



## Evaluation of dihydropyrimidin-(2H)-one analogues and rhodanine derivatives as tyrosinase inhibitors

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### ABSTRACT

A series of dihydropyrimidin-(2H)-one analogues and rhodanine derivatives were synthesized and their inhibitory effects on the diphenolase activity of mushroom tyrosinase were evaluated. The results showed that some of the synthesized compounds exhibited significant inhibitory activities. Especially, compound **15** bearing a hydroxyethoxyl group at position-4 of phenyl ring exhibited most potent tyrosinase inhibitory activity with IC<sub>50</sub> value of 0.56 mM. The inhibition mechanism analysis of compound **15** demonstrated that the inhibitory effect of the compound on the tyrosinase was irreversible. These results suggested that such compounds might be served as lead compounds for further designing new potential tyrosinase inhibitors.

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Dihydropyrimidinones (DHPMs) and their derivatives have attracted interest in medicinal chemistry, exhibiting pharmacological and therapeutic properties. In the past decades, a broad range of biological effects including antiviral, antitumor, antibacterial and anti-inflammatory activities have been described for these compounds.<sup>1,2</sup> Compounds containing the 2-thioxothiazolidin-4-one ring have showed a wide range of pharmacological activities, which includes antimicrobial, antiviral, and anticonvulsant effects.<sup>3</sup> Prominent amongst the resultant hits was a series of derivatives containing a 5-benzylidenerhodanine substructure (Fig. 1). Benzylidenerhodanines have been reported as small molecule inhibitors of numerous targets such as cyclooxygenase and 5-lipoxygenase,  $\beta$ -lactamase, cathepsin D, HCV NS3 protease, aldose reductase, protein mannosyl transferase and JNK-stimulating phosphatase.<sup>4</sup> Although, during the last years extensive studies on the pharmacology of DHPMs and rhodanine derivatives have been reported, the tyrosinase inhibitor activities of this kind of compounds have never appeared in the literature.

Tyrosinase (monophenol or *o*-diphenol, oxygen oxidoreductase, EC 1.14.18.1, syn. polyphenol oxidase), also known as polyphenol oxidase (PPO), is a copper-containing monooxygenase that is widely distributed in microorganisms, animals, and plants.<sup>5</sup> Tyrosinase could catalyze two distinct reactions involving molecular oxygen in the hydroxylation of monophenols to *o*-diphenols (monophenolase) and in the oxidation of *o*-diphenols to *o*-qui-

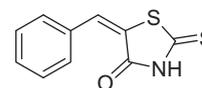
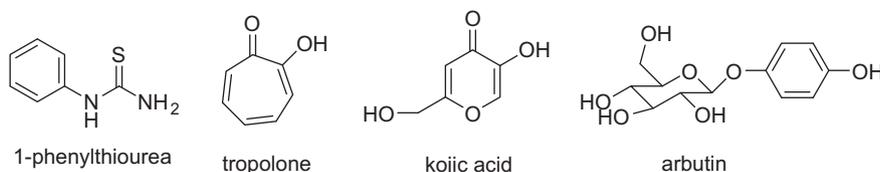


Figure 1. The substructure of 5-benzylidenerhodanine.

nonnes (diphenolase).<sup>6</sup> Due to the high reactivity, quinines could polymerize spontaneously to form high molecular weight brown-pigments (melanins) or react with amino acids and proteins to enhance brown color of the pigment produced.<sup>7,8</sup> Previous reports confirmed that tyrosinase not only was involved in melanizing in animals, but also was one of the main causes of most fruits and vegetables quality loss during post harvest handling and processing, leading to faster degradation and shorter shelf life.<sup>9</sup> Recently, investigation demonstrated that various dermatological disorders, such as age spots and freckle, were caused by the accumulation of an excessive level of epidermal pigmentation.<sup>10,11</sup> Tyrosinase has also been linked to Parkinson's and other neurodegenerative diseases.<sup>12</sup> In insects, tyrosinase is uniquely associated with three different biochemical processes, including sclerotization of cuticle, defensive encapsulation and melanization of foreign organism, and wound healing.<sup>13</sup> These processes provide potential targets for developing safer and effective tyrosinase inhibitors as insecticides and ultimately for insect control. Thus, the development of safe and effective tyrosinase inhibitors is of great concern in the medical, agricultural, and cosmetic industries. However, only a few such as kojic acid, arbutin, tropolone, and 1-phenyl-2-thiourea

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**Figure 2.** Chemical structure of known tyrosinase inhibitors.

(PTU) (Fig. 2) are used as therapeutic agents and cosmetic products.<sup>11,14</sup>

On the other hand, it was reported that phenyl thioureas and alkyl thioureas could exhibit weak to moderate depigmenting activity<sup>15</sup> and Ley and Bertram reported that benzaldoximes and benzaldehyde-*o*-alkyloximes possess higher tyrosinase inhibitory ability.<sup>16</sup> Tyrosinase belongs with catechol oxidase to the type-3 copper protein group. Crystal structure of phenyl thioureas bound catechol oxidase was reported. The sulfur atom of this compound binds to both copper ions in the active site of the enzyme.<sup>8</sup> More recently, our investigation also demonstrated that condensation products of aldehyde or ketone and thiosemicarbazide derivatives exhibited potent inhibitory activities against mushroom tyrosinase.<sup>17,18</sup> Stimulated by these results, in the present investigation, we synthesized a series of dihydropyrimidin-(2*H*)-ones analogues and rhodanine derivatives, and their inhibitory effects on the diphenolase activity of mushroom tyrosinase were investigated.

According to the general procedure shown in Scheme 1,<sup>19,20</sup> we carried out the preparation of 3,4-dihydropyrimidin-2-(1*H*)-ones (DHPMs) and thione analogs from the corresponding aldehydes, pentane-2,4-dione and urea or thiourea in the presence of magnesium bromide under solvent-free conditions. The synthesis is usually complete within 0.5–3 h at 50 °C or 100 °C, and the target compounds could be purified by recrystallization from 95% alcohol. The yields for these reactions are from moderate to good, and all the target compounds were characterized by IR, NMR and MS. The Schiff base of 3,4-dihydropyrimidin-2(1*H*)-one was prepared according to Scheme 2.<sup>21</sup> Rhodanine derivatives were synthesized for this study by means of the Knoevenagel condensation of the suitable aldehydes with 2-thioxo-4-thiazolidinone, respectively, in refluxing acetic acid in the presence of sodium acetate (Scheme 3).<sup>22,23</sup> The target compounds were also characterized by IR, NMR and MS.

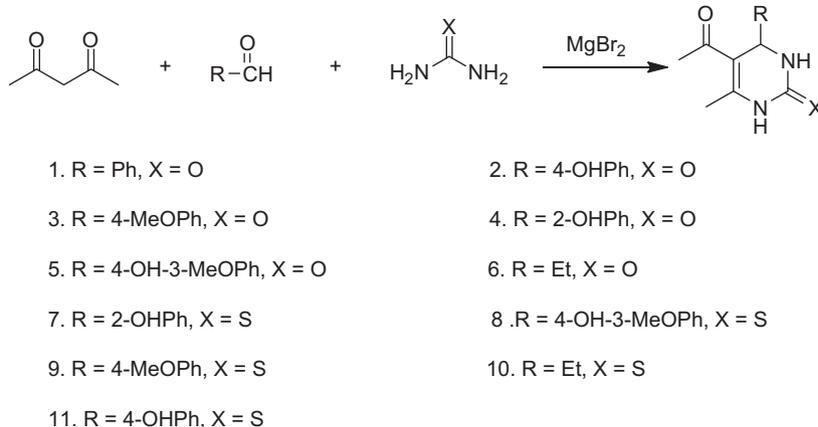
For evaluating the tyrosinase inhibitory activity, all the synthesized compounds were subjected to tyrosinase inhibition assay with *L*-DOPA as substrate, according to the method reported by our groups with some slight modifications.<sup>9,18</sup> The tyrosinase inhibitory activities of arbutin and 4-methoxycinnamic acid were ever reported, therefore, they were selected as comparing

substances. Figure 3 showed that the remaining enzyme activity rapidly decreased with the increasing concentrations of compound 15. The IC<sub>50</sub> values of dihydropyrimidin-(2*H*)-one analogues and rhodanine derivatives against tyrosinase were summarized in Table 1, and IC<sub>50</sub> values of all these compounds were determined from logarithmic concentration–inhibition curves (at least eight points) and are given as means of three experiments.

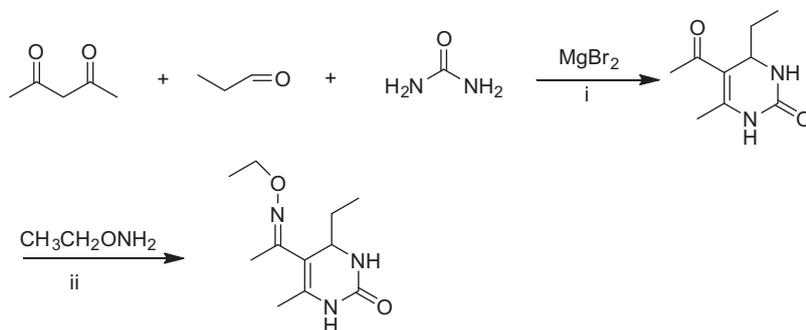
Our results showed that compounds 7–16 exhibited potent inhibitory on mushroom tyrosinase with IC<sub>50</sub> values ranged from 0.56 to 25.11 mM. Some of the synthesized compounds demonstrated more potent inhibitory activities than the reference standard inhibitor arbutin. Compounds 1–6 and 17 did not show any significant activities at 50 mM concentration. The result may be related to the structure of tyrosinase contained a type-3 copper center with a coupled dinuclear copper active site in the catalytic core. Tyrosinase inhibition of compounds 7–16 depended on the competency of the sulfur atom to chelate with the dicopper nucleus in the active site, and tyrosinase would lose its catalyzing ability after forming complex.

Comparing to the tyrosinase inhibitory activities of 3,4-dihydropyrimidin-2-(1*H*)-thione analogs, compounds with hydroxyl group in the benzene ring exhibited more potent inhibitory activities. This suggested that hydroxyl group might play an important role in determining their inhibitory activities. The result was consistent with the Kim's propose that the hydroxyl groups might inhibit the tyrosinase enzyme by participating in a metal–ligand binding interaction with the dicopper nucleus.<sup>27</sup> The introduction of a hydroxy group in the *para* position led to appreciably better inhibitory activity than the hydroxyl group in the *ortho* position, when the substituents introduced in the *meta* position on the phenyl rings may have hindered docking of the inhibitor to tyrosinase, and led to the decrease in inhibitory activities. Interestingly, compound 10 was found to be the most potent inhibitor with an IC<sub>50</sub> value of 10.67 mM. These results suggested that benzene ring might cause steric hindrance for the inhibitors approaching the active site of the enzyme.

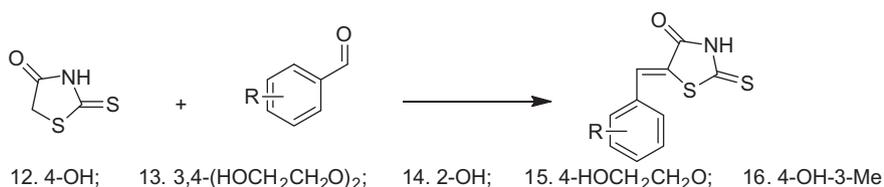
As shown in Table 1, rhodanine derivatives exhibited more potent inhibitor activities than dihydropyrimidin-(2*H*)-ones



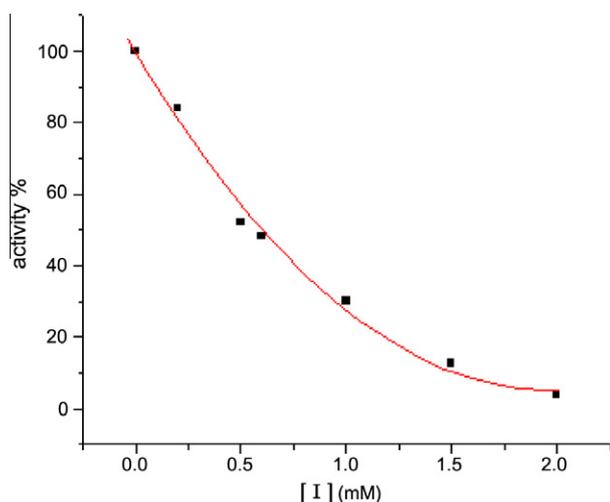
**Scheme 1.** Synthesis of 3,4-dihydropyrimidin-2-(1*H*)-ones (DHPMs) and thione analogs. Reagents and conditions: MgBr<sub>2</sub>, 50 °C or 100 °C, 0.5–3 h.



**Scheme 2.** Synthesis of the Schiff base of 3,4-dihydropyrimidin-2(1H)-ones (DHPMs). Reagents and conditions: (i)  $\text{MgBr}_2$ , 50 °C, 3 h. (ii) anhydrous ethanol, rt, 24 h.



**Scheme 3.** Synthesis of Rhodanine derivatives. Reagents: acetic acid, sodium acetate, reflux.



**Figure 3.** Effect of compound **15** on the diphenolase activity of mushroom tyrosinase for the catalysis of L-DOPA at 25 °C.

analogues, which indicated that the smaller size of the ring in volume would be beneficial to the molecules to approach the active centre of the enzyme. Compound **15** bearing a hydroxyethoxyl group at position-4 of phenyl ring exhibited most potent tyrosinase inhibitory activity with  $\text{IC}_{50}$  value of 0.56 mM. These results suggested that introducing appropriate hydrophobic subunits into position-4 of benzaldehyde might be facilitated their inhibitory effects on the diphenolase activity of mushroom tyrosinase. Interestingly, hydroxyethoxyl group introduced to the position-3,4 of phenyl ring (compound **13**,  $\text{IC}_{50}$  = 5.69 mM), led to a dramatic decrease in inhibitory activities. These results further confirmed that the substituents introduced in the *meta* position on the phenyl rings may hinder the complex formation between inhibitors unit and the metallic center of the active site in enzyme due to the steric hindrance.

The inhibitory mechanism of the selected compound **15** on mushroom tyrosinase for the oxidation of L-DOPA was determined. Figure 4 showed the relationship between enzyme activity and

**Table 1**  
Tyrosinase inhibitory activities and yields of the synthesized compounds

Compounds	Yield (%)	$\text{IC}_{50}^a$ (mM)
<b>1</b>	58.5	NA <sup>b</sup>
<b>2</b>	48.9	NA <sup>b</sup>
<b>3</b>	55.0	NA <sup>b</sup>
<b>4</b>	53.7	NA <sup>b</sup>
<b>5</b>	87.2	NA <sup>b</sup>
<b>6</b>	57.0	NA <sup>b</sup>
<b>7</b>	51.2	16.25
<b>8</b>	52.6	20.12
<b>9</b>	58.4	25.11
<b>10</b>	47.2	10.67
<b>11</b>	70.1	12.25
<b>12</b>	33.2	10.06
<b>13</b>	35.8	5.69
<b>14</b>	23.6	9.55
<b>15</b>	35.3	0.56
<b>16</b>	37.2	11.08
<b>17</b>	77.7	NA <sup>b</sup>
4-Methoxycinnamic acid		0.41 (mM) <sup>c</sup>
Arbutin		10.40 (mM) <sup>d</sup>

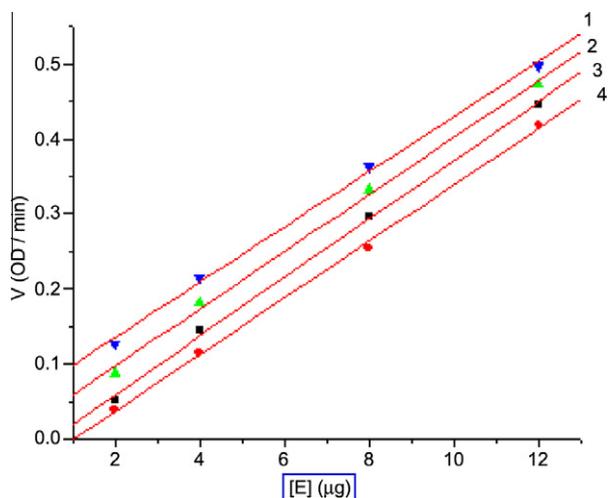
<sup>a</sup> Values were determined from logarithmic concentration–inhibition curves (at least eight points) and are given as means of three experiments

<sup>b</sup> Not active at 50 mM concentration.

<sup>c</sup>  $\text{IC}_{50}$  values in the literature is 0.34–0.43 mM.<sup>24,25</sup>

<sup>d</sup> The concentration of 10.4 mM corresponding to inhibition percentage, determined in this work, is 30%. The reported  $\text{IC}_{50}$  value of arbutin was more than 30 mM.<sup>26</sup>

concentration in the presence of different concentration of the above-mentioned compound. The results displayed that 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 **15** on the tyrosinase was irreversible, suggesting that 2-thioxothiazolidin-4-one moiety of 5-(4-(2-hydroxyethoxy)-benzylidene)-2-thioxothiazolidin-4-one effectively inhibited the enzyme by binding to its binuclear active site irreversibly. The result may be related to the structure of tyrosinase, within the structure, there are two copper ions in the active centre of tyrosinase and a lipophilic long-narrow gorge near to the active centre. Hydrophobic subunit and sulfur atom exist in the structure of



**Figure 4.** The effect of concentrations of tyrosinase on its activity for the catalysis of L-DOPA at different concentration of compound **15**. The concentrations of compound **15** for curves 1–4 are 0, 0.1, 0.2 and 3.0 mmol/L, respectively.

compound **15**, the compound could exhibit strong affinity for copper ions in the active centre and form a reversible non-covalent complex with the tyrosinase, and this then reacts to produce the covalently modified ‘dead-end complex’.

The present investigation reported that dihydropyrimidin-(2H)-one analogues and rhodanine derivatives had potent inhibitory effects on the diphenolase activity of mushroom tyrosinase. Interestingly, compound **15** was found to be the most potent inhibitor with  $IC_{50}$  value of 0.56 mM. Preliminary structure–activity relationships (SARs) analysis indicated that (1) hydroxyl group in the benzene ring might play an important role in determining their inhibitory activities, big groups might cause steric hindrance for the inhibitors approaching the active site of the enzyme resulting in the decrease of inhibitory activities, the substituents introduced in the *meta* position of the phenyl rings may have hindered docking of the inhibitor to tyrosinase; (2) the smaller size of the ring in volume would be beneficial to the molecules to approach the active centre of the enzyme; (3) rhodanine derivatives bearing a hydroxyethoxyl group at position-4 of phenyl ring might be beneficial to increase the inhibitory activities. The inhibition mechanism analysis of compound **15** demonstrated that the inhibitory effect of the compound on the tyrosinase was irreversible.

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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.2011.02.076.

## References and notes

- Kappe, C. O. *Eur. J. Med. Chem.* **2000**, *35*, 1043.
- Liang, B.; Wang, X.; Wang, J. X. *Tetrahedron* **2007**, *63*, 1981.
- Sortino, M.; Delgado, P. *Bioorg. Med. Chem.* **2007**, *15*, 484.
- Irvine, M. W.; Patrick, G. L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2032.
- Song, K. K.; Huang, H.; Han, P.; Zhang, C. L.; Shi, Y.; Chen, Q. X. *Biochem. Biophys. Res. Commun.* **2006**, *342*, 1147.
- Chen, Q. X.; Liu, X. D.; Huang, H. *Biochemistry (Moscow)* **2003**, *68*, 644.
- Kubo, I.; Nihei, K. I.; Shimizu, K. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 5343.
- Matsuura, R.; Ukeda, H.; Sawamura, M. *J. Agric. Food. Chem.* **2006**, *54*, 2309.
- Yi, W.; Cao, R. *Eur. J. Med. Chem.* **2010**, *45*, 639.
- Gupta, A. K.; Gover, M. D.; Nouri, K. N.; Taylor, S. J. *Am. Acad. Dermatol.* **2006**, *55*, 1048.
- Thanigaimalai, P.; Hoang, T. A. L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2991.
- Zhu, Y. J.; Zhou, H. T.; Hu, Y. H. *Food Chem.* **2011**, *124*, 298.
- Ashida, M.; Brey, P. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 10698.
- Battaini, G.; Monzani, E.; Casella, L.; Santagostini, L.; Pagliarini, R. *J. Biol. Inorg. Chem.* **2000**, *5*, 262.
- Klabunde, T.; Eicken, C. *Nat. Struct. Biol.* **1998**, *5*, 1084.
- Ley, J. P.; Bertram, H. J. *Bioorg. Med. Chem.* **2001**, *9*, 1879.
- Liu, J. B.; Yi, W.; Wan, Y. Q.; Ma, L.; Song, H. C. *Bioorg. Med. Chem.* **2008**, *14*, 1096.
- Liu, J. B.; Cao, R. H.; Yi, W.; Ma, C. M.; Wan, Y. Q.; Zhou, B. H.; Ma, L.; Song, H. C. *Eur. J. Med. Chem.* **2009**, *44*, 1773.
- Ahmed, N.; Lier, J. E. *Tetrahedron Lett.* **2007**, *48*, 5407.
- General procedures for the synthesis of 3,4-dihydropyrimidin-2-(1H)-ones (DHPMs) compounds:* To a 50 mL flame dried round-bottom flask were added 20 mmol of benzaldehyde, 20 mmol of acetophenone, 20 mmol of urea or thiourea and 3.5 mmol of  $MgBr_2$ . The resulting mixture was heated to 100 °C. After completion of the reaction as indicated by TLC, and then the mixture was cooled to room temperature and poured into 50 mL ice-water. The solid product was filtered, washed with ice-water and ethanol (95%), dried, and recrystallized from 95% ethanol. **Compound 11:** Yellow solid power, mp 279.6–281.3; IR (KBr,  $cm^{-1}$ ): 3267, 2998, 2889, 1608, 1566, 1505, 1463, 1377, 1329, 1252, 1191, 832;  $^1H$  NMR (300 MHz,  $DMSO-d_6$ ):  $\delta$  10.54 (bs, 1H, NH), 9.38 (br s, 1H, NH), 9.16 (s, 1H, OH), 7.10 (d,  $J = 8.4$  Hz, 2H, Ph), 6.71 (d,  $J = 8.4$  Hz, 2H, Ph), 5.19 (d,  $J = 2.7$  Hz, 1H, CH), 2.31 (s, 3H,  $CH_3$ ), 2.24 (s, 3H,  $CH_3$ );  $^{13}C$  NMR (75 MHz,  $DMSO-d_6$ ):  $\delta$  196.5, 175.1, 158.9, 149.2, 135.8, 128.5, 115.8, 108.1, 55.8, 31.2, 19.1; MS (ESI):  $m/z$  (100%) 263 (M+1).
- Liu, J. B.; Cao, R. H.; Wang, Z. H.; Peng, W. L.; Song, H. C. *Eur. J. Med. Chem.* **2009**, *44*, 1737.
- Irvine, M. W.; Patrick, G. L.; Kewney, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2032.
- General procedures for the synthesis of Rhodanine derivatives:* The mixture of 2-thioxo-4-thiazolidinone (2.48 mmol), suitable aldehydes (2.54 mmol) and sodium acetate (7.32 mmol) in acetic acid (30 mL) was heated to reflux for 3–6 h. The reaction was monitored by TLC. After completion of the reaction, upon cooling of the mixture to room temperature, a precipitate formed which was collected by filtration, washed with cold ethanol, and dried under vacuum to provide the target compound. **Compound 15:** Yellow solid power, mp 197.7–198.4; IR (KBr,  $cm^{-1}$ ): 3068, 2837, 1726, 1684, 1579, 1502, 1438, 1230, 1201, 1172, 1060, 800;  $^1H$  NMR (300 MHz,  $CDCl_3$ ):  $\delta$  7.63 (s, 1H, =CH), 7.46 (d,  $J = 8.7$  Hz, 2H, Ph), 7.03 (d,  $J = 8.7$  Hz, 2H, Ph), 4.75 (t,  $J = 4.5$  Hz, 2H,  $OCH_2$ ), 4.26 (t,  $J = 4.5$  Hz, 2H,  $OCH_2$ );  $^{13}C$  NMR (75 MHz,  $CDCl_3$ ):  $\delta$  196.2, 170.0, 161.8, 143.7, 129.6, 126.4, 117.0, 114.8, 70.3, 60.8; MS (ESI):  $m/z$  (100%) 282 (M+1).
- Shi, Y.; Chen, Q. X.; Wang, Q.; Song, K. K.; Qiu, L. *Food Chem.* **2005**, *92*, 707.
- Lee, H. S. *J. Agric. Food. Chem.* **2002**, *50*, 1400.
- Kazuhisa, S.; Koji, N.; Takahisa, N.; Taro, K.; Kenji, S. *J. Biosci. Bioeng.* **2005**, *99*, 272.
- Kim, D.; Park, J.; Kim, J.; Han, C.; Yoon, J.; Kim, N.; Seo, J.; Lee, C. *J. Agric. Food. Chem.* **2006**, *54*, 935.