Stilbenes with tyrosinase inhibitory activity

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Tyrosinase (EC 1.14.18.1) is a multicopper monooxygenase enzyme with wide distribution. In mammals, it is responsible for pigmentation of skin, eyes and hair. The enzyme is involved in the undesired browning of bruised or cut fruits and vegetables. In insects, tyrosinase is one of the key enzymes in the molting process. Investigation of inhibitors of this enzyme may lead to development of novel skin whitening agents, anti-browning substances or insect control compounds. A number of naturally occurring stilbenes have been shown to possess strong tyrosinase inhibitory potential. Results from the studies on several natural and synthetic analogues have indicated that the number of free OH groups and their positions on the aromatic rings are important for the activity. This review gives a brief overview of the enzyme and describes tyrosinase-inhibiting stilbenoids regarding their botanical origin, structure–activity relationships, mechanisms of action and practical applications.

Keywords: Anti-browning agents, inhibitory activity, stilbenes, tyrosinase.

TYROSINASE (EC 1.14.18.1), also known as polyphenol oxidase (PPO), is a multifunctional copper-containing enzyme widely distributed in nature, including bacteria, fungi, higher plants and animals[1,2]. Its functions include monophenolase (cresolase) and diphenolase (catecholase) activity. It is involved in the biosynthesis of melanin and catalyses the *ortho*-hydroxylation of tyrosine (monophenol) to 3,4-dihydroxyphenylalanine or DOPA (*o*-diphenol), and the oxidation of DOPA to dopaquinone (*o*-quinone). This *o*-quinone can then be transformed into melanin pigments through a series of enzymatic and non-enzymatic reactions (Figure 1)[3–5].

In mammals, tyrosinase is responsible for pigmentation of the skin, eyes and hair[3]. In plants, it causes undesired enzymatic browning of farm products, such as bruised or cut fruits and vegetables, which subsequently leads to a significant decrease in nutritional and market values[6,7]. In insects, the enzyme is essential for the sclerotization of the exoskeleton, wound healing and parasite encapsulation[8,9]. It is apparent that inhibitors of this enzyme should have broad applications. In fact, tyrosinase inhibitors have been used as depigmenting agents for the treatment or prevention of pigmentation disorders[10]. In the food industry, they could be used as preservatives for foods and beverages of plant origin. Tyrosinase inhibitors may also be used as alternative insect control agents.

Biochemical properties of tyrosinase

So far, experimental data have suggested that tyrosinases from all sources are monomeric proteins with more than one isoform[4,5,11,12]. It is known that both monophenolase and diphenolase activities of the enzyme require molecular oxygen[5,13].

To date, the properties of the active site and the reactivity of tyrosinase have been elucidated mostly by correlations to hemocyanins, the bluish copper-containing proteins of mollusks and arthropods[4,14]. Two copper atoms are situated in the active site, and it is the geometric and electronic structures of this binuclear copper structure that control the exertion of the enzyme activity. There exist three different types of arrangement of the binuclear

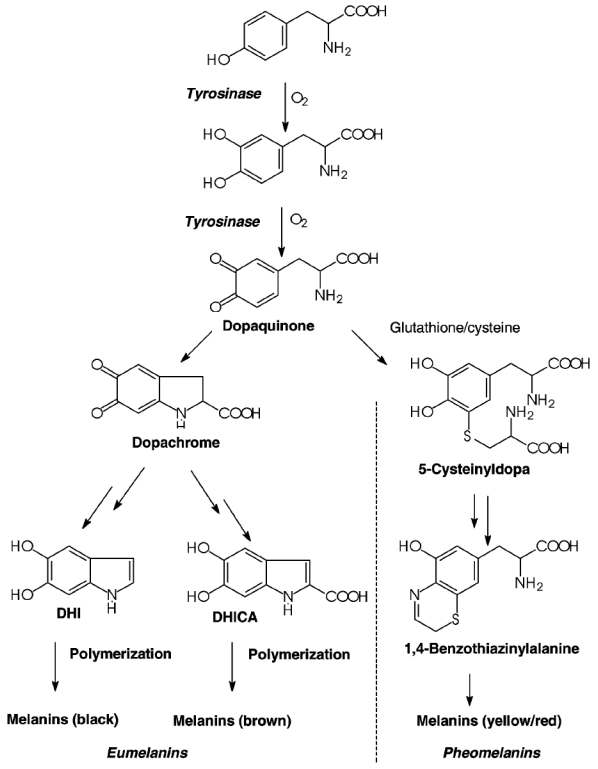

Figure 1. Functions of tyrosinase and melanognesis.

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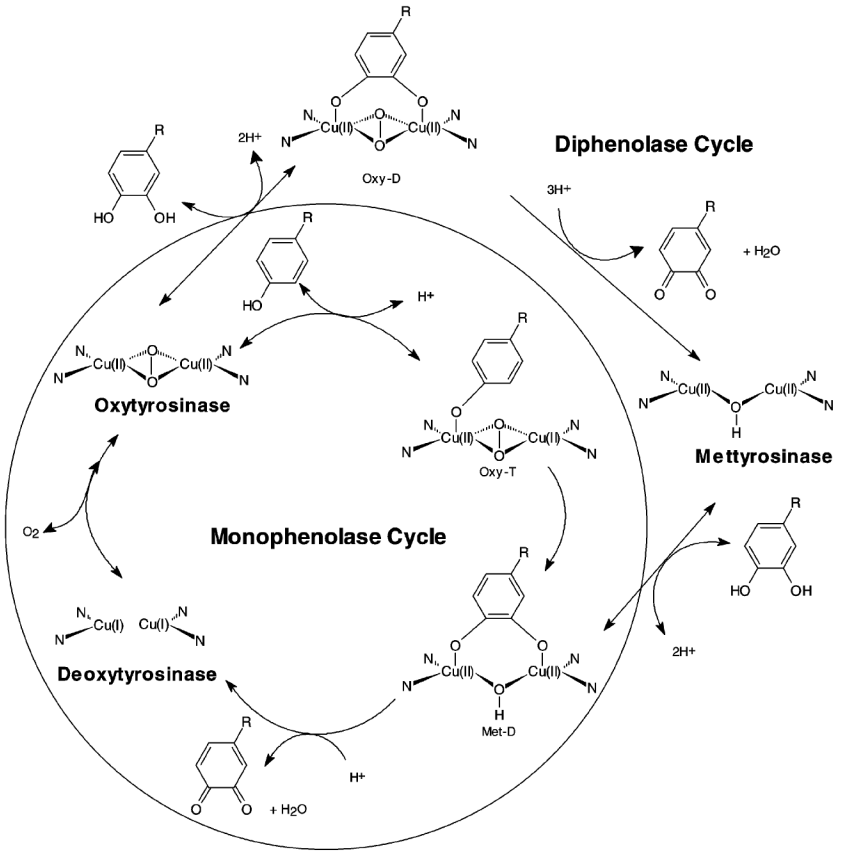


Figure 2. Mechanism for monophenolase and diphenolase activity of tyrosinase.

copper structure of tyrosinase, i.e. three isoforms, namely oxytyrosinase, mettyrosinase and deoxytyrosinase (Figure 2).

Oxytyrosinase has two tetragonal Cu(II) atoms. Each Cu atom is coordinated by three N_{His} ligands, consisting of a weak axial and two strong equatorial bondings. The exogeneous oxygen molecule (O_2) is bound to this site as peroxide, and bridges the two Cu ions. Mettyrosinase has a tetragonal bicupric structure with an endogenous oxygen bridge. The met derivative is the contributor to the resting activity of tyrosinase[4]. The enzyme as obtained after purification is in the resting form, and is a mixture of mettyrosinase and oxytyrosinase in the ratio 85 : 15. Deoxytyrosinase has a bicuprous structure Cu(I)–Cu(I). No oxygen bridge is present in this structure. Two-electron reduction to the deoxy site followed by binding of molecular oxygen regenerates oxytyrosinase[3].

Mechanism for monophenolase and diphenolase activity

Monophenolase activity

As seen in Figure 2, the monophenolase activity begins with the binding of the substrate monophenol to one of the Cu atoms of the oxygenated form (oxytyrosinase)[4,15], to generate Oxy-T. Then, *o*-hydroxylation of the monophenol by the bound peroxide occurs, and an enzyme-coordinated *o*-diphenol structure (Met-D) is formed. Next, Met-D is further oxidized to give *o*-quinone and the enzyme in the deoxy form (deoxytyrosinase), which is to be transformed to the oxygenated form via oxygen molecular binding. Alternately, *o*-diphenol is released, and the enzyme is converted to the met form (mettyrosinase), which will be involved in the diphenolase cycle. It should be noted that monophenol can react with oxytyrosinase, but not with mettyrosinase, to form the product *o*-quinone. Monophenolase activity shows a characteristic lag period. This may be due to the fact that tyrosinase in the resting form contains 15% oxy sites, which is the only form that can react with monophenol substrates.

Diphenolase activity

In the diphenolase cycle, the *o*-diphenol can react with both the oxy and the met forms to produce *o*-quinone[4]. Regardless of the substrates, the diphenolase activity ($k = 10^7$ per s) proceeds at a rate faster than the monophenolase ($k = 10^3$ per s)[16,17]. The reaction of diphenol

with mettyrosinase converts the enzyme to the deoxy form, bringing it into the monophenolase cycle[4].

Biosynthetic pathway of melanin in mammalia

Melanins are biosynthesized in the melanosomes of melanocytes, which are specialized cells situated in the basal layer of the skin epidermis. Once mature, melanosomes are transferred from melanocytes to kerationocytes[18]. Melanogensis begins with the formation of dopaquinone from L-tyrosine or L-DOPA (Figure 1). Two types of melanin are produced, i. e. eumelanins (black or brown) and pheomelanins (yellow or reddish-brown)[1,3]. In eumelanogenesis, dopaquinone undergoes cyclization and oxidation reactions to form dopachrome. 5,6-Dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) are then generated and polymerized to give eumelanins. During pheomelanogenesis, dopaquinone is attacked by the nucleophilic thiol group of glutathione or cysteine, leading to the formation of 1,4-benzothiazinylalanine. Polymerization of the monomeric units forms pheomelanins[20,21].

Enzymatic browning of plant-derived foods

In plants, tyrosinase is localized in the chloroplasts of healthy plant tissues, whereas its substrates are contained in the vacuole[2,22]. Brushing, peeling or crushing of the plant tissues leads to the loss of this compartment, allowing tyrosinase-mediated browning reactions to take place. Chlorogenic acid, a phenolic compound widely distributed in fruits and vegetables, is also oxidized by this enzyme to form brown or black spots[6].

Insect melanogenesis

Melanogenesis is involved in the insect molting process. It also represents a unique and innate immune system of arthropods, responding to the invasion of pathogens and parasites[23–25]. The biosynthesis of melanin pigments in insects parallels that in mammals, except for the lack of ability to convert dopachrome to DHICA[24].

Assays for tyrosinase inhibitory activity

Most of the preliminary studies on tyrosinase inhibitors are based on *in vitro* assays using commercially available mushroom tyrosinase with L-tyrosine or L-DOPA as substrate, although reports using murine tyrosinase prepared from B16 melanoma cells have also been described[26–30]. The progress of the reaction of the substrate and the enzyme is monitored by spectrophotometric measurement of the amount of the product dopachrome[31–34]. Kojic acid, a well-known tyrosinase inhibitor, is usually used as the positive control. Compounds with potential applications may be further studied *in vivo* in cultured B16 melanoma cells[35].

Tyrosinase inhibitors from natural sources

Tyrosinase inhibitors have been identified from several organisms, ranging from fungi to higher plants. Examples of fungal metabolites showing tyrosinase inhibitory activity are azelaic acid (1,7-heptanedicarboxylic acid)[36], kojic acid [5-hydroxy-2-(hydroxymethyl)-γ-pyrone][37] and metallothionein[38]. In higher plants, most of them are polyphenolic compounds[10,39], although non-aromatic tyrosinase inhibitors have been recently reported[40–42]. This review focuses on tyrosinase inhibitors of stilbene-type.

Stilbenes with tyrosinase inhibitory activity

Stilbenes are C_6 (aromatic)-C_2-C_6 (aromatic) compounds that are biogenetically produced through the mixed shikimate–acetate pathway. They have been found in nature as monomers and oligomers. Data on naturally occurring stilbenoids with tyrosinase inhibitory activity are summarized in Table 1.

Monomeric stilbenes

A number of naturally occurring stilbenes with polyoxygenation have been reported to possess tyrosinase inhibitory activity. They can be classified according to the degree of oxygenation as di-, tri- and tetra-oxygenated stilbenes (Figure 3 and Table 1).

Di-oxygenated stilbenes

So far, only one dioxygenated stilbene, i. e. pinosylvin (**1**) has been studied for tyrosinase inhibitory potential. It showed only weak activity[43], probably due to the lack of 4-alkyl resorcinol structure (*vide infra*).

Tri-oxygenated stilbenes

In this group, resveratrol (**2**), better known for its cancer-chemopreventive potential[44], represents the simplest structure, with free phenolic groups at positions 4, 3′ and 5′. Compound **2** showed stronger DOPA oxidase inhibitory activity than kojic acid[45]. Compounds **3** and **4**, having less free phenolic groups, were found[27,28,34] to be less active than **2**. Dihydroresveratrol (**7**) was also less active than the parent compound (**2**)[28]. This implied that the number of free OH groups, as well as their arrangement in space, played an important role in determining the activity. In another report, the resveratrol galloylglucosides **5** and **6** were evaluated for mushroom tyrosinase inhibitory

Table 1. Naturally occurring stilbenes studied for tyrosinase inhibitory activity

Stilbene	Botanical origin	Substrate	Enzyme source	Type of inhibition	Reference
(**1**) Pinosylvin	Unspecified source	L-tyrosine	Mushroom	NR	43
(**2**) Resveratrol	*Artocarpus lakoocha*	L-dopa	Mushroom	NR	47
	A. gomezianus	L-dopa	Mushroom	NR	45
	Morus alba	L-dopa	Mushroom	NR	34
	Veratrum album var. *grandiflorum*	L-tyrosine	Mushroom	NR	27
			Murine	NR	27
	Vatica rassak	L-dopa	Murine	NR	28
(**3**) Resveratrol-4-*O*-methyl ether	*Rheun undulatum*	L-tyrosine	Mushroom	NR	27
			Murine	NR	27
		L-dopa	Mushroom	NR	34
(**4**) Piceid	*Polygonum cuspidatum*	L-tyrosine	Mushroom	NR	27
			Murine	NR	
		L-dopa	Mushroom	NR	34
(**5**) Resveratrol-4-*O*-(2″-*O*-galloyl)glucoside	*Rheum officinale*	L-tyrosine	Mushroom	NR	46
		L-dopa	Mushroom	Competitive	46
(**6**) Resveratrol-4-*O*-(6″-*O*-galloyl)glucoside	*R. officinale*	L-tyrosine	Mushroom	NR	46
		L-dopa	Mushroom	Competitive	46
(**7**) Dihydroresveratrol	*V. rassak*	L-dopa	Murine	NR	28
(**8**) Oxyresveratrol	*A. lakoocha*	L-dopa	Mushroom	Non-competitive	47, 51
	M. alba	L-tyrosine	Mushroom	Non-competitive	27
			Murine	NR	27
		L-dopa	Mushroom	Non-competitive	34
	Unspecified source	DL-dopa	Mushroom	Competitive	43
(**9**) Chlorophorin	*Artocarpus incisus*	L-tyrosine	Mushroom	NR	35
		DL-dopa	Mushroom	Competitive	43
	Chlorophora excelsa	L-dopa	Mushroom	NR	48
(**10**) 4-Prenyloxyresveratrol	*A. incisus*	L-tyrosine	Mushroom	NR	35
		DL-dopa	Mushroom	Competitive	43
(**11**) 4-[(2″*E*)-7″-hydroxy-3″,7″-dimethyloct-2″-enyl]-2′,3,4′,5-tetrahydroxy-*trans*-stilbene	*C. excelsa*	L-dopa	Mushroom	NR	48
(**12**) Artocarbene	*A. incisus*	L-tyrosine	Mushroom	NR	35
(**13**) Mulberroside F	*M. alba*	L-tyrosine	Mushroom	NR	50
			Human	NR	50
(**14**) Rhapontigenin	*R. undalatum*	L-tyrosine	Mushroom	NR	27
			Murine	NR	27
(**15**) Rhaponticin	*R. undalatum*	L-tyrosine	Mushroom	NR	27
			Murine	NR	27
(**16**) Gnetol	*Gnetum gnemon*	L-dopa	Murine	NR	29
(**17**) Artogomezianol	*Artocarpus gomezianus*	L-dopa	Mushroom	NR	33
(**18**) Andalasin A	*A. gomezianus*	L-dopa	Mushroom	NR	33
(**19**) (–)-ε-Viniferin	*V. rassak*	L-dopa	Murine	NR	28
(**20**)–(**23**) Vaticanols A–C and G	*V. rassak*	L-dopa	Murine	NR	28
(**24**) (+)-α-Viniferin	*Shorea hemsleyana*	L-dopa	Murine	NR	28
(**25**) (–)-Hopeaphenol	*Hopea utilis*	L-dopa	Murine	NR	28

NR, Not reported.

activity with L-DOPA as substrate and were found to be competitive inhibitors (Table 1)[46].

Tetra-oxygenated stilbenes

Oxyresveratrol (**8**) displayed a nine-fold stronger inhibitory effect on tyrosinase than resveratrol (**2**), in view of the IC_{50} value (1.5 vs 14.4 μM)[45,47]. It appeared that the additional OH at C-2 in **8** enhanced the activity.

The stilbenes chlorophorin (**9**), 4-prenyloxyresveratrol (**10**), 4-[(2″*E*)-7″-hydroxy-3″,7″-dimethyloct-2″-enyl]-2′,3,4′,5-tetrahydroxy-*trans*-stilbene (**11**), artocarbene (**12**) and mulberoside F (**13**) are similar to **8** in that their structures are oxygenated at the 2, 4, 3′ and 5′ positions. Compounds **9** and **10** were found to be as potent as **8**, whereas **11** and **12** were less active[43,48]. This was probably due to the decreased binding affinity caused by the bulkiness of the R group at C-4′. Compound **13** was reported to exhibit stronger L-tyrosine oxidase activity than kojic acid[49].

Analysis of the structures of **8**–**13** indicated that ring A of each of these compounds resembled the structure of 4-substituted resorcinol. This 4-substituted resorcinol structure is important for the tyrosinase inhibitory activity of several stilbenes and flavonoids[33,35,43,47,48]. The argument is in accordance with the lack of activity observed for **14** and **15**, the structures of which were devoid of 4-substituted resorcinol moiety[27]. In fact, synthetic 4-hexyl resorcinol has been known to be a safe and effective tyrosinase inhibitor for prevention of shrimp black spots[50]. It should be mentioned, however, that gnetol (**16**), despite the absence of 4-substituted resorcinol-like structure, was reported to be a stronger inhibitor than kojic acid[29].

Oxyresveratrol (**8**) has been a target for detailed investigation due to its potent tyrosinase inhibitory activity[27,34,43,45,47]. Over the years, conflicting reports on the inhibition mechanism were described for this compound (Table 1). Data obtained from a study on the effect of **8** on mushroom tyrosinase with DL-DOPA as substrate suggested that it was a competitive inhibitor[43]. However, in experiments with L-DOPA or L-tyrosine, **8** was reported to be a non-competitive inhibitor[27,34]. To dissipate this confusion, we re-investigated the kinetic properties of this compound[51]. The Lineweaver–Burk plots of mushroom tyrosinase in the presence of oxyresveratrol (**8**) with different concentrations of L-DOPA revealed that the maximum velocity (V_{max}) decreased, but the Michaelis constant (K_m) was not affected. This confirmed that **8** was a non-competitive inhibitor of mushroom tyrosinase with respect to L-DOPA.

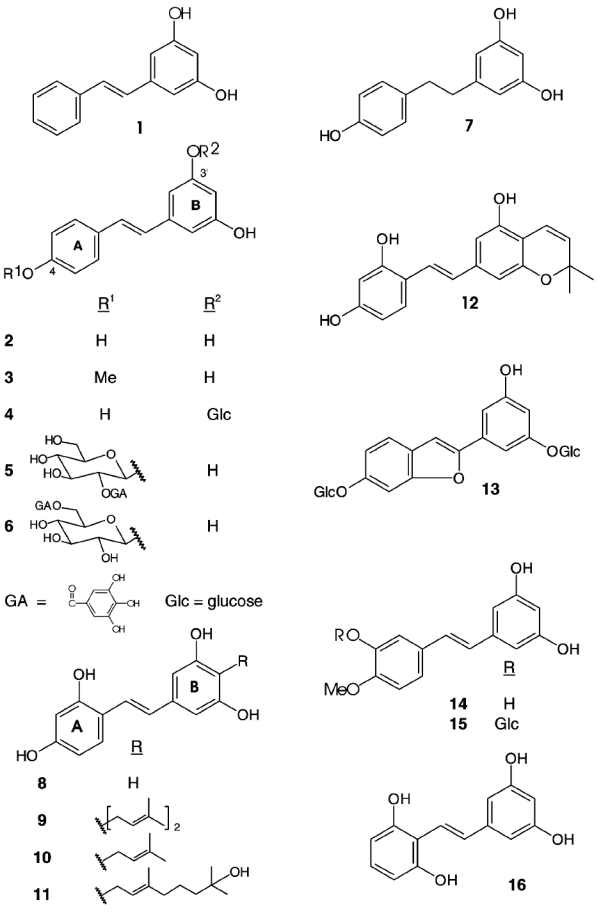


Figure 3. Naturally occurring monomeric stilbenes with tyrosinase inhibitory activity.

Oligomeric stilbenes

To date, only a few natural oligomeric stilbenes have been studied for tyrosinase inhibitory activity (Figure 4) and no kinetic studies have beeen reported (Table 1).

All of them showed much less inhibitory effect than their corresponding monomers. For example, artogomezianol and andalasin A (**17** and **18**), which were dimers of oxyresveratrol (**8**), showed lower activity than **8** (refs 33 and 47). The di-, tri- and tetramers of resveratrol, including (–)-ε-viniferin (**19**), vaticanols A, B, C and G (**20**–**23**), (+)-α-viniferin (**24**) and (–)-hopeaphenol (**25**), were found to be weak inhibitors of murine tyrosinase[28].

Semi-synthetic and synthetic stilbenes

Several polyoxygenated stilbenes (**26**–**42**) were prepared and studied for tyrosinase inhibitory activity (Figure 5 and Table 2). The trioxygenated stilbenes **26** and **27** were found to be weak inhibitors of both mushroom and murine tyrosinases[27]. Studies on the inhibitory effects of stilbenes **28**–**40** on murine and mushroom tyrosinases revealed that these polyoxygenated stilbenes possessed only marginal activity[30,51]. These data indicated that *O*-methylation destroyed the activity, re-emphasizing the importance of 4-substituted resorcinol structure with free OH groups. It should be noted that **32** was reported to have higher activity than its *cis*-isomer[52], but the opposite was described for **33** and its *cis* form[30].

Dihydrognetol (**41**) was prepared from **16** and found to have less inhibitory activity against murine tyrosinase than the parent compound[30]. However, dihydrooxyresveratrol (**42**) exhibited an eight-fold stronger inhibitory effect on L-DOPA oxidase activity of mushroom tyrosinase[51], than did oxyresveratrol (**8**). Kinetic studies revealed that the presence of **42** did not affect the K_m value, but decreased V_{max}. This suggested that **42**, similar to **8**, was a non-competitive inhibitor of mushroom tyrosinase. The K_i value of **42** was found to be five-fold higher than that of **8**, implying that **42** had higher affinity to the enzyme. Dihydrooxyresveratrol (**42**) has been identified in minute amounts from some members of the genus *Morus*[53], but it can be easily prepared in a large amount from **8**, the main constituent of the heartwood of *Artocarpus lakoocha*[51,54].

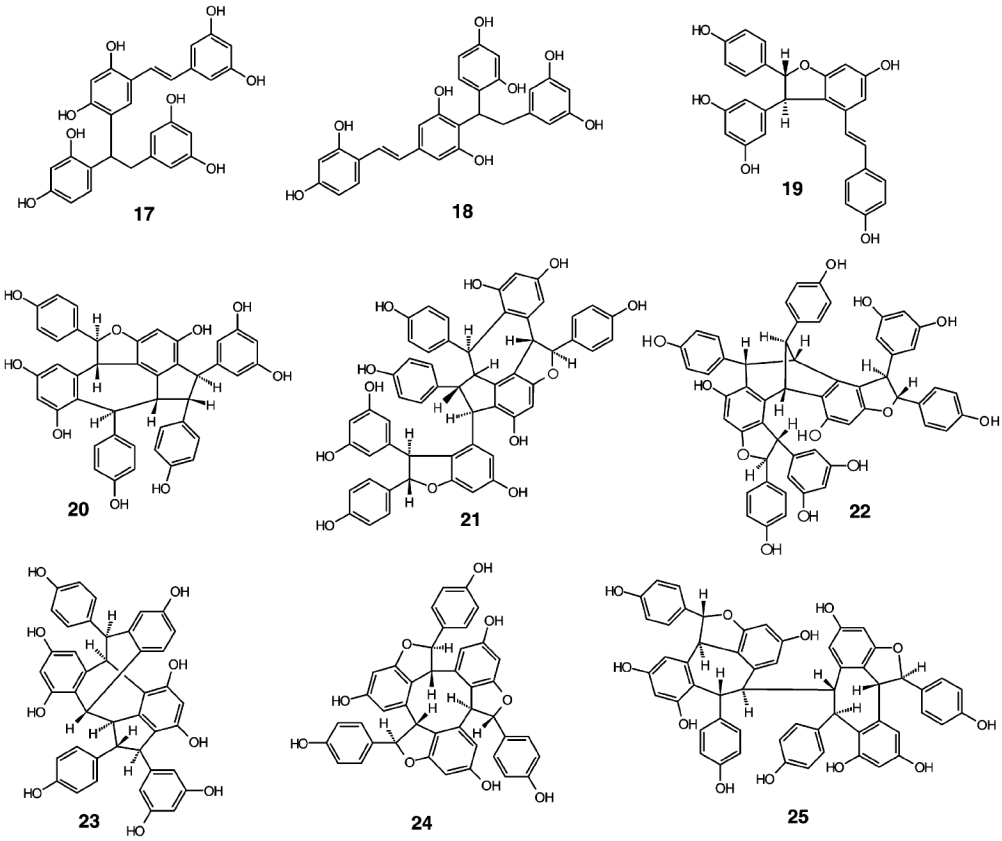
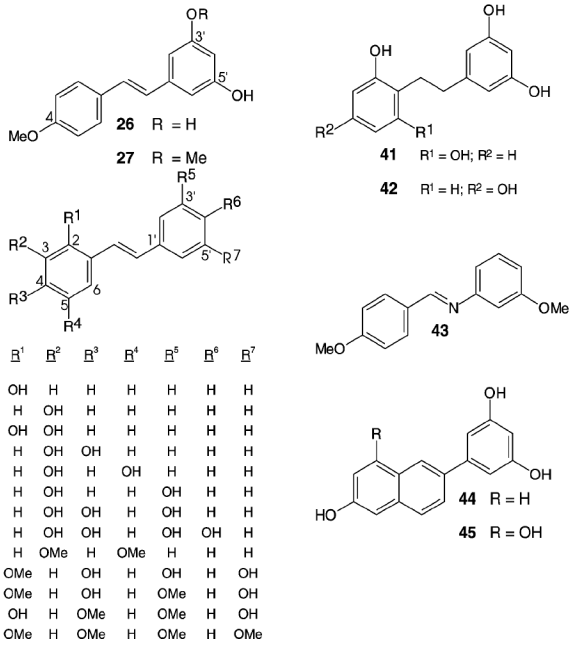


Figure 4. Naturally occurring oligomeric stilbenes with tyrosinase inhibitory activity.



	R1	R2	R3	R4	R5	R6	R7
28	OH	H	H	H	H	H	H
29	H	OH	H	H	H	H	H
30	OH	OH	H	H	H	H	H
31	H	OH	OH	H	H	H	H
32	H	OH	H	OH	H	H	H
33	H	OH	H	H	OH	H	H
34	H	OH	OH	H	OH	H	H
35	H	OH	OH	H	OH	OH	H
36	H	OMe	H	OMe	H	H	H
37	OMe	H	OH	H	OH	H	OH
38	OMe	H	OH	H	OMe	H	OH
39	OH	H	OMe	H	OMe	H	OH
40	OMe	H	OMe	H	OMe	H	OMe

Figure 5. Synthetic stilbenes with tyrosinase inhibitory activity.

Attempts to prepare tyrosinase inhibitors with other types of stilbene-related structure were also made. A stilbene-like compound containing a nitrogen atom (**43**) was prepared and found to have moderate activity[55]. Recently, hydroxy-2-phenylnaphthalenes (**44** and **45**), which were isosteric with resveratrol (**2**) and oxyresveratrol (**8**) respectively, were synthesized and shown to be potent tyrosinase inhibitors[56].

Potential applications

So far, among tyrosinase inhibitors of stilbene-type, oxyresveratrol (**8**) has been the most well-studied compound. A recent study indicated that it could be a promising anti-browning agent for food products[57]. The compound was shown to inhibit browning in cloudy apple juices at a concentration as low as 0.01%. Moreover, a solution of 0.001 M oxyresveratrol, in the presence of isoascorbic acid, calcium chloride and acetylcysteine, could suppress the browning of fresh-cut apple slices.

Oxyresveratrol (**8**) was also shown to have the ability to suppress dermal melanogenesis in animals[58]. In an experiment using guinea pigs, oxyresveratrol was evaluated

Table 2. Synthetic stilbenes studied for tyrosinase inhibitory activity

Stilbene	Substrate	Enzyme source	Type of inhibition	Reference
(**26**) 3′,5′-Dihydroxy-4-methoxystilbene	L-tyrosine	Mushroom	NR	27
		Murine	NR	27
(**27**) 5′-Hydroxy-4,3′-dimethoxystilbene	L-tyrosine	Mushroom	NR	27
		Murine	NR	27
(**28**) 2-Hydroxystilbene	L-dopa	Murine	NR	30
(**29**) 3-Hydroxystilbene	L-dopa	Murine	NR	30
(**30**) 2,3-Dihydroxystilbene	L-dopa	Murine	NR	30
(**31**) 3,4-Dihydroxystilbene	L-dopa	Murine	NR	30
(**32**) 3,5-Dihydroxystilbene	L-dopa	Murine	NR	30
		Mushroom	Competitive	56
(**33**) 3,3′-Dihydroxystilbene	L-dopa	Murine	NR	30
(**34**) 3,4,3′-Trihydroxystilbene	L-dopa	Murine	NR	30
(**35**) 3,4,3′,4′-Tetrahydroxystilbene	L-dopa	Murine	NR	30
(**36**) 3,5-Dimethoxystilbene	L-dopa	Murine	NR	30
(**37**) 2-Methoxy-4,3′,5′-trihydroxystilbene	L-dopa	Mushroom	NR	51
(**38**) 2,3′-Dimethoxy-4,5′-dihydroxystilbene	L-dopa	Mushroom	NR	51
(**39**) 4,3′-Dimethoxy-2,5′-dihydroxystilbene	L-dopa	Mushroom	NR	51
(**40**) 2,4,3′,5′-Tetramethoxystilbene	L-dopa	Mushroom	NR	51
(**41**) Dihydrognetol	L-dopa	Murine	NR	30
(**42**) Dihydrooxyresveratrol	L-dopa	Mushroom	Non-competitive	51

NR, Not reported.

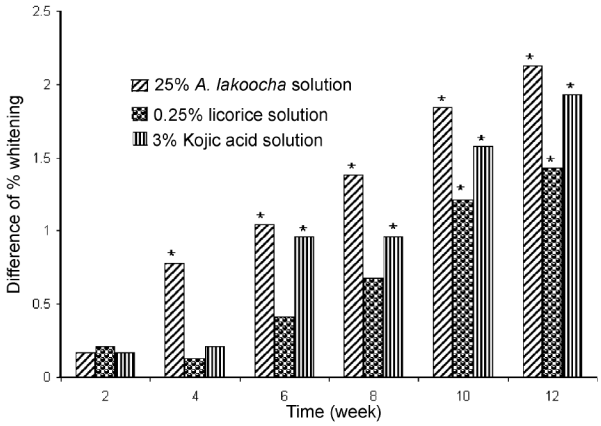


Figure 6. Whitening effect of *Artocarpus lakoocha* extract (80% oxyresveratrol) in propylene glycol in comparison with licorice and kojic acid (adapted from Tengamnuay *et al.*[59]). *Significantly different from control within group, $P < 0.05$ by Student's *t*-test.

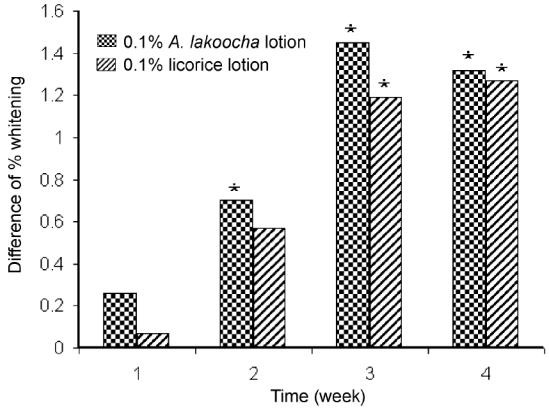


Figure 7. Whitening efficacy of *A. lakoocha* (80% oxyresveratrol) lotion in comparison with licorice lotion (adapted from Tengamnuay *et al.*[59]). *Significantly different from control within group, $P < 0.05$ by Student's *t*-test.

for depigmenting activity in comparison with kojic acid. After four weeks of daily application, it demonstrated higher effect than kojic acid on the skin of animals that was previously darkened by photo-induced pigmentation.

The whitening effect of oxyresveratrol (**8**) was also studied in humans[59]. In a 12-week comparative study using sixty female volunteers, an *A. lakoocha* extract (80% oxyresveratrol content), a licorice extract and kojic acid, in the form of 0.25, 0.25 and 3% solution in propylene glycol respectively, were examined for their whitening effect (Figure 6). Each volunteer applied a designated test sample onto one of her upper arms daily, with the other upper arm as control. Whitening effect could be observed for the *A. lakoocha* extract after four weeks of application, whereas those for kojic acid and licorice extracts were recognized after 6 and 10 weeks respectively. The *A. lakoocha* extract also displayed higher magnitude of whitening than the others, after week 4 and throughout the remaining period of the experiment.

In another study the *A. lakoocha* extract (80% oxyresveratrol content) and the licorice extract were each formulated as a 0.1% lotion, and the corresponding products were assessed for whitening efficacy in fifty female volunteers in a similar manner[59]. It was found that the *A. lakoocha* lotion had a shorter onset and showed higher bleaching effect than the licorice lotion (Figure 7).

Conclusion

Studies have shown that several stilbenoids are strong tyrosinase inhibitors. With regard to the mechanisms of action, both competitive and non-competitive inhibitions were observed for this class of compounds. Oxyresveratrol (**8**) appears to be the most promising inhibitor. Its potential use as an anti-browning agent for plant-derived foods has been demonstrated. Judging from the number of patent registrations related to its tyrosinase inhibitory activity since 2002, oxyresveratrol could soon become a widely used skin lightening agent, substituting for kojic acid which has recently been banned in some countries such as Korea, Japan and Switzerland due to mutagenicity concerns[60]. It could be envisaged that more applications will be reported for other tyrosinase inhibitors of stilbene-type in the near future.

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