Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 5027–5029

Synthesis and evaluation of antiplatelet activity of trihydroxychalcone derivatives

Li-Ming Zhao,[a,b] Hai-Shan Jin,[a] Liang-Peng Sun,[a] Hu-Ri Piao[a] and Zhe-Shan Quan[a,*]

[a]*College of Pharmacy, Yanbian University, 121 Juzi Street, Yanji 133000, Jilin, China*
[b]*College of Pharmacy, Shanghai Jiaotong University, Minhang, Shanghai 200240, China*

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.

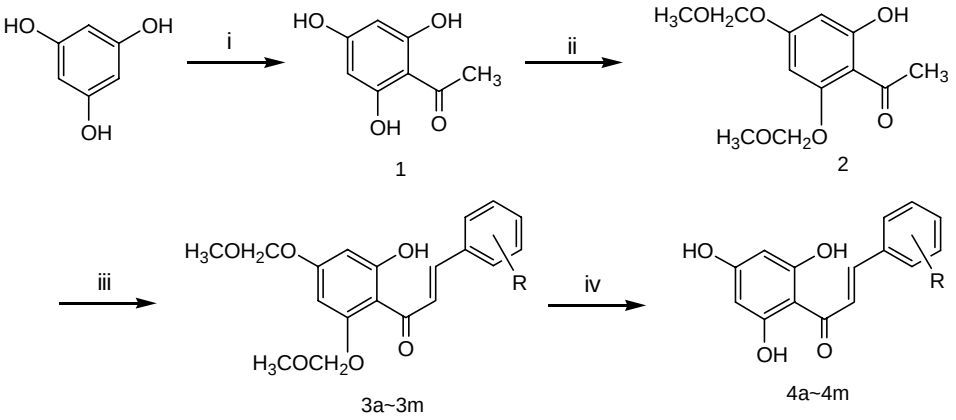
It is generally accepted that platelets play an important role in the progress and development of thrombotic disorders, especially cerebro vascular diseases such as transient ischemic attack,[1] ischemic heart diseases such as myocardial infarction[2] and peripheral vascular diseases.[3] Consequently, 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] antiulcerative,[8] analgesic,[9] antiviral,[10] antifungal,[11] antimalarial,[12] and antibacterial activities.[13] Nakadate et al.[14] reported that 2′-hydroxychalcones, 4′-hydroxychalcones, and 2′,4′-dihydroxychalcones inhibited 12-lipoxygenase and cyclooxygenase in the mouse epidermis. Ballesteros et al.[15] reported that two synthetic 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. All

Keywords: Hydroxychalcones; Antiplatelet; Structure–activity relationship.
* Corresponding author. Tel.: +86 43 32 66 06 06; fax: +86 43 326 60 568; e-mail: zsquan@ybu.edu.cn

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.08.039



Scheme 1. Reagents and conditions: (i) $HCl/CH_3CN/ZnCl_2$, 0 °C, 24 h and reflux, 2 h; (ii) $ClCH_2OCH_3/K_2CO_3$/acetone, rt, 2 h; (iii) aromatic aldehyde/KOH/EtOH, rt, 24 h; (iv) 3 M HCl in MeOH, reflux, 0.5 h.

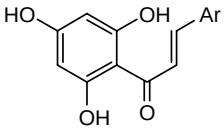
spectral data (IR, ^1H NMR, and MS) obtained were consistent with the structures proposed.[18]

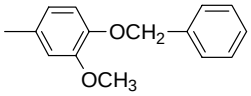
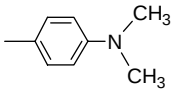
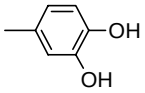
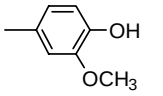
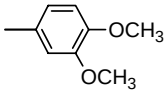
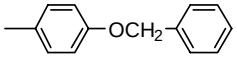
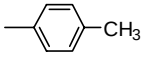
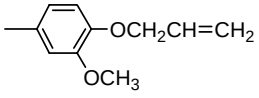
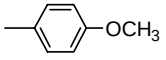
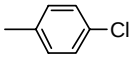
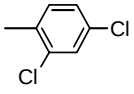
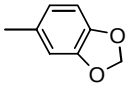
The antiplatelet activity was studied by measuring the aggregation of washed rabbit platelet applying Born's turbidimetric method.[19] The washed platelet suspension of rabbits was incubated at 37 °C for 4 min in an aggregometer with stirring at 1000 rpm and the aggregation was stimulated by adding arachidonic acid (100 μM) or collagen (10 μg/ml) at concentrations giving maximal aggregatory response, as Jin et al.[20] described in detail. The antiplatelet activity was expressed as percent inhibition with respect to control. Anti-aggregating potency of the compounds was indicated by IC_{50} 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 comparative to aspirin, a COX inhibitor which was used as a positive control. Comparing with compound **4l**, compounds **4a**, **4e**, **4h**, **4i**, and **4j** had potent antiplatelet effects. It seemed that the substituent 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 IC_{50} 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*-phenylmethylation 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 methoxyl 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 **4l** (i.e., **4j**) or dichloro substituted at C-2 and C-4 of **4l** (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 IC_{50} 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 cycloxygenase activity and reduced thromboxane formation as it had been reported that some chalcone derivatives inhibited arachidonic acid-induced platelet aggregation by inhibition of cycloxygenase activity and thromboxane formation.[16] 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

Table 1. In vitro antiplatelet activity of compounds **4a**–**4m** and ASA in washed rabbit platelets



Compound	Ar	IC_{50} value (μM)	
		Arachidonic acid	Collagen
4a		28.5 ± 2.2	37.4 ± 4.4
4b		>100	>100
4c		>100	>100
4d		>100	>100
4e		15.2 ± 5.4	27.4 ± 4.4
4f		>100	>100
4g		>100	>100
4h		19.8 ± 3.5	28.4 ± 5.3
4i		25.5 ± 2.7	33.3 ± 4.6
4j		45.2 ± 6.7	66.5 ± 5.3
4k		70.6 ± 3.5	>100
4l		>100	>100
4m		>100	>100
Aspirin		28.5 ± 3.2	72.4 ± 4.6

IC_{50} values were calculated from at least three separate experiments. Data are presented as means ± SE ($n = 3$).

studies. Further studies are in progress in our laboratory and will be reported in detail in a series of forthcoming papers.

References and notes

1. Sherman, D. G.; Hart, R. G. *J. Am. Coll. Cardiol.* **1986**, *8*, 88.
2. Trip, M. D.; Cats, V. M.; Capelle, F. J. L.; Vreeken, J. *N. Engl. J. Med.* **1990**, *322*, 1549.
3. Genton, E.; Clagett, G. P.; Salzman, E. W. *Chest* **1986**, *89*, 75S.
4. Schror, K. *Drugs* **1996**, *50*, 7.
5. Bennett, J. S. *Annu. Rev. Med.* **2001**, *52*, 161.
6. Kumar, S. K.; Hager, E.; Pettit, C.; Gurulingappa, H.; Davidson, N. E.; Khan, S. R. *J. Med. Chem.* **2003**, *46*, 2813.
7. Hsieh, H. K.; Lee, T. H.; Wang, J. P.; Wang, J. J.; Lin, C. N. *Pharm. Res.* **1998**, *15*, 39.
8. Murakami, S.; Muramatsu, M.; Aihara, H.; Otomo, S. *Biochem. Pharmacol.* **1991**, *42*, 1447.
9. Viana, G. S.; Bandeira, M. A.; Matos, F. J. *Phytomedicine* **2003**, *10*, 189.
10. Wu, J. H.; Wang, X. H.; Yi, Y. H.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1813.
11. Lopez, S. N.; Castelli, M. V.; Zacchino, S. A.; Dominguez, J. N.; Lobo, G.; Charris-Charris, J.; Cortes, J. C.; Ribas, J. C.; Devia, C.; Rodriguez, A. M.; Enriz, R. D. *Bioorg. Med. Chem.* **2001**, *9*, 1999.
12. Liu, M.; Wilairat, P.; Go, M. L. *J. Med. Chem.* **2001**, *44*, 4443.
13. Bekhit, A. A.; Habib, N. S.; el-Din, A.; Bekhit, A. *Boll. Chim. Farm.* **2001**, *140*, 297.
14. Nakadate, T.; Aizu, E.; Yamamoto, S.; Kato, R. *Prostaglandins* **1985**, *30*, 357.
15. Ballesteros, J. F.; Sanz, M. J.; Ubeda, A.; Miranda, M. A.; Iborra, S.; Paya, M.; Alcaraz, M. J. *J. Med. Chem.* **1995**, *38*, 2794.
16. Lin, C. N.; Hsieh, K. H.; Ko, H. H.; Hsu, M. F.; Lin, H. C.; Chang, Y. L.; Chung, M. I.; Kang, J. J.; Wang, J. P.; Teng, C. M. *Drug Dev. Res.* **2001**, *53*, 9.
17. Blatt, A. H. *Org. Syn.* New York, 1950; Vol 2, pp 522–523.
18. Selected data for compound **4e**: IR (KBr) cm^{-1} 1624 (C=O). ^1H NMR (300 MHz, DMSO-d_6)δ:3.84 (6H, s, OCH_3), 5.99–7.19 (5H, m, Ar-H), 7.68 (1H, d, $J = 15.6$ Hz, H_α), 8.03 (1H, d, $J = 15.6$ Hz, H_β). MS (MH^+) 317.
19. Born, G. V.; Cross, M. J. *J. Physiol.* **1963**, *168*, 178.
20. Jin, Y. R.; Cho, M. R.; Ryu, C. K.; Chung, J. H.; Yuk, D. Y.; Hong, J. T.; Lee, K. S.; Lee, J. J.; Lee, M. Y.; Lim, Y.; Yun, Y. P. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 214.