



Research article

Synthesis of biflavonoid frameworks using Suzuki-Miyaura coupling with boronic acids

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Abstract

Importance of the work: Boronic acids were used in the Suzuki-Miyaura coupling reaction to construct biflavonoid compounds.

Objectives: To investigate the use of boronic acids in the Suzuki-Miyaura coupling reaction for biflavonoid synthesis.

Materials and Methods: Various methods were used, depending on molecular symmetry. A symmetrical 3',3'''-biapigenin framework was constructed using a center-to-edge strategy. With the amentoflavone framework, the initial preparation of monoflavonoid intermediates was followed by a sequence of reactions to form biflavonoid frameworks.

Results: A biapigenin structure, characterized by both A–B and B–B linkages, was synthesized under the specified conditions. A comparative study with amentoflavone revealed that boronic acids ($\text{ArB}(\text{OH})_2$) were effective under standard aqueous-basic Suzuki-Miyaura coupling conditions.

Main finding: In the examined systems, Suzuki-Miyaura coupling reactions using boronic acids proceeded, whereas the corresponding reactions using pinacol boronates were unsuccessful.

Introduction

Biflavonoids, which consist of two monoflavonoid units, were discovered by Baker et al. (1940) when they isolated them from *Ginkgo biloba* L. They are widely distributed in angiosperms, bryophytes, ferns and gymnosperms (He et al., 2021) and by the end of 2025, the number of biflavonoids with

known structures from natural sources is exceeded 600 (Zhang et al., 2011; Zhang et al., 2026). Biflavonoids have been shown to possess a range of biological activities with relatively low toxicity, including anti-inflammatory, antioxidant, antibacterial, antiviral, antidiabetic, antitumor and cytotoxic properties (Lin et al., 2001; Mangmool et al., 2024; Liu et al., 2025). Owing to their unique dimeric structure and biological activity, there has been increasing interest in their application in drug discovery

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in recent years. However, it is extremely challenging to extract pure biflavonoids from natural sources; typically, only small amounts of compounds with limited purity can be obtained (Duman et al., 2025). Hence, researchers have refocused from natural extraction to laboratory syntheses. Biflavonoids can be divided into two main types: C–C-linked (such as amentoflavone) and C–O–C-linked (such as hinokiflavone) bioflavonoids, where these different linkages may affect molecular flexibility, target binding and biological activity (Karmakar et al., 2026).

Biapigenin, a biflavonoid formed by C–C dimerization, is denoted by two apigenin units and several positional isomers are known depending on the interflavonyl linkage (for example I-3/II-8, I-6/II-3' and I-3'/II-3'). Among them, the I-3/II-8 isomer, amentoflavone (Fig. 1), is common in plants. Its isolation from *Hypericum perforatum* L. and the diverse biological activities reported for biflavones (including anti-inflammatory, antiviral, anti-tumor) are well documented (Berghöfer and Hölzl, 1987; He et al., 2021). In contrast, the B-Ring–B-Ring (3'–3'') linked biapigenin (3',3''-biapigenin (Fig. 1)) has been reported rarely in nature; related B–B-linked biflavonoids have been detected in *Selaginella* extracts; however, systematic studies of their synthesis and evaluation remain scarce relative to AA/AB-type linkages (Li et al., 2014; Kumar et al., 2021).

Biflavonoids can be synthesized through various methodologies, including oxidative coupling, metal-catalyzed cross-coupling and condensation-based approaches. Among these, the Suzuki-Miyaura coupling reaction has emerged as a particularly effective method for constructing C–C interflavonyl bonds due to its functional group tolerance and mild reaction conditions. Several research groups have used this strategy for biflavonoid synthesis. For example, Sagrera and Seoane (2010, 2011) reported the total synthesis of 3',3''-binaringenin and related biflavonoids using a symmetry-based Suzuki-Miyaura approach, while Park et al. (2015) reported the total synthesis of amentoflavone. However, these methods often relied on pinacol boronate esters as boron-coupling partners. Although

pinacol boronates offer good stability and ease of handling, they require prior hydrolysis to the corresponding boronic acid before transmetalation can occur and their increased steric bulk and reduced Lewis acidity can decrease coupling efficiency, particularly in sterically congested or strongly conjugated flavonoid systems (Zhang et al., 2012). Indeed, in the synthesis of amentoflavone, attempts to use bis(pinacolato)diboron ($B_{2}pin_{2}$) for the borylation of the C-8-halogenated flavonoid monomer were unsuccessful, yielding only debrominated byproducts and unreacted starting material, likely due to the low oxidative addition reactivity at the hindered C-8 site and strong conjugation within the chromenone framework (Park et al., 2015). In contrast, boronic acids can participate directly in the catalytic cycle without an additional hydrolysis step, offering superior reactivity and atom economy under standard aqueous-basic conditions (Lennox and Lloyd-Jones, 2014). These considerations motivated the present study, in which boronic acids were adopted as coupling partners for the construction of biflavonoid frameworks.

Herein, the application is described of boronic acids in Suzuki-Miyaura coupling reactions for the synthesis of a B–B-linked biapigenin skeleton and compared with the synthesis of amentoflavone hexamethyl ether.

Materials and Methods

General methods

Thin-layer chromatography (TLC) was conducted using silica gel 60 F₂₅₄ pre-coated plates (0.25 mm) and visualized using a combination of ultraviolet (UV) staining. Silica gel 60N (neutral, sphere, particle size 0.063–0.210 mm) was used for column chromatography. Nuclear magnetic resonance (1H NMR) spectra were recorded on a JEOL JNM-ECZ400 (400 MHz) spectrometer at room temperature. The data for 1H spectra were: chemical shift (δ ppm), multiplicity, coupling

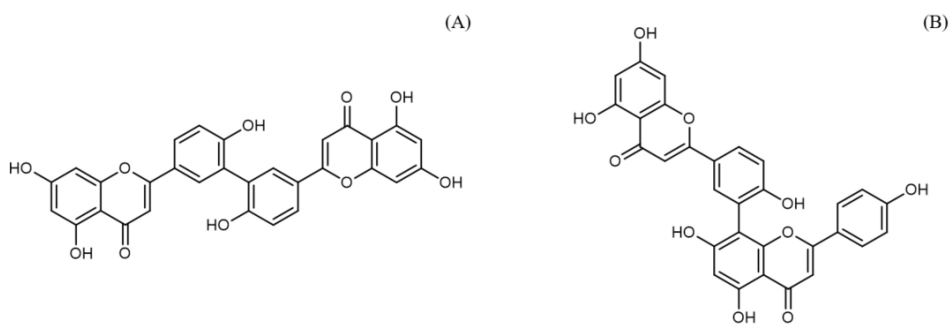


Fig. 1 Elemental structure of: (A) 3',3''-biapigenin and (B) amentoflavone.

constant (J) (Hz) and integration and were referenced to the residual solvent peak at 7.26 ppm for CDCl_3 or 0.00 ppm for tetramethylsilane. ^{13}C NMR spectra were reported regarding chemical shift (at 100 MHz) and referenced to the residual solvent peak at 77.0 ppm for CDCl_3 . Mass spectra (MS) were measured using a Thermo Scientific Orbitrap Exploris 240 with an ion transfer tube temperature of 320°C and a static spray voltage of 3,400 V (positive mode). MS samples were dissolved in MeOH and injected using a syringe pump at a flow rate of $10\ \mu\text{L}/\text{min}$.

Retrosynthesis analysis

As shown in Fig. 2, the retrosynthetic analysis of biflavonoids focused on Suzuki-Miyaura coupling, which was used to introduce aryl groups into the flavonoid skeletons. Differentiated retrosynthetic strategies were designed based on the distinct linkage configurations and symmetries of the biflavonoid skeletons. For the symmetrical 3',3'''-biapigenin skeleton, a center-to-periphery strategy was adopted. First, a symmetric dialdehyde intermediate was constructed via Suzuki-Miyaura coupling using boronic acid, followed by Claisen-Schmidt condensation, cyclization and deprotection reactions for the efficient production of the target product (Sagrera et al., 2010; Sagrera et al., 2011). In contrast, the retrosynthetic design for the amentoflavone skeleton prioritized the construction of the monoflavonoid skeleton, followed by the introduction of the aryl group via a Suzuki-Miyaura coupling using boronic acid (Wang et al., 2025) and finally the completion of the biflavonoid skeleton through aldol condensation and cyclization reactions.

Synthesis

1-(2-Hydroxy-4,6-dimethoxyphenyl)ethan-1-one (8)

Methyl *p*-toluenesulfonate (8.00 mmol, 1.48 g) and 2,4,6-trihydroxyacetophenone (2.00 mmol, 336 mg) were added to a 100 mL round-bottomed flask equipped with a magnetic stir bar. Acetone (16 mL) was added to the mixture and stirred. Then, sodium carbonate (8.00 mmol, 848 mg) was added. The reaction mixture was refluxed overnight at 60°C in an argon atmosphere using a condenser. The reaction progress was monitored using TLC. After complete consumption of the starting material, the reflux setup was removed and water (16 mL) was added. Then, the mixture was heated to 90°C and stirred for 1.5 hr. Next, the reaction mixture was cooled to room temperature and the pH was adjusted to approximately 9 using 6 M hydrochloric acid, resulting in the formation of a white precipitate. The precipitate was collected using filtration, washed with water and dried to yield a grayish-white crude product. Recrystallization from methanol produced a white solid with parameters: 87% yield, ^1H NMR (CDCl_3 , 400 MHz): δ 6.00 (d, $J = 2.4$ Hz, 1H), 5.87 (d, $J = 2.4$ Hz, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 2.56 (s, 3H). These data were consistent with those reported in the literature (Park et al., 2015).

3-Bromo-4-methoxybenzaldehyde (9)

p-Anisaldehyde (0.12 mL, 1.0 mmol) was added to a dry 30 mL round-bottomed flask. Methanol (4 mL) was added and the mixture was stirred in an argon atmosphere at 0°C . A solution of bromine (1.0 M in CH_2Cl_2 , 1.5 mL, 1.5 mmol) was placed in another dry flask. Then, the bromine solution was added dropwise during 20 min in an ice bath. Subsequently,

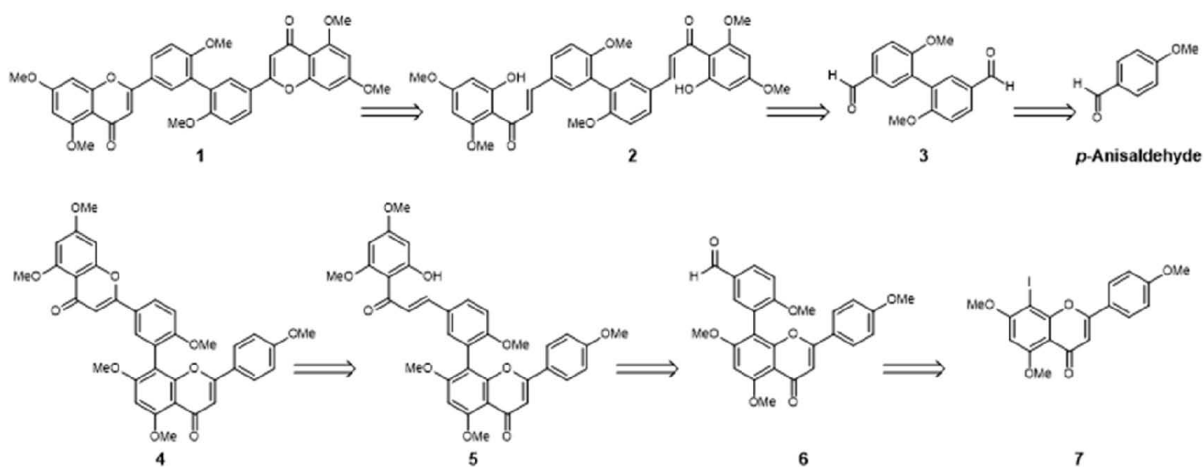


Fig. 2 Retrosynthesis of 3',3'''-biapigenin hexamethyl ether (1) and amentoflavone hexamethyl ether (4).•

acetic acid (0.10 mL) was added and the mixture was stirred at 45°C in an argon atmosphere for 24 hr. The reaction progress was monitored using TLC. After complete consumption of the starting material, the reaction mixture was cooled to room temperature and approximately two-thirds of the solvent was removed under reduced pressure. The reaction was quenched with a saturated aqueous solution of sodium thiosulfate until the color no longer changed. The mixture was extracted using ethyl acetate and the aqueous phase was re-extracted using ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain a brownish-white crude product. Purification using column chromatography (ethyl acetate (EA)/hexane = 1:10) produced a white solid with parameters: 96% yield, ^1H NMR (CDCl_3 , 400 MHz): δ 9.84 (s, 1H), 8.08 (d, J = 2.0 Hz, 1H), 7.82 (dd, J = 8.4 Hz, 1.6 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.99 (s, 3H). The ^1H NMR data were consistent with the spectral data from SpectraBase (<https://spectrabase.com/>; Spectrum ID: 22Xkida0qfW).

6,6'-Dimethoxy-[1,1'-biphenyl]-3,3'-dicarbaldehyde (3)

3-Bromo-4-methoxybenzaldehyde (74 mg, 0.35 mmol), 5-formyl-2-methoxyphenylboronic acid (93 mg, 0.52 mmol) and potassium carbonate (190 mg, 1.38 mmol) were added to a 30 mL round-bottomed flask. Then, degassed DMF/ H_2O (4.5 mL/0.5 mL, degassed for 30 min) was added, followed by PPh_3 (3.2 mg, 0.069 mmol) and PdCl_2 (3.1 mg, 0.017 mmol). The reaction mixture was stirred at 80°C in an argon atmosphere for 2 hr. After completion, the reaction mixture was cooled to room temperature, filtered through a Celite pad and washed with ethyl acetate. The filtrate was extracted using ethyl acetate and the organic layer was washed with water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain a white, crude product. Purification by column chromatography (EA/hexane = 1:2) produced a white solid with parameters: 93% yield, ^1H NMR (CDCl_3 , 400 MHz): δ 9.92 (s, 1H), 7.92 (dd, J = 8.8 Hz, 2.2 Hz, 1H), 7.79 (d, J = 2.4 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 3.87 (s, 3H). These data were consistent with those reported in the literature (Sagrera et al., 2010).

(2E,2'E)-3,3'-(6,6'-Dimethoxy-[1,1'-biphenyl]-3,3'-diyl)bis(1-(2-hydroxy-4,6-dimethoxyphenyl)prop-2-en-1-one) (2)

6,6'-Dimethoxy-[1,1'-biphenyl]-3,3'-dicarbaldehyde (114 mg, 0.442 mmol) and 1-(2-hydroxy-4,6-dimethoxyphenyl)ethan-1-one (166 mg, 0.844 mmol) were added to a 100 mL

round-bottomed flask equipped with a magnetic stirrer. Ethanol (13 mL) was added and the mixture was stirred. Barium hydroxide octahydrate ($\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, 200 mg, 0.634 mmol) was added and the reaction mixture was stirred at 60°C for 2 d. Subsequently, the reaction mixture was cooled to room temperature and 1 M HCl was added under vigorous stirring to obtain pH 3, which resulted in the formation of a yellow precipitate. Then, water was added and the mixture was stirred and filtered to collect the precipitate obtained. The solid was washed with water 1–2 times to produce a yellow crude product. Recrystallization from MeOH produced a yellow solid with parameters: 97% yield, ^1H NMR (CDCl_3 , 400 MHz): δ 7.81 (d, J = 4.8 Hz, 2H), 7.63 (dd, J = 8.6 Hz, 2.4 Hz, 1H), 7.53 (d, J = 2.4 Hz, 1H), 7.03 (d, J = 15.6 Hz, 1H), 6.10 (d, J = 2.4 Hz, 1H), 5.94 (d, J = 2.4 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 192.5, 168.3, 166.0, 162.4, 158.8, 142.4, 131.4, 129.9, 128.0, 127.6, 125.3, 111.2, 106.3, 93.7, 91.2, 55.88, 55.83, 55.6. These data were consistent with those reported in the literature (Sagrera et al., 2010).

2,2'-(6,6'-Dimethoxy-[1,1'-biphenyl]-3,3'-diyl)bis(5,7-dimethoxy-4H-chromen-4-one) (1)

(2E,2'E)-3,3'-(6,6'-Dimethoxy-[1,1'-biphenyl]-3,3'-diyl)bis(1-(2-hydroxy-4,6-dimethoxyphenyl)prop-2-en-1-one) (99 mg, 0.16 mmol) was added to a Schlenk tube and dissolved in dimethyl sulfoxide (DMSO, 4 mL). Then, a solution of I_2 in DMSO (0.010 M, 1.6 mL, 0.016 mmol) was added. The reaction mixture was stirred at 190°C in an argon atmosphere for 1 hr. After completion, the reaction mixture was cooled to room temperature and quenched with a saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ solution until a yellowish-white precipitate formed. Then, water was added and the mixture was stirred and subsequently filtered. The solid was washed with water 1–2 times to obtain a brown crude product. Purification using column chromatography (3% MeOH in CHCl_3) produced a white-yellow solid with parameters: 84% yield, ^1H NMR (CDCl_3 , 400 MHz): δ 7.90 (dd, J = 8.6 Hz, 2.4 Hz, 1H), 7.80 (d, J = 2.4 Hz, 1H), 7.10 (d, J = 8.8 Hz, 1H), 6.65 (s, 1H), 6.56 (d, J = 2.4 Hz, 1H), 6.37 (d, J = 2.4 Hz, 1H), 3.95 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 177.7, 163.9, 160.8, 160.6, 159.8, 159.5, 129.0, 127.4, 127.3, 111.2, 109.2, 107.8, 96.1, 56.4, 55.9, 55.7. HRMS (ESI), m/z calcd for $\text{C}_{36}\text{H}_{31}\text{O}_{10}$ [$\text{M}+\text{H}$] $^+$ 623.1912, found 623.1906. These data were consistent with those reported in the literature (Sagrera et al., 2010).

1-(6-Hydroxy-3-iodo-2,4-dimethoxyphenyl)ethan-1-one (10)

1-(2-Hydroxy-4,6-dimethoxyphenyl)ethan-1-one (110 mg, 0.560 mmol) was added to a 10 mL round-bottomed flask and dissolved in acetic acid (AcOH, 2 mL). The reaction mixture was cooled to 0°C in an argon atmosphere and a solution of I₂ in CHCl₃ (185 mg, 0.730 mmol) was added dropwise. After the addition, 60% HNO₃ (0.043 mL, 0.56 mmol) was introduced and the reaction mixture was stirred at 0°C in an argon atmosphere for 3.5 hr. The reaction was quenched with a saturated aqueous Na₂S₂O₃ solution until the color no longer changed. The mixture was extracted with ethyl acetate and the organic layer was washed with water and brine, dried over Na₂SO₄ and concentrated under reduced pressure to obtain a crude product. The crude product was used directly in the next step without further purification with parameters: 65% yield, ¹H NMR (CDCl₃, 400 MHz): δ 6.00 (s, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 2.63 (s, 3H). These data were consistent with those reported in the literature (Park et al., 2015).

(E)-1-(2-hydroxy-3-iodo-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (11)

1-(6-Hydroxy-3-iodo-2,4-dimethoxyphenyl)ethan-1-one (75 mg, 0.23 mmol) and *p*-anisaldehyde (0.030 mL, 0.23 mmol) were added to a dry 50 mL round-bottomed flask. Ethanol (5 mL) was added and the mixture was stirred. Then, Ba(OH)₂·8H₂O (95 mg, 0.30 mmol) was introduced and the reaction mixture was stirred at 60°C in an argon atmosphere overnight. After completion, the reaction mixture was cooled to room temperature and the pH was adjusted to approximately 7 using 1 M HCl. Next, water was added, resulting in the formation of a yellow precipitate. The solid was collected using filtration and washed with water to obtain a yellow, crude product. The crude product was used directly in the next step without further purification and in a quantitative yield, with parameters: ¹H NMR (CDCl₃, 400 MHz): δ 7.86 (m, 2H), 7.57 (d, *J* = 8.8 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 6.07 (s, 1H), 4.00 (s, 3H), 3.98 (s, 3H), 3.86 (s, 3H). These data were consistent with those reported in the literature (Park et al., 2015).

8-Iodo-5,7-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (7)

(*E*)-1-(2-Hydroxy-3-iodo-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (118 mg, 0.270 mmol) was added to a 50 mL round-bottomed flask and dissolved in DMSO (5 mL). Then, iodine (I₂, 48 mg, 0.19 mmol) was added and the reaction mixture was stirred at 120°C in an argon atmosphere overnight. After completion, the reaction mixture

was cooled to room temperature and quenched with a saturated aqueous Na₂S₂O₃ solution until the color no longer changed. The mixture was filtered and the solid was washed with water and air-dried to obtain a crude product. Purification using column chromatography (CHCl₃/MeOH = 20:1) produced a yellow solid with parameters: 71% yield, ¹H NMR (CDCl₃, 400 MHz): δ 8.00 (dd, *J* = 6.8 Hz, 2.0 Hz, 2H), 7.01 (dd, *J* = 5.2 Hz, 2.0 Hz, 2H), 6.63 (s, 1H), 6.41 (s, 1H), 4.02 (s, 6H), 3.88 (s, 3H). These data were consistent with those reported in the literature (Park et al., 2015).

3-(5,7-dimethoxy-2-(4-methoxyphenyl)-4-oxo-4H-chromen-8-yl)-4-methoxybenzaldehyde (6)

8-Iodo-5,7-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (38 mg, 0.090 mmol) and 5-formyl-2-methoxyphenylboronic acid (30 mg, 0.17 mmol) were added to a 20 mL round-bottomed flask. Potassium carbonate (60 mg, 0.44 mmol) and PPh₃ (5.8 mg, 0.022 mmol) were added, followed by degassed DMF/H₂O (1.8 mL/0.2 mL). Catalytic PdCl₂ was added and the reaction mixture was subjected to three vacuum-argon refill cycles. The reaction mixture was stirred at 80°C in an argon atmosphere for 4 hr. The reaction progress was monitored using TLC. After completion, the mixture was cooled to room temperature, filtered through a Celite pad and washed with ethyl acetate. The filtrate was extracted with ethyl acetate and the organic layer was washed with water and brine, dried over Na₂SO₄ and concentrated under reduced pressure to obtain a crude product. Purification using preparative thin-layer chromatography (PTLC; CHCl₃/MeOH = 20:1) produced the desired product with parameters: 45% yield, ¹H NMR (CDCl₃, 400 MHz): δ 9.96 (s, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.86 (s, 1H), 7.33 (d, *J* = 8.8 Hz, 2H), 7.17 (d, *J* = 8.8 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 2H), 6.58 (s, 1H), 6.52 (s, 1H), 4.07 (s, 3H), 3.89 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 190.8, 177.9, 162.5, 161.8, 160.9, 160.8, 160.3, 156.0, 134.8, 131.7, 129.5, 127.1, 123.4, 122.3, 114.1, 110.7, 108.6, 106.5, 106.3, 91.5, 56.0, 55.9, 55.4, 55.3. HRMS (ESI), *m/z* calcd for C₂₆H₂₃O₇ [M+H]⁺ 447.1438, found 447.1436.

(E)-8-(5-(3-(2-hydroxy-4,6-dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-2-methoxyphenyl)-5,7-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (5)

3-(5,7-Dimethoxy-2-(4-methoxyphenyl)-4-oxo-4H-chromen-8-yl)-4-methoxybenzaldehyde (17.5 mg, 0.0390 mmol) and 1-(2-hydroxy-4,6-dimethoxyphenyl)ethan-1-one (15 mg, 0.078 mmol) were added to a 20 mL round-bottomed

flask. EtOH (1.5 mL) was added and the mixture was stirred. Then, Ba(OH)₂·8H₂O (25 mg, 0.078 mmol) was introduced and the reaction mixture was stirred overnight at 65°C in an argon atmosphere. After completion, the reaction mixture was cooled to room temperature and the pH was adjusted to approximately 6 using 1 M HCl. Next, water was added and the mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over Na₂SO₄ and concentrated under reduced pressure to obtain a crude product. Purification using PTLC (2% MeOH in CHCl₃) produced the desired product with parameters: 48% yield, ¹H NMR (CDCl₃, 400 MHz): δ 7.89–7.79 (m, 2H), 7.70 (dd, *J* = 6.4 Hz, 2.4 Hz, 1H), 7.62 (d, *J* = 2.0 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 2H) 7.08 (d, *J* = 8.8 Hz, 1H), 6.80 (d, *J* = 8.8 Hz, 2H), 6.60 (s, 1H), 6.53 (s, 1H), 6.09 (d, *J* = 2.4 Hz, 1H), 5.91 (d, *J* = 2.4 Hz, 1H), 4.07 (s, 3H), 3.91 (s, 3H), 3.82 (s, 3H), 3.76 (s, 3H), 3.75 (s, 6H). ¹³C NMR (CDCl₃, 100 MHz): δ 192.6, 178.2, 168.4, 166.1, 162.5, 162.0, 161.1, 160.8, 160.5, 159.5, 156.2, 142.6, 132.7, 130.6, 128.0, 127.4, 125.3, 123.6, 122.1, 114.4, 114.3, 111.1, 108.9, 107.2, 106.6, 106.4, 93.8, 91.8, 91.2, 56.6, 56.2, 55.9, 55.8, 55.7, 55.5. HRMS (ESI), *m/z* calcd for C₃₆H₃₃O₁₀ [M+H]⁺ 625.2068, found 625.2066.

8-(5-(5,7-dimethoxy-4-oxo-4H-chromen-2-yl)-2-methoxyphenyl)-5,7-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (4)

(E)-8-(5-(3-(2-Hydroxy-4,6-dimethoxyphenyl)-3-oxoprop-1-en-1-yl)-2-methoxyphenyl)-5,7-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (6.0 mg, 0.010 mmol) was added to a 20 mL round-bottomed flask. Then, a solution of I₂ in DMSO (0.7 mL, 0.007 mmol) was added and the reaction mixture was stirred at 100°C in an argon atmosphere for 2.5 hr. After completion, the reaction mixture was cooled to room temperature and quenched with a saturated aqueous Na₂S₂O₃ solution until the color no longer changed. The mixture was extracted with ethyl acetate and the organic layer was washed with water and brine, dried over Na₂SO₄ and concentrated under reduced pressure to obtain a crude product. Purification using PTLC (1% MeOH in CHCl₃) produced the desired product with parameters: 47% yield, ¹H NMR (CDCl₃, 400 MHz): δ 7.97 (dd, *J* = 6.4 Hz, 2.4 Hz, 1H), 7.86 (d, *J* = 2.4 Hz, 1H), 7.40 (d, *J* = 8.8 Hz, 2H), 7.15 (d, *J* = 8.8 Hz, 1H), 6.79 (d, *J* = 9.2 Hz, 2H), 6.66 (s, 1H), 6.61 (s, 1H), 6.54 (s, 1H), 6.50 (d, *J* = 2.4 Hz, 1H), 6.36 (d, *J* = 2.0 Hz, 1H), 4.08 (s, 3H), 3.95 (s, 3H), 3.91 (s, 3H), 3.85 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 178.0, 177.6, 163.8, 161.8, 160.9, 160.8, 160.7, 160.6, 160.4, 160.0, 159.8, 156.1, 130.5,

127.4, 127.2, 123.5, 122.1, 114.2, 111.0, 109.1, 108.7, 107.8, 106.7, 106.6, 96.1, 92.7, 91.6, 56.4, 56.3, 56.1, 55.8, 55.7, 55.3. HRMS (ESI), *m/z* calcd for C₃₆H₃₁O₁₀ [M+H]⁺ 623.1912, found 623.1904. These data were consistent with those reported in the literature (Park et al., 2015).

Results and Discussion

As shown in Fig. 2, to construct the 3',3'''-biapigenin skeleton, commercially available 2,4,6-trihydroxyacetophenone was first protected with methyl *p*-toluenesulfonate (TsOMe) to produce compound 8, with 87% yield. In addition, *p*-anisaldehyde was brominated with liquid bromine to give compound 9, with 96% yield. Subsequently, a key Suzuki-Miyaura coupling between compounds 9 and 5-formyl-2-methoxyphenylboronic acid was performed in the presence of K₂CO₃, PdCl₂ and PPh₃, producing compound 3, with 93% yield. Then the condensation of compound 3 with compound 8 was performed under basic conditions (Ba(OH)₂·8H₂O) to yield the corresponding product, with 97% yield. Finally, cyclization catalyzed using iodine produced 3',3'''-biapigenin hexamethyl ether (1), with 84% yield (Fig. 3). The overall yield over the five steps was 63%.

As summarized in Fig. 4, the synthesis of amentoflavone hexamethyl ether, compound 8 was first iodinated with iodine to produce compound 10, with 65% yield. Subsequently, compound 10 was condensed with *p*-anisaldehyde to produce compound 11 in a quantitative yield. Cyclization catalyzed using iodine produced the iodinated flavonoid monomer (compound 7), with 71% yield. Compound 7 was subjected to a Suzuki-Miyaura coupling with 5-formyl-2-methoxyphenylboronic acid to produce compound 6, with 45% yield. Next, aldol condensation of compound 6 produced compound 5, with 48% yield. Finally, iodine-catalyzed cyclization produced amentoflavone hexamethyl ether (4), with 47% yield. The overall yield over the six steps was approximately 5%.

Different strategies were explored in biflavonoid synthesis to construct 3',3'''-biapigenin hexamethyl ether and amentoflavone hexamethyl ether. For the symmetrical 3',3'''-biapigenin skeleton, a symmetrical intermediate was constructed via the Suzuki-Miyaura coupling as a key step, followed by Claisen-Schmidt condensation and cyclization, which produced the target compound with a high yield. The substantial overall yield of 63% achieved over five steps underscored the efficacy of the center-to-periphery strategy. This approach leverages the molecular symmetry of the target,

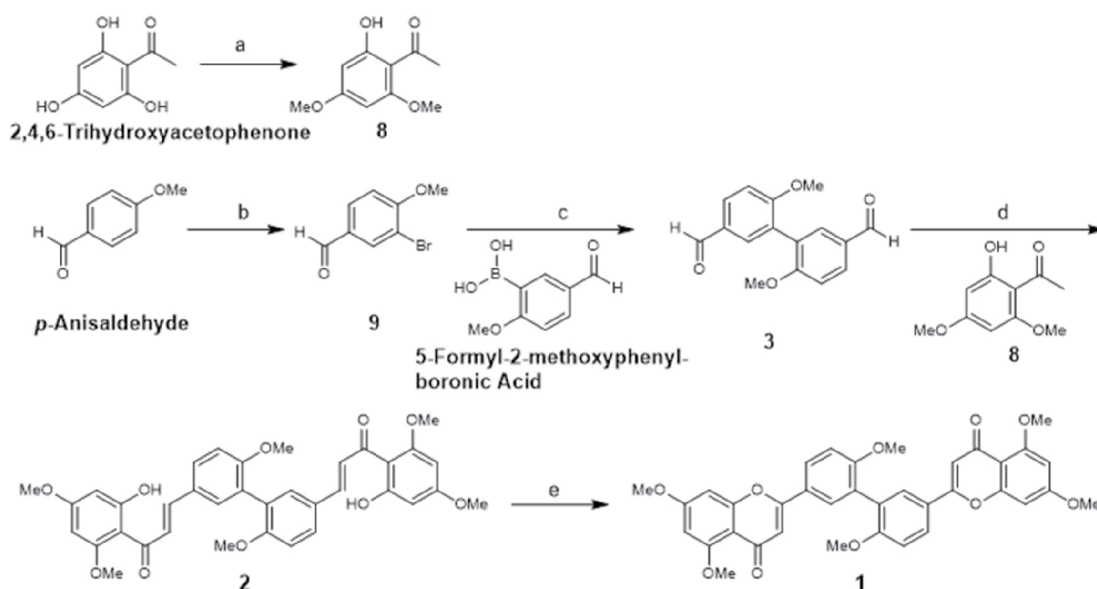


Fig. 3 Synthesis of 3',3'''-biapiogenin hexamethyl ether (1), using reagents and conditions: (a) TsOMe, Na₂CO₃, acetone, 60 °C, reflux overnight (87%); (b) Br₂, AcOH, MeOH, 0 °C to 45 °C, 24 hr (96%); (c) PdCl₂, PPh₃, K₂CO₃, DMF/H₂O, 80 °C, 2 hr (93%); (d) Ba(OH)₂•8H₂O, EtOH, 60 °C, 2 d (97%); (e) I₂, DMSO, 190 °C, 1 hr (84%). 8H₂O, EtOH, 60 °C, 2 d (97%); (e) I₂, DMSO, 190 °C, 1 hr (84%).

enabling a single Suzuki-Miyaura coupling step to introduce concurrently both the aryl substituents. Consequently, this effectively reduced the number of bond-forming events required by one-half.

In the synthesis of the amentoflavone skeleton, a linear stepwise approach was necessitated by the asymmetrical nature of the I-3/II-8 interflavonyl linkage. As discussed

in the Introduction, attempts to borylate the C-8-iodinated monomer 7 using B₂pin₂ were unsuccessful. Conversely, the direct application of 5-formyl-2-methoxyphenylboronic acid in the Suzuki-Miyaura coupling of 7 successfully yielded aldehyde 6, with 45% yield, demonstrating that the boronic acid partner effectively engaged at this sterically hindered position without requiring a prior hydrolysis step. Subsequent

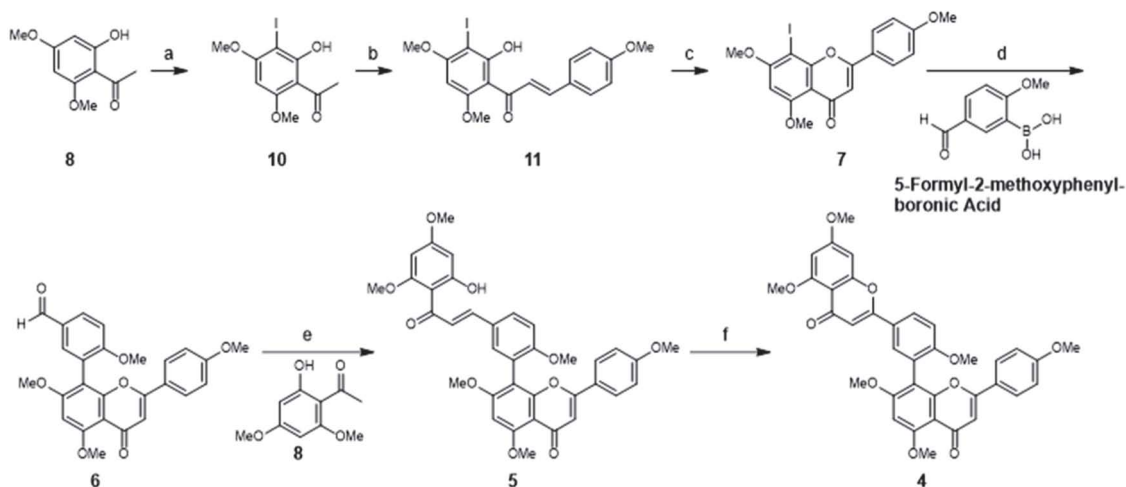


Fig. 4 Synthesis of amentoflavone hexamethyl ether (4), using reagents and conditions: (a) I₂/CHCl₃, HNO₃, AcOH, 0 °C, 3.5 hr (65%); (b) Ba(OH)₂–8H₂O, EtOH, 60 °C, overnight (quant); (c) I₂, DMSO, 120 °C, overnight (71%); (d) PdCl₂, PPh₃, K₂CO₃, DMF/H₂O, 80 °C, 4 hr (45%); (e) Ba(OH)₂–8H₂O, EtOH, 65 °C, overnight (48%); (f) I₂, DMSO, 100 °C, 2.5 hr (47%).

aldol condensation and iodine-catalyzed cyclization produced amentoflavone hexamethyl ether (4), completing the sequence in six steps, with an overall yield of approximately 5%. The reduced overall yield compared to the biapigenin route was due primarily to the increased number of steps and moderate yields of the coupling (45%) and cyclization (47%) stages, rather than any inherent limitation of the boronic acid strategy itself. Notably, the convergent nature of the biapigenin route, wherein the two flavonoid units were assembled simultaneously from a common biphenyl precursor, greatly enhanced its synthetic efficiency compared to the linear, sequential construction required for the amentoflavone framework. Collectively, these findings illustrated that boronic acids were effective coupling partners in both symmetrical and asymmetrical biflavonoid frameworks, providing direct reactivity under standard aqueous-basic conditions. Although boronic acids are known to be prone to protodeboronation under extended reaction or storage conditions, no major side reactions of this nature were observed under the optimized conditions applied here, further supporting their practical utility in complex flavonoid systems. These findings offer practical guidelines for selecting boron reagents in Suzuki-Miyaura coupling reactions involving structurally complex flavonoid systems. The choice of boron reagents played a crucial role in determining coupling efficiency and reaction outcomes, as demonstrated in other mechanistic and synthetic studies (Lennox and Lloyd-Jones, 2014; Hurtová et al., 2022).

The structural characteristics and substituent configurations of biflavonoids play a crucial role in determining their biological activities, as documented by Lin et al. (2001). Biflavonoid 12 (Fig. 5), featuring a C6–C6''' linkage and a hexamethyl ether substitution pattern, exhibited the most potent antibacterial activity against *Mycobacterium tuberculosis* Mtb H37Rv among a series of natural and synthetic biflavonoids. Primarily, this effect was attributed to the increased lipophilicity conferred by the hexamethyl ether, which improved interactions with biological targets.

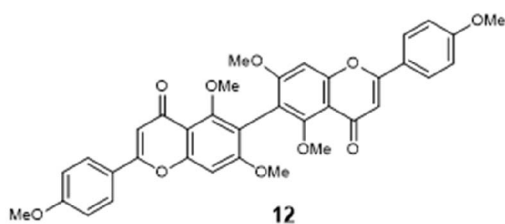


Fig. 5 Most active biflavonoid (12) against *Mycobacterium tuberculosis* Mtb H37Rv.

In addition, in a study on Alzheimer's disease by Sum et al. (2017), methylated derivative 13 (Fig. 6) had the most potent inhibitory activity against A β ₄₂ aggregation among a series of bioflavonoids.

Furthermore, Chan et al. (2009) evaluated a series of biflavonoids using a permeability glycoprotein (P-gp, a drug transport protein)-expressing human breast cancer cell line *in vitro*. They reported that compound 14 (Fig. 7), bearing methyl substitutions at C-6 and C-6''', had the highest activity in reversing multidrug resistance.

Their findings suggested that enhanced molecular lipophilicity improved the capacity to regulate P-glycoprotein activity. Overall, these studies indicated that introducing lipophilic substituents, such as methyl or methoxy groups, was an effective strategy for enhancing biflavonoid biological activity. The hexamethyl ether derivatives synthesized in the present study, namely 3',3'''-biapigenin hexamethyl ether (1) and amentoflavone hexamethyl ether (4), are classified as lipophilic biflavonoid analogs. Their efficient synthesis may facilitate future biological evaluations of methylated biflavonoid frameworks.

Conclusion

This study delineated the application of boronic acids in the Suzuki-Miyaura coupling reactions for the construction of

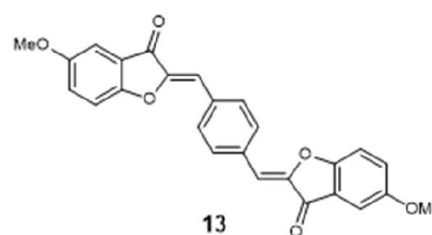


Fig. 6 Methylated derivative (13) inhibited A β ₄₂ aggregation.

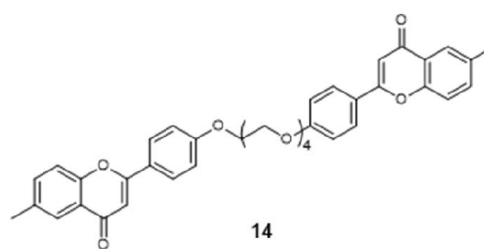


Fig. 7 C-6 and C-6''' dimethyl-substituted biflavonoid (14) having highest multidrug resistance reversal activity.

biflavonoid structures. These results exemplified the utilization of boronic acid-based coupling for the synthesis of biflavonoids. This study provided practical guidance for selecting boron reagents in Suzuki-Miyaura coupling reactions, particularly for the efficient synthesis of complex biflavonoid frameworks.

Conflict of Interest

The authors declare no conflicts of interest.

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