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Heterocyclic Compounds Against the Enzyme Tyrosinase Essential for Melanin Production: Biochemical Features of Inhibition

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Abstract Tyrosinase is a copper-containing bifunctional metalloenzyme, widely distributed around the phylogeny. This enzyme is involved in the production of melanin and some other pigments in humans, animals, etc. Abnormal accumulation of melanin, which is due to the overexpression of the enzyme, is called hyperpigmentation and underexpression is called vitiligo, which is a major skin problem around the world. The inhibitors of this enzyme have been utilized in cosmetics, especially as depigmenting agents in the case of hyperpigmentation. They are also involved in several other disease conditions. In the last few decades a large number of tyrosinase inhibitors have been discovered and reported by several groups including ours. This chapter principally emphasizes the discovery of some interesting inhibitors, mainly of heterocyclic origin, and their impacts on drug discovery; some of the inhibitors might not be heterocyclic but their chemistry is quite interesting in terms of the inhibition.

Keywords Tyrosinase inhibitors · *Agaricus bisporus* · Polyphenol oxidase · Hyperpigmentation · Vitiligo · Melanogenesis · Alkaloid

Abbreviations

CO Catechol oxidases
EC Enzyme Commission
PO Phenol oxidases
PPO Polyphenol oxidases

1 Introduction

Tyrosinase or polyphenol oxidase (EC 1.14.18.1) is a bifunctional, copper-containing enzyme widely distributed on the phylogenetic tree. This enzyme uses molecular oxygen to catalyze the oxidation of monophenols to their corresponding *o*-diphenols (cresolase activity) as well as their subsequent oxidation to *o*-quinones (catecholase activity). The *o*-quinones thus generated polymerize to form melanin, through a series of subsequent enzymatic and nonenzymatic reactions [1–3].

This enzyme is involved in many biological processes, such as defense, mimetism, protection from UV light, hardening of cell walls in fungi or exoskeleton in Arthropods, and in general the production of melanins [4]. This process is involved in abnormal accumulation of melanin pigments (hyperpigmentation, melasma, freckles, ephelide, senile lentigines, etc.). Therefore, tyrosinase inhibitors have been established as important constituents of cosmetic materials, as well as depigmenting agents for hyperpigmentation [5].

Tyrosinase may also play an important role in neuromelanin formation in the human brain, particularly in the substantia nigra, and could be central to dopamine neurotoxicity as well as contributing to the neurodegeneration associated with Parkinson's disease [6]. Melanoma-specific anticarcinogenic activity is known to be linked with tyrosinase activity [7].

Tyrosinases are often referred to as phenolases, phenol oxidases (PO), polyphenol oxidases (PPO), or catechol oxidases (CO), etc., depending on the particular source and also on the authors who have described any particular enzyme. The term tyrosinase is usually adopted for the animal and human enzymes, and refers to the "typical" substrate, tyrosine. PPO is perhaps the most suitable general definition [8]. The enzyme extracted from the edible (champignon) mushroom *Agaricus bisporus* is usually referred to as tyrosinase, and its high homology with the mammalian ones renders it well suited as a model for studies on melanogenesis. This enzyme has been thoroughly characterized [8]. Nowadays, mushroom tyrosinase has become popular because it is readily available and useful in a number of applications [9].

Additionally, the enzyme is also responsible for the detrimental enzymatic browning of fruits and vegetables [10] that takes place during senescence or damage at the time of postharvest handling, which makes the identification of novel tyrosinase inhibitors extremely important. Nevertheless, besides this

role in undesired browning, the activity of tyrosinase is needed in other cases (raisins, cocoa, fermented tea leaves) where it produces distinct organoleptic properties [9].

Tyrosinase oxidizes phenol in two steps [11]:

- Phenol is oxidized to catechol (*o*-benzenediol).
- This catechol is subsequently 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*-, and *p*-benzenediols to the corresponding *o*-, *m*-, and *p*-quinones, is used for the detection of these benzenediols. Thus, coimmobilization of tyrosinase and laccase allows the detection of several phenolic compounds [11]. Several authors have presented a large number of research and review papers on the structural and kinetic aspects of the enzyme tyrosinase [12–14].

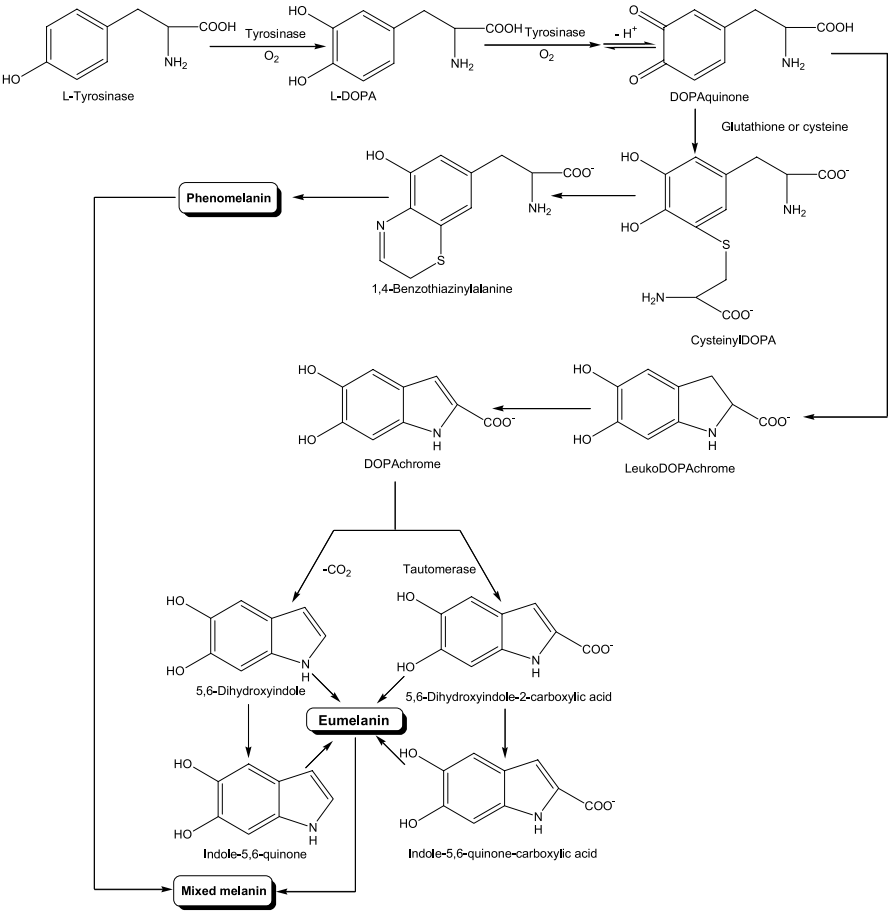
2 Biosynthesis of Melanin

The biosynthetic pathway for melanin formation, operating in insects, animals, and plants, has largely been elucidated by Raper [15], Mason [16], and Lerner et al. [17]. The first two steps in the pathway are the hydroxylation of monophenol to *o*-diphenol (monophenolase or cresolase activity) and the oxidation of diphenol to *o*-quinones (diphenolase or catecholase activity), both using molecular oxygen followed by a series of nonenzymatic steps resulting in the formation of melanin [15, 18, 19]. The whole pathway for melanin biosynthesis is shown in Scheme 1.

3 Tyrosinase Inhibitors

Tyrosinase inhibition may be a potential approach to prevent and control the enzymatic browning reactions and improve the quality and nutritional value of food products [20]. Tyrosinase also plays a major key role in the developmental and defensive functions of insects. Tyrosinase is involved in melanogenesis, wound healing, parasite encapsulation, and sclerotization in insects [21–23]. For these reasons, in recent years the development of tyrosinase inhibitors has become an active alternative approach to control insect pests [20]. Additionally, it is now well-recognized that tyrosinase inhibitors are important for their potential applications in medical and cosmetic products [24–26].

Furthermore, the inhibitors may be clinically used for the treatment of some skin disorders associated with melanin hyperpigmentation and are also important in cosmetics for skin whitening effects [27–42], so there is a need



Scheme 1 Melanin biosynthetic pathway [15, 18, 19]

to identify the compounds that inhibit mushroom tyrosinase activity [9]. The molecular structures of some mushroom tyrosinase inhibitors are shown in Fig. 1.

In the next sections some of the promising classes of tyrosinase inhibitors are discussed with especial emphasis on heterocyclic origin; a few of them may not be heterocyclic but their inhibition pattern and chemistry is highly interesting.

3.1 Xanthates

Very recently Saboury et al. (2007) reported the tyrosinase inhibitory potentials of four sodium salts of *N*-alkyl xanthates [43] (see Fig. 2). The xanthates

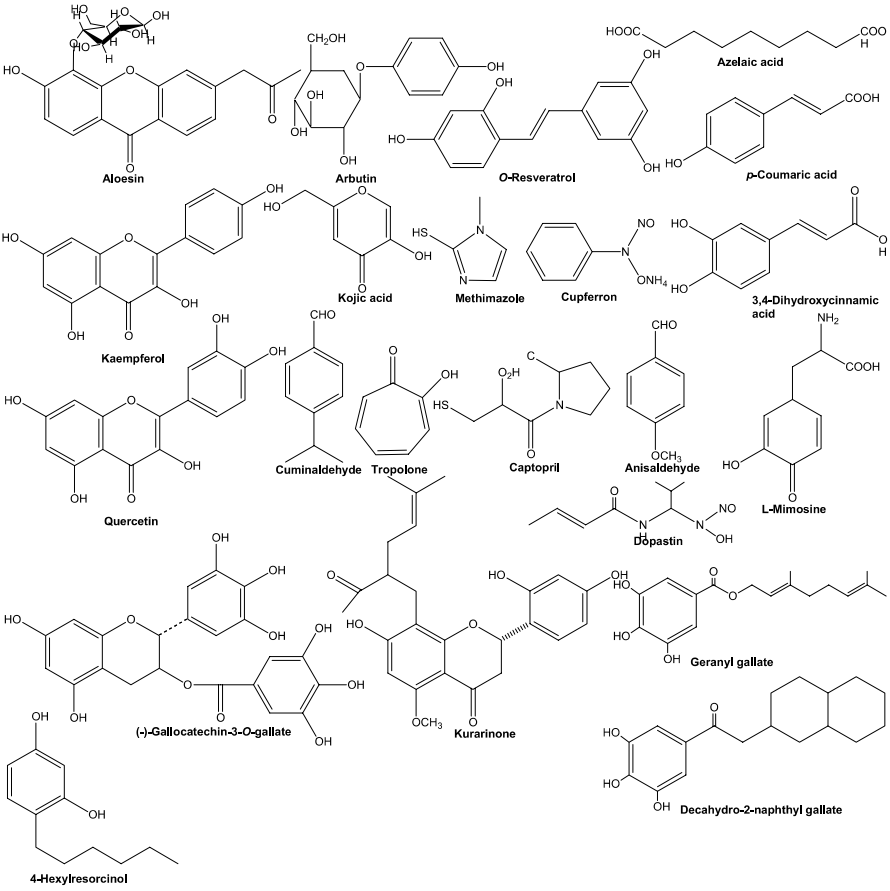


Fig. 1 Molecular structures of some mushroom tyrosinase inhibitors

have been synthesized and examined for their inhibition of both cresolase and catecholase activities against mushroom tyrosinase, taking 4-[(4-methylbenzo)azo]-1,2-benzenediol (MeBACat) and 4-[(4-methylphenyl)azo]-phenol (MePAPh) as substrates (for structures see Fig. 3) [43].

By using Lineweaver–Burk plots the authors found that four xanthates exhibited different patterns of mixed, competitive, or uncompetitive inhibition. For the cresolase activity, **1** and **2** demonstrated uncompetitive inhibition but **3** and **4** exhibited competitive inhibition [43]. For the catecholase activity, **1** and **2** showed mixed inhibition but **3** and **4** showed competitive inhibition against tyrosinase [43]. The xanthates (compounds **1, 2, 3** and **4**) have been classified as potent inhibitors against tyrosinase due to their K_i values of 13.8, 11.0, 8.0, and 5.0 μM, respectively, for the cresolase activities, and 1.4, 5.0, 13.0, and 25.0 μM, respectively, for the catecholase activities [43]. The authors concluded that, for the catecholase activity, both substrate and inhibitor can

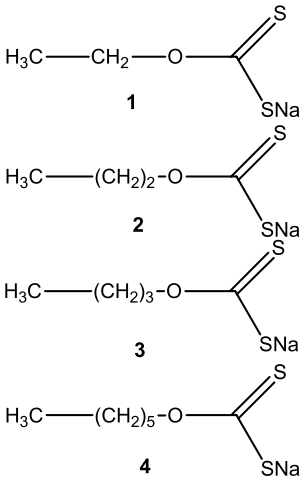


Fig. 2 Molecular structures of four *N*-alkyl xanthates reported by Saboury et al. [43]

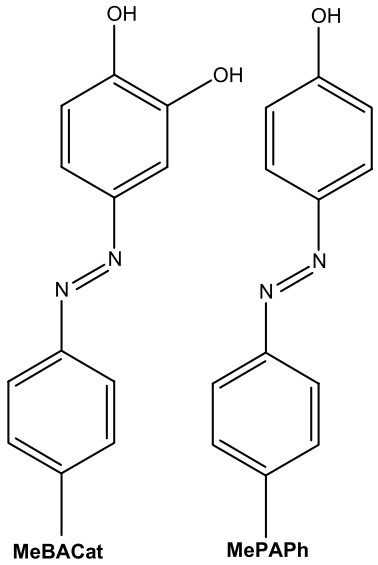


Fig. 3 Structures of 4-[(4-methylbenzo)azo]-1,2-benzenediol (MeBACat) and 4-[(4-methylphenyl)azo]-phenol (MePAPh) used by Saboury et al. as synthetic substrate in their studies against mushroom tyrosinase [43]

be bound to the enzyme with negative cooperativity between the binding sites, and this negative cooperativity increases with increasing length of the aliphatic tail of these compounds. The length of the hydrophobic tail of the xanthates has a stronger effect on the K_i values for catecholase inhibition than for cresolase inhibition. Increasing the length of the hydrophobic tail leads to

a decrease of the K_i values for cresolase inhibition and an increase of the K_i values for catecholase inhibition [43].

3.2
N,*N*-Unsubstituted Selenourea Derivatives

Recently, Ha et al. (2005) reported the inhibitory potentials of *N*,*N*-unsubstituted selenourea derivatives **5–8** on tyrosinase. Three types of *N*,*N*-unsubstituted selenourea derivatives exhibited an inhibitory effect on the DOPAoxidase activity of mushroom tyrosinase. For the structures of these selenourea derivatives (**5–8**), see Fig. 4. Compound **8** exhibited 55.5% inhibition at a concentration of 200 μM (IC_{50} = 170 μM). This inhibitory effect was higher than that of reference compound kojic acid (39.4%, for structure see Fig. 1) [44]. Interestingly, this compound (**8**) was identified as a noncompetitive inhibitor by Lineweaver–Burk plot analysis. In addition, **8** also inhibited melanin production in melan-a cells [44].

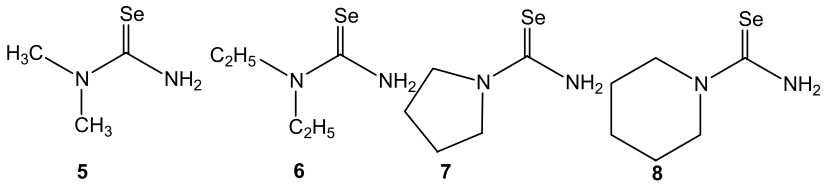


Fig. 4 Molecular structures of *N*,*N*-unsubstituted selenourea derivatives exhibiting tyrosinase inhibitory activities [44]

3.3
Selenium-Containing Carbohydrates

Ahn and coworkers recently (2006) reported the potency of selenium-containing carbohydrates on depigmentation, based on the direct inhibition of mushroom tyrosinase [45]. The structures of these selenium-containing compounds are shown in Fig. 5. Among them two selenoglycosides, **11** (bis(2,3,4-tri-*O*-acetyl-β-D-arabinopyranosyl) selenide) and **16** (4′-methylbenzoyl 2,3,4,6-tetra-*O*-acetyl-D-selenomanopyranoside), have been found to be effective depigmenting compounds against mushroom tyrosinase [45]. In enzyme kinetic studies, compound **11** exhibited a competitive inhibition effect which was found to be similar to that of kojic acid [45]. At 100 and 150 μM concentration, **16** exhibited an uncompetitive inhibition pattern [45].

The authors also performed studies of the same compounds in melan-a cell-originated tyrosinase inhibition assays, which showed that **16** was a less potent inhibitor than the kojic acid [45]. Compound **11** showed a similar kind of inhibitory effect as kojic acid in the melan-a cell-originated tyrosinase inhibitory assay [45].

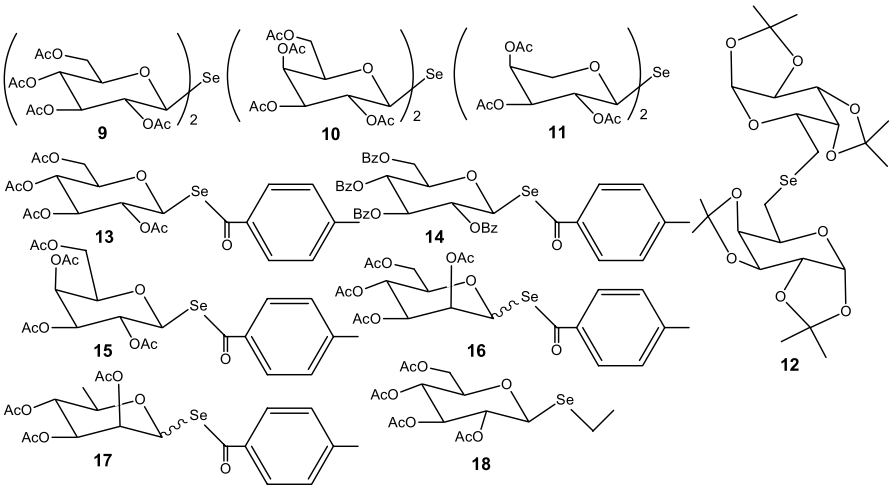


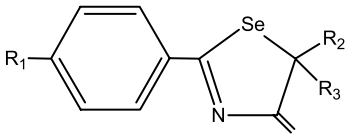
Fig. 5 Structures of the selenium-containing carbohydrates: **9**, bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) selenide; **10**, bis(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) selenide; **11**, bis(2,3,4-tri-*O*-acetyl-β-D-arabinopyranosyl) selenide; **12**, bis(1,2 : 3,4-di-*O*-isopropylidene-6-deoxy-β-D-galactopyranosyl) selenide; **13**, 4′-methylbenzoyl 2,3,4,6-tetra-*O*-acetyl-D-selenoglucopyranoside; **14**, 4′-methylbenzoyl 2,3,4,6-tetra-*O*-benzoyl-D-selenoglucopyranoside; **15**, 4′-methylbenzoyl 2,3,4,6-tetra-*O*-acetyl-β-D-selenogalactopyranoside; **16**, 4′-methylbenzoyl 2,3,4,6-tetra-*O*-acetyl-D-selenomanopyranoside; **17**, 4′-methylbenzoyl 2,3,4,6-tetra-*O*-acetyl-D-selenorhamnopyranoside; **18**, ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-selenoglucopyranoside [45]

Compound **16** showed dose-dependent cytotoxicity in a study of inhibition of melanin synthesis by melan-a cell lines. The cellular survival rate was found to be low after treatment with 20 μM of **16** [45]. Compound **11** inhibited melanin synthesis in the melan-a cells at a concentration of 10 μM. The inhibition of melanin synthesis by **11** was found to be similar to that of phenylthiourea, which is a well-known melanin synthesis inhibitor [45]. The authors concluded that compound **11** is a new candidate for the development of depigmenting agents [45].

3.4
1,3-Selenazol-4-one Derivatives

Koketsu et al. (2002) reported the DOPAoxidase activities of the 1,3-selenazol-4-one derivatives **19–24** against mushroom tyrosinase [46]. All of these compounds exhibited 33.4–62.1% inhibition of DOPAoxidase activity at a concentration of 500 μM. Their inhibitory effects were higher than that of kojic acid (31.7%) [46]. 2-(4-Methylphenyl)-1,3-selenazol-4-one (**19**) exhibited the most potent inhibitory effect among them in a dose-dependent manner. Enzyme kinetic studies showed that compound **19** showed competitive in-

Table 1 The structure–activity relationships of the 1,3-selenazol-4-one derivatives **19–24** against mushroom tyrosinase [46]



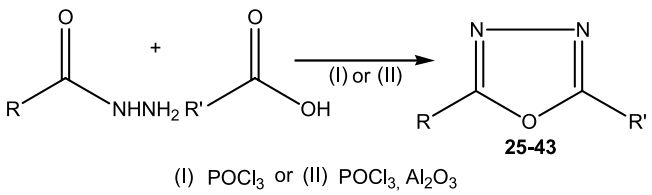
Compound	Substitutions			IC_{50} (in μM)
	R_1	R_2	R_3	
19	$-CH_3$	– H	– H	333.2
20	$-CH_3$	$-CH_2CH_3$	– H	384.3
21	$-CH_3$	$-CH_3$	$-CH_3$	> 500
22	– H	– H	– H	478.1
23	– Cl	– H	– H	498.0
24	$-OCH_3$	– H	– H	> 500

hibition against tyrosinase [46]. The structure–activity relationships of the 1,3-selenazol-4-one derivatives **19–24** are shown in Table 1.

3.5 Oxadiazole Derivatives

Khan et al. (2005) performed and reported tyrosinase inhibition studies of a combinatorial library of 2,5-disubstituted-1,3,4-oxadiazoles (**25–43**) [47]. The library of oxadiazoles was synthesized under microwave irradiation [47]. The synthetic steps involved for these compounds are shown in Scheme 2. Among the compounds from the library, **29** (30-[5-(40-bromophenyl)-1,3,4-oxadiazol-2-yl]pyridine, for structure see Fig. 6) exhibited the most potent (IC_{50} = 2.18 μM) inhibition against tyrosinase, which has found to be more potent than the standard potent inhibitor L-mimosine (IC_{50} = 3.68 μM, for structure see Fig. 1) [47].

Table 2 shows the structure–activity relationships of the compound library of the 2,5-disubstituted 1,3,4-oxadiazoles **25–43** against the enzyme



Scheme 2 The steps involved in the synthetic process of the library of 2,5-disubstituted 1,3,4-oxadiazoles **25–43** [47]

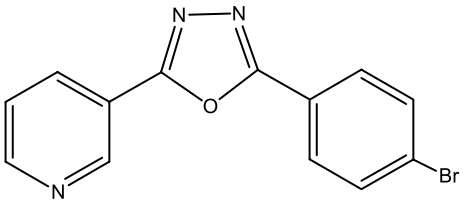


Fig. 6 Structural features of compound **29** (30-[5-(40-bromophenyl)-1,3,4-oxadiazol-2-yl]pyridine) [47]

Table 2 The structure–activity relationships of the compound library of 2,5-disubstituted 1,3,4-oxadiazoles (**25–43**) [47]

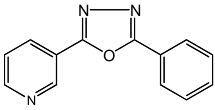
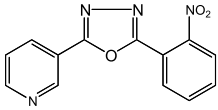
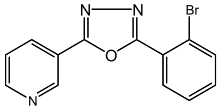
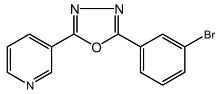
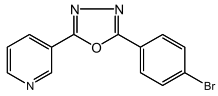
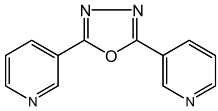
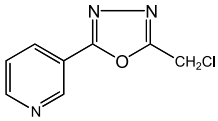
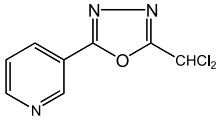
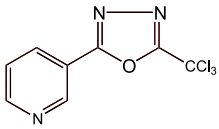
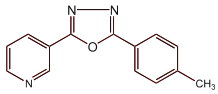
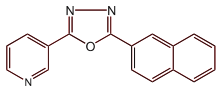
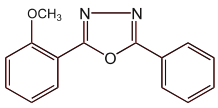
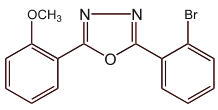
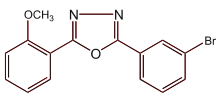
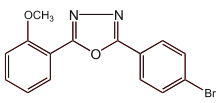
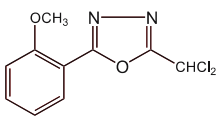
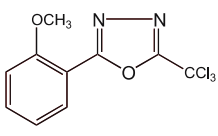
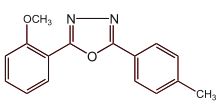
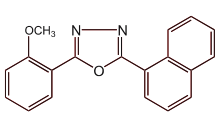
Compound	R′	Structure of the compound	IC_{50} (in μM)
25	C_6H_5		5.15
26	*o*-$NO_2C_6H_4$		3.18
27	*o*-BrC_6H_4		5.23
28	*m*-BrC_6H_4		6.04
29	*p*-BrC_6H_4		2.18
30	3-Pyridinyl		3.29
31	CH_2Cl		4.18
32	$CHCl_2$		4.01

Table 2 (continued)

Compound	R′	Structure of the compound	IC_{50} (in μM)
33	CCl_3		3.98
34	*p*-$CH_3C_6H_4$		10.40
35	1-$C_{10}H_7$		3.23
36	C_6H_5		8.71
37	*o*-BrC_6H_4		5.16
38	*m*-BrC_6H_4		7.18
39	*p*-BrC_6H_4		7.82
40	$CHCl_2$		7.28
41	CCl_3		6.21
42	*p*-$CH_3C_6H_4$		6.43
43	2$C_{10}H_7$		7.81

tyrosinase. The authors deduced that for a better inhibition, electronegative substitution is essential, as most probably the active site(s) of the enzyme contains some hydrophobic site and the position of the substitution also plays a very important role in inhibition, maybe due to the conformational space. The electronegativities of the compounds have been found to be proportional to the inhibitory activity [47].

3.6 Diterpenoid Alkaloids

Shaheen et al. (2005) reported lycoctonine-type norditerpenoid alkaloids isolated from the aerial parts of *Aconitum laeve* Royle, swatinine, delphatine, lappaconitine, puberanine, and *N*-acetylsepaconitine [48]. They performed and reported the anti-inflammatory, antioxidant, and tyrosinase inhibition studies of all these compounds, in which lappaconitine (IC_{50} = 93.33 μM) and puberanine (IC_{50} = 205.21 μM) were found to be active against the enzyme tyrosinase [48].

In another report Sultankhodzhaev et al. discussed the tyrosinase inhibitory potentials and structure–activity relationships of 15 diterpenoid alkaloids with the lycoctonine skeleton, and their semisynthetic derivatives [49]. At least three of them, lappaconitine hydrobromide (**44**, IC_{50} = 13.3 μM), methyllycaconitine perchlorate (**45**, IC_{50} = 477.84 μM), and aconine (**46**, IC_{50} = 220.7 μM), were found to be active against tyrosinase [49]. Their structures are shown in Fig. 7.

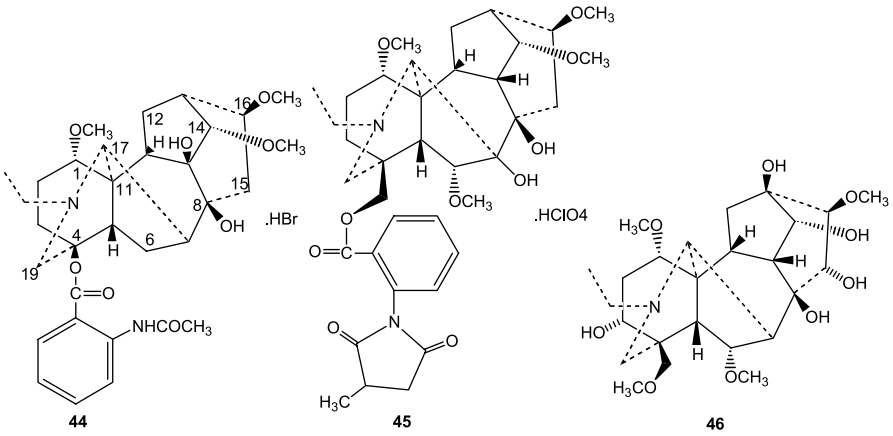


Fig. 7 Structures of the tyrosinase inhibitory diterpenoid alkaloids: lappaconitine hydrobromide (**44**), methyllycaconitine perchlorate (**45**), and aconine (**46**) [49]

3.7
Napelline-Type Alkaloids

Sultankhodzhaev et al. reported the tyrosinase inhibitory pattern of six napelline-type alkaloids [49], and only two compounds exhibited some inhibition against tyrosinase. Napelline (**47**) exhibited moderate inhibition (IC_{50} = 167.66 μM) of the enzyme. The presence of a benzoyloxy group at C-1, as in compound **48** (1-*O*-benzoylnapelline), maybe potentiated the inhibitory activity (IC_{50} = 33.10 μM) against tyrosinase [49]. It is interesting to note that free hydroxyl groups at C-12 and C-15 were crucial for better potency. Acylation at C-12 and C-15 of other compounds (not shown here), or replacement of the hydroxy function (not shown here), results in total inactivation against tyrosinase [49]. The structures of the compounds are shown Fig. 8.

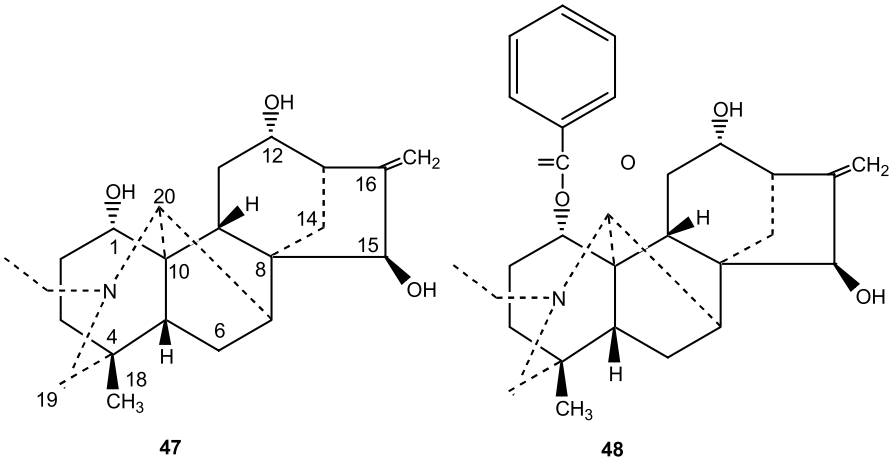


Fig. 8 Structures of the two tyrosinase inhibitory napelline-type alkaloids (napelline, **47**, and 1-*O*-benzoylnapelline, **48**) [49]. The structures of the inactive compounds are not shown

Recently (2006), the quantitative structure–activity relationship (QSAR) modeling of the same compounds based on their atomic linear indices, for finding functions that discriminate between the tyrosinase inhibitor compounds and inactive ones, has been reported by the same group [50].

3.8
Coumarinolignoids

In 2004, Ahmad and coworkers reported a new coumarinolignoid, 8′-epicleomiscosin A (**49**), together with the new glycoside 8-*O*-*β*-D-glucopyranosyl-6-hydroxy-2-methyl-4*H*-1-benzopyran-4-one (**50**), isolated from the aerial

parts of *Rhododendron collettianum* [51]. These authors also reported the isolation of cleomiscosin A (**51**), aquillochin (**52**), and 5,6,7-trimethoxycoumarin (**53**) from the same plant. Their structures are shown in Fig. 9.

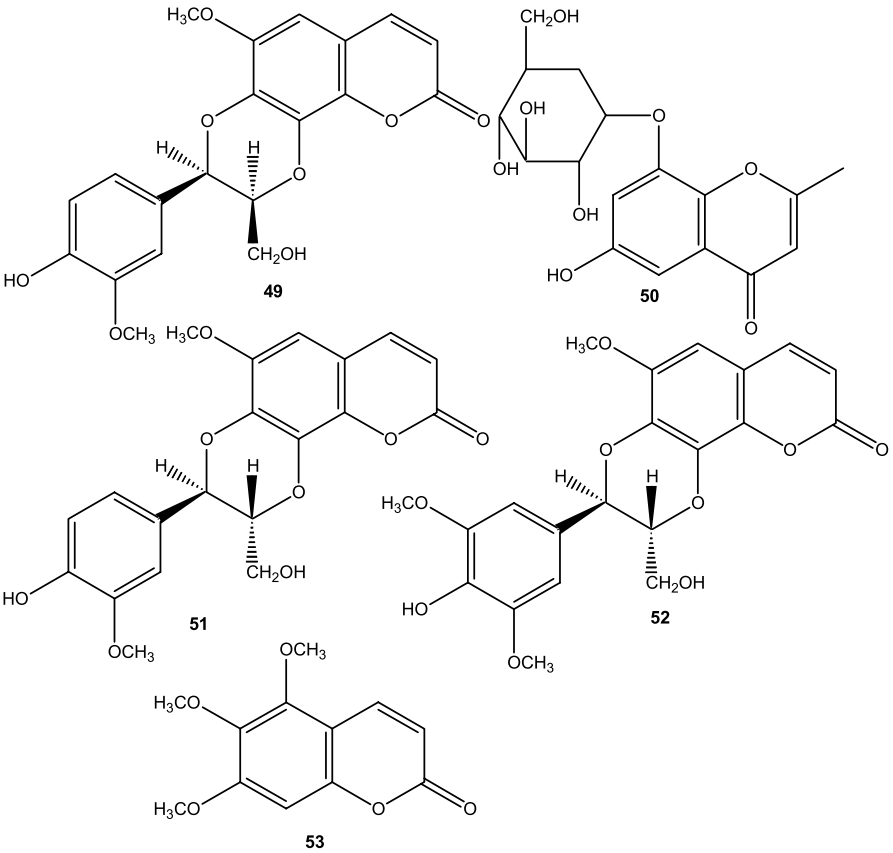


Fig. 9 Molecular structures of 8′-epi-cleomiscosin A (**49**), 8-*O*-*β*-D-glucopyranosyl-6-hydroxy-2-methyl-4*H*-1-benzopyran-4-one (**50**), cleomiscosin A (**51**), aquillochin (**52**), and 5,6,7-trimethoxycoumarin (**53**), isolated from the aerial parts of *Rhododendron collettianum* [51]

Tyrosinase inhibition studies of the same compounds and their structure–activity relationships have also been investigated and reported. The compounds exhibited potent to mild inhibition activity against the enzyme. Especially, compound **49** showed strong inhibition (IC_{50} = 1.33 μM) against the enzyme tyrosinase, as compared to the standard tyrosinase inhibitors kojic acid (IC_{50} = 16.67 μM) and L-mimosine (IC_{50} = 3.68 μM), indicating its potential use for the treatment of hyperpigmentation associated with the overexpression of melanocytes.

3.9 Lignans

Recently, Haq and coworkers (2006) isolated eight lignans from the methanol extract of *Vitex negundo* [52]: negundin A (**54**), negundin B (**55**), 6-hydroxy-4-(4-hydroxy-3-methoxy)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde (**56**), vitrofolal E (**57**), (+)-lyoniresinol (**58**), (+)-lyoniresinol-3α-*O*-β-D-glucoside (**59**), (+)-(–)-pinoresinol (**60**), and (+)-diasyringaresinol (**61**). The structures of these compounds (shown in Fig. 10) were elucidated unambiguously by spectroscopic methods including 1D and 2D NMR analysis, and also by comparing experimental results with literature data [52].

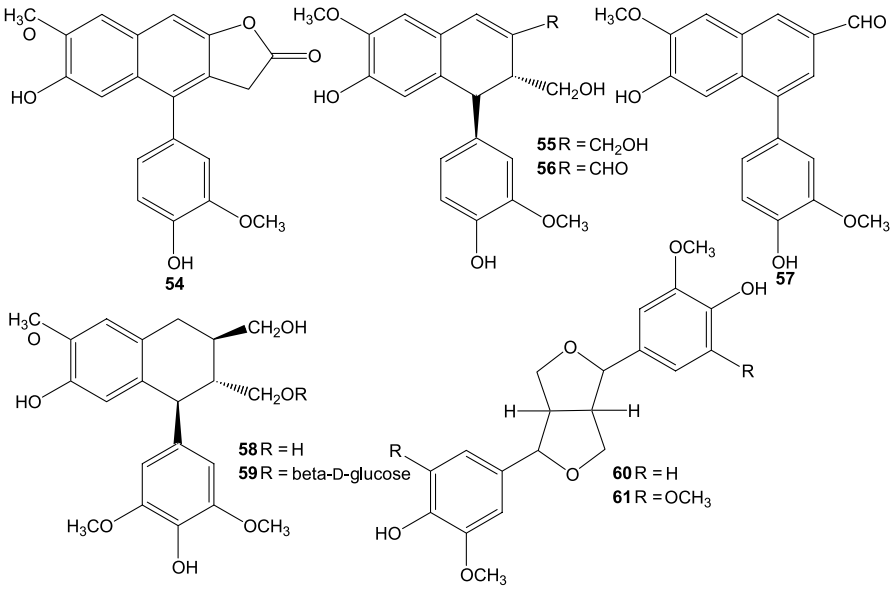


Fig. 10 Structures of the lignans (**54–61**) reported by Haq and coworkers [52]

The tyrosinase inhibitory potency of these compounds was also evaluated by the authors and attempts were made to justify their structure–activity relationships [52]. Their inhibitory potential is shown in Table 3. Compound **58** was found to be the most potent (IC_{50} = 3.21 μM), while other compounds demonstrated moderate to potent inhibitions [52]. It was reported that the substitution of functional group(s) at the C-2 and C-3 positions and the presence of the – CH_2OH group play a vital role in the potency of these compounds. Compound **58** can act as a potential lead molecule to develop new drugs for the treatment of hyperpigmentation associated with the high production of melanocytes [52].

Table 3 The structure–activity relationships of the lignans reported by Haq and coworkers against the enzyme tyrosinase [52]

Lignans	IC_{50} (in μM)
54	10.06
55	6.72
56	7.81
57	9.76
58	3.21
59	Not active
60	15.13
61	5.61

4 Conclusion

Most of the above mentioned research results suggest that there is extensive ongoing research work on mushroom tyrosinase. This is due to the vast range of clinical and industrial applications and also to the easy availability of the enzyme. To accomplish these targets, diverse molecules from both natural and synthetic sources have been investigated in the last few decades. Perceptibly, much more endeavor is still desirable in this direction for the discovery of better and potent inhibitors. Overall, much more research work on mushroom tyrosinase is required to find the role of this enzyme in other unknown fields, which will be accommodating in designing or improving enzymatic activities for various applications [9].

Besides being used in the treatment of some dermatological disorders associated with melanin hyperpigmentation, tyrosinase inhibitors have found an important role in the cosmetics industry for their skin whitening effect and depigmentation after sunburn [5, 37, 41, 53–70]. However, more tangible research work with human tyrosinase is essential from a clinical point of view. An additional important clinical application of mushroom tyrosinase includes its role in the treatment of vitiligo, as the enzyme acts as the marker of this disease [65, 71, 72]. A number of studies have been conducted on animal models, but still more research has to be done to cure vitiligo in human beings [9].

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