Malaysian Journal of Chemistry, 2025, Vol. 27(1), 220-228
(Special Issue: *International Conference in Organic Synthesis, ICOS 2024*)

Synthesis and Characterisation of Pinocembrin and Pinostrobin Derivatives

Nyotia Nyokat[1]*, Khong Heng Yen[1], Isabel Fong Lim[2], Mohd Fazli Mohamat[3] and Fong Siat Yee[4]

[1]School of Chemistry & Environment, Faculty of Applied Sciences, Universiti Teknologi MARA, Sarawak Branch, 94300 Kota Samarahan, Sarawak, Malaysia

[2]Department of Preclinical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak (UNIMAS), Malaysia

[3]Organic Synthesis Laboratory, Institute of Science, Universiti Teknologi MARA, UiTM Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

[4]Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah (UMS), Malaysia

*Corresponding author (e-mail: nyotia@uitm.edu.my)

Pinocembrin and pinostrobin are flavonoid molecules widely utilized in the pharmaceutical industry for their multifunctional properties. These compounds exhibit a broad range of pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects. This study aimed to synthesize derivatives of pinocembrin and pinostrobin, and to enhance their pharmacological properties. By modifying their structures, the goal is to improve their therapeutic efficacy and selectivity against specific diseases, such as cancer, inflammation, and neurodegenerative disorders. In the synthesis and derivatisation, eight compounds have been synthesised. The synthesis was accomplished in three steps. The first step is the synthesis of chalcone via Claisen–Schmidt condensation. The reaction involved 2-hydroxy-4,6-dimethoxyacetophenone and substituted benzaldehydes. 4'-chloro-2-hydroxy-4,6-dimethoxychalcone (**1**) and 4'-bromo-2-hydroxy-4,6-dimethoxychalcone (**2**) were successfully synthesized. 4'-chloro-5,7-dimethoxyflavanone (**3**) and 4'-bromo-5,7-dimethoxyflavanone (**4**) were obtained in step two via the cyclization of chalcones and in the last step is demethylation of aryl methyl ethers. 4'-chloropinostrobin (**5**), 4'-chloropinocembrin (**6**), 4'-bromopinostrobin (**7**), and 4'-bromopinocembrin (**8**) have been synthesized successfully, but in low yield.

Keywords: Synthesis; pinocembrin; pinostrobin; derivatives

Received: September 2024; Accepted: December 2024

Pinocembrin and pinostrobin are naturally occurring flavonoids found in a variety of plants, particularly within the *Zingiberaceae* and *Fabaceae* families. These flavonoids belong to the flavanone subclass and have gained significant attention in recent years due to their diverse pharmacological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. These bioactivities make them promising candidates for drug development, but their poor bioavailability and stability in biological systems often limit their therapeutic potential. Consequently, the synthesis of novel derivatives with modified structures to enhance bioactivity and pharmacokinetic properties has become a major research focus [1-2].

Natural products contain abundant bioactive compounds, making them an essential source for discovering and developing cancer prevention and therapy drugs. Pinocembrin and pinostrobin are biologically active flavonoids in many plants [3]. They have been reported to possess anticancer activity against different types of cancer [4, 5, 6], including breast cancer [7, 8]. However, the anticancer mechanism of these compounds, particularly in CRC, is still unknown.

Our previous study isolated two flavonoids, pinocembrin and pinostrobin, and 15 triterpenoids from *A. odoratissimus* and *A. sarawakensis*. [9]. Twenty-one synthesised compounds derived from these isolated compounds exhibited strong anticancer activity against human breast cancer (MCF-7), in which 4'-ethyl-2-hydroxy-4,6-dimethoxychalcone showed the most potent activity against MCF-7. These compounds, together with about another 100 synthesised derivatives from the isolated flavonoids, have registered for a patent with the Intellectual Property Corporation of Malaysia (MyIPO), entitled "An Anti-Inflammatory Compound" with Application Number PI 2018704123 [10]. These compounds inhibit targets of inflammatory disorders and, therefore, are useful anti-inflammatory agents. Thus, our research team continued synthesising different derivatives of pinocembrin and pinostrobin that are expected to inhibit targets for cancer cells and may be further developed into new chemical entities to treat cancer illness.

†Paper presented at the International Conference in Organic Synthesis 2024 (ICOS 2024)

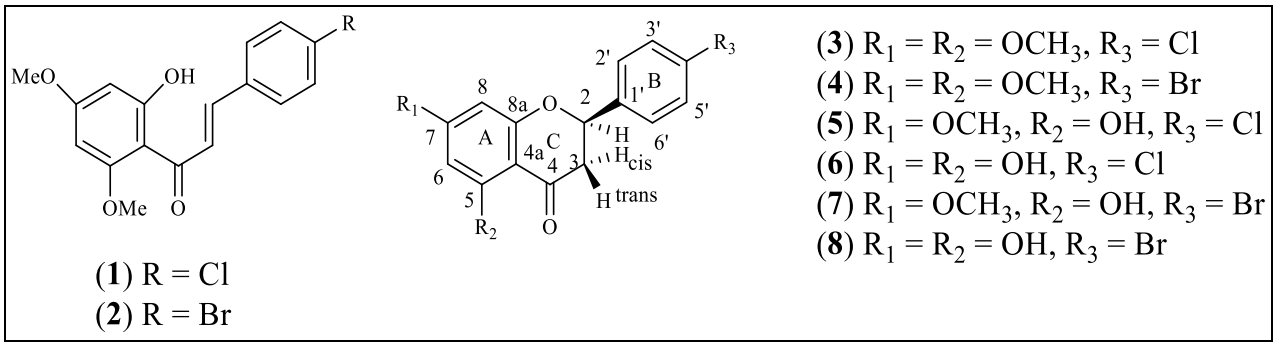


Figure 1. Synthesised Compounds.

EXPERIMENTAL

Synthesis of Pinostrobin and Pinocembrin Derivatives

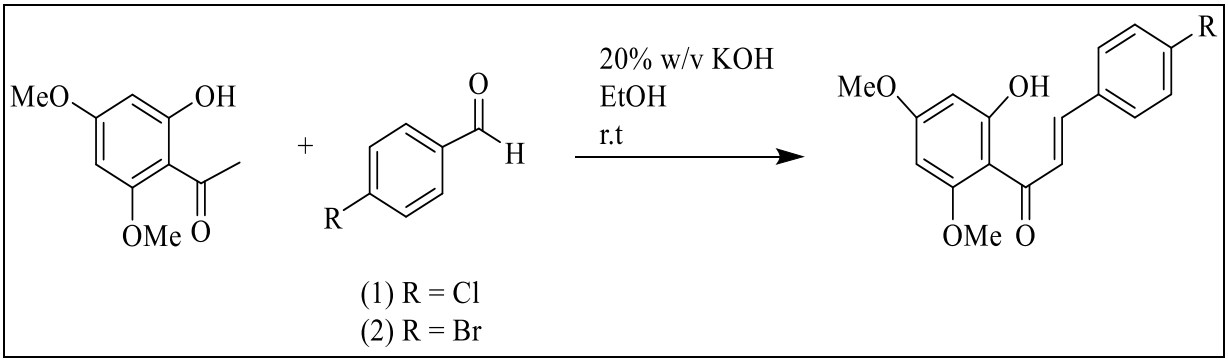
The synthesis of pinocembrin and pinostrobin derivatives was accomplished in three steps: the synthesis of chalcone via Claisen–Schmidt condensation, the synthesis of flavanone derivatives via cyclization of chalcone derivatives, and the demethylation of aryl methyl ethers. Eight compounds were successfully synthesized (Figure 1).

Step 1: Synthesis of Chalcones

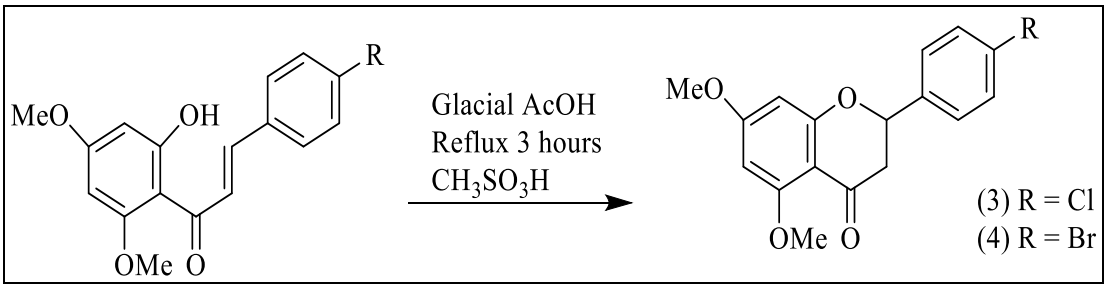
5.0 mL of KOH (50 %) was added into a solution of 2-hydroxy-4,6-dimethoxyacetophenone (1.0 g, 5.10 mmol) in ethanol (30 mL) followed by the addition of 4-chlorobenzaldehyde or 4-bromobenzaldehyde (1.0 g, 4.23 mmol). The reaction mixture was stirred occasionally for 24 h at room temperature until the end of the reaction. Completion of the reaction was monitored by TLC. The reaction mixture was acidified with aqueous 10% HCl solution and then poured into crushed ice. The precipitate was filtered and washed with an excess of distilled water. The products were purified by recrystallisation. Two chalcones, namely 4'-chloro-2-hydroxy-4,6-dimethoxy chalcone (**1**) and 4'-bromo-2-hydroxy-4,6-dimethoxy chalcone (**2**), were successfully synthesised as yellow crystal.

Step 2: Synthesis of Flavanones

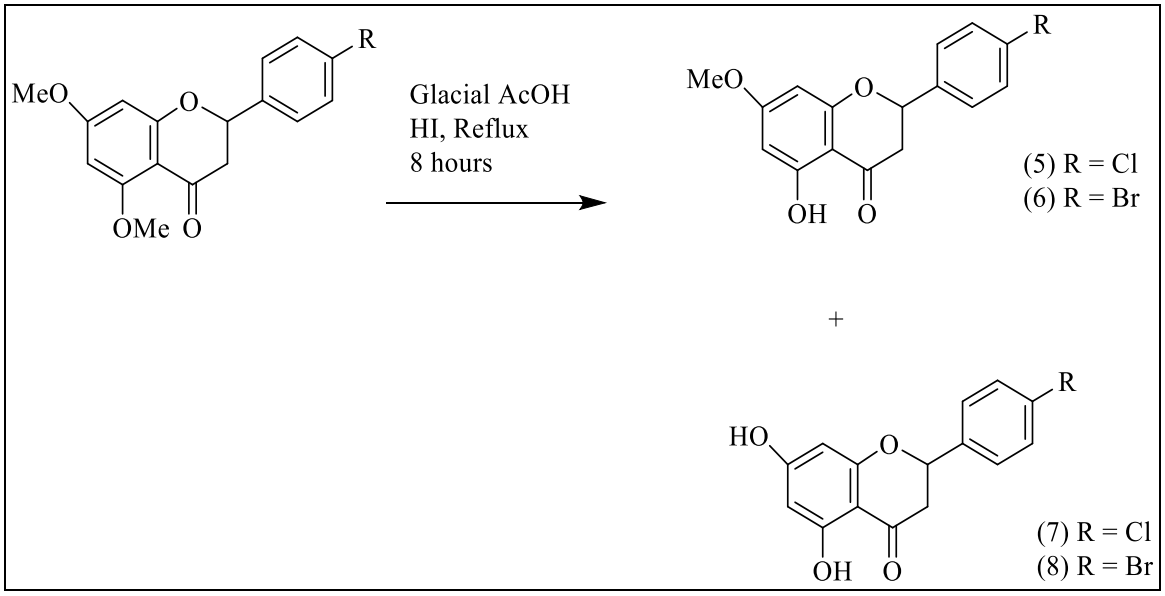
Chalcone from step 1 (0.55 g) was dissolved in glacial AcOH (6 mL). Methane sulfonic acid was added as a catalyst. The solution was heated to reflux for 3 hours. Then, the mixture was poured into cold water and extracted with EtOAc (2 x 25 mL). EtOAc layer was washed with brine, and the organic layer was dried over $MgSO_4$. The solvent evaporated to dryness. The product was purified by column chromatography performed on silica gel (230–400 mesh) using hexane, followed by an increasing gradient of ethyl acetate up to 100% gave 4'-chloro-5,7-dimethoxyflavanone **(3)** and 4'-bromo-5,7-dimethoxyflavanone **(4)** as white needles crystal.



Scheme 1. Synthesis of chalcones.



Scheme 2. Synthesis of flavanones.



Scheme 3. Demethylation of aryl methyl ether.

Step 3: Synthesis of Pinostrobin and Pinocembrin Derivatives

Flavanone (**3**) from step 2 (0.95 g, 2.62 mmol) was dissolved in glacial AcOH (5 mL), and 1 mL of HI was added to the solution. The solution was heated to reflux for 8 hours. Then, the mixture was poured into cold water and extracted with EtOAc (2x25 mL). EtOAc layer was washed with brine, and the organic layer was dried over $MgSO_4$. The solvent evaporated to dryness. The product was purified by column chromatography performed on silica gel (230–400 mesh) using ethyl acetate and hexane mixture (1:4) as the mobile phase to give 4'-chloropinostrobin **(5)** and 4'-chloropinocembrin **(6)** as white needles crystals. The same procedure was repeated for flavanone (**4**) to produce 4'-bromopinostrobin (**7**) and 4'-bromopinocembrin (**8**).

Characterisation

4'-chloro-2-hydroxy-4,6-dimethoxychalcone (1)

Yellow needles $C_{17}H_{15}O_4Cl$. Melting point: 328-330 °C. EI-MS m/z: 318.75; IR ν_{max} (ATR) cm^{-1}: 1625 (C=O), 1562 and 1439 (C=C aromatic), 1212 (C-O), 757 (C-Cl); ^1H NMR (400 MHz; $CDCl_3$): δ_H 3.85 (3H, *s*, -OCH_3), 3.93 (3H, *s*, -OCH_3), 5.98 (1H, *d*, *J* = 2.4 Hz, H-3), 6.12 (1H, *d*, *J* = 2.4 Hz, H-5), 7.47 (2H, *d*, *J* = 8.4 Hz, H-3', H-5'), 7.55 (2H, *d*, *J* = 8.4 Hz, H-2', H-6'), 7.70 (1H, *d*, *J* = 15.6 Hz, H-α), 7.90 (1H, *d*, *J* = 15.6 Hz, H-β), 14.26 (OH); ^{13}C APT NMR (100 MHz; $CDCl_3$): δ_C 55.6 (-OCH_3, CH_3), 55.9 (-OCH_3, CH_3), 91.3 (C-5, C-H), 93.8 (C-3, C-H), 106.2 (C-1, C-4°), 124.2 (C-α, C-H), 128.0 (C-4', C-4°), 129.7 (C-2', C-6', C-H), 131.6 (C-3', C-5', C-H), 134.5 (C-1', C-4°), 140.8 (C-β, C-H), 162.5 (C-2, C-4°), 166.4 (C-6, C-4°), 168.5 (C-4, C-4°) and 192.3 (C=O, C-4°).

4'-bromo-2-hydroxy-4,6-dimethoxy chalcone (2)

Yellow needles $C_{17}H_{15}O_4Br$. Melting point: 388-390 °C. EI-MS *m/z* 363.18; IR ν_{max} (ATR) cm^{-1}: 1625 (C=O), 1562 and 1439 (C=C aromatic), 1212 (C-O), 757 (C-Br); ^1H NMR (400 MHz; $CDCl_3$): δ_H 3.82 (3H, *s*, -OCH_3), 3.90 (3H, *s*, -OCH_3), 5.94 (1H, *d*, *J* = 2.4 Hz, H-3), 6.09 (1H, *d*, *J* = 2.4 Hz, H-5), 7.44 (2H, *d*, *J* = 8.4 Hz, H-3', H-5'), 7.51 (2H, *d*, *J* = 8.4 Hz, H-2', H-6'), 7.67 (1H, *d*, *J* = 15.6 Hz, H-α), 7.85

(1H, *d*, *J* = 15.6 Hz, H-β); ^{13}C APT NMR (100 MHz; $CDCl_3$): δ_C 55.7 (-OCH_3, CH_3), 55.9 (-OCH_3, CH_3), 91.4 (C-5, C-H), 93.9 (C-3, C-H), 106.3 (C-1, C-4°), 124.3 (C-α, C-H), 124.3 (C-4', C-4°), 128.2 (C-4', C-4°), 129.8 (C-2', C-6', C-H), 132.2 (C-3', C-5', C-H), 134.6 (C-1', C-4°), 140.9 (C-β, C-H), 162.5 (C-2, C-4°), 166.5 (C-6, C-4°), 168.5 (C-4, C-4°) and 192.4 (C=O, C-4°).

4'-chloro-5,7-dimethoxyflavanone (3)

White needles. $C_{17}H_{15}O_4Cl$. Melting point: 293-295 °C. EI-MS m/z : M^+ 318.07; IR ν_{max} (ATR) cm^{-1}: 1696 (C=O), 1603 and 1463 (C=C aromatic), 1234 (C-O), 758 (C-Cl); ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.78 (1H, *dd*, *J* = 16.4 and 2.8 Hz, H-3a), 2.99 (1H, *dd*, *J* = 16.4 and 13.2 Hz, H-3b), 3.84 (3H, *s*, -OCH_3), 3.91 (3H, *s*, -OCH_3), 5.39 (1H, *dd*, *J* = 13.2 and 3.2 Hz, H-2), 6.12 (1H, *d*, *J* = 2.4 Hz, H-8), 6.17 (1H, *d*, *J* = 2.4 Hz, H-6), 7.41 (4H, *m*, *J* = 6.8 Hz, H-2', H-3', H-5', H-6'); ^{13}C APT NMR (100 MHz; $CDCl_3$): δ_C 45.5 (C-3, CH_2), 55.6 (-OCH_3, CH_3), 56.2 (-OCH_3, CH_3), 78.4 (C-2, C-H), 93.3 (C-6, C-H), 93.6 (C-8, C-H), 106.0 (C-1', C-4°), 126.2 (C-2', C-6', C-H), 128.8 (C-4', C-H), 128.9 (C-3', C-5', C-H), 138.8 (C-4a, C-4°), 162.3 (C-8a, C-4°), 165.1(C-7, C-4°), 166.1 (C-5, C-4°) and 189.3 (C=O, C-4°).

4'-bromo-5,7-dimethoxyflavanone (4)

White needles. $C_{17}H_{15}O_4Br$. Melting point: 323-325 °C. EI-MS m/z : M^+ 363.18; IR ν max (ATR) cm^{-1}: 1677 (C=O), 1600 and 1563 (C=C aromatic), 1260 (C-O), 751 (C-Br); ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.76 (1H, *dd*, *J* = 16.4 and 3.2 Hz, H-3a), 2.93 (1H, *dd*, *J* = 16.8 and 12.8 Hz, H-3b), 3.80 (3H, *s*, -OCH_3), 3.87 (3H, *s*, -OCH_3), 5.35 (1H, *dd*, *J* = 12.8 and 2.8 Hz, H-2), 6.08 (1H, *d*, *J* = 2.4 Hz, H-8), 6.12 (1H, *d*, *J* = 2.4 Hz, H-6), 7.31 (2H, *d*, *J* = 8.4 Hz, H-2', H-6'), 7.52 (2H, *d*, *J* = 8.4 Hz, H-3', H-5'); ^{13}C APT NMR (100 MHz; $CDCl_3$): δ_C 45.5 (C-3, CH_2), 55.7 (-OCH_3, CH_3), 56.3 (-OCH_3, CH_3), 78.5 (C-2, C-H), 93.4 (C-6, C-H), 93.6 (C-8, C-H), 105.9 (C-4a, C-4°), 122.7 (C-4', C-H), 127.8 (C-2', C-6', C-H), 132.0 (C-3', C-5', C-H), 137.9 (C-1', C-4°), 162.4 (C-8a, C-4°), 164.8 (C-7, C-4°), 166.1 (C-5, C-4°) and 188.8 (C=O, C-4°).

4'-chloropinostrobin (5)

White needles. $C_{16}H_{13}O_4Cl$. Melting point: 358-360 °C. EI-MS m/z : M^+ 304.05; IR ν_{max} (KBr) cm^{-1}: 3168 (-OH), 1636 (C=O), 1595 and 1459 (C=C aromatic), 1157 (C-O) and 959 (=C-H); ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.80 (1H, *dd*, *J* = 17.2 and 3.2 Hz, H-3a), 3.00 (1H, *dd*, *J* = 17.2 and 13.2 Hz, H-3b), 3.83 (3H, *s*, -OCH_3), 5.39 (1H, *dd*, *J* = 13.2 and 3.2 Hz, H-2), 6.06 (1H, *d*, *J* = 2.4 Hz, H-8), 6.07 (1H, *d*, *J* = 2.4 Hz, H-6), 6.09 (2H, *d*, *J* = 8.8 Hz, H-2', H-6'), 7.42 (2H, *m*, *J* = 6.8 Hz, H-3', H-5'), 12.00 (OH); ^{13}C APT NMR (100 MHz; $CDCl_3$): δ_C 43.3 (C-3, CH_2), 55.7 (-OCH_3, CH_3), 78.4 (C-2, C-H), 94.4 (C-6, C-H), 95.3 (C-8, C-H), 103.1 (C-4a, C-4°), 122.9 (C-4', C-H), 127.9 (C-2', C-6', C-H), 134.7 (C-3', C-5', C-H), 136.9 (C-1', C-4°), 162.5 (C-8a, C-4°), 164.2 (C-5, C-4°), 168.1 (C-7, C-4°) and 195.3 (C=O, C-4°).

4'-chloropinocembrin (6)

White needles. $C_{15}H_{11}O_4Cl$. Melting point: 424-426 °C. EI-MS m/z : M^+ 290.70; IR ν_{max} (KBr) cm^{-1}: 3160 (-OH), 1641 (C=O), 1600 and 1490 (C=C aromatic), 1160 (C-O), 706 (C-Cl); ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.80 (1H, *dd*, *J* = 17.0 and 3.2 Hz, H-3a), 3.12 (1H, *dd*, *J* = 17.2 and 12.8 Hz, H-3b), 5.56 (1H, *dd*, *J* = 12.8 and 3.2 Hz, H-2), 5.94 (1H, *d*, *J* = 2.0 Hz, H-8), 5.98 (1H, *d*, *J* = 2.0 Hz, H-6), 7.51 (2H, *m*, H-2', H-6'), 7.61 (2H, *m*, H-3', H-5'), 12.10 (OH); ^{13}C APT NMR (100 MHz; $CDCl_3$): δ_C 42.6 (C-3, CH_2), 78.4 (C-2, C-H), 95.1 (C-6, C-H), 96.3 (C-8, C-H), 102.4 (C-4a, C-4°), 122.0 (C-4', C-H), 128.6 (C-2', C-6', C-H), 131.8 (C-3', C-5', C-H), 138.5 (C-1', C-4°), 163.0 (C-8a, C-4°), 164.4 (C-5, C-4°), 166.6 (C-7, C-4°) and 195.5 (C=O, C-4°).

4'-bromopinostrobin (7)

White needles. $C_{16}H_{13}O_4Br$. Melting point: 389-391 °C. EI-MS m/z : M^+ 349.18; IR ν_{max} (KBr) cm^{-1}: 3168 (-OH), 1636 (C=O), 1595 and 1459 (C=C aromatic), 1157 (C-O) and 959 (=C-H); ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.79 (1H, *dd*, *J* = 17.2 and 3.2 Hz, H-3a), 3.01 (1H, *dd*, *J* = 17.2 and 13.2 Hz, H-3b), 3.80 (3H, *s*, -OCH_3), 5.37 (1H, *dd*, *J* = 13.2 and 3.2 Hz, H-2), 6.04 (1H, *d*, *J* = 2.4 Hz, H-8), 6.07 (1H, *d*, *J* = 2.4 Hz, H-6), 7.32 (2H, *d*, *J* = 8.8 Hz, H-2', H-6'), 7.55 (2H, *m*, *J* = 6.8 Hz, H-3', H-5'), 11.96 (OH); ^{13}C APT NMR (100 MHz; $CDCl_3$): δ_C 43.3 (C-3, CH_2), 55.8 (-OCH_3, CH_3), 79.5 (C-2, C-H), 94.4 (C-6, C-H), 95.3 (C-8, C-H), 103.2 (C-4a, C-4°), 122.9 (C-4', C-H), 127.9 (C-2', C-6', C-H), 132.1 (C-3', C-5', C-H), 137.5 (C-1', C-4°), 162.5 (C-8a, C-4°), 164.2 (C-5, C-4°), 168.1 (C-7, C-4°) and 195.4 (C=O, C-4°).

4'-bromopinocembrin (8)

White needles. $C_{15}H_{11}O_4Br$. Melting point: 455-457 °C. EI-MS m/z : M^+ 335.15. IR ν_{max} (KBr) cm^{-1}: 3160 (-OH), 1641 (C=O), 1590 and 1491 (C=C aromatic), 1165 (C-O), 708 (C-Br); ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.80 (1H, *dd*, *J* = 17.2 and 3.2 Hz, H-3a), 3.12 (1H, *dd*, *J* = 17.2 and 12.8 Hz, H-3b), 5.56 (1H, *dd*, *J* = 12.8 and 3.2 Hz, H-2), 5.94 (1H, *d*, *J* = 2.0 Hz, H-8), 5.98 (1H, *d*, *J* = 2.0 Hz, H-6), 7.51 (2H, *m*, H-2', H-6'), 7.61 (2H, *m*, H-3', H-5'), 12.10 (OH); ^{13}C APT NMR (100 MHz; $CDCl_3$): δ_C 42.6 (C-3, CH_2), 78.4 (C-2, C-H), 95.1 (C-6, C-H), 96.3 (C-8, C-H), 102.4 (C-4a, C-4°), 122.0 (C-4', C-H), 128.6 (C-2', C-6', C-H), 131.8 (C-3', C-5', C-H), 138.6 (C-1', C-4°), 163.1 (C-8a, C-4°), 164.5 (C-5, C-4°), 166.6 (C-7, C-4°) and 195.7 (C=O, C-4°).

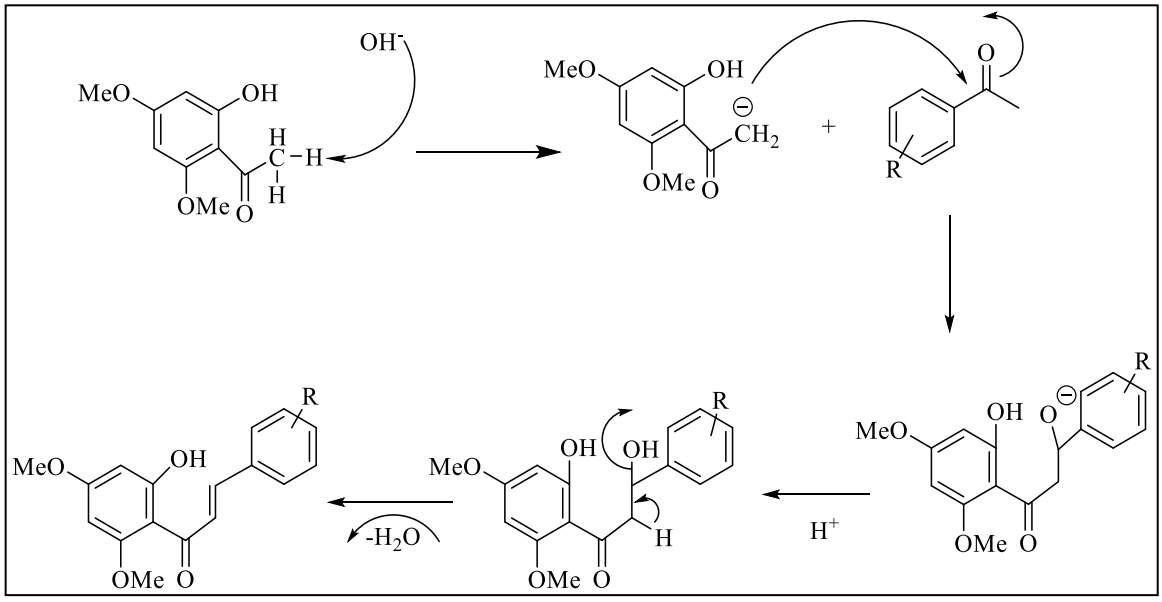
RESULTS AND DISCUSSION

Eight compounds were synthesised during the synthesis and derivatisation. The synthesis was accomplished in three steps. In the first step of the mechanism (**Scheme 4**), the step was the synthesis of chalcone via Claisen–Schmidt condensation. The reaction involved 2-hydroxy-4,6-dimethoxyacetophenone and substituted benzaldehydes. 4'-chloro-2-hydroxy-4,6-dimethoxy-chalcone (**1**) and 4'-bromo-2-hydroxy-4,6-dimethoxy-chalcone (**2**) were successfully synthesised.

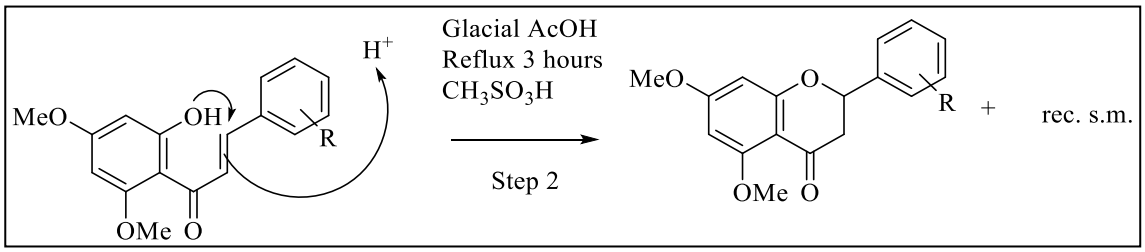
Compound (**1)** was obtained in excellent yield, 93.8% as bright yellow crystals with melting point 328-330ºC. The mass spectrum showed the molecular ion peak at m/z 318.75, indicating the molecular formula $C_{17}H_{15}O_4Cl$. In the IR spectral analysis of chalcone (**2**), the peak at 1625 cm^{-1} indicates the presence of carbonyl group (C=O), The peaks at 1562 and 1439 cm^{-1} indicate the presence of aromatic C=C group, C-O (1212 cm^{-1}) and 757 (C-Cl) functionalities. In ^{1}H NMR, two singlets at δ_H 3.85 (3H) and δ_H 3.90 (3H) due to the two methoxy groups at C-4 and C-6, respectively. The *meta-coupled* protons of the A-ring appeared at δ_H 5.98 (1H, *d*, *J*= 2.4 Hz, H-3) and δ_H 6.12 (1H, *d*, *J*= 2.4 Hz, H-5). The four aromatic protons of the B-ring were observed at δ_H 7.47 (2H, *d*, *J*= 8.4 Hz, H-3', 5') and 7.55 (2H, *d*, *J*= 8.4 Hz) assigned to H-2' and H-6' respectively. The characteristics signals for a chalcone moiety appeared as two doublets at δ_H 7.70 (1H, *d*, *J*= 15.6 Hz, H-α) and δ_H 7.90 (1H, *d*, *J*= 15.6 Hz, H-β). The ^{13}C APT NMR chalcone **(2)** showed the presence of 15 signals attributed to 17 different carbons. The signals for methyl carbons were observed at δ_C 55.6 and 55.9. The spectrum also confirmed the presence of seven quaternary carbons at δ_C 106.2 (C-1), 134.5 (C-1'), 162.5 (C-2), 166.4 (C-6), 168.5 (C-4), 192.3 (C=O) and 128.0 carbon 4' where chlorine atom attached to. Eight methine carbons were observed at δ_C 91.3 (C-5), 93.8 (C-3), 124.2 (C-α), 129.7 (C-2', C-6'), 131.6 (C-3', C-5') and 140.8 (C-β).

Compound (**2)** was obtained in excellent yield, 97.1% as bright yellow crystals with melting point 388-390ºC. The mass spectrum showed the molecular ion peak at m/z 363.18, indicating the formal formula $C_{17}H_{15}O_4Br$. In the IR spectral analysis of chalcone (**2**), the peak at 1625 cm^{-1} indicates the presence of carbonyl group (C=O), The peaks at 1562 and 1439 cm^{-1} indicate the presence of aromatic C=C group, C-O (1212 cm^{-1}) and 757 (C-Br) functionalities. In ^{1}H NMR, two singlets at δ_H 3.82 (3H) and δ_H 3.93 (3H) due to the two methoxy groups at C-4 and C-6, respectively. The *meta-coupled* protons of the A-ring appeared at δ_H 5.94 (1H, *d*, *J*= 2.4 Hz, H-3) and δ_H 6.09 (1H, *d*, *J*= 2.4 Hz, H-5). The four aromatic protons of the B-ring were observed at δ_H 7.44 (2H, *d*, *J*= 8.4 Hz, H-3', 5') and 7.51 (2H, *d*, *J*= 8.4 Hz) assigned to H-2' and H-6' respectively. The characteristics signals for a chalcone moiety appeared as two doublets at δ_H 7.67 (1H, *d*, *J*= 15.6 Hz, H-α) and δ_H 7.85 (1H, *d*, *J*= 15.6 Hz, H-β). The ^{13}C APT NMR chalcone **(2)** showed the presence of 15 signals attributed to 17 different carbons. The signals for methyl carbons were observed at δ_C 55.7 and 55.9. The spectrum also confirmed the presence of seven quaternary carbons and eight methine carbons.

4'-chloro-5,7-dimethoxyflavanone (**3**) and 4'-bromo-5,7-dimethoxyflavanone (**4**) were obtained in step two via the cyclisation of chalcones (**Scheme 5**).



Scheme 4. Mechanism for Synthesis of Chalcone.

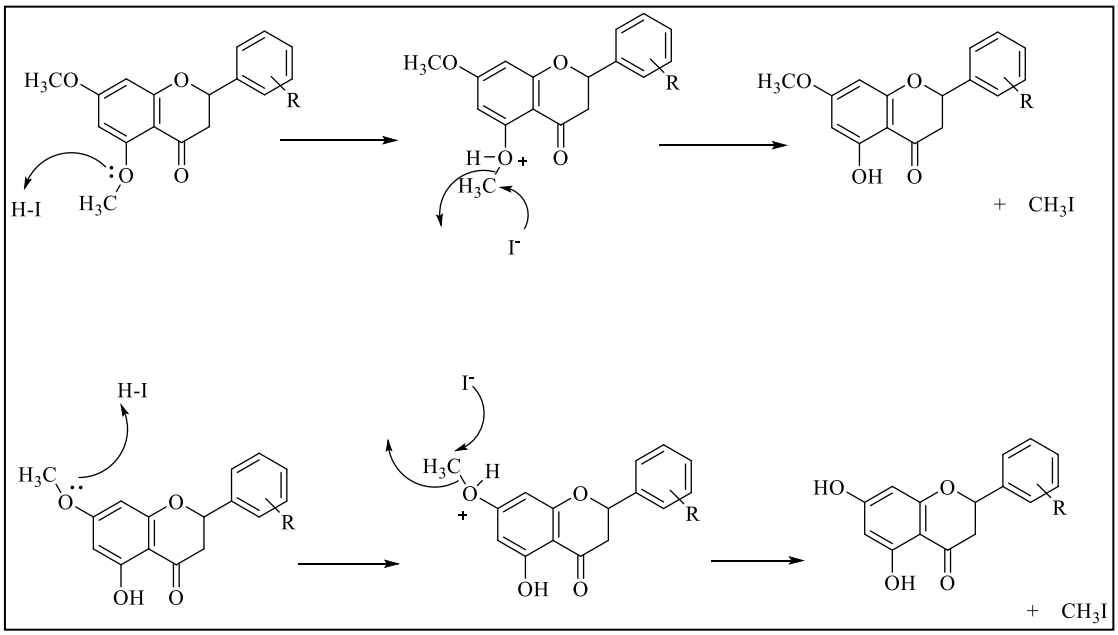


Scheme 5. Mechanism for Synthesis of Flavanone.

Compound (**3**) was obtained as white needle crystals with melting points 293-295ºC. The percentage yield was 30.5%. The mass spectrum showed the molecular ion peak at m/z 318.07, indicating the formal formula $C_{17}H_{15}O_4Cl$. In the IR spectral analysis of chalcone (**3**), the peak at 1696 cm^{-1} indicates the presence of the carbonyl group (C=O); the peaks at 1463 and 1603 cm-1 indicate the presence of aromatic C=C group, C-O (1234 cm^{-1}) and 758 (C-Cl) functionalities. In ^{1}H NMR, two doublet-doublet of one proton each at δ 2.78 and δ 2.99 attributable to H-3a (*cis*) and H-3b (*trans*), respectively. two singlets at δ_H 3.84 (3H) and δ_H 3.91 (3H) due to the two methoxy groups at C-5 and C-7 respectively. The *meta-coupled* protons of the A-ring appeared at δ_H 6.12 (1H, *d*, *J*= 2.4 Hz, H-8) and δ_H 6.17 (1H, *d*, *J*= 2.4 Hz, H-6). The four aromatic protons of the B-ring were observed at δ_H 7.41 (4H, H-2', H-3', H-5'and H-6'). The ^{13}C APT NMR chalcone **(2)** showed the presence of 15 signals attributed to 17 different carbons. The signals for methyl carbons were observed at δ_C 55.6 and 56.2. The spectrum also confirmed the presence of seven quaternary carbons and eight methine carbons.

Compound (**4)** was obtained as white needle crystals with melting points 388-390ºC. The percentage yield was 27.3%. The mass spectrum showed the molecular ion peak at m/z 363.18, indicating the formal formula $C_{17}H_{15}O_4Br$. In the IR spectral analysis of compound (**4**), the peak at 1677 cm^{-1} indicates the presence of carbonyl group (C=O), The peaks at 1563 and 1600 cm^{-1} indicate the presence of aromatic C=C group, C-O (1260 cm^{-1}) and 751 (C-Br) functionalities. In ^{1}H NMR, two singlets at δ_H 3.80 (3H) and δ_H 3.87 (3H) due to the two methoxy groups at C-5 and C-7, respectively. The characteristics of the 1H NMR were the absence of the typical flavanone H-3 proton and the presence of an ABX system. The X part appeared as a doublet-doublet for a proton centre at *δ* 5.35 (*dd*, *J* = 13.2 and 3.2 Hz) attributed to H-2 and the AB portion, as two doublet-doublet of one proton each at *δ* 2.76 (*dd*, *J* = 16.4 and 3.2 Hz) and 2.93 (*dd*, *J* = 16.8 and 12.8 Hz) attributed to H-3. Two doublets at δ 6.08 (1H, *d*, *J*= 2.4 Hz) and δ 6.12 (1H, *d*, *J*= 2.4 Hz) attributed to *meta*, H-8 and H-6. The four aromatic protons of the B-ring were observed at δ 7.31 (2H, *d*, *J*= 8.4 Hz, H-2', 6') and 7.52 (2H, *d*, *J*= 8.4 Hz) assigned to H-3' and H-5' respectively. The ^{13}C NMR spectrum of flavanone **(4)** showed the presence of 15 signals attributed to 17 different carbons. The signals for methyl carbons were observed at δ 55.7 and 56.3. The spectrum also confirmed the presence of two methyl carbons, one methylene, six quaternary carbons and eight methine carbons in this compound. Signals for C-2 and C-3 at 78.5 and 45.5 ppm, respectively, and a low field signal at 188.8 ppm for the C=O.

Compound (**5)** was obtained as white needles crystal with melting point 358-360ºC. The percentage yield was 20.5%. The mass spectrum showed the molecular ion peak at m/z 304.15, indicating that the compound had the formula $C_{16}H_{13}O_4Cl$. The IR spectrum displayed a broad band at 3168 cm^{-1} corresponding to O-H stretching, besides bands due to an ester of acetic acid at 1636 cm^{-1} for C=O and 1157 cm^{-1} for C-O, C=C aromatic (1595 and 1459 cm^{-1}) and 706 cm^{-1} (C-Br) stretching. In the ^{1}H NMR, one-proton singlet signal at *δ* 12.00 due to the hydroxyl group (position 5) with an H-bridge to O at the carbonyl at position 4. The spectrum also displayed one singlet at δ 3.83 (3H) due to the methoxy groups at C-7. Two doublets at *δ* 6.07 (*J* = 2.4 Hz) and 6.06 (*J* = 2.4 Hz) attributed to *meta*, H-6 and H-8. The AB portion, as two doublet-doublet of one proton each at *δ* 2.80 (*J* = 17.2, 3.2 Hz) and 3.00 (*J* = 17.2, 3.2 Hz), attributed to H-3 (*cis*) and H-3 (*trans*), respectively. The four aromatic protons of the B-ring were observed at δ 6.09 (2H, *d*, *J*= 8.4 Hz, H-2', 6') and 7.42 (2H, *m*, *J* = 6.8 Hz) assigned to H-3' and H-5' respectively. The ^{13}C-NMR spectrum showed 14 carbon signals, including one methyl, one methylene, seven methine and seven quaternary carbon atoms. The carbon signal of the methoxy group is assigned due to chemical shift 55.7. At ring C, the chemical shift of a carbonyl carbon was assigned at *δ* 195.3.



Scheme 6. Mechanism for Synthesis of Flavanone.

Compound (**6)** was obtained as white needle crystals with melting points 424-426ºC. The percentage yield was 22.4%. The mass spectrum showed the molecular ion peak at m/z 290.70, indicating the formal formula $C_{15}H_{11}O_4Cl$. In the IR spectral analysis of compound (**8**), a broad band at 3160 cm^{-1} corresponds to O-H stretching, besides bands due to an ester of acetic acid at 1641 cm^{-1} for C=O and 1160 cm^{-1} for C-O. C=C aromatic (1490 and 1490 cm^{-1}) and 706 (C-Cl) stretching cm^{-1}. In the ^1H NMR, one-proton singlet signal at δ 12.10 due to the hydroxyl group (position 5) with an H-bridge to O at the carbonyl (position 4). Two doublets at δ 5.94 (J = 2.0 Hz) and 5.98 (J = 2.0 Hz) attributed to meta, H-8 and H-6. The four aromatic protons of the B-ring were observed at δ 7.51 (2H, *m*, H-2', 6') and 7.61 (2H, *m*) assigned to H-3' and H-5', respectively. The doublet-doublet for a proton centre at δ 5.56 (*dd*, J = 12.8, 3.2 Hz) attributed to H-2 and the AB portion, as two doublet-doublet of one proton each at δ 2.80 (J = 17.2, 3.2 Hz) and 3.12 (J = 17.2, 12.8 Hz) attributed to H-3. The ^{13}C-NMR showed 13 carbons signals attributed to 15 different carbons, including one methylene, seven methine and seven quaternary carbon atoms.

In the last step is demethylation of aryl methyl ethers. 4'-chloropinostrobin (**5**), 4'-chloropinocembrin (**6**), 4'-bromopinostrobin (**7**), and 4'-bromopinocembrin (**8**) had been synthesised successfully. The mechanisms for the dimetylation of aryl methyl ether are summarized in **Scheme 6**. The mechanism involves strong acid (HI) protonates the ether oxygen, which turns it into a better leaving group. Next, the iodide ion attacks the carbon in an S_N2 reaction the alcohol and methyl iodide.

Compound (**7)** was obtained as a colourless crystal with a melting point of 389-391ºC. The percentage yield was 13.2%. The mass spectrum showed the molecular ion peak at m/z 348.13, indicating that the compound had the formula $C_{16}H_{13}O_4Br$. The IR spectrum displayed a broad band at 3168 cm^{-1} corresponding to O-H stretching, besides bands due to an ester of acetic acid at 1636 cm^{-1} for C=O and 1157 cm^{-1} for C-O, C=C aromatic (1595 and 1459 cm^{-1}) and 706 cm^{-1} (C-Br) stretching. In the ^1H NMR, one-proton singlet signal at δ 11.96 due to the hydroxyl group (position 5) with an H-bridge to O at the carbonyl at position 4. The spectrum also displayed one singlet at δ 3.80 (3H) due to the methoxy groups at C-7. Two doublets at δ 6.07 (J = 2.4 Hz) and 6.04 (J = 2.4 Hz) attributed to *meta*, H-6 and H-8. The X part appeared as a doublet-doublet for a proton centre at δ 5.37 (J = 13.2, 3.2 Hz) attributed to H-2 and the AB portion, as two doublet-doublet of one proton each at δ 2.79 (J = 17.2, 3.2 Hz) and 3.01 (J = 17.2, 3.2 Hz) attributed to H-3 (*cis*) and H-3 (*trans*), respectively. The four aromatic protons of the B-ring were observed at δ 7.32 (2H, *d*, *J*= 8.4 Hz, H-2', 6') and 7.55 (2H, *m*, J = 6.8 Hz) assigned to H-3' and H-5' respectively. The ^{13}C-NMR spectrum showed 14 carbon signals, including one methyl, one methylene, seven methine and seven quaternary carbon atoms. The carbon signal of the methoxy group is assigned due to chemical shift 55.8. Six carbon atoms of the mono-substituted aromatic ring (ring B) are located at δ 137.5 (C-1'), 127.9 (C-2', C-6') and 132.1 (C-3', C-5'). The spectrum showed six signals of carbon in ring A, located at δ 103.2 (C-4a), 164.2 (C-5), 94.4 (C-6), 168.1 (C-7), 95.3 (C-8) and 162.5 (C-8a). At ring C, the chemical shift of a carbonyl carbon was assigned at δ 195.4 (C-4) and two carbon signals (C-2 and C-3) were located at δ 79.5 and 43.3 (C-3), respectively.

Compound (**8)** was obtained as white needle crystals with melting points 455-457ºC. The percentage yield was 17.1%. The mass spectrum showed the molecular ion peak at m/z 335.15, indicating the formal formula $C_{15}H_{11}O_4Br$. In the IR spectral analysis of compound (**8**), a broad band at 3160 cm^{-1} corresponds to O-H stretching, besides bands due to an ester of acetic acid at 1641 cm^{-1} for C=O and 1165 cm^{-1} for C-O. C=C aromatic (1590 and 1491 cm^{-1}) and 708 (C-Br) stretching cm^{-1}. In the ^{1}H NMR, one-proton singlet signal at δ 12.10 due to the hydroxyl group (position 5) with an H-bridge to O at the carbonyl (position 4). Two doublets at δ 5.94 (J = 2.0 Hz) and 5.98 (J = 2.0 Hz) attributed to meta, H-8 and H-6. The four aromatic protons of the B-ring were observed at δ 7.51 (2H, *m*, H-2', 6') and 7.61 (2H, *m*) assigned to H-3' and H-5', respectively. The doublet-doublet for a proton centre at δ 5.56 (*dd*, J = 12.8, 3.2 Hz) attributed to H-2 and the AB portion, as two doublet-doublet of one proton each at δ 2.80 (J = 17.2, 3.2 Hz) and 3.12 (J = 17.2, 12.8 Hz) attributed to H-3. The ^{13}C-NMR showed 13 carbon signals attributed to 15 different carbons, including one methylene, seven methine and seven quaternary carbon atoms. Six carbon atoms of the mono-substituted aromatic ring (ring B) are located at δ 122.0 (C-4'), 138.6 (C-1'), 128.6 (C-2', C-6') and 131.8 (C-3', C-5'). The spectrum showed six signals of carbon in ring A, located at δ 102.4 (C-4a), 164.5 (C-5), 95.1 (C-6), 166.6 (C-7), 96.3 (C-8) and 163.1 (C-8a). At ring C, the chemical shift of a carbonyl carbon was assigned at δ 195.7 (C-4) and two carbon signals (C-2 and C-3) were located at δ 78.4 and 42.6 (C-3), respectively.

CONCLUSION

Eight compounds were synthesised during the synthesis and derivation. The synthesis was accomplished in 3 steps. The first step is the synthesis of chalcone via Claisen–Schmidt condensation. The reaction involved 2-hydroxy-4,6-dimethoxyacetophenone and substituted benzaldehydes. 4'-chloro-2-hydroxy-4, 6-dimethoxy-chalcone (**1)** and 4'-bromo-2-hydroxy-4, 6-dimethoxy-chalcone (**2**) were successfully synthesized. 4'-chloro-5, 7-dimethoxyflavanone (**3**) and 4'-bromo-5, 7-dimethoxyflavanone (**4**) had been synthesised successfully in step two via cyclisation of chalcones and in the last step is demethylation of aryl methyl ethers. Four compounds, namely, 4'-chloropinostrobin (**5**), 4'-chloropinocembrin (**6**), 4'-bromopinostrobin (**7**), and 4'-bromopinocembrin (**8**), had been synthesised successfully.

The results from this study suggest that, the synthesis and characterisation of pinocembrin and pinostrobin derivatives is a promising avenue for developing new drug candidates with enhanced therapeutic properties. The work is crucial not only for expanding the applications of these natural products but also for advancing the development of flavonoid-based therapies in the pharmaceutical industry.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial assistance provided by the SDG-BORNEO Grant Scheme entitled Apoptosis Signal Pathways for Cancer Treatment Using the Synthesized Derivatives of Pinocembrin and Pinostrobin (600-RMC/SDG-BORNEO 5/3 (018/2020)). The authors also thank the Research Management Centre, Universiti Teknologi MARA (UiTM), Malaysia, for their excellent support and assistance.

REFERENCES

1. Jiang, M., Zhang, Y. and Li, T. (2017) Anti-oxidant activity of pinocembrin and pinostrobin derivatives. *Phytochemistry Reviews*, **16(4)**, 849–865.

2. Xu, J., Wang, Q. and Yang, H. (2019) Flavonoids as antioxidants: Mechanisms and structure-activity relationships. *Frontiers in Pharmacology*, **10**, 872.

3. Ruttanapattanakul, J., Wikan, N., Potikanond, S. and Nimlamool, W. (2022) Molecular targets of pinocembrin underlying its regenerative activities in human keratinocytes. *Pharmaceuticals*, **15**, 954.

4. Rasul, A., Millimouno, F. M., Ali Eltayb, W., Ali, M., Li, J. and Li, X. (2013) Pinocembrin: a novel natural compound with versatile pharmacological and biological activities. *BioMed Research International*, **2013**, 1–9.

5. Roman Junior, W. A., Gomes, D. B., Zanchet, B., Schönell, A. P., Diel, K. A. P., Banzato, T. P., Ruiz, A. L. T. G., Carvalho, J. E., Neppel, A., Barison, A. and Santos, C. A. M. (2017) Anti-proliferative effects of pinostrobin and 5,6-dehydrokavain isolated from leaves of *Alpinia zerumbet*. *Revista Brasileira de Farmacognosia (Brazilian Society of Pharmacognosy)*, **27(5)**, 592–598.

6. Jaudan, A., Sharma, S., Malek, S. and Dixit, A. (2018) Induction of apoptosis by pinostrobin in human cervical cancer cells: Possible mechanism of action. *PloS One*, **13(2)**, e0191523.

7. Fadilah, F., Edina, B. C., Rahmawati, R. A., Wiyono, L., Erlina, L., Paramita, R. I. and Tedjo, A. (2018) The molecular dynamic of pinostrobin and pinocembrin from kaempferia pandurate roxb. towards estrogen receptor positive (ESR) and estrogen receptor negative (VEGFR) of breast cancer. *Asian Journal of Pharmaceutics*, **12(4)**, S1473.

8. Mohammed, I. A., Akhtar, M. N., Biau, F. J., Tor, Y. S., Zareen, S., Shahabudin, S., Abd Hamid, H., Haq, Z. U., Khalil, R. and Khalaf, R. M. (2019)

Isolation of cardamonin and pinostrobin chalcone from the rhizomes of *Boesenbergia rotunda* (L.) Mansf. and their cytotoxic effects on H-29 and MDA-MB-231 cancer cell lines. *The Natural Products Journal*, **9**, 341.

9. Nyokat, N., Khong, H. Y., Hamzah, A. S., Fong, L. I. and Saidin, A. S. (2017) Isolation and Synthesis of Pinocembrin and Pinostrobin from *Artocarpus odoratissimus*. *Malaysian Journal of Analytical Sciences*, **21(5)**, 1156–1161.

10. MYIPO https://iponlineext.myipo.gov.my/SPHI/Extra/IP/Mutual/Browse.aspx?sid=637550536653982775