Fitoterapia 103 (2015) 192–196

Contents lists available at ScienceDirect

Fitoterapia

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

Isoquinoline alkaloids as prolyl oligopeptidase inhibitors

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ARTICLE INFO

Article history:
Received 20 February 2015
Accepted in revised form 2 April 2015
Available online 9 April 2015

Chemical compounds studied in this article:
Argemonine (CID: 442168)
Berberine (PubChem CID: 2353)
Californidine (PubChem CID: 45266443)
Canadine (PubChem CID: 443422)
Corypalmine (PubChem CID: 185605)
Corynoline (PubChem CID: 177014)
Dihydrosanquinarine (PubChem CID: 124069)
N-Methyllaurotetanine (PubChem CID: 16573)

Keywords:
Isoquinoline alkaloids
Prolyl oligopeptidase
Californidine
Dihydrosanquinarine

ABSTRACT

Prolyl oligopeptidase is a cytosolic serine peptidase that hydrolyses proline-containing peptides at the carboxy terminus of proline residues. It has been associated with schizophrenia, bipolar affective disorder, and related neuropsychiatric disorders and therefore may have important clinical implications. Thirty-one isoquinoline alkaloids of various structural types, previously isolated in our laboratory, were screened for their ability to inhibit prolyl oligopeptidase. Promising results have been showed by alkaloids californidine (IC_{50} = 55.6 ± 3.5 μM), dihydrosanquinarine (IC_{50} = 99.1 ± 7.6 μM), corypalmine (IC_{50} = 128.0 ± 10.5 μM) and *N*-methyllaurotetanine (IC_{50} = 135.0 ± 11.7 μM).

1. Introduction

Prolyl oligopeptidase (POP) is a cytosolic serine peptidase that cleaves peptide bonds at the carboxyl end of proline, and is widely distributed in the organs of the body, including the brain [1,2]. Previous studies indicate that POP activity is involved in key physiological functions, such as learning and memory, cell division and differentation, and signaling transduction, as well as in some psychiatric disorders [3]. In recent years, POP has gained importance as a target for the treatment of schizophrenia (SZ), bipolar affective disorder (BD) and cognitive disturbances, such as those present in Alzheimer's disease (AD), mainly due to its involvement in the metabolism of inositol-1,4,5-P_3 (IP_3), which is a key molecule in the transduction cascade of neuropeptide signaling. Neuropeptides induce and increase IP_3 levels by binding to their receptor in the membrane of the endoplasmatic reticulum and inducing the release of Ca^{2+}, which is believed to play a crucial role in learning and memory [4]. POP has also been involved in the processing of neuropeptide precursors [5]. Moreover, neuroprotective and cognition-enhancing effects of POP inhibitors in experimental animals have been reported [3,6].

The use of natural products for medical purposes is gaining international popularity. Medicinal plants are an attractive

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http://dx.doi.org/10.1016/j.fitote.2015.04.004
0367-326X/

source for drug research and development as they produce chemically-varying molecules with a wide range of biological activities. Some natural products are known POP inhibitors in the micromolar range such as the flavonoids baicalin [7] and oroxylin [8], the alkaloid berberine [9], and 6-(8′Z-pentadecenyl)salicylic acid [10].

Alkaloids are without doubt the most potent therapeutic compounds of natural origin and often have significant pharmacological effects on humans and animals [11]. Isoquinoline alkaloids belong to a large group of nitrogenous compounds with over 400 members. Their biological effects include antimalarial, anti-HIV, antitumor, and antimicrobial activities. They are divided into several different classes including aporphines, protopines, protoberberines, phthalideisoquinolines, benzophenantheidines, benzylisoquinolines, morphinans and spirobenzylisoquinolines [12]. Some of these compounds possess promising biological/pharmacological properties for the treatment of important diseases including cancer, AD, and microbial infections. Aporphines, benzylisoquinolines and protoberberines showed a higher cytotoxicity than other structural types of isoquinoline alkaloids. A typical example is berberine with a protoberberine skeleton that showed a remarkable cytotoxicity on a wide range of cancer cell lines (e.g. A549, SK-OV-3, SK-MEL-2 and others) [12]. As mentioned above, it has also been reported to be a potent inhibitor of POP [9], but other isoquinoline alkaloids have not been tested until now. On the other hand, a wider range of Amaryllidaceae alkaloids has been tested for POP inhibition activity so far. Important results have been shown by 9-*O*-demethylgalanthin (IC_{50} = 150 ± 20 μM) [13] and by further Amaryllidaceae alkaloids narwedine and incartine isolated from *Narcissus poeticus* cv. Brackenhurst and *N. jonquila* var. *henriquesii*, respectively [14].

The aim of this study was to elucidate the possible POP inhibitory effect of diverse types of isoquinoline alkaloids isolated in our laboratory from plant sources.

2. Results and discussion

In the current study, 31 isoquinoline alkaloids of six structural types: aporphine, benzophenanthridine, benzylisoquinoline, pavinane, protoberberine and protopine, previously isolated in our laboratory, were screened for their ability to inhibit prolyl oligopeptidase (Fig. 1).

Five of the tested compounds showed inhibition activity either stronger than or comparable with berberine, which has been used as one of the standards (Table 1). The most interesting inhibition activity has been demonstrated by the quaternary pavinane alkaloid californidine with an IC_{50} value of 55.6 ± 3.5 μM, isolated from *Eschscholtzia californica*. It seems that quaternary nitrogen could play an important role in the POP inhibition activity. It is known that compounds with quaternary nitrogen are also active AChE inhibitors, such as sanquinarine, chelerythrine [15], berberine [16] and californidine [17]. The quaternary nitrogen plays an important role in binding with the enzyme [18]. However, it is reported that these compounds might have problems with crossing the blood–brain barrier (BBB) [19], on the other hand, there is a general view that permeability of the BBB is increased in AD and also that quaternary compounds could cross the BBB [20]. For example, the protoberberine quaternary alkaloid berberine passes through the BBB in rats and is quickly distributed to the thalamus [21]. AD is the most predominant cause of dementia in the elderly, affecting more than 20 million people worldwide and it is estimated that this figure will increase to 114 million by 2040 [22]. Currently, acetylcholinesterase inhibition is the most used therapeutic treatment for the symptoms of AD [20]. In recent studies, some POP inhibitors have been found to be efficacious antidementia drugs [23]. Thus, POP inhibition can represent an important supporting approach in AD treatment, and, therefore, the search for new compounds influencing more therapeutic targets connected with AD is required.

Recent evidence also pointed to the involvement of POP in cancer and tumor growth. The pattern of POP activity was studied in a large series of human neoplastic tissues. The increased POP activity in Clear Cell Renal Cell Carcinoma (CCRCC), Urothelial Carcinoma of the Renal Pelvis (UCRP), Head and Neck Squamous Cell Carcinoma (HNSCC) and colorectal adenomatous polyp suggest that this enzyme could be involved also in these malignancies [24].

Further important POP inhibitions have also been shown by the benzophenanthridine alkaloid dihydrosanquinarine (IC_{50} value 99.1 ± 7.6 μM; Fig. 2) isolated from *Macleaya cordata* [25], two protoberberines corypalmine (IC_{50} value 128.0 ± 10.5 μM; Fig. 2) and canadine (IC_{50} value 152.0 ± 12.5 μM) from *Corydalis cava* [26], and finally by the aporphine type alkaloid *N*-methyllaurotetanine (IC_{50} value 135.0 ± 11.7 μM) from *E. californica* [17]. All these activities are comparable with those reported and our POP inhibition activity of berberine (IC_{50} = 145 μM; Table 1) [9].

In conclusion, although the mechanism of the POP inhibition of the tested compounds has not been elucidated, the findings of this study indicate the potential of isoquinoline alkaloids as POP inhibitors. Although these inhibitors are not excellent concerning the inhibitory potency compared to known inhibitors (e.g. Z-prolyl-prolinal), these compounds are of interest since they can be used as leads in the development of new potent therapeutic drugs to treat neuropsychiatric disorders. The mechanism of the POP inhibition and prepared semi-synthetic derivatives of the reported active compounds will be studied in our future experiments.

3. Material and methods

3.1. Isoquinoline alkaloids

(−)-Californidine iodide, (−)-eschscholtzine, (−)-caryachine, (−)-argemonine, (−)-*O*-methylcaryachine, (−)-O-methylneocaryachine, (+)-reticuline, (+)-salutaridine and (+)-*N*-methyllaurotetanine were isolated from aerial parts and roots of *E. californica* [17]. Protopine, allocryptopine, (+)-chelidonine, (+)-homochelidonine and (−)-stylopine were isolated from aerial parts and roots of *Chelidonium majus* [15]. Cryptopine, (+)-bulbocapnine, (+)-canadine, (+)-canadaline, (−)-corycavamine, (±)-corycavidine, (+)-corynoline, (+)-corydine, (−)-corypalmine, (+)-isocorydine, (−)-isocorypalmine, (−)-sinoacutine, (+)-tetrahydropalmatine, (−)-scoulerine and (+)-corydaline were isolated from tubers of *C. cava* [26]. Dihydrosanguinarine was isolated from a commercial extract of *M. cordata* [25]. (−)-Platycerine was isolated from aerial parts of *Argemone platyceras*.