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Molecular design of tyrosinase inhibitors: A critical review of promising novel inhibitors from synthetic origins*

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Abstract: The enzyme tyrosinase is known to be a multifunctional copper-containing enzyme from the oxidase superfamily, which is the key protein involved in the biosynthesis of the large biological pigment, melanin. The enzyme catalyzes two distinct reactions of melanin biosynthesis, the hydroxylation of a monophenol and the conversion of an *o*-diphenol to the corresponding *o*-quinone. Inhibitors of this protein have a huge impact on industry and economy. So a number of research groups around the world are engaged and are expending much effort in the discovery of these inhibitors. In this report, we review the importance and applications of the recently designed synthetic tyrosinase inhibitors from our and other leading laboratories of the world, which have been published in recent years. In our continuing search for tyrosinase inhibitors from natural resources to semi- and full synthetic approaches, until now we discovered and reported a large number of mild to potent inhibitors of several classes, such as phenolics, terpenes, steroids, chalcones, flavonoids, alkaloids, long-chain fatty acids, coumarins, sildenafil analogs, bipiperidines, biscoumarins, oxadiazole, tetraketones, etc. The structure–activity relationships (SARs) of different classes of synthetic tyrosinase inhibitors have also been discussed in this review.

Keywords: tyrosinase; tyrosinase inhibitors; melanin; structure–activity relationships; melanoma; molecular mechanics; molecular dynamics; sildenafil; hyperpigmentation.

INTRODUCTION

The enzyme tyrosinase (EC 1.14.18.1, syn. polyphenol oxidase, PPO; monophenol; dihydroxy-L-phenylalanin; oxidoreductase) is known to be a multifunctional copper-containing enzyme from the oxidase superfamily. This is the key enzyme involved in the biosynthesis of the large biological pigment, melanin. This enzyme catalyzes two divergent reactions of melanin biosynthesis, the hydroxylation of a monophenol and the conversion of an *o*-diphenol to the corresponding *o*-quinone [1]. This protein is widely distributed in nature [2] and on the phylogenetic scale.

The biosynthetic pathway for melanin formation operating in various life forms has largely been elucidated by Raper (1928) [3], Mason (1948) [4], and Lerner et al. (1949) [5]. The first two steps in the pathway are hydroxylation of monophenol to *o*-diphenol (monophenolase or cresolase activity) and oxidation of diphenol to *o*-quinones (diphenolase or catecholase activity), both using molecular oxygen

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followed by a series of non-enzymatic steps resulting in the formation of melanin, which plays a crucial protective role against skin photocarcinogenesis, but abnormal melanin pigmentation (melasma, freckles, ephelide, senile lentigines, etc.) is a serious aesthetic problem in human beings [6].

The modifications in melanin biosynthesis occur in many disease states. Melanin pigments are also found in the mammalian brain. Tyrosinase may play a role in neuromelanin formation in the human brain and could be central to dopamine neurotoxicity as well as contribute to the neurodegeneration associated with Parkinson's disease [7]. Melanoma-specific anticarcinogenic activity is also known to be linked with tyrosinase activity [8].

Figure 1 shows the binuclear copper active site of the enzyme tyrosinase, where "C" is the copper ion, "O" is the oxygen, and "H" is the His-N [2,9]. Six conserved histidine residues (H in the figure) [10] bind a pair of copper ions in the active site of the enzyme tyrosinase, which interacts with both molecular oxygen (O_2, O in the figure) and its phenolic substrate.

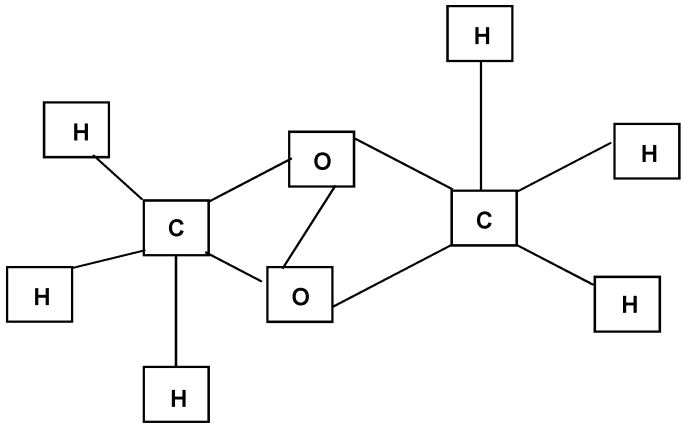


Fig. 1 Bionuclear copper (C) active site of the enzyme tyrosinase (from [2]).

This enzyme uses molecular oxygen to catalyze the oxidation of monophenols to their corresponding *o*-diphenols (monophenolase or cresolase activity) and their subsequent oxidation to *o*-quinones (diphenolase or catecholase activity). The *o*-quinones thus generate polymerization to form melanin through a series of subsequent enzymatic and nonenzymatic reactions. Although the physiological function of tyrosinase in fungi is not yet understood, melanin synthesis is correlated with the differentiation of reproductive organs and spore formation, the virulence of pathogenic fungi, and tissue protection after injury [11–14].

Tyrosinase is at the moment a well-characterized enzyme. It oxidizes phenol in two steps (Fig. 2) [15]: phenol is oxidized to catechol (*o*-benzenediol), which is consequently oxidized (by tyrosinase) to *o*-quinone.

Tyrosinase shows no activity for the oxidation of *p*- and *m*-benzenediols. Laccase, which catalyzes the oxidation of *o*-, *m*-, *p*-benzenediols to the corresponding *o*-, *m*-, *p*-quinones, is used for the detection of these benzenediols. Thus, co-immobilization of tyrosinase and laccase allows the detection of several phenolic compounds [15].

In this review, recent advances in the discovery of synthetic inhibitors from other laboratories and from some case studies from our work are discussed.

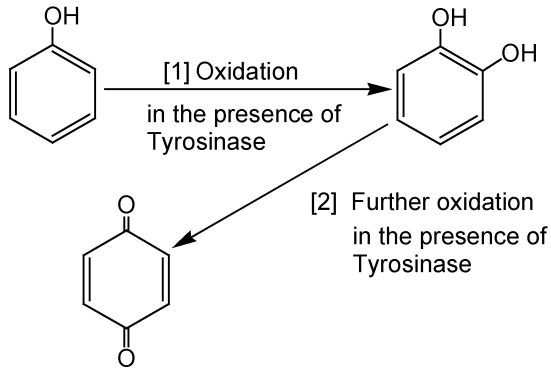


Fig. 2 Reaction steps involved in the tyrosinase-mediated oxidation of phenol (from [15]).

PHARMACOLOGY OF THE ENZYME TYROSINASE AND ITS INHIBITORS

The enzyme tyrosinase is responsible for melanization in animals and is the key enzyme for the regulation of melanogenesis in mammals. Melanin is synthesized in epidermal melanocytes and then is transferred into epidermal keratinocytes via the melanocytes' dendrites. Melanogenesis is the process by which melanin is produced and subsequently distributed by melanocytes within the skin and hair follicles. This process results in the synthesis of melanin pigments, which play a protective role against skin photocarcinogenesis. The main physiological stimulus of melanogenesis is the UV radiation of solar light, which can act directly on melanocytes or indirectly through the release of keratinocyte-derived factors such as MSH (α-melanocyte stimulating hormone). One of the biggest causative agents of hyperpigmentation is probably UV light. However, the skin darkening can be suppressed, at least partially, by deactivating of tyrosinase. Therefore, tyrosinase inhibitors have become increasingly important in the cosmetic and medicinal products used in the prevention of hyperpigmentaion [13]. A large number of compounds, such as hydroquinone (HQ), kojic acid, and benzaldehyde-*O*-alkyloximes, etc., have been reported as tyrosinase inhibitors [16].

The established murine B16-F10 melanoma cell line (B16 cells) offers a model system with readily quantifiable markers that are characteristic of differentiation, including melanogenesis. In B16 cells, α-tocopheryl ferulate was shown to have a depigmenting effect [16]. Although 44'-dihydroxybiphenyl (4,4'-BP), a bisphenol derivative, was known as a radical scavenger for methacrylate polymerization, its efficacy on tyrosinase inhibition and melanin biosynthesis has not been reported [16].

Kubo et al. reported the molecular design and synthesis of the antibrowning agents, i.e., antioxidative tyrosinase inhibitors [17]. They found that dodecyl (C12) gallate (3,4,5-trihydroxybenzoate) inhibited the oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA) catalyzed by the enzyme tyrosinase. The tyrosinase-mediated inhibition was found to be a slow and reversible reaction with residual enzyme activity. The inhibition kinetics analyzed by Lineweaver–Burk plots revealed that dodecyl (lauryl) gallate is an uncompetitive inhibitor (K_i = 0.636 mM). The parent compound of dodecyl gallate (gallic acid) acts as a reducing agent for the *o*-quinone produced by the enzymatic oxidation [17].

The molecular structures of dodecyl gallate and its chemical analogs are shown in Fig. 3.

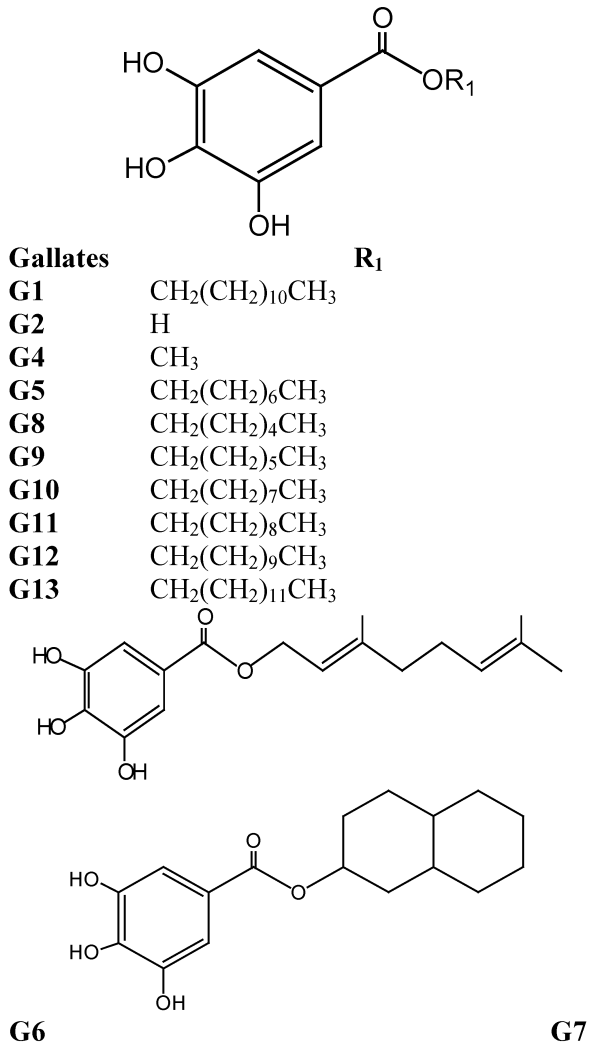


Fig. 3 Chemical structures of dodecyl (C12) gallate and its chemical analogs (from [17]).

EXAMPLES OF SYNTHETIC TYROSINASE INHIBITORS FROM SEVERAL CHEMICAL CLASSES

Figure 4 shows structures of some synthetic tyrosinase inhibitors and derivatives of naturally found tyrosinase inhibitors.

Interestingly, some of these synthetic inhibitors are simple chemicals and some are already used clinically as drug(s), but for other purposes, such as captopril, as an antihypertensive drug.

In this review, we discuss some of these tyrosinase inhibitors recently reported from synthetic origins.

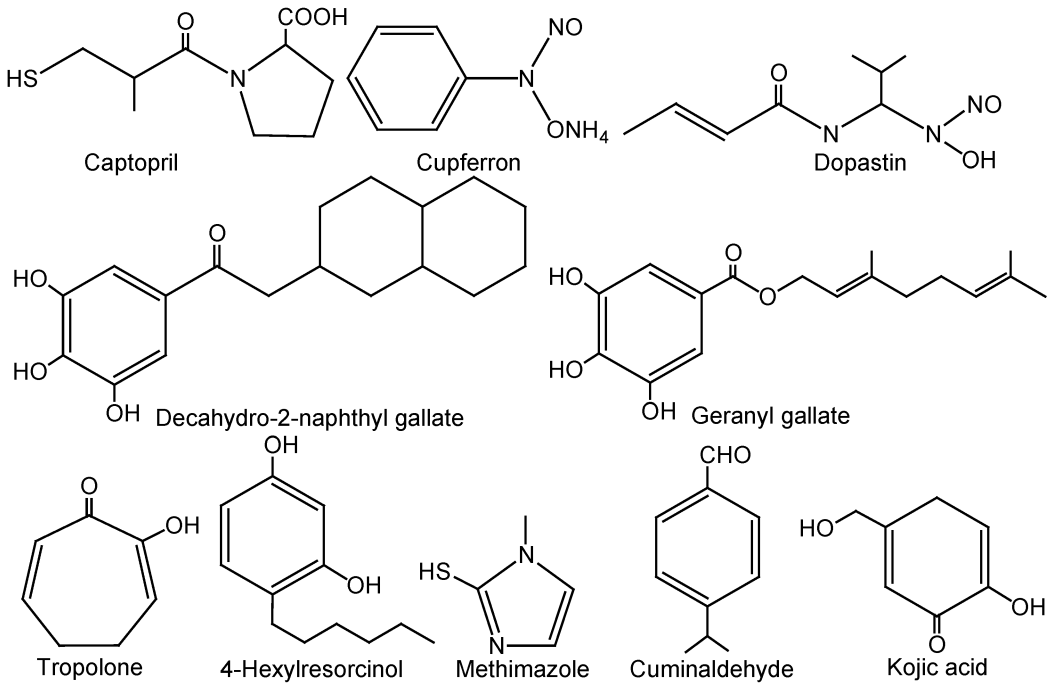
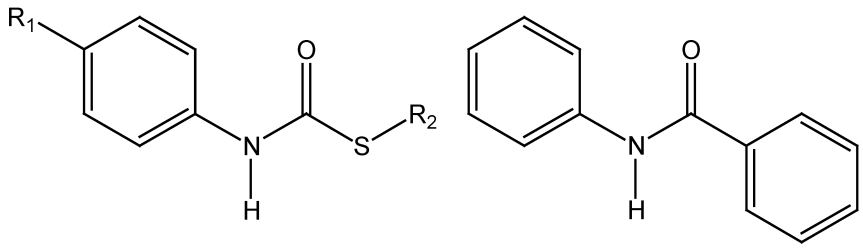


Fig. 4 Structures of some synthetic tyrosinase inhibitors.

Carbamate derivatives

In a recent scientific article, Lee and coworkers reported the potent inhibitory effects of *N*-aryl *S*-alkylthiocarbamate derivatives on the enzyme mushroom tyrosinase [18]. The *N*-aryl *S*-alkylthiocarbamate derivatives were found to exhibit potent inhibitory effect on the DOPAoxidase activity of tyrosinase. Most of the *N*-aryl *S*-alkylthiocarbamate derivatives (**1–10**) exhibited higher inhibitory effects than kojic acid (IC_{50} = 318 μM), a well-known tyrosinase inhibitor [18]. Compound **5** exhibited the most potent inhibitory activity against the enzyme tyrosinase (IC_{50} = 7.25 μM), and this inhibition was 44 times stronger than that of kojic acid. Compound **5** exhibited 95.0 % of inhibition at 100 μM. Kinetic studies using the Lineweaver–Burk plots analysis showed that compound **5** is a competitive inhibitor against tyrosinase [18].

Figure 5 shows the structural features of compounds **1–11**, whereas Table 1 shows the inhibitory profiles of these compounds against the enzyme tyrosinase when their activity was compared with reference compounds DETC (diethyldithiocarbamate), oxyresveratrol, and kojic acid (modified from [18]).



Compounds 1-10 **Compound 11**

Fig. 5 Structural features of compounds **1–11**. For the substitutions at R_1 and R_2 positions for compounds **1–10**, see Table 1 (from [18]).

Table 1 Substitutions of compounds (**1–10**) and the tyrosinase inhibitory activities of *N*-aryl *S*-alkylthiocarbamate derivatives (**1–11**) compared with reference compounds DETC, oxyresveratrol, and kojic acid (modified from [18]).

Compounds	Substitutions		IC_{50} (in μM)
	R_1	R_2	
1	–H	$-CH_2CH_3$	>100
2	–H	$-(CH_2)_2CH_3$	>100
3	–H	$-(CH_2)_2CH(CH_3)_2$	38.1
4	–H	$-CH_2C_6H_5$	18.4
5	–H	$-(CH_2)_2C_6H_5$	7.3
6	–H	$-(CH_2)_3C_6H_5$	46.6
7	$-CH_3$	$-CH_3$	48.5
8	$-CH_3$	$-(CH_2)_2CH_3$	87.5
9	$-CH_3$	$-(CH_2)_2CH(CH_3)_2$	47.4
10	–Cl	$-CH_2CH_3$	>100
11			>100
DETC			61.2
Oxyresveratrol			53.7
Kojic acid			318.0

Selenourea derivatives

Ha et al. investigated the inhibitory effects of *N*,*N*-unsubstituted selenourea derivatives on the enzyme tyrosinase [19]. Three types of *N*,*N*-unsubstituted selenourea derivatives exhibited an inhibitory effect on DOPAoxidase activity of mushroom tyrosinase. Compound **S4** exhibited 55.5 % of inhibition at a concentration of 200 μM. This inhibitory effect was higher than that of reference compound kojic acid (39.4 %). The IC_{50} value of compound was found to be 170 μM [19]. Interestingly, this compound (**S4**) was identified as a noncompetitive inhibitor by Lineweaver–Burk plot analysis. In addition, compound **S4** also inhibited the melanin production in melan-a cells [19]. For the structures of *N*,*N*-unsubstituted selenourea derivatives (**1–4**), see Fig. 6.

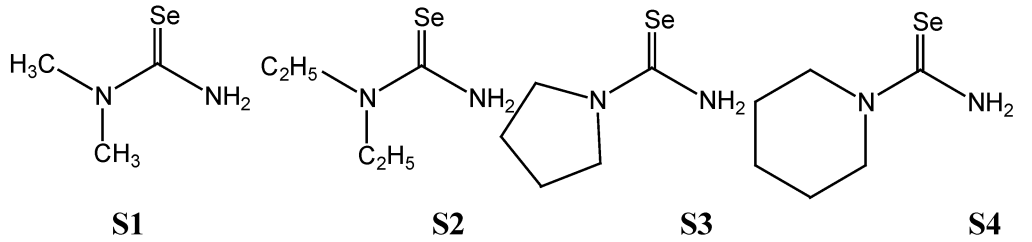


Fig. 6 Structures of *N*,*N*-unsubstituted selenourea derivatives exhibited tyrosinase inhibitory activities (modified from [19]).

Benzaldehyde analogs

Recently, Nihei and coworkers reported the synthesis and tyrosinase inhibitory studies of chamaecin (2-hydroxy-4-isopropylbenzaldehyde) [20]. Figure 7 shows the structure of chamaecin.

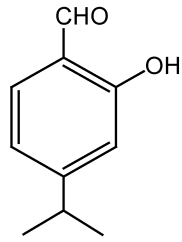
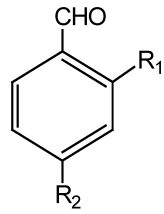


Fig. 7 Molecular structure of chamaecin [20].

Kubo and Kinst-Hori (1999) reported that 2-hydroxy-4-methoxybenzaldehyde (B5) has been isolated as a potent tyrosinase inhibitor from an African medicinal plant that inhibits the oxidation of L-DOPA catalyzed by mushroom tyrosinase with an IC_{50} of 30 μM, and kinetic studies showed that it is a mixed-type inhibitor [21]. Whereas compound B6 (known as chamaecin) exhibited much potency compared to the B1 with a similar type of reaction kinetics [21]. Kubo and Kinst-Hori (1999) also stated that the primary amino group very likely plays an important role in the tertiary structure of tyrosinase. For instance, the amino group in the enzyme may be involved with hydrogen bonding, which is essential to maintain the tertiary structure of the enzyme. Compound B6 is a relatively nonpolar molecule and may form a Schiff base with an amino group possibly located near the hydrophobic region of the enzyme. However, native proteins form a sort of intramolecular micelle in which the nonpolar portion is likely to be out of contact in the water-based test solution. Hence, some substrates first approach the binuclear active site and form the enzyme-substrate complex, and then B6 forms a Schiff base with a free primary amino group of the enzyme. 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 [21].

Table 2 summarizes the structure–activity relationships (SARs) of these benzaldehyde analogs against the enzyme tyrosinase.

Table 2 SARs of the tyrosinase inhibitory benzaldehydes (extracted from [20]).



Benzaldehyde compounds	Substitutions		IC_{50} (in μM)
	R_1	R_2	
B1	—H	—H	820.0
B2	—OH	—H	3300.0
B3	—H	—OCH_3	320.0
B4	—H		50.0
B5	—OH	—OCH_3	30.0
B6	—OH		2.3

A number of aromatic aldehydes have been studied for their structural criteria and anti-tyrosinase activity. It has been reported that the presence of an electron-donating group [21–23] at *para* position increases the inhibitory activity, however, contradictory results were obtained in the case of cinnamaldehyde [24], where the activity decreased by *para* methoxy substitution. Thus, the presence of an electron-donating group is not the sole criteria for better inhibitory activity, but the chemical structure of the compound also plays an important role in determining the inhibitory strength [25].

Jimenez et al. reported a kinetic study of the inhibition of mushroom tyrosinase by seven 4-substituted benzaldehydes (**1–7**, for structures, see Fig. 8) and found that these benzaldehydes behave as classical competitive inhibitors, inhibiting the oxidation of L-DOPA by mushroom tyrosinase (*o*-diphenolase activity) [26].

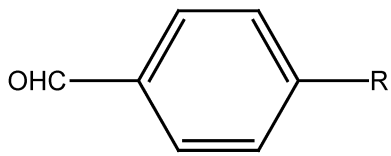


Fig. 8 Structural features of the (**1**) benzaldehyde (R = –H) and its related derivatives: (**2**) 4-ethylbenzaldehyde (R = $–CH_2CH_3$), (**3**) 4-*tert*-butylbenzaldehyde [R = $–C(CH_3)_3$], (**4**) 4-isopropyl benzaldehyde or cuminaldehyde (R = $–CH(CH_3)_2$), (**5**) 4-methoxybenzaldehyde or anisaldehyde (R = $–OCH_3$), (**6**) 4-propoxybenzaldehyde (R = $–OCH_2CH_2CH_3$), and (**7**) 4-butoxybenzaldehyde (R = $–O\ CH_2CH_2CH_2CH_3$) (modified from [26]).

The kinetic parameter (K_i) characterizing this inhibition was evaluated for all of these seven benzaldehydes, where Jimenez et al. reported that cuminaldehyde (**4**) exhibited the most potent inhibitory activity (K_i = 9 μM) [26]. This compound also inhibited the oxidation of L-tyrosine by mushroom tyrosinase (*o*-monophenolase activity) in a competitive manner, and the corresponding kinetic parameter for this inhibition was evaluated (K_i = 0.12 μM) [26].

Phenolic amines

Melanogenesis provides a unique target for the development of antitumor agents specific for malignant melanoma. Yukitake and coworkers examined and found most promising antimelanoma effects from 4-*S*-cysteaminylphenol (4-*S*-CAP), a phenolic amine [27]. For further improvement, they also synthesized the *R*- and *S*-enantiomers (99 % enantiomer excess) of α-methyl-4-*S*-cysteaminylphenol (α-Me-4-*S*-CAP) and α-ethyl-4-*S*-cysteaminylphenol (α-Et-4-*S*-CAP), whereas they found that enantiomers of α-Me-4-*S*-CAP and α-Et-4-*S*-CAP were better substrates for tyrosinase than the natural substrate, L-tyrosine [27]. They also studied them in vitro, which showed that all four enantiomers were highly cytotoxic to pigmented B16-F1 melanoma cells, the effect being 70- and 160-fold greater than that on nonpigmented B16-G4F melanoma cells and 3T3 fibroblasts, respectively. The cytotoxic effect against B16-F1 cells was completely inhibited by phenylthiourea, a tyrosinase inhibitor, or by *N*-acetyl-L-cysteine, which increases the intracellular reduced glutathione (GSH) level. 4-*S*-CAP and the enantiomers were taken up into B16-F1 cells at comparable rates, but showed varying rates of GSH depletion that were inversely correlated to the cytotoxicity. These results suggest that the use of enantiomers would increase the efficacy of tyrosinase-dependent cytotoxic phenols [27].

Chalcone and its analogs

Khatib and coworkers have introduced groups of mono-, di-, tri-, and tetra-substituted hydroxychalcones as effective tyrosinase inhibitors. They have also shown that the most important factor determining tyrosinase inhibition efficiency is the position of the hydroxyl group(s) rather their number [28]. So we can say that, for the tyrosinase inhibition, –OH groups play the most important role, then the position and the number of the –OH groups have importance. These statements are also supported by the recent findings of Khatib and coworkers [28]. Their findings are summarized in Table 3, where chalcones C2 and C4 are shown to be the most potent inhibitors, having IC_{50} values 0.2 and 0.02 μM, respectively, when L-tyrosine have been used as substrate. The values IC_{50} for the same compounds were 7.5 and 90 μM, respectively, when L-DOPA has been used as substrate [28].

Nerya and coworkers tested nine mono-, di-, tri-, and tetrahydroxychalcones as inhibitors of tyrosinase mono- and diphenolase activities [29]. They showed that the most important factor in the efficacy of those chalcones was the location of the hydroxyl groups on both aromatic rings, with a significant preference to a 4-substituted B ring, rather than a substituted A ring. Neither the number of hydroxyls nor the presence of a catechol moiety on ring B correlated with increasing tyrosinase inhibition potency [29]. They also reported that 4-hydroxychalcone (4-HC), ILC, and butein inhibited tyrosinase [29]. As pigmentation also results from auto-oxidation, they also tested the antioxidant activities of 4-HC, ILC, and butein [29]. Their results showed that chalcones are also potent antioxidants, with butein the most potent one. Nerya et al. concluded that chalcones are strongly potent new depigmentation agents, with their double effect of reduction and antioxidant activity [29]. Table 4 shows some of the activities (IC_{50} values) of chalcone and its analogs against tyrosinase.

Table 3 Inhibitory effect of chalcones against mushroom tyrosinase activities with different substrates (extracted from [28]).

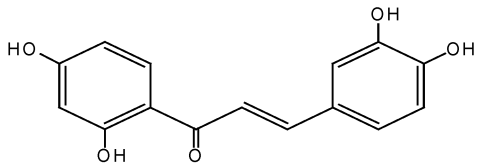
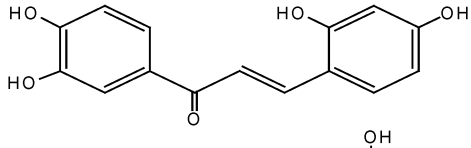
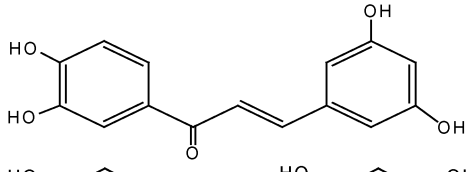
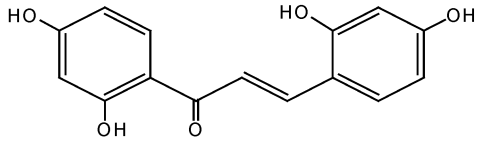
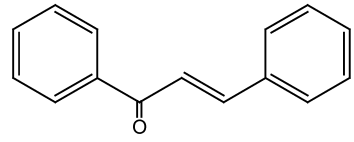
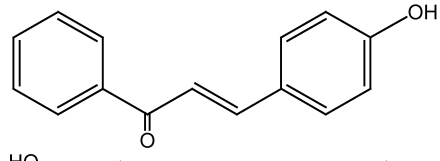
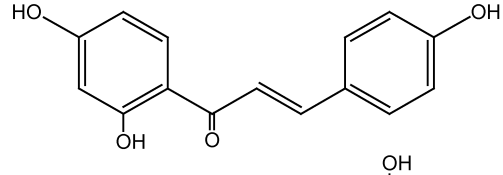
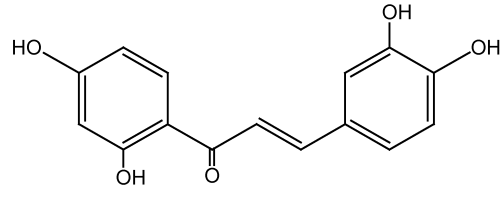
Chalcones	Structures	IC_{50} (in μM)	
		L-Tyrosine	L-DOPA
C1		29.3	>100
C2		0.2	7.5
C3		32.68	>1000
C4		0.02	90

Table 4 Tyrosinase-inhibiting activities (IC_{50} values) of chalcone and its analogs (modified from [29]).

Name of the chalcones	Structures	IC_{50} (in μM)
Chalcone		Not active
4-Hydroxychalcone		21.8
2',4',4-Trihydroxychalcone		8.1
2',4',3,4-Tetrahydroxychalcone (Butein)		29.3

Biphenyl compound

Recently, Kim et al. reported the inhibition of tyrosinase by 44'-BP (for structure, see Fig. 9). This molecule was also assessed for the melanin biosynthesis in B16 melanoma cells [16]. They found that 44'-BP exhibited strong tyrosinase inhibitory potential with an IC_{50} value of 1.91 μM. The kinetic analysis of tyrosinase inhibition revealed that 44'-BP acts as a competitive inhibitor ($K_i = 4.0 \times 10^{-4}$ M at 2.5 μM and 21×10^{-5} M at 5 μM). Additionally, the data found on melanin biosynthesis indicate that the amount of melanin was clearly suppressed by 44'-BP [16].

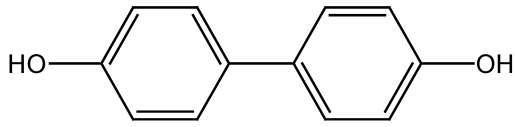


Fig. 9 Structure of 44'-BP, a potent inhibitor of the enzyme tyrosinase [16].

Thiohydroxyl compounds

Park et al. detected and reported an unusual thioether bridge (Cys-His) at the active site of mushroom tyrosinase, and the effects of thiohydroxyl compounds such as dithiothreitol (DTT) and β-mercaptoethanol (β-ME) on Cu^{2+} at the active site have also been elucidated [30]. DTT and β-ME exhibited completely inactivated DOPAoxidase activity of mushroom tyrosinase in a dose-dependent manner [30].

Sequential kinetic studies of Park et al. revealed that DTT and β-ME caused different mixed-type inhibition mechanisms: the slope-parabolic competitive inhibition ($K_i = 0.143$ mM) by DTT and slope-hyperbolic noncompetitive inhibition ($K_i = 0.0128$ mM) by β-ME, respectively [38]. Kinetic Scatchard analysis consistently showed that mushroom tyrosinase had multiple binding sites for DTT and β-ME with different affinities [30]. A reactivation study of inactivated enzyme by addition of Cu^{2+} confirmed that DTT and β-ME directly bound with Cu^{2+} at the active site [30].

The results found by Park et al. provide useful information regarding the interactions of tyrosinase inhibitor for designing an effective whitening agent targeted to the tyrosinase active site [30].

Copper chelator: Ammonium tetrathiomolybdate

Ammonium tetrathiomolybdate (ATTM) is known as a drug for the treatment of Wilson's disease. Recently, Park et al. reported the inhibitory properties of ATTM against mushroom tyrosinase [31]. They found that ATTM completely inactivated the enzyme activity of mushroom tyrosinase in a dose-dependent manner. Progress-of-substrate reaction kinetics using the two-step kinetic pathway and dilution of the ATTM revealed that ATTM is a tight-binding inhibitor and a high dose of ATTM irreversibly inactivated tyrosinase [31].

They also performed the enzyme kinetic studies of ATTM against the enzyme tyrosinase, where they found that progress-of-substrate reaction kinetics and activity restoration with a dilution of the ATTM indicated that the copper-chelating ATTM may bind slowly but reversibly to the active site without competition with substrate, and the enzyme-ATTM complex subsequently undergoes reversible conformational change, leading to complete inactivation of the tyrosinase activity [31]. The inhibition by ATTM on tyrosinase could be categorized as a complex type of inhibition with a slow and reversible binding. Detailed analysis of inhibition kinetics provided IC_{50} at the steady state, and inhibitor K_i value found for ATTM as 1.0 μM and 10.65 μM, respectively [31].

Carboxylic acid derivative

Yu has reported the inhibition patterns of the (*R*)-, (*S*)-, and (+/–)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acids (HTCCAs) against mushroom tyrosinase [32]. They found that all the HTCCAs inhibited the tyrosinase activity. The ID_{50} values were found to be 1.88, 1.84, and 1.88 for the (*R*)-, (*S*)-, and (+/–)-HTCCAs, respectively. For the structural features of *S*- and *R*-HTCCAs, see Fig. 10.

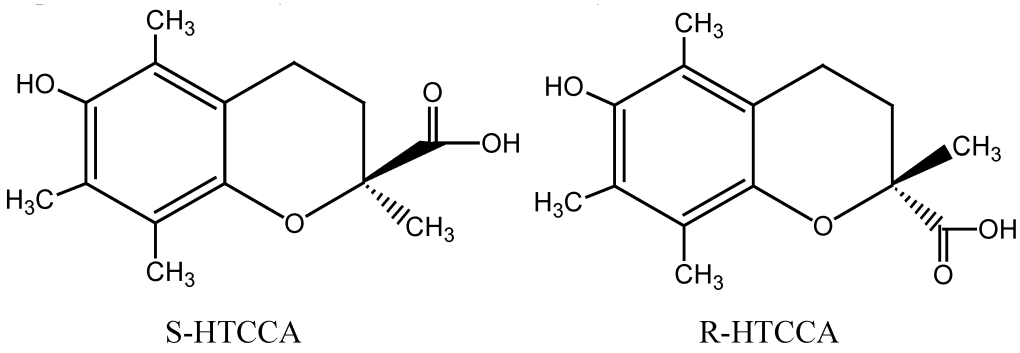


Fig. 10 Structures and stereochemistry of *S*- and *R*-HTCCAs (from [32]).

Yu has also done the inhibition kinetics by Hanes–Woolf plots, which indicate that both the (*R*)- and (*S*)-HTCCAs are competitive inhibitors of the enzyme tyrosinase, with K_i values of 0.83 and 0.61 mM, respectively [32].

Yu also tested dimethyl sulfoxide (DMSO) for its direct inhibitory activity against tyrosinase. Interestingly, he found that DMSO has potential influence on the tyrosinase inhibitory effects of both the (*R*)- and (*S*)-HTCCAs, whereas it is a widely used solvent for many compounds, including tyrosinase inhibitors, and was found to dose-dependently inhibit tyrosinase activity [32].

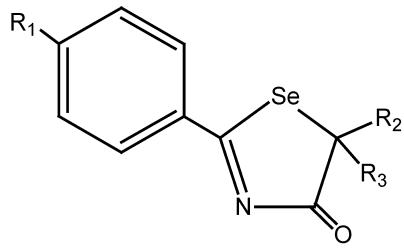
Yu concluded that the addition of DMSO in a tyrosinase digest containing either the (*R*)- or (*S*)-HTCCA further dose-dependently reduced tyrosinase activity. These data indicated a potential to use a HTCCA as a tyrosinase inhibitor in food, cosmetic, and medicinal products and a need to improve the solvent system for the studies of tyrosinase inhibitions [32].

1,3-Selenazol-4-one derivatives

Koketsu and coworkers performed and reported the DOPAoxidase activity of six derivatives of 1,3-selenazol-4-one (A, B, C, D, E, and F) on mushroom tyrosinase [41]. All of these compounds exhibited 33.4–62.1 % of inhibition of DOPAoxidase activity at a concentration of 500 μM. Their inhibitory effects were higher than that of kojic acid (31.7 %), a well-known tyrosinase inhibitor. 2-(4-Methylphenyl)-1,3-selenazol-4-one (A) exhibited the strongest inhibitory effect among them dose-dependently and in a competitive inhibition manner [33].

The structures of the six derivatives of 1,3-selenazol-4-one (A, B, C, D, E and F) and their SARs are shown in Table 5.

Table 5 Structures of the six derivatives of 1,3-selenazol-4-one (A, B, C, D, E, and F) and their SARs (modified from [33]).



Compounds	Substitutions			IC_{50} (in μM)
	R_1	R_2	R_3	
A	$-CH_3$	–H	–H	333.2
B	$-CH_3$	$-CH_2CH_3$	–H	384.3
C	$-CH_3$	$-CH_3$	$-CH_3$	>500
D	–H	–H	–H	478.1
E	–Cl	–H	–H	498.0
F	$-OCH_3$	–H	–H	>500
Kojic acid				934.3

Sildenafil analogs

Recently, we reported the tyrosinase inhibitory potentials of the sildenafil and its several analogs [34,35].

First, we synthesized sildenafil (Viagra™) (**21**) and its 10 (**11–20**) analogs, employing microwave (MW) irradiations utilizing S_NAr reaction taking bromopyrazole as precursor [34]. And then these compounds (**11–21**) have been subjected to tyrosinase inhibition assays and their SARs [34].

Compounds **14** and **16** exhibited moderate to mild tyrosinase inhibition with their IC_{50} values of 19.95 and 54.43 μM, respectively [34].

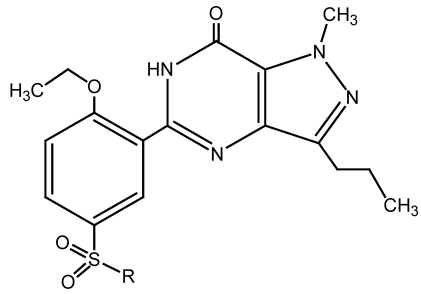
On the other hand, compounds **15** and **17** showed potent inhibition of tyrosinase with low IC_{50} values of 8.69 and 3.54 μM, respectively, comparable to standard tyrosinase inhibitors, such as KA and LM, the IC_{50} values were found to be 16.92 and 3.68 μM, respectively [34].

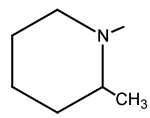
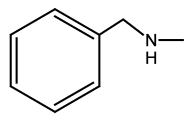
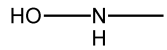
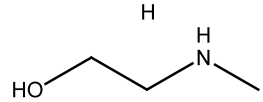
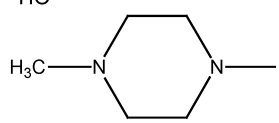
Table 6 shows sildenafil and some of its analogs (**14–17**), which exhibited inhibition against the enzyme tyrosinase.

This study reflected that the inhibition was enhanced with an increase of the carbon chain. In the case of compound **17**, the –OH group was replaced with $-CH_2-CH_2-OH$ with a resulting increase in inhibition against tyrosinase. Compound **17** was found to be more potent than the potent reference inhibitors LM and KA [34].

Compound **14**, where *R* is 2-methylpiperidine, showed good tyrosinase inhibitory activity with IC_{50} value of 19.95 μM, whereas 3- and 4-methyl piperidinyl derivatives **12** and **13** were found to be inactive. The decrease in activity of compound **12** and **13** was apparently due to a change of position of the methyl group (not shown in Table 6). In other words, the substitution of the methyl group at position-2 is a best fit for the activity and may help in the interaction of the molecule with enzyme. The activity of compound **15** (IC_{50} = 8.69 μM) may be rationalized by its secondary amino function and its aromatic characters. Both of these effects may help to bind the molecule with the enzyme. The decrease in activity of compound **16** (IC_{50} = 54.43 μM) may be due to direct hydroxyl attachment with amino function, which may decrease the binding affinity of hydroxyl group with the enzyme [34,36].

Table 6 Sildenafil and some of its analogs showed tyrosinase inhibitory potentials (adopted from [34]).



Compounds	Substitutions (*R*)	IC_{50} values (in μM)
14		19.95
15		8.69
16		54.43
17		3.54
21 (sildenafil)		NA[a]
KA[b]		16.67
LM[b]		3.68

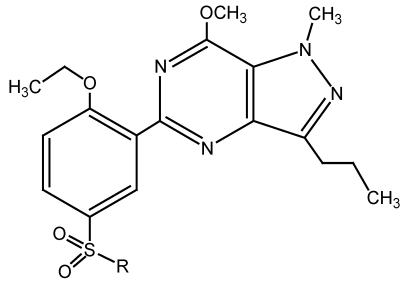
[a]Not active against the enzyme tyrosinase.
[b]Used as reference inhibitors in these experiments.

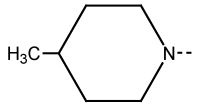
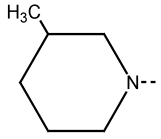
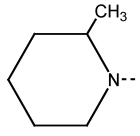
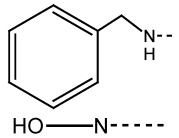
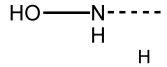
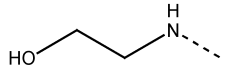
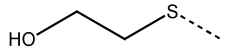
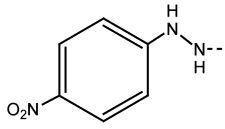
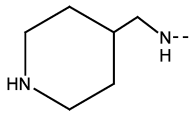
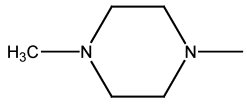
The most active compound of the series was compound **17**, which was the ethanolamine derivative of compound **11**. The enhanced activity of compound **17** (IC_{50} = 3.54 μM) compared to compound **16** may be explained on the basis of the electronegativity differences in carbon and oxygen. In compound **16**, the nitrogen atom was directly attached with oxygen, which causes lowering of the electron density on nitrogen atom, which may bind with the copper of the enzyme to inhibit its unfavorable activities. However, in the case of compound **17**, oxygen is present two carbons away from the nitrogen, which may additionally enhance the ability to bind the molecule to the enzyme by either the oxygen or nitrogen of molecule [34,36]. The α-effect of –NHOH might play some important role in this as well.

In the same paper, we also reported some in silico energy minimizations using molecular mechanics (MM), single-molecule molecular dynamic (MD) simulations, and their different energy calculations, 2D and 3D hydrogen bonding thermodynamic (HYBOT) descriptors, etc., studies of these molecules besides the synthetic and enzyme inhibition studies [34].

More recently, we reported another group of a similar class of compounds against the enzyme tyrosinase [35]. In this paper, we again reported the MW-assisted synthesis of 10 new sildenafil analogs (**6–15**). We have also discussed their detailed SARs in this report [35]. Table 7 shows these 10 new sildenafil analogs (**6–15**) those exhibited inhibition against the enzyme tyrosinase.

Table 7 The tyrosinase inhibitory potentials of the 10 new sildenafil analogs (**6–15**) (adopted from [35]).



Compounds	Substitutions (*R*)	IC_{50} values (in μM)
6		8.76
7		16.37
8		10.36
9		21.39
10		3.59
11		9.65
12		2.15
13		12.31
14		19.36
15		NA[a]
KA[b]		16.67
LM[b]		3.68

[a]Not active against the enzyme tyrosinase.
[b]Used as reference inhibitors in these experiments.

In this group of sildenafil analogs, compounds **10** and **12** exhibited most potent inhibition against the enzyme tyrosinase, and their IC_{50} values were 3.59 and 2.15 μM, respectively. These compounds contained hydroxylamino and (2-hydroethyl)sulfanyl groups at the *R* positions, respectively [34]. As substitutions in this position, most of the above-mentioned cases that drastically change the potential activities of the molecules against tyrosinase show that this position might play a vital role in the inhibition [34]. We shall be able to confirm this concept in our advance communications with the molecular docking interaction studies with the active ligands and the 3D structure of the protein.

Oxadiazole derivatives

Khan et al. performed the tyrosinase inhibition studies of the library of 2,5-disubstituted-1,3,4-oxadiazoles, which have been reported, and their SARs also have been discussed in this report. The library of the oxadiazoles was synthesized under MW irradiation [37].

Compound **3e** {30-[5-(40-bromophenyl)-1,3,4-oxadiazol-2-yl]pyridine} exhibited most potent (IC_{50} = 2.18 μM) inhibition against the enzyme tyrosinase, which is more potent than the standard potent inhibitor L-mimosine (IC_{50} = 3.68 μM) [37].

Table 8 shows the substitutions at the *R* position for different oxadiazoles and their tyrosinase inhibitory potentials (IC_{50} values, in μM).

Table 8 SARs of the oxadiazoles [37].

Sr.	R	IC_{50} (in μM)
3a	C_6H_5	5.15
3b	*o*-$NO_2C_6H_4$	3.18
3c	*o*-BrC_6H_4	5.23
3d	*m*-BrC_6H_4	6.04
3e	*p*-BrC_6H_4	2.18
3f	3-Pyridinyl	3.29
3g	CH_2Cl	4.18
3h	$CHCl_2$	4.01
3i	CCl_3	3.98
3j	*p*-$CH_3C_6H_4$	10.40
3l	1-$C_{10}H_7$	3.23
4A	C_6H_5	8.71
4c	*o*-BrC_6H_4	5.16
4d	*m*-BrC_6H_4	7.18
4e	*p*-BrC_6H_4	7.82
4h	$CHCl_2$	7.28
4i	CCl_3	6.21
4j	*p*-$CH_3C_6H_4$	6.43
4m	2-$C_{10}H_7$	7.81

In this report, Khan et al. conclude that for a better inhibition of tyrosinase, electronegative substitution is essential as most probably the active site(s) of the enzyme contains some hydrophobic site and position is also very important for the inhibition purposes due to the conformational space [37]. We shall be able to prove this theory as well in our next communications by further molecular docking interaction studies.

The electronegativity of the compounds is somewhat proportional to the inhibitory activity [37].

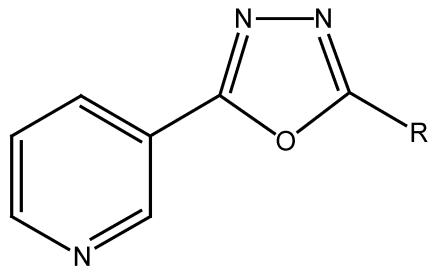


Fig. 11 Structural features of the compounds shown in Table 8 (from [37]).

4-Vinyl derivatives

Song et al. recently investigated the inhibitory effects of 4-vinylbenzaldehyde and 4-vinylbenzoic acid on the activity of mushroom tyrosinase. Their results exhibited that both the 4-vinylbenzaldehyde and 4-vinylbenzoic acid could inhibit both monophenolase activity and diphenolase activity of the enzyme [38].

Song et al. found that for the monophenolase activity, 4-vinylbenzoic acid could lengthen the lag time, but 4-vinylbenzaldehyde could not. Both 4-vinylbenzaldehyde and 4-vinylbenzoic acid decreased the steady-state activity, and the IC_{50} values were estimated as 93 μM and 3.0 mM for monophenolase activity, respectively. For the diphenolase activity, the inhibitory capacity of 4-vinylbenzaldehyde was stronger than that of 4-vinylbenzoic acid, and the IC_{50} values were estimated as 23 μM and 0.33 mM, respectively [38].

The authors also performed the kinetic analyses, which showed that the inhibitions by both compounds were reversible and their mechanisms were mixed-II type; their inhibition constants were also determined and compared [38].

CONCLUSION AND RECOMMENDATIONS

Tyrosinase is a melanogenic copper-containing enzyme that catalyzes the transformation of tyrosine to dopaquinone [39,40]. This enzyme is responsible for melanization in plants and animals, which leads to the undesirable browning of farm products and the coloring of an animal's skin, eyes, inner ear, and hair [41,42]. Various dermatological disorders, such as melasma, age spots, and sites of actinic damage, arise from the accumulation of an excessive level of epidermal pigmentation [43].

The exploration and characterization of new inhibitors are not only useful for the medicinal purposes, but their potential applications in improving food quality and nutritional value, controlling insect pests, etc., are also important.

So it is very important to discover novel and potent inhibitors of the enzyme tyrosinase. We also recommend preclinical studies, such as in vitro toxicity studies for short- and long-term effects, as well as pharmacokinetic and pharmacodynamic studies, which are important for the development of improved clinically active inhibitors.

Unfortunately, we do not yet have the 3D structural information and the folding natures of the enzyme tyrosinase. This kind of structural information can shed more light on the mechanism of action of the enzyme and will be helpful in in vitro and/or in silico (point) mutation studies, including antisense RNA techniques and gene silencing, which will help to decrease production of tyrosinase in vivo. The X-ray crystallographic or solution NMR structure of tyrosine will play a vital role in this research.

To unearth the role of this enzyme in further unexplored and new fields, much more research on the enzyme tyrosinase is required. This will be helpful for designing and/or improving enzymatic activities for diverse appliances.

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