Steroids 77 (2012) 91–99

Contents lists available at SciVerse ScienceDirect

Steroids

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

Brassinosteroids and analogs as neuroprotectors: Synthesis and structure–activity relationships

Jihane Ismaili, Martin Boisvert, Fanny Longpré, Julie Carange, Céline Le Gall, Maria-Grazia Martinoli, Benoit Daoust *

Département de Chimie-Biologie, Groupe de Recherche en Neuroscience, Université du Québec à Trois-Rivières, 3351 Boulevard des Forges, C.P. 500, Trois-Rivières, Québec, Canada G9A 5H7

ARTICLE INFO

Article history:
Received 4 August 2011
Received in revised form 18 October 2011
Accepted 20 October 2011
Available online 28 October 2011

Keywords:
Brassinosteroids
Structure–activity relationship
Neuroprotection
MPP^+
Dopaminergic cells
Parkinson's disease

ABSTRACT

We have demonstrated previously that the brassinosteroid (BR) 24-epibrassinolide exerts neuroprotective effects deriving from its antioxidative properties. In this study, we synthesized 2 natural BRs and 5 synthetic analogs and analyzed their neuroprotective actions in neuronal PC12 cells, against 1-methyl-4-phenylpyridinium (MPP^+), a neurotoxin known to induce oxidative stress and degenerescence of dopaminergic neurons characteristic of Parkinsonian brains. We also tested the neuroprotective potential of 2 commercially available BRs. Our results disclosed that 6 of the 9 BRs and analogs tested protected neuronal PC12 cells against MPP^+ toxicity. In addition, our structure–activity study suggests that the steroid B-ring and lateral chain play an important role for their neuroprotective action.

1. Introduction

BRs are highly oxygenated steroids isolated from several vegetables, including *Vicia faba* seeds and pollen [1–3]. To date, about 60 natural BRs have been identified, such as 24-epibrassinolide (**1**) and homocastasterone (**2a**) (Fig. 1), both oxygenated in positions 2, 3, 6, 22 and 23 [4]. Several BR non-natural analogs, such as 22S,23S-homocastasterone (**2b**) and 22S,23S-homobrassinolide (**3**) (Fig. 1), have been synthesized [5,6]. Owing to their peculiar structural features, their extremely low abundance in natural sources and potent biological activity [7], BRs have been the subject of numerous synthetic efforts [5,6,8–10]. Their 4 contiguous chiral centers (C-20, C-22, C-23 and C-24) represent major challenges in the synthesis of these steroids. The methods developed by McMorris et al. [5], Mori et al. [6] and Brosa et al. [8] are especially efficient and versatile, allowing the swift preparation of a variety of 29-carbon BRs. All these protocols use stigmasterol as the starting product, and their synthetic scheme can be summarized in 2 main reaction sequences: (i) transformation of the homoallylic alcohol functionality of stigmasterol into 2,3,6- and 3,6-oxygenated moieties, and (ii) standard osmylation of the 22,23-alkene, resulting in 22,23-dihydroxylated steroidal side-chains.

BRs are being studied intensively to understand their role in plant metabolism. Their main physiological effects in plants include regulation of hormonal balance, activation of protein and nucleic acid synthesis, enzyme activity, growth promotion, and, most interestingly, increased resistance to unfavorable environmental factors, stress and diseases (for review see [7]). BRs have also been reported to exert anti-oxidative actions [11–17]. Exogenous application of natural BRs, such as 24-epibrassinolide (**1**), to plants enhances activities of the enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT) and peroxidase, reducing lipid peroxidation [11–15]. Oral administration of homobrassinolide evokes anti-oxidative outcomes in mammals [16]. Recently, we determined that 24-epibrassinolide (**1**) modulates SOD, CAT and glutathione peroxidase (GPx) in mammalian cells [17].

Several neurodegenerative diseases, e.g. Parkinson's disease (PD), are associated with oxidative stress [18]. PD is characterized by the selective degeneration of nigrostriatal dopaminergic neurons, resulting in dopamine (DA) depletion [19]. Numerous studies have demonstrated that, in *post mortem* samples of *substantia nigra pars compacta*, DAergic neurons exhibit markers of oxidative stress, such as lipid peroxidation, DNA oxidative damage, and carbonyl modifications of soluble proteins [20,21]. L-3,4-Dihydroxyphenylalanine (L-dopa), the amino acid precursor of DA, is nowadays the most effective symptomatic treatment of PD [22]. Clinical reports indicate that consumption of *V. faba* beans and seedlings, which contain L-dopa [23,24], has beneficial effects on PD patients

* Corresponding author. Tel.: +1 819 376 5011x3349; fax: +1 819 376 5084.
E-mail address: benoit.daoust@uqtr.ca (B. Daoust).

0039-128X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2011.10.009

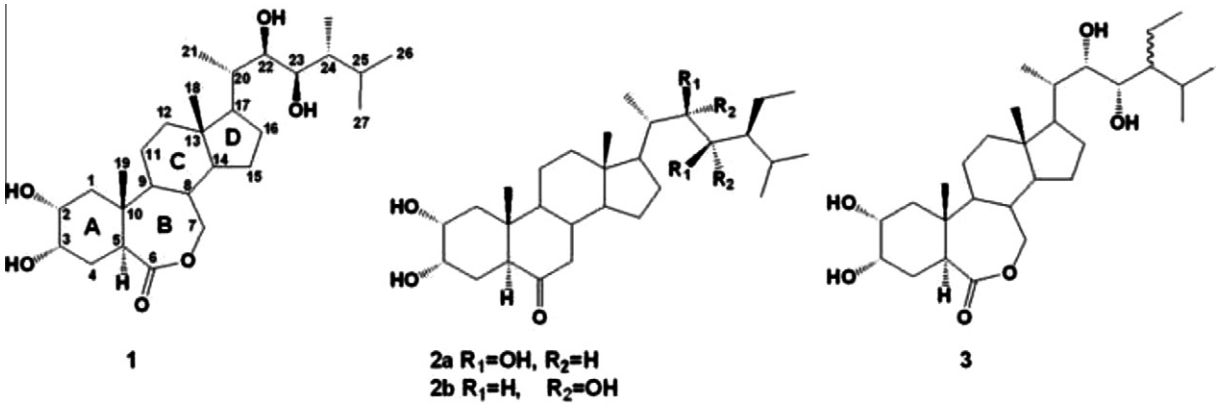

Fig. 1. Structure of 24-epibrassinolide (**1**), homocastasterone (**2**), and 22S,23S-homobrassinolide (**3**).

[25,26]. However, the concentration of L-dopa in *V. faba* is not sufficient to explain the magnitude of the responses observed in PD patients [26]. Thus, it raises the possibility that other compounds in *V. faba* may complement the effect of L-dopa. It is known that *V. faba* contains BRs such as 24-epibrassinolide (**1**), castasterone and brassinolide [1–3]. Recently, we established that 24-epibrassinolide (**1**) is neuroprotective of nerve growth factor (NGF)-differentiated PC12 (neuronal) cells against MPP^+-induced toxicity [17]. MPP^+ is the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a toxin serving extensively to reproduce PD in animal models [27]. This neurotoxin is known to act as an in vitro and in vivo oxidant [28]. MPP^+ is selectively taken up by DAergic neurons via high-affinity DA transporters expressed in NGF-differentiated PC12 cells [29,30]. The neurotoxin is actively transported into mitochondria where it interferes with mitochondrial respiration through complex I inhibition [31–33]. It evokes elevated levels of reactive oxygen species (ROS) in MPP^+-treated neuronal cells [34–36] and neuronal cell death by apoptosis [37,38]. We recently reported that 24-epibrassinolide (**1**) modulates SOD, CAT and GPx activities and reduces MPP^+-induced apoptosis and intracellular ROS in neuronal PC12 cells [17].

The aim of the present investigation study was to evaluate the neuroprotective effects of natural BRs and synthetic analogs and provide new insights into their structure–activity relationships as neuroprotective molecules in a well-known in vitro model of PD, NGF-differentiated PC12 cells [29,39]. We followed the protocol of Brosa et al. to prepare BRs for this study [8]. This strategy was particularly well-suited for our work since it allowed us to freely functionalize steroid A and B rings as well as C20–C29 side-chain. We were, therefore, able to synthesize 7 BRs with different levels of oxygenation. We demonstrated that some of these molecules were neuroprotective against MPP^+-evoked toxicity. Structure–activity analysis revealed the importance of lateral chain and B ring functionalization for neuroprotection. We also noted that BR hydroxyl group configurations were not crucial for neuroprotection. Overall, our findings clearly indicate that BRs and analogs are new protective molecules against MPP^+-induced toxicity. Therefore, they might be regarded as novel candidates to investigate the outcomes of complementary and/or preventive therapies in neurodegenerative diseases.

2. Experimental

2.1. General

All reagents, including 24-epibrassinolide (**1**) and 22S,23S-homobrassinolide (**3**), were purchased from Sigma–Aldrich (Oakville, ON, Canada) unless noted otherwise. All solvents (Fisher Scientific, Ottawa, ON, Canada) were ACS-certified, distilled and dried prior to use. Reactions requiring anhydrous conditions were conducted under positive nitrogen atmosphere in oven-dried glassware, and reaction flasks were fitted with rubber septa for the introduction of substrates and reagents via standard syringe techniques. Flash chromatography was performed on Merck silica gel 60 on Siliaflash P60 (0.040–0.063 mm, Silicycle, Quebec, QC, Canada) under compressed air pressure. Analytical thin-layer chromatography (TLC) was carried out on pre-coated (0.25 mm) Merck silica gel F54 plates (VWR, Ville Mont-Royal, Qc, Canada) or on silica gel 60 (0.25 mm, Silicycle, Quebec, QC, Canada) and developed with an acid solution of ammonium phosphomolybdate. ^{1}H NMR spectra were recorded on a Varian 200 MHz NMR spectrometer with $CDCl_3$ (δ = 7.26 ppm) as reference. ^{13}C NMR spectra were traced at 50.3 MHz with $CDCl_3$ (δ = 77.1 ppm) as reference. For acetate **9**, NMR spectra were charted on a Varian 600-MHz spectrometer. The data reflect the following: chemical shift in ppm, multiplicity (d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets), number of protons, coupling constants in Hertz and assignation (if possible). IR spectra were recorded by Nicolet Impact 420 spectrophotometer. Low resolution mass spectra (LRMS) were recorded on an Agilent Technologies GC system 6890 N and mass detector 5973 with helium as carrier gas. High resolution mass spectra (HRMS) were measured in the electrospray (ESI) mode on an HPLC 1200 system with a TOF 6210 detector from Agilent Technologies. Melting points (mp) were recorded on an Electrothermal apparatus and were uncorrected. If compounds were recrystallized prior to determination of their melting points, the re-crystallization solvent is indicated in brackets.

2.2. (22E,24S)-3α,5-cyclo-stigmast-22-en-6-one (*5*)

Compound **5** was prepared in 3 steps from stigmasterol (**4**) according to Brosa's method [8]. Under N_2 atmosphere and anhydrous conditions, 5.25 g (12.1 mmol) of stigmasterol (**4**) 95% (Acros Organics, Fisher Scientific, Ottawa, ON, Canada) were dissolved in 60 mL of toluene. To this solution, 13.0 mL (93.1 mmol, 7.7 eq) of triethylamine were added with a syringe. The mixture was then cooled to 0 °C and 2.8 mL (36.1 mmol, 3.0 eq) of $MeSO_2Cl$ were included drop-wise over 10 min. After stirring at 0 °C for 1.5 h, the resulting yellow solution was diluted with water and extracted with toluene (3X). The organic layers were washed with saturated aq. $NaHCO_3$ (2X) and brine, then dried over $MgSO_4$. Evaporation of the solvent provided 5.84 g (97%) of the mesylate. This step was repeated several times, and the yields obtained were 95–97%. ^{1}H- and ^{13}C NMR data on the crude compound were consistent with data in the literature [8].