Steroids 85 (2014) 58–64

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Biological activities of new monohydroxylated brassinosteroid analogues with a carboxylic group in the side chain

Miroslav Kvasnica [a], Jana Oklestkova [a,*], Vaclav Bazgier [a,b], Lucie Rarova [a], Karel Berka [c], Miroslav Strnad [a]

[a] Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR & Palacký University, Šlechtitelů 11, 78371 Olomouc, Czech Republic

[b] Department of Physical Chemistry, Faculty of Science, Palacký University, tř. 17. Listopadu 12, 77146 Olomouc, Czech Republic

[c] Regional Centre of Advanced Technologies and Material, Department of Physical Chemistry, Faculty of Science, Palacký University, 17. Listopadu 12, 77146 Olomouc, Czech Republic

ARTICLE INFO

Article history:
Received 16 December 2013
Received in revised form 3 April 2014
Accepted 10 April 2014
Available online 21 April 2014

Keywords:
Anticancer activity
Brassinosteroids
Organic synthesis
Molecular docking
Receptor kinase BRI1
Plant bioassays

ABSTRACT

Thirteen monohydroxylated brassinosteroids analogues were synthesized and tested for their biological activity in plant and animal systems. The cytotoxic activity of the products was studied using human normal and cancer cell lines with 28-homocastasterone as positive control, their brassinolide type activity was established using the bean second-internode test with 24-epibrassinolide as standard.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Brassinosteroids (BRs) represent a large group of plant steroids which include more than 70 structurally and functionally related compounds [1]. BRs have been found in a wide range of plant species, including higher and lower plants, and have been detected in various plant parts such as pollen, seeds, leaves, stems, roots, flowers and insect galls. They demonstrate various kinds of regulatory action on the growth and development of plants, such as the stimulation of cell enlargement and cell division, improvement of the biomass formation, yield and quality of seeds, and plant adaptability [2]. 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 hormones. Plants perceive steroids at cell membrane, using the membrane-integral receptor kinase brassinosteroid insensitive 1 (BRI1) [3–5]. 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 [6]. 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 [6,7]. 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 have indicated that BRs have antiviral, antiproliferative and antibacterial activity [8]. BRs analogues have been reported to have antiviral activity against herpes simplex virus type 1, arenaviruses as well as against replication of vesicular stomatitis virus in Vero cells [9–11]. Natural types of BRs and their analogues affected the viability, proliferation, apoptosis and expression of some cell cycle related proteins in cancer and normal human cell lines [12–14]. It was shown that natural BRs, 24-epibrassinolide and 28-homocastasterone, inhibited *in vitro* angiogenesis of human endothelial cells [15].

The aim of our study relates to the synthesis of new brassinosteroid monohydroxylated derivatives and to study of their biological and anticancer properties. The plant growth promoting activity of synthetic analogs was assayed using the bean second internode bioassay. The antiproliferative activity of the new BRs analogs was evaluated *in vitro* using cancer cell lines of different histopathological origins and normal human fibroblasts. All derivative

* Corresponding author. Tel.: +420 58563483; fax: +420 585634870.
E-mail address: jana.oklestkova@upol.cz (J. Oklestkova).

http://dx.doi.org/10.1016/j.steroids.2014.04.007
0039-128X/© 2014 Elsevier Inc. All rights reserved.

structures were further subjected to docking studies using AutoDock Vina [16] in order to find structural patterns responsible for the results.

2. Experimental

2.1. General methods

The melting points were determined on a Hund H 600 apparatus (Helmut Hund, Germany). The elemental analyses (C, H, N) were carried out on a Perkin-Elmer 2400 II elemental analyzer. Optical rotations were measured on an Autopol IV polarimeter (Rudolf Research Analytical, Flanders, USA) at 25 °C in chloroform and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g. The infrared spectra were recorded on a Bruker IFS 55 spectrometer in chloroform. The wave numbers are given in cm^{-1}. The ^{1}H NMR spectra were taken in $CDCl_3$ on a Bruker AVANCE-400 (at 400 MHz) instrument with tetramethylsilane as an internal reference. Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. All of the values were obtained by first-order analysis. The mass spectra (ESI) were obtained with a LTQ Orbitrap XL (Thermo Fisher Scientific). For column chromatography, neutral silica gel (60 μm, Fluka) was used. The HPLC system consisted of a Gilson semi-preparative HPLC system including a quaternary pump, liquid handler, UV–VIS, RI, and ELSD detectors. The semi preparative column was based on silica gel (Labio Biosphere PSI 200).

Reagents and solvents were purchased from Sigma–Aldrich and were not purified.

2.2. Hydroboration of **1**

Solution of borane in THF (1 M, 20 mL) was added dropwise to the solution of methyl ester **1** (1 g, 2.49 mmol) in dry THF (30 mL). The reaction mixture was stirred at room temperature for 6 h. Then, the reaction mixture was quenched by water (10 mL) and cooled to 10 °C. Aqueous solution of NaOH (10%, 20 mL) was added followed by dropwise addition of 30% hydrogen peroxide (30 mL). This reaction mixture was stirred overnight at room temperature. It was then poured into a mixture of sodium sulfite (2 g) in water (50 mL) and acetic acid (1 mL) and stirred for additional 30 min. After then, the reaction mixture was diluted with ethyl acetate, extracted twice with water, the extract was dried over anhydrous sodium sulfate, and solvents evaporated under reduced pressure. A crude mixture of products was dissolved in a mixture of THF and acetone (5:1, 10 mL) and 5% aqueous HCl was added. The reaction mixture was heated under reflux for 3 h. Then it was diluted with ethyl acetate, extracted twice with water, the extract was dried over anhydrous sodium sulfate, and the solvents were evaporated under reduced pressure. Two main products were separated on HPLC in ethyl acetate–hexane (1:1). Two hydroxy compounds **2a** and **2b** were obtained.

2.2.1. Methyl (20S)-3α-hydroxy-6-oxo-5α-pregnane-20-carboxylate (***2a***)

Yield: 380 mg (40%), m.p. 198–200 °C (MeOH), $[\alpha]_D = -6°$ (c 0.27). IR ν (cm^{-1}) 3615, 1727, 1706, 1165. ^{1}H NMR ($CDCl_3$) δ 0.68, 0.73 (both s, 3H, CH_3), 1.19 (d, 3H, J = 6.9 Hz, CH_3), 1.97 (m, 1H), 2.02 (dd, 1H, J = 12.8, J' = 1.0 Hz, H-7β), 2.29 (dd, 1H, J = 12.8, J' = 4.5 Hz, H-7α), 2.43 (dq, 1H, J = 10.3, J' = 6.8 Hz, H-20), 2.73 (dd, 1H, J = 8.9, J' = 6.9 Hz, H-5α), 3.65 (s, 3H, OCH_3), 4.16 (t, 1H, J = 2.1 Hz, H-3β). ^{13}C NMR δ 12.17 (CH_3), 12.30 (CH_3), 17.06 (CH_3), 21.01 (CH_2), 23.96 (CH_2), 26.95 (CH_2), 27.69 (CH_2), 28.16 (CH_2), 31.67 (CH_2), 37.90 (CH), 39.27 (CH_2), 41.49 (C), 42.35 (CH), 43.00 (C), 46.75 (CH_2), 51.34 (CH), 51.66 (CH_3), 52.79 (CH), 53.78 (CH), 56.37 (CH), 65.35 (CH), 177.13 (C), 212.37 (C). HRMS: (ESI+) calculated for $C_{23}H_{36}O_4Na$ (M^++Na) 399.25058. Found 399.25059. Anal. Calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64. Found: C, 73.45; H, 9.60%.

2.2.2. Methyl (20S)-2α-hydroxy-6-oxo-5α-pregnane-20-carboxylate (***2b***)

Yield: 400 mg (46%), m.p. 153–155 °C (MeOH), $[\alpha]_D = -8°$ (c 0.62). IR ν (cm^{-1}) 3608, 1727, 1708, 1168. ^{1}H NMR ($CDCl_3$) δ 0.68, 0.75 (both s, 3H, CH_3), 1.20 (d, 3H, J = 6.9 Hz, CH_3), 1.95–2.10 (m, 4H), 2.15 (dd, 1H, J = 11.9, J' = 2.6 Hz, H-5α), 2.29 (dd, 1H, J = 13.1, J' = 4.6 Hz, H-7α), 2.43 (dq, 1H, J = 10.3, J' = 6.8 Hz, H-20), 3.65 (s, 3H, OCH_3), 3.77 (m, 1H, $W_{½}$ = 31.5 Hz, H-2β). ^{13}C NMR δ 12.16 (CH_3), 14.14 (CH_3), 17.06 (CH_3), 19.49 (CH_2), 21.20 (CH_2), 23.96 (CH_2), 26.95 (CH_2), 34.57 (C), 37.57 (CH), 39.18 (CH_2), 42.31 (CH), 42.98 (CH_2), 46.56 (CH_2), 47.07 (C), 51.35 (CH_3), 52.76 (CH), 54.12 (CH), 56.26 (CH), 57.95 (CH), 66.93 (CH), 177.13 (C), 211.58 (C). HRMS: (ESI+) calculated for $C_{23}H_{36}O_4Na$ (M^++Na) 399.25058. Found 399.25057. Anal. Calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64. Found: C, 73.42; H, 9.57%.

2.3. General procedure for Baeyer–Villiger oxidation

A solution of trifluoroperoxyacetic acid, freshly prepared from trifluoroacetic anhydride (0.45 mL, 3.2 mmol) and hydrogen peroxide (30%; 0.1 mL) in dichloromethane (3 mL), was added to a solution of 6-oxocompound (0.40 mmol) in dichloromethane (8 mL). After standing at room temperature for 5 h, the reaction mixture was poured into water and taken up in chloroform (50 mL). The chloroform extract was washed with water, a saturated solution of potassium hydrogen carbonate, water, and dried over anhydrous sodium sulfate. Solvents were evaporated under reduced pressure and products were separated and purified on HPLC in ethyl acetate–hexane 7:3.

2.4. Baeyer–Villiger oxidation of ***2a***

Ketone **2a** (150 mg; 0.40 mmol) was used for Baeyer–Villiger oxidation as described in general procedure. Reaction afforded two lactones **3a** and **3b**.

2.4.1. Methyl (20S)-3α-hydroxy-7-oxa-7a-homo-6-oxo-5α-pregnane-20-carboxylate (***3a***)

Yield: 69 mg (44%), m.p. 185–187 °C (MeOH), $[\alpha]_D = +10°$ (c 0.27). IR ν (cm^{-1}) 3615, 1723, 1182. ^{1}H NMR ($CDCl_3$) δ 0.72, 0.90 (both s, 3H, CH_3), 1.19 (d, 3H, J = 6.9 Hz, CH_3), 1.93 (dt, 1H, J = 6.6, J' = 3.0 Hz), 2.14 (ddd, 1H, J = 15.0, J' = 12.3, J'' = 2.6 Hz, H-4a), 2.43 (dq, 1H, J = 10.3, J' = 6.9 Hz, H-20), 3.19 (dd, 1H, J = 12.2, J' = 4.4 Hz, H-5α), 3.65 (s, 3H, OCH_3), 4.07–4.11 (m, 2H, H-7α, H-7β), 4.17 (m, 1H, $W_{½}$ = 10.9 Hz, H-3β). ^{13}C NMR δ 11.97 (CH_3), 14.54 (CH_3), 17.02 (CH_3), 22.11 (CH_2), 24.88 (CH_2), 26.92 (CH_2), 28.27 (CH_2), 32.53 (CH_2), 32.92 (CH_2), 36.34 (C), 39.47 (CH_2), 39.50 (CH), 41.79 (CH), 42.35 (CH), 42.68 (C), 51.15 (CH), 51.39 (CH_3), 52.80 (CH), 58.36 (CH), 64.87 (CH), 70.32 (CH_2), 176.60 (C), 177.02 (C). HRMS: (ESI+) calculated for $C_{23}H_{36}O_5Na$ (M^++Na) 415.24550. Found 415.24540. Anal. Calcd for $C_{23}H_{36}O_5$: C, 70.38; H, 9.24. Found: C, 70.29; H, 9.30%.

2.4.2. Methyl (20S)-3α-hydroxy-6-oxa-7a-homo-7-oxo-5α-pregnane-20-carboxylate (***3b***)

Yield: 50 mg (32%), m.p. 225–227 °C (MeOH), $[\alpha]_D = +10°$ (c 0.22). IR ν (cm^{-1}) 3614, 1723, 1168. ^{1}H NMR ($CDCl_3$) δ 0.71, 0.90 (both s, 3H, CH_3), 1.18 (d, 3H, J = 6.9 Hz, CH_3), 1.83 (ddd, 1H, J = 14.2, J' = 11.4, J'' = 2.7 Hz, H-4a), 1.92 (d, 1H, J = 12.3, J' = 3.0 Hz), 2.03 (ddd, 1H, J = 14.2, J' = 5.1, J'' = 3.1 Hz, H-4b), 2.43 (dq, 1H, J = 10.3, J' = 6.9 Hz, H-20), 2.46–2.53 (m, 2H, H-7α, H-7β), 3.65 (s, 3H, OCH_3), 4.22 (m, 1H, H-3β), 4.62 (dd, 1H, J = 11.4, J' = 5.1 Hz,

Download English Version:

https://daneshyari.com/en/article/2027963

Download Persian Version:

https://daneshyari.com/article/2027963

Daneshyari.com