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Synthesis of phospholipase A_2 inhibitory biflavonoids

Jianjun Chen,[a] Hyeun Wook Chang,[b,*] Hyun Pyo Kim[a] and Haeil Park[a,*]

[a]*College of Pharmacy, Kangwon National University, Chunchon 200-701, Republic of Korea*
[b]*College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Republic of Korea*

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Abstract—A series of C–C biflavones was designed to investigate the relationship between structural array of different flavone–flavone subunit linkage and the inhibitory activity against phospholipase A_2 (PLA_2). Among six classes of C–C biflavones designed, four classes of C–C biflavones, which have flavone–flavone subunit linkages at A ring–A ring, A ring–B ring, B ring–B ring, and B ring–C ring, were synthesized. The synthetic biflavones exhibited somewhat different inhibitory activities against $sPLA_2$-IIA. Among them, the biflavone **a** having a C–C 4′–4′ linkage showed comparable inhibitory activity with that of the natural biflavonoid, ochnaflavone, and 7-fold stronger activity than that of amentoflavone. Further chemical modification is being carried out in order to obtain the chemically optimized biflavonoids.

Biflavonoids are flavonoid dimers connected with a C–C or C–O–C bond. Although a wealth of biflavonoids have been discovered from various plant species, their biological and pharmacological data are limited. Previously, certain biflavonoids were reported to inhibit phosphodiesterase,[1] lens aldose reductase,[2] and mast cell histamine release,[3] and to show anticancer activity.[4] Recently, some C–C biflavonoids were synthesized and their antimicrobial activities were demonstrated.[5] During our investigations to find potential anti-inflammatory plant drugs, several biflavones such as amentoflavone and ochnaflavone (Fig. 1) were for the first time demonstrated as inhibitors of group II secretory phospholipase A_2 ($sPLA_2$IIA).[6] Later, morelloflavone, a flavone–flavanone dimer, was also revealed as $sPLA_2$ inhibitors[7] (Fig. 1).

PLA_2 is a growing family of distinct enzymes that exhibit different substrate specificities, cofactor requirement, subcellular localization, and cellular functions.[8] $sPLA_2$ has low molecular weights (14–18 kDa) with a rigid tertiary structure configured by 6–8 disulfide bridges. Thus so far, 10 genes coding for structurally related and enzymatically active $sPLA_2$s have been identified in mammals (groups IB, IIA, IIC, IID, II, IIF, III, V, X, and XII).[9] Since $sPLA_2$ is a pivotal enzyme to generate arachidonic acid that is converted further to proinflammatory eicosanoids, $sPLA_2$ inhibitors may show favorable anti-inflammatory activity. Actually, some of these biflavonoids were found to possess promising anti-inflammatory activity in vivo.[7,10] In this respect, several basic biflavonoids have been synthesized and their inhibitory activities on $sPLA_2$-IIA were evaluated in this investigation.

A series of C–C biflavones was designed (Fig. 2) to investigate the relationship between structural array of a different flavone–flavone subunit linkage and the inhibitory activity against $sPLA_2$-IIA. Among six classes of C–C biflavones designed, four classes of C–C biflavones, which have flavone–flavone subunit linkages at A ring–A ring, A ring–B ring, B ring–B ring, and B ring–C ring, were synthesized.

The total synthesis of C–C biflavones was approached via construction of two flavone analogs, one substituted with halogen atom (bromo) and the other substituted with groups that could be coupled using transition metal-catalyzed cross-coupling methodology.

Two typical methods, Suzuki coupling reaction[11] and Stille coupling reaction,[12] were applied to connect two flavone units via a biaryl linkage (Scheme 1). Halogenoflavones (Ar-X) were prepared from halogen-substituted 2-hydroxyacetophenones or flavones by reacting with halogenating reagents following the general procedures

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* Corresponding authors. Tel.: +82 33 250 6920; fax: +82 33 255 7865 (H.P.); tel.: +82 53 810 2811; fax: +82 53 810 4654 (H.W.C.); e-mail addresses: hwchang@yu.ac.kr; haeilp@kangwon.ac.kr

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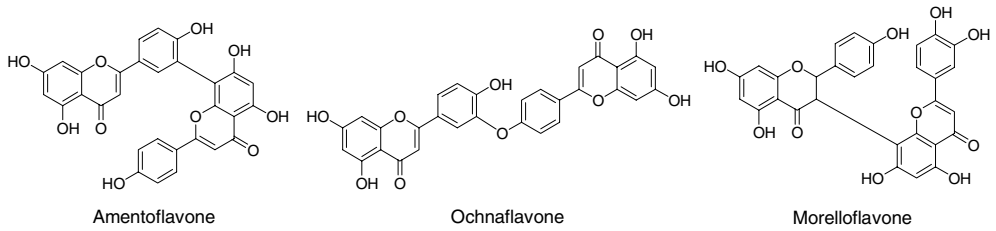


Figure 1. Structures of amentoflavone, ochnaflavone, and morelloflavone.

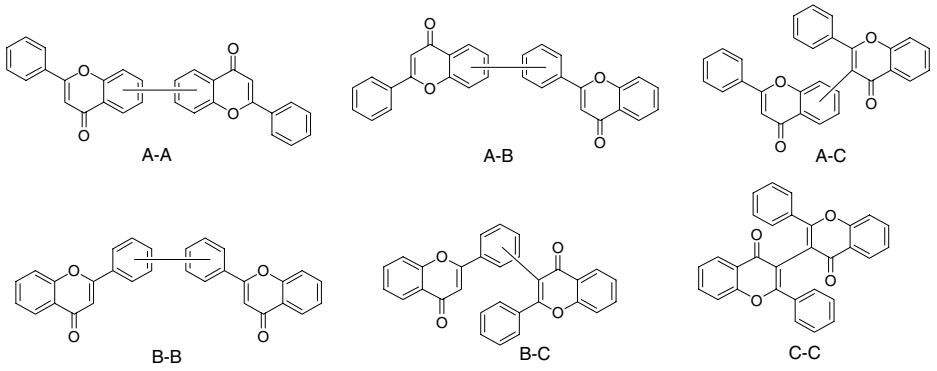
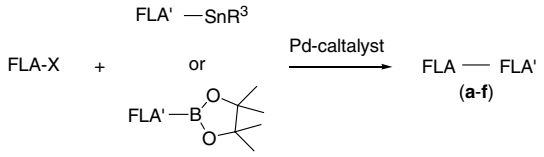


Figure 2. Biflavones with different flavone–flavone subunit linkages.



Scheme 1. Typical cross-coupling reactions for C–C biflavones.

as described in the earlier publications.[13–18] Also tributyltinflavones and boronates of flavones were prepared from the corresponding halogenoflavones following the procedures and conditions as described in the previous literatures.[13,17]

Treatment of bromoflavones (4′-, 6-, and 3′-) with commercially available hexa(*n*-butyl)ditin in the presence of catalytic $Pd(PPh_3)_4$ in refluxing toluene afforded the tributyltinflavones. Stille coupling of tributyltinflavones (1.3 equiv) with bromoflavones (3-, 6-, 3′-, and 4′-, 1.0 equiv) in the presence of 5 mol% $Pd(PPh_3)_4$ in refluxing toluene gave C–C biflavones (**a**, **b**, **d**, and **e**) in 25–50% yields. Treatment of 4-bromoflavone with bis(pinacolato)diboron in the presence of catalytic $PdCl_2$(dppf) and K_2CO_3 in DMF at 90 °C provided the corresponding pinacolato boronate. Suzuki coupling reactions of the pinacolato boronate (1.2 equiv) with bromoflavones (3′- and 3-, 1.0 equiv) in standard conditions [$Pd(PPh_3)_4$ (5 mol%), NaOH (4.0 equiv)$_3$, DMF–water (9:1), 90 °C] gave C–C biflavones (**c** and **f**) in 31% and 21% yields, respectively. Thus six C–C biflavones (Fig. 3) were prepared and evaluated for their inhibitory activity against phospholipase A_2.

The cDNA for human sPLA$_2$-IIA was cloned into an expression vector and transfected into human embryonic kidney 293 cells (HEK293 cells) using LipofectAMINE PLUS (Gibco-BRL, Gaithersburg, MD, USA) as described previously.[19,20]

The standard reaction mixture (200 μl) contained 100 mM Tris–HCl (pH 9.0), 6 mM $CaCl_2$, 1% bovine serum albumin, 2.5 μM of radiolabeled 1-acyl-2-[1-^{14}C]-arachidonyl-*sn*-glycerol phosphoethanolamine (48 mCi/mmole, NEN, Boston, MA, USA), and synthetic biflavonoids. The reaction was started by the addition of an aliquot of the culture medium as an enzyme source and carried out at 37 °C for 20 min, and [^{14}C]arachidonic acid released was extracted by the method described previously.[21] Under these conditions, the reaction mixture without synthetic biflavonoids released 10% free fatty acid. Inhibition was expressed as a percentage. Synthetic biflavonoids were dissolved in dimethylsulfoxide (DMSO) and added to the enzyme assay tubes at 2% of the final volume. Control experiments showed that DMSO at concentrations up to 2% had no effect on enzymatic activity. All determinations were duplicated and the 50% inhibitory concentration was obtained by linear regression analysis at 1–100 μM biflavones tested.

As demonstrated in Table 1, the synthetic biflavonoids exhibited somewhat different inhibitory activities against sPLA$_2$-IIA depending on their chemical structures. Among them, the biflavone a having a C–C 4′–4′ linkage showed a potent inhibition. The inhibitory potency of **a** was comparable with that of the natural biflavonoid, ochnaflavone, and 7-fold stronger than that of amentoflavone. The biflavones, **b**, **d**, and **f**, possess the similar inhibitory potency with amentoflavone. However, the potency of inhibition of **c** and **e** was weaker.

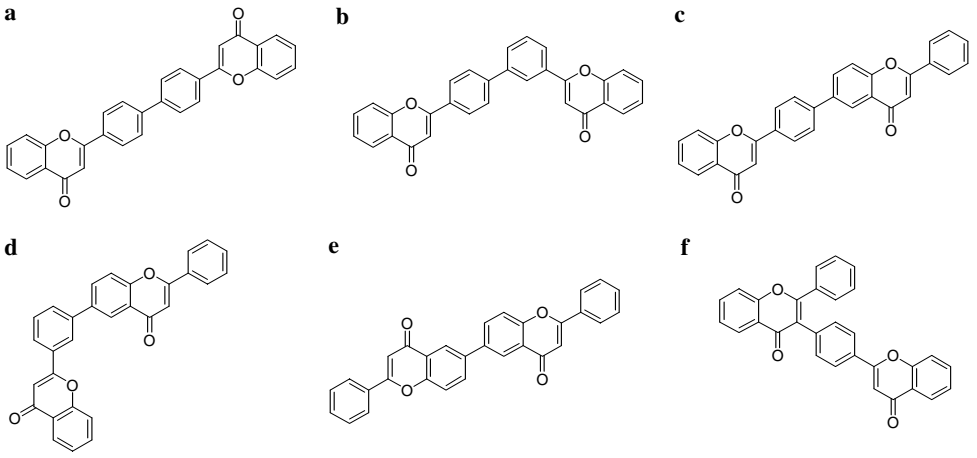


Figure 3. Structures of synthesized C–C biflavones (**a**–**f**).

Table 1. Inhibition of $sPLA_2$-IIA by synthetic biflavonoids **a**–**e**

Compound	IC_{50} (μM)[a]
Amentoflavone	23.8 ± 3.4
Ochnaflavone	3.5 ± 0.6
a	3.0 ± 0.9
b	15.5 ± 3.7
c	63.9 ± 4.2
d	19.9 ± 4.6
e	69.3 ± 5.7
f	23.2 ± 3.1

[a] All data are arithmetic means ± SD ($n = 3$).

Further chemical modification of these basic structures is being carried out for increasing their pharmacological activities.

Acknowledgments

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