Fitoterapia 130 (2018) 251–258

Contents lists available at ScienceDirect

Fitoterapia

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

New diterpenes from *Salvia pseudorosmarinus* and their activity as inhibitors of monoacylglycerol lipase (MAGL)

Marinella De Leo[a,d], Conny Guillen Huallpa[a], Britt Alvarado[b], Carlotta Granchi[a], Giulio Poli[a], Nunziatina De Tommasi[c,*], Alessandra Braca[a,d]

[a] Dipartimento di Farmacia, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy
[b] Facultad de Farmacia y Bioquímica, Universidad Nacional Mayor de San Marcos, CIRNA, NATURE Jardín Botánico, Jr. Puno, 1002 Lima, Peru
[c] Dipartimento di Farmacia, Università di Salerno, Via Giovanni Paolo II, 84084 Fisciano, SA, Italy
[d] Centro Interdipartimentale di Ricerca "Nutraceutica e Alimentazione per la Salute", Università di Pisa, via del Borghetto 80, 56124 Pisa, Italy

ARTICLE INFO

Keywords:
Salvia pseudorosmarinus
Lamiaceae
Clerodane diterpenes
Cancer
Monoacylglycerol lipase
Lactate dehydrogenase

ABSTRACT

As a part of our ongoing research program on compounds from higher plants with lactate dehydrogenase (LDH) and monoacylglycerol lipase (MAGL) inhibitory activities, three new neoclerodane diterpene 12-deacetylsplendidin C (**1**), pseudorosmaricin (**2**), and 2-dehydroxysalvileucanthsin A (**3**) along with six known compounds were isolated from *Salvia pseudorosmarinus* aerial part extracts. Their structures were determined by spectroscopic and spectrometric techniques including 1D- and 2D NMR, and MS analyses. The isolated diterpenes were assayed for their inhibitory activity on LDH5 and MAGL, two enzymes covering key roles in the peculiar energetic metabolism of malignant tumours. All the assayed diterpenes showed negligible activity on LDH5, whereas the known jewenol A (**4**) displayed a moderate inhibition activity on MAGL, showing an IC_{50} value of 46.8μM and it proved to be a reversible MAGL inhibitor. Docking and molecular dynamic simulation studies where thus performed to evaluate the binding mode of **4** within MAGL.

1. Introduction

It is widely known that most aggressive tumours are characterized by a dysregulated energetic metabolism which affects both glucose and lipid metabolism. Tumour cells are characterized by an addiction to glucose and a marked preferential use of the glycolytic pathway to produce energy even in the presence of abundant oxygen, despite the lower efficiency of glycolysis in the production of ATP from glucose compared to the oxidative phosphorylation. This metabolic shift (Warburg effect) results in an increased rate of glycolysis to compensate the moderate energetic production: the high glucose uptake and the augmented lactate production furnish an adequate energetic refueling to rapidly growing tumour cells [1]. An aberrant lipid metabolism is frequently observed in cancer cells, and many evidences highlight the important role played by lipid metabolism in the development of cancer. The *de novo* fatty acid synthesis is significantly increased in cancer to the detriment of the use of exogenous dietary fatty acids, since the huge amount of internalized glucose is also utilized to synthesize fatty acids by providing building blocks, such as acetyl-CoA. Many of the produced fatty acids are immediately used or they are stored in the cells and they are used on demand, thanks to the enzymatic activity of lipases. Lipids are necessary for the synthesis of membranes of growing tumour cells and for the production of oncogenic signaling molecules [2].

In this context, many proteins are upregulated in tumours, such as enzymes, receptors and effectors of the involved deregulated metabolic pathways. For example, the last enzyme of the glycolytic cascade, the isoform 5 of lactate dehydrogenase (LDH5) plays a pivotal role in the maintenance and progression of tumours. LDH5 is a homotetrameric enzyme composed of four A subunits and it is localized in the cytoplasm. LDH5 catalyzes the conversion of pyruvate, the end product of glycolysis, to lactate, with the concomitant oxidation of the cofactor NADH to NAD^+. In this way, lactate is excreted out of the cells, and the acidic extracellular environment facilitates the spread of metastasis, and the regenerated NAD^+ allows the glycolytic pathway to continue to produce energy for tumour cells [3]. In the lipid metabolism, the serine hydrolase monacylglycerol lipase (MAGL) is the enzyme responsible for the hydrolysis of monoacyglycerols to free fatty acids and glycerol in peripheral tissues and it is also the enzyme designated to the catabolism of the endocannabinoid 2-arachidonylglcyerol in the central nervous system. The increased release of stored fatty acids in tumours generated by the intensified MAGL activity is necessary to support cancer growth

* Corresponding author.
E-mail address: detommasi@unisa.it (N. De Tommasi).

https://doi.org/10.1016/j.fitote.2018.09.010
Received 5 July 2018; Received in revised form 11 September 2018; Accepted 17 September 2018
Available online 18 September 2018
0367-326X/

and the synthesis of lipid messengers, triggering cell migration and aggressiveness [4].

The metabolic reprogramming occurring in most tumours is a therapeutic opportunity: both LDH5 and MAGL represent two attractive anti-cancer targets, since it was demonstrated that their inhibition led to antiproliferative effects *in vitro* and/or *in vivo* [5,6]. Moreover, the inhibition of LDH5 or MAGL can be considered a harmless therapeutic strategy since healthy cells are unaffected, because they do not completely rely on the dysregulated energetic metabolic pathways for their survival. In the last decades, many synthetic and natural compounds showing inhibitory activities on LDH5 [7,8] or MAGL [9,10] were discovered, and there is a great interest for the discovery of new inhibitors of these two enzymes, highlighting that cancer metabolism is an attractive area of investigation. In addition, the interest in the identification of novel MAGL inhibitors is also due to their potential use in the treatment of other pathologies such as chronic and neuropathic pain [11].

The genus *Salvia* (Lamiaceae) consist of over 900 species widely distributed in different regions around the world such as the Mediterranean area, Central Asia, Africa, and America. Some members of this genus have economic importance for their use as flavoring agents in perfumery and cosmetics but are also interesting for their biologically active constituents, particularly diterpenes and phenolics, many of which showed antitumor properties [12]. Moreover, diterpenoids and phenolic derivatives isolated from different *Salvia* species showed antioxidant, anticoagulant, antihypertensive, anti-fibrotic, anti-ischemia-reperfusion injury, and antiviral activities [13]. Although the interest in the genus has increased, only a relatively small number of the known species have been chemically and biologically explored. In the course of our ongoing research program on compounds from higher plants with LDH5 and MAGL inhibitory activity, *Salvia pseudorosmarinus* Epling was selected as the subject of this investigation. The plant is a perennial shrub up to 150 cm high with purple flowers growing in Peruvian Ande at 3500–4000 m above sea level, where the leaves are used in folk traditional medicine against stomach ache and diarrhea [14]. To the best of our knowledge, this species has been never investigated. In this paper, we describe the isolation and structural characterization of three new neoclerodanes (**1–3**) (Fig. 1), together with six known compounds from *S. pseudorosmarinus* aerial parts, and LDH5 and MAGL inhibitory activity of the isolated diterpenes.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on an Atago AP-300 digital polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. NMR experiments were performed on a Bruker DRX-600 spectrometer (Bruker BioSpin GmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI CryoProbe at 300 K. All 2D NMR spectra were acquired in methanol-d_4 (99.95%, Sigma-Aldrich), and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra. ESI-MS were obtained using a Finnigan LC-Q Advantage Termoquest spectrometer, equipped with Xcalibur software. HRESIMS were acquired in the positive ion mode on a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific). TLC were performed on precoated Kieselgel 60 F_{254} plates (Merck); compounds were detected by spraying with $Ce(SO_4)_2/H_2SO_4$ solution. Column chromatographies (CC) were performed over Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden) and Isolera® Biotage® purification system (flash Silica gel 60 SNAP 340 g cartridge, flow rate 100 mL/min); reversed-phase (RP) HPLC separations were conducted on a Shimadzu LC-20AT series pumping system equipped with a Shimadzu RID10A refractive index detector and a Shimadzu injector, using a C_{18}μ-Bondapak column (30 cm × 7.8 mm, 10 μm, Waters-Milford) and a mobile phase consisting of MeOH-H_2O mixtures at a flow rate of 2 mL/min.

2.2. Plant material

Aerial parts of *S. pseudorosmarinus* were collected near Cotaparaco, Ancash Province, Peru, in November 2016. The plant was identified by Hamilton Beltram of the Universidad Nacional Mayor de San Marcos, Lima, Peru where a voucher specimen (142-USM-2016) was deposited.

2.3. Extraction and isolation

The dried aerial parts of *S. pseudorosmarinus* (1 kg) were exhaustively extracted with solvents of increasing polarity: *n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1), and MeOH to give 12.8, 41.3, 12.2, and 65.9 g of the respective residues. Part of the $CHCl_3$ residue (5.0 g) was subjected to Isolera Biotage column chromatography (340 g silica SNAP cartridge, flow rate 100 mL/min), eluting with $CHCl_3$ followed by increasing concentrations of MeOH in $CHCl_3$ (between 1% and 100%). Fractions of 27 mL were collected, analyzed by TLC and grouped into six major fractions (A-F). Fraction C contained oleanolic acid. Fractions B (872 mg) and D (500 mg) were subjected to RP-HPLC with MeOH-H_2O (4.5:5.5) to give compound **2** (2.0 mg, t_R = 9 min) from fraction B; compound **3** (1.3 mg, t_R = 17 min), from fraction D. Fraction F (646 mg) was purified by RP-HPLC with MeOH-H_2O (1:1) to give compounds **1** (3.1 mg, t_R = 12 min) and **4** (10 mg, t_R = 15 min). Part of the $CHCl_3$-MeOH residue (10.0 g) was submitted to Sephadex LH-20 column chromatography (5 × 75 cm, flow rate 1.5 mL/min) using MeOH as eluent and collecting five major fractions (A-E) grouped by TLC. Fraction C (137 mg) was chromatographed over RP-HPLC with MeOH-H_2O (3.5:6.5) to give protocatechualdehyde (1.5 mg, t_R = 8 min) and caffeic acid methyl ester (1.6 mg, t_R = 12 min). Fraction E (140 mg) was subjected to RP-HPLC with MeOH-H_2O (1:1) to yield eriodictyol (1.7 mg, t_R = 10 min) and pedalitin (2.5 mg, t_R = 16 min). All the compounds met the criteria of ≥94% purity, as inferred by HPLC and NMR analyses.

2.3.1. 12-deacetylsplendidin C (1)

Pale yellow amorphous powder; $[\alpha]_D^{25}$-16.3 (*c* 0.1, MeOH); ^1H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; ESI-MS *m/z* 529 $[M + Na]^+$, 369 $[M + Na - 160]^+$; HR-ESIMS *m/z* 529.2045 $[M + Na]^+$ (calcd for $C_{26}H_{34}O_{10}Na$ 529.2050).

2.3.2. Pseudorosmaricin (2)

Pale yellow amorphous powder; $[\alpha]_D^{25}$ +44.6 (*c* 0.1, MeOH); ^1H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; ESI-MS *m/z* 423 $[M - H]^-$, 259 $[M - H - 164]^-$; HR-ESIMS *m/z* 425.2164 $[M + H]^+$ (calcd for $C_{22}H_{33}O_8$ 425.2175).

2.3.3. 2-Dehydroxysalvileucanthsin A (3)

Pale yellow amorphous powder; $[\alpha]_D^{25}$ +16.2 (*c* 0.1, MeOH); ^1H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; HR-ESIMS *m/z* 357.1339 $[M + H]^+$ (calcd for $C_{20}H_{21}O_6$ 357.1338).

2.4. Enzymatic assays

Compounds **1–4** were tested on purified human LDH5 (Lee Biosolution Inc.) and MAGL (Cayman Chemical), as previously reported [15,16]. Compound (4-(4-chlorobenzoyl)piperidin-1-yl)(4-methoxyphenyl)-methanone, named CL6a, is the reference MAGL inhibitor previously synthesized in our laboratory (purity percentage 99% by HPLC analysis). DMSO stock solutions of the compounds (the concentration of DMSO did not exceed 4% during the measurements) were diluted to obtain seven different concentrations (from 400 to 0.064 μM, in duplicate for each concentration), that were dispensed in 96-well plates to generate the concentration-response curves. Maximum and minimum controls were present in each plate. Both the assays were run