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Discovery of 4-functionalized phenyl-*O*-β-D-glycosides as a new class of mushroom tyrosinase inhibitors

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ABSTRACT

A series of 4-functionalized phenyl-*O*-β-D-glycosides were designed, synthesized and evaluated as a new class of mushroom tyrosinase inhibitors. The results demonstrated that compounds **6a**–**13a** bearing a thiosemicarbazide moiety exhibited potent activities with IC_{50} values range from 0.31 to 52.8 μM. Particularly, compound **9a** containing acetylated glucose moiety was found to be the most active molecule with an IC_{50} value of 0.31 μM. SARs analysis suggested that (1) the thiosemicarbazide moiety remarkably contributed to the increase of inhibitory effects on tyrosinase; (2) the configuration and bond type of sugar moiety also played a very important role in determining their inhibitory activities. The inhibition kinetics and inhibition mechanism study revealed that compound **9a** was reversible and competitive type inhibitor, whereas compound **13a** was reversible and competitive–uncompetitive mixed-II type inhibitor.

Tyrosinase, a multifunctional type-3 copper-containing metalloenzyme, is widely distributed in microorganism, plants and animals with slightly different forms.[1] It catalyzes two distinct reactions of melanin biosynthesis: the *o*-hydroxylation of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) (cresolase/monophenolase activity; EC 1.14.18.1), and subsequently the oxidation of L-DOPA to the corresponding *o*-dopaquinone (catecholase/diphenolase activity; EC 1.10.3.1), which are the rate-limiting steps in the pathway.[2,3] Recent investigations showed that the tyrosinase process was responsible not only for the undesired enzymatic browning of fruits and vegetables but also for the formation of various dermatological disorders, such as freckles, melasma, ephelide and senile lentigines.[4,5] Therefore, tyrosinase inhibitors have become increasingly important in food industry as well as in the medicinal and cosmetic products. In the last decades, a large number of naturally occurring and synthetic compounds acting as tyrosinase inhibitors were reported,[6–14] and the most representative tyrosinase inhibitor so far is tropolone **1** (Fig. 1) exhibiting an IC_{50} of 0.40 μM.[14] However, only few of them are put into practical use due to their weak individual activities or safety concerns. Undoubtedly, more efforts are still needed to search and develop more effective and safe tyrosinase inhibitors.

β-Arbutin, hydroquinone-*O*-β-D-glucopyranoside **5** (Fig. 1), has been widely used as a whitening agent in cosmetics.[6] It was reported to show a diphenolase activity of tyrosinase with an IC_{50} of 8.4 mM, while its isomer α-arbutin had no inhibitory effect on mushroom tyrosinase activity.[7] Recently, 4-hydroxyphenyl β-maltoside (β-Ab-α-G1) and 4-hydroxyphenyl β-maltotrioside (β-Ab-α-G2) were reported to exhibit more potent inhibitory effects on human tyrosinase than arbutin.[8] More recently, we found that the inhibitory effect of helicid, 4-formylphenyl-*O*-β-D-allopyranoside **6** (Fig. 1), and its analogues on mushroom tyrosinase

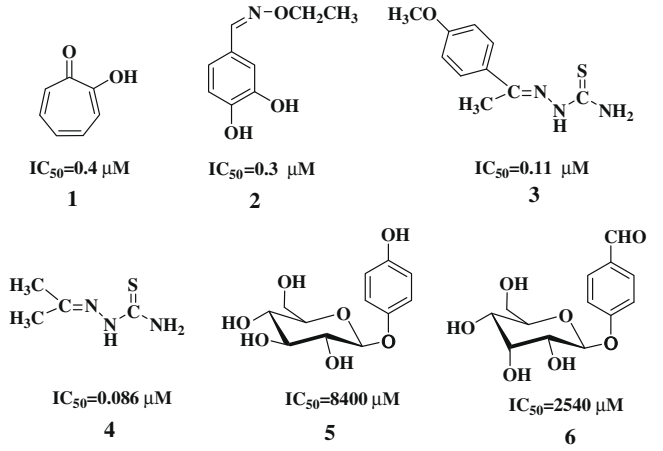

Figure 1. Chemical structures of some known tyrosinase inhibitors tropolone (**1**), 3,4-dihydroxybenzaldehyde-*O*-ethyloxime (**2**), 1-(propan-2-ylidene)thiosemicarbazide (**3**), 1-[1-(4-methoxyphenyl)ethylidene]thiosemicarbazide (**4**), arbutin (**5**) and helicid (**6**).

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were superior to arbutin.[9] These results indicated that the modification of glycosyl moiety of arbutin might facilitate their inhibitory effects on tyrosinase.

In addition, Ley and Bertram reported[15] that benzaldoximes and benzaldehyde-*O*-alkyloximes exhibited significant inhibitory activities against mushroom tyrosinase. Especially, the inhibitory effect on tyrosinase of 3,4-dihydroxybenzaldehyde-O-ethyloxime **2** (Fig. 1) was comparable to tropolone. Our group also reported that alkylidenethiosemicarbazides[10] and 1-(1-arylethylidene)thiosemicarbazides[11] displayed remarkable inhibitory activities, and the inhibitory effects on mushroom tyrosinase of 1-(propan-2-ylidene)thiosemicarbazide **3** (Fig. 1) and 1-[1-(4-methoxyphenyl)ethylidene]thiosemicarbazide **4** (Fig. 1) were superior to tropolone. These observations suggested that the thiosemicarbazide significantly facilitated the increase of inhibitory effect on mushroom tyrosinase.

Inspired by these results, a series of novel 4-functionalized phenyl-*O*-β-D-glycosides bearing thiosemicarbazide, oxime and methyloxime were designed, synthesized and evaluated as a new class of mushroom inhibitors. The purpose of this investigation was to investigate the inhibitory effects on mushroom tyrosinase of 4-functionalized phenyl-*O*-β-D-glycosides, with the ultimate aim of developing novel potent tyrosinase inhibitors. To the best of our knowledge, this is the first time to report that the inhibitory effect of 4-functionalized phenyl-*O*-β-D-glycosides on the diphenolase activity of mushroom tyrosinase. We hope that these findings may lead to the discovery of therapeutically potent agents against clinically dermatological disorders including hyperpigmentation as well as skin melanoma.

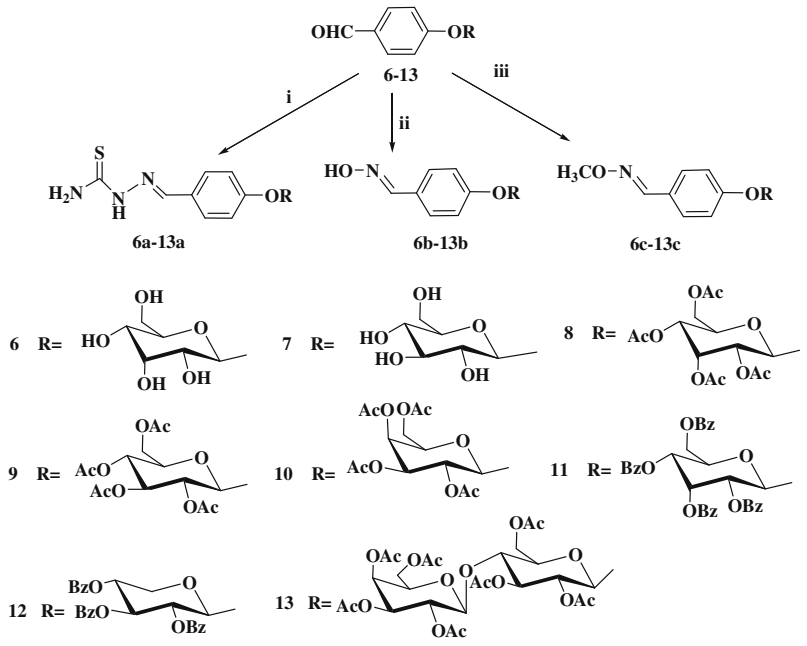
The synthesis of compounds **6–13**, **7a–c** and **9a–c** has been described in our previous report.[16] The procedure for the preparation of compounds **6a–13a**, **6b–13b** and **6c–13c** was outlined in Scheme 1. Briefly, helicid **6** reacted with acetyl anhydride in the presence of anhydrous pyridine to provide 4-formylphenyl (2,3,4,6-tetra-*O*-acetyl)-β-D-allopyranoside **8**. In the presence of NaOH and tetrabutylammonium bromide (TBAB), the corresponding glycosyl bromide, prepared according to the methods described elsewhere,[17] reacted with 4-hydroxybenzaldehyde to afford the corresponding benzaldehyde glycosides **9–13** in 27–59% yield. Hydrolyzation of **9** with sodium methoxide in methanol solution gave compound **7** in 56% yield. Subsequently, the benzaldehyde glycosides **6–13** reacted with thiosemicarbazide, hydroxylamine and methoxyamine to produce Schiff base derivatives **6a–13a**, **6b–13b** and **6c–13c**, respectively. All compounds synthesized were characterized by chemical and spectral methods such as ^{1}H NMR, EI-MS and IR, and purities were confirmed by elemental analysis.[18,19]

The inhibition of our synthetic 4-functionalized phenyl-*O*-β-D-glycosides on the diphenolase activity of mushroom tyrosinase was investigated by usual procedure[11] and compared with Arbutin. The IC_{50} values of all obtained compounds were summarized in Table 1. The benzaldehyde glycosides **6–13** were inactive to mushroom tyrosinase at the concentration of 200 μM (data not shown). Similarly, compounds **6b–13b** and **6c–13c** bearing hydroxylamine and methoxamine moiety, respectively, also exhibited no inhibitory effects on mushroom tyrosinase at the concentration of 200 μM. As predicted, compounds **6a–13a** bearing a

Table 1
Inhibitory effects on mushroom tyrosinase of 4-functionalized phenyl-*O*-β-D-glycosides as compared with arbutin

Compounds	IC_{50}[a] (μM)	Compounds	IC_{50} (μM)
6a	3.61 ± 0.55	**10b**	>200
7a	2.96 ± 0.62	**11b**	>200
8a	3.41 ± 0.28	**12b**	>200
9a	0.31 ± 0.12	**13b**	>200
10a	0.41 ± 0.09	**6c**	>200
11a	52.8 ± 2.2	**7c**	>200
12a	36.5 ± 3.8	**8c**	>200
13a	0.65 ± 0.21	**9c**	>200
6b	>200	**10c**	>200
7b	>200	**11c**	>200
8b	>200	**12c**	>200
9b	>200	**13c**	>200
Arbutin	7300 ± 600[b]		

[a] IC_{50} = mean ± SEM. SEM: standard error of mean.
[b] Values in the literature[2,7] is 5.3–8.4 mM.



Scheme 1. Synthesis of 4-functionalized phenyl-*O*-β-D-glycosides **6a–13a**, **6b–13b** and **6c–13c**. Reagents and conditions: (i) $H_2NHCSNH_2$/EtOH/reflux, 5–12 h; (ii) $NH_2OH{\cdot}HCl$/EtOH, pH 6–7, 45 °C, 2–6 h; (iii) $NH_2OCH_3{\cdot}HCl$/EtOH, pH 6–7, 45 °C, 2–6 h.

thiosemicarbazide moiety demonstrated significant tyrosinase inhibitory effects with IC_{50} values range from 0.31 to 52.8 μM. These results suggested that the thiosemicarbazide moiety contributed to the increase of inhibitory effects.

Of all glycoside thiosemicarbazides **6a**–**13a**, compounds **6a** (IC_{50} = 3.61 μM) and **7a** (IC_{50} = 2.96 μM) bearing a β-D-allopyranoside and β-D-glucopyranoside moiety, respectively, demonstrated similar inhibitory effects on mushroom tyrosinase, whereas, their tetra-*O*-acetyl congeners **8a** (IC_{50} = 3.41 μM) and **9a** (IC_{50} = 0.31 μM) displayed distinct inhibitory activities, and compound **9a** exhibited 10-fold inhibitory potential comparable to compound **8a**. Obviously, tetra-*O*-aetyl-β-D-glucopyranoside moiety was more favourable. Replacement of tetra-*O*-acetyl-β-D-glucopyranoside of compound **9a** with tetra-*O*-aetyl-β-D-galactopyranoside moiety gave compound **10a** (IC_{50} = 0.41 μM), which exhibited comparative inhibitory potency. Unfortunately, replacement of acetyl substituents of compound **9a** with benzoyl groups to afford compound **11a** exhibited sharply decreased inhibitory effect with IC_{50} value of 52.8 μM. Interestingly, compound **12a** (IC_{50} = 36.5 μM) bearing a tri-*O*-benzoyl-β-D-xylopyranoside moiety had more potent inhibitory activity than compound **11a**.

To further investigate the inhibitory effect of the molecular dimension of glycosides, compound **13a** bearing a hepta-*O*-acetyl-β-D-lactoside was examined for its tyrosinase inhibitory activity. As shown in Figure 3, compound **13a** also exhibited significant inhibitory potency with IC_{50} value of 0.65 μM. Particularly, compounds **9a**, **10a** and **13a** exhibited remarkable inhibitory activities with an IC_{50} value in the nanomolar range, and compound **9a** was found to be the most active molecule with an IC_{50} value of 0.31 μM comparable to tropolone (IC_{50} = 0.40 μM),[14] one of the well-known tyrosinase inhibitor. All these observations indicated that (1) the configuration and bond type of sugar moiety played a very important role in determining inhibitory activities; (2) the lipophilic property of acetylated sugar moiety facilitated the inhibitory potency.

The inhibition mechanism on the mushroom tyrosinase by **9a** and **13a** for the oxidation of L-DOPA was investigated. Figure 2 showed the relationship between enzyme activity and concentration in the presence of different concentrations of **9a** and **13a**, respectively. The plots of the remaining tyrosinase activity versus the concentrations of tyrosinase in the presence of different concentrations of **9a** and **13a** gave a family of straight lines, which all passed through the origin. Increasing the concentration of **9a** and **13a** resulted in the decreasing slope of the lines, indicating that the inhibition of **9a** and **13a** on the mushroom tyrosinase was reversible. Previous literature[20] reported that 1-phenylthiourea was defined as an irreversible inhibitor of tyrosinase, and suggesting that it could exhibit strong affinity for copper ions in the active centre. Our recent investigation[11] also indicated that the inhibitory effect of 1-(1-phenylethylidene)thiosemicarbazide was an irreversible inhibitor. Obviously, the inhibition mechanism of **9a** and **13a** was different from 1-phenylthiourea and 1-(1-phenylethylidene)thiosemicarbazide. By analogy with their chemical structures, these results suggested that the introduction of sugar moiety might affect the binding mode of the substrate and the active site of tyrosinase.

The kinetic behaviours of **9a** and **13a** on the mushroom tyrosinase, during the oxidation of L-DOPA, were determined from

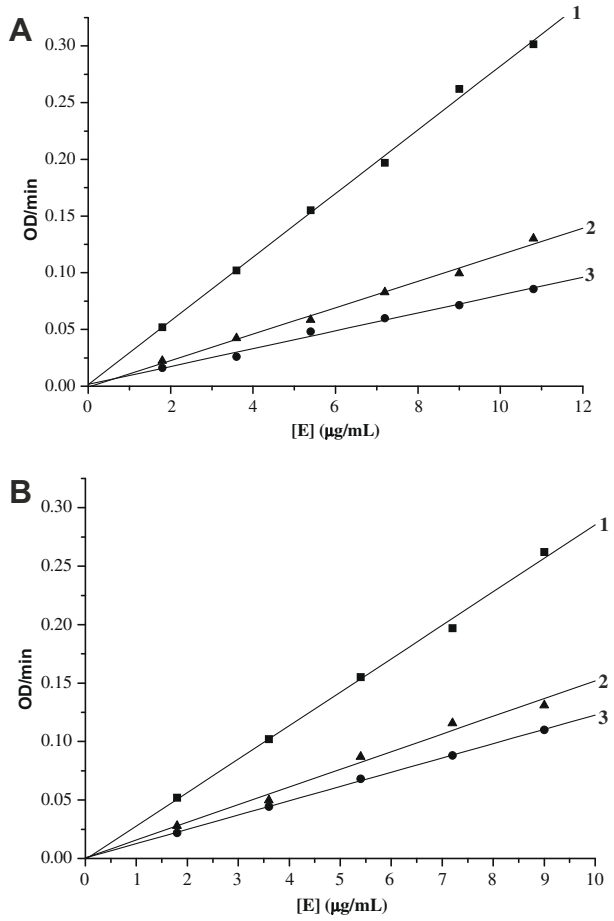


Figure 2. Effects of concentrations of tyrosinase on its activity for the catalysis of L-DOPA at different concentrations. A and B represented the inhibition effectory of compounds **4a** and **6a**, respectively. The concentrations of compound **4a** for curves 1–3 are 0, 1.20 and 2.40 μM, respectively. And the concentrations of compound **6a** for curves 1–3 are 0, 1.02 and 2.04 μM, respectively.

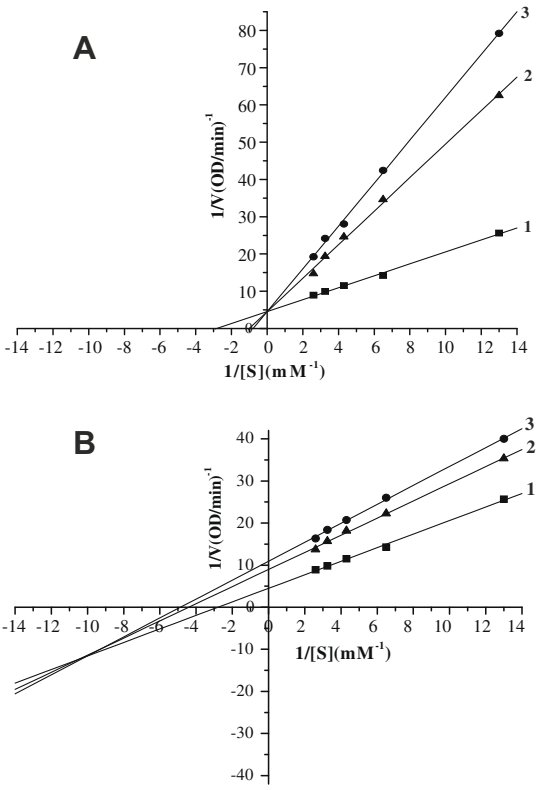


Figure 3. Lineweaver–Burk plots for inhibition of helicid analogues on mushroom tyrosinase for the catalysis of L-DOPA. A and B represented inhibition effect of compounds **4a** and **6a**, respectively. The concentrations of compound **4a** for curves 1–3 are 0, 0.06 and 0.12 μM, respectively. And the concentrations of compound **6a** for curves 1–3 are 0, 0.05 and 0.10 μM, respectively.

Lineweaver–Burk double reciprocal plots. The results (Fig. 3A) showed that the plots of $1/V$ versus 1/[S] gave a family of straight lines with different slopes that intersected one another in the *Y*-axis. The value of V_m remained the same and the value of K_m increased with the concentrations of **9a**, indicating that it was a competitive inhibitor of mushroom tyrosinase. Interestingly, Figure 3B showed that the inhibition behaviour of **13a** was different from that of **9a**. The Lineweaver–Burk double reciprocal plots in the presence of **13a** yielded a family of straight lines with different slopes that intersected one another in the third quadrant, indicating that it was a competitive–uncompetitive mixed-II type inhibitor.

Walker and Wilson[21] reported that tyrosinase had two distinct sites of combination for the binding of the substrate and the inhibitor. Recently, the crystallographic structure of tyrosinase has been established,[22] enabling a close look at its three-dimensional structure and a better understanding of its mechanism of action. This three-dimensional structure revealed that one site of combination was the binuclear copper ions active centre and the other site of combination was the hydrophobic enzyme pocket active site adjoining the binuclear copper ions active site, which is composed of some bioactive amino acids, such as Arg 55, Trp 184, Glu 182, Ile 42, His 190 and Ala 202.[23] The inhibition kinetics of **9a** and **13a** led us to hypothesize that **9a** interacted not only with the binuclear copper ions active site, but also with the hydrophobic protein domain surrounding the binuclear copper active site. The sulfur atom of the thiosemicarbazide moiety chelate the binuclear copper of tyrosinase, and such interaction acted as a bridge to link the acetylated glucose moiety and the hydrophobic protein pocket, which facilitated the acetylated glucose moiety to approach the hydrophobic protein pocket. Such interactions resulted in inhibiting the combination of the substrate L-DOPA and the binuclear copper active site, whereas the large size of lactoside moiety of **13a** hindered it to approach the activity centre of enzyme, and compound **13a** could only bind with free enzyme.

In conclusion, the present investigation reported for the first time the inhibitory effects of 4-functionalized phenyl-*O*-β-D-glycosides on the diphenolase activity of mushroom tyrosinase for the oxidation of L-DOPA. Compound **9a** bearing acetylated glucose moiety was found to be the most active molecule with an IC_{50} value of 0.31 μM. SARs analysis suggested that (1) the thiosemicarbazide moiety contributed to the increase of inhibitory effects on tyrosinase; (2) the configuration and bond type of sugar moiety also played a very important role in determining their inhibitory activities. The inhibition kinetics and inhibition mechanism study revealed that compound **9a** was reversible and competitive type inhibitor, whereas compound **13a** was reversible and competitive–uncompetitive mixed-II type inhibitor. All these data suggested that these molecules might be served as candidates for further development of drug for the treatment of dermatological disorders.

Acknowledgements

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18. *General procedures for the synthesis of compounds* **6a–13a**: The appropriate compounds **6–13** (1 mmol) were dissolved in anhydrous ethanol (15 mL), thiosemicarbazide (1 mmol) was added to the above solution. The reaction mixture was refluxed for 5–12 h and cooled to room temperature. The precipitate was filtered, washed with ethyl ether, and recrystallized from 95% alcohol to give compounds **6a–13a** in 56–91% yields. Compound **8a**: White solid powder, yield 79%, mp 177–178 °C. IR (KBr, cm^{-1}) *v*: 3452, 3301, 1753, 1579, 1503, 1409, 1226, 1092, 1046, 714; ^1H NMR (DMSO-d_6, 300 MHz) *δ*: 10.04 (s, 1H, NH), 7.89 (s, 1H, –CH=N–), 7.61 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.19 (s, 1H, NH), 7.04 (d, *J* = 8.7 Hz, 2H, Ar-H), 6.49 (s, 1H, NH), 5.75 (t, *J* = 3.0 Hz, 1H, CH), 5.42 (d, *J* = 8.1 Hz, 1H, CH), 5.17 (dd, *J* = 8.1 Hz, 3.1 Hz, 1H, CH), 5.07 (dd, *J* = 9.6 Hz, 2.7 Hz, 1H, CH); 4.28 (d, *J* = 4.2 Hz, 1H, CH), 4.27–4.23 (m, 2H, CH), 2.18, 2.10, 2.06, 2.05 (4 × s, 4 × 3H, 4 × CH_3CO); ^{13}C NMR (DMSO-d_6, 75 MHz) *δ*: 178.5, 171.3, 171.2, 170.3, 158.3, 143.2, 129.8, 129.6, 158.3, 117.4, 96.5, 70.8, 69.7, 68.1, 66.7, 62.6, 21.3, 21.2; ESI-MS: *m/z* 526 (M+1, 33.5), 548 (M+Na, 100). Anal. Calcd for $C_{22}H_{27}N_3O_{10}S$: C, 50.28; H, 5.18; N, 8.00. Found: C, 49.95; H, 4.91; N, 7.75.
19. *General procedures for the synthesis of compounds* **6b–13b** *and* **6c–13c**. To the solution of compounds **6–13** (1 mmol) in 15 mL of ethanol was added hydroxylamine hydrochloride or methoxylamine hydrochloride (1 mmol) which had been adjusted with 2 M NaOH to pH 6–7. The mixture was stirred for 6 h at 45 °C. After being cooled, the formed precipitate was filtered off, washed with ether and recrystallized from 95% ethanol to afford compounds **6b–13b** and **6c–13c** in 46–85% yields. Compound **6c**: White solid powder, yield 66%, mp 83–84 °C. IR (KBr, cm^{-1}) *v*: 3355, 2930, 1608, 1509, 1236, 1046, 916, 539; ^1H NMR (DMSO-d_6, 300 MHz) *δ*: 8.15 (s, 1H, –CH=N–), 7.53 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.03 (d, *J* = 8.7 Hz, 2H, Ar-H), 5.15 (d, *J* = 7.2 Hz, 1H), 3.93–3.91 (m, 1H, CH × 2), 3.86 (s, 3H, OCH_3), 3.69–3.65 (m, 1H, CH), 3.46–3.40 (m, 3H, CH × 3); ^{13}C NMR (DMSO-d_6, 75 MHz) *δ*: 159.8, 149.0, 129.1, 126.5, 117.2, 99.5, 75.4, 72.5, 71.0, 67.7, 62.2, 61.8; ESI-MS: *m/z* 314 (M+1, 100). Anal. Calcd for $C_{14}H_{19}NO_7$: C, 53.67; H, 6.11; N, 4.47. Found: C, 53.38; H, 6.32; N, 4.55.
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