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Synthesis of novel aryl brassinosteroids through alkene cross-metathesis and preliminary biological study

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ABSTRACT

A series of phenyl analogues of brassinosteroids was prepared via alkene cross-metathesis using commercially available styrenes and 24-nor-5α-chola-2,22-dien-6-one. All derivatives were successfully docked into the active site of BRI1 using AutoDock Vina. Plant growth promoting activity was measured using the pea inhibition biotest and Arabidopsis root sensitivity assay and then was compared with naturally occuring brassinosteroids. Differences in the production of plant hormone ethylene were also observed in etiolated pea seedlings after treatment with the new and also five known brassinosteroid phenyl analogues. Antiproliferative activity was also studied using normal human fibroblast and human cancer cell lines.

1. Introduction

Brassinosteroids (BRs, Fig. 1) represent a large group of plant steroids with more than 70 structurally and functionally related compounds [1]. BRs have been found at low concentrations throughout the plant kingdom, widely distributed in higher and lower plants, and have been detected in various plant parts such as pollen, seeds, leaves, stems, roots, and flowers. They are essential for many aspects of plant growth and development, such as cell division, elongation and differentiation, pollen tube growth, seed germination, regulation of gene expression, enzyme activation and photosynthesis [2–5]. At the molecular level, BRs change the gene expression and the metabolism of nucleic acids and proteins. BRs have structures similar to those of animal steroid hormone. Unlike animals, plants perceive steroids at cell membrane, using the membrane-integral receptor kinase brassinosteroid insensitive 1 (BRI1) [6–8]. The encoded protein, BRI1, belongs to a large family of plant LRR (leucine-rich repeat) receptor-like kinases, characterized by an extracellular LRR domain, a single-pass transmembrane segment and a cytoplasmic kinase domain. BRI1 has been established as an authentic brassinosteroid receptor by genetic and biochemical investigations [9]. Crystal structures of BRI1 in both free (PDB ID: 3RIZ, 3RGX), and brassinolide-bound (PDB ID: 3RJ0, 3BRZ), forms are available, following independent X-ray diffraction structural determinations by two groups [9,10]. The structure of the ligand-binding domain resembles a superhelix of 25 twisted LRRs. A 70-amino acid island domain between LRRs 21 and 22 folds back into the interior of the superhelix, creating a surface pocket where the brassinosteroids bind. These recently published structures of *Arabidopsis thaliana* BRI1 enable the rational design of brassinosteroid-like antagonists and agonists. Recent studies [11,12] have indicated that molecular docking is a powerful tool to predict how effective incorporation of different functional groups into brassinosteroid skeleton is and to design new types of BRs with biological activities comparable to natural BRs [11].

The aim of our study is related to the synthesis of new aryl analogues of BRs by alkene cross-metathesis and to study of their biological properties. Alkene cross-metathesis was chosen for preparation of all aryl analogues as an efficient method for construction of the new side chains using different commercially available substituted styrenes. The biological activities of newly prepared derivatives were evaluated using plant bioassays (pea inhibition biotest and Arabidopsis root sensitivity bioassay) and Calcein AM cytotoxicity assay. All derivative structures were subjected to docking studies using AutoDock Vina [13] in order to analyze the results with theoretical studies.

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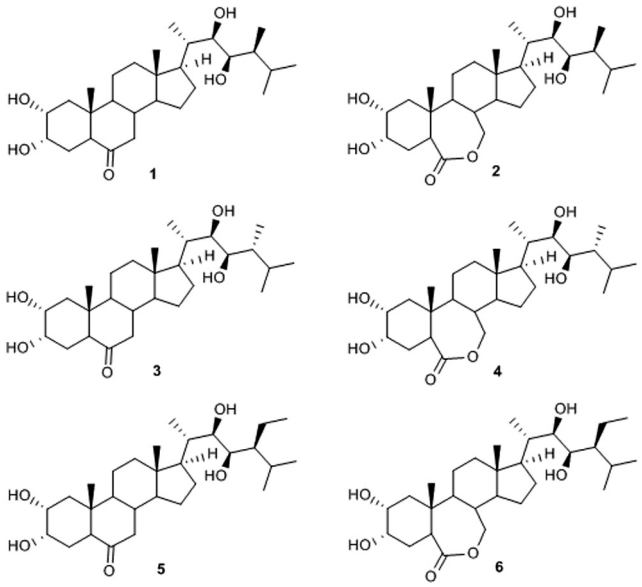

Fig. 1. Structures of most common natural brassinosteroids; castasterone (1), brassinolide (2), 24-epicastasterone (3), 24-epibrassinolide (4), 28-homocastasterone (5), 28-homobrassinolide (6).

2. Experimental

2.1. General methods

The melting points were determined on a Stuart SMP30 instrument (Bibby Scientific Ltd., UK). Elemental analyses were performed using an EA 1108 elemental analyzer (Fison Instruments); the values (C, H, N) agreed with the calculated values within acceptable limits. The infrared spectra were recorded on a Thermo Scientific Nicolet spectrometer iZ10 using the ATR technique. The wave numbers are given in cm^{-1}. The NMR spectra were taken on a JEOL JNM-ECA 500 (JEOL, Tokyo, Japan; 1H, 500 MHz; 13C, 125 MHz) spectrometer equipped with a 5 mm JEOL Royal probe. 1H NMR and 13C NMR chemical shifts (δ) were calibrated using tetramethylsilane (TMS, 1H δ = 0 ppm) or solvents: CDCl3 (1H δ = 7.26 ppm, 13C δ = 77.00 ppm) or DMSO-d_6 (1H δ = 2.46 ppm, 13C δ = 40.00 ppm). Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. All values were obtained by first-order analysis. For API HRMS analysis, the samples were dissolved in chloroform (or chloroform: methanol; 1:1; v/v, in case of hydroxylated compounds) to a concentration 10 μg.mL^{-1}. The ASAP (Atmospheric Solids Analysis Probe) was dipped into the sample solution, placed into the ion source and analysed in fullscan mode. The source of the Synapt G2-Si Mass Spectrometer (Waters, Manchester, UK) was operated in positive ionisation mode (ASAP+), if not stated otherwise, at source temperature of 120 °C. The Corona needle current was kept at 5 μA and the collision energy at value 4. The probe temperature was ramped up from 50 °C to 600 °C in 3 min. Data were acquired from 50 to 1000 Da with 1.0 s scan time in High Resolution Mode. The data were processed using the Masslynx 4.1 software (Waters). Mass accuracy of 1 ppm or less was achieved with the described instrumentation for all compounds. Merck silica gel Kieselgel 60 (230–400 mesh) was used for column chromatography. The HPLC system consisted of a Waters semi-preparative HPLC system including quaternary pump, liquid handler, UV–VIS and ELSD detectors. The semi preparative column was filled with silica gel. Reagents and solvents were purchased from Sigma–Aldrich and were not purified.

2.1.1. General procedure for cross metathesis

Hoveyda-Grubbs 2nd generation catalyst (19 mg; 0.03 mmol) was added to a solution of dien **7** (100 mg; 0.31 mmol) and styrene (2.48 mmol) in dichloroethane (5 mL). The reaction mixture was heated at 80 °C for 5 h. Then, another portion of H-G catalyst (19 mg; 0.03 mmol) was added and the reaction mixture was heated at 80 °C for additional 5 h. Then, the solvent was evaporated and crude solid was purified by column chromatography on silica gel (mobile phase – 3% ethyl acetate in cyclohexane, R_f of products 0.18–0.25). In some cases, stated in each experiment, HPLC had to be used due to very close retention time of product and starting material (mobile phase – 0.5% ethyl acetate in cyclohexane).

2.1.2. (22E)-23-phenyl-24-nor-5α-chola-2,22-dien-6-one (8a)

The general procedure with styrene afforded 120 mg (81%) of the title compound **8** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.72, 0.74 (both s, 3H, CH_3), 1.14 (d, 3H, J = 6.4 Hz, CH_3), 1.69–1.80 (m, 2H), 1.96–2.04 (m, 4H), 2.07 (dt, 1H, J = 12.6, J' = 3.3 Hz), 2.23–2.31 (m, 2H), 2.34–2.37, (m, 2H), 5.57 (m, 1H, H-3), 5.69 (m, 1H, H-2), 6.06 (dd, 1H, J = 15.9, J' = 8.9 Hz, H-22), 6.30 (d, 1H, J = 15.9 Hz, H-23), 7.19 (m, 1H, Ar-H), 7.27–7.35 (m, 4H, 4 × Ar-H). ^{13}C NMR δ 12.19 (C-18), 13.50 (C-19), 20.38 (C-21), 21.10, 21.70, 23.92, 28.21, 37.68, 39.35, 39.37, 40.04, 40.39, 42.92, 46.96, 53.40, 53.83, 55.83, 56.75, 124.49 (C-3), 124.95 (C-2), 125.92 (2 × C), 126.72, 127.42 (C-23), 128.45 (2 × C), 136.94 (C-22), 137.98, 211.98 (C-6). Spectral data in agreement with literature [11].

2.1.3. (22E)-23-(2-fluorophenyl)-24-nor-5α-chola-2,22-dien-6-one (8b)

The general procedure with *o*-fluorostyrene afforded 84 mg (65%) of the title compound **9a** as a colorless oil: IR ν (cm^{-1}) 2930, 1702, 1655, 1593, 1560, 965. ^{1}H NMR ($CDCl_3$) δ 0.73, 0.75 (both s, 3H, CH_3); 1.15 (d, 3H, J = 6.7 Hz, CH_3); 1.72–1.81 (m, 2H); 1.97–2.06 (m, 4H); 2.08 (dt, 1H, J = 12.6, J' = 3.5 Hz); 2.24–2.33 (m, 2H); 2.34–2.38 (m, 2H); 5.58 (m, 1H, H-3); 5.70 (m, 1H, H-2); 6.14 (dd, 1H, J = 15.9, J' = 8.6 Hz, H-22); 6.47 (d, 1H, J = 15.9 Hz, H-23); 7.01 (ddd, J = 10.9, J' = 7.8, J'' = 0.9 Hz, Ar-H); 7.07 (td, 1H, J = 7.8, J' = 1.2 Hz, Ar-H); 7.16 (m, 1H, Ar-H); 7.41 (td, 1H, J = 7.8, J' = 1.8 Hz, Ar-H). ^{13}C NMR δ 12.19 (C-18), 13.48 (C-19), 20.27 (C-21), 21.08, 21.70, 23.91, 28.17, 37.65, 39.32, 39.35, 40.01, 40.76, 42.92, 46.93, 53.37, 53.80, 55.68, 56.71, 115.56 (d, J = 22.8 Hz), 119.79 (d, J = 3.6 Hz), 123.91 (d, J = 3.6 Hz), 124.48 (C-3), 124.94 (C-2), 125.62 (d, J = 13.2 Hz), 126.92 (d, J = 3.6 Hz), 127.86 (d, J = 8.4 Hz), 139.50 (d, J = 3.6 Hz), 159.94 (d, J = 248.3 Hz, C-F), 211.90 (C-6). ^{19}F NMR {^{1}H} δ −118.83 (s, 1F). HRMS: (API+) calculated for $C_{29}H_{38}FO$ ($[M+H]^+$) 421.2907, Found 421.2910.

2.1.4. (22E)-23-(3-fluorophenyl)-24-nor-5α-chola-2,22-dien-6-one (8c)

The general procedure with *m*-fluorostyrene afforded 94 mg (73%) of the title compound **10a** as a colorless oil: IR ν (cm^{-1}) 2933, 1705, 1656, 1593, 1560, 966. ^{1}H NMR ($CDCl_3$) δ 0.73, 0.74 (both s, 3H, CH_3); 1.14 (d, 3H, J = 6.7 Hz, CH_3); 1.71–1.80 (m, 2H); 1.97–2.04 (m, 4H); 2.07 (dt, 1H, J = 12.5, J' = 3.2 Hz); 2.23–2.31 (m, 2H); 2.34–2.38 (m, 2H); 5.58 (m, 1H, H-3); 5.70 (m, 1H, H-2); 6.08 (dd, 1H, J = 15.7, J' = 8.7 Hz, H-22); 6.28 (d, 1H, J = 15.7 Hz, H-23); 6.88 (td, 1H, J = 8.3, J' = 2.6 Hz, Ar-H); 7.03 (m, 1H, Ar-H); 7.08 (b d, 1H, J = 8.3 Hz, Ar-H); 7.24 (td, 1H, J = 7.8, J' = 6.1 Hz, Ar-H). ^{13}C NMR δ 12.16 (C-18), 13.46 (C-19), 20.22 (C-21), 21.06, 21.68, 23.87, 28.16, 37.62, 39.30, 39.32, 39.98, 40.30, 42.90, 46.90, 53.33, 53.77, 55.67, 56.66, 112.25 (d, J = 21.6 Hz), 113.44 (d, J = 21.6 Hz), 121.81 (d, J = 2.4 Hz), 124.45 (C-3), 124.92 (C-2), 126.46 (d, J = 2.4 Hz), 129.79 (d, J = 8.4 Hz), 138.28, 140.35 (d, J = 7.2 Hz), 163.08 (d, J = 244.7 Hz, C-F), 211.82 (C-6). ^{19}F NMR {^{1}H} δ −113.78 (s, 1F). HRMS: (API+) calculated for $C_{29}H_{38}FO$ ($[M+H]^+$) 421.2907, Found 421.2910.

2.1.5. (22E)-23-(4-fluorophenyl)-24-nor-5α-chola-2,22-dien-6-one (8d)

The general procedure with *p*-fluorostyrene afforded 96 mg (75%) of the title compound **8** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.72, 0.73 (both s, 3H, CH_3), 1.13 (d, 3H, J = 6.7 Hz, CH_3), 1.71–1.80 (m, 2H),