RESEARCH ARTICLE

Synthesis of Triazole Schiff’s Base Derivatives and Their Inhibitory Kinetics on Tyrosinase Activity

Feng Yu1*, Yu-Long Jia1*, Hui-Fang Wang1, Jing Zheng1, Yi Cui1, Xin-Yu Fang1, Lin-Min Zhang1, Qing-Xi Chen1,2*

1 State Key Laboratory of Cellular Stress Biology, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, School of Life Sciences, Xiamen University, Xiamen, 361005, China, 2 Key Laboratory for Chemical Biology of Fujian Province, Xiamen University, Xiamen, 361005, China

☯ These authors contributed equally to this work.
*
chenqx@xmu.edu.cn

Abstract

In the present study, new Schiff’s base derivatives: (Z)-4-amino-5-(2-(3-fluorobenzylidene)hydrazinyl)-4H-1,2,4-triazole-3-thiol (Y1), (Z)-3-((2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)hydrazono)methyl)phenol (Y2), (Z)-2-((2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)hydrazono)methyl)phenol (Y3) and 3-((Z)-(2-(4-(((E)-3-hydroxybenzylidene)amino)-5-mercapto-4H-1,2,4-triazol-3-yl)hydrazono)methyl)phenol (Y4) were synthesized and their structures were characterized by LC-MS, IR and 1H NMR. The inhibitory effects of these compounds on tyrosinase activities were evaluated. Compounds Y1, Y2 and Y3 showed potent inhibitory effects with respective IC₅₀ value of 12.5, 7.0 and 1.5 μM on the dipheno-lase activities. Moreover, the inhibition mechanisms were determined to be reversible and mixed types. Interactions of the compounds with tyrosinase were further analyzed by fluorescence quenching, copper interaction, and molecular simulation assays. The results together with the anti-tyrosinase activities data indicated that substitution on the second position of benzene ring showed superior ant-ityrosinase activities than that on third position, and that hydroxyl substitutes were better than fluorine substitutes. In addition, two benzene rings connecting to the triazole ring would produce larger steric hindrance, and affect the bonding between tyrosinase and inhibitors to decrease the inhibitory effects. The anti-tyrosinase effects of these compounds were in contrast to their antioxidant activities. In summary, this research will contribute to the development and design of antityrosinase agents.

Introduction

Melanin existed in bacteria, fungi, plants and keratinocytes of skin and hair of animals, catalyzed by tyrosinase, made the surface coloring, which played an important role in protecting the skin and eye from ultraviolet radiation and preventing overheating of internal organization.
But overexpression of epidermal pigmentation may lead to some dermatological disorders, such as melasma, freckles, and senile lentigines [3]. Tyrosinase, a kind of multifunctional enzyme, mainly contributes to the melanin biosynthesis [4]. The enzyme could catalyze two distinct reactions involving the hydroxylation of monophenols and oxidation of diphenols to quinones [5]. The quinones could polymerize spontaneously to form macromolecular dark pigments or aggregate with amino acids and proteins to increase brown color of the pigment [6,7]. In addition, tyrosinase is involved in the process of insect molting, and fresh-keeping of fruits and vegetables [8–10]. In recent years, studies of tyrosinase mainly focus on pigment obstructive disease, melanoma, albino, early onset alzheimer’s disease [11]. Therefore, it is of pressing need to acquire new tyrosinase inhibitors from different sources. Hydroquinone, kojic acid, azelaic acid, and arbutin as tyrosinase inhibitors have been applied in pharmaceuticals and cosmetics [12–15]. However, hydroquinone is prohibited for its irritation, mutagenesis and cytotoxic effects [16,17]. The use of kojic acid and arbutin are also limited because of their low efficacy in vivo, unsatisfactory formulation stability, and poor skin penetration [18]. Safe and efficient tyrosinase inhibitors will provide theoretical basis for the treatment of pigment disorders and enrich whitening cosmetics markets [19,20]. Mushroom tyrosinase as a mature model has been widely used in estimating of potential antityrosinase agents [21].

The copper ions in the active center of tyrosinase were the central part of catalytic activities of tyrosinase and it were found in the enzyme from different species [22,23]. So synthesis and screening of antityrosinase agents with copper chelating ability have become current research focus [24,25]. Heterocyclic compounds containing triazole ring have extensive biological activities such as antibacterial, antispasmodic, anti-inflammatory, especially a large number of derivatives have been synthesized as antibacterial drugs [26,27]. Because N and S atoms of the compounds played a key role in the coordination of metals at the active site of metalloprotein [28], they may have the ability to chelate the copper ions in active center of tyrosinase. So 1,2,4-triazole was widely used as mother nucleus to synthesize a series of special biological molecules, but few applications in the synthesis of tyrosinase inhibitor were reported. The structure of hydroxyl group on the benzene ring is similar to the enzyme substrate which can competitively inhibit the activity of enzyme. Therefore, using 4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole (AHMZ, CAS No.1750-12-5, the IC_{50} is 32.5 μM), a derivative of triazole, and benzaldehyde as potential moiety to make up a series of new Schiff’s base molecules and explore their antityrosinase activities is the aims of our current work. The results could provide references for developing tyrosinase inhibitor as addition agent for use in fields of whitening cosmetics or fruit and vegetable preservation.

Materials and Methods

Materials

The tyrosinase, from mushroom, was bought from Sigma Chemical Co. (St. Louis, MO, USA) and the activity was 6680 U/mg. 4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole, 3-hydroxy benzaldehyde, 2-hydroxy benzaldehyde and 3-fluorobenzene formaldehyde were obtained from Aladdin Industrial Co. (Shanghai, China). Other reagents were all analytical grade.

Synthesis

Schiff’s base derivatives Y₁, Y₂, Y₃ and Y₄ have been synthesized by reactions between 4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole and benzaldehyde derivatives in ethanol under reflux [29]. The products were filtrated and washed several times with ethanol and dried by suction filter. The synthetic procedures were described in Fig 1.
The structures of compounds Y1 to Y4 were verified by $^1$H NMR, IR and MS. The mass spectroscopy of compounds showed molecular ions peak [M+H]$^+$.  

### Enzyme assay

L-Tyr and L-DOPA were used as the substrate to test the monophenolase and diphenolase activities of tyrosinase, respectively [30]. The reaction system (3 ml) contained 1.1 mM L-Tyr or 0.5 mM L-DOPA, 25 mM PBS buffer (pH 6.8) and different concentrations of the inhibitors [31]. For monophenolase and diphenolase activities, the final concentration of tyrosinase was $33.3\, \mu g/ml$ and $6\, \mu g/ml$, respectively. The increased optical density with the oxidation of the substrates at 475 nm (the absorption coefficient was 3700 $M^{-1}cm^{-1}$) within a certain time were measured by DU800 spectrophotometer [32]. IC$_{50}$ was defined as the concentration of inhibitor inhibited 50% of the enzyme activity, which directly reflects the effect of the inhibitors. The Michaelis-Menten constant $K_m$ of tyrosinase was determined by Lineweaver-Burk plot with different concentrations of L-DOPA as substrate and the inhibition constants $K_i$ and $K_{IS}$ were determined by the secondary plots of the apparent $K_m/V_m$ or $1/V_m$ versus the concentration of the inhibitors [33]. The inhibition mechanism was reflected by the plots of $1/v$ versus $1/[S]$ with various concentrations of mushroom tyrosinase [34].

### Fluorescence quenching experiments

Fluorescence quenching means the decrease of fluorescence intensity between the fluorescent molecules and solute molecules. This method was usually used to study the interaction between conformation of protein molecules and small molecules [35]. Cary Eclipse fluorescence spectrophotometer was used to record the fluorescence intensities with an excitation wavelength of 280 nm and emission slit widths of 5 nm [33]. $Y_1$, $Y_2$ and $Y_3$ do not haven fluorescence phenomenon at the excitation wavelength. In this study, the inhibitor was added in 0.3 mg/ml tyrosinase solution to detect the fluorescence intensity changes and the final concentrations of inhibitor range from 10 to 70 $\mu M$.

### Copper interaction

The method to study the relationship between the copper ions and the compounds was similar to that described by Xiao-Xin Chen et al with slight modification [36, 37]. The reaction media include 0.5 mM PBS buffer, $33.3\, \mu M$ inhibitor solutions and different concentrations of Cu$^{2+}$. The DU800 spectrophotometer was used to record the spectra ranged from 300 nm to 450 nm 30 seconds after the addition of CuSO$_4$. 

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**Fig 1. Synthetic processes of compound $Y_1$, $Y_2$, $Y_3$ and $Y_4$.**

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Antioxidant assay

ABTS free-radical scavenging assay was used to test the antioxidant capacity of compounds Y1 to Y4. The method can be found in many articles [38, 39]. 7 mM ABTS and 2.45 mM ammonium persulfate were blended and stored in a dark place 16 hours, and then diluted by 80% ethanol to obtain the working solution which gave an absorbance of 0.69 at 734 nm. The working solution and compounds were mixed and allowed to react for ten minutes, and then measured the absorbance at 734 nm (A0) was measured using spectrophotometer. L-ascorbic acid and 80% ethanol was used as a positive and blank control, respectively. The A0 value was the blank absorbance. The antioxidant rate (%) = \((A_0 - A_I)/A_0 \times 100\%\)

Molecular docking

The molecular docking technique allows better understanding of the potency of all compounds as inhibitors and the structure and activity relationship. We proceeded to examine the interactions of tyrosinase and compound Y1, Y2 and Y3. In this study, ChemDraw software was used to display the molecular models of the compounds and tyrosinase-inhibitors docking were demonstrated by molecular operation environment software (MOE). The polyphenol oxidase 3 (ppo3, PDB code: 2Y9W) without the structure of the exogenous protein, the PEG and water molecules was used as the protein model [40,41]. The bonding site of the enzyme in hydrophobic pocket nearby the copper ions was screened by software for the highest score. Before docking, the structure of tyrosinase molecule and ligand were energy minimized by MOE software.

Results

IR, 1H NMR and mass spectrometry

The products were white powders and dissolved in DMSO or water with alkali promoting. The followings were the data of IR, 1H NMR and LC-MS spectra of the compounds.

(Z)-4-amino-5-(2-(3-fluorobenzylidene)hydrazinyl)-4H-1,2,4-triazole-3-thiol (Y1): IR (S1 Fig) (KBr, vmax, cm-1) 3289, 3269(-NH2), 3197(-NH), 1648(C = N); 1H NMR (S2 Fig) (600 MHz, DMSO-d6) δ 10.84 (s, 1H), 8.32 (d, J = 2.3 Hz, 1H), 7.51–7.38 (m, 3H), 7.24–7.07 (m, 1H), 5.53 (d, J = 2.0 Hz, 2H), 5.28 (d, J = 2.1 Hz, 1H); LC-MS (m/z) (S3 Fig): observed, 253.35 [M+H]+; calculated, 252.27 [M]+.

(Z)-3-((2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)hydrazono)methyl)phenol (Y2): IR (S4 Fig) (KBr, vmax, cm-1) 3351(-OH), 3244, 3200(-NH2), 3153(-NH), 1651(C = N); 1H NMR (S5 Fig) (600 MHz, DMSO-d6) δ 12.94 (s, 1H), 10.60 (s, 1H), 9.53 (s, 1H), 8.23 (s, 1H), 7.21 (t, J = 7.8 Hz, 1H), 7.09 (t, J = 1.9 Hz, 1H), 6.99 (dt, J = 7.6, 1.2 Hz, 1H), 6.77 (ddd, J = 8.1, 2.6, 1.0 Hz, 1H), 5.50 (s, 2H); LC-MS (m/z) (S6 Fig): observed, 251.33 [M+H]+; calculated, 250.28 [M]+.

(Z)-3-((Z)-(2-(4-(((E)-3-hydroxybenzylidene)amino)-5-mercapto-4H-1,2,4-triazol-3-yI)hydrazono)methyl)phenol (Y4): IR (S7 Fig) (KBr, vmax, cm-1) 3448(-OH), 3283, 3247(-NH2), 3176(-NH), 1646(C = N); 1H NMR (S8 Fig) (600 MHz, DMSO-d6) δ 13.01 (s, 1H), 11.00 (s, 1H), 10.91 (s, 1H), 8.50 (d, J = 2.1 Hz, 1H), 7.48–7.37 (m, 1H), 7.24 (t, J = 7.7 Hz, 1H), 6.95–6.84 (m, 2H), 5.54 (d, J = 2.1 Hz, 2H); LC-MS (m/z) (S9 Fig): observed, 251.33 [M+H]+; calculated, 250.28 [M]+.

3-((Z)-(2-(4-(((E)-3-hydroxybenzyldiene)amino)-5-mercapto-4H-1,2,4-triazol-3-yl)hydrazono)methyl)phenol (Y): IR (S10 Fig) (KBr, vmax, cm-1) 3755(-OH), 3187, 3110(-NH2), 3020(-NH), 1637(C = N); 1H NMR (S11 Fig) (600 MHz, DMSO-d6) δ 10.68 (s, 1H), 9.98 (s, 1H), 9.84 (s, 1H), 9.57 (s, 1H), 8.25 (s, 1H), 7.45–7.28 (m, 3H), 7.22 (t, J = 7.8 Hz, 1H), 7.13 (t, J = 1.9 Hz, 1H), 7.03 (dt, J = 7.8, 1.7 Hz, 2H), 6.84–6.74 (m, 1H); LC-MS (m/z) (S12 Fig): observed, 355.42 [M+H]+; calculated, 354.39 [M]+.
Effects of the compounds on mushroom tyrosinase

L-DOPA as the diphenolase substrate of tyrosinase was used to screen the compounds with good antityrosinase activity. The results showed that compounds Y1 to Y3 had good inhibitory activity on diphenolase. Their IC₅₀ values were determined to be 12.5, 7.0 and 1.5 μM, respectively. However, compound Y4 almost do not have antityrosinase activity. Then the inhibitory effects of the three compounds on monophenolase activity of tyrosinase were also determined. The results for the oxidation of the L-Tyr were shown in Fig 2AII, 2BII and 2CII, respectively.

In Fig 2, curves 0 to 4 expressed the monophenolase kinetics with different concentrations of inhibitors, and the activities of tyrosinase dropped significantly with the concentrations of the inhibitors increasing. After a certain period of time, the reaction slopes were constant and enzyme catalysis reached steady-state. The different slopes represented the stable activity of monophenolase, which reflected the oxidation rates of L-Tyr. The curves of Fig 2AII, 2BII and 2CII represented the dynamic trend of the steady-state activity of monophenolase with various concentrations of the inhibitors. The concentration of Y1, Y2 and Y3 to decrease half of the steady-state activities of monophenolase was 185, 245 and 95 μM, respectively. The results showed that the inhibitory effects of the compounds on the reaction rates of diphenolase and monophenolase were dose-dependent. A conclusion could be drawn from Fig 2 that inhibitory effects of inhibitors on diphenolase were higher than that of monophenolase inhibition.

Inhibitory mechanisms, types, and constants of the compounds on mushroom tyrosinase

Under different concentrations of the enzyme, the residual activity of tyrosinase with a fixed amount of substrate was tested. The results were showed in Fig 3AI, 3BI and 3CI. The straight lines 1 to 5 all passed through the origin, which indicated that the inhibitory mechanisms of Y1, Y2 and Y3 on tyrosinase activity were reversible.

In order to explore the inhibitory types of the Schiff’s bases Y1 to Y3, the concentration of tyrosinase was kept constant and measured the initial reactions velocity (v₀) under various concentrations of substrate were measured. A set of straight lines (Fig 3AII, 3BII and 3CII) of 1/v₀ versus 1/[S] all passed through the second quadrant or third quadrant. The results indicated that the inhibitory types between the inhibitors and tyrosinase were of mixed type including competitive, non-competitive and anti-competitive inhibition. The constants Kᵢ, for the inhibitor bonding with the free enzymes, were shown in Fig 3AIII, 3BIII and 3CIII. Furthermore, the constants KᵢS, for the inhibitors bonding with enzyme-substrate complexes, were shown in Fig 3AIV, 3BIV and 3CIV. Kᵢ and KᵢS were acquired from the straight lines of the slopes and vertical intercepts versus the concentrations of inhibitors, respectively.

KI and KᵢS Its values of Y₁, Y₂ and Y₃ were determined to be 6.67 and 4 μM, 7.94 and 27.8 μM, 15.47 and 21.04 μM, respectively. The parameters of inhibition were summarized in Table 1.

Fluorescence quenching

Fluorescence quenching can be divided into static quenching and dynamic quenching. Dynamic quenching is a process, which has energy or electron transfer, while the static quenching process will generate complexes without fluorescence [42]. The quenching rate between the fluorescent
molecules and quenching agents followed the Stern-Volmer curve equation [43]:

$$\frac{F_0}{F} = 1 + K_{SV} [Q]$$

In the formula, $F_0$ is the intensity of fluorescence without quenching agent, $F$ is the fluorescence intensity after adding quencher, $K_{SV}$ (L/mol) represents the relationship of dynamic equilibrium between biological macromolecules and fluorescence quencher molecules after diffusing and colliding, and $[Q]$ is the concentration of quencher. From Fig 4D, the $K_{SV}$ of Y1, Y2 and Y3 was determined to be 94437, 7160983 and 159186 L/mol, respectively. The rate constants of $K_{SV} < 100$ L/mol in dynamic quenching process indicated that the quenching process was not controlled by diffusion but perhaps a static quenching process which will have an impact on protein secondary structure and physiological activity.

In addition, the fluorescence intensity decreased and the emission wavelength values slightly increased with the concentration of inhibitors increasing, which further illustrated the generation of complexes between inhibitors and tyrosinase (Fig 4A, 4B and 4C).

Copper interaction

Tyrosinase has two copper ions in its active center, which catalyzes the adjacent hydroxylation of monophenol into diphenol and then catalyzes the reaction of diphenol into quinones [44]. The whole wavelength scanning experiment detected the bonding ability of the three compounds and copper ions, which was used to infer the intensity of the inhibitors to combine with tyrosinase and reveal inhibitory mechanisms. The results were shown in Fig 5.

As observed in Fig 5a, 5b and 5c, a new absorption peak was generated at about 380 nm in Fig 5c, thus only compound Y3 could combine with copper ion. A conclusion could be speculated: Y3 could combine with the copper ions in the active center of tyrosinase and the ability...
of the substituted group to chelate the copper ions was related to its location on the benzene ring. And the hypothesis will be further confirmed by molecular docking analysis.

Antioxidant assay

Triazole derivatives generally have extensive biological activities, because of its thiol and hydroxyl groups, it may have strong antioxidant activity. With vitamin C (Vc) and AHMZ as

Table 1. The Inhibition parameters of compounds Y₁-Y₃ on tyrosianse.

| parameters         | Y₁     | Y₂     | Y₃    |
|--------------------|--------|--------|-------|
| IC₅₀(μM)           | 12.5   | 7      | 1.52  |
| Kᵢ                | 6.67   | 7.49   | 15.47 |
| Kᵢₛ               | 4      | 28     | 21.04 |
| Inhibition type    | Mixed  | Mixed  | Mixed |
| Inhibitory mechanisms | Reversible | Reversible | Reversible |

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control, the antioxidation abilities of the compounds Y1 to Y4 were assayed and the results were shown in Fig 6.

The determination of antioxidant activity achieved the desired results and the properties were dose-dependent. The antioxidant capacities were in the range of $Y_1 > Y_2 > Y_3 > Y_4 > Vc > AHMZ$. It can be concluded that the combination of ATMZ and benzaldehyde could improve the antioxidant capacity. This result may be explained by the superimposition effect of reducing groups (hydroxyl, thiol and amino).

**Molecular docking**

Molecular simulations further clarified the underlying mechanisms of compounds in the active center, which would give a more convincing conclusion by combining the results of copper ions mutual effect. Fig 7 depicted the docking conformation of the four compounds in the tyrosinase catalytic center.
For the substituent on the benzene ring, only the hydroxyl of compound Y₃ could interact with the copper ion in the active center of enzyme. Some atoms on compound Y₁ to Y₃ act as the acceptor or donor generate hydrogen bonds with the center of tyrosinase. Compound Y₁ generated hydrogen bonds with His₂₄₄ and Glu₂₅₆, Y₂ with Glu₂₅₆, His₂₅₉, His₈₅, His₉₄, Y₃ with His₂₄₄ and Glu₂₅₆. In addition compound Y₁ could also form interactions with tyrosinase.
residues: Ala286, His263, Phe264, Asn260, Val283, His259, His85, His94, Phe90, His61, which was shown in Fig 7AIII. Compound Y2 formed interactions with tyrosinase residues: Val283, Ser282, His263, Ala286, Phe292, His61, His296, Tyr97, Trp93, Cy83, Phe90, Phe264, Asn260, His244, which was shown in Fig 7B. Compound Y3 form interactions with tyrosinase residues: Val283, Phe90, Asn260, His85, His61, His296, Ala286, His259, Met257, with the result was shown in Fig 7C. Moreover, compound Y4 could also form interaction with tyrosinase residues: Val248, Val283, His259, His61, Phe292, Met280, Phe264, Ala286, His263, Asn260, His244, Met257, Glu256, which was shown in Fig 7D.

Discussion

The methods of decreasing the activity of melanocytes or avoiding UV radiation all could effectively reduce the accumulation of melanin. However, inhibiting the activities of tyrosinase was most effective when the damage has been done [45,46]. For the purpose of acquiring efficient antityrosinase agents, we synthesized 10 compounds and 3 from them were found to exhibit antityrosinase activities. This article reported the synthesis of tyrosinase inhibitors and screening on monophenolase and diphenolase of tyrosinase, tested the inhibitory types, mechanisms, fluorescence quenching, copper ions interaction, molecular simulation to further unravel the mechanisms, and its oxidation resistance as a supplement of biological activity.

Compounds Y1 to Y3 have inhibitory effect on monophenolase and diphenolase activities of mushroom tyrosinase, and the inhibition effects on enzyme were dose-dependent. For monophenolase activity, the compounds suppressed the steady-state activities drastically to decrease the process of enzyme catalysis. For the diphenolase activity, reversible inhibition indicated that the inhibitors suppressed tyrosinase not through reducing the number of the dynamic enzymes. The mixed inhibitory types showed that Y1, Y2 and Y3 suppressed the enzyme activities through bonding with the free enzymes and the enzyme-substrate complexes. Fluorescence quenching results reflected the tyrosinase molecular conformation could be changed by the quencher, which directly showed that tyrosinase could combine with inhibitors to form. The result revealed that the inhibition on tyrosinase did not include the anti-competitive inhibition and the bonding between inhibitors and enzyme may alter the conformations of bonding sites of the substrate to make the bonding difficult or the inhibitor may combine with enzyme-substrate complex and changed the conformation of enzyme to form the inactive inhibitor-enzyme-substrate complexes. Copper ions interaction and molecular docking experiment further exposed the reason of the inhibitory effect on tyrosinase. The inhibition abilitise of the inhibitors on tyrosinase were closely related to the structures of the inhibitors. From the slight difference of molecular structures in the docking information, the following information could be get: the free amino hydrogen on imidazole ring had the potential ability to inhibit the activity of tyrosinase by forming hydrogen bonds with residues of the enzyme active center. The molecular structures of compounds Y1 to Y4 proved that the inhibitory effects of 2-substitutes on the benzene ring were better than 3-substitutes and hydroxyl substitutes were better than that of fluorine substitutes. In addition, two free amino groups on the imidazole ring connected with two benzene rings respectively, the groups would generate greater steric hindrance and affect the bonding between tyrosinase and inhibitors resulting in reduced inhibitory effect. The differences of structure affect the degree of inhibition on the enzyme and the way of interaction with the enzyme which is in agreement with previous reports [47,48].

The antioxidant abilities of the compounds Y1 to Y4 as a supplement of biological activity were assayed. Comparing the differences of molecular structures, the position and type of the substituent on the benzene ring affected the antioxidant capacity. The results that fluorine substitutes were superior to hydroxyl substitutes, and that 3-substituted on the benzene ring were better than
2-substitutes were in contrast to their antityrosinase effects. The antioxidant mechanisms of the compounds are different from their antityrosinase mechanisms, which needs further studies.

In summary, three novel compounds were synthesized as antityrosinase agents. Through many primary determinations, the inhibitors showed effective inhibitory effects on mushroom tyrosinase and the inhibition mechanisms were unraveled. Our current work offered some guidance to the design of novel tyrosinase inhibitors. However, further studies are needed to explore their inhibitory effect on excessive synthesis of melanin, their usage in fresh-keeping, or their other biological activities.

Supporting Information

S1 Fig. The IR spectrum of Compound Y₁.
(TIF)
S2 Fig. The ¹H NMR spectrum of Compound Y₁.
(TIF)
S3 Fig. The LC-MS spectrum of Compound Y₁.
(TIF)
S4 Fig. The IR spectrum of Compound Y₂.
(TIF)
S5 Fig. The ¹H NMR spectrum of Compound Y₂.
(TIF)
S6 Fig. The LC-MS spectrum of Compound Y₂.
(TIF)
S7 Fig. The IR spectrum of Compound Y₃.
(TIF)
S8 Fig. The ¹H NMR spectrum of Compound Y₃.
(TIF)
S9 Fig. The LC-MS spectrum of Compound Y₃.
(TIF)
S10 Fig. The IR spectrum of Compound Y₄.
(TIF)
S11 Fig. The ¹H NMR spectrum of Compound Y₄.
(TIF)
S12 Fig. The LC-MS spectrum of Compound Y₄.
(TIF)
S13 Fig. The article summary. Synthesis of triazole Schiff’s base derivatives and the study of tyrosinase inhibitory mechanism.
(TIF)

Author Contributions

Conceived and designed the experiments: FY YLJ QXC. Performed the experiments: FY YLJ HFW JZ LMZ. Analyzed the data: FY YC XYF. Contributed reagents/materials/analysis tools: FY YC LMZ XYF. Wrote the paper: FY YLJ HFW QXC.
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