Synthesis and evaluation of antiplatelet activity of trihydroxychalcone derivatives

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Received 30 April 2005; revised 16 July 2005; accepted 1 August 2005
Available online 19 September 2005

Abstract—In an effort to develop potent antiplatelet agents, a series of trihydroxychalcones was synthesized and screened in vitro for their inhibitory effects on washed rabbit platelet aggregation induced by arachidonic acid (100 μM) and collagen (10 μg/ml). Of five compounds with potent inhibitory effects on arachidonic acid- and collagen-induced platelet aggregation, compound 4e was found to be the most potent. The structure–activity relationships suggested that antiplatelet activity was governed to a greater extent by the substituent on B ring of the chalcone template, and most of the active compounds had methoxy or dimethoxy groups on B ring.

It is generally accepted that platelets play an important role in the progress and development of thrombotic disorders, especially cerebro vascular diseases such as transient ischemic attack,1 ischemic heart diseases such as myocardial infarction2 and peripheral vascular diseases.3 Consequently, the inhibition of platelet function represents a promising approach for the prevention of these diseases. For this reason, many antiplatelet drugs have been used clinically. However, the antiplatelet agents currently used have certain disadvantages such as notable side effects and inefficient therapy.4,5 Therefore, searching for antiplatelet drugs which are more effective and safer with fewer side effects is very important.

The compounds with the backbone of chalcone have been reported to exhibit a wide variety of pharmacological effects, including antioncogenic,6 anti-inflammatory,7 antulcerative,8 analgesic,9 antiviral,10 antifungal,11 antimalarial,12 and antibacterial activities.13 Nakadate et al.14 reported that 2-hydroxychalcones exerted topical anti-inflammatory effects in mice. Lin et al.16 reported that 2, 5-dihydroxychalcones had good selective inhibitory effects on arachidonic acid-induced platelet aggregation. These reports suggested that some hydroxychalcones might be the promising antithrombotic or anti-inflammatory agents. The antiplatelet activity of trihydroxychalcones has not been reported previously. So in this study, we designed and synthesized a series of new 2', 4', 6-trihydroxychalcones and varied the substituent of B ring to screen for their antiplatelet effects on washed rabbit platelets in vitro. The structure–activity relationships were also discussed.

The route followed for the preparation of trihydroxychalcones is illustrated in Scheme 1. 1-(2,4,6-Trihydroxy-phenyl)-ethanone 1, prepared as reported previously,17 was treated with chloromethyl methyl ether and potassium carbonate in acetone at room temperature to produce 1-(2-hydroxy-4,6-bis-methoxymethoxy-phenyl)-ethanone 2. Intermediates 3a-3m were prepared by Claisen-Schmidt condensation of 2 with appropriate aromatic aldehydes or hydroxyaromatic aldehydes, protected as chloromethyl methyl ether. Then intermediates 3a-3m were treated with 10% HCl in methanol at reflux temperature to yield the corresponding hydroxychalcones 4a-4m in good yield.
The antiplatelet activity was studied by measuring the aggregation of washed rabbit platelet applying Born’s turbidimetric method. The washed platelet suspension of rabbits was incubated at 37 °C for 4 min in an aggregometer with stirring at 1000 rpm and the aggregation was stimulated by adding arachidonic acid (100 μM) or collagen (10 μg/ml) at concentrations giving maximal aggregatory response, as Jin et al. described in detail. The antiplatelet activity was expressed as percent inhibition with respect to control. Anti-aggregating potency of the compounds was indicated by IC₅₀ values that were calculated by linear regression analysis of the concentration–response curves obtained for each compound. Data are reported in Table 1.

As shown in Table 1, among the tested compounds, six compounds showed potent inhibitory effects on arachidonic acid-induced washed rabbit platelet aggregation, and the potencies of some compounds were better or comparable to aspirin, a COX inhibitor which was used as a positive control. Comparing with compound 4i, compounds 4a, 4e, 4h, 4i, and 4j had potent antiplatelet effects. It seemed that the substitution on chalcone B ring might be important in the inhibition of platelet aggregation. However, compounds 4b, 4c, 4d, 4f, 4g, and 4m that bore substituent(s) on the B ring did not show any inhibitory effect at a concentration of 100 μM. These results indicated that the character of substituent on the B ring had a significant influence on the antiplatelet activity. The compounds 4a, 4e, and 4h–4k concentration-dependently inhibited washed rabbit platelet aggregation induced by arachidonic acid, with the IC₅₀ values of 28.5 ± 2.2, 15.2 ± 5.4, 19.8 ± 3.5, 25.5 ± 2.7, 45.2 ± 6.7, and 70.6 ± 3.5 μM, respectively. It seemed that the increase of hydroxy group on chalcone A ring could influence the inhibitory effect on platelet aggregation, but the potency depended on the variation of the substituent of the B ring. Compound 4e was the most potent in inhibiting arachidonic acid-induced platelet aggregation; the demethylation at C-4 of 4e (i.e., 4d) and the demethylation at C-3 and C-4 of 4e (i.e., 4c) did not contribute so much to the antiplatelet effect.

These results suggested that substitution of the methoxy group on chalcone B ring significantly increased the antiplatelet activity. The O-phenylmethylolation at C-4 of 4d (i.e., 4a), O-allyloxylation at C-4 of 4d (i.e., 4h), and the demethoxylation at C-3 of 4e (i.e., 4i) enhanced the inhibitory effects on arachidonic acid-induced platelet aggregation, although they were not as potent as that of compound 4e. The replacement of the methoxy at C-4 of 4i with a methyl group (i.e., 4g) produced no effect on the inhibition of platelet aggregation at a concentration of 100 μM. These results indicated that etherifying the phenolic hydroxyl of the B ring was required for the inhibition of platelet aggregation induced by arachidonic acid. On the other hand, compound 4b had no significant inhibitory effect, indicating that introduction of a strong electron-donating group at the C-4 might attenuate its inhibitory effect. In addition, lipophilicity did not appear to be an important factor for the antiplatelet activity, because a chloro group substituted at C-4 of 4i (i.e., 4j) or dichloro substituted at C-2 and C-4 of 4i (i.e., 4k) was not more potent than that of compound 4e. A similar inhibitory pattern was also observed from the tested compounds in the collagen-induced platelet aggregation, except for compound 4k which did not show any inhibitory effect at a concentration of 100 μM. The IC₅₀ values of compounds 4a, 4e, 4h, 4i, and 4j against collagen-induced platelet aggregation were 37.4 ± 4.4, 27.4 ± 4.4, 28.4 ± 5.3, 33.3 ± 4.6, and 66.5 ± 5.3 μM, respectively.

In conclusion, some compounds showed good inhibitory effects on platelet aggregation induced by arachidonic acid and collagen. Some of them exhibited even better potency than the reference drug, aspirin. The antiplatelet effects of these compounds were probably mediated through the suppression of cyclooxygenase activity and reduced thromboxane formation as it had been reported that some chalcone derivatives inhibited arachidonic acid-induced platelet aggregation by inhibition of cyclooxygenase activity and thromboxane formation. Further experiments were needed to elucidate their mechanism of action. Compound 4e exhibited the most potent antiplatelet effect among the tested compounds. Therefore, it merits further investigation as the lead compound in continuing
Further studies are in progress in our laboratory and will be reported in detail in a series of forthcoming papers.

### References and notes


11. Selected data for compound 4e: IR (KBr) cm⁻¹ 1624 (C=O), 1H NMR (300 MHz, DMSO-d₆): δ 5.99–7.19 (5H, m, Ar-H), 7.68 (1H, d, J = 15.6 Hz, Hₐ), 8.03 (1H, d, J = 15.6 Hz, Hₐ) MS (MH⁺) 317.


16. Selected data for compound 4e: IR (KBr) cm⁻¹ 1624 (C=O), 1H NMR (300 MHz, DMSO-d₆): δ 5.99–7.19 (5H, m, Ar-H), 7.68 (1H, d, J = 15.6 Hz, Hₐ), 8.03 (1H, d, J = 15.6 Hz, Hₐ) MS (MH⁺) 317.


### Table 1

In vitro antiplatelet activity of compounds 4a-4m and ASA in washed rabbit platelets

<table>
<thead>
<tr>
<th>Compound Ar</th>
<th>IC₅₀ value (μM) Arachidonic acid</th>
<th>Collagen</th>
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<tbody>
<tr>
<td>4a</td>
<td>OCH₃</td>
<td>28.5 ± 2.2</td>
</tr>
<tr>
<td>4b</td>
<td>NCH₃</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4c</td>
<td>OH</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4d</td>
<td>OCH₃</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4e</td>
<td>OCH₃</td>
<td>15.2 ± 5.4</td>
</tr>
<tr>
<td>4f</td>
<td>OCH₂CH₂CH₂</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4g</td>
<td>CH₃</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4h</td>
<td>OCH₂CH=CH₂</td>
<td>19.8 ± 3.5</td>
</tr>
<tr>
<td>4i</td>
<td>OCH₃</td>
<td>25.5 ± 2.7</td>
</tr>
<tr>
<td>4j</td>
<td>Cl</td>
<td>45.2 ± 6.7</td>
</tr>
<tr>
<td>4k</td>
<td>Cl</td>
<td>70.6 ± 3.5</td>
</tr>
<tr>
<td>4l</td>
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</tr>
<tr>
<td>4m</td>
<td>O</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Aspirin | 28.5 ± 3.2 | 72.4 ± 4.6 |

IC₅₀ values were calculated from at least three separate experiments. Data are presented as means ± SE (n = 3).