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Synthesis and biological evaluation of helicid analogues as mushroom tyrosinase inhibitors

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ABSTRACT

A series of helicid analogues were synthesized and evaluated as tyrosinase inhibitors. The results demonstrated that some compounds had more potent inhibitory activities than arbutin (IC_{50} 7.3 mM). In particular, compound **1c** bearing 4,6-*O*-benzylidene substituent on the sugar moiety was found to be the most potent inhibitor with IC_{50} value of 0.052 mM. The inhibition kinetics analyzed by Lineweaver–Burk plots revealed that helicid analogues were competitive inhibitors. The Circular dichroism spectra indicated that those compounds induced conformational changes of mushroom tyrosinase upon binding.

Tyrosinase (EC 1.14.18.1), a multifunctional copper-containing enzyme, is widely distributed in nature. It catalyzes two distinct reactions of melanin synthesis: the *o*-hydroxylation of monophenols and the oxidation of *o*-diphenols to *o*-quinones. Quione is a highly reactive compound and can polymerize spontaneously to form melanin by a series of non-enzymatic reactions. Although melanin is principally responsible for skin color and plays a crucial role against skin photocarcinogenesis, abnormal melanin pigmentation can cause some dermatological disorders associated with freckles, melasma, ephelide and senile lentigines.[1]

Tyrosinase inhibitors are clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation and also important in the cosmetic industry for whitening and depigmentation after sunburn.[2–4] Many efforts have been spent in the search for effective and safe tyrosinase inhibitors, and a large number of naturally occurring and synthetic tyrosinase inhibitors have already been reported.[5–10] But some of their individual activities are either not potent enough to be considered of practical use or not compatible with safety regulations for food and cosmetic additives. Therefore, it is still necessary to search and develop novel tyrosinase inhibitors with potent activities and lower side effects.

Helicid, 4-formylphenyl-*O*-β-D-allopyranoside (Fig. 1), was originally isolated as one of the main active constituents from *Helicid nilgrinica* Bedd, a plant indigenous to western China, which has been used clinically as antalgic and hypnotic for a long time in China and no obvious side effect has been reported.[11,12] More recently, our group reported that helicid analogues exhibited potent acetylcholinesterase (AChE) inhibitory effects.[13] Arbutin, 4-hydroxylphenyl-*O*-β-D-glucopyranoside (Fig. 1), was reported to show a dose-dependent inhibitory effect on the oxidation of L-DOPA catalyzed by mushroom tyrosinase with an IC_{50} of 8.4 mM.[14] It has been widely used as a whitening agent in cosmetics.[15] By analogy with arbutin's chemical structure, helicid and its analogues are thought to be potential inhibitors of mushroom tyrosinase.

It is well known that, within the structure of tyrosinase, there are two copper ions in the active center of tyrosinase and a lipophilic long-narrow gorge near to the active center, and introducing the proper lipophilic group into the inhibitor can exhibited potent tyrosinase inhibitory activity. Therefore, in the present investigation, a series of helicid analogues bearing various lipophilic group in glycosyl moiety were synthesized and their tyrosinase inhibitory capacity were also evaluated. In addition, the inhibition kinetics and mechanisms of action of the selected compounds were also

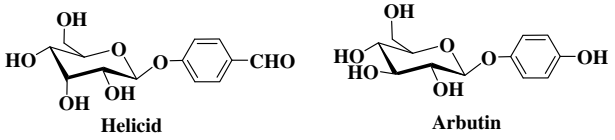

Figure 1. Chemical structures of helicid and arbutin.

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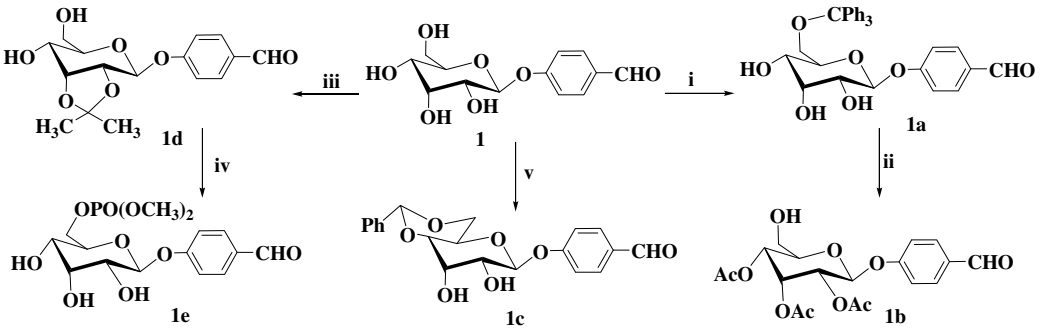

Scheme 1. Synthesis of compounds **1a–e**. Reagents and conditions: (i) $C(Ph)_3Cl$, helicid, pyridine, rt, 20 h, 68%; (ii) a—Ac_2O, pyridine; b—I_2, NaS_2O_3, rt, 12 h, 59%; (iii) helicid, I_2, NaS_2O_3, rt, 4 h, 56%; (vi) $POCl_3$, Et_3N, $CHCl_2$, CH_3OH, CH_3ONa, 0 °C, 2 h, 83%; (v) helicid, benzaldehyde, dimethyl acetal, 4-methylbenzenesulfonic acid, DMF, 50 °C, 12 h, 70%.

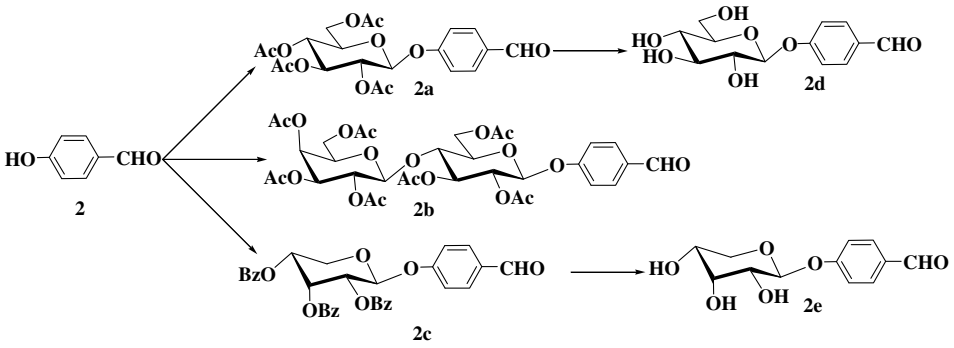

Figure 2. Synthesis of compounds **2a–e**.

investigated. To the best of our knowledge, this is the first report about inhibitory effects of helicid analogues on the diphenolase activity of mushroom tyrosinase.

The synthesis of helicid analogues **1a–e** and **2a–e** was summarized in Scheme 1 and Figure 2. The reaction of helicid with chlorotriphenylmethane in anhydrous pyridine gave the expected derivatives **1a** in 68% yield. Compound **1a** was first acetylated then selectively deprotected to provide the target compound **1b**. Helicid was reacted with benzaldehyde and dimethyl acetal in anhydrous DMF in the presence of 4-methylbenzenesulfonic acid to afford the derivative **1c** in good yield. Compound **1d** was prepared from the starting material helicid and anhydrous acetone using iodine as catalyst. The synthesis of compound **1e** was carried out by reacting compound **1d** with $POCl_3$ in anhydrous dichloromethane, followed by esterification with anhydrous methanol in the presence of sodium methoxide. The preparation of compounds **2a–e** has been described in our previous report.[7] The structures of the synthesized compounds were determined by different spectroscopic techniques, like ^1H NMR, EIMS and IR, and purities were confirmed by elemental analysis.

The inhibition of our synthetic helicid analogues and helicid on the mushroom tyrosinase was investigated by usual procedure[16] and compared with those of 4-methoxycinnamic acid and arbutin. The IC_{50} value of all compounds investigated is summarized in Table 1. As shown in Table 1, parent compounds helicid **1** and 4-hydroxybenzaldehyde **2** exhibited weak inhibitory activities with IC_{50} of 2.54 and 1.12 mM, respectively. Compounds **1a–b** and **2a–c** had no clear inhibitory effect on tyrosinase activity at concentration of 3.0 mM, while compounds **1c–e** and **2d–e** demonstrated potent inhibitory activity with IC_{50} value of less than 1 mM. Interestingly, compound **1c** bearing 4,6-*O*-benzylidene substituent on the sugar moiety showed the most potent inhibitory effect on mushroom tyrosinase with IC_{50} value of 0.052 mM. Preliminary structure–activity relationships (SARs) analysis indicated that (1) introducing 4,6-*O*-benzylidene (compound **1c**), 2,3-*O*-isopropylidene (compound **1d**) and 6-*O*-dimethyl phosphate (compound **1e**) into sugar moiety facilitated inhibitory activity on tyrosinase, These results confirmed that the lipophilic property might play a vital role in determining their inhibitory activities. Among these compounds, 4,6-*O*-benzylidene was more favorable; (2) the configuration of sugar moiety affected greatly inhibitory activity on mushroom tyrosinase, compound **2d** bearing a β-D-glucopyranoside (IC_{50} = 0.94 mM) was more active than helicid **1** bearing a β-D-allopyranoside (IC_{50} = 2.54 mM), which suggested that the bind type in sugar moiety can make the inhibitory activities of tyro-

Table 1
Inhibitory effects on mushroom tyrosinase of helicid and its analogues as compared with the standard inhibitors.

Compound	IC_{50}[a] (mM)	Inhibition rate (%) at 0.1 mM
1	2.54	10
1a	>3.0	Ni
1b	>3.0	Ni
1c	0.052	77
1d	0.62	13
1e	0.43	24
2	1.12[b]	15
2a	>3.0	Ni
2b	>3.0	5.0
2c	>3.0	Ni
2d	0.94	30
2e	0.28	42
Arbutin	7.3[c]	Ni
4-Methoxycinnamic acid	0.41[d]	22

[a] Assay performed using mushroom tyrosinase. Values are means of three different experiments.
[b] Values in the literature[4] is 1.2 mM.
[c] Values in the literature[14,17] is 5.3–8.4 mM.
[d] Values in the literature[17] is 0.42 mM.

sianse change obviously; (3) the ribose moiety (compound **2e**) was more preferable than glucose (compound **2d** and helicid **1**). These results indicated that the increase of hydroxyl group might cause stereo-hindrance for the inhibitors approaching to the active site of the enzyme.

The kinetic behavior of helicid and its analogues **1c** and **2d** on the mushroom tyrosinase, during the oxidation of L-DOPA, were determined from Lineweaver–Burk double reciprocal plots. The Lineweaver–Burk plots from Figure 3 indicated that they were competitive inhibitors of the mushroom tryrosinase. These results suggested that helicid and its analogues may only bind with the free enzyme.

The influence of helicid **1** and its analogues **1c** and **2d** on the secondary structure of mushroom tyrosinase was also investigated by Circular dichroism spectroscopy.[18,19] The results were summarized in Table 2. As shown in Table 2, the α-helical content of tyrosinase was 48.4% in the absence of inhibitor and the β-sheet was not found. However, the β-sheet was noticed in the presence of inhibitors, and its contents were increased gradually with the increase of the concentration of the inhibitors. On the other hand, it was observed that the content of rigidity structure (α-helix + β-sheet) of tyrosinase was increased while that of loop structure (β-turn + random) of tyrosinase was decreased accompanying the increasing of concentration of helicid and its analogues. These results indicated that helicid and its analogues induced conformational changes of mushroom tyrosinase upon binding.

Safety is a primary consideration for tyrosinase inhibitors, especially for those materials used in food and cosmetic products. Compounds **1c** and **2d** were selected to evaluate acute toxicity in mice. Clinical symptom was measured for 20 days after the oral single gavage administration of 1200 mg/kg. The results showed that all of the mice after administration at dose of 1200 mg/kg body weight/day did not show any mortality, and autopsy of the animals at the end of the experimental period (20 days) revealed no apparent changes in any organs. The results indicated that the compound **1c** and **2d** were safe at dose of 1200 mg/kg in mice.

In conclusion, the present investigation reported the effects of helicid analogues on the diphenolase activity of mushroom tyrosinase for the oxidation of L-DOPA. SARs analysis indicated that the nature of substituents on sugar moiety, the configuration and type of sugar moiety affected inhibitory activity on mushroom tyrosinase. The inhibition kinetics analyzed by Lineweaver–Burk plots revealed that helicid and its analogues were competitive inhibitors. Preliminary acute toxicity assay demonstrated that helicid analogues were safe as tyrosinase inhibitors. All these data suggested that these molecules might be served as candidates for further development of drug for the treatment of dermatological disorders.

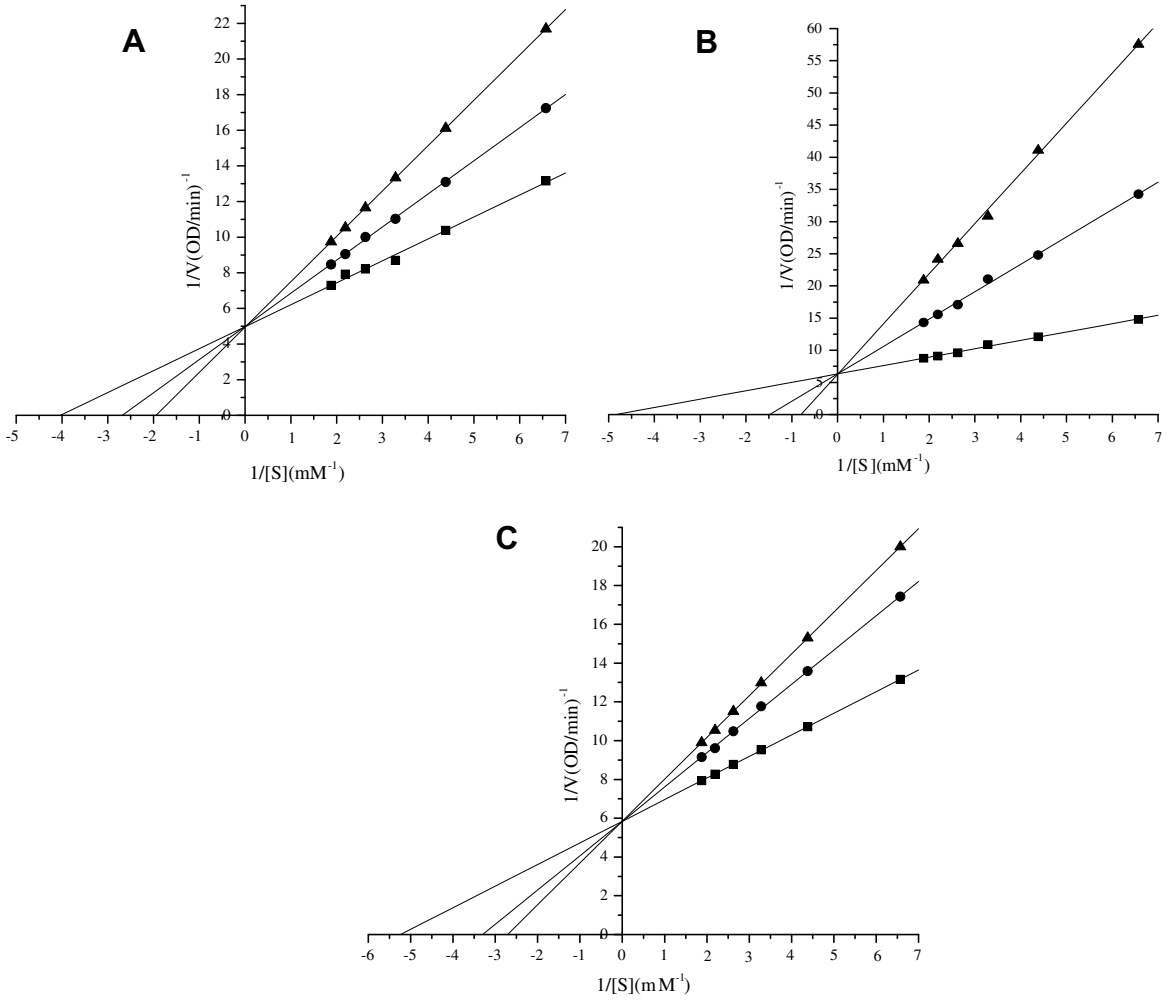

Figure 3. Lineweaver–Burk plots for inhibition of helicid analogues on mushroom tyrosinase for the catalysis of L-DOPA. (A), (B),. and (C) represents inhibition effectory of compounds **1, 1c** and **2d**, respectively.

Table 2
The influence of helicid and its analogues on the secondary structure of tyrosinase.

Compound	Concentration (μM)	α-Helix (%)	β-Sheet (%)	α-Helix+β-Sheet (%)	β-Turn (%)	Random (%)	β-Turn+Random (%)
1	0	48.4	0	48.4	18.6	33.0	51.6
	80	33.9	19.3	53.2	17.1	29.7	46.8
	160	40.3	20.5	60.8	13.5	25.7	39.2
1c	80	62.2	17.0	79.2	8.5	12.3	20.8
	160	49.2	29.5	78.7	7.1	14.2	21.3
2d	80	28.4	33.4	61.8	10.8	27.4	38.2
	160	31.1	34.7	65.8	9.5	24.7	34.2

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.056.

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