Fitoterapia 81 (2010) 552–556

Contents lists available at ScienceDirect

Fitoterapia

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

Flavonoids with prolyl oligopeptidase inhibitory activity isolated from *Scutellaria racemosa* Pers

Micaela R. Marques [a], Caroline Stüker [a], Nessim Kichik [b], Teresa Tarragó [b], Ernest Giralt [b,c], Ademir F. Morel [a], Ionara I. Dalcol [a,*]

[a] *Núcleo de Pesquisa de Produtos Naturais (NPPN), Departamento de Química, Universidade Federal de Santa Maria, 97105-900, Santa Maria – RS, Brazil*
[b] *Institute for Research in Biomedicine, Barcelona Science Park, E-08028, Barcelona, Spain*
[c] *Departament de Química Orgánica, Universitat de Barcelona, Martí i Franqués 1-11 08028, Barcelona, Spain*

ARTICLE INFO

Article history:
Received 9 September 2009
Accepted in revised form 14 January 2010
Available online 1 February 2010

Keywords:
Scutellaria racemosa Pers
Prolyl oligopeptidase
POP inhibition
Dipeptidyl peptidase
DPP IV inhibition

ABSTRACT

Prolyl oligopeptidase (POP) is a serine protease highly expressed in the brain that hydrolyses peptide bonds at the carboxyl terminal of prolyl residues. There is evidence that this enzyme participates in several functions of the central nervous system. *Scutellaria racemosa* Pers demonstrated significant and selective POP inhibition. Fractionation of the hydroalcoholic extract resulted in the isolation of four main constituents identified for the first time from *S. racemosa* Pers, the triterpenoid lupeol (**1**) and the flavonoids oroxylin A (5,7-dihydroxy-6-methoxyflavone, **2**), hispidulin (4′,5,7-trihydroxy-6-methoxyflavone, **3**), and oroxyloside (oroxylin A 7-*O*-glucuronide, **4**). Inhibitory assays indicated that **3** and **4** at a concentration of 100 μM inhibit 43 and 34% of total POP activity, respectively.

1. Introduction

The genus *Scutellaria* (Labiatae) is formed by approximately 350 species. Some of these are collectively known as "skullcap", medicinal plants used popularly in Europe, the United States and Eastern Asia. *Scutellaria lateriflora* L. and *Scutellaria galericulata* L. are used as skullcaps in Europe and United States while the roots of *Scutellaria baicalensis* Georgi (*Scutellaria radix* in the Japanese pharmacopeia; common name "Baikal skullcap") is largely administered in folk medicine in the countries of Eastern Asia, as China, Korea and Japan [1]. In general, the aerial parts of skullcap are used as a sedative, a tonic for the nerves, an anti-spasmodic agent (for treatment of epilepsy) and to treat sleeplessness, anxiety and as substitute in chronic treatments with barbiturates and tranquillizers [2–4]. In Canada, skullcap is used as tonic or in combination with other medicinal plants, as the *Valeriana*, in the induction of sleep [3,4]. Like another medicinal species of the *Scutellaria* genus, *S. racemosa* Pers is used in South America folk medicine as a neurologically active plant [5]. To date, only the North American native *S. racemosa* has been studied with regard to its antioxidant potential. The study on *S. racemosa* native from Florida, USA, described the presence of the indoleamines serotonin and melatonin, and the flavonoids wogonin, baicalin and baicalein [6]. Phytochemical analyses of *S. baicalensis* and *S. lateriflora* detected the same flavonoids, besides the neurohormones melatonin and serotonin [7], while oroxyloside and wogonoside were identified from *Scutellaria phyllostachya* [8]. In general, flavonoids are the principal class of compounds that have been isolated from several species of *Scutellaria* [9]. To our knowledge there are no reports about phytochemical studies carried out on *S. racemosa* Pers native from Brazil.

Prolyl oligopeptidase (POP, EC 3.4.21.26) and dipeptidyl peptidase IV (DPP IV, EC 3.4.14.5) cleave bioactive peptides after proline residues, and are therapeutic targets for several diseases. POP may be involved in the catabolism of the neuropeptides arginine-vasopressin (AVP) and thyrotrophin-releasing hormone (TRH) [10,11]. Research on the features of

* Corresponding author. Tel.: +55 3220 8869; fax: +55 3220 8031.
E-mail address: iidalcol@gmail.com (I.I. Dalcol).

0367-326X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.fitote.2010.01.018

POP has indicated that this enzyme participates in several aspects of central nervous system (CNS) function. Depression, anxiety and schizophrenia are associated with a significant increase in the serum POP activity [12–17]. Several potent POP inhibitors, such as Z-Pro-Prolinal and S-17092-1, have been proposed for potential use in the prevention and treatment of neurological diseases [10,11]. However, few studies have addressed POP inhibitors derived from medicinal plants [18–21]. Like POP, DPP IV belongs to the same family of serine proteases. Therapeutically, DPP IV is a key target in the treatment of type 2 diabetes mellitus since it inactivates glucagon-like peptide-1, a hormone that stimulates insulin secretion in a glucose-dependent manner. Despite their low amino acid sequence homology, POP and DPP IV share similar three-dimensional structures. Thus, selectivity is highly relevant in the discovery of new inhibitors since these enzymes are involved in several types of illnesses and show distinct mechanisms of action. Therefore, in the study of new POP inhibitors it is also of interest to evaluate in parallel the capacity of compounds to inhibit DPP IV.

In the course of our continuing search for POP inhibitors of natural origin, the present paper describes the isolation of the triterpenoid lupeol (**1**) and of three flavonoids (**2**–**4**) from *S. racemosa* Pers. The isolated compounds have been evaluated as POP and DPP IV inhibitors.

2. Material and methods

2.1. General experimental procedures

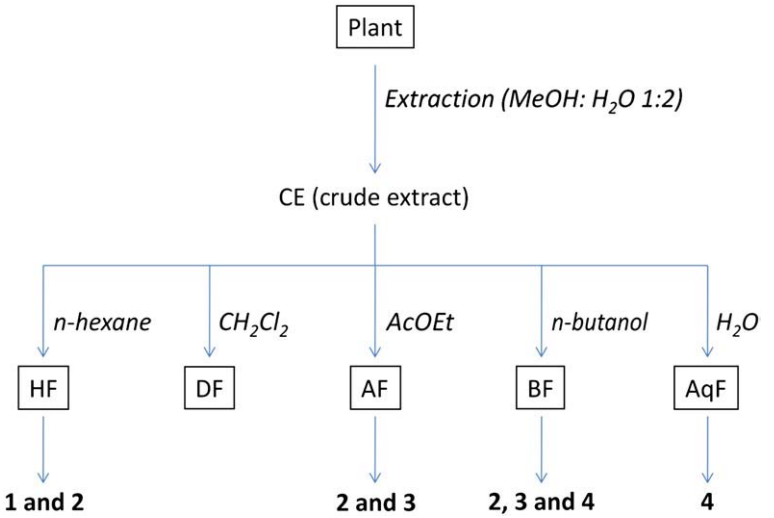
Melting points were determined on an MQAPF-301 Digital (Micro-Química, Florianópolis, Brazil) apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 400 spectrometer at 400.11/100.6 MHz and a Varian 400 NMR spectrometer operating at 400.12/100.61 MHz using $CDCl_3$, CD_3OD or DMSO-d_6 as solvent and TMS as an internal standard. Column chromatography (CC) was carried out on silica gel 60 230–400 mesh (Merck) or reversed-phase C18. Preparative thin-layer chromatography (PTLC) was carried out on silica gel PF_{254} (Merck) in glass blades. Thin-layer chromatography was performed on precoated TLC plates (Merck, silica 60F-254), and visualized by UV light and by spraying with $FeCl_3$ 1% in EtOH and anisaldehyde in H_2SO_4. HPLC was performed using a Waters Alliance 2695 (Waters, Massachusetts, USA) chromatography system with a PDA 995 detector, a reverse-phase Symmetry C18 (4.6×100 mm, 3.5 μm column), and mobile phases of H_2O with trifluoroacetic acid (TFA, 0.045%), and MeCN with TFA (0.036%) at a flow rate of 10 ml min^{-1}. HPLC-MS analysis was carried with a Waters 2998 Photodiode Array Detector; Waters 2695 Separations Module; Waters Micromass ZQ ESCi Multi mode ionization Enabled. Mass spectra were recorded on a MALDI Voyager DE RP time-of-flight (TOF) spectrometer (PE Biosystems, Foster City, CA), using an ACH matrix. Fluorescence was measured using a Bio-Tek FL600 fluorescence plate reader (Bio-Tek Instruments, Vermont, USA). POP was obtained by expression in *E. coli* and affinity purification using a His tail fusion according to a literature procedure [21]. Porcine DPP IV was purchased from Sigma-Aldrich (Deisenhofen, Germany). ZGP-AMC (*N*-benzyloxycarbonyl-Gly-Pro-methylcoumarinyl-7-amide) and GP-AMC (H-Gly-Pro-methylcoumarinyl-7-amide) were obtained from Bachem (Bubendorf, Switzerland).

2.2. Plant material

The material was collected in October 2006 in Santa Maria, state of Rio Grande do Sul, Brazil. *S. racemosa* Pers (Labiatae) was identified by Prof. Dr. Thais Scotti do Canto-Dorow and deposited in the herbarium (no. 11461) of the Universidade Federal de Santa Maria, Santa Maria – RS, Brazil.

2.3. Extraction and isolation

Dried roots and aerial parts of *S. racemosa* Pers (170.25 g) were subjected to exhaustive extraction in Soxhlet apparatus (Scheme 1) with methanol: water (1:2), affording 37.40 g of crude extract (CE). The CE was fractioned with solvent of different degrees of polarity, thereby resulting in *n*-hexane



Scheme 1. Fractionation protocol for the isolation of lupeol (**1**),oroxylin A (**2**), hispidulin (**3**) and oroxyloside (**4**) from *Scutellaria racemosa* Pers.