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Synthesis and bioactivity of novel isoxazole chalcone derivatives on tyrosinase and melanin synthesis in murine B16 cells for the treatment of vitiligo

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ABSTRACT

A new series of chalcone derivatives **1**–**18**, bearing isoxazole moieties were designed and synthesized, and biologically evaluated for their activity on mushroom tyrosinase and melanin synthesis in murine B16 cells. The result indicated that most of prepared compounds **1**–**18** showed potent activating effect on tyrosinase, especially for **1**–**2**, **4**, **6**–**7**, **9** and **15**. Among them, compounds **2**, **4** and **9** demonstrated the best activity with EC_{50} = 1.3, 2.5 and 3.0 μmol·L^{-1} respectively, much better than the positive control 8-methoxypsoralan (8-MOP, EC_{50} = 14.8 μmol·L^{-1}); In B16 cells, all the tested compounds exhibited a stronger activity on melanogenesis than 8-MOP (with the value of 115%). It was interesting that derivatives substituted with halogen (**1**, **2**, **4**, **5**, **7**, **9**) were generally more potent. Compounds **2** (463%) and **18** (438%) with 3 and 4-fold potency compared with 8-MOP respectively, were recognized as the most promising candidate hits for further pharmacological study of anti-vitiligo.

1. Introduction

Melanin, derived from dopaquinone and synthesized in the melanosomes of melanocytes, serves number of valuable physiological functions with the most important being photoprotection of the human skin from ultra-violet (UV) radiation.[1] More than 100 distinct genes have been found being involved in the regulation of melanogenesis.[2] Melanin synthesis is regulated by enzymatic cascade, such as tyrosinase, tyrosinase-related protein 1 (TRP-1) and tyrosinase-related protein 2 (TRP-2).[3,4] Among them, tyrosinase is regarded as the rate-limiting enzyme of melanogenesis, which modulates this process by catalyzing the hydroxylation of tyrosine into 3,4-dihydroxyphenylalanine (DOPA) and the further oxidation of DOPA into dopaquinone.[5–7]

Vitiligo also named leukoderma, is an acquired, progressive, depigmenting disorder characterized by the development of white macules in the skin due to the chronic, selective loss of functional melanocytes in the epidermis.[8] Depigmentation may be the symptom of severe psychological distress, autoimmune abnormality, and increased risk of psychiatric morbidity. So far, several theories have been put forward to explain the pathogenesis of vitiligo.[9] However, none of them is generally accepted by academia, and the clinical treatment of this disease was largely absent from the effective medicines. The *Vernohia anthelmintica* L. (Fig. 1) is a kind of plant growing only in the high altitude localities of southern Xinjiang and limited regions of Pakistan and India. Its fruits extract is one of the most popular Uygur medicines used for vitiligo and initially recorded in "*Yao Yong Zong Ku*" around 300 years ago.[10–13] Some important flavonoid compounds are isolated from the plant (Fig. 2),[14–17] and it is suggested that they play an important role in this treatment. Since these flavonoid compounds may activate tyrosinase and improve the melanin production.[18,19]

Unfortunately, few flavonoids as activator of tyrosinase had been reported. Some derivatives of carbazole chalcone uera[20] and 4′-(phenylurenyl/thiourenyl)chalcone[21] (Fig. 3), which bearing a poor activator effect on banana tyrosinase were first synthesized and investigated. In addition, Dubois et al.[22] synthesized several aurones (**1a**–**1e**) and discovered that **1c**, **1d** can behave as hyperbolic activators of mushroom tyrosinase (Fig. 4); Haudecoeur et al.[23] prepared a series of 24 aurones with different hydroxylation patterns on A, B rings, evaluated their abilities on tyrosinase

Abbreviations: 8-MOP, 8-methoxypsoralan; UV, ultra-violet; TRP-1, tyrosinase-related protein 1; TRP-2, tyrosinase-related protein 2; DOPA, 3,4-dihydroxyphenylalanine; EWG, electron-withdrawing group.

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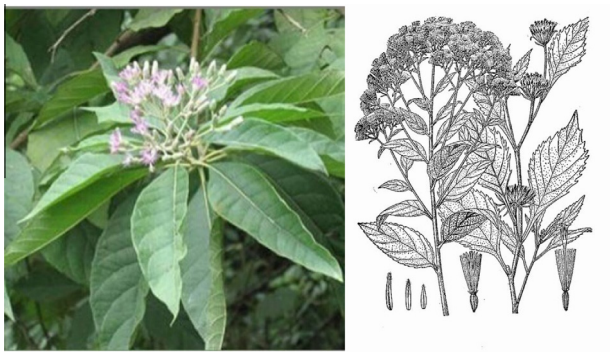


Figure 1. The plant of the *Vernohia anthelmintica* L.

from mushroom to bacterial respectively, and the result showed that **6b–6c**, **7b–7c**, **8b–8c** and **9b–9c** can improve the activity of mushroom tyrosinase (Fig. 5).

Our group has been dedicated on the drug development of the vitiligo for many years.[24,25] In our previous research,[26] three novel benzothiazole chalcones and two amide chalcones with potent activating effect on mushroom tyrosinase were exploited (Fig. 6).

In addition, 1,2,3-triazole was introduced to A ring of chalcone instead of thiazole. Fortunately, it was found that six novel chalcone derivatives were able to activate the mushroom tyrosinase (Fig. 7), especially for compounds **10a** and **14a**, which possessed a better activity than the positive control 8-MOP.[27]

The isoxazole was widely use in medicinal chemistry, can be regarded as isostere of the thiazole or triazole and possessed an extensive range of biological activities.[28–34] Beside, it could actively participate in hydrogen bonding and was very stable in most reaction conditions. Inspired by above results, the active molecules were further optimized by replacing the 1,2,3-triazole with isoxazole with the aim of developing a better medication for the vitiligo. In this paper, eighteen novel chalcone derivatives bearing substituted isoxazole on ring A were synthesized, and the activity of them on mushroom tyrosinase in cell-free and melanin content in murine B16 cells was studied and the SAR was summarized.

2. Result and discussion

2.1. Synthesis

The synthetic procedure of the target compounds was shown in Scheme 1. Commercially available aromatic aldehydes were condensed with hydroxyl amine hydrochloride to obtain their

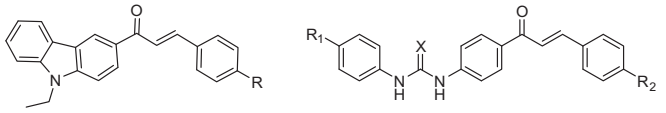


Figure 3. The structure of the carbazole and phenylurenyl/thiourenyl chalcone derivatives.

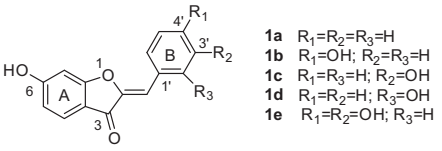


Figure 4. The structure of the synthetic aurones.

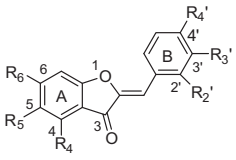
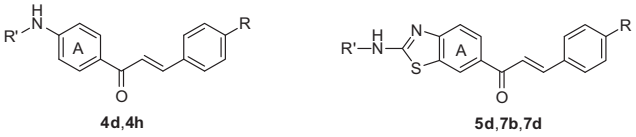


Figure 5. The structure of 24 hydroxyl aurones.



Compound	Substituent	EC_{50}(μ M)
4d	R=Cl,R'=Bz	21.1
4h	R=Cl,R'=Ts	25.8
5d	R=Cl,R'=H	30.6
7b	R=CH_3,R'=Bz	17.1
7d	R=Cl,R'=Bz	9.6
8-MOP(positive control)	-	14.8

Figure 6. The structure of the benzothiazole and amide chalcones.

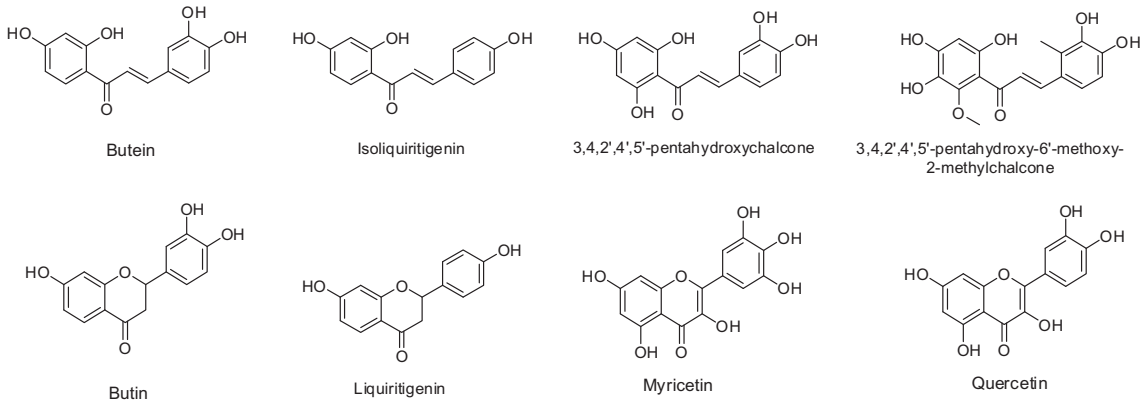
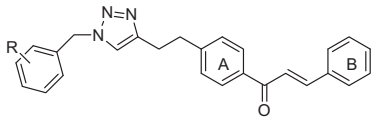


Figure 2. The structure of the flavonoid compounds isolated from the *Vernohia anthelmintica* L.



Compound	Substituent	EC_{50}(μ M)
3a	R=3-OCH_3	37.4±2.2
8a	R=3-Cl	11.9±0.9
9a	R=4-Cl	23.9±2.6
10a	R=3,4-Cl	1.71±0.23
14a	R=3-F	5.6±0.5
15a	R=4-F	14.6±1.4
8-MOP(positive control)	-	14.80± 1.5

Figure 7. The structure of the chalcones with 1,2,3-triazole.

corresponding oxime derivatives (**a**′). They were converted to their corresponding hydroxymethylisoxazoles (**b**′) by intermolecular cycloaddition with propynol in presence of *N*-chlorosuccinimide. The intermediates **b**′ were finally brominated to give the desired **c**′ by PBr_3 in dichloromethane at 0 °C.[35]

The 4′-hydroxyl chalcone was prepared through the Claisen–Schmidt condensation of the corresponding acetophenones and benzaldehydes using NaOH as base,[36,37] followed by deprotection with HCl in ethanol. The 4′-hydroxyl chalcone was then refluxed with **c**, catalyzed by K_2CO_3 in acetone to yield the final compounds **1**–**18**.

All new compounds were characterized by ^1H NMR, ^{13}C NMR, IR and HRMS (ESI). As shown in Figure 8, the single crystals of the compounds **1** and **5** (**1**: CCDC No. 1476858; **5**: CCDC No. 1476859) used for X-ray diffraction analysis were obtained by slow evaporation of acetone-ethanol (*V*/*V* = 1:3) mixed solution at room temperature.

2.2. Tyrosinase activity evaluation in cell-free system

The activities on tyrosinase of synthesized compounds **1**–**18** were performed according to a modified method,[38] with 8-MOP[39–41] as positive control. The effects of the compounds were summarized in Table 1. As shown in Table 1, most of prepared compounds **1**–**18** showed good to excellent activating effect on tyrosinase. The potencies of compound **1** (with the value of EC_{50} = 6.4 μmol·L^{-1}), **2** (EC_{50} = 1.3 μmol·L^{-1}), **4** (EC_{50} = 2.5 μmol·L^{-1}) and **6** (EC_{50} = 8.1 μmol·L^{-1}), **7** (EC_{50} = 12.7 μmol·L^{-1}), **9** (EC_{50} = 3.0 μmol·L^{-1}) and **15** (EC_{50} = 11.4 μmol·L^{-1}) were better to the positive control 8-MOP, of which EC_{50} was 14.8 μmol·L^{-1}.

The position, number and nature of the substituent on benzene ring were varied in order to identify the most appropriate group. The compounds with halogen (**1**–**7**, **9**) and -CF_3O (**11**) showed higher activity compared with -$N(CH_3)_2$ (**13**), -OCH_3 (**8**, **10**) and un-substituted (**12**), which suggested that the electron-withdrawing group (EWG) may be favorable to enhance the activity. However, the activity dropped rapidly when substituted with -NO_2 (**14**), indicating that substituents with too strong electron-withdrawing ability must have no benefit to the activity.

Among these halogenated compounds **1**–**7** and **9**, the most important factor in their efficacy was the location of the group. The shift of -F substituent from the *para* (**1**, EC_{50} = 6.4 μmol·L^{-1}) into the *meta* position led to **5** (EC_{50} = 21.3 μmol·L^{-1}), which had a 3.3-fold lower activity. The similar result was observed in compounds **6** and **3** which were substituted with Cl group as well. It is apparent that the presence of a halogen group in the *meta* or *para* position of the phenyl ring was crucial for activity.

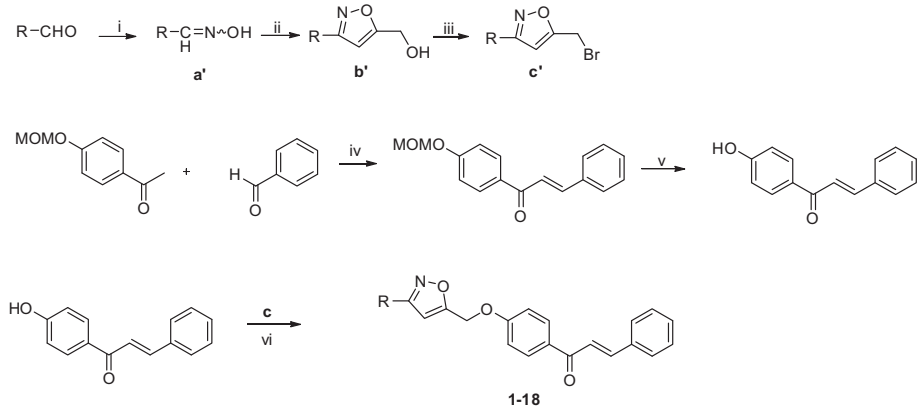
The replacement of the -F group with -Cl contributed no enhancement to the activity. Nevertheless, the number of the halogen atom on benzene made a great influence on activity. Introduction of a second -F or -Cl to the benzene strongly increased activity, such as **2**, **4**, **7** and **9** compared with **1**, **6**, **3** and **5**. The interesting thing was that 3,5-disubstituted compound **7** was less potent than 3,4-disubstituted compound **2** (EC_{50} = 1.3 μmol·L^{-1}), **4** (EC_{50} = 2.5 - μmol·L^{-1}) and **9** (EC_{50} = 3.0 μmol·L^{-1}), which exhibited a most promising activity than any other compounds.

However, the activity decreased dramatically when the benzene on isoxazole was substituted by furan (**15**, **16**), thiophen (**17**) and 1,4-benzodioxane (**18**), which suggested that benzene was fundamental for the activity.

Compared with the 1,2,3-triazole compounds in our previous research[21] (Fig. 3), the active isoxazole compounds in this work (**1**, **2**, **4**, **6**, **7**, **9** and **15**) generally showed a better activity, which may be influenced by the shortened distance between benzene and isoxazole, characteristics of the isoxazole as well.

2.3. Melanin synthesis evaluation in B16 cells

In order to avoid the possibility that inhibition of melanin synthesis was due to cytotoxicity, we first performed CCK-8 assay to



1	R=4-fluorophenyl	**2**	R=3,4-difluorophenyl	**3**	R=3-chlorophenyl
4	R=3,4-dichlorophenyl	**5**	R=3-fluorophenyl	**6**	R=4-chlorophenyl
7	R=3,5-dichlorophenyl	**8**	R= 2-methoxyphenyl	**9**	R=4-chloro-3-fluorophenyl
10	R=4-methoxyphenyl	**11**	R=3-trifluoromethoxyphenyl	**12**	R=phenyl
13	R=4-dimethylaminophenyl	**14**	R=4-nitrophenyl	**15**	R=5-iodofuran-2-yl
16	R=5-methylfuran-2-yl	**17**	R=thiophen-2-yl	**18**	R=2,3-dihydrobenzo[b][1,4]dioxin-6-yl

Scheme 1. Synthetic route for the chalcones with isoxazole. (i) $NH_2OH{\cdot}HCl$, Na_2CO_3, 30% methanol aqueous solution, rt (ii) NCS, propynol, triethylamine, DCM, reflux (iii) PBr_3, DCM, 0 °C (iv) 10% NaOH, ethanol, rt (v) 3 M HCl, ethanol, rt (vi) K_2CO_3, acetone, reflux.

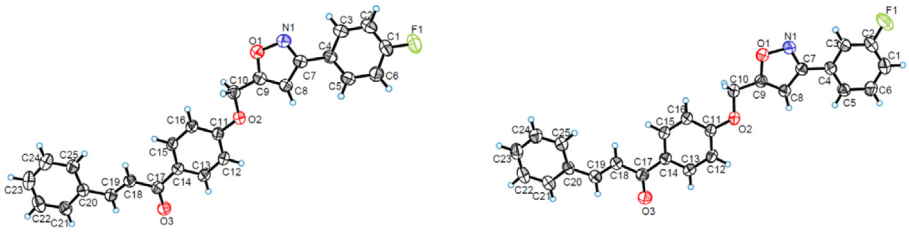


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Figure 8. Chemical structure of the crystals of **1** and **5**.

Table 1
Activator effect on tyrosinase of chalcone derivatives **1**–**18**

Compd	Substrate	R	EC_{50}[a] (μmol/L)
1	L-Tyrosine	4-Fluorophenyl	6.4 ± 0.3
2	L-Tyrosine	3,4-Difluorophenyl	1.3 ± 0.1
3	L-Tyrosine	3-Chlorophenyl	23.8 ± 1.0
4	L-Tyrosine	3,4-Dichlorophenyl	2.5 ± 0.2
5	L-Tyrosine	3-Fluorophenyl	21.3 ± 0.8
6	L-Tyrosine	4-Chlorophenyl	8.1 ± 0.4
7	L-Tyrosine	3,5-Dichlorophenyl	12.7 ± 0.3
8	L-Tyrosine	2-Methoxyphenyl	65.3 ± 3.4
9	L-Tyrosine	4-Chloro-3-fluorophenyl	3.0 ± 0.3
10	L-Tyrosine	4-Methoxyphenyl	47.2 ± 2.3
11	L-Tyrosine	3-Trifluoromethoxyphenyl	25.5 ± 0.7
12	L-Tyrosine	Phenyl	78.1 ± 4.7
13	L-Tyrosine	4-Dimethylaminophenyl	28.9 ± 0.8
14	L-Tyrosine	4-Nitrophenyl	>100
15	L-Tyrosine	5-Iodofuran-2-yl	11.4 ± 0.4
16	L-Tyrosine	5-Methylfuran-2-yl	35.6 ± 2.1
17	L-Tyrosine	Thiophen-2-yl	56.2 ± 3.2
18	L-Tyrosine	2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl	>100
8-MOP	L-Tyrosine	—	14.8

[a] EC_{50} is the concentration of compounds that gave a 50% activator effect (when the rate of activation = 50%). results are the mean ± SD from three independent experiments.

determined whether these chalcone derivatives (**1**–**18**) were cytotoxic to B16 cells. The result showed that the cells treated with compounds for 24 h caused mild cytotoxicity when compared with the control at the dosage of 50 μM (Fig. 9); and the protein concentration of B16 cells were examined as well in Figure 10.

After that, all the compounds were further studied for their activity on melanin synthesis, using a modified method.[42] According to the screening data (Fig. 11), all the tested compounds from **1** to **18** exhibited a stronger activity than the control drug (8-MOP). Among chalcones **1**–**14**, compounds substituted with halogen (**1**, **2**, **4**, **5**, **7**, **9**) and -CF_3O (**11**) were generally more potent than that substituted with -OCH_3 (**8**, **10**) and -$N(CH_3)_2$ (**13**), It is apparent that introduction of a EWG was accompanied by a noticeable increase in activity, except -NO_2 (**14**).

Different halogen were introduced into the different positions of the benzene of isoxazole chalcone led to **1**–**7** and **9**. Two mono fluoro-substituted compounds **1**, **5** and 3,4-dichloro substituted **2** possessed a better activity than monochloro-substituted **3**, **6** and 4-chloro-3-fluoro substituted **9**, especially for **2** (with the value of 463%) and **5** (330%), which were separately 3 and 4-fold potent than the control (8-MOP, 115%). Considering the *meta*-substituted group (**5**) contributed more than the *para* one (**1**), it can be inferred that the type and the position of the halogen was the most important factor for their efficacy. The introduction of a second halogen to benzene (**2**, **4**, **7**, **9**) greatly improved the activity compared with the mono. However, in the case of **7**, a serious decrease (57%) was

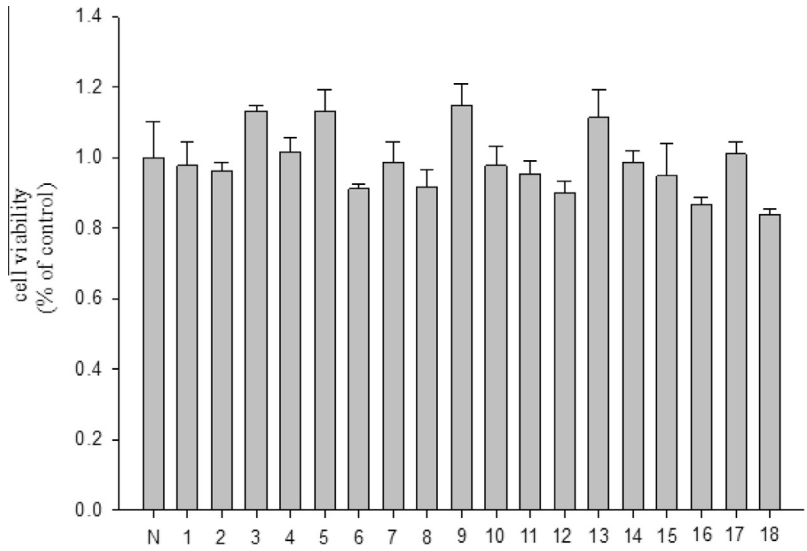

Figure 9. Effect of chalcone derivatives on B16 cells viability. *N* means negative control; the B16 cells were incubated with 50 μM of different chalcone derivatives for 24 h and the cell viability was assayed by adding CCK-8 solution. Values are expressed as the mean ± SD of three separate experiments. $P > 0.05$.

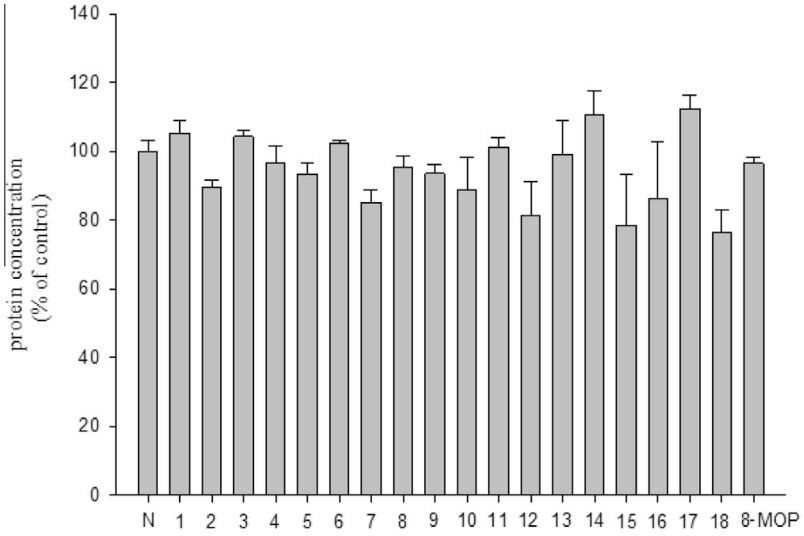

Figure 10. Effect of chalcone derivatives on protein concentration of B16 cells. *N* means negative control; 8-MOP as positive control. The B16 cells were incubated with 50 μM of different chalcone derivatives for 48 h and the protein concentration was assayed by the method described below. Values are expressed as the mean ± SD of three separate experiments. $P > 0.05$.

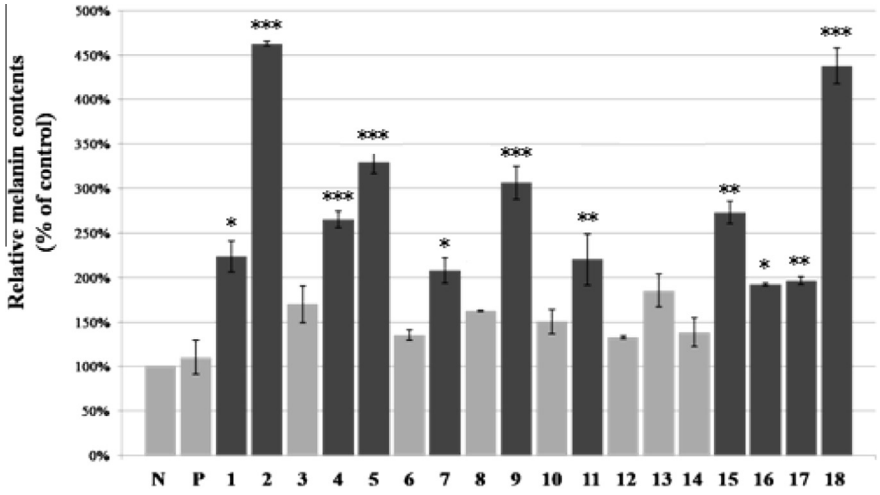

Figure 11. Effect of chalcone derivatives on melanin content in B16 cells. N means negative control; P means positive control (8-MOP); The B16 cells were treated with 50 μM of different chalcone derivatives for 48 h. After that, melanin content was measured directly. Values are expressed as the mean ± SD of three separate experiments. The statistical analysis was performed with one-way ANOVA followed by Tukey's *post hoc* test for multiple comparison tests. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control.

observed compared with **4**, which indicated that position of two halogen substituent on benzene was essential for the activity as well.

When the benzene ring was substituted with furan, thiophen and 1,4-benzodioxane (**15**–**18**), a remarkable increase was noticed, which suggested that the benzene ring was not required for maintaining the activity. The introduction of -I (**15**) also enhanced the melanogenesis as same as we discovered above. It was impressed that compound **18** (438%) with [1,4]dioxine demonstrated the second most promising activity of all chalcone derivatives, which was nearly 4-fold potent than the control 8-MOP.

3. Conclusion

In summary, eighteen chalcone compounds as novel activator of tyrosinase was developed and biologically evaluated, It was found that the introduction of substituted-phenyl isoxazole was an effective way to improve the activity of tyrosinase and the melanogenesis of murine B16 cells: Most of compounds (**1**–**18**) had potent effect on tyrosinase of mushroom, especially for the derivatives with two halogen atoms on phenyl (**2**, **4** and **9**); In cell system, compounds from **1** to **18** exhibited a stronger activity on melanogenesis than 8-MOP (positive control). Among them, **2** and **18** were 3 and 4-fold potent than the 8-MOP, respectively. Additional experiments are also needed on human tyrosinase or melanocytes to support the potential of these compounds for human-directed applications. Currently, further studies on action mechanism of these chalcone derivatives and animal experiment on vitiligo transgenic mouse were under way. This research may provide some new suggestion for the design of the novel activator of tyrosinase based on chalcone.

4. Experimental section

4.1. Chemistry

Reagents and solvents were purchased from Sigma, and used without further purification. Thin-layer chromatography (TLC)

was carried out on glass plates coated with silica gel (Qingdao Haiyang Chemical Co., G60F-254) and visualized by UV light (254 nm). The products were purified by column chromatography over silica gel (Qingdao Haiyang Chemical Co., 200–300 mesh). Melting points were determined on a Buchi B-540 apparatus and uncorrected. All the NMR spectra were recorded with a Varian400 MHz NMR spectrometer in $CDCl_3$, using TMS as an internal standard. High-resolution mass spectra (HRMS) were recorded on AB SCIEX QSTAR Elite quadrupole time-of-flight mass spectrometry. The IR data were recorded on a Thermo Fisher Scientific Nilolet 6700 FT-IR infrared spectrometer (KBr).

4.1.1. General procedure of preparation of intermediate a′

To a mixture of aldehyde (10.0 mmol) in 30% methanol aqueous solution, $NH_2OH{\cdot}HCl$ (0.695 g, 10.0 mmol) was added slowly. After the $NH_2OH{\cdot}HCl$ was fully dissolved, Na_2CO_3 (0.53 g 5.0 mmol) was added and then the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic phase was dried to afford intermediate **a′** as white solids.

4.1.2. General procedure of preparation of intermediate b′

A mixture of **a′** (10.0 mmol) in CH_2Cl_2 was stirred at room temperature, NCS (1.60 g, 12.0 mmol) was then added. The resulted mixture was stirred at reflux and propynol (0.56 g, 10.0 mmol) was added dropwise. After 0.5 h, triethylamine (1.01 g, 10 mmol) was added dropwise and refluxed for another 4 h. After cooling, the reaction mixture was washed with water and evaporated under reduced pressure. The obtained residue was purified by silica gel chromatography with petroleum ether/ethyl acetate as eluent to give intermediate **b′**.

4.1.2.1. 3-(4-Dimethylaminophenyl)-5-(hydroxymethyl)isoxazole. ^1H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.9 Hz, 2H), 6.76 (d, J = 8.3 Hz, 2H), 6.48 (s, 1H), 4.79 (s, 2H), 3.02 (s, 6H).

4.1.2.2. 3-(4-Methoxyphenyl)-5-(hydroxymethyl)isoxazole. ^1H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 8.9 Hz, 2H), 6.95 (d, J = 8.9 Hz, 2H), 6.49 (s, 1H), 4.78 (s, 2H), 3.84 (s, 3H).

4.1.2.3. 3-(3,5-Dichlorophenyl)-5-(hydroxymethyl)isoxazole. ^1H NMR (400 MHz, cdcl$_3$) δ 7.69 (d, J = 1.9 Hz, 2H), 7.44 (t, J = 1.9 Hz, 1H), 6.55 (s, 1H), 4.84 (s, 2H).

4.1.3. General procedure of preparation of intermediate c′

A mixture of **b′** (10 mmol) in anhydrous CH_2Cl_2 (25 mL) was cooled to 0 °C, a solution of PBr_3 (0.95 mL, 10 mmol) in CH_2Cl_2 (2 mL) was added dropwise and stirred in ice-bath until the raw material consumed. When warmed up to room temperature, the reaction mixture was neutralized with 10% NaOH and extracted with CH_2Cl_2 two times, The organic phase was evaporated under reduced pressure and purified by silica gel chromatography with petroleum ether/ethyl acetate to give **c′**.

4.1.3.1. 3-(4-Methoxyphenyl)-5-(bromomethyl)isoxazole. ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.7 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H), 6.57 (s, 1H), 4.50 (s, 2H), 3.86 (s, 3H).

4.1.3.2. 3-(Thiophen-2-yl)-5-(bromomethyl)isoxazole. ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 (ddd, J = 6.1, 4.3, 1.0 Hz, 2H), 7.11 (dd, J = 5.0, 3.7 Hz, 1H), 6.50 (s, 1H), 4.80 (s, 2H).

4.1.3.3. 3-(5-Methylfuran-2-yl)-5-(bromomethyl)isoxazole. ^1H NMR (400 MHz, $CDCl_3$) δ 6.73 (d, J = 3.3 Hz, 1H), 6.43 (s, 1H), 6.09–6.06 (m, 1H), 4.75 (s, 2H), 2.34 (s, 3H).

4.1.3.4. 3-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(bromomethyl)isoxazole. ^1H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, J = 2.0 Hz, 1H), 7.29–7.26 (m, 1H), 6.93 (d, J = 8.4 Hz, 1H), 6.53 (s, 1H), 4.49 (s, 2H), 4.30 (s, 4H).

4.1.4. Preparation of 4′-hydroxy chalcone

To the mixture of 4′-methoxymethoxy acetophenone (0.18 g, 1.0 mmol) and benzaldehyde (1.06 g, 1.0 mmol) in ethanol (20 mL) was added dropwise a solution of KOH (11.2 mg mmol, 0.2 mmol) in ethanol (2 mL) at 0–5 °C. The resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated under reduced pressure, and the residue was diluted with water (35 mL). The aqueous mixture was neutralized by the addition of aqueous 10% HCl solution and extracted with ethyl acetate (2 × 30 mL). The organic phase was washed with aqueous saturated NH_4Cl solution and brine. The organic layer was separated and dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product, which was purified by silica gel chromatography to produce the pure compound 4′-methoxymethoxy chalcone.

A stirred mixture of 4′-methoxymethoxychalcone (0.27 g, 1.0 mmol) in 20 mL 3 M HCl ethanol solution was refluxed for 2 h. After cooling, water was added, the resulted mixture was neutralized with 10% NaOH and extracted with chloroform (2 × 30 mL). The organic phase was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product, which was purified by silica gel chromatography with petroleum ether/ethyl acetate to give 4′-hydroxychalcone.

4.1.4.1. 4′-Hydroxy chalcone. Yield 41%, white solid, mp 178–181 °C, ^1H NMR (400 MHz, CD_3OD) δ 8.01 (d, J = 8.4 Hz, 2H), 7.81 (d, J = 15.7 Hz, 1H), 7.72–7.60 (m, 2H), 7.54 (d, J = 15.7 Hz, 1H), 7.47–7.37 (m, 3H), 6.94 (d, J = 8.4 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 188.82, 159.84, 144.23, 131.29, 131.16, 130.40, 128.93, 128.38, 121.79, 115.44, 110.00; ESI-MS (m/z, %): 223 ([M−H]$^-$, 100).

4.1.5. General procedure of preparation of 1–18

4′-Hydroxychalcone (0.224 g, 1.0 mmol), **c** (1.0 mmol) and K_2CO_3 (0.69 g, 5 mmol) were dissolved in 20 mL of acetone. This solution was refluxed for 5 h, the progress of the reaction was monitored by TLC. When the reaction was complete, 100 mL of water was poured into the solution, the reaction mixture was extracted with $CHCl_3$ for three times. The organic layer was separated and washed with distilled water and dried over anhydrous $MgSO_4$. The solvent was removed at reduced pressure, and the residue was purified by a silica gel column eluted with petroleum ether/ethyl acetate to give the corresponding **c**.

4.1.5.1. 1-(4-((3-(4-Fluorophenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (1). Yield 89%, light yellow solid, mp 163–166 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.7 Hz, 2H), 7.84–7.77 (m, 3H), 7.67–7.62 (m, 2H), 7.53 (d, J = 15.6 Hz, 1H), 7.45–7.39 (m, 3H), 7.15 (t, J = 8.6 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H), 6.64 (s, 1H), 5.29 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.78, 167.98, 165.30, 162.81, 161.44, 144.54, 135.09, 132.36, 131.05, 130.61, 129.09, 128.90, 128.54, 121.84, 116.36, 116.14, 114.71, 101.69, 61.47; IR (KBr) ν: 2920, 2359, 1726, 1657, 1604, 1527, 1343, 1226, 839 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{19}FNO_3$ $[M+H]^+$ 400.1349, found 400.1338.

4.1.5.2. 1-(4-((3-(3,4-Difluorophenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (2). Yield 85%, white solid, mp 160–162 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.7 Hz, 2H), 7.82 (d, J = 15.7 Hz, 1H), 7.70–7.62 (m, 3H), 7.58–7.49 (m,

2H), 7.45–7.40 (m, 3H), 7.08 (d, J = 8.6 Hz, 2H), 6.64 (s, 1H), 5.30 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.44, 161.39, 161.06, 144.62, 135.09, 131.11, 130.65, 129.11, 128.57, 123.45, 118.26, 118.08, 116.32, 116.13, 114.71, 101.62, 61.46; IR (KBr) ν: 2921, 2357, 1760, 1640, 1600, 1545, 1488, 1323, 1215 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{18}F_2NO_3$ $[M+H]^+$ 418.1255, found 418.1270.

4.1.5.3. 1-(4-((3-(3-Chlorophenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (3). Yield 87%, white solid, mp 116–119 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 7.85–7.78 (m, 2H), 7.70 (d, J = 7.2 Hz, 1H), 7.67–7.62 (m, 2H), 7.53 (d, J = 15.7 Hz, 1H), 7.47–7.37 (m, 5H), 7.09 (d, J = 8.8 Hz, 2H), 6.67 (s, 1H), 5.31 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.81, 168.25, 161.68, 161.41, 159.24, 144.56, 135.18, 135.11, 132.41, 131.08, 130.62, 130.42, 129.10, 128.55, 127.13, 125.11, 121.86, 114.72, 101.75, 61.48; IR (KBr) ν: 2922, 2360, 1726, 1658, 1600, 1467, 1257, 1189, 1081 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{19}ClNO_3$ $[M+H]^+$ 416.1053, found 416.1070.

4.1.5.4. 1-(4-((3-(3,4-Dichlorophenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (4). Yield 90%, white solid, mp 161–163 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 1.9 Hz, 1H), 7.85–7.78 (m, 2H), 7.69–7.60 (m, 4H), 7.57–7.50 (m, 3H), 7.45–7.38 (m, 3H), 7.08 (d, J = 8.8 Hz, 2H), 6.66 (s, 1H), 5.31 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.80, 168.53, 161.30, 144.60, 139.18, 134.62, 132.42), 131.19, 131.08, 130.63, 130.56, 129.10, 128.84, 128.55, 128.10, 126.59, 126.08, 121.81, 114.69, 101.62, 61.43; IR (KBr) ν: 2923, 2359, 1805, 1726, 1666, 1599, 1548, 1357, 1219 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{18}Cl_2NO_3$ $[M+H]^+$ 450.0664, found 450.0657.

4.1.5.5. 1-(4-((3-(3-Fluorophenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (5). Yield 83%, light yellow solid, mp 118–121 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 15.7 Hz, 1H), 7.67–7.62 (m, 2H), 7.59 (d, J = 7.7 Hz, 1H), 7.53 (d, J = 15.5 Hz, 2H), 7.47–7.39 (m, 4H), 7.15 (td, J = 8.3, 2.3 Hz, 1H), 7.08 (d, J = 8.8 Hz, 2H), 6.67 (s, 1H), 5.30 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.78, 168.19, 164.35, 161.41, 144.53, 135.08, 132.37, 131.05, 130.82, 130.60, 129.09, 128.54, 122.73, 121.83, 117.42, 117.21, 114.70, 114.12, 113.89, 101.81, 61.44; IR (KBr) ν: 2922, 1834, 1726, 1659, 1604, 1573, 1370, 1286, 1187, 978 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{19}FNO_3$ $[M+H]^+$ 400.1349, found 400.1361.

4.1.5.6. 1-(4-((3-(4-Chlorophenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (6). Yield 88%, white solid, mp 135–139 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 15.6 Hz, 1H), 7.75 (d, J = 8.4 Hz, 2H), 7.67–7.62 (m, 2H), 7.53 (d, J = 15.6 Hz, 1H), 7.46–7.40 (m, 5H), 7.08 (d, J = 8.8 Hz, 2H), 6.66 (s, 1H), 5.30 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.80, 168.14, 161.81, 161.43, 144.57, 136.46, 135.10, 132.40, 131.07, 130.62, 129.42, 129.10, 128.55, 128.27, 127.23, 121.85, 114.71, 101.68, 61.48; IR (KBr) ν: 2924, 1726, 1657, 1605, 1530, 1431, 1345, 1256, 1173, 1094, 826 cm^{-1}; HRMS (ESI) calcd for C_{25}-$H_{19}ClNO_3$ $[M+H]^+$ 416.1053, found 416.1038.

4.1.5.7. 1-(4-((3-(3,5-Dichlorophenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (7). Yield 92%, light yellow solid, mp 159–161 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 15.6 Hz, 1H), 7.72–7.62 (m, 4H), 7.53 (d, J = 15.6 Hz, 1H), 7.46–7.39 (m, 4H), 7.08 (d, J = 8.8 Hz, 2H), 6.66 (s, 1H), 5.31 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.64, 168.43, 161.11, 144.45, 139.04, 135.73, 130.92, 130.40, 130.09, 128.93, 128.38, 127.94, 126.42, 125.23, 121.68, 114.49, 101.48, 61.17; IR (KBr) ν: 2923, 2360, 1726, 1630, 1599, 1547, 1367, 1304, 1171, 975 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{18}Cl_2NO_3$ $[M+H]^+$ 450.0664, found 450.0669.

4.1.5.8. 1-(4-((3-(2-Methoxyphenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (8). Yield 80%, white solid, mp 132–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.8 Hz, 2H), 7.89 (dd, J = 7.7, 1.7 Hz, 1H), 7.81 (d, J = 15.7 Hz, 1H), 7.67–7.61 (m, 2H), 7.54 (d, J = 15.7 Hz, 1H), 7.45–7.38 (m, 4H), 7.09 (d, J = 8.8 Hz, 2H), 7.04 (td, J = 7.6, 0.7 Hz, 1H), 7.00 (d, J = 8.3 Hz, 1H), 6.89 (s, 1H), 5.28 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.77, 166.22, 161.66, 160.36, 157.32, 144.38, 135.09, 132.12, 131.54, 130.97, 130.53, 129.59, 129.05, 128.50, 121.87, 121.08, 114.73, 111.57, 105.51, 61.41, 55.68; IR (KBr) ν: 2925, 2360, 1711, 1641, 1606, 1444, 1347, 1309, 824 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{22}NO_4$ $[M+H]^+$ 412.1549, found 412.1559.

4.1.5.9. 1-(4-((3-(4-Chloro-3-Fluorophenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (9). Yield 90%, white solid, mp 130–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 15.7 Hz, 1H), 7.67–7.59 (m, 3H), 7.57–7.47 (m, 3H), 7.45–7.40 (m, 3H), 7.08 (d, J = 8.8 Hz, 2H), 6.66 (s, 1H), 5.31 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.76, 168.52, 161.35, 161.04, 159.71, 157.23, 144.57, 135.07, 132.42, 131.42, 131.06, 130.62, 129.09, 128.54, 123.34, 121.81, 115.24, 115.01, 114.68, 101.63, 61.41; IR (KBr) ν: 2924, 2359, 1726, 1657, 1604, 1579, 1450, 1256, 1176, 982 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{18}ClFNO_3$ $[M+H]^+$ 434.0959, found 434.0951.

4.1.5.10. 1-(4-((3-(4-Methoxyphenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (10). Yield 86%, white solid, mp 145–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 15.7 Hz, 1H), 7.75 (d, J = 8.8 Hz, 2H), 7.68–7.62 (m, 2H), 7.54 (d, J = 15.7 Hz, 1H), 7.44–7.39 (m, 3H), 7.09 (d, J = 8.9 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 6.62 (s, 1H), 5.29 (s, 2H), 3.86 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.63, 167.33, 162.19, 161.40, 161.15, 144.31, 134.96, 132.11, 130.89, 130.44, 128.94, 128.40, 128.25, 121.72, 121.07, 114.58, 114.36, 101.49, 61.35, 55.35; IR (KBr) ν: 2924, 2359, 1726, 1678, 1620, 1573, 1483, 1378, 1256 824 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{22}NO_4$ $[M+H]^+$ 412.1549, found 412.1563.

4.1.5.11. 1-(4-((3-(3-Trifluoromethoxyphenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylpr-op-2-en-1-one (11). Yield 74%, light yellow solid, mp 138–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 15.7 Hz, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.70–7.62 (m, 3H), 7.57–7.48 (m, 2H), 7.44–7.40 (m, 3H), 7.35–7.29 (m, 1H), 7.09 (d, J = 8.9 Hz, 2H), 6.69 (s, 1H), 5.32 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.81, 168.41, 161.60, 161.41, 144.58, 135.12, 132.45, 131.09, 130.65, 130.64, 129.11, 128.56, 125.36, 122.71, 121.86, 119.61, 114.72, 101.76, 61.48; IR (KBr) ν: 2923, 1657, 1607, 1468, 1450, 1290, 1222, 1174, 983 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{19}F_3NO_4$ $[M+H]^+$ 466.1266, found 466.1275.

4.1.5.12. 1-(4-((3-Phenylisoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (12). Yield 85%, white solid, mp 131–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 7.85–7.78 (m, 3H), 7.67–7.63 (m, 2H), 7.53 (d, J = 15.7 Hz, 1H), 7.48–7.40 (m, 6H), 7.09 (d, J = 8.8 Hz, 2H), 6.68 (s, 1H), 5.30 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.78, 167.74, 162.72, 161.49, 144.47, 135.08, 132.28, 131.02, 130.56, 130.34, 129.07, 129.08, 128.51, 126.96, 121.85, 114.71, 101.84, 61.47; IR (KBr) ν: 2923, 2360, 1678, 1603, 1450, 1343, 1253, 1194, 977 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{19}F_3NO_4$ $[M+H]^+$ 382.1443, found 382.1452.

4.1.5.13. 1-(4-((3-(4-Dimethylaminophenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (13). Yield 87%, yellow solid, mp 106–110 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.9 Hz, 2H), 7.81 (d, J = 15.7 Hz, 1H), 7.72–7.62 (m, 5H), 7.53 (d, J = 15.7 Hz, 1H), 7.44–7.39 (m, 3H), 7.09 (d, J = 8.9 Hz, 2H), 6.60 (s, 1H), 5.27 (s, 2H), 3.03 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.84, 167.00, 162.61, 161.61, 144.43, 135.10, 132.18, 131.01, 130.55, 129.06, 128.52, 128.02, 121.89, 115.65, 114.73, 101.43, 77.48, 77.16, 76.84, 61.55, 40.64; IR (KBr) v: 2924, 1725, 1599, 1547, 1483, 1334, 1217, 975 cm^{-1}; HRMS (ESI) calcd for $C_{27}H_{25}F_3N_2O_3$ $[M+H]^+$ 425.1865, found 425.1852.

4.1.5.14. 1-(4-((3-(4-Nitrophenyl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (14). Yield 70%, yellow solid, mp 121–123 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 15.7 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.70–7.62 (m, 3H), 7.57–7.47 (m, 2H), 7.44–7.40 (m, 3H), 7.34–7.29 (m, 1H), 7.09 (d, J = 8.8 Hz, 2H), 6.69 (s, 1H), 5.31 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ188.74, 168.37, 161.46, 159.42, 149.77, 144.51, 135.06, 132.36, 131.03, 130.60, 129.06, 128.51, 125.33, 122.65, 121.81, 119.54, 114.68, 101.75, 61.40; IR (KBr) v: 2923, 1654, 1608, 1467, 1449, 1289, 1260, 1174, 995 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{19}N_2O_5$ $[M+H]^+$ 427.1294, found 427.1303.

4.1.5.15. 1-(4-((3-(5-Iodofuran-2-yl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (15). Yield 74%, yellow solid, mp 151–154 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 15.7 Hz, 1H), 7.67–7.62 (m, 2H), 7.53 (d, J = 15.7 Hz, 1H), 7.44–7.40 (m, 3H), 7.06 (d, J = 8.9 Hz, 2H), 6.84 (d, J = 3.4 Hz, 1H), 6.68 (d, J = 3.5 Hz, 1H), 6.64 (s, 1H), 5.28 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.72, 167.62, 161.22, 157.96, 154.14, 149.00, 144.45, 134.94, 132.23, 130.93, 130.47, 128.95, 128.41, 122.45, 121.70, 114.57, 113.04, 101.15, 90.64, 61.19; IR (KBr) v: 2922, 2360, 1654, 1604, 1449, 1254, 1018, 978 cm^{-1}; HRMS (ESI) calcd for $C_{23}H_{17}INO_4$ $[M+H]^+$ 498.0202, found 498.0216.

4.1.5.16. 1-(4-((3-(5-Methylfuran-2-yl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (16). Yield 73%, yellow solid, mp 115–118 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.9 Hz, 2H), 7.81 (d, J = 15.7 Hz, 1H), 7.67–7.62 (m, 2H), 7.53 (d, J = 15.7 Hz, 1H), 7.44–7.39 (m, 3H), 7.06 (d, J = 8.9 Hz, 2H), 6.79 (d, J = 3.3 Hz, 1H), 6.57 (s, 1H), 6.11 (dd, J = 3.2, 0.8 Hz, 1H), 5.26 (s, 2H), 2.37 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.85, 167.20, 161.45, 155.08, 154.64, 144.53, 142.14, 135.09, 132.30, 131.04, 130.59, 129.08, 128.54, 121.86, 114.72, 112.06, 108.12, 101.13, 61.39, 13.85; IR (KBr) v: 2921, 1655, 1603, 1499, 1253, 1171, 1025, 890 cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{20}NO_4$ $[M+H]^+$ 386.1392, found 386.1380.

4.1.5.17. 1-(4-((3-(Thiophen-2-yl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (17). Yield 67%, yellow solid, mp 136–138 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.9 Hz, 2H), 7.81 (d, J = 15.7 Hz, 1H), 7.67–7.62 (m, 2H), 7.53 (d, J = 15.6 Hz, 1H), 7.47 (dd, J = 3.6, 1.1 Hz, 1H), 7.45–7.40 (m, 4H), 7.14–7.10 (m, 1H), 7.10–7.05 (m, 2H), 6.61 (s, 1H), 5.28 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.83, 167.79, 161.43, 157.97, 144.54, 135.12, 132.38, 131.06, 130.61, 129.10, 128.55, 128.07, 127.90, 127.84, 127.23, 126.92, 121.88, 114.72, 101.85, 61.44. IR (KBr) v: 2922, 1655, 1603, 1448, 1344, 1256, 1227, 1032, 977 cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{20}NO_4$ $[M+H]^+$ 388.1007, found 388.1021.

4.1.5.18. 1-(4-((3-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)isoxazol-5-yl)methoxy)phenyl)-3-phenylprop-2-en-1-one (18). Yield 63%, yellow solid, mp 133–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 15.7 Hz, 1H), 7.67–7.62 (m, 2H), 7.53 (d, J = 15.7 Hz, 1H), 7.44–7.40 (m, 3H), 7.33 (d, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.4, 2.0 Hz, 1H), 7.08 (d, J = 8.9 Hz, 2H), 6.93 (d, J = 8.4 Hz, 1H), 6.58 (s, 1H), 5.27 (s, 2H), 4.29 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.81, 167.48, 162.23, 161.52, 145.51, 144.48, 143.98, 135.11, 132.27, 131.04, 130.58, 129.08, 128.54, 121.89, 122.06, 120.37, 117.95, 116.03, 114.72, 101.72, 64.64, 64.42, 61.49; IR (KBr) v: 2924, 1655, 1420, 1384, 1260, 1172, 1023, 890 cm^{-1}; HRMS (ESI) calcd for $C_{27}H_{22}NO_5$ $[M+H]^+$ 440.1498, found 440.1488.

4.2. Biological activity

4.2.1. Tyrosinase activity assay

Mushroom tyrosinase purchased from Sigma was stored in refrigerator below −20 °C before use. Potassium phosphate buffer (0.06 mL, 50 mM) at pH 6.5, 0.04 mL tyrosinase (250 U/mL) and 2 μL of the test compounds (0.5–300 μM), dissolved in DMSO were inserted into 96-well plates. After 5 min incubation at 25–30 °C, 0.1 mL of L-tyrosine (2 mM) was added and incubated for additional 30 min. After that, the optical density of the systems at 490 nm was measured on ELASA and the rate of activation (RA) was calculated according the formula: RA = [(C − D) − (A − B)]/(A − B) * l00, The A was optical density of the system at 490 nm with only tyrosinase; B was the one with neither compounds nor tyrosinase; C was the one with both compounds and tyrosinase; And the D was the one with only compounds. Finally, the activity of the compounds was expressed as the compounds concentration that gave a 50% activator effect (when RA = 50%) in the enzyme activity (EC_{50}).

4.2.2. Cell culture

Murine B16 melanoma cell lines (B16F10) were obtained from CAS (Chinese Academy of Sciences, China). The B16F10 cells were grown in DMEM medium (GIBICO, USA) supplemented with 10% heat-inactivated fetal bovine serum (GIBICO, USA), 100 U/mL penicillin and 100 mg/mL streptomycin (GIBCO, USA) in a humidified atmosphere with 5% CO_2 at 37 °C.

4.2.3. Cell viability measurement

Cell viability was assayed by adding CCK-8 (TransGen Biotech, Beijing, China) solution. B16 cells were plated in 96-well dishes at a density of 5×10^3 cells per well. After 12 h incubation at 37 °C, the culture medium was removed and replaced with fresh medium containing the candidate compounds in indicated concentrations. The cells were incubated for another 24 h, washed with ice cold PBS, 10 μl of CCK-8 solution was added into each well and cells were incubated at 37 °C for another 2 h. The absorbance was determined at 450 nm. The cell proliferation of each group was calculated as the absorbance of the treatment group relative to the control.

4.2.4. Protein concentration measurement

Cells were treated with different compounds at the indicated concentrations for 48 h, after treatment according to the method previously described, melanin amount was measured spectro-photometrically at 405 nm by a multi-plate reader, total protein concentration was measured by the Bradford (Biomed, Beijing, China)assays, measured spectro-photometrically at 595 nm by a multi-plate reader.

4.2.5. Melanin contents assay

Exponentially growing cells were seeded into 6-well plates at a concentration of 5×10^5 cells per well. After 24 h incubation at 37 °C, the culture medium was removed and replaced with fresh medium containing the candidate compounds in different concentrations. The cells were incubated for another 48 h, washed with ice cold PBS, followed by lysis with RIPA buffer for 40 min on ice,

and the lysates were centrifuged at 10,000 g for 20 min. Supernatants containing protein were subject to the protein assay and the pellets with intracellular melanin were solubilized in 200 µl of 1 M NaOH for 2 h at 60 °C. Melanin amount was determined spectrophotometrically at 405 nm by a multi-plate reader. The melanin amount was calculated by normalizing the total melanin values with protein content (abs melanin/µg protein).

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