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Synthesis and evaluation of 5-benzylidene(thio)barbiturate-β-D-glycosides as mushroom tyrosinase inhibitors

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ABSTRACT

A series of 5-benzylidene(thio)barbiturate-β-D-glycosides were designed, synthesized and evaluated as a new class of mushroom tyrosinase inhibitors. The results demonstrated that most of compounds had more potent inhibitory activities than arbutin (IC_{50} 8.4 mmol/L). Compound **12b** was found to be the most potent inhibitor with IC_{50} value of 0.05 mmol/L. SARs analysis suggested that (1) 5-benzylidenethiobarbiturate substructures were efficacious for the inhibitory activity; (2) the lipophilic property of acetylated sugar moiety facilitated the inhibitory potency; (3) the hydroxyl group of 3′-configuration contributed to the increase of inhibitory effects. In addition, the inhibition mechanism study revealed that 5-benzylidene(thio)barbiturate-β-D-glycosides were irreversible inhibitors.

Tyrosinase is a copper-containing enzyme widely distributed in nature. It catalyzes two distinct reactions, the hydroxylation of a monophenol (monophenolase activity) and the oxidation of *o*-diphenols to *o*-quinones (diphenolase activity).[1] Tyrosinase is responsible, not only for melanization in animals, but also for browning in plants. The unfavorable enzymatic browning of fruits and vegetables generally results in a loss of nutritional and commercial value.[2] The accumulation of an excessive level of epidermal pigmentation can cause some dermatological disorders associated with freckles, melasma, ephelide and senile lentigines.[3] In addition, tyrosinase has been reported to be involved in the molting process of insects[4] and it may be characterized as a potential target site for the control of insect pests. Therefore, the control of the tyrosinase is important in relation to browning control of vegetables and fruits, and potent tyrosinase inhibitors have also become increasing important in medicinal and cosmetic products in relation to hyperpigmentation. To date, numerous natural and synthetic compounds acting as tyrosinase inhibitors were reported, and the most potent tyrosinase inhibitors so far is tropolone exhibiting an IC_{50} of 0.00013 mmol/L.[5] However, only few of them are used as skin-whitening agents due to safety concerns. Obviously, more efforts are still needed to search and develop novel tyrosinase inhibitors with better activities and lower side effects.

Arbutin, hydroquinone-*O*-β-D-glucopyranoside (Fig. 1), has been widely used as a whitening agent in cosmetics.[6] It was previously reported to show a diphenolase activity of tyrosinase with an IC_{50} of 8.4 mmol/L.[7] Recently, Sugimoto et al.[8] reported that 4-hydroxyphenyl β-maltoside (β-Ab-α-G1) and 4-hydroxyphenyl β-maltotrioside (β-Ab-α-G2) exhibited more potent inhibitory effects on human tyrosinase than arbutin. These results indicated that the modification of glycosyl group of arbutin might facilitate their inhibitory effects on tyrosinase.

More recently, our group described the syntheses and the inhibitory effects on mushroom tyrosinase of numerous helicid analogues.[9] The chemical structure of these compounds is similar to arbutin, but their inhibitory activities on mushroom tyrosinase are supior to arbutin, for example helicid (4-formylphenyl-*O*-β-D-allopyranoside) and 4-formylphenyl-*O*-β-D-glucopyranoside

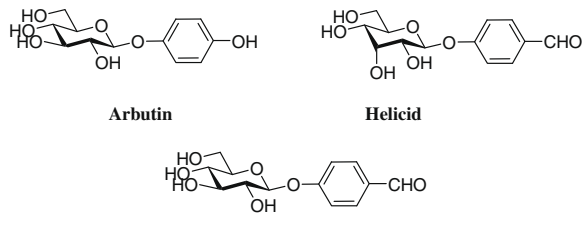

Figure 1. Chemical structures of arbutin, helicid and 4-formylphenyl-*O*-β-D-glucopyranoside.

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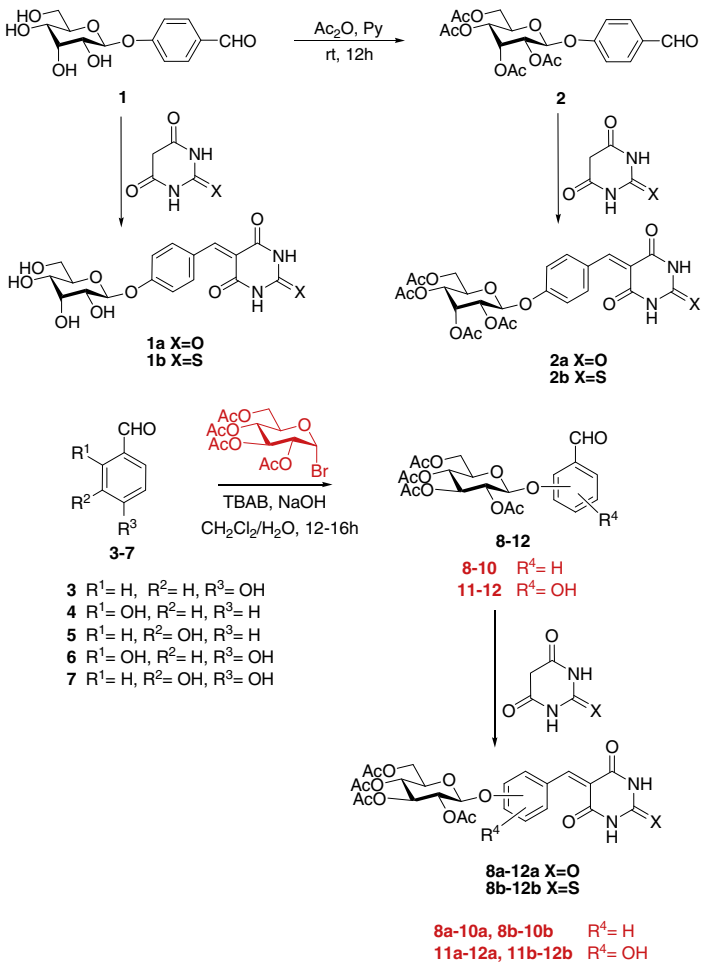
(Fig. 1) with IC_{50} value of 2.54 mmol/L and 0.94 mmol/L, respectively. It is worthy noted that helicid exhibited no obvious side effects[10] and 4-formylphenyl-*O*-β-D-glucopyranoside was also safe at dose of 1200 mg/kg in mice.[9] The structure–activity relationships (SARs) analysis provided evidence that the presence of a lipophilic group on sugar moiety facilitated inhibitory effect on mushroom tyrosinase. In addition, our group also reported that the linear chain thiourea moiety, thiosemicarbazide significantly increased the inhibitory activity on mushroom tyrosinase.[11]

Taking advantage of above information, in the present investigation, we designed and synthesized a series of 5-benzylidene(thio)barbiturate-β-D-glycosides bearing lipophilic glycoyl group and cyclic urea or thiourea moiety. The purpose of this study was to investigate the inhibitory effect on mushroom tyrosinase of 5-benzylidene(thio)barbiturate-β-D-glycosides, with the ultimate aim of developing novel potent tyrosinase inhibitors.

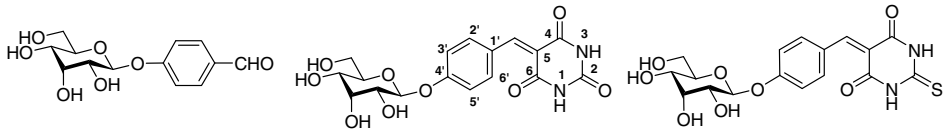
The synthesis of compounds **2** and **8** has been described in our previous report.[12] The procedure for the preparation of compounds **1a–2a**, **1b–2b**, **8–12, 8a–12a** and **8b–12b** was outlined in Scheme 1. Firstly, the 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide was prepared according to the methods described elsewhere,[13,14] and then the above mentioned glucosyl bromide reacted with hydroxybenzaldehydes **4–7** in the presence of NaOH and tetrabutylammonium bromide (TBAB) to afford the corresponding formylphenyl glucosides **9–12**. Thereinto, the β-D-glucoside was obtained as a major product using TBAB as phase-transfer catalyst.[15,16] The X-ray crystallographic data of compound **12** confirmed that the glucoside was β-D-glucopyranoside and also showed that the glucoside moiety was on *para*-position. Subsequently, reaction of **1–2**, **8–12** with barbituric or thiobarbituric acid in ethanol via well-known Knoevenegal condensation afforded 5-benzylidenebarbiturate-β-D-glycosides **1a–2a** and **8a–12a** and 5-benzylidenethiobarbiturate -β-D-glycosides **1b–2b** and **8b–12b** in good yields.[17] All compounds synthesized were characterized by chemical and spectral methods.

The inhibition of our synthetic 5-benzylidene(thio)barbiturate-β-D-glycosides on the diphenolase activitiy of mushroom tyrosinase was investigated by usual procedure[8] and compared with helicid and arbutin. As shown in Figure 2, helicid **1** only exhibited weak inhibitory activity with IC_{50} value of 2.62 mmol/L, while its tetra-*O*-acetyl congener **2** was nearly four-fold more active than helicid **1** with IC_{50} value of 0.78 mmol/L. Unfortunately, replacement of β-D-allopyranosyl moiety of compound **2** with β-D-glucopyranosyl moiety to afford compound **8** showed no inhibitory effects at the concentration of 3.0 mmol/L. Interestingly, incorporation of additional hydroxyl group onto position-3 of aromatic ring of compound **8** to give compound **12** (IC_{50} = 0.43 mmol/L) exhibited excellent inhibitory activity, whereas compound **11** (IC_{50} >3.0 mmol/L) having additional hydroxyl group of *ortho*-configuration displayed no activity.

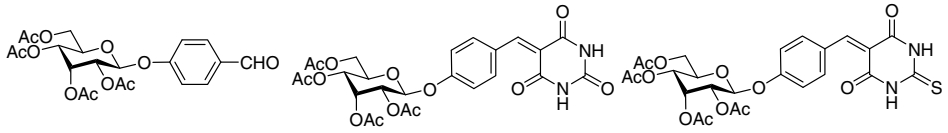
Coupling glycoside benzaldehydes **1**, **2** and **8–12** with (thio)barbituric acid to provide the corresponding 5-benzylidene(thio)barbiturate-β-D-glycosides **1a–2a**, **8a–12a**, **1b–2b** and **8b–12b** all (except **2a**) demonstrated more potent inhibitory activities than their parent β-D-glycoside benzaldehydes. Moreover, compared with 5-benzylidenebarbiturate-β-D-glycosides **1a–2a** and **8a–12a**,



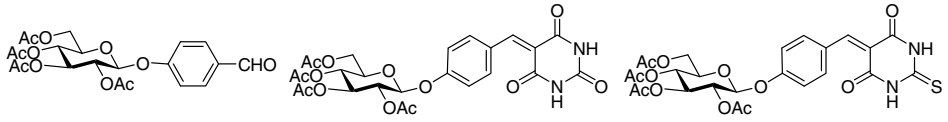
Scheme 1. Synthesis of 5-benzylidene(thio)barbiturate-β-D-glycosides.



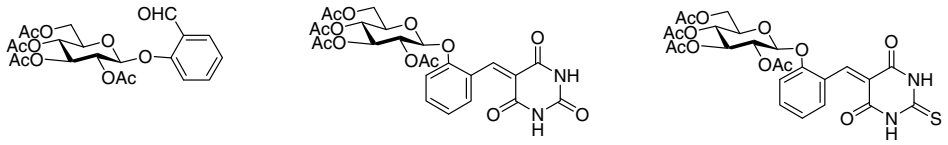
1 IC_{50}=2.62±0.055 mmol/L [a,b] **1a** IC_{50}=1.63±0.029 mmol/L **1b** IC_{50}=1.16±0.021 mmol/L



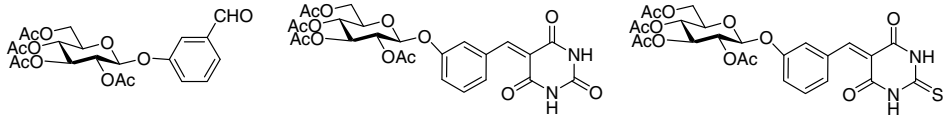
2 IC_{50}=0.78±0.015 mmol/L **2a** IC_{50}=1.50±0.028 mmol/L **2b** IC_{50}=0.34±0.007 mmol/L



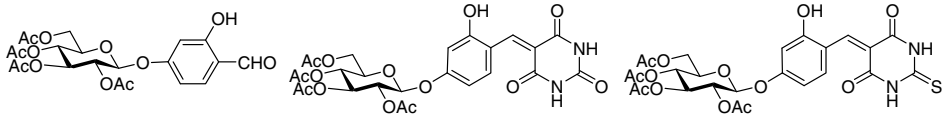
8 IC_{50} >3.0 mmol/L[9] **8a** IC_{50}=1.27±0.039 mmol/L **8b** IC_{50}=0.23±0.005 mmol/L



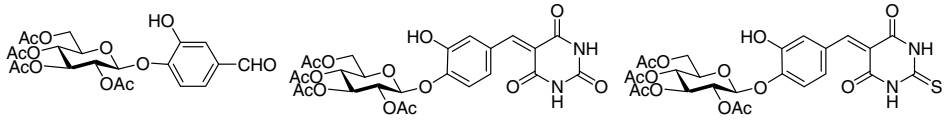
9 IC_{50} >3.0 mmol/L **9a** IC_{50} >3.0 mmol/L **9b** IC_{50}=0.43±0.010 mmol/L



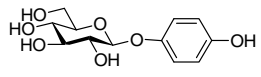
10 IC_{50} >3.0 mmol/L **10a** IC_{50} >3.0 mmol/L **10b** IC_{50}=0.87±0.019 mmol/L



11 IC_{50} >3.0 mmol/L **11a** IC_{50}=1.73±0.034 mmol/L **11b** IC_{50}=0.28±0.006 mmol/L



12 IC_{50}=0.43±0.011 mmol/L **12a** IC_{50}=0.13±0.025 mmol/L **12b** IC_{50}=0.05±0.002 mmol/L



Arbutin IC_{50}=8.4 mmol/L[c]

Figure 2. Structures and inhibitory activities against mushroom tyrosinase (diphenolase) of 5-benzylidene(thio)barbiturate-β-D-glycosides. [a]IC_{50} = mean ± SEM. SEM: standard error of mean. [b]Value in the literature[9] is 2.54 mmol/L. [c]Value in the literature[9] is 7.3 mmol/L.

their thiobarbiturate analogues **1b–2b** and **8b–12b** exhibited more significant inhibitory effects on tyrosinase, for example, compound **12a** exhibited inhibitory activity on tyrosinase with IC_{50} value of 0.13 mmol/L, and its thiobarbiturate analogue **12b** displayed 2.6-fold activity with IC_{50} value of 0.05 mmol/L. These results suggested that (1) 5-benzylidene(thio)barbiturate moiety facilitated inhibitory activity on mushroom tyrosinase; and (2) the thiobarbiturate moiety was more favorable.

Of all 5-benzylidenethiobarbiturate-β-D-glycosides **1b–2b** and **8b–12b**, compound **1b** (IC_{50} = 1.16 mmol/L) showed moderate inhibitory effect on mushroom tyrosinase, however, its tetra-*O*-acetyl congener **2b** (IC_{50} = 0.34 mmol/L) exhibited more potent

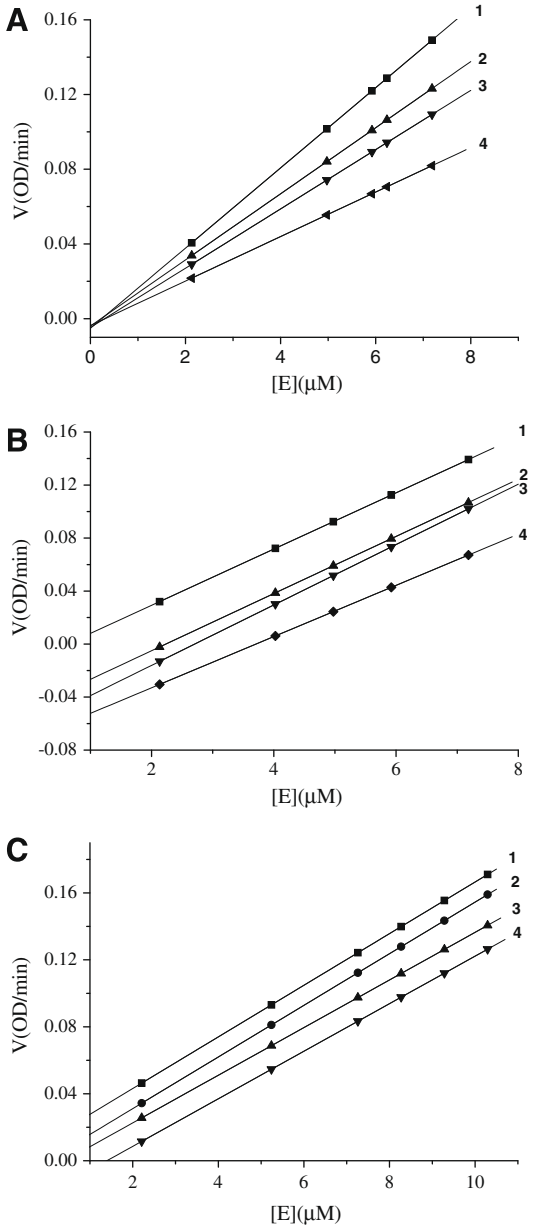


Figure 3. The effect of concentrations of tyrosinase on its activity for the catalysis of L-DOPA at different concentration of compounds **12** (A), **12a** (B) and **12b** (C). The concentrations of compounds **12**, **12a** and **12b** for curves **1**–**4** are 0, 0.5, 0.8 and 1.0 mmol/L, respectively.

inhibitory activity. These results, together with the above-mentioned inhibitory effects of compounds **1–2**, **1a** and **2a**, suggested that the lipophilic property of acetylated sugar moiety facilitated inhibitory potency on mushroom tysosinase. To further investigate the inhibitory effect of the position of glycoside at the aromatic ring of 5-benzylidenethiobarbiturate, compounds **8b**, **9b** and **10b** bearing a glycoside moiety of 4′-, 2′- and 3′-configuration were examined for their tyrosinase inhibitory activities. As shown in Figure 3, compounds **8–10b** all exhibited potent inhibitory effects with the tendency of 4′-(**8b**, IC_{50} = 0.23 mmol/L) > 2′-(**9b**, IC_{50} = 0.43 mmol/L) > 3′-configuration (**10b**, IC_{50} = 0.87 mmol/L). In addition, incorporation of additional hydroxyl group onto position-2 of aromatic ring of compound **8b** to give compound **11b** (IC_{50} = 0.28 mmol/L) showed slightly lower inhibitory activity, whereas compound **12b** having additional hydroxyl group of 3′-configuration was found to be the most potent tyrosinase inhibitor with IC_{50} value of 0.05 mmol/L. These observations, together with the inhibitory effects of compounds **8**, **11** and **12**, indicated that a 3′-configuration of hydroxyl group might contribute to the increase of inhibitory effects on mushroom tyrosinase.

The inhibition mechanism of compounds **12**, **12a** and **12b** on mushroom tyrosinase for the oxidation of L-DOPA was determined. Figure 3A showed the relationship of enzyme activity with its concentration in the presence of different concentrations of compound **12**. The results showed that the plots of *V* versus [*E*] gave a family of straight lines with different slopes but they intersected one another in the *Y*-axis. In the presence of compounds **12a** and **12b**, the kinetics of the enzyme, were shown in Figure 3B and C. The plots of *V* versus [*E*] gave a family of parallel straight lines with the same slopes. These results demonstrated that the inhibitory effect of compound **12** on the tyrosinase was reversible, whereas the inhibition of compounds **12a** and **12b** were irreversible, suggesting that barbiturate and thiobarbiturate moieties of 5-benzylidene(thio)barbiturate-β-D-glycosides effectively inhibited the enzyme by binding to its binuclear active site irreversibly.

In conclusion, the present investigation reported the inhibitory effects of 5-benzylidene(thio)barbiturate-β-D-glycosides on the diphenolase activity of mushroom tyrosinase for the oxidation of L-DOPA. Compound **12b** was found to be the most potent tyrosinase inhibitor with IC_{50} value of 0.05 mmol/L. SARs analysis indicated that (1) 5-benzylidene thiobarbiturate substructures were efficacious for the inhibitory activity; (2) the lipophilic property of acetylated sugar moiety facilitated inhibitory potency; (3) the hydroxyl group of 3′-configuration contributed to the increase of inhibitory effects. The inhibition mechanism study revealed that 5-benzylidene(thio)barbiturate-β-D-glycosides were irreversible inhibitors. All these results suggested that 5-benzylidene(thio)barbiturate-β-D-glycosides might be utilized for the development of new candidate for treatment of dermatological disorders related to tyrosinase.

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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.2009.06.018.

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