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Lichen-derived compounds show potential for central nervous system therapeutics

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ABSTRACT

Background: Natural products from lichens are widely investigated for their biological properties, yet their potential as central nervous system (CNS) therapeutic agents is less explored.
Purpose: The present study investigated the neuroactive properties of selected lichen compounds (atranorin, perlatolic acid, physodic acid and usnic acid), for their neurotrophic, neurogenic and acetylcholine esterase (AChE) activities.
Methods: Neurotrophic activity (neurite outgrowth) was determined using murine neuroblastoma Neuro2A cells. A MTT assay was performed to assess the cytotoxicity of compounds at optimum neurotrophic activity. Neuro2A cells treated with neurotrophic lichen compounds were used for RT-PCR to evaluate the induction of genes that code for the neurotrophic markers BDNF and NGF. Immunoblotting was used to assess acetyl H3 and H4 levels, the epigenetic markers associated with neurotrophic and/or neurogenic activity. The neurogenic property of the compounds was determined using murine hippocampal primary cultures. AChE inhibition activity was performed using a modified Ellman's esterase method.
Results: Lichen compounds atranorin, perlatolic acid, physodic acid and (+)-usnic acid showed neurotrophic activity in a preliminary cell-based screening based on Neuro2A neurite outgrowth. Except for usnic acid, no cytotoxic effects were observed for the two depsides (atranorin and perlatolic acid) and the alkyl depsidone (physodic acid). Perlatolic acid appears to be promising, as it also exhibited AChE inhibition activity and potent proneurogenic activity. The neurotrophic lichen compounds (atranorin, perlatolic acid, physodic acid) modulated the gene expression of BDNF and NGF. In addition, perlatolic acid showed increased protein levels of acetyl H3 and H4 in Neuro2A cells.
Conclusion: These lichen depsides and depsidones showed neuroactive properties in vitro (Neuro2A cells) and ex vivo (primary neural stem or progenitor cells), suggesting their potential to treat CNS disorders.

Introduction

Central nervous system (CNS) disorders account for 12% of deaths worldwide, and those who survive are reported to have a poor quality of life (Aarli, 2006). Neurodegenerative conditions associated with the diverse neurological and psychiatric disorders impair neural integrity, neuroglial plasticity and CNS homeostasis (Dawbarn and Allen, 2003). Restoring CNS homeostasis after an injury or a disease is a major challenge. Neurotrophins or neurotrophic factors, like Brain-Derived Neurotrophic Factor (BDNF) and Glial-Derived Neurotrophic Factor (GDNF), have shown promising outcomes in these restoration processes (Sofroniew et al., 2001). However, administered exogenous neurotrophins may have poor or no bioavailability. This has galvanized research into new compounds, including natural products, that mimic neurotrophic factors or act as neuromodulators to accelerate neuritogenesis and result in neuroprotection (McAllister, 2001). Recently, we and others have reported several compounds from natural sources that have good neuroactive and neuroprotective properties, yet no

Abbreviations: AChE, acetylcholine esterase; AKT, protein kinase B; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; ERK, extracellular signal-regulated kinase; FBS, foetal bovine serum; GDNF, glial-derived neurotrophic factor; HDACi, histone deacetylase inhibitor; MTT, (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NGF, nerve growth factor; NPCs, neural progenitor cells; PBS, phosphate-buffered saline; SEM, standard error of the mean; THF, tetrahydrofuran.

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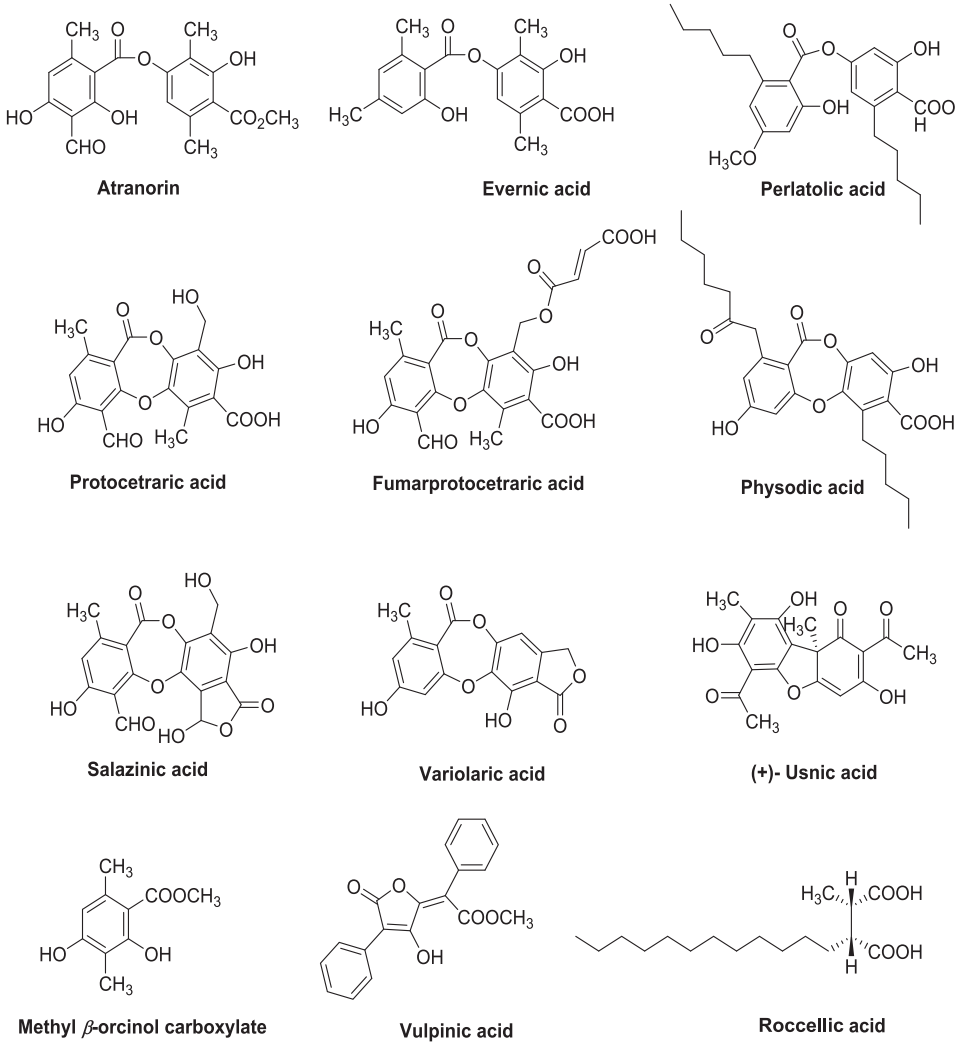

Fig. 1. Structures of lichen compounds.

cytotoxicity, with regard to their biocompatibility at neurotrophically active doses (Smriga and Saito, 2000; Hur et al., 2004; Mehta et al., 2012; Praveen Kumar et al., 2012). Lichens, composite organisms of a fungal and a photosynthetic (algal or cyanobacterium) partner growing together in a symbiotic relationship, are a source of unique specialized metabolites (Shrestha and Clair, 2013). Diverse lichen compounds and secondary metabolites, identified from different sources and environmental conditions, are reported to have a number of bioactive properties (Behera et al., 2012; Singh et al., 2013). Furthermore, lichen extracts are still being used in Asian countries as traditional and alternative medicine (Shukla et al., 2010). However, their potential benefit to the CNS is less explored (Fernandez-Moriano et al., 2015). Here, we report the *in vitro* neuroactive properties of selected lichen-derived compounds (Fig. 1) with respect to their neurotrophic, neurogenic and acetylcholine esterase (AChE) inhibition activities.

Materials and methods

Source and identification of lichen compounds

Evernic acid and vulpinic acid were purchased from Extrasynthese (Genay, France) and methyl ß-orcinol carboxylate from Sigma-Aldrich. The other lichen compounds were isolated and identified from lichen thalli mainly collected in France and identified at the Université de Rennes1. Atranorin, fumarprotocetraric acid and (+)-usnic acid were isolated from *Usnea articulata* as previously reported (Lohézic-Le Dévéhat et al., 2007). Perlatolic acid (Oettl et al., 2013) and physodic acid were obtained from a diethyl ether extract of *Cladonia portentosa* and an acetonic extract of *Pseudevernia furfuracea*, respectively (Bauer et al., 2012). Salazinic acid and variolaric acid were obtained from *Parmotrema tinctorum* (Eifler-Lima, 2000) and *Ochrolechia parella* (Millot et al., 2007), respectively, and roccellic acid was from *Roccella phycopsis* (Parrot et al., 2014). Protocetraric acid (200 mg) was isolated from *Pertusaria albescens* (18 g) under microwave-irradiations (Synthewave® 402 Prolabo) (Bonny et al., 2009), using a 5 min successive extraction with *n*-heptane (2×50 ml) and THF (4×50 ml).

All compounds (Fig. 1) were checked for >95% purity by HPLC, and spectroscopic data were in agreement with those reported (Huneck and Yoshimura, 1996) (HPLC and ^{1}H NMR spectra are available in Supplementary Figs. 1 and 2).

Maintenance of neuronal cells

The Neuro2A (mouse neuroblastoma) cell line obtained from the American Type Culture Collection (ATCC) was maintained in Eagle's Minimum Essential Medium (Sigma-Aldrich) supplemented with 10% FBS (Gibco), penicillin/streptomycin (1% of 100x), Non-