SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF THREE NOVEL 23,24-DINORCHOLANE BRASSINOSTEROID ANALOGS BEARING BENZOATE GROUPS AND HALOGEN SUBSTITUENTS

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ABSTRACT

Brassinosteroids are an important family of plant hormones involved in various processes of plant growth and development. They also play a crucial role in plant stress responses, improving tolerance to abiotic factors such as temperature extremes, drought, and salinity, and contributing to resistance against biotic stresses. This work describes the synthesis and full structural characterization of three new 23,24-dinorcholane-type brassinosteroid analogs bearing benzoate groups at C-22 and substituted with fluorine and chlorine atoms, while maintaining the structural features of the A/B rings present in active natural brassinosteroids such as castasterone. The synthesis was achieved in two steps: an esterification reaction with acyl chlorides, followed by a stereospecific Sharpless dihydroxylation. Both reactions proceeded with good yields.

Keywords: Brassinosteroids, analogs, 23,24-dinorcholane, synthesis.

1. INTRODUCTION

Brassinosteroids (BRs) are a class of steroidal phytohormones essential for plant growth and development, regulating processes such as cell elongation and division, photomorphogenesis, and reproduction [1]. They also play a crucial role in plant stress responses, improving tolerance to abiotic factors such as temperature extremes, drought, and salinity, and contributing to resistance against biotic stresses [2–4]. BRs enhance overall plant productivity by improving traits such as fruit size and yield and influencing flowering time and inflorescence architecture [5].

Within the large family of natural brassinosteroids, brassinolide (**1**), 24-epibrassinolide (**2**), castasterone (**3**), and 24-epicastasterone (**4**) (Figure 1) are among the most studied molecules and have demonstrated potent biological activities in various bioassays. Consequently, extensive structure–activity relationship (SAR) studies have been conducted to determine the structural requirements for enhanced biological activity. These studies have shown that brassinolide (**1**) is the most active compound among this class of phytohormones [6–11].

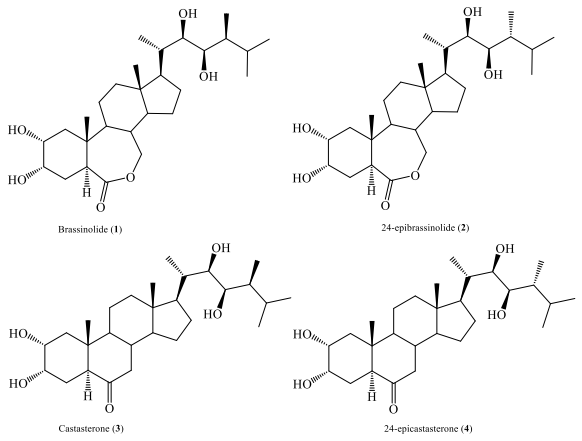

Figure 1. Structure of Brassinolide (**1**), 24-epibrassinolide (**2**), Castasterone (**3**) and 24-epicastasterone (**4**), some more important naturally occurring BRs.

BRs occur in roots, stems, leaves, flowers, anthers, pollen, seeds, and grains, but their concentrations are extremely low: 1–100 ng/g fresh weight in young tissues and 0.01–0.1 ng/g in mature tissues [12]. Therefore, considerable effort has been devoted to developing efficient synthetic methods for brassinolide (**1**) and its analogs. Partial syntheses starting from abundant steroids with functional groups at positions amenable to chemical modification have been explored to obtain BR derivatives in sufficient amounts for both scientific and practical studies. Several approaches focus on stereoselective synthesis targeting functionalization of the A/B rings in the steroid nucleus, as well as side-chain modifications of naturally occurring BRs and their synthetic analogs [13–15].

Our research group has recently reported the synthesis of brassinosteroid analogs with shortened 23,24-dinorcholane-type side chains, bearing a benzoate group at C-22 and various substituents on the aromatic ring, differing in size and electronic distribution, while preserving the structural features of the A/B rings present in active natural brassinosteroids such as teasterone (**5**) [16] and castasterone (**3**) [17].

Brassinosteroid analogs **6**–**17** (Figure 2) were evaluated in different bioassays, including the Rice Lamina Inclination Test (RLIT), Bean Second Internode (BSI) assay, and *Arabidopsis thaliana* brassinosteroid sensitivity assay. Results from RLIT indicated that at the lowest tested concentration (1×10^{-8} M), analogs **8**, **12**, and **13** were more active than brassinolide (**1**). In the *A. thaliana* root sensitivity assay, analogs **12** and **13** were the most active [16]. Among analogs **14**–**16**, only compound **15** showed activity comparable to brassinolide (**1**) at 1×10^{-7} M in RLIT, while analog **16** exhibited significantly higher activity than brassinolide (**1**) in the BSI assay [17]. Compound **17** was synthesized and characterized; however, its biological activity will be reported later [18].

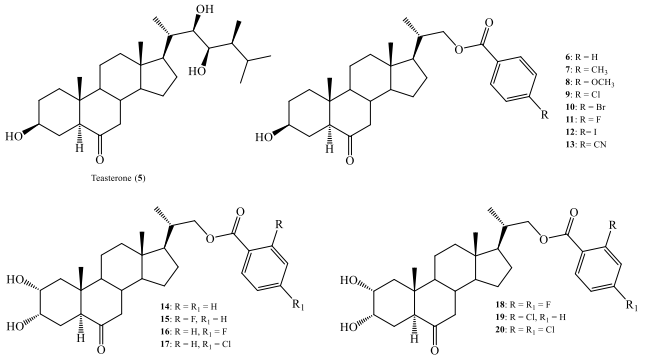

Figure 2. Structure of Teasterone (**5**), synthetic BRs analogs with 23,24-dinorcholan type with 3β-hydroxyl and 22-benzoate having a different substituent at *"para"* position on aromatic ring (compounds **6**-**13**), 2α,3α-dihydroxy with 4-substituted benzoate at C-22, (compounds **14**-**17**) and new BRs analogs with fluorine and chlorine atoms **18**-**20**.

2. MATERIALS AND METHODS

2.1 General Chemical and methods

All chemical reagents were obtained from commercial suppliers and used without further purification. Melting points were determined on an SMP3 apparatus (Stuart-Scientific, now Merck KGaA, Darmstadt, Germany) and are uncorrected. Detailed conditions for recording Fourier-transform infrared (FT-IR) spectra, ^{1}H, ^{13}C, ^{13}C DEPT-135, and gradient-selected 2D ^{1}H–^{13}C HSQC and HMBC NMR spectra have been reported elsewhere [17, 19, 20]. High-resolution mass spectra (HRMS-ESI) were recorded on a Bruker Daltonik instrument under the following conditions: dry temperature, 180 °C; nebulizer pressure, 0.4 bar;

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dry gas flow, 4 L/min; spray voltage, 4.5 kV in positive or negative ion mode. Accurate mass measurements were performed at a resolving power of 140,000 FWHM over an m/z range of 50–1300.

Analytical TLC was performed on silica gel 60 plates (0.25 mm layer), and spots were visualized by heating after spraying with 25% H_2SO_4 in water. Column chromatography was carried out on glass columns packed with silica gel 60 (230–400 mesh) using EtOAc–hexane mixtures with increasing polarity. Organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure below 40 °C.

2.2 Synthesis

2.2.1 General procedure with acyl chloride esterification

Precursor **21** was dissolved in CH_2Cl_2 and pyridine, followed by the addition of DMAP and the corresponding ArCOCl under stirring at room temperature. Reaction progress was monitored by TLC (2 h). After partial solvent evaporation to ~10 mL, EtOAc (30 mL) was added, and the organic layer was washed with 5% $KHSO_4$ (2 × 15 mL) and water (2 × 15 mL), dried over Na_2SO_4, and filtered. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel using hexane/EtOAc mixtures *6-Oxo-23,24-dinor-5α-Cholan-2-en-22-yl-(2,4-difluoro)-benzoate (**22**).*

Precursor **21** (0.10 g, 0.303 mmol); CH_2Cl_2 (30 mL), pyridine (1.20 mL), DMAP (50 mg), 2,4-difluorobenzoyl chloride (0.5 mL, *d* = 1.44 g/mL, 4.07 mmol). Compound **22** was obtained as a colorless solid (0.105 g, 73.7% yield; m.p. = 160.8 - 161.9 °C, Et_2O/EtOAc). FT-IR_{vmax} (cm^{-1}): 3082 and 3017 (=C-H); 2957 and 2943 (-CH_3); 2902 and 2866 (CH_2-); 1725 (C=O); 1703 (C=O); 1656 and 1613 (C=C); 1433 (CH_2-); 1387 (CH_3-); 1263 and 1132 (C-O); 963, 866, 773, 672 and 604 (=C-H). ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 7.98 (1H, td, *J* = 8.8 and 6.5 Hz, H-6′); 6.94 (1H, dddd, *J* = 8.8, 7.7, 2.5 and 1.1 Hz, H-5′); 6.87 (1H, ddd, *J* = 8.8, 8.5 and 2.5 Hz, H-3′); 5.71-5.66 (1H, m, H-3); 5.59-5.54 (1H, m, H-2); 4.33 (1H, dd, *J* = 10.8 and 3.4 Hz, H-22a); 4.06 (1H, dd, *J* = 10.8 and 6.9 Hz, H-22b); 2.38-2.33 (2H, m, H-5 and H-7); 1.76 (1H, ddd, *J* = 10.3, 6,7 and 4.6 Hz, H-20); 1.18-1.07 (1H, m, H-15); 1.12 (3H, d, *J* = 6.7 Hz, H-21); 0.723 (3H, s, H-19); 0.715 (3H, s, H-18). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 211.82 (C-6); 165.62 (dd, $^1J_{CF}$ = 254.5 and $^3J_{CF}$ = 11.7 Hz, C-4′); 163.88 (d, $^3J_{CF}$ = 4.4 Hz, ArCO); 162.86 (dd, $^1J_{CF}$ = 262.2 and $^3J_{CF}$ = 11.7 Hz, C-2′); 133.93 (dd, $^3J_{CF}$ = 10.6 and $^4J_{CF}$ = 2.5 Hz, C-6′); 124.96 (C-3); 124.46 (C-2); 115.45 (d, $^2J_{CF}$ = 13.4 Hz, C-1′); 111.60 (dd, $^2J_{CF}$ = 21.7 and $^4J_{CF}$ = 3.9 Hz, C-5′); 105.21 (t, $^2J_{CF}$ = 25.4 Hz, C-3′); 70.41 (C-22); 56.42 (C-14); 53.82 (C-5); 53.33 (C-9); 52.58 (C-17); 46.92 (C-7); 42.95 (C-13); 39.99 (C-10); 39.34 (C-1); 39.30 (C-12); 37.67 (C-20); 35.83 (C-8); 27.49 (C-16); 24.00 (C-15); 21.70 (C-4); 21.08 (C-11); 17.25 (C-21); 13.49 (C-19); 11.98 (C-18).

*6-Oxo-23,24-dinor-5α-Cholan-2-en-22-yl-(2-chloro)-benzoate (**23**).*

Precursor 21 (0.15 g, 0.454 mmol); DCM (40 mL), Py (1.60 mL), DMAP (50 mg), 2-chlorobenzoyl chloride (0.5 mL, *d* = 1.38 g/mL, 3.94 mmol). Compound 23 (0.146 g, 68.5% yield) was obtained as a colorless solid (m.p. = 163.8 – 167.7 °C, Et2O/EtOAc). FT-IRvmax (cm-1): 3087 and 3018 (=C-H); 2970 and 2944 (-CH3); 2866 (CH2-); 1708 (C=O); 1583 (C=C); 1430 (CH2-); 1392 (CH3-); 1277 and 1106 (C-O); 837, 773 and 669 (=C-H). 1H NMR (400.1 MHz, CDCl3) d (ppm): 7.80 (1H, d, J = 8.2 Hz, H-6′); 7.48 (1H, d, J = 2.1 Hz, H-3′); 7.31 (1H, dd, J = 8.2 and 2.1 Hz, H-5′); 5.70-5.66 (1H, m, H-3); 5.59-5.54 (1H, m, H-2); 4.33 (1H, dd, J = 10.7 and 3.2 Hz, H-22a); 4.07 (1H, dd, J = 10.7 and 7.1 Hz, H-22b); 2.38-2.32 (2H, m, H-5 and H-7); 2.30-2.20 (1H, m, H-4); 1.75 (1H, ddd, J = 10.9, 6,4 and 4.4 Hz, H-20); 1.18-1.08 (1H, m, H-15); 1.12 (3H, d, J = 6.4 Hz, H-21); 0.718 (3H, s, H-19); 0.711 (3H, s, H-18). 13C NMR (100.6 MHz, CDCl3) □ (ppm): 211.78 (C-6); 165.07 (ArCO); 138.20 (C-4′); 134.77 (C-2′); 132.54 (C-6′); 131.00 (C-3′); 128.63 (C-1′); 127.00 (C-5′); 124.95 (C-3); 124.44 (C-2); 70.83 (C-22); 56.42 (C-14); 53.80 (C-5); 53.30 (C-9); 52.58 (C-17); 46.90 (C-7); 42.95 (C-13); 39.97 (C-10); 39.32 (C-1); 39.30 (C-12); 37.65 (C-20); 35.81 (C-8); 27.52 (C-16); 23.99 (C-15); 21.69 (C-4); 21.07 (C-11); 17.38 (C-21); 13.48 (C-19); 11.98 (C-18).

*6-Oxo-23,24-dinor-5α-Cholan-2-en-22-yl-(2,4-dichloro)-benzoate (**24**).*

Precursor **21** (0.10 g, 0.303 mmol); DCM (30 mL), Py (1.20 mL), DMAP (50 mg), 2,4-dichloro chloride (0.3 mL, *d* = 1.494 g/mL, 2.14 mmol). Compound **24** (0.121 g, 79.3% yield) was obtained as a colorless solid (m.p. = 153.1 - 154.1 °C, Et_2O/EtOAc). FT-IR_{vmax} (cm^{-1}): 3087 and 3018 (=C-H); 2970 and 2944 (-CH_3); 2866 (CH_2-); 1708 (C=O); 1583 (C=C); 1430 (CH_2-); 1392 (CH_3-); 1277 and 1106 (C-O); 837, 773 and 669 (=C-H). ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 7.80 (1H, d, *J* = 8.2 Hz, H-6′); 7.48 (1H, d, *J* = 2.1 Hz, H-3′); 7.31 (1H, dd, *J* = 8.2 and 2.1 Hz, H-5′); 5.70-5.66 (1H, m, H-3); 5.59-5.54 (1H, m, H-2); 4.33 (1H, dd, *J* = 10.7 and 3.2 Hz, H-22a); 4.07 (1H, dd, *J* = 10.7 and 7.1 Hz, H-22b); 2.38-2.32 (2H, m, H-5 and H-7); 2.30-2.20 (1H, m, H-4); 1.75 (1H, ddd, *J* = 10.9, 6,4 and 4.4 Hz, H-20); 1.18-1.08 (1H, m, H-15); 1.12 (3H, d, *J* = 6.4 Hz, H-21); 0.718 (3H, s, H-19); 0.711 (3H, s, H-18). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 211.78 (C-6); 165.07 (ArCO); 138.20 (C-4′); 134.77 (C-2′); 132.54 (C-6′); 131.00 (C-3′); 128.63 (C-1′); 127.00 (C-5′); 124.95 (C-3); 124.44 (C-2); 70.83 (C-22); 56.42 (C-14); 53.80 (C-5); 53.30 (C-9); 52.58 (C-17); 46.90 (C-7); 42.95 (C-13); 39.97 (C-10); 39.32 (C-1); 39.30 (C-12); 37.65 (C-20); 35.81 (C-8); 27.52 (C-16); 23.99 (C-15); 21.69 (C-4); 21.07 (C-11); 17.38 (C-21); 13.48 (C-19); 11.98 (C-18).

2.2.2 General Procedure for Sharpless Dihydroxylation.

A solution of OsO_4 in *t*-BuOH (1 g per 20 mL) was added to a mixture of olefin (**23**–**24**), hydroquinidine 4-chlorobenzoate (DHQD-CLB), methanesulfonamide ($CH_3SO_2NH_2$), K_2CO_3, and $K_3[Fe(CN)_6]$ in *t*-BuOH/H_2O (1:1 v/v). The reaction mixture was stirred at room temperature for 36 h, then quenched with a saturated Na_2SO_3 solution and stirred for an additional 30 min. The mixture was extracted with EtOAc (30 mL) and water (2 × 20 mL). Combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography on silica gel using hexane/EtOAc/MeOH mixtures (6:4:0 → 4.8:4.8:0.4) afforded the desired products [17, 22].

*2α,3α-Dihydroxy-6-oxo-23,24-dinor-5α-cholan-22-yl-(2,4-difluoro)-benzoate (**18**).*

Compound **22** (0.08 g, 0.700 mmol); DHQD-CLB (0.03 g, 0.06 mmol); $CH_3SO_2NH_2$ (0.04 g, 0.42 mmol); K_2CO_3 (0.19 g, 1.36 mmol); $K_3[Fe(CN)_6]$ (0.68 g, 2.07 mmol); *t*-BuOH/H_2O (15.0 mL); OsO_4 (0.15 mL, 0.03 mmol). Compound **18** (0.0691 g, 83.3 % yield) was obtained as a colorless solid (m.p. = 216.2 - 217.6 °C, Et_2O/MeOH). FT-IR $_{vma}$x (cm^{-1}): 3485 and 3385 (O-H); 3077 (=C-H); 2945 (-CH_3); 2892 and 2862 (CH_2-); 1727 (C=O); 1703 (C=O); 1616 and 1596 (C=C); 1427 (CH_2-); 1382 (CH_3-); 1262 and 1147 (C-O); 972, 956, and 877 (=C-H). ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 7.97 (1H, td, *J* = 8.5 and 6.5 Hz, H-6′); 6.94 (1H, dddd, *J* = 9.0, 7.5, 0.9 and 1.1 Hz, H-5′); 6.87 (1H, ddd, *J* = 9.0, 8.5 and 2.3 Hz, H-3′); 4.32 (1H, dd, *J* = 10.8 and 3.3 Hz, H-22a); 4.05 (1H, 1H, dd, *J* = 10.8 and 7.0 Hz, H-22b); 4.04 (1H, bd, *J* = 3.3 Hz, H-3); 3.76 (1H, dt, *J* = 8.4 and 3.3 Hz, H-2); 2.68 (1H, dd, *J* = 12.8 and 3.4 Hz, H-5); 2.29 (1H, dd, *J* = 13.1 and 4.7 Hz, H-7α); 2.26 (1H, bs, OH); 1.91 (1H, dt, *J* = 15.0 and 3.7 Hz, H-4b); 1.17-1.06 (1H, m, H-15); 1.11 (3H, d, *J* = 6.6 Hz, H-21); 0.754 (3H, s, H-19); 0.705 (3H, s, H-18). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 211.96 (C-6); 165.64 (dd, $^1J_{CF}$ = 255.6 and $^3J_{CF}$ = 11.9 Hz, C-4′); 163.85 (d, $^3J_{CF}$ = 4.0 Hz, ArCO); 162.81 (dd, $^1J_{CF}$ = 261.1 and $^3J_{CF}$ = 12.5 Hz, C-2′); 133.87 (dd, $^3J_{CF}$ = 10.5 and $^4J_{CF}$ = 1.7 Hz, C-6′); 115.39 (dd, $^2J_{CF}$ = 9.8 and $^4J_{CF}$ = 3.4 Hz, C-1′); 111.57 (dd, $^2J_{CF}$ = 21.6 and $^4J_{CF}$ = 3.7 Hz, C-5′); 105.22 (t, $^2J_{CF}$ = 26.0 Hz, C-3′); 70.37 (C-22); 68.36 (C-3); 68.24 (C-2); 56.32 (C-14); 53.63 (C-9); 52.52 (C-17); 50.69 (C-5); 46.67 (C-7); 43.08 (C-13); 42.52 (C-10); 40.16 (C-1); 39.20 (C-12); 37.63 (C-8); 35.80 (C-20); 27.42 (C-16); 26.30 (C-4); 24.00 (C-15); 21.15 (C-11); 17.22 (C-21); 13.53 (C-19); 12.06 (C-18). HRMS-ESI (negative mode): m/z calculated for $C_{29}H_{38}F_2O_5$: 504.2687 $[M]^+$; found 503.2566 $[M - H]^+$.

*2α,3α-Dihydroxy-6-oxo-23,24-dinor-5α-cholan-22-yl-(2-chloro)-benzoate (**19**).*

Compound **23** (0.113 g, 0.241 mmol); DHQD-CLB (0.03 g, 0.06 mmol); $CH_3SO_2NH_2$ (0.04 g, 0.42 mmol); K_2CO_3 (0.19 g, 1.36 mmol); $K_3[Fe(CN)_6]$ (0.68 g, 2.07 mmol); *t*-BuOH/H_2O (15.0 mL); OsO_4 (0.15 mL, 0.03 mmol). Compound **19** (0.104 g, 85.8 % yield) was obtained as a colorless solid (m.p. = 203.6 - 204.1 °C, Et_2O/MeOH). FT-IR ν_{max} (cm^{-1}): 3426 (O-H); 3062 (=C-H); 2947 (-CH_3); 2862 (CH_2-); 1732 (C=O); 1705 (C=O); 1591 (C=C); 1436 (CH_2-); 1384 (CH_3-); 1250 and 1049 (C-O); 715 (=C-H). ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 7.84 (1H, dd, *J* = 7.4 and 1.5 Hz, H-6′); 7.49-7.42 (2H, m, H-3′and H-4′); 7.34 (1H, dd, *J* = 7.5 and 7.4 Hz, H-5′); 4.36 (1H, dd, *J* = 11.0 and 3.5 Hz, H-22a); 4.08 (1H, dd, *J* = 11.0 and 6.8 Hz, H-22b); 4.06 (1H, bd, *J* = 2.9 Hz, H-3); 3.77 (1H, bd, *J* = 11.3 Hz, H-2); 2.70 (1H, dd, *J* = 12.4 and 3.2 Hz, H-5);

2.40 (1H, bs, OH); 2.31 (1H, dd, J = 13.5 and 4.9 Hz, H-7α); 2.18 (1H, bs, OH); 1.19-1.08 (1H, m, H-15); 1.15 (3H, d, J = 6.4 Hz, H-21); 0.770 (3H, s, H-19); 0.724 (3H, s, H-18). ^{3}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 212.04 (C-6); 166.04 (ArCO); 133.55 (C-2′); 132.44 (C-4′); 131.38 (C-3′); 131.06 (C-6′); 130.39 (C-1′); 126.56 (C-5′); 70.59 (C-22); 68.33 (C-3); 68.21 (C-2); 56.31 (C-14); 53.60 (C-9); 52.50 (C-17); 50.68 (C-5); 46.66 (C-7); 43.06 (C-13); 42.51 (C-10); 40.13 (C-1); 39.18 (C-12); 37.62 (C-8); 35.81 (C-20); 27.50 (C-16); 26.26 (C-4); 23.98 (C-15); 21.13 (C-11); 17.36 (C-21); 13.52 (C-19); 12.05 (C-18). HRMS-ESI (positive mode): m/z calculated for $C_{29}H_{39}ClO_5$: 502.2486 $[M]^+$; found 503.2566 $[M + H]^+$.

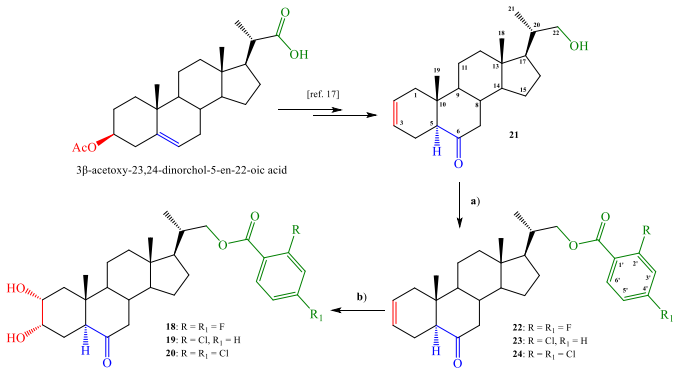
*2α,3α-Dihydroxy-6-oxo-23,24-dinor-5α-cholan-22-yl-(2,4-dichloro)-benzoate (**20**).*

Compound **24** (0.094 g, 0.187 mmol); DHQD-CLB (0.03 g, 0.06 mmol); $CH_3SO_2NH_2$ (0.04 g, 0.42 mmol); K_2CO_3 (0.19 g, 1.36 mmol); $K_3[Fe(CN)_6]$ (0.68 g, 2.07 mmol); t-BuOH/H_2O (15.0 mL); OsO_4 (0.15 mL, 0.03 mmol). Compound **20** (0.0798 g, 79.8 % yield) was obtained as a colorless solid (m.p. = 195.3 - 197.4°C, Et_2O/MeOH). FT-IR ν_{max} (cm^{-1}): 3398 (O-H); 3067 (=C-H); 2947 (-CH_3); 2900 and 2867 (CH_2-); 1733 (C=O); 1708 (C=O); 1584 (C=C); 1470 (CH_2-); 1377 (CH_3-); 1244 and 1104 (C-O); 926, and 770 (=C-H). ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm): 7.80 (1H, d, J = 8.4 Hz, H-6′); 7.47 (1H, d, J = 1.9 Hz, H-3′); 7.30 (1H, dd, J = 8.4 and 1.9 Hz, H-5′); 4.33 (1H, dd, J = 10.8 and 3.2 Hz, H-22a); 4.06 (1H, dd, J = 10.8 and 7.1 Hz,H-22b); 4.04-4.03 (1H, m, H-3); 3.75 (1H, dt, J = 10.9 and 4.1 Hz, H-2); 2.67 (1H, dd, J = 12.7 and 3.1 Hz, H-5); 2.33 (1H, bs, OH); 2.29 (1H, dd, J = 13.1 and 4.3 Hz, H-7α); 2.10 (1H, bs, OH); 1.16-1.06 (1H, m, H-15); 1.11 (3H, d, J = 6.8 Hz, H-21); 0.747 (3H, s, H-19); 0.699 (3H, s, H-18). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 211.97 (C-6); 165.09 (ArCO); 138.22 (C-4′); 134.75 (C-2′); 132.52 (C-6′); 131.00 (C-3′); 128.59 (C-1′); 127.01 (C-5′); 70.80 (C-22); 68.34 (C-3); 68.22 (C-2); 56.32 (C-14); 53.60 (C-9); 52.52 (C-17); 50.68 (C-5); 46.65 (C-7); 43.07 (C-13); 42.51 (C-10); 40.14 (C-1); 39.19 (C-12); 37.61 (C-8); 35.79 (C-20); 27.50 (C-16); 26.27 (C-4); 23.98 (C-15); 21.13 (C-11); 17.36 (C-21); 13.52 (C-19); 12.05 (C-18). HRMS-ESI (positive mode): m/z calculated for $C_{29}H_{38}Cl_2O_5$: 536.2096 $[M]^+$; found 537.2159 $[M + H]^+$.

3. RESULTS AND DISCUSSION

Compound **21** (Scheme 1) was obtained from commercially available 3β-acetoxy-23,24-dinorchol-5-en-22-oic acid according to the reported methodology [17]. Subsequent benzoylation of the primary alcohol **21** with the corresponding benzoyl chlorides [17, 21] afforded compounds **22**–**24** in 73.7%, 68.5%, and 79.3% yields, respectively (Scheme 1). These compounds were fully characterized using combined FT-IR and 1D/2D NMR spectroscopic techniques (Supplementary Material, Figures S1–S15).

To illustrate the structural elucidation process, the 2D ^{1}H–^{13}C HMBC spectrum of ester **24** is shown in Figure 3, highlighting the key heteronuclear correlations used to confirm the ester function at C-22 and to achieve unambiguous structural determination.



Scheme 1. Conditions: **a.** ArCOCl, DMAP/pyridine/CH_2Cl_2, r.t., 2 h; compounds **22**, **23**, and **24** obtained in 73.7%, 68.5%, and 79.3% yields, respectively. **b.** Sharpless dihydroxylation: DHQD-CLB/$CH_3SO_2NH_2$, K_2CO_3/$K_3[Fe(CN)_6]$, t-BuOH/H_2O (1:1 v/v), OsO_4/t-BuOH, 36 h; compounds **18**–**20** obtained in 83.3%, 85.8%, and 79.8% yields, respectively.

The formation of the ester group at C-22 was confirmed by the characteristic signals in the ^{1}H NMR spectrum of compound **24**, observed at δ = 4.33 ppm (1H, dd, J = 10.7 and 3.2 Hz) and δ = 4.07 ppm (1H, dd, J = 10.7 and 7.1 Hz), corresponding to H-22a and H-22b, respectively. These signals correlated in the 2D HSQC spectrum with the carbon resonance at δ = 70.83 ppm (CH_2-22), as observed in the ^{13}C and DEPT-135 spectra (Supplementary Material, Figures S11–S15). The ester linkage was further confirmed by the long-range $^{3}J_{HC}$ correlation between H-22a/b and the carbonyl carbon at δ = 165.07 ppm (ArCO) in the HMBC spectrum (Figure 3). Additional heteronuclear correlations in the A/B rings and side chain supported the complete structural assignment of compound **24**.

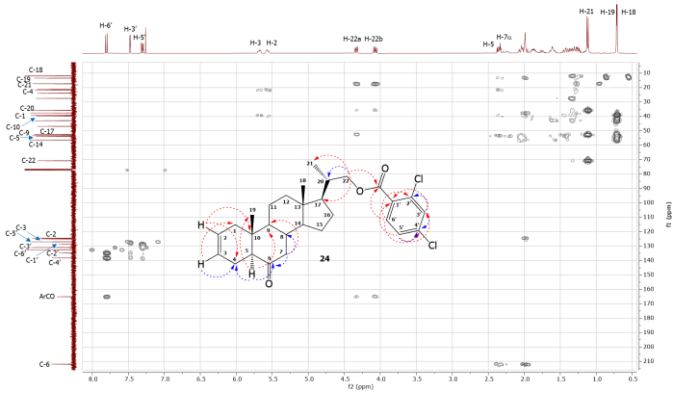


Figure 3. Structure of compound **24**, 2D ^{1}H-^{13}C HMBC spectrum. Main $^{2}J_{HC}$ (blue) and $^{3}J_{HC}$ (red) correlations observed for hydrogens H-22 of side chain, indole moiety ring and A/B junction rings.

Sharpless dihydroxylation [17, 22] of the C-2–C-3 double bond in compounds **22**–**24** (Scheme 1) afforded the new BR analogs **18**–**20**, each bearing a 2α,3α-glycol function, in 83.3%, 85.8%, and 79.8% yields, respectively. Structural determination of these compounds was achieved using combined FT-IR, 1D and 2D NMR, and HRMS techniques (Supplementary Material, Figures S16–S33).

As an example, the 2D ^{1}H–^{13}C HSQC spectrum of compound **18** is shown in Figure 4. The presence of the glycol function at C-2 and C-3 was confirmed by the carbinolic proton signals at δ = 4.04 ppm (1H, bd, J = 3.3 Hz, H-3) and δ = 3.76 ppm (1H, dt, J = 8.4 and 3.3 Hz, H-2). These correlated with carbon signals at δ = 68.36 and 68.24 ppm, assigned to C-3 and C-2, respectively, in the HSQC spectrum. Additional key correlations and assignments are indicated in Figure 4. Similar analyses were performed for analogs **19** and **20**, with complete spectroscopic data provided in the Supplementary Material (Figures S22–S33).

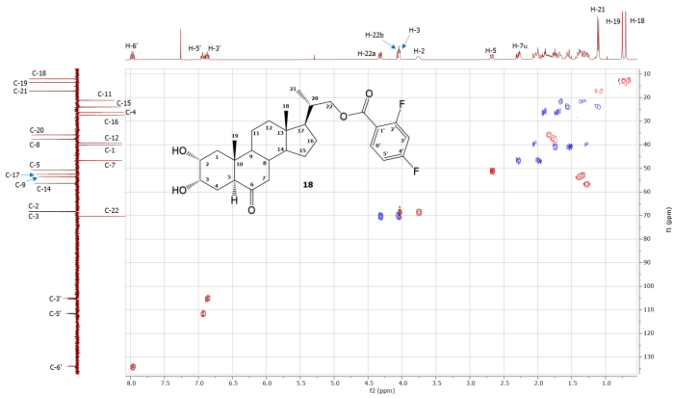


Figure 4. Structure of compound **18**, phase-edited 2D ^{1}H-^{13}C HSQC spectrum with ^{13}C DEPT-135 projection.

CONCLUSIONS

The synthesis and full characterization of three new brassinosteroid analogs with shortened 23,24-dinorcholane-type side chains, bearing a benzoate group at C-22 and substituted with fluorine and chlorine atoms on the aromatic ring, while preserving the structural features of the A/B rings present in active natural

brassinosteroids such as castasterone (**3**), have been described. These new analogs were obtained in two steps from the known compound **21**, involving an esterification reaction (acylation with acyl chlorides) followed by stereospecific Sharpless dihydroxylation. Both reactions proceeded with good yields. Biological evaluation of these compounds in plant growth assays will be reported in future work.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization, Luis Espinoza-Catalán; María Núñez. and Lautaro Taborga; methodology, María Núñez; Juana Yufla. and Luis Espinoza-Catalán; formal analysis, Luis Espinoza-Catalán; María Núñez and Lautaro Taborga; investigation, Luis Espinoza-Catalán; María Núñez and Lautaro Taborga; writing-original draft preparation, Luis Espinoza-Catalán and Lautaro Taborga; writing-review and editing, Luis Espinoza-Catalán; María Núñez and Lautaro Taborga; project administration, Luis Espinoza Catalán; funding acquisition, Luis Espinoza-Catalán. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF COMPETING INTERESTS

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this article.

SUPPORTING INFORMATION

Copies of 1D and 2D NMR spectra for compounds **18**–**24** and HRMS spectra for compounds **18**–**20** are provided in the Supplementary Materia.

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REFERENCES

1. Bajguz, A. Brassinosteroids-Occurrence and chemical structures in plants. In Brassinosteroids: A Class of Plant Hormone. Hayat, S., Ahmad, A., Eds.; Springer: Dordrecht, The Netherlands, pp. 1-27, 2016.
2. Nolan, T. M.; Vukašinović, N.; Liu, D.; Russinova, E.; Yin, Y. Brassinosteroids: Multidimensional Regulators of Plant Growth, Development, and Stress Responses. *Plant Cell* **2020**, *32*(2), 295-318.
3. Li, S.; Zheng, H.; Lin, L.; Wang, F.; Sui, N. Roles of brassinosteroids in plant growth and abiotic stress response. *Plant Growth Regul*. **2021**, *93*(1), 29-38.
4. Hafeez, M. B.; Zahra, N.; Zahra, K.; Raza, A.; Batool, A.; Shaukat, K.; Khan, S. Brassinosteroids: Molecular and physiological responses in plant growth and abiotic stresses. *Plant Stress 2*, **2021**, 100029.
5. Aryal, D. and Alferez, F. Brassinosteroids: Biosynthesis, Signaling, and Hormonal Crosstalk as Related to Fruit Yield and Quality. *Plants* **2025**, *14*(12), 1865.
6. Thompson M. J.; Meudt W. J.; Mandava N. B.; Dutky S. R.; Lusby W. R.; Spaulding D. W. Synthesis of Brassinosteroids and Relationship of Structure to Plant Growth-Promoting Effects. *Steroids* **1982**, *39*(1) 89-105.
7. Takatsuto S.; Yazawa N.; Ikekawa N.; Morishita T.; Abe H. Synthesis of (24R)-28-homobrassinolide analogues and structure-activity relationships of brassinosteroids in the rice-lamina inclination test. *Phytochemistry* **1983**, *22*(6), 1393-1397.
8. Takatsuto, S.; Yazawa, N.; Ikekawa, N.; Takematsu, T.; Takeuchi, Y.; Koguchi, M. Structure Activity Relationship of Brassinosteroids. *Phytochemistry* **1983**, *22*(11), 2437-2441.
9. Takatsuto, S.; Ikekawa, N.; Morishita, T.; Abe, H. Structure Activity Relationship of Brassinosteroids with Respect to the A/B-Ring Functional-Groups. *Chem. Pharm. Bull*. **1987**, *35*(1), 211-216.
10. Brosa, C.; Capdevila, J. M.; Zamora, I. Brassinosteroids: A new way to define the structural requirements. *Tetrahedron* **1996**, *52*(7), 2435-2448.
11. Zullo M. A. T.; Adam G. Brassinosteroid phytohormones: Structure, bioactivity and applications. *Braz. J. Plant Physiol*. **2002**, *14*(3), 143-181.
12. Hayat, S. & Ahmad, A. Brassinosteroids Bioactivity and Crop Productivity. 1st edition. Dordrecht: Springer, 2003.
13. Kovganko, N. V.; Ananich, S. K. Advances in the chemical synthesis of brassinosteroids. *Chem. Nat. Compd*. **1997**, *33*(4), 389-416.
14. Khripach, V. A.; Zhabinskii, V. N.; de Groot, A. E. Basic synthetic methods and formal syntheses. In Brassinosteroids. A New Class of Plant Hormones. Eds. Academic Press: San Diego, Chapter VI, pp 73-135, 1999.
15. Khripach, V.; Zhabinskii, V.; Ermolovich, Y. Synthetic Aspects of Brassinosteroids. Studies in Natural Products Chemistry, Atta-ur-Rahman FRS, University of Karachi, Karachi, Pakistan, Chapter 6, 44, pp. 309-352, 2015.
16. Nuñez, M.; Wang, Y.; Russinova, E.; Estévez-Braun, A.; Amesty, A.; Olea, A. F.; Mellado, M.; Díaz, K.; Espinoza-Catalán, L. Synthesis, Biological Activity, and Molecular-Docking Studies of New Brassinosteroid Analogs. *Int. J. Mol. Sci*. **2024**, *25*(18), 10158.
17. Aitken, V.; Diaz, K.; Soto, M.; Olea, A. F.; Cuellar, M. A.; Nuñez, M.; Espinoza-Catalán, L. New Brassinosteroid Analogs with 23,24-Dinorcholan Side Chain, and Benzoate Function at C-22: Synthesis, Assessment of Bioactivity on Plant Growth, and Molecular Docking Study. *Int. J. Mol. Sci*. **2024**, *25*(1), 419.
18. Núñez, M. Síntesis de nuevos análogos de brasinoesteroides con cadena lateral del tipo 23,24-bisnorcolánicos, benzoilados en C-22 y su evaluación como reguladores de crecimiento de plantas. Tesis de Doctorado, Departamento de Química, Universidad Técnica Federico Santa María, Chile. 2024.
19. Jorquera, S.; Soto, M.; Díaz, K.; Nuñez, M.; Cuellar, M. A.; Olea, A. F.; Espinoza-Catalán L. Novel Brassinosteroid Analogues with 3,6 Dioxo Function, 24-Nor-22(*S*)-Hydroxy Side Chain and *p*-Substituted Benzoate Function at C-23-Synthesis and Evaluation of Plant Growth Effects. *Int. J. Mol. Sci*. **2024**, *25*(14), 7515.
20. Valdés, E.; Díaz, K.; Núñez, M.; Olea, A.F.; Moral, J.F.Q.d.; Carvajal, R.; Cuellar, M.A.; Espinoza-Catalán, L. Novel 3-Dehydroteasterone Derivatives with 23,24-Dinorcholanic Side Chain and Benzoate Groups at C-22: Synthesis and Activity Evaluation by Rice Lamina Inclination Test and Bean Second-Internode Bioassay. *Int. J. Mol. Sci*. **2025**, *26*(17), 8710.
21. Carvajal, R.; Gonzalez, C.; Olea, A. F.; Fuentealba, M.; Espinoza, L. Synthesis of 2-Deoxybrassinosteroids Analogs with 24-nor, 22(S)-23-Dihydroxy-Type Side Chains from Hyodeoxycholic Acid. Molecules **2028**, *23*(6), 1306.
22. Kvasnica, M.; Oklestkova, J.; Bazgier, V.; Rárová, L.; Korinkova, P.; Mikulík, J.; Budesinsky, M.; Béres, T.; Berka, K.; Lu, Q. Design, synthesis and biological activities of new brassinosteroid analogues with a phenyl group in the side chain. *Org. Biomol. Chem*. **2016**, *14*(37), 8691-8701.