the discovery of safe and efficient skin depigmenting agents in the future [167].

Conclusions

Browning of plant-derived foods and beverages occurs due to enzymatic oxidation of phenols by tyrosinases, which can cause the destruction of essential amino acids, the impairment of digestibility and nutritional quality, and the formation of toxic compounds. This unfavorable browning has been of great concern, and it is necessary to search for potent tyrosinase inhibitors. Besides being used in the treatment of some dermatological disorders associated with melanin hyperpigmentation, tyrosinase inhibitors have found an important role in the cosmetic industry for their skin-whitening effect and depigmentation after sunburn. However, only a few antimelanogenic reagents, typically kojic acid and arbutin, are currently commercially available. Furthermore, they provide some cause for concern, e.g. high toxicity toward cells, and low stability toward oxygen and water, resulting in their limited application. More concrete studies of human tyrosinase from a clinical point of view are required.

Safety is a primary consideration for tyrosinase inhibitors, especially those in food and cosmetic products, which may be utilized in unregulated quantities on a regular basis. Hence, inhibitors from natural sources have great potential in the food industry, as they are considered to be safe and largely free from adverse side effects. However, some inhibitors from natural sources such as (2E)-alkenals were reported to exhibit potent mutagenic activity [168], though aromatic aldehydes such as cinnamaldehyde and benzaldehyde are known to be generally recognized as safe.

The discovery and characterization of new tyrosinase inhibitors are useful for their potential applications in improving food quality and nutritional value, controlling insect pests, and preventing pigmentation disorders and other melanin-related health problems in human beings. Additionally, understanding tyrosinase activators and inhibitors is of crucial importance in finding novel and more consumer-compatible approaches toward regulation of the discoloration process. To obtain better inhibitors, different types of compounds from both natural and synthetic sources have been investigated. However, on balance, much more research on tyrosinase inhibitors is needed to confirm their structure and activity, and to improve their safety and effectiveness in various applications.

- 1 Protá G. (1988) Progress in the chemistry of melanins and related metabolites. *Med. Res. Rev.* **8**: 525–556
- 2 Spritz R. A. and Hearing V. J. Jr (1994) Genetic disorders of pigmentation. *Adv. Hum. Genet.* **22**: 1–45
- 3 Frenk E. (1995) Treatment of melasma with depigmenting agents. In: *Melasma: New Approaches to Treatment*, pp. 9–15, Martin Dunitz, London
- 4 García-Cánovas F., García-Carmona F., Vera-Sánchez J., Iborra-Pastor J. L. and Lozano-Teruel J. A. (1982) The role of pH in the melanin biosynthesis pathway. *J. Biol. Chem.* **257**: 8738–8744
- 5 Rodríguez-López J. N., Tudela J., Varón R. and García-Cánovas F. (1991) Kinetic study on the effect of pH on the melanin biosynthesis pathway. *Biochim. Biophys. Acta* **1076**: 379–386
- 6 Cooksey C. J., Garratt P. J., Land E. J., Pavel S., Ramsden C. A., Riley P. A. et al. (1997) Evidence of the indirect formation of the catecholic intermediate substrate responsible for the autoactivation kinetics of tyrosinase. *J. Biol. Chem.* **272**: 26226–26235
- 7 Pawelek J. M. and Körner A. M. (1982) The biosynthesis of mammalian melanin. *AMSCA* **70**: 136–145
- 8 Mayer A. M. (1987) Polyphenol oxidases in plants: recent progress. *Phytochemistry* **26**: 11–20
- 9 Whittaker J. R. (1995) In: *Food Enzymes, Structure and Mechanism*, pp. 271–307, Wong D. (ed.), Chapman and Hall, New York
- 10 Friedman M. (1996) Food browning and its prevention: an overview. *J. Agric. Food Chem.* **44**: 631–653
- 11 Barrett F. M. (1984) Wound-healing phenoloxidase in larval cuticle of *Calpodex ethulius* (Lepidoptera: Hesperidae). *Can. J. Zool.* **62**: 834–838
- 12 Sugumaran M. (1988) Molecular mechanism for cuticular sclerotization. *Adv. Insect Physiol.* **21**: 179–231
- 13 Sugumaran M. (1991) Molecular mechanisms for mammalian melanogenesis comparison with insect cuticular sclerotization. *FEBS Lett.* **293**: 4–10
- 14 Mosher A. M., Pathak M. A. and Fitzpatrick T. B. (1983) In: *Dermatology in General Medicine*, pp. 205–225, Fitzpatrick T. B., Eisen A. Z., Wolff K., Freedberg I. M. and Austern K. F. (eds), Mc-Graw-Hill, New York
- 15 Maeda K. and Fukuda M. (1991) In vitro effectiveness of several whitening cosmetic components in human melanocytes. *J. Soc. Cosmet. Chem.* **42**: 361–368
- 16 Sánchez-Ferrer Á., Villalba J. and García-Carmona F. (1989) Triton X-114 as a tool for purifying spinach polyphenol oxidase. *Phytochemistry* **28**: 1321–1325
- 17 Sánchez-Ferrer Á., Bru R. and García-Carmona F. (1990) Partial purification of a thylakoid-bound enzyme using temperature-induced phase partitioning. *Anal. Biochem.* **184**: 279–282
- 18 Himmelwright R. S., Eickman N. C., Lubein C. D., Solomon E. I. and Lerch K. (1980) Chemical and spectroscopic studies of the binuclear copper active site of *Neurospora* tyrosinase: comparison to hemocyanins. *J. Am. Chem. Soc.* **102**: 7339–7344
- 19 Solomon E. I., Sundaram U. M. and Machonkin T. E. (1996) Multicopper oxidases and oxygenases. *Chem. Rev.* **96**: 2563–2605
- 20 Mason H. S., Fowlks W. L. and Peterson E. (1955) Oxygen transfer and electron transport by the phenolase complex. *J. Am. Chem. Soc.* **77**: 2914–2915
- 21 Nishioka K. (1978) Particulate tyrosinase of human malignant melanoma. Solubilization, purification following trypsin treatment and characterization. *Eur. J. Biochem.* **85**: 137–146
- 22 Lerch K. (1982) Primary structure of tyrosinase from *Neurospora crassa*. II. Complete amino acid sequence and chemical structure of a tripeptide containing an unusual thioether. *J. Biol. Chem.* **257**: 6414–6419
- 23 Kwon B. S., Haq A. K., Pomerantz S. H. and Halaban R. (1987) Isolation and sequence of a cDNA clone for human ty-

- rosinase that maps at the mouse c-albino locus. *Proc. Natl. Acad. Sci. USA* **84**: 7473–7477
- 24 Kupper U., Niedermann D. M., Travaglini G. and Lerch K. (1989) Isolation and characterization of the tyrosinase gene from *Neurospora crassa*. *J. Biol. Chem.* **264**: 17250–17258
 - 25 Oetting W. S. and King R. A. (1994) Molecular basis of oculocutaneous albinism. *J. Invest. Dermatol.* **103**: 131S–136S
 - 26 Robb D. A. (1984) In: *Copper Proteins and Copper Enzymes*, vol. 2, pp. 207, Lontie R. (ed.), CRC Press, Boca Raton, FL
 - 27 Barrett F. M. (1991) In: *Physiology of the Insect Epidermis*, pp. 195, Binnigton K. and Retnakaram A. (eds), CISRO, East Melbourne, Australia
 - 28 Schoot Uiterkamp A. J. M. (1972) Monomer and magnetic dipole-coupled Cu^{2+} EPR signals in nitrosylhemocyanin. *FEBS Lett.* **20**: 93–96
 - 29 Hepp A. F., Himmelwright R. S., Eickman N. C. and Solomon E. I. (1979) Ligand displacement reactions of oxyhemocyanin: comparison of reactivities of arthropods and mollusks. *Biochem. Biophys. Res. Commun.* **89**: 1050–1057
 - 30 Himmelwright R. S., Eickman N. C., Lubein C. D. and Solomon E. I. (1980) Chemical and spectroscopic comparison of the binuclear copper active site of mollusc and arthropod hemocyanins. *J. Am. Chem. Soc.* **102**: 5378–5388
 - 31 Volbeda A. and Hol W. G. (1989) Crystal structure of hexameric haemocyanin from *Panulirus interruptus* refined at 3.2 Å resolution. *J. Mol. Biol.* **209**: 249–279
 - 32 Magnus K. A., Hazes B., Ton-That H., Bonaventura C., Bonaventura J. and Hol W. G. (1994) Crystallographic analysis of oxygenated and deoxygenated states of arthropod hemocyanin shows unusual differences. *Proteins* **19**: 302–309
 - 33 Lerch K. (1981) In: *Metal Ions in Biological Systems*, pp. 143–186, Sigel H. (ed.), Marcel Dekker, New York
 - 34 Wilcox D. E., Porras A. G., Hwang Y. T., Lerch K., Winkler M. E. and Solomon, E. I. (1985) Substrate analogue binding to the coupled binuclear copper active site in tyrosinase. *J. Am. Chem. Soc.* **107**: 4015–4027
 - 35 Sánchez-Ferrer Á., Rodríguez-López J. N., García-Cánovas F. and García-Carmona F. (1995) Tyrosinase: a comprehensive review of its mechanism. *Biochim. Biophys. Acta* **1247**: 1–11
 - 36 Himmelwright R. S., Eickman N. C. and Solomon E. I. (1979) Reactions and interconversion of met and dimer hemocyanin. *Biochem. Biophys. Res. Commun.* **86**: 628–634
 - 37 Solomon E. I. (1981) In: *Copper Proteins*, vol. III, pp. 41–108, Spiro T. G. (ed.), Wiley-Interscience, New York
 - 38 Kim Y. J., Chung J. E., Kurisawa M., Uyama H. and Kobayashi S. (2004) New tyrosinase inhibitors, (+)-catechin-aldehyde polycondensates. *Biomacromolecules* **5**: 474–479
 - 39 Jackman M. P., Hajnal A. and Lerch K. (1991) Albino mutants of *Streptomyces glaucescens* tyrosinase. *Biochem. J.* **274**: 707–713
 - 40 van Gelder C. W. G., Flurkey W. H. and Wichers H. J. (1997) Sequence and structural features of plant and fungal tyrosinase. *Phytochemistry* **45**: 1309–1323
 - 41 Beltramini M., Salvato B., Santamaria M. and Lerch K. (1990) The reaction of CN^- with the binuclear copper site of *Neurospora* tyrosinase: its relevance for a comparison between tyrosinase and hemocyanin active sites. *Biochim. Biophys. Acta* **1040**: 365–372
 - 42 Burton S. G. (1994) Biocatalysis with polyphenol oxidase: a review. *Catal. Today* **22**: 459–487
 - 43 Sanjust E., Cecchini G., Sollai F., Curreli N. and Rescigno A. (2003) 3-Hydroxykynurenine as a substrate/activator for mushroom tyrosinase. *Arch. Biochem. Biophys.* **412**: 272–278
 - 44 Laskin J. D. and Piccinini L. A. (1986) Tyrosinase isozyme heterogeneity in differentiating B16/C3 melanoma. *J. Biol. Chem.* **261**: 16626–16635
 - 45 Cabanes J., García-Cánovas F., Lozano J. A. and García-Carmona F. (1987) A kinetic study of the melanization pathway between L-tyrosine and dopachrome. *Biochim. Biophys. Acta* **923**: 187–195
 - 46 Ros J. R., Rodríguez-López J. N. and García-Cánovas F. (1993) Effect of ferrous ions on the monophenolase activity of tyrosinase. *Biochim. Biophys. Acta* **1163**: 303–308
 - 47 Fenoll L. G., Rodríguez-López J. N., García-Sevilla F., García-Ruiz P. A., Varón R., García-Cánovas F. et al. (2001) Analysis and interpretation of the action mechanism of mushroom tyrosinase on monophenols and diphenols generating highly unstable *o*-quinones. *Biochim. Biophys. Acta* **1548**: 1–22
 - 48 Protá G. (1992) In: *Melanins and Melanogenesis*, pp. 1–290, Academic Press, New York
 - 49 Ito S. (1993) Biochemistry and physiological of melanin. In: *Pigmentation and Pigmentary Disorders*, pp. 33–59, Levine N. (ed.), CRC Press, Boca Raton, FL
 - 50 Hirobe T. (1991) Control of melanocyte proliferation and differentiation in the mouse epidermis. *J. Invest. Dermatol.* **5**: 1–11
 - 51 Hearing V. J. and King R. A. (1993) Determinants of skin color: melanocytes and melanization. In: *Pigmentation and Pigmentary Abnormalities*, pp. 3–32, Levine N. (ed.), CRC Press, New York
 - 52 Kobayashi T., Vieira W. D., Protterf B., Sakai C. and Imokawa G. (1995) Modulation of melanogenic protein expression during the switch from eu- to pheomelanogenesis. *J. Cell Sci.* **108**: 2301–2309
 - 53 Brun A. and Rosset R. (1974) Étude électrochimique de l'oxydation de la dihydroxy-3,4 phénylalanine (Dopa): mécanisme d'oxydation des catéchols en milieu acide. *J. Electroanal. Chem.* **49**: 287–300
 - 54 Pawelek J. M. (1991) After dopachrome? *Pigment Cell Res.* **4**: 53–62
 - 55 Aroca P., García-Borrón J. C., Solano F. and Lozano J. A. (1990) Regulation of mammalian melanogenesis I: partial purification and characterization of a dopachrome converting factor: dopachrome tautomerase. *Biochim. Biophys. Acta* **1035**: 266–275
 - 56 Lambert C., Chacon J. N., Chedekel M. R., Land E. J., Riley P. A., Thompson A. et al. (1989) A pulse radiolysis investigation of the oxidation of indolic melanin precursors: evidence for indolequinones and subsequent intermediates. *Biochim. Biophys. Acta* **993**: 12–20
 - 57 Sugumaran M. and Semesi V. (1991) Quinone methide as a new intermediate in eumelanin biosynthesis. *J. Biol. Chem.* **266**: 6073–6078
 - 58 Hurrell R. F. and Finot P. A. (1984) Nutritional consequences of the reactions between proteins and oxidized polyphenolic acids. *Adv. Exp. Med. Biol.* **177**: 423–435
 - 59 Deshpande S. S., Sathe S. K. and Salunkhe D. K. (1984) Chemistry and safety of plant polyphenols. In: *Nutritional and Toxicological Aspects of Food Safety*, pp. 457–495, Friedman M. (ed.), Plenum, New York
 - 60 Martinez M. V. and Whitaker J. R. (1995) The biochemistry and control of enzymatic browning. *Trends Food Sci. Technol.* **6**: 195–200
 - 61 Wong D. W. S. (1989) *Mechanism and Theory in Food Chemistry*, AVI-Van Nostrand Reinhold, New York
 - 62 Route-Mayer M. A., Ralambosa J. and Philippon J. (1990) Roles of *o*-quinones and their polymers in the enzymic browning of apples. *Phytochemistry* **29**: 435–440
 - 63 Fulcrand H., Cheminat A., Brouillard R. and Cheynier V. (1994) Characterization of compounds obtained by chemical oxidation of caffeic acid in acidic conditions. *Phytochemistry* **35**: 499–505
 - 64 Friedman M. and Bautista F. F. (1995) Inactivation of polyphenol oxidase by thiols in the absence and presence of potato tissue suspensions. *J. Agric. Food Chem.* **43**: 69–76

- 65 Dao L. and Friedman M. (1992) Chrologenic acid content of fresh and processed potatoes determined by ultraviolet spectrophotometry. *J. Agric. Food Chem.* **40**: 2152–2156
- 66 Sapers G. M. (1993) Browning of foods: control by sulfites, antioxidants and other means. *Food Technol.* **10**: 75–84
- 67 Priestley G. C. (1993) *Molecular Aspects of Dermatology*, Wiley, Chichester, UK
- 68 Wick M. M., Byers L. and Frei F. (1977) L-DOPA: selective toxicity for melanoma cells in vitro. *Science* **197**: 468–469
- 69 Graham D. G., Tiffany S. M. and Vogel S. F. (1978) The toxicity of melanin precursors. *J. Invest. Dermatol.* **70**: 113–116
- 70 Korytowski W., Sarna T., Kalyanaraman B. and Sealy R. C. (1987) Tyrosinase-catalyzed oxidation of dopa and related catechol(amine)s: a kinetic electron spin resonance investigation using spin-stabilization and spin label oximetry. *Biochim. Biophys. Acta* **924**: 383–392
- 71 Wick M. M., Byers L. and Ratliff J. (1979) Selective toxicity of 6-hydroxydopa for melanoma cells. *J. Invest. Dermatol.* **72**: 67–69
- 72 Sachs C. and Jonsson G. (1975) Mechanisms of action of 6-hydroxydopamine. *Biochem. Pharmacol.* **24**: 1–8
- 73 Heikkilä R. E. and Cohen G. (1973) 6-Hydroxydopamine: evidence for superoxide radical as an oxidative intermediate. *Science* **181**: 456–457
- 74 Saner A. and Thoenner H. (1971) Model experiments on the molecular mechanism of action of 6-hydroxydopamine. *Mol. Pharmacol.* **7**: 147–154
- 75 Rothman A., Daly J. W. and Creveling C. R. (1976) Oxygen-dependent reaction of 6-hydroxydopamine, 5,6-dihydroxytryptamine and related compounds with proteins in vitro: a model for cytotoxicity. *Mol. Pharmacol.* **12**: 887–899
- 76 Riely P. A., Sawyer B. and Wolff M. A. (1975) The melanocytotoxic effect of 4-hydroxyanisole. *J. Invest. Dermatol.* **64**: 86–89
- 77 Morgan B. D. G., O'Neill T., Dewey D. L., Galpine A. R. and Riely P. A. (1981) Treatment of malignant melanoma by intravascular 4-hydroxyanisole. *Clin. Oncol.* **7**: 227–234
- 78 Naish S., Holden J. L., Cooksey C. J. and Riely P. A. (1988) The major primary cytotoxic product of 4-hydroxyanisole oxidation by mushroom tyrosinase is 4-methoxy ortho benzoquinone. *Pigment Cell Res.* **1**: 382–385
- 79 Riely P. A. (1991) Melanogenesis: a realistic target for anti-melanoma therapy. *Eur. J. Cancer* **27**: 1172–1177
- 80 Schiller C. D., Gescher A. and Jheeta P. (1991) Mechanism of toxicity of the antimelanoma drug 4-hydroxyanisole in mouse hepatocytes. *Eur. J. Cancer* **27**: 1017–1022
- 81 Stolze K. and Nohl H. (1991) Formation of methemoglobin and phenoxyl radicals from *p*-hydroxyanisole and oxyhemoglobin. *Free Radical Res. Commun.* **11**: 321–327
- 82 Seiberg M., Paine C., Sharlow E., Andrade-Gordon P., Costanzo M., Eisinger M. et al. (2000) Inhibition of melanosome transfer results in skin lightening. *J. Invest. Dermatol.* **115**: 162–167
- 83 Seiberg M., Paine C., Sharlow E., Andrade-Gordon P., Costanzo M., Eisinger M. et al. (2000) The protease-activated receptor 2 regulates pigmentation via keratinocyte-melanocyte interactions. *Exp. Cell. Res.* **254**: 25–32
- 84 Tücmantel W., Kozikowski A. P. and Romanczyk, L. J., Jr. (1999) Studies in polyphenol chemistry and bioactivity. 1. Preparation of building blocks from (+)-catechin. Procyandin formation. Synthesis of the cancer cell growth inhibitor, 3-*O*-Galloyl-(2R,3R)-epicatechin-4 β ,8-[3-*O*-galloyl-(2R,3R)-epicatechin]. *J. Am. Chem. Soc.* **121**: 12073–12081
- 85 Harborne J. B. and Williams C. A. (2000) Advances in flavonoid research since 1992. *Phytochemistry* **55**: 481–504
- 86 Hammerstone J. F., Lazarus S. A. and Schmitz H. H. (2000) Procyandin content and variation in some commonly consumed foods. *J. Nutr.* **130**: 2086S–2092S
- 87 Stewart A. J., Bozonnet S., Mullen W., Jenkins G. I., Lean M. E. and Crozier A. (2000) Occurrence of flavonols in tomatoes and tomato-based products. *J. Agric. Food Chem.* **48**: 2663–2669
- 88 Lopez M., Martinez F., Del Valle C., Orte C. and Miro M. (2001) Analysis of phenolic constituents of biological interest in red wines by high-performance liquid chromatography. *J. Chromatogr. A* **922**: 359–363
- 89 Kubo I. and Kinst-Hori I. (1999) Flavonols from saffron flower: tyrosinase inhibitory activity and inhibition mechanism. *J. Agric. Food Chem.* **47**: 4121–4125
- 90 No J. K., Soung D. Y., Kim Y. J., Shim K. H., Jun Y. S., Rhee S. H. et al. (1999) Inhibition of tyrosinase by green tea components. *Life Sci.* **65**: 241–246
- 91 Kubo I., Kinsy-Hori I., Chaudhuri S. K., Kubo Y., Sánchez Y. and Ogura T. (2000) Flavonols from *Heterotheca inuloides*: tyrosinase inhibitory activity and structural criteria. *Bioorg. Med. Chem.* **8**: 1749–1755
- 92 Gómez-Cordovés C., Bartolomé B., Vieira W. and Virador V. M. (2001) Effects of wine phenolics and surghum tannins on tyrosinase activity and growth of melanoma cells. *J. Agric. Food Chem.* **49**: 1620–1624
- 93 Kermasha S., Bao H. and Bisakowski B. (2001) Biocatalysis of tyrosinase using catechin as substrate in selected organic solvent media. *J. Mol. Catal. B: Enz.* **11**: 929–938
- 94 Badria F. A. and El Gayyar M. A. (2001) A new type of tyrosinase inhibitors from natural products as potential treatments for hyperpigmentation. *Boll. Chim. Farma.* **140**: 267–271
- 95 Kubo I., Kinst-Hori I., Ishiguro K., Chaudhuri S. K., Sanchez Y. and Oruga T. (1994) Tyrosinase inhibitory flavonoids from *Heterotheca inuloides* and their structural functions. *Bioorg. Med. Chem. Lett.* **4**: 1443–1446
- 96 Cabanes J., Chazarra S. and García-Carmona F. (1994) Kojic acid, a cosmetic skin whitening agent, is a slow-binding inhibitor of catecholase activity of tyrosinase. *J. Pharm. Pharmacol.* **46**: 982–985
- 97 Shimizu K., Kondo R. and Sakai K. (2000) Inhibition of tyrosinase by flavonoids, stilbenes and related 4-substituted resorcinols: structure-activity investigation. *Planta Med.* **66**: 11–15
- 98 Nerya O., Vaya J., Musa R., Izrael S., Ben-Arie R. and Tamir S. (2003) Glabrene and isoliquiritigenin as tyrosinase inhibitors from licorice roots. *J. Agric. Food Chem.* **51**: 1201–1207
- 99 Shahidi L. M. and Nacz M. (1995) *Food Phenolics*, Lancaster, Technomic Publishing
- 100 Sayre L. M. and Nadkarni D. V. (1994) Direct conversion of phenols to *o*-quinones by copper(I) dioxygen: questions regarding the monophenolase activity. *J. Am. Chem. Soc.* **116**: 3157–3158
- 101 Kim J. H., Sapers G. M. and Choi S. W. (1998) Identification of tyrosinase inhibitor from *Galla rhois*. *Food Sci. Biotechnol.* **7**: 56–59
- 102 Menon S., Fleck R. W., Yong G. and Strothkamp K. G. (1990) Benzoic acid inhibition of α , β and γ isozymes of *Agaricus bisporus* tyrosinase. *Arch. Biochem. Biophys.* **280**: 27–30
- 103 Kermasha S., Goetghebeur M., Monfette A., Metchet M. and Rovelt M. (1993) Inhibitory effects of cysteine and aromatic acids on tyrosinase activity. *Phytochemistry* **34**: 349–353
- 104 Kubo I. and Kinst-Hori I. (1998) Tyrosinase inhibitors from anise oil. *J. Agric. Food Chem.* **46**: 1268–1271
- 105 Kubo I. and Kinst-Hori I. (1998) Tyrosinase inhibitors from cumin. *J. Agric. Food Chem.* **46**: 5338–5341
- 106 Kubo I. and Kinst-Hori I. (1999) Tyrosinase inhibitory activity of the olive oil flavor compounds. *J. Agric. Food Chem.* **47**: 4574–4578
- 107 Kubo I. and Kinst-Hori I. (1999) 2-Hydroxy-4-methoxy benzaldehyde: a potent tyrosinase inhibitor from african medicinal plants. *Planta Med.* **65**: 19–22
- 108 Lee S. E., Kim M. K., Lee S. G., Ahn Y. J. and Lee H. S. (2000) Inhibitory effects of *Cinnamomum cassia* bark-derived mate-

- rials on mushroom tyrosinase. *Food Sci. Biotechnol.* **9**: 330–333
- 109 Conrad J. S., Dawso S. R., Hubbard E. R., Meyer T. E. and Strothkamp K. G. (1994) Inhibitor binding to the binuclear active site of tyrosinase: temperature, pH, and solvent deuterium isotope effects. *Biochemistry* **33**: 5739–5744
 - 110 Nazzro-Porro M., Passi S., Morpurgo G. and Breathnach A. S. (1979) Identification of tyrosinase inhibitors in cultures of *Pytirosporium*, and their melanocytotoxic effect. In: *Pigment Cell*, vol. 1, pp. 234–243, Klaus S. N. (ed.), Basel, Karger
 - 111 Schallreuter K. U. and Wood J. W. (1990) A possible mechanism of action for azelaic acid in the human epidermis. *Arch. Dermatol. Res.* **282**: 168–171
 - 112 Kahn V. (1995) Effect of kojic acid on the oxidation of DL-DOPA, norepinephrine and dopamine by mushroom tyrosinase. *Pigment Cell Res.* **8**: 234–240
 - 113 Kahn V. and Zakin V. (1995) Effect of kojic acid on the oxidation of trihydroxyphenols by mushroom tyrosinase. *J. Food Biochem.* **18**: 427–443
 - 114 Parrish F. W., Wiley B. J., Simmons E. G. and Long L. Jr (1966) Production of aflatoxins and kojic acid by species of *Aspergillus* and *Penicillium*. *Appl. Microbiol.* **14**: 139
 - 115 Wiley J. W., Tyson G. N. and Steller J. S. (1942) The configuration of complex kojates formed with some transition elements as determined by magnetic susceptibility measurements. *J. Am. Chem. Soc.* **64**: 963–964
 - 116 Niwa Y. and Akamatsu H. (1991) Kojic acid scavenges free radicals while potentiating leukocyte functions including free radical generation. *Inflammation* **15**: 303–315
 - 117 Hider R. C. and Lerch K. (1989) The inhibition of tyrosinase by pyridinones. *Biochem. J.* **257**: 289–290
 - 118 Tanaka T., Takeuchi M. and Ichishima E. (1989) Inhibition study tyrosinase from *Aspergillus oryzae*. *Agric. Biol. Chem.* **53**: 557–558
 - 119 Chen J. S., Wei C. I. and Marshall M. R. (1991) Inhibition mechanism of kojic acid on polyphenol oxidase. *J. Agric. Food Chem.* **39**: 1897–1901
 - 120 Chen J. S., Wei C. I., Rolle R. S., Otwell W. S., Balaban M. O. and Marshall M. R. (1991) Inhibitory effect of kojic acid on some plant and crustacean polyphenol oxidases. *J. Agric. Food Chem.* **39**: 1396–1401
 - 121 Lee M. J. and Anstee J. (1995) Phenoloxidase and its zymogen from the haemolymph of larvae of the lepidopteran *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Comp. Biochem. Physiol.* **110B**: 379–384
 - 122 Lerch K. (1980) Copper metallothionein, a copper-binding protein from *Neurospora crassa*. *Nature* **284**: 368–370
 - 123 Byrd J., Berger R. M., McMillin D. R., Wright C. F., Hamer D. and Winge D. R. (1988) Characterization of the copper-thiolate cluster in yeast metallothionein and two truncated mutants. *J. Biol. Chem.* **263**: 6688–669
 - 124 Bremner I., Mehra R. K. and Sato M. (1988) Metallothionein in blood, bile and urine. *Experientia Suppl.* **52**: 507–517
 - 125 Goetghebeur M. and Kermasha S. (1996) Inhibition of polyphenol oxidase by copper-metalllothionein from *Aspergillus niger*. *Phytochemistry* **42**: 935–94
 - 126 Hamer D. H. (1986) Metallothionein 1,2. *Annu. Rev. Biochem.* **55**: 913–951
 - 127 Bremner I. (1991) Nutritional and physiologic significance of metallothionein. *Methods Enzymol.* **205**: 25–35
 - 128 Madhosingh C. and Sundberg L. (1974) Purification and properties of tyrosinase inhibitor from mushroom. *FEBS Lett.* **49**: 156–158
 - 129 Espín J. C., Jolivet S. and Wichers H. J. (1998) Inhibition of mushroom polyphenol oxidase by agaritine. *J. Agric. Food Chem.* **46**: 2976–2980
 - 130 Kubo I., Chen Q. X. and Nihei K. (2003) Molecular design of antibrowning agents: antioxidative tyrosinase inhibitors. *Food Chem.* **81**: 241–247
 - 131 Yu L. (2003) Inhibitory effects of (S)- and (R)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acids on tyrosinase activity. *J. Agric. Food Chem.* **51**: 2344–2347
 - 132 Kubo I. (1999) Molecular design of antioxidative and antimicrobial agents. *Chemtech* **11**: 37–42
 - 133 Tanford C. (1980) *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Wiley, New York
 - 134 Cleland J. G., Dargie H. J., Hodsman G. P., Ball S. G., Robertson J. I., Morton J. J. et al. (1984) Captopril in heart failure. A double blind controlled trial. *Br. Heart J.* **52**: 530–535
 - 135 Cleland J. G. (1994) The clinical course of heart failure and its modification by ACE inhibitors: insights from recent clinical trials. *Eur. Heart J.* **15**: 125–130
 - 136 Espín J. C. and Wichers H. J. (2001) Effect of captopril on mushroom tyrosinase activity in vitro. *Biochim. Biophys. Acta* **1544**: 289–300
 - 137 Jay D., Cuellar A., Zamorano R., Muñoz E. and Gleason R. (1991) Captopril does not scavenge superoxide: captopril prevents O₂[•] production by chelating copper. *Arch. Biochem. Biophys.* **290**: 463–467
 - 138 Bartosz M., Kedziora J. and Bartosz G. (1996) The copper complex of captopril is not a superoxide dismutase mimic. Artefacts in DMPO spin trapping. *Free Radic. Res.* **24**: 391–396
 - 139 Lovstad R. A. (1976) Effect of penicillamine on the conversion of dopa to dopachrome in the presence of tyrosinase or ceruloplasmin. *Biochem. Pharmacol.* **25**: 533–535
 - 140 Andrawis A. and Kanh V. (1986) Effect of methimazole on the activity of mushroom tyrosinase. *Biochem. J.* **235**: 91–96
 - 141 Kahn V. and Andrawis A. (1985) Inhibition of mushroom tyrosinase by tropolone. *Phytochemistry* **24**: 905–908
 - 142 Espín J. C. and Wichers H. J. (1999) Slow-binding inhibition of mushroom (*Agaricus bisporus*) tyrosinase isoforms by tropolone. *J. Agric. Food Chem.* **47**: 2638–2644
 - 143 Sculley M. J., Morrison J. F. and Cleland W. W. (1996) Slow-binding inhibition: the general case. *Biochim. Biophys. Acta* **1298**: 78–86
 - 144 Cabanes J., García-Carmona F., García-Cánovas F., Iborra J. L. and Lozano J. A. (1984) Kinetic study of on the slow inhibition of frog epidermis tyrosinase by *m*-coumaric acid. *Biochim. Biophys. Acta* **790**: 101–107
 - 145 Cabanes J., García-Cánovas F., Tudela J., Lozano J. A. and García-Carmona F. (1987) L-Mimosine, a slow-binding inhibitor of mushroom tyrosinase. *Phytochemistry* **26**: 917–919
 - 146 Jiménez M. and García-Carmona F. (1997) 4-Substituted resorcinols (sulfite alternatives) as slow-binding inhibitors of tyrosinase catecholase activity. *J. Agric. Food Chem.* **45**: 2061–2065
 - 147 McEvily J. A., Iyengar R. and Otwell W. S. (1992) Inhibition of enzymatic browning in foods and beverages. *Crit. Rev. Food Sci. Nutr.* **32**: 253–273
 - 148 Frankos V. H., Schmitt D. F., Haws L. C., McEvily A. J., Iyengar R. and Miller S. A. (1991) Generally recognized as safe (GRAS) evaluation of 4-hexylresorcinol for use as a processing aid for prevention of melanosis in shrimp. *Regul. Toxicol. Pharmacol.* **14**: 202–212
 - 149 Iyengar R., Bohmont C. W. and McEvily A. (1991) 4-Hexylresorcinol and prevention of shrimp black spot: residual analysis. *J. Food Compos. Anal.* **4**: 148–157
 - 150 Shiino M., Watanabe Y. and Umezawa K. (2001) Synthesis of N-substituted N-nitrosohydroxylamines as inhibitors of mushroom tyrosinase. *Bioorg. Med. Chem.* **9**: 1233–1240
 - 151 Skotland T. and Ljones T. (1980) Inactivation of dopamine β -monooxygenase by hydrogen peroxide and by ascorbate. *Arch. Biochem. Biophys.* **201**: 81–87
 - 152 Andrawis A. and Kahn V. (1985) Inactivation of mushroom tyrosinase by hydrogen peroxide. *Phytochemistry* **24**: 397–405

- 153 Carpenter K. J. (1981) Individual amino acid levels and bioavailability. In: Protein Quality in Humans: Assessment and In Vitro Estimation, pp. 239–160, Bodwell C. E., Adkins J. S. and Hopkins D. T. (eds), AVI, Westport, CT
- 154 Konanayakam M. and Sastry S. K. (1988) Kinetics of shrinkage of mushrooms during blanching. *J. Sci. Food Agric.* **53**: 1406–1411
- 155 Decareau R. V. (1985) *Microwaves in the Food Processing Industry*, Academic Press, London
- 156 Devece C., Catalá-Civera J. M., Sánchez-Heránández D. and de los Reyes E. (1997) Polyphenoloxidase (PPO) inactivation in industrial mushroom blanching processes employing microwave heating techniques. 14th ICECOM Int. Conf. 1: 115–119
- 157 Langdon T. T. (1987) Preventing of browning in fresh prepared potatoes without the use of sulfiting agents. *Food Tech.* **41**: 47–64
- 158 Santerre C. R., Cash J. N. and van Norman D. J. (1988) Ascorbic acid/citric acid combinations in the processing of frozen apple slices. *J. Food Sci.* **53**: 1713–1716
- 159 Dudley E. D. and Hotchkiss J. H. (1989) Cysteine as an inhibitor of polyphenol oxidase. *J. Food Biochem.* **13**: 65–75
- 160 Eskin N. A., Henderson H. M. and Townsend R. J. (1971) *Biochemistry of Foods*, pp. 69–103, Academic Press, New York
- 161 FDA. (1990) Sulfiting agents; revocation of GRAS status for use on ‘fresh’ potatoes served or sold unpackaged or unlabeled to consumers. FDA. Federal Registration **55**: 9826–9833
- 162 Hsu A. F., Sheih J. J., Bill D. D. and White K. (1988) Inhibition of mushroom polyphenol oxidase by ascorbic acid derivatives. *J. Food Sci.* **53**: 765–767
- 163 Dooley T. P. (1997) Topical skin depigmentation agents: current products and discovery of novel inhibitors of melanogenesis. *J. Dermatol. Treat.* **7**: 188–200
- 164 Hermanns J. F., Petit L., Martalo O., Pierard-Franchimont C., Cauwenbergh G. and Pierard G. E. (2000) Unraveling the patterns of subclinical pheomelanin-enriched facial hyperpigmentation: effect of depigmentation agents. *Dermatology* **201**: 118–122
- 165 Funayama M., Arakawa H., Yamamoto R., Nishino T., Shin T. and Murao S. (1995) Effects of α and β arbutin on the activity of tyrosinase from mushroom and mouse melanoma. *Biosci. Biotechnol. Biochem.* **59**: 143–144
- 166 Jin Y. H., Lee S. J., Chung M. H., Park J. H., Park Y. I., Cho T. H. et al. (1999) Aloesin and arbutin inhibit tyrosinase activity in a synergistic manner via a different action mechanism. *Arch. Pharmacol. Res.* **22**: 232–236
- 167 Kasraee B., Hügin A., Tran C., Sorg O. and Saurat J. -H. (2004) Methimazole is an inhibitor of melanin synthesis in cultured B16 melanocytes. *J. Invest. Dermatol.* **122**: 1338–1341
- 168 Marnett L. J., Hurd H. K., Hollstein M. C., Levin D. E., Esterbauer H. and Ames B. N. (1985) Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA 104. *Muta. Res.* **148**: 25–34



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