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REVIEW ARTICLE

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Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors

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ABSTRACT

Melanogenesis is a process to synthesize melanin, which is a primary responsible for the pigmentation of human skin, eye and hair. Although numerous enzymatic catalyzed and chemical reactions are involved in melanogenesis process, the enzymes such as tyrosinase and tyrosinase-related protein-1 (TRP-1) and TRP-2 played a major role in melanin synthesis. Specifically, tyrosinase is a key enzyme, which catalyzes a rate-limiting step of the melanin synthesis, and the downregulation of tyrosinase is the most prominent approach for the development of melanogenesis inhibitors. Therefore, numerous inhibitors that target tyrosinase have been developed in recent years. The review focuses on the recent discovery of tyrosinase inhibitors that are directly involved in the inhibition of tyrosinase catalytic activity and functionality from all sources, including laboratory synthetic methods, natural products, virtual screening and structure-based molecular docking studies.

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Introduction

It is estimated approximately 15% of the world population invest in skin whitening agents with Asia is being dominated[1]. Global industry analysts (GIA) have predicted that the universal market for skin lighteners will reach $23 billion by 2020, driven by new markets in Asia, particularly India, Japan and China[2]. According to the SIRONA biochem report[1], approximately $13 billion spent on skin care products and cosmetics in Asia Pacific's. In India alone, it is estimated that $432 million was spent in 2010 on skin lightening creams and skin care agents. A recent survey showed that 80% of Indian men use fairness creams and the number of consumer's are growing 18% annually[1]. The molecular mechanism of these skin lightening agents are to reduce the melanin, which is the main source of skin color.

Melanin is primarily responsible for the pigmentation of human skin, eyes and skin, which is produced from epidermis melanocytes in an approximate ratio of 1:36 with basal keratinocytes[3]. In response to ultraviolet B (UVB)-irradiation, melanocyte synthesizes melanin through the process called melanogenesis. The synthesized melanin in melanosomes is transported to neighboring keratinocytes in epidermis[4]. Under normal physiological conditions, pigmentation has a beneficial effect on the photo-protection of human skin against harmful UV injury and plays an important evolutionary role in camouflage and animal mimicry[5]. However, an excessive production of melanin causes dermatological problems such as freckles, solar lentigo (age spots) and melasma[6–9], as well as cancer[10] and postinflammatory melanoderma[11]. In addition, continuous UV-irradiation can result in DNA damage, gene mutation, cancer development and impairment of the immune system or photoaging[12].

Regulation of melanogenesis

Melanogenesis is a complex pathway involving a combination of enzymatic and chemical catalyzed reactions. Melanocytes produce two types of melanin: eumelanin (brown-black) and pheomelanin (red-yellow) formed by the conjugation of cysteine or glutathione (Figure 1)[13–15].

The melanogenesis process is initiated with the oxidation of *L*-tyrosine to dopaquinone (DQ) by the key enzyme, tyrosinase (TYR). The resulting quinone will serve as a substrate for the synthesis of eumelanin and pheomelanin[16,17]. The formation of DQ is a rate-limiting step in the melanin synthesis because remainder of the reaction sequence can proceed spontaneously at a physiological pH value[17]. After DQ formation, it undergoes intramolecular cyclization to produce indoline, leukodopachrome (cyclodopa). The redox exchange between leukodopachrome and DQ give rise to dopachrome and *L*-3,4-dihydroxyphenylalanine (*L*-DOPA), which is also a substrate for TYR and oxidized to DQ again by the enzyme. Dopachrome gradually decomposes to give dihydroxyindole (DHI) and dihydroxyindole-2-carboxylicacid (DHICA). The later process is catalyzed by TRP-2, now known as dopachrome tautomerase (DCT). Ultimately, these dihydroxyindoles (DHI and DHICA) are oxidized to eumelanin. TRP-1 is believed to catalyze the oxidation of DHICA to eumelanin. Alongside, DQ is converted to 5-*S*-cysteinyldopa or glutothionyldopa in the presence of cysteine or glutathione. Subsequent oxidation gives benzothiazine intermediates and finally to produce pheomelanin. Although three enzymes, TYR, TRP-1 and TRP-2 are involved in the melanogenesis pathway, tyrosinase is exclusively necessary for melanogenesis.

Tyrosinase and its key role in melanin synthesis

Tyrosinase (monophenol or *o*-diphenol, oxygen oxidoreductase, EC 1.14.18.1, syn.polyphenol oxidase), a multifunctional membrane bound type-3 copper-containing glycoprotein is located in the membrane of the melanosome[18]. Tyrosinase is produced only by melanocyte cells. Following its production and consequent

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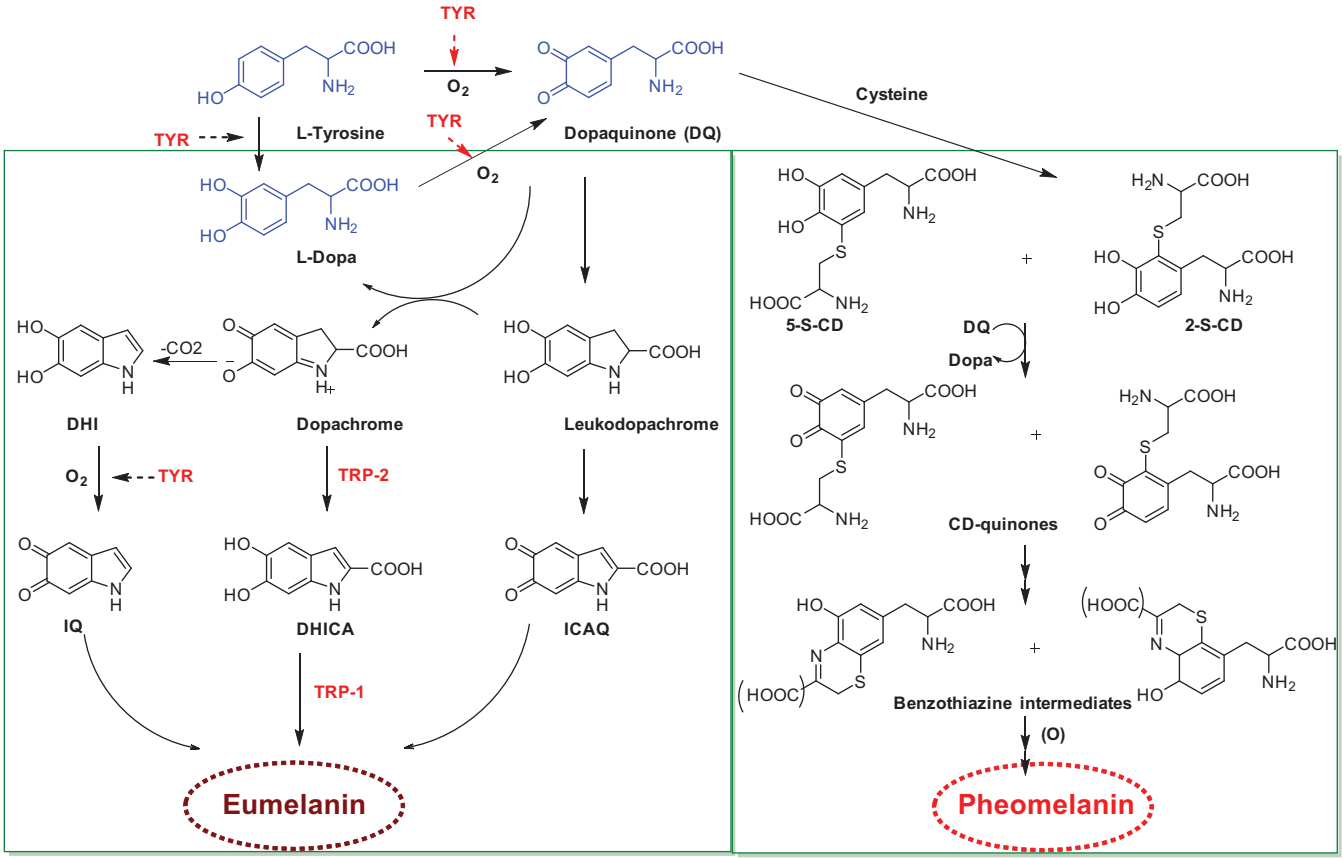


Figure 1. Melanogenesis pathway (production of eumelanin and pheomelanin)[13]. (Tyr: tyrosinase; DQ: dopaquinone; L-Dopa: L-3:4-dihydroxyphenylalanine; DHICA: 5,6-dihydroxyindole-2 carboxylic acid; DHI: 5,6-dihydroxyindole; ICAQ: indole-2-carboxylic acid-5,6-quinone; IQ: indole-5,6-quinone; HBTA: 5-hydroxy-1,4-benzothiazinylalanine).

processing in the endoplasmic reticulum and Golgi, it is trafficked to melanosomes, wherein the pigment melanin is synthesized. From the structural point of view, two copper ions are surrounded by three histidine residues that are responsible for the catalytic activity of tyrosinase (Figure 2)[19]. The active site has three states; oxy, met and deoxy forms in the formation of pigmentation. More specifically, at the active site, two copper ions interact with dioxygen to form a highly reactive chemical intermediate that directly participate in the hydroxylation of monophenols to diphenols (monophenolase activity) and in oxidation of *o*-diphenols to *o*-quinones (diphenolase activity)[20,21]. Tyrosinase is also catalyzing the process of neuromelanin production in which the oxidation of dopamine produces dopaquinones. However, excessive production of dopaquinones results in neuronal damage and cell death. This suggests that tyrosinase might play a significant role in neuromelanin formation in the human brain and responsible for the neurodegeneration associated with Parkinson's disease and Huntington's diseases[22–26]. Tyrosinase has also been linked in the browning of vegetables and fruits during postharvest and handing process, leading to quick degradation[27–29]. The application of tyrosinase was further extended in the molting process of insects[30]. Thus, the regulation of melanin synthesis by inhibiting the tyrosinase enzyme is a major motivation for researchers in the context of preventing hyperpigmentation.

Tyrosinase inhibitors

Since tyrosinase is a crucial enzyme in synthesizing melanin through melanogenesis, it becomes the most prominent and successful target for melanogenesis inhibitors that directly inhibit the tyrosinase catalytic activity. Most of cosmetics or skin lightening agents commercially available are tyrosinase inhibitors. The fact that the inhibitors target tyrosinase may specifically inhibit the melanogenesis in cells without side effects, as tyrosinase is exclusively produced only by melanocytes. Many tyrosinase inhibitors such as hydroquinone (HQ)[31–34], arbutin, kojic acid[35–37], azelaic acid[38,39], *L*-ascorbic acid[40–42], ellagic acid[43–45], tranexamic acid[46–48] have been used as skin-whitening agents, with certain drawbacks (Figure 3).

HQ is potentially mutagenic to mammalian cells and linked to a number of adverse reactions including contact dermatitis, irritation, transient erythema, burning, prickling sensation, leukoderma, chestnut spots on the nails, hypochromia and ochronosis[49–52]. Arbutin, a prodrug of hydroquinone, is a natural product and reduces or inhibits melanin synthesis by inhibiting tyrosinase. However, natural forms of arbutin are chemically unstable and can release hydroquinone which is catabolized to benzene metabolites with the potential toxicity for bone marrow[53]. Kojic acid use in cosmetics has been limited, due to its carcinogenicity and its instability during storage[54]. *L*-Ascorbic acid is sensitive to heat and degrades easily[55]. Ellagic acid is insoluble and thus poorly bioavailable[56], and for the tranexamic acid the melanogenic pathway remains undetermined[47]. Thus, it is in great need of developing new tyrosinase inhibitors with drug-like properties.

The review focuses on the recent discovery of tyrosinase inhibitors that are directly involved in the inhibition of tyrosinase catalytic activity and functionality from all sources, including laboratory synthetic methods, natural products, virtual screening and structure-based molecular docking studies. In the present review, the inhibitors were mainly categorized into two parts,

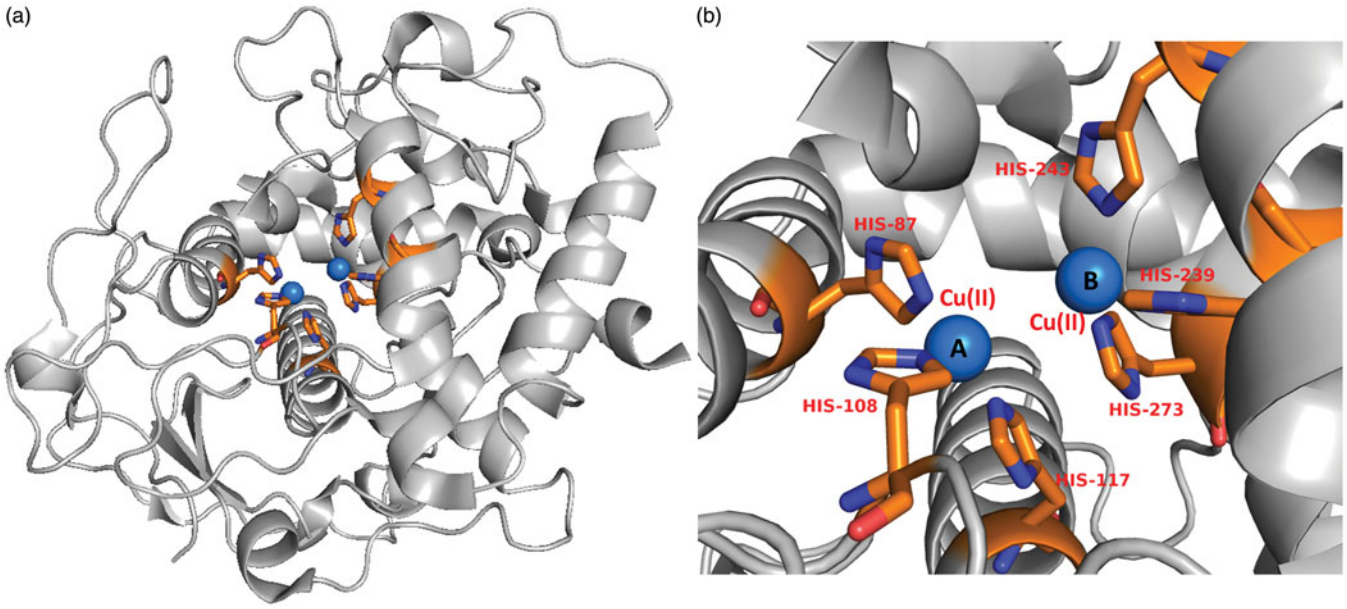


Figure 2. (a) A recent high resolution (1.8 Å) crystal structure of a plant tyrosinase (PDB ID: 5CE9, walnut leaves, *Juglans regia*)[19]. (b) The active site of the enzyme contains two copper ions A and B which are coordinated by six histidine residues His87, His108, His117 for CuII (A) and His239, His243, His273 for CuII(B), respectively and represented in stick model.

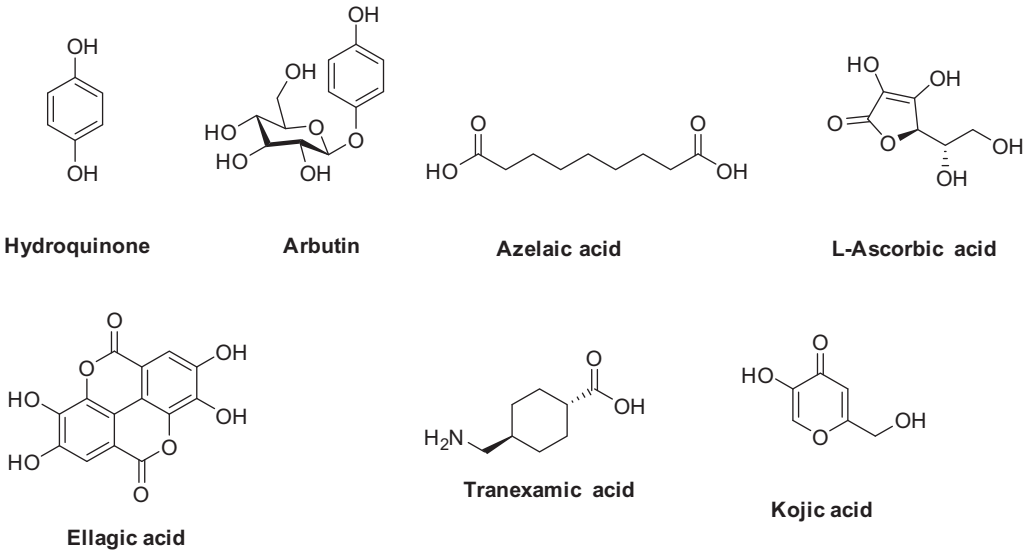


Figure 3. Chemical structure of well-known tyrosinase inhibitors as skin lightening agents.

mushroom and human tyrosinase inhibitors and further classified on the basis of their chemical functionalities (Figure 4). We believe that this perspective will comprise a cumulative source of melanogenesis inhibitors for researchers and further understanding of anti-melanogenesis chemotherapy.

Mushroom tyrosinase inhibitors

Tyrosinase from the mushroom *Agaricus bisporus* is frequently used as an enzymatic *in vitro* model for developing the skin-whitening substances targeting human tyrosinase. Because of the commercial availability of mushroom tyrosinase (mTAR), most of the research has been studied with this enzyme. For screening of the compounds, the popular whitening agents, such as kojic acid, arbutin or hydroquinone, were used as a positive control. As a result, in recent years, numerous mushroom tyrosinase inhibitors from natural and synthetic sources have been identified. The inhibitory strength was expressed as IC_{50} value, which is the concentration of the inhibitor needed to inhibit half of the enzyme activity in the tested condition. The K_i value is the reflective of ligand-binding affinity to the enzyme. The lower K_i value means higher binding affinity, whereas higher K_i value means lower binding affinity. The K_i value for noncompetitive inhibitors is essentially the same numerical value as the IC_{50} of the inhibitors, whereas for competitive inhibitors, the K_i is about one-half that of the numerical values of IC_{50}. For *in vitro* analysis, B16 cells were usually used because they are relatively easy to culture *in vitro* and shares the melanogenesis mechanisms of normal human melanocytes.

Chalcones and flavanone inhibitors

Chalcones belong to the class of natural products widely distributed in fruits, vegetables, spices, tea and soya-based foodstuff and

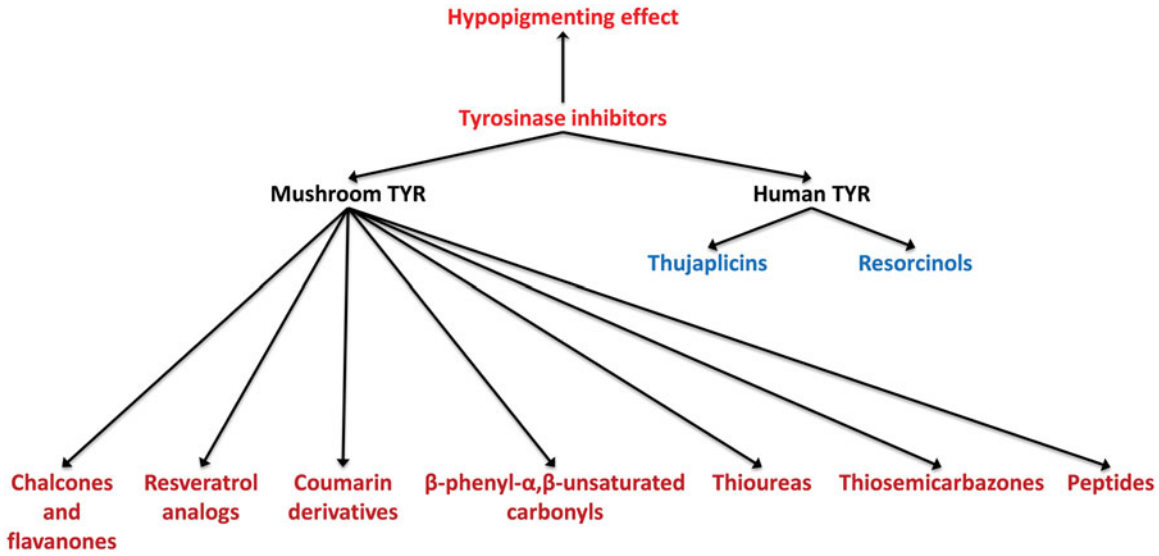


Figure 4. Chemical classification chart of tyrosinases (mushroom and human) inhibitors.

exhibited inhibitory effect on tyrosinase with varying biological activities. A new series of 4-(phenylurenyl)chalcone (**1**) derivatives were synthesized and their inhibitory effects on the diphenolase activity of banana tyrosinase were evaluated (Figure 5(a), **1a**–**1j**)[57]. The results showed that compounds **1a**–**1j** inhibit the tyrosinase enzyme and in particular **1e** and **1i** were effectively inhibited with IC_{50} values of 0.133 and 0.134 μM, respectively. Furthermore, the compounds were identified as competitive inhibitors.

In another study, chalcones **2a**–**2d** isolated from *Morus australis* were evaluated for their inhibitory activity on mushroom tyrosinase using *L*-tyrosine as the substrate[58]. The results showed that all the four chalcone derivatives were extremely potent inhibitors for tyrosinase in comparison to the standard compound arbutin (Figure 5(a), **2a–2d**). Especially, compound **2a** was 700-fold potent inhibition in comparison to arbutin. The structure–activity relationship (SAR) analysis showed that resorcinol construction at both ring A and ring B could be the reason for strong tyrosinase inhibition. In addition, the substitution at 3′ position of ring A plays an important role in enhancing the inhibitory potency. For example, the steric bulky substituent at ring A on **2c** reduced the potency compared to the unsubstituted **2a**, this is due to chelating ability towards the copper ions. Interestingly, compound **2d** exhibited stronger inhibitory activity than **2a** and **2c**, indicate that 4-hydroxy-1-pentene group at 3′-position of **2d** is responsible for the enhancement. Further, the effects of chalcones on melanin synthesis were tested in melanin-producing B16 murine melanoma cells and compounds **2a**, **2b** and **2d** were strongly inhibited with little or no cytotoxicity.

Recently, Radhakrishnan et al., reported a library of azachalcones and[59] the inhibitory effects on mushroom tyrosinase using *L*-DOPA as substrate was investigated. Among the investigated compounds in the study, two compounds **3a** and **3b,** congeners (carbonyl reduction) of the pyridyl azachalcones were found to be potent enzyme inhibitors than kojic acid (IC_{50} = 27.30 μM) (Figure 5(a), **3a**–**3b**). Furthermore, kinetic analysis identified both **3a** and **3b** were competitive inhibitors with K_i values of 2.62 and 8.10 μM, respectively. The structure–function analysis showed that the nitrogen atom in the pyridine skeleton of the inhibitors could complex with the copper ions present in the tyrosinase active site. The same research group has reported another chalcone series with oxime functionality as inhibitors of tyrosinase and melanin formation in melanoma B16 cells.[60] Two of the compounds (**4a**: IC_{50} = 4.77 μM and **4b**: IC_{50} = 7.89 μM) exhibited potent tyrosinase inhibitory activities (Figure 5(a), **4a**–**4b**) than the kojic acid (IC_{50} = 22.25 μM). Kinetic studies revealed as competitive inhibitors with K_i values of 5.25 and 8.33 μM. In α-melanocyte-stimulating hormone (α-MSH) induced B16 melanoma cells, these two compounds **4a** and **4b** inhibited the melanin formation and tyrosinase without cytotoxicity. In terms of SAR analysis, it was identified that the presence of *ortho*-methoxy with para-nitro substituents (ring B) were responsible for potent tyrosinase inhibition (**4a**). In addition, an electron donating para-dimethyl amino ring (ring B) exhibited the second most potent inhibition (**4b**).

In another study, a novel series of 2,3-dihydro-1*H*-inden-1-one chalcone-like derivatives were reported.[61] Two of them, **5a** and **5b**, were identified as potent inhibitors of diphenolase activity of tyrosinase with IC_{50} values of 12.3 and 8.2 μM (Figure 5(a)). Further exploration of the mechanism, both the inhibitors **5a** and **5b** were found to be reversible and competitive.

Wang et al. isolated dihydrochalcones (Figure 5(a), **6a**–**6c**) and flavanones (Figure 5(b), **7a**–**7c**) from *Flemingia philippinensis* and investigated for their inhibitory activities on tyrosinase[62]. The results showed that they inhibit the monophenolase (IC_{50} = 1.01 to 18.4 μM) and diphenolase (IC_{50} = 5.22 to 84.1 μM) actions of tyrosinase. In particular, dihydrochalcone (**6c**) effectively inhibited both monophenolase and diphenolase activities of tyrosinase with IC_{50} values of 1.28 and 5.22 μM, respectively. The SAR analysis is very interesting because the pharmacophore is not associated with tyrosinase inhibition and it lacks the α,β-unsaturated ketone motif which present in most of the inhibitors. In the case of flavanones, compound containing resorcinol group (**7a**) were competitive and significantly inhibited monophenolase (IC_{50} = 1.79 μM) and diphenolase (IC_{50} = 7.48 μM) of tyrosinase.

In search of new tyrosinase inhibitors, it was found that the extracts of *Camylotropis hirtella* show tyrosinase inhibition[63]. After successful purification and isolation of fourteen compounds, four compounds (Figure 5(b), **8a**–**8d**) showed potent inhibitory activities against tyrosinase. The most potent compound was found to be neorauflavane **8c** exhibiting IC_{50} values of 30 and 500 nM against monophenolase and diphenolase activity of tyrosinase. Furthermore, comparing with kojic acid (13.2 μM), **8c** was 400-fold more potent against monophenolase activity of tyrosinase. The second most potent compound was geranylated isoflavanone **8a** inhibited monophenolase and diphenolase with IC_{50} values 2.9

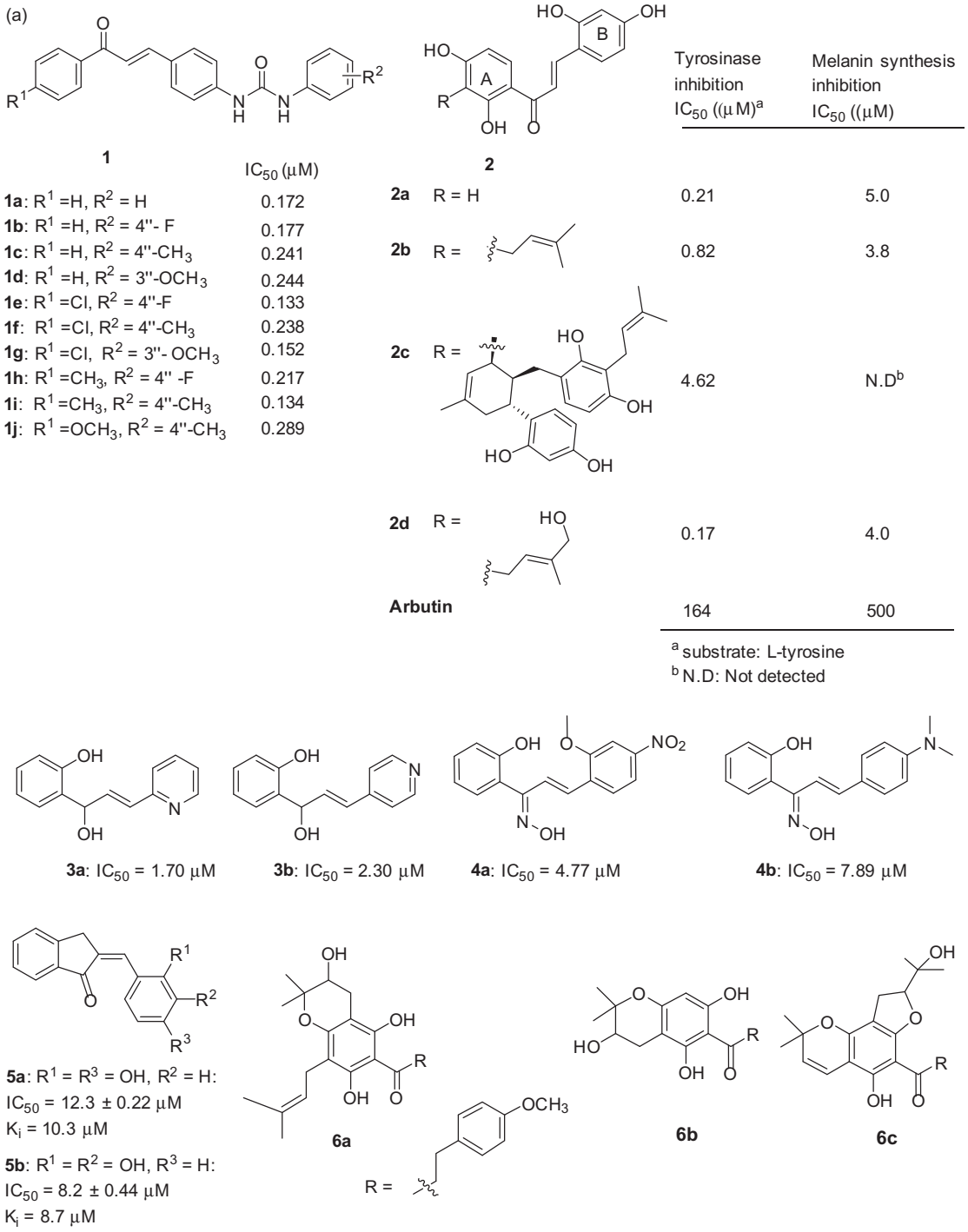


Figure 5. Chemical structure of chalcones, **1a–1j**,[57] **2a–2d**,[58] **3a–3b**,[59] **4a–4b**,[60] **5a–5b**[61] and **6a–6c**[62] (a) and flavanone inhibitors, **7a–7c**[62] and **8a–8d**[63] (b).

and 128.2 μM, respectively, and identified as a competitive and reversible inhibitor. In addition, compounds **8a** and **8c** efficiently reduced the melanin content in α-MHS-induced B16 melanoma cells, without influencing cell viability. From the structural point of view, reduction of geranyl side chain improves the tyrosinase inhibitory activity.

Resveratrol analogs

Resveratrol (3,5,4′-trihydroxy-trans-stilbene, **9**) a widely distributed stilbenoid in nature such as in grapes, exhibited the inhibitory activity against mushroom tyrosinase through the mechanism of K_{cat} (suicide substrate) type inhibition[64]. *In vitro* analysis in α-MSH-stimulated B16 murine melanoma cells, resveratrol inhibited the cellular melanin production *via* suppression of melanogenesis-related proteins such as tyrosinase, TRP-1, TRP-2 and microphthalmia-associated transcription factor (MITF) expression[65] without any cytotoxicity up to 200 μM.[64] The inhibitory effects of resveratrol have been confirmed in an *in vivo* model using UVB-irradiated brownish guinea pigs. In this study, treatment of resveratrol with UVB-irradiated dorsal skin of guinea pigs visually decreased the hyperpigmentation.

In an effort to improve the activity of resveratrol, Fenco et al., demonstrated a study with a series of resveratrol analogs

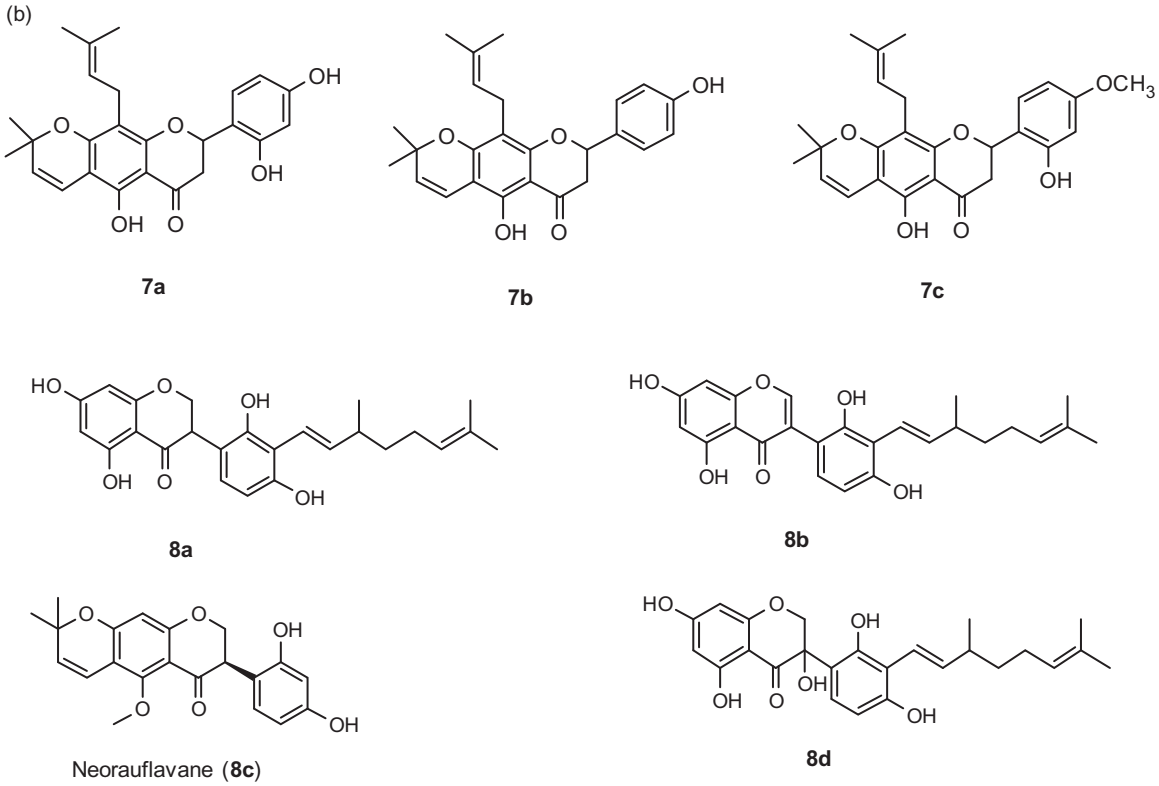


Figure 5. Continued.

(Figure 6, **10a**–**10f**), where one of -CH group was replaced with nitrogen atom[66]. Among the tested analogs, compounds with 4-methoxy (**10a**), 4-hydroxy (**10d**) or 2-hydroxy (**10e**) substitutions show higher potent inhibition, suggest that changes to the basic resveratrol structure by a nitrogen atom resulted an increase in tyrosinase inhibitory effects. Moreover, the hydroxyl group at *para* position (**10d**) was found to be important for the potency. In another study, Bae et al designed, synthesized and evaluated a series of (*E*)-*N*-substituted benzylidene-hydroxy or methoxy-aniline derivatives (aza-resveratrol type) for their inhibitory effect on tyrosinase activity (Figure 6, **11a**–**11e**)[67]. Derivatives with a 4-methoxy- or 4-hydroxy-anilino group (**11a–11d)** showed more potent inhibition against mushroom tyrosinase than 2-hydroxyanilino group **11e**. (*E*)-4-((4-Hydroxyphenylimino)methyl)benzene-1,2-diol **11c** exhibited the most potent and noncompetitive inhibition on mushroom tyrosinase showing an IC_{50} value of 17.22 μM and effective than kojic acid (51.11 μM). Compound **11a** and **11c**–**11e** were identified to be as noncompetitive inhibitors, whereas compound **11b** as a competitive inhibitor. The study was further extended by synthesizing a series of (*E*)-2-((substitutedphenyl)diazenyl)phenyl-4-methylbenzenesulfonate (**12a**–**b**) and (E)-2-((substituted phenyl)diazenyl)phenol derivatives (**12c**–**d**) (Figure 6) and most of the compounds were strongly inhibit the tyrosinase in a dose-dependent manner. In particular, the novel compound, (*E*)-2-((2,4-dihydroxyphenyl)diazenyl)phenyl-4-methylbenzenesulfonate (**12b**) showed the best result with an IC_{50} of 17.85 μM[68]. Lineweaver–Burk plot assay using L-tyrosine as substrate indicated **12b** as a competitive inhibitor. Moreover, the compounds **12a**–**12d** inhibited cellular tyrosinase activity and melanin synthesis in in murine B16F10 melanoma cells.

Recently, a series of azo-resveratrol (**13a**–**13e** and **13 g**) and azo-oxyresveratrol (**13f**) were reported (Figure 6)[69]. Among these compounds, **13a** and **13b** exhibited high tyrosinase inhibitory activity of 56.25% and 72.75% at 50 μM, respectively[69]. The 4-hydroxyphenyl moiety was found to be essential for higher inhibition and 3,5-dihydroxyphenyl or 3,5-dimethoxyphenyl derivatives showed better tyrosinase inhibition than 2,5-dimethoxyphenyl derivatives. Particularly, introduction of hydroxyl or methoxy group into the 4-hydroxyphenyl moiety diminished or significantly reduced mushroom tyrosinase inhibition. Among the synthesized azo compounds, azo-resveratrol (**13b**) was the most potent mushroom tyrosinase inhibition with an IC_{50} value of 36.28 μM. The results indicate that azo-resveratrol with high Log *p* value might be superior to resveratrol for the development of whitening agents and pharmaceutical drugs in the treatment of hyperpigmentation.

Coumarin derivatives

Coumarins are large family of benzopyrone compounds available from natural and synthetic origins with different pharmacological activities[70]. In recent studies, few coumarins proved to inhibit the mushroom tyrosinase, which includes esculetin and umbelliferone with stronger tyrosinase inhibitory activity[71,72]. In a continuous effort, Matos et al., have demonstrated a series of coumarin-resveratrol hybrid compounds, 3-phenyl coumarins with hydroxyl or alkoxy and bromo substituent at various positions in the scaffold[73] (Figure 7). Among the series, compound with bromo atom and two hydroxyl groups in the 3-phenylcoumarin moiety (**14**), was identified as a best inhibitor with an IC_{50} value of 215 μM. This compound is a noncompetitive tyrosinase inhibitor with a K_i value of 0.189 mM.

In another study, a series of umbelliferone analogs were reported for their inhibitory effects on mushroom tyrosinase[74]. Specifically, compounds **15a** and **15b** possessing 3,4-dihydroxy and 3,4,5-dihydroxy phenyl scaffold showed more potent inhibitory activities against mushroom tyrosinase activity (Figure 7).

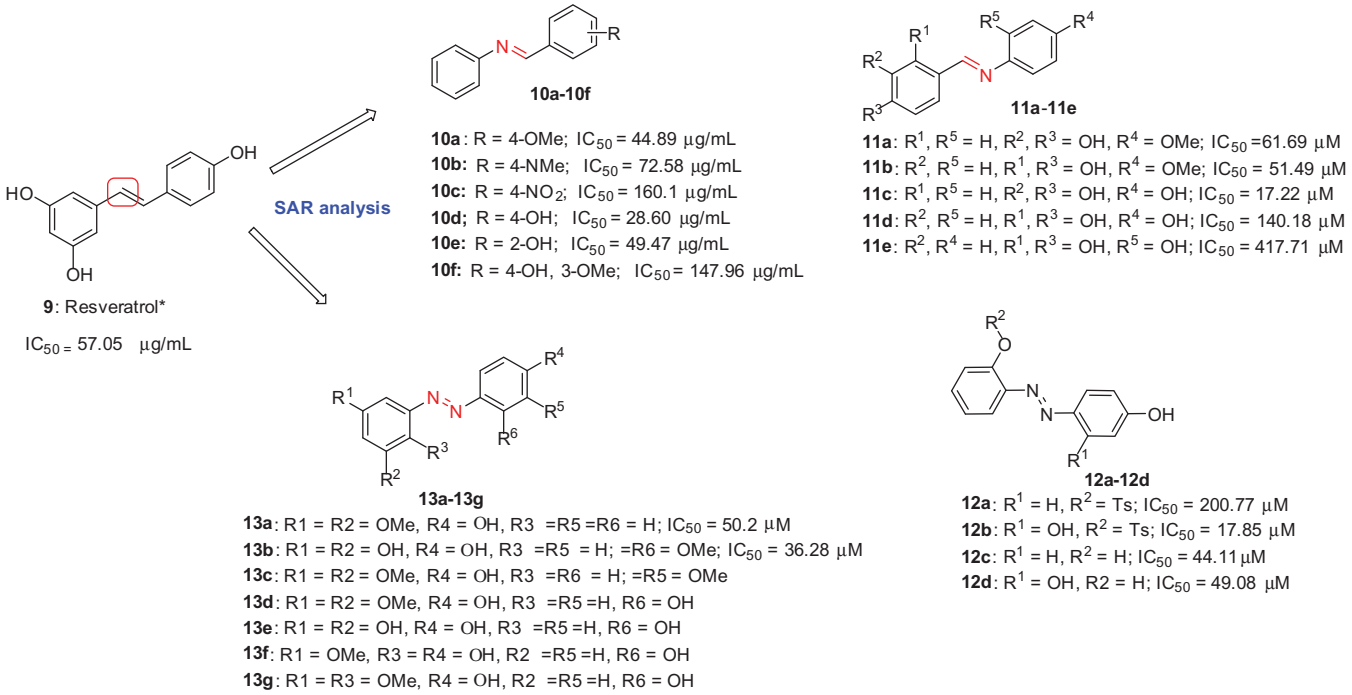


Figure 6. Resveratrol[64] and its analogs, **10a–10f**,[66] **11a–11e**,[67] **12a–12d**[68] and **13a–13g**[69]. *The IC_{50} value of resveratrol is 26.63 ± 0.55 µM[65] and 57.05 µg/mL[66] according to the references 65 and 66.

Asthana et al., demonstrated a series of hydroxycoumarins (**16a**–**16d**)[75] (Figure 7). The SAR studies suggested that the position of hydroxyl substituent on the coumarin plays a role in enzymatic inhibition; the compounds with aromatic hydroxylated, 6-hydroxycoumarin (**16c**) and 7-hydroxycoumarin (**16d**), found to be weak substrates of the enzyme. Especially, 7-hydroxycoumarin strongly inhibited the dopachrome concentration in a range of 0.3 – 0.9 mM. At a maximum 7-hydroxycoumarin concentration, the inhibition reached 88%. The authors found the phenomenon was due to the specific inhibition of L-tyrosine conversion. On the other hand, the compounds with pyrone hydroxylated, 3-hydroxycoumarin (**16a**) and 4-hydroxycoumarin (**16b**) were not substrates of tyrosinase. 3-hydroxycoumarin (**16a**) was found to inhibit tyrosinase but not the compound with 4-hydroxycoumarin (**16b**), indicate the pyrone ring cannot be hydroxylated by tyrosinase.

Recently, a series of thiophosphonic acid diamides were screened for their tyrosinase inhibition activity[76]. The results showed that the substituent attached to C-5 and stereochemistry of the two stereogenic centers (C-5 and phosphorus atom) were important for the tyrosinase inhibition (**17a**–**17d**). Diastereomers with unsubstituted phenyl did not show any inhibitory activity against tyrosinase (**17a** and **17a′**). In contrast, compounds with a substituted phenyl showed various effects on tyrosinase activity, for example compound **17b** with p-chlorophenyl (80.65% tyrosinase inhibition) moderately inhibited the tyrosinase but its diastereomer **17b′** 16.5% tyrosinase inhibition) was inactive. In another case, **17c** (58.54% tyrosinase inhibition) and **17c′** contain *p*-methylphenyl (61.80% tyrosinase inhibition) exhibited good tyrosinase inhibition. Compound **17d** consist of a 2-pyridinyl (97.40% tyrosinase inhibition) fragment was found to be the most potent tyrosinase inhibitor of the above study.

Inhibitors with β-phenyl-α,β-unsaturated carbonyl functionality

Recently, it was reported that benzylidenehydantoin **18a**, benzylidenepyrrolidinedione **18b**, and benzylidenethiazolidine-2,4-dione **18c** (Figure 8(a)) derivatives as potential tyrosinase inhibitors and using *in vivo* studies the compounds were proved to be an effective skin whitening agents[77–80]. They exhibited strong inhibitory activities than kojic acid and arbutin. In fact, the compound **18a** was designed by mimicking the chemical structure of the *L*-tyrosine and *L*-DOPA, tyrosinase substrates. The SAR studies revealed that the amide NH at 1-position of hydantoin **18a** has the ability to form hydrogen bonds with amino acids at the active site of tyrosinase. Furthermore, the imido group of **18a** mimics the carboxylic acid group of the substrates. On the basis of this background, Kim et al. recently synthesized and evaluated a series of 5-(hydroxyl- or alkoxysubstituted benzylidene)thiohydantoin analogs possessing β-phenyl-α,β-unsaturated carbonyl scaffolds.[81] Among them, three compounds, **19a**–**19c**, exhibited high inhibitory activities than kojic acid or resveratrol (Figure 8(a)). Especially, 2,4-dihydroxybenzylidene-2-thiohydantoin **19c** (IC_{50} = 1.07 µM) was found to be the best inhibitor of this study. In addition, **19c** inhibited the cellular tyrosinase activity in B16 cells without any significant cytotoxicity.

In a continuation, (*E*)-2-benzoyl-3-(substituted phenyl)acrylonitriles (BPA analogs) with a linear β-phenyl-α,β-unsaturated carbonyl scaffold were synthesized and evaluated as potential tyrosinase inhibitors[82]. Among them, three compounds **20a**–**20c** effectively inhibited the mushroom tyrosinase activity (Figure 8(a)). Especially, compound **20c** significantly suppressed the melanin biosynthesis and inhibited intracellular tyrosinase activity in B16 cells without influencing the cell viability. The SAR analysis revealed that all active compounds have 4-hydroxy group on the phenyl ring, and substitution of Br at 3-position or at 3 and 5-position were found to be associated with potent tyrosinase inhibitory activity.

Recently, the same research group continued to explore the SAR of 3-(substituted phenyl)acrylonitriles. Accordingly, a series of (*E*)-2-cyano-3-(substituted phenyl)acrylamide derivatives possessing a linear β-phenyl-α,β-unsaturated carbonyl scaffold showed inhibitory activity against mushroom tyrosinase[83]. Among the

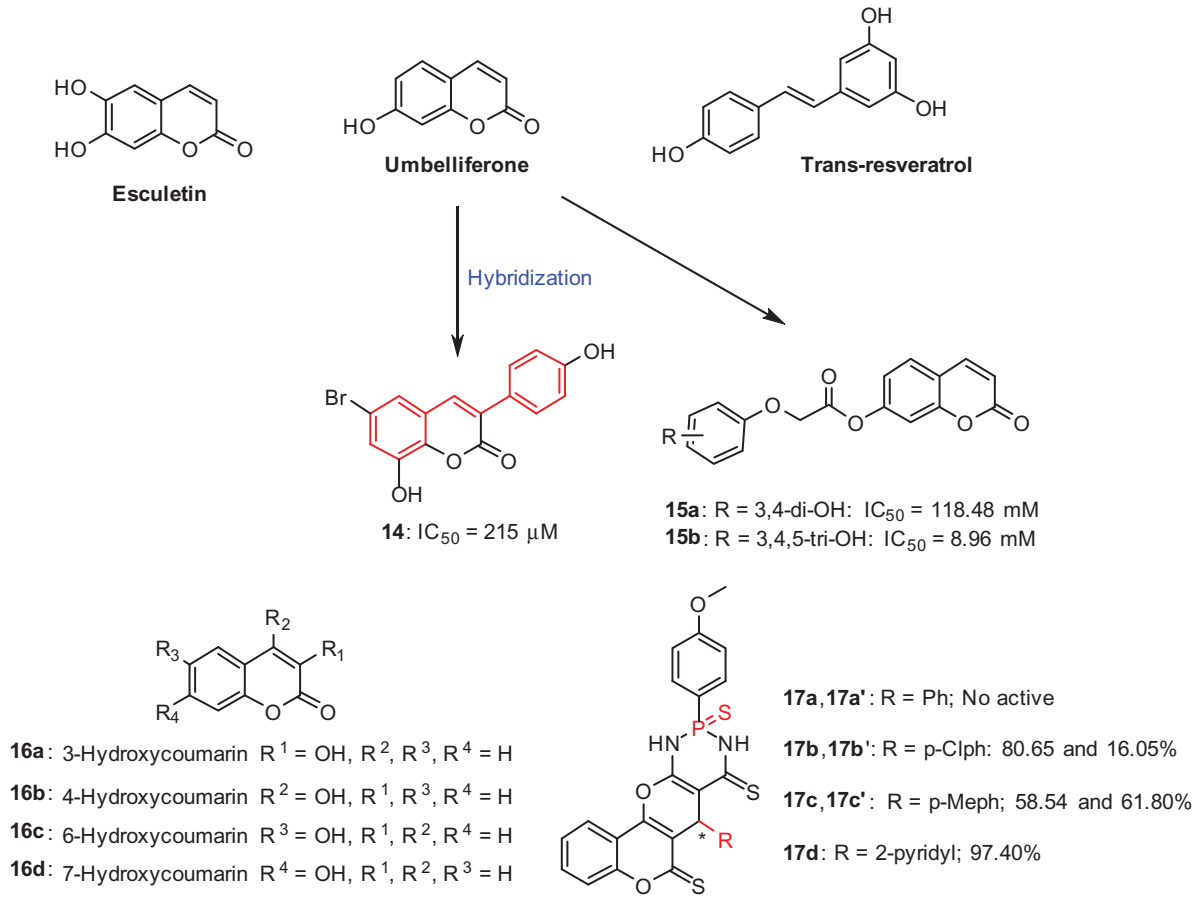


Figure 7. Chemical structure of coumarin derivatives, **14**,[73] **15a–15b**,[74] **16a–16d**[75] and **17a–17d**[76].

compounds, **21a** and **21b** exerted inhibitory activity against mushroom tyrosinase (Figure 8(a)). Especially, compound **21a** showed excellent inhibitory activity. In B16 cells, **21a** significantly suppressed tyrosinase activity in a dose-dependent manner without any influence of cytotoxic effect. From the structural point of view, a "linear" β-phenyl-α,β-unsaturated carbonyl scaffold plays an essential role in showing anti-melanogenic effect through the direct inhibition of tyrosinase enzyme.

It has been a long time known that cinnamaldehyde was able to inhibit L-DOPA oxidation by mushroom tyrosinase[84]. Recently, Cui et al., reported a series of α-substituted derivatives of cinnamaldehyde derivatives[85]. The SAR studies showed that α-bromocinnamaldehyde **22a**, α-chlorocinnamaldehyde **22b**, and α-methylcinnamaldehyde **22c** compounds reduced both monophenolase and diphenolase activity on tyrosinase (Figure 8(a)). The IC_{50} values of **22a**–**22c** were 0.075, 0.140 and 0.440 mM on monophenolase and 0.049, 0.110 and 0.450 mM on diphenolase, respectively. Furthermore, it was suggested that the α-substituted cinnamaldehyde derivative were more potent compared to cinnamaldehyde.

Recently, thio/barbiturates have drawn attention in the field of tyrosinase inhibitors[86], due to their attractive structural unit of β-phenyl-α,β-unsaturated carbonyl scaffold responsible for tyrosinase inhibitory function. In the literature, few 5-benzylidene (thio) barbiturates with hydroxyl substituent at 4-position of the phenyl ring had excellent inhibitory activities, for example, **23a** and **23b** inhibited with IC_{50} values of 13.98 and 14.49 μM, respectively[87] (Figure 8(b)). Inspired by this work, Chen et al., recently explored the SAR of thio/barbiturates emphasizing the position and the number of hydroxyl substituents for the influence of tyrosinase inhibitory activity. Accordingly, a series of hydroxy- or methoxy-substituted 5-benzylidene(thio)barbiturates were reported for their inhibitory effects on the diphenolase activity of mushroom tyrosinase[88]. The results show that compounds (**23c**–**23 g**) had potent tyrosinase inhibitory activities compared to kojic acid (IC_{50} = 18.25 μM). In particular, compound with 3,4-dihydroxy substituents **23e** was identified as a best inhibitor with an IC_{50} value of 1.52 μM. The SAR studies revealed that barbiturates were potent than thiobarbiturates and 3,4-dihydroxyl groups on the phenyl ring improved the potency. Furthermore, these inhibitors were found to reversible type.

Thiourea derivatives

Phenylthiourea (PTU) is one of the most well-known tyrosinase inhibitors studied in the literature[89–91]. Sulfur atom of PTU binds to both copper ions at the active site of tyrosinase and blocks the enzyme activity. Professor Jung et al. recently explored the SAR of PTU derivatives on mushroom tyrosinase[92,93]. Although the effects of PTU derivatives were primarily evaluated for their melanogenesis inhibition in B16 melanoma cells, later it was confirmed that it was due to inhibition of tyrosinase activity. The SAR studies has highlighted the important structural requirements for both melanogenesis and tyrosinase inhibition (Figure 9(a)): (i) direct connection of π-planar structure to thiourea (**24a**–**24b**), (ii) hydrophobic substituent at the para- or meta-position of the phenyl ring was accepted (**24c**–**24d**), substituent at the ortho-position was not tolerated (**24e**), suggested that C2-substituted phenyl may hinder the complex formation of thiourea with copper ions at the active site of tyrosinase. Moreover, (iii) free 3-amino hydrogens were

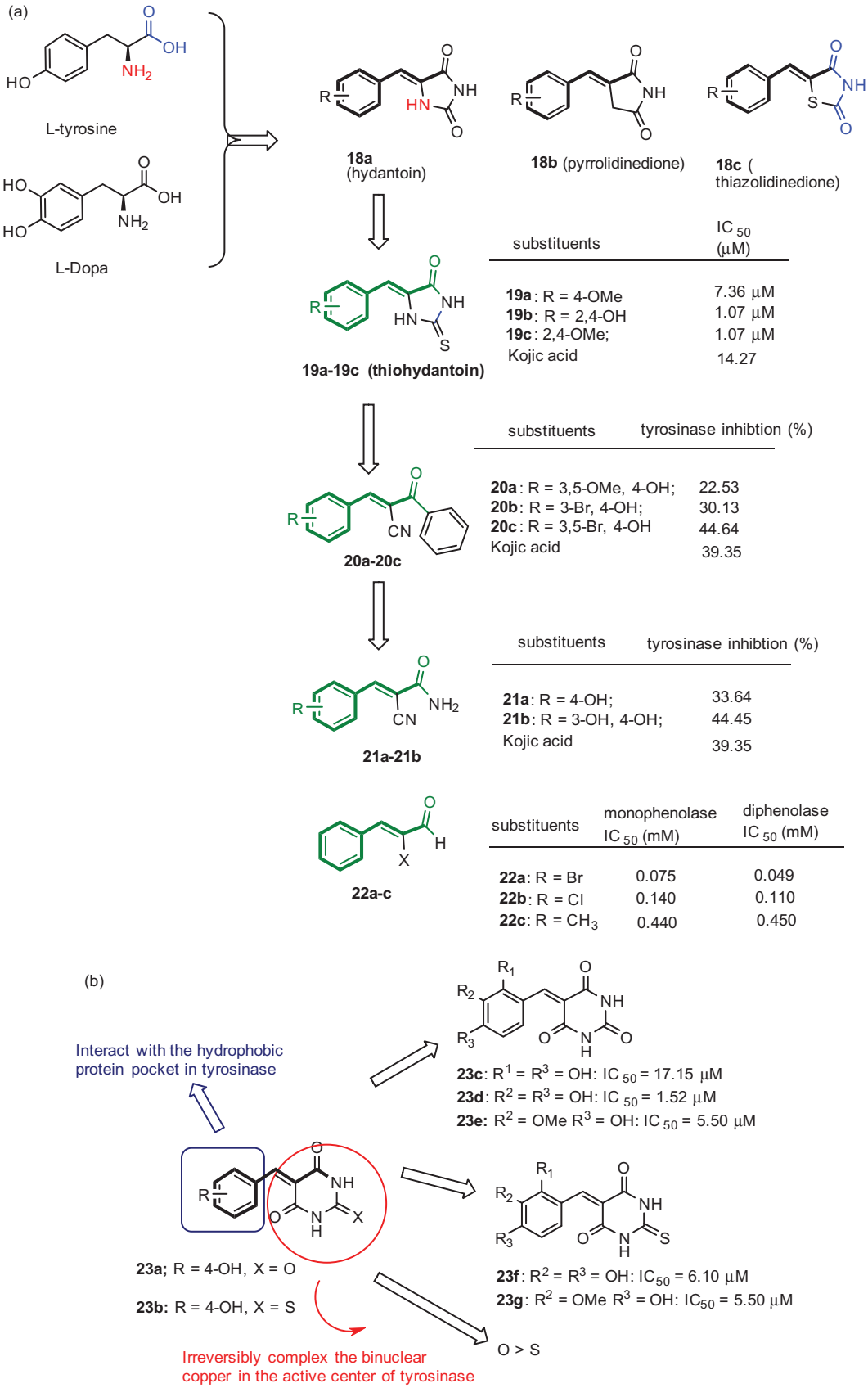
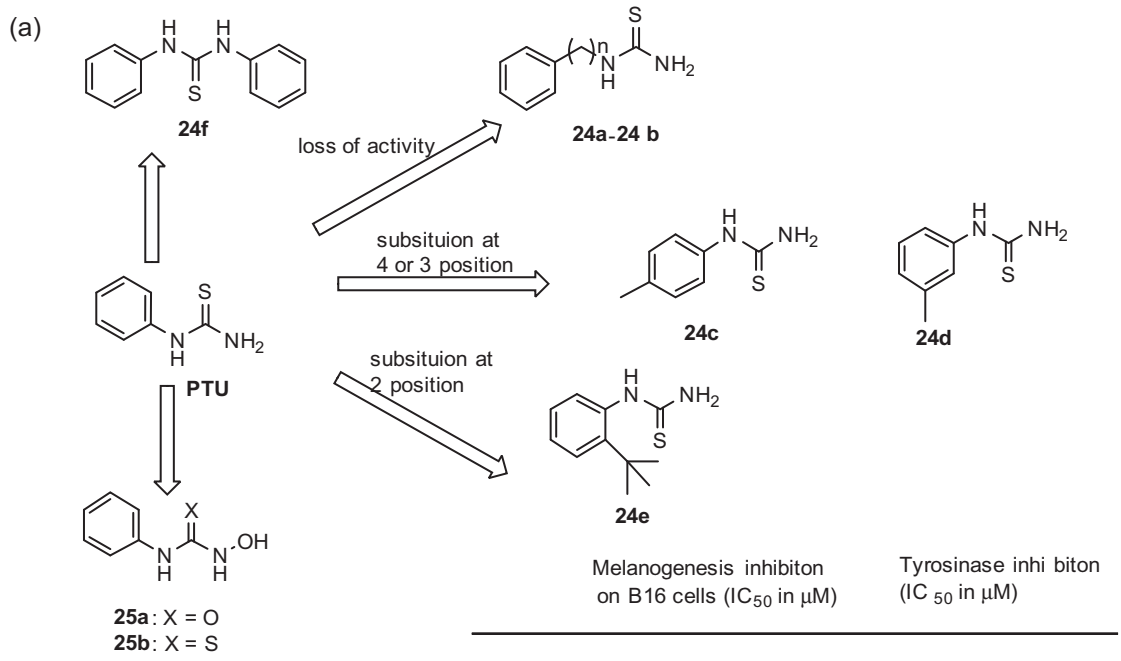


Figure 8. Chemical structure of inhibitors with β-phenyl-α,β-unsaturated carbonyl functionality, **18a–18c**,[77–80] **19a–19c**,[81] **20a–20c**,[82] **21a–21b**,[83] **22a–22c**[85] (a); **23a–23b**[85] and **23c–23g**[88] (b).



	Melanogenesis inhibiton on B16 cells (IC_{50} in μM)	Tyrosinase inhi biton (IC_{50} in μM)
PTU	5.4	1.5
24a	>10	25.4
24b	>10	>100
24c	5.5	1.4
24d	5.0	0.6
24e	>10	>10
24f	4.5	>100
25a	-	0.29
25b	-	>1000
26a	-	4.0
26b	-	4.5
26c	-	>1 mM
26d	-	14.4
26e	-	2.8

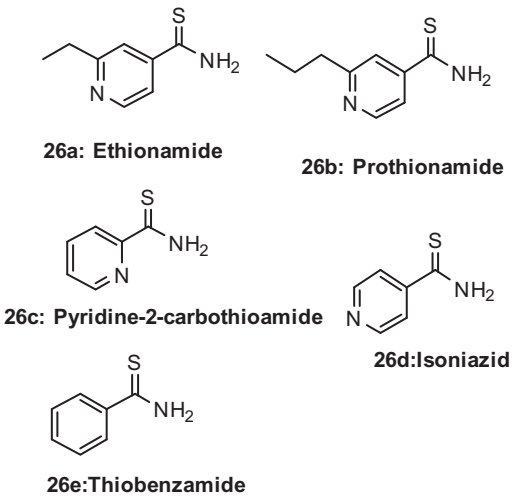


Figure 9. Chemical structure of thiourea derivatives, **24a–24f**,[92], [93] **25a–25b**[91] and **26a–26e**[95] (a); **27a–27e**,[97] **28a–28d**,[98] **29a–29d**[98] and **30a–30b**[99] (b).

important for tyrosinase inhibition but not for the melanogenesis inhibition on B16 cells, 1,3-disubsituted derivatives showed greater potency in melanogenesis inhibition (**24f**). These findings suggested that 1,3-disubsituted derivatives act through a pathway different from the tyrosinase catalytic activity to prevent melanogenesis. Molecular docking analysis of 1-phenylthiourea and 1,3,-diphenylthiourea revealed that direct connection of planar phenyl to thiourea unit and free 3-NH_2 were prerequisite for the tyrosinase activity (Figure 10).

On the other hand, Crinton et al reported a series of *N*-hydroxy-*N*′-phenylurea derivatives, replacing sulfur with oxygen and 3-NH_2 with *N*-hydroxylamine in PTU[91]. The results showed the reported derivatives were more potent, in particular, *N*-hydroxy-*N*′-phenylurea (**25a**) showed 6-time more potent than PTU. In contrast *N*-hydroxy-*N*′-phenylthiourea (**25b**) derivatives showed no inhibition, suggested that the chelating ability of *N*-hydroxyurea is important for the tyrosinase inhibition (Figure 9(a)).

Apart from synthetic or natural sources, screening is another alterative strategy to find new inhibitors. Mainly screening of drugs that are clinically approved has become increasing for many biological targets. The data associated with an existing drug will reduce the time and cost associated with the intellectual rights for developing the novel pharmaceutics. This approach has several advantages; including availability, lower cost and safety/tolerability. Phenylthiourea has long been known as a tyrosinase inhibitor[89–91,94]. The chemical similarity analysis performing by ligand-based virtual or HTS screening identified ethionamide (**26a**) and its analogs (**26c**–**26e**), including prothionamide (**26b**), as tyrosinase inhibitors[95] (Figure 9(a)). Ethionamide is an approved second-line antituberculosis drug used for the treatment of multi-drug-resistant tuberculosis. In contrast, isoniazid, a structural analog and first-line antituberculosis drug was a poor inhibitor of tyrosinase. In B16 cells, inhibitors pyridine-2-carbothioamide and thiobenzamide substantially reduced the melanin content 44% and 37%, respectively. After an extensive structural analysis, the

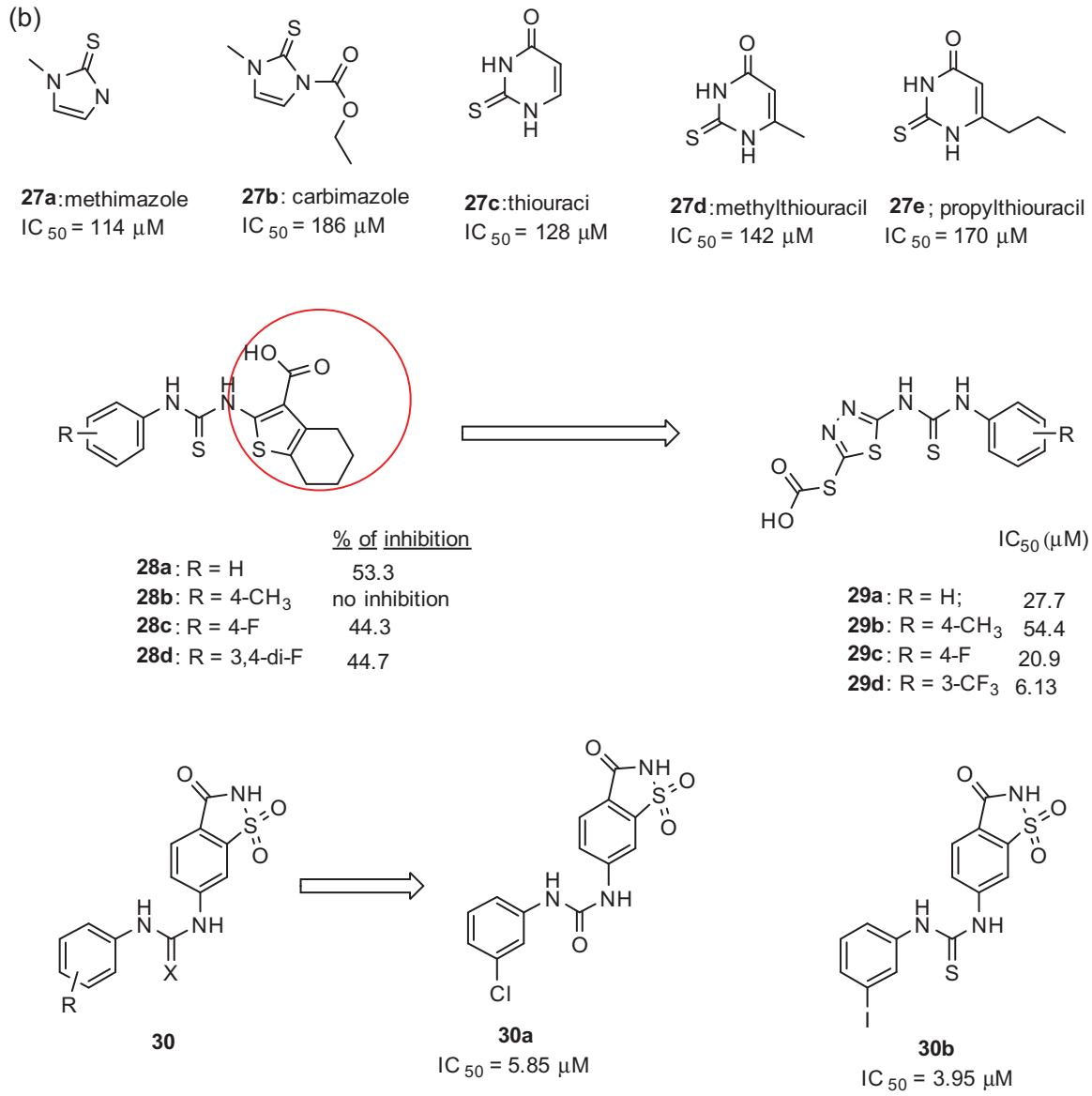


Figure 9. Continued.

SAR data suggest that carbothioamide was a central moiety for the development of new and potent tyrosinase inhibitors.

In a recent study, the researchers retrieved the thiourea-derived drugs in clinical use and investigated their effect on tyrosinase activities by using enzyme- and cell-based assays[96]. It was observed that the antithyroid agents methimazole **27a**,[97] carbimazole **27b**,[97] thiouracil **27c**,[97] methylthiouracil **27d**[97] and propylthiouracil **27e**[97] inhibited mushroom tyrosinase (Figure 9(b)). In addition, kinetic studies assigned thiourea-containing drugs as noncompetitive inhibitors. The SAR studies explained that thiourea itself inhibits tyrosinase enzyme activity in a concentration-dependent manner. This shows the inhibitory activity of thiourea analogs must be originated from the sulfur and the nitrogen atoms.

In another study, a class of novel *N*-aryl-*N*′-substituted phenylthiourea derivatives was evaluated on the diphenolase activity of mushroom tyrosinase[98]. The results showed few 4,5,6,7-tetrahydro-2-[[(phenylamino)thioxomethyl]amino]-benzo[*b*]thiophene-3-carboxylic acid derivatives (**28a**–**28d,** (Figure 9(b))) exhibited moderate inhibitory potency on diphenolase activity of tyrosinase. When the scaffold of 4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic acid was replaced with 2–(1,3,4-thiadiazol-2-yl)thio acetic acid, the inhibitory activity of compounds (e.g. **29a**–**29d**, Figure 9(b))[98] against tyrosinase was improved. Especially, **29d** ($IC_{50} = 6.13$ μM) exhibited potent inhibitory activity than kojic acid ($IC_{50} = 33.3$ μM).

Since 1885, saccharin, 1,2-benzisothiazole-3-one-1,1-dioxide is a well-known heterocyclic compound and used as a sweetener in the form of its sodium salt. Besides, it is reported for many biological targets and thus can be viewed as a privileged scaffold. Recently, a novel series of 6-(phenylurenyl/thiourenyl) conjugated saccharin derivatives were evaluated for inhibitory effects on the diphenolase activity of banana tyrosinase enzyme[99]. The results showed that all the compounds inhibited the tyrosinase activity. Among them, 6–(3-chlorophenylurenyl) saccharin **30a** ($K_i = 5.85$ μM) and 6–(3-iodophenylthiourenyl) saccharin **30b** ($K_i = 3.95$ μM) were found to be the most active compounds. The SAR studies showed 6-(phenylthiourenyl) saccharin derivatives exhibited higher inhibitory activity than 6-(phenylurenyl) saccharin

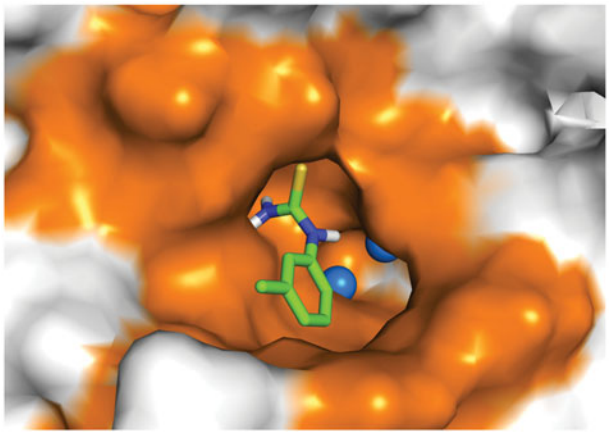


Figure 10. The docked pose of **24d**[93] (stick model) is shown with the two copper ions (sphere representation) and the binding pocket (surface model) of tyrosinase from *Bacillus megaterium* (PDB ID: 3NQ1).

derivatives. An electron withdrawing group at 3-position of phenylurenyl/thiourenyl-ring increased in activity, in particular, halogen series showed higher inhibitory activities.

Thiosemicarbazone-type inhibitors

Thiosemicarbazones occupy one of the major classes of tyrosinase inhibitors due to their classical structural unit and the ability to chelate copper ions at the active site of tyrosinase enzyme. Recently, a huge number of thiosemicarbazones by different research groups have been reported[99–101] as potent tyrosinase inhibitors.

The SAR studies revealed that the thiosemicarbazide scaffold was a key unit for determining the tyrosinase inhibitory activity because it was able to effectively complex the two copper ions at the active site of tyrosinase. In an effort to improve the activity, a series of 4/3-aminoacetophenones and derived thiosemicarbazones were reported for their inhibitory activity of mushroom tyrosinase[102]. Results from the biological evaluation, the acylamino compounds (**31a**–**31g**, Figure 11) exhibited potent tyrosinase inhibitors than kojic acid ($IC_{50} = 28.5\ \mu M$), and in contrast, the aminophenones (**31 h** and **31i**) showed activation of tyrosinase activity. Compound **31d** was found to be the most active compound with an IC_{50} value of 0.291 μM. The studies with SAR analysis also revealed that thiosemicarbazide group played a very vital role for tyrosinase inhibition, acylamino moieties crucial for enhancing the tyrosinase inhibitory activity and the acylamide substituent at 4-position on the phenyl ring increased the tyrosinase inhibitory potency. Moreover, the inhibition mechanism and kinetics study revealed that compound **31a** was reversible and a noncompetitive inhibitor.

In continuing to search for potent compounds as highly efficient tyrosinase inhibitors, and understanding the SARs of thiosemicarbazone compounds, a series of novel 4-alkoxy- and 4-acyloxy-phenylethylenethiosemicarbazone analogs (**32a**–**32i**, Figure 11) were synthesized and evaluated for tyrosinase inhibitory activity[103]. The results showed that most of the inhibitors displayed remarkable potency inhibiting tyrosinase with an IC_{50} value lower than 1.0 μM. In particular, compound **32d** exhibited a remarkable tyrosinase inhibitory potency compared to other analogs from these series. The SAR studies revealed that the thiosemicarbazone moiety played a key role in determining the tyrosinase inhibitory activity. The length of methylene linker between the phenyl ring and the thiosemicarbazone moiety (**32f**–**g**) had no influence on the tyrosinase inhibitory potency. The introduction of thiocarbonohydrazide moiety (**32 h**–**32i**) was unfavorable for the tyrosinase inhibitory activity.

Peptide type inhibitors

In an effort to discover new tyrosinase inhibitors, the recent attention has been drawn to apply peptide sequences for tyrosinase inhibition. Several tyrosinase inhibitory peptides such as dipeptides[104], cyclic peptides[105], short-sequence oligopeptides[106] and kojic acid tripeptide compounds[107], have been investigated. Especially, oligopeptides have proven to be effective tyrosinase inhibitors. Two oligopeptides **P3**, an octapeptide (Arg-Ala-Asp-ser-Arg-Ala-Asp-Cys) and a decapeptide **P4** (Tyr-Arg-ser-Arg-Lys-Tyr-Ser-Ser-Trp-Tyr) showed substantial inhibitory effects on mushroom and human tyrosinase with IC_{50} value of 123 and 40 μM, compared with hydroquinone. These oligopeptides did not show any effect on melanocytes cytotoxicity[108]. After successful formulation into topical cream and favorable clinical results, a decapeptide **P4** also known as decapeptide-12 has been commercialized as the main active ingredient in a skin-lightening product[109].

In a recent study, Hsiao et al., discovered dipeptide-like compound (A5) and tripeptides RCY and CRY effectively inhibit the tyrosinase activity[109]. Especially, a novel tripeptide CRY showed the most striking inhibitory potency against mushroom tyrosinase ($IC_{50} = 6.16\ \mu M$). This tripeptide is more potent than the known oligopeptides and comparable with kojic acid-tripeptides. CRY and RCY used the thiol group of the cysteine residue to coordinate with Cu ions at the active site of tyrosinase, thereby showing low tyrosinase activity. In another study, the cysteine-containing dipeptides were reported to inhibit the tyrosinase activity in an effective manner[110]. The authors suggested that these cysteine-containing dipeptides could directly block the active site of tyrosinase and thereby leading to potent inhibition. In particular, N-terminal cysteine-containing dipeptides markedly outperform the C-terminal and the cysteine-containing dipeptides, CE, CS, CY and CW show comparative bioactivities and tyrosine-containing dipeptides are substrate-like inhibitors. In addition, these dipeptides do not show significant cytotoxicity in melanocytes, and CA and PD attenuated 5.6 and 16.5% melanin content, respectively, at 100 μM (Table 1).

Li et al., reported a set of hydroxypyridinone-*L*-phenylalanine conjugates (**33a** and **33b**) starting from kojic acid (Figure 12)[111] for their inhibitory activities on tyrosinase. Evaluation against tyrosinase activity revealed that one of the compounds ((S)-(5-(benzyloxy)-1-octyl-4-oxo-1,4- dihydropyridin-2-yl)methyl 2-amino-3-phenylpropanoate, (**33b**) showed IC_{50} values 12.6 and 4.0 μM for monophenolase and diphenolase activities, respectively. Moreover, these conjugates were mixed-type inhibitors, suggesting they could bind to both the free enzyme and the enzyme – substrate complexes.

In another study, hydroxypyridinone-*L*-amino acid conjugates were designed and evaluated for their inhibitory activities on mushroom tyrosinase[112]. Among the investigated compounds, only two compounds exhibited both monophenolase (**34**, $IC_{50} = 1.95\ \mu M$; **35**, $IC_{50} = 2.79\ \mu M$) and diphenolase inhibitory (**34**, $IC_{50} = 8.97\ \mu M$; **35**, $IC_{50} = 26.20\ \mu M$) activity of tyrosinase (Figure 12). Moreover, the compounds **34** and **35** were identified to be reversible and mixed-type inhibitors.

Miscellaneous mushroom tyrosinase inhibitors

Recently, the extracts and isolated compounds from natural sources have attracted much attention as tyrosinase inhibitors and

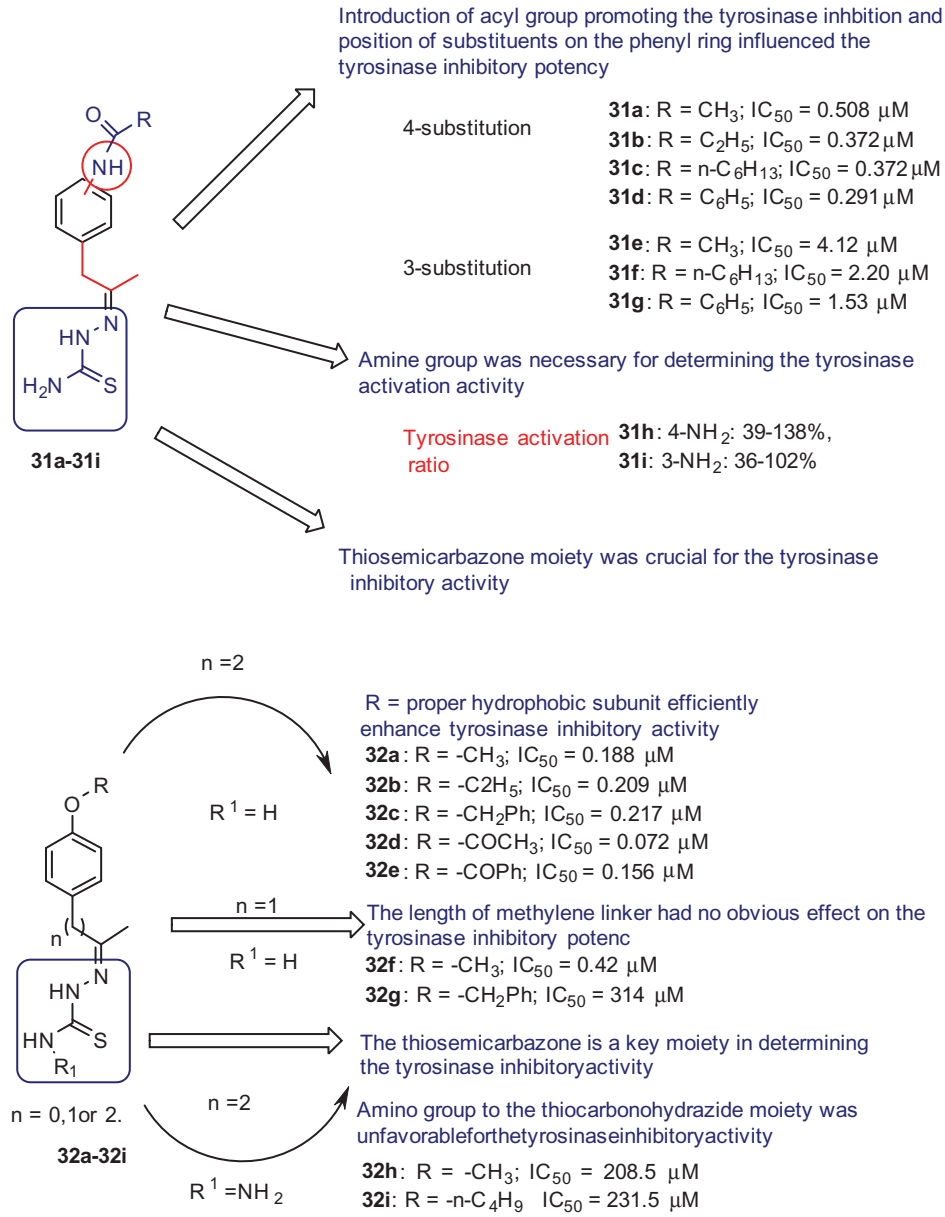


Figure 11. Chemical structure of thiosemicarbazone analogs, **31a–31i**[102] and **32a–32i**[103].

Table 1. Inhibition of melanin content in melanocytes by dipeptides[111].

	Melanin content (%)		
Dipeptide	(1 μM)	(10 μM)	(100 μM)
CA	94.5 ± 1.8	23.64 ± 0.31	23.64 ± 0.31
CY	85.9 ± 3.3	23.64 ± 0.31	23.64 ± 0.31
PD	88.6 ± 1.1	23.64 ± 0.31	23.64 ± 0.31
DY	91.1 ± 3.4	23.64 ± 0.31	23.64 ± 0.31
Kojic acid (50 μM)	97.7 ± 1.8		23.64 ± 0.31
β-arbutin (50 μM)	102.1 ± 4.6		

have been accepted as popular skin whitening agents[113–123]. In an effort to find a safe and effective whitening substance, Chen et al., screened a number of natural products from herbal plants and isolated compounds **36** and **37** from the rhizome of *Gastrodia elata*, as mushroom tyrosinase inhibitors[124]. Subsequent SAR studies have identified analogs **38–40** (Figure 13). Bis(4-hydroxybenzyl)sulfide **36** showed outstanding inhibitory potency against tyrosinase with an IC_{50} value of 0.5 μM and K_i value of 58 nM. The compound **37** connected through an ether linkage show 713-fold decrease in the inhibitory ability (IC_{50} = 378.11 μM) indicate the sulfur atom is very important in chelating with the copper ions and contributes in a greater inhibition tyrosinase activity. On the other hand, shortening the carbon linker, which connects the sulfur to benzene rings, resulted in the moderate tyrosinase inhibition of **38**. The removal of hydroxyl group, **39** lead to poor tyrosinase inhibition, indicating that two hydroxyl groups are important. With the methoxy substitution, an analog of **36**, reduces potency to IC_{50} value of 40.02 μM, suggesting that hydrogen bond interactions were favorable than the hydrophobic interactions provided by methoxide groups.

Compound **36** treated with 50 μM reduced 20% melanin content in the human melanocytes system without significant cytotoxicity. In addition, the zebrafish *in vivo* assay reveals that **36** effectively reduce melanogenesis with no adverse effect. Moreover, the acute oral toxicity study confirmed that the compound **36** was free of discernable cytotoxicity in mice. Thus compound **36** is a potential candidate in developing safe and effective

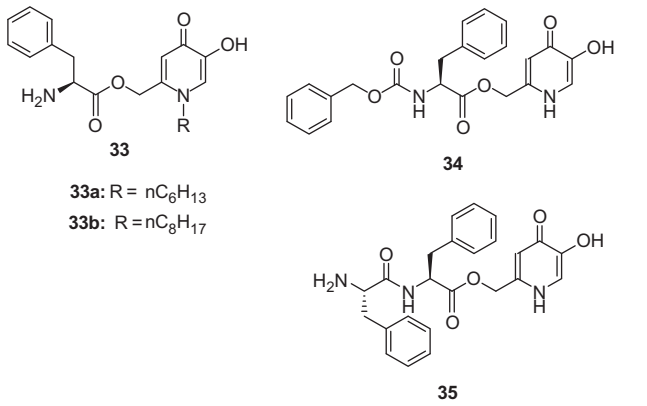


Figure 12. Chemical structure of peptide conjugates, **33a–33b**,[112] **34** and **35**[113].

pharmacological agent for skin-whitening. Recently, Ai et al., screened a chemical library using a virtual screening approach and identified a compound **41** as a potent mushroom tyrosinase inhibitor[125], with an IC_{50} value of 8 μM and yielded a 29%±17.64% blockage of melanin biosynthesis in B16 cells at a concentration of 0.002% that was equal to 27.5 μM.

The SAR studies examined around structure **41** showed the aromaticity of the ring B of compound **41** appears essential for activity, as replacement of ring B with cyclohexyl ring (compound **42b**) losses the inhibitory activity of melanin synthesis in B16 cells. On the other hand, the substitution at 4-position of the ring A had a negligible effect on the inhibitory activity (**42a**–**42d**). These compounds were dose dependent and inhibit the melanin synthesis in B16 cells. However, further development of compound **41**, lead to a potential formulation problem and eliminated as a candidate for cosmetic purposes. A further substructural analysis identified a compound **43** exhibited 79%±5.34% inhibition on melanin biosynthesis of B16 cells at a concentration of 0.001% (33.6 μM). These two compounds **41** and **43** possess good biochemical properties and satisfy Lipinski's "rule of five" and exhibited a substantial inhibitory effect on melanin synthesis in B16 cells. This melanin synthesis inhibition was shown not to affect the cellular viability, which further underscores the potential commercial utility of these compounds.

It was reported that vanillin and vanillic acid isolated from *Origanum vulgare* may serve as agents for antimelanogenesis[126]. Based on this background, a series of vanillin esters incorporating benzoic acid, cinnamic acid and piperazine have been reported for tyrosinase inhibitory activity[127]. The results showed that compounds **44a**–**44d** exhibited good to excellent inhibition of mushroom tyrosinase activity (Figure 14). In particular, **44b** exhibited the most potent inhibitory activity with an IC_{50} value of 16.13 μM. From the structural point of view, the substituted cinnamic acid esters and hydroxyl substitution played an important role in tyrosinase inhibition. The kinetic studies revealed compound **44b** was a mixed-type tyrosinase inhibitor with K_i 13 μM and K_i' 53 μM and formed reversible enzyme inhibitor complex.

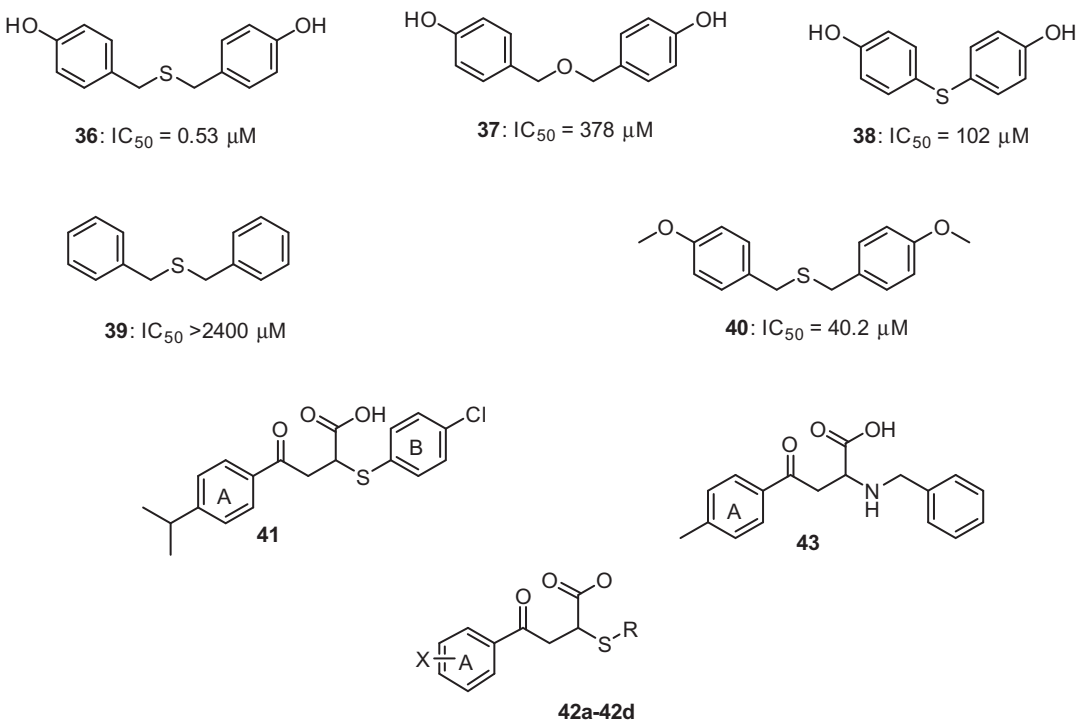
In another study, 2-hydroxytyrosol **45** (2-HT)[128] was found to inhibit mushroom tyrosinase with an IC_{50} value of 13.0 μmol/L which was equally potent as kojic acid (IC_{50} = 14.8 μmol/L). Furthermore, 2-HT dose-dependently inhibited tyrosinase activity (IC_{50} = 32.5 μmol/L) in the cell-free extract of B16 melanoma cells and α-MSH-stimulated melanin formation in intact B16 melanoma cells. Methimazole (2-mercapto-1-methylimidazole) derivatives were reported to inhibit mushroom tyrosinase[129]. 2-Mercaptoimidazole (**46a**), mercapto-1-methylimidazole (**46b**) and tert-butyl 3-methyl-2-sulfanylidene-2,3-dihydro-1*H*-imidazole-1-carboxylate (**46c**) significantly inhibited tyrosinase activity exhibiting an IC_{50} value of 4.11 mM, and 1.43 mM and 1.45 mM, respectively, (Figure 14). Kinetic analysis indicated that compounds **46a** and **46b** as competitive tyrosinase inhibitors, while **46c** as a noncompetitive tyrosinase inhibitor. Further, *in vitro* analysis on B16 cells, compounds of **46a**–**46c** exerted potent inhibitory effect on intracellular melanin production without influencing the cytotoxicity.

A series of triazole Schiff's base derivatives have been demonstrated for their inhibitory activity against mushroom tyrosinase activity[130]. The results showed from the biological evaluation that only three compounds **47a**–**47c** exhibit potent inhibitory effects with IC_{50} values of 12.5, 7.0 and 1.5 μM (Figure 14). Kinetic analysis revealed that inhibitors are reversible and mixed type. Fluorescence quenching and copper interaction studies confirmed that interaction of inhibitors with tyrosinase and chelation ability with copper ions in the active site. From structural point of view, the substitution at 2-position of the benzene ring showed superior activity (**47c**) than 3-position (**47b**). A novel series of thymol analogs incorporating hydroxylated benzoic acids and cinnamic acids were reported as mushroom tyrosinase inhibitors[131]. In general, the cinnamic acid-derived thymol derivatives showed better tyrosinase inhibitory than the benzoic acid derivatives, exemplified with the comparison of **48a** (IC_{50} 15.20 μM) versus **48b** (IC_{50} 91.5 μM).

Recently, the effect of picrionoside A **49** isolated from the leaves of Korean ginseng (*P. ginseng* C.A. Mayer) was examined[132] and it has inhibited mushroom tyrosinase activity with an IC_{50} value of 9.8 μM, about 6.8 to 10-fold higher than kojic acid and arbutin, respectively (Figure 14). In melan-Ab cells, **49** reduced 17.1% melanin content in a dose-dependent manner without inducing the cell viability. In addition, Picrionoside A-treated zebrafish showed a remarkable inhibitory effect on body's pigmentation. Taken together these results show that Picrionoside A may be an effective skin-whitening agent[133]. In the course of screening, melanogenesis inhibitors *in Streptomyces bikiniensis*, it was found that *S*-(−)-10,11-dihydroxyfarnesoic acid methyl ester (dhFAME, **50**), an insect juvenile hormone produced by *Beauveria bassiana* CS1029[134] directly inhibited the tyrosinase activity. In addition, **50** significantly reduce the melanin content, inhibited the cellular tyrosinase activity as well as the intracellular accumulation of cAMP levels in melan-a cells without inducing the cell viability.

A new class of potent tyrosinase inhibitors was identified by structure-based virtual screening prediction[135]. The structure of mushroom tyrosinase (PDB ID: 2Y9X) was used as a template for molecular dynamics (MD) simulation. Initially, an ensemble of 10 000 structures using molecular dynamics simulation was generated. Consequent screenings yielded top 61 molecules for evaluating against mushroom tyrosinase activity and the selective inhibitors (**51a**–**51e**) are indicated in Figure 14. The results show that the moieties of tetrazole and triazole were able to interact with the di-copper catalytic center of the tyrosinase. In particular, a tetrazole compound **51b** exhibiting the strongest activity. The authors found that many compounds displayed good reduction in melanin production in B16 melanoma cells with no cytotoxicity. Specifically, a thiosemicarbazone-containing compound **51e** reduced melanin content by 55%. The results provide valuable insight into the modulation of the functions of type-3 copper enzymes.

Captopril ([2*S*]-*N*-[3-mercapto-2-methylpropionyl]-*L*-proline**)** is an angiotensin converting enzyme inhibitor[136,137] which are widely used in the treatment of hypertension and heart failure. In order to identify novel and effective tyrosinase inhibitors, the inhibitory



Compound	X	R	B16 inhibition at 0.001% n = 6[a]	Molarity of 0.001%
42a	4-ethoxy	4-chlorobenzyl	85% ± 94%	26.39 μM
42b	4-t-buty	cyclohexy	47% ± 5.33%	28.73 μM
42c	4-chloro	4-chlorobenzyl	78% ± 5.22%	27.1 μM
42d	4-chloro	cyclohexyl	56% ± 10.55%	30.67 μM

Note: [a]six independent experiments

Figure 13. Chemical structures of miscellaneous tyrosinase inhibitors **36–40125** and **41–43**[126].

effect of captopril (**52**) was experimented for tyrosinase inhibitory activity. The result showed that **52** inhibited tyrosinase activity with an IC_{50} value of 590 μg/mL (Figure 14)[138]. Further *in vitro* studies in B16 cells, **52** was found to inhibit the tyrosinase activity[139,140] in dose-dependent manner that leads to the inhibition of melanin formation without cytotoxicity.

Human tyrosinase inhibitors

Although a huge number of tyrosinase inhibitors were available and several of them with potent inhibitory activities were discussed earlier, almost all the inhibitors were evaluated against mushroom tyrosinase. In an effort to find novel inhibitors against human tyrosinase, Yoshimori et al demonstrated thujaplicins (**52–54**; α, β and γ isomers, Figure 15) for their inhibitory effects on both mushroom tyrosinase and human tyrosinase (hTYR), with comparison[141]. The results showed that β-and γ-thujaplicins (**53** and **54**) effectively inhibited the human tyrosinase activity in a dose-dependent manner with IC_{50} values of 8.98 and 1.15 μM, respectively. Especially, γ-thujaplicin **54** was extremely superior to kojic acid (IC_{50} = 17 μM). The SAR studies revealed the position of isopropyl on the tropolone scaffold was the determinant factor for the potency of thujaplicines. The potency of thujaplicins is in the following order: γ > β > α-thujaplicin.

Researchers further evaluated the inhibitory effects of thujaplicins on mushroom tyrosinase inhibitory activities and compared with hTYR inhibitory activity. The results showed that huge differences in the inhibitory activities of thujaplicins against hTYR and mTYR were observed: kojic acid was found to be 10.64-fold weaker inhibition against hTYR than mTYR. In thujaplicins, α,β- and γ-thujaplicins were approximately >104.93-, 99.78-, and 16.43-fold, respectively, weaker inhibition against hTYR than mTYR. The activity differences of thujaplicins were explained by comparing the values obtained for hTYR and mTYR, and the amino acid compositions in the active sites of these enzymes.

In order to understand the inhibitory mechanism, the binding mode of γ-thujaplicin was predicted using homology model of hTYR. It showed the carbonyl and hydroxyl group of the γ-thujaplicin chelate with two copper ions at the active site of hTYR. Tropolone scaffolds of thujaplicin forms stacking interaction with imidazole ring of His367 and hydrophobic interaction with isopropyl of Val377. The isopropyl group of thujaplicin forms hydrophobic interaction with Ile368. In addition, the results from comparative studies on the inhibitory effects of the other thujaplicins (α and β) indicated that van der Waals (VdW) clashes of the isopropyl group of α-thujaplicin with Val377 and S380 might reduce the inhibitory activity against hTYR. The main reason for higher inhibitory activity of γ–thujaplicin against hTYR among thujaplicins is considered to be the hydrophobic interaction of the isopropyl group with Ile368. In contrast, in mTYR, the Val377 and Ser380 are replaced with proline (P257) and alanine (A260), respectively, therefore it can be considered that there is little VdW clashes of the isopropyl group of α-thujaplicin with A260 and P257 (Figure 16). This explains why the inhibitory effect of

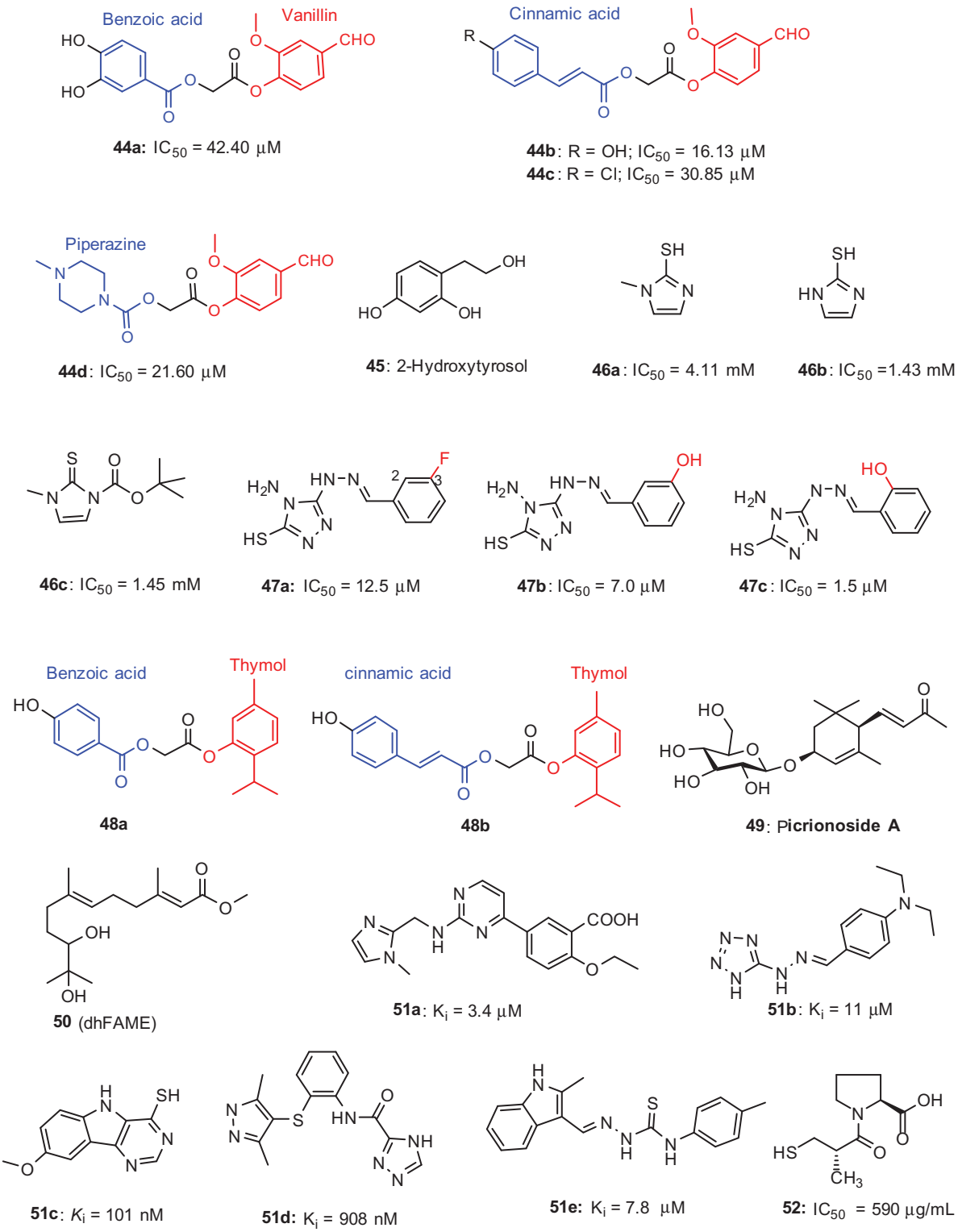
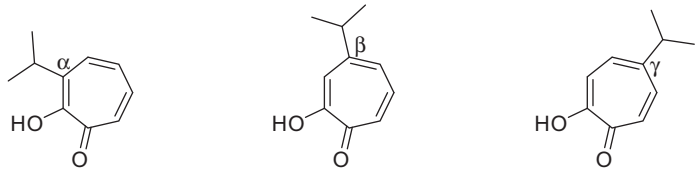


Figure 14. Chemical structure of miscellaneous tyrosinase inhibitors, **44a–44c**,[128] **45** [129] **46a–46c**,[130] **47a–47c**,[131] **48a–48b**,[132] **49** [133] **50** [135] **51a–51e**[136] and **52**.[139].

α-thujaplicin against mTYR (IC_{50} = 9.53 μM) is more than two-orders of magnitude stronger than hTYR (IC_{50} > 1000 μM). Accordingly, it was suggested that the difference of Ala260 in mTYR and Ser380 in hTYR dramatically affect the inhibitory profile of α-thujaplicin.

Wang and coworkers have recently found the natural products linderanolide B (**55**) and subamolide A (**56**) isolated from the stems of *Cinnamomum* were proved to have good *in vitro* inhibitory abilities of mushroom tyrosinase at low doses (Figure 15)[142]. Both compounds showed cell viability of human keratinocytes, melanocytes and fibroblasts treated with various concentrations of two compounds. Treatment at a low dose (0.01–1.0 μM) did not show significant cytotoxicity to human skin cells. The study revealed both compounds could reduce 50% of human tyrosinase activities at a dose of 1 μM after 48-h treatment and effectively inhibited (40% reduction) the melanin formation in HEMn-MP cells. Both **55** and **56** showed a remarkable inhibitory potential on zebrafish *in vivo* pigmentation even at low doses without

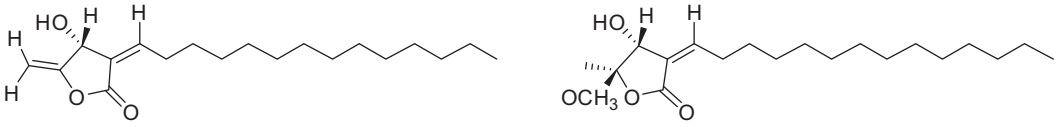
(a)



α-Thujaplicin (52) **β-Thujaplicin (53)** **γ-Thujaplicin (54)**

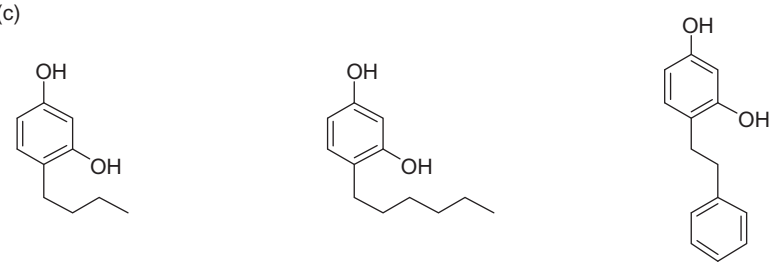
Compound	Tyrosinase inhibition IC50 (μM)	
	Human	Mushroom
α-Thujaplicin	>1000	9.53
β-Thujaplicin	8.98	0.09
γ-Thujaplicin	1.15	0.07
Kojic acid	571.17	53.70

(b)



Linderanolide B (55) **Subamolide A (56)**

(c)



4-butyl resorcinol (57) **4-hexyl resorcinol (58)** **4-phenyethyl resorcinol (59)**

Figure 15. Chemical structure of tyrosinase inhibitors; (a) thujaplicin analagoues (**52–54**),[142] (b) linderanolide B and subamolide A[143] and (c) resorcinol derivatives [144].

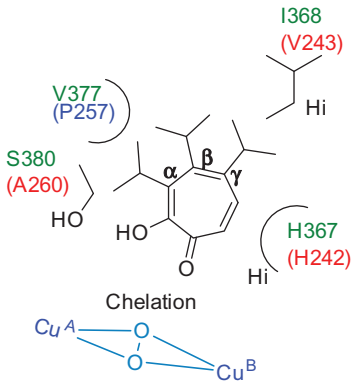


Figure 16. Schematic representation of binding interaction of thujaplicins (α, β and γ) with hTYR (V377, I368, H367 and S380) and mTYR (P257, V243, H242 and A260)[142].

observable toxicity. Therefore these two compounds are effective novel tyrosinase inhibitors to be considered as skin-whitening agent. In another study, Kolbe et al., examined the inhibitory effects of kojic acid, hydroquinone, arbutin with the other well-known class of compound 4-butyl resorcinol (**57**) on human tyrosinase as well as inhibition of production of MelanoDerm™ skin model culture[143]. In 1995, 4-butyl resorcinol introduced a resorcinol derivative has inhibitory effect on both tyrosinase[144] and TRP-1[145,146]. The results showed that 4-butyl resorcinol proved to be highly effective inhibitor of human tyrosinase with an IC_{50} value of 21 μmol/L and complete inhibition at concentrations above 100 μmol/L. 4-Butyl resorcinol exhibited 20 times more potent inhibitory activity than kojic acid, which showed an IC_{50} of 500 μmol/L and maximum inhibition (89%) was achieved at 5.6 mmol/L concentration. Arbutin and hydroquinone are poor inhibitors of human tyrosinase with IC_{50} values in the millimolar

range, that is, 6500 μmol/L for arbutin and 4400 μmol/L for hydroquinone. However, none of both have completely inhibited the human tyrosinase.

In melanoDerm skin model, arbutin showed only marginal efficacy on melanin production with an IC_{50} value of 500 μmol/L, while kojic acid inhibited with an IC_{50} of 400 μmol/L. Interestingly, hydroquinone inhibited melanin production with an IC_{50} value below 40 μmol/L, which is probably due to different mechanisms of tyrosinase inhibition. 4-Butyl resorcinol was the most potent inhibitor with an IC_{50} of 13.5 μmol/L. The *in vivo* efficacy of 4-butyl resorcinol was confirmed in clinical studies. Patients with age spots on the forearm treated twice daily two age spots with a formula containing 4-n-butylresorcinol (**57**), 4-hexylresorcinol (**58**) and 4-phenylethylresorcinol (**59**). Within 8 weeks, 4-butylresorcinol (**57**) significantly reduced the appearance of age spots while 4-hexylresorcinol, 4-phenylethylresorcinol showed significant effects after 12 weeks. A second study showed that 4-butylresorcinol was more effective than 4-hexylresorcinol and 4-phenylethylresorcinol. The resulting clinical output on hyperpigmentation reveals that 4-butylresorcinol could be a valuable active compound for the management of pigmentation disorders. In fact, 4-butylresorcinol has been used for the treatment of melasma. In all published literatures, 4-butylresorcinol 0.1% cream showed rapid efficacy, safety and tolerability when it is used for the melasma treatment[147,148]. The recent study reported that 4-butylresorcinol 0.3% cream is safe, effective and well tolerated in Indian patients with melisma[149].

Conclusions

Catalyzing the rate-limiting step of melanin synthesis, tyrosinase has become one of the most important targets for the development of hypopigmenting agents. In fact, tyrosinase is the most studied target for inhibiting the melanogenesis. Therefore, the inhibitors that target tyrosinase may specifically inhibit the melanogenesis in cells without other side effects. As a result, in recent years, numerous inhibitors have been developed and an overview of the inhibitors discussed in this review is shown in Figure 4. Different classes of inhibitors include chalcones, resveratrols and flavanones were discussed in this review. Very interestingly, inhibitors with β-phenyl-α,β-unsaturated carbonyl scaffold were newly classified in this report and showed remarkable tyrosinase inhibitory activities. Especially, benzylidene-2-thiohydantoins and 5-benzylidene(thio)barbiturates showed greater inhibitory potency (Figure 7). More medicinal chemistry efforts and structure activity relationships on these scaffolds would bring novel inhibitors in future. Another new scaffold bis(4-hydroxybenzyl)sulfide **36** showed outstanding inhibitory potency against tyrosinase with an IC_{50} value of 0.5 μM and K_i value of 58 nM. Compound **36** treated with 50 μM reduced 20% melanin content in the human melanocytes system without significant cytotoxicity. In addition, the zebrafish *in vivo* assay revealed that **36** effectively reduce melanin formation without adverse effects. Moreover, the acute oral toxicity study confirmed that the compound **36** was free of discernable cytotoxicity in mice. Thus, compound **36** is a potential candidate in developing safe and effective pharmacological agent for skin-whitening.

Repurposing of existing drugs has become one of the important approaches in the drug discovery program of developing potent melanogenesis inhibitors. The data associated with an existing drug will reduce the time and cost associated with the intellectual right for developing the novel pharmaceutics. This approach has several advantages; including availability, lower cost and safety/tolerability. Phenylthiourea has long been known as a tyrosinase inhibitor. The researchers retrieved the thiourea-derived drugs in clinical use and investigated their effect on tyrosinase activities. Ethionamide (**26a**) and its analogs (**26c**–**26e**), including prothionamide (**26b**), were identified as tyrosinase inhibitors (Figure 9). Ethionamide is an approved second-line antituberculosis drug used for the treatment of multidrug-resistant tuberculosis. Many antithyroid drugs were identified as potent tyrosinase inhibitors; especially, methimazole **27a**, carbimazole **27b**, thiouracil **27c**, methylthiouracil **27d**, and propylthiouracil **27e** inhibited mushroom tyrosinase (Figure 9).

In general, mushroom tyrosinase is the most frequently used *in vitro* model for screening the hypopigmenting agents in the development of skin-whitening substance, while human and mouse melanocytic lysates were used to a lesser extent. This is because of the tyrosinase from the mushroom *Agaricus bisporus* is abundantly available and can be easily purified. However, in several aspects, the tyrosinase from mushroom is very different from the human tyrosinase. A secreted form of a mushroom tyrosinase is a tetramer enzyme present in the cytosol of the cells, while human tyrosinase is a monomeric and inactive glycosylated membrane bound form. Furthermore, it has been reported that human tyrosinase showed 6-fold higher affinity for *L*-DOPA oxidation activity than the mushroom tyrosinase, the K_m value of human and mushroom tyrosinase for *L*-DOPA were 0.31 mM and 1.88 mM, respectively. In addition, the amino acid sequence identity between human and mushroom tyrosinase is 23%. These structural discrepancies were well correlated in the tyrosinase inhibitory activities assayed by AbTYR and hTYR. In fact, it was found that many melanogenesis inhibitors did not exhibit inhibitory effects on mushroom tyrosinase activity (see comparison of thujaplicins, section human tyrosinase inhibitors).

In conclusion, we hope that this review will be useful to medicinal chemists working on melanogenesis, especially on tyrosinase proteins, to identify novel inhibitors with drug-like properties.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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