Industrial Crops and Products 85 (2016) 287–299

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Regiospecific synthesis, anti-inflammatory and anticancer evaluation of novel 3,5-disubstituted isoxazoles from the natural maslinic and oleanolic acids

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ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form 19 January 2016
Accepted 10 March 2016
Available online 21 March 2016

Keywords:
Maslinic acid
Oleanolic acid
Isoxazoles
Cu-catalyzed
Anti-inflammatory
Anti-proliferative

ABSTRACT

Maslinic acid (**1**) and oleanolic acid (**2**) were isolated from *Olea europaea* L. under ultra-sonication conditions with large amounts (8.5 and 3.4 mg/g DW, respectively). Copper-catalyzed microwave-assisted 1,3-dipolar cycloaddition reactions between natural pentacyclic triterpenoid-alkyne derivatives and a series of aryl nitrile oxides, regiospecifically afforded 3,5-disubstituted isoxazoles in quantitative yields. The reaction times were shorter than those reported in the literature. Most of the compounds were evaluated for their anti-inflammatory and anti-proliferative activities. Compounds **12c**, **12e**, **12g**, **13c** and **13d** found to have higher anti-inflammatory activity. The anti-proliferative evaluation towards EMT-6 and SW480 cancer cell lines indicated that most of the compounds exhibited significant anti-cancer activity. Among all triterpenic derivatives, isoxazole **12g** bearing a furfuryl ring in its isoxazole moiety was found to have highest anti-inflammatory and anti-cancer activity. Therefore, this derivative can serve as a promising lead candidate for further study.

1. Introduction

Maslinic acid (2α,3β-dihydroxyolean-12-en-28-oic acid) **1** has been reported to possess several biological activities, such as antitumor (Taniguchi et al., 2002) antioxidant (Montilla et al., 2003), antiviral (anti-HIV) (Xu et al., 1996) and anti-inflammatory (Sheng and Sun, 2011) activities. In this sense, recently some studies have demonstrated the role of triterpenoid **1** as a glycogen phosphorylase inhibitor (Wen et al., 2005, 2006). Also, this compound inhibits the increase in plasma glucose induced in diabetic mice by adrenaline (Wen et al., 2005). Moreover, oleanolic acid (3β-hydroxyolean-12-en-28-oic acid) **2** is one of the most popular compounds of pentacyclic oleananes which exhibit anticancer (Srivastava et al., 2010), gastroprotective (Sánchez et al., 2006), antibacterial (Fontanay et al., 2008), antidiabetic (Chen et al., 2006), hepatoprotective (Yim et al., 2001) and many other important activities. Thus, an interesting report from Hichri et al. (2003) suggests that an acyl motif incorporated at C-28 position of oleanolic acid was a useful structural modification to improve its antibacterial inhibition against two Gram-positive and two Gram-negative bacteria. Recently, we studied in our laboratory the anti-acetylcholinesterase evaluation on new series of oleanolic acid derivatives designed to contact the peripheral sites of AChE. These molecules have been shown to be potent inhibitors of acetylcholinesterase (AChE) and the greatest acetylcholinesterase inhibitory activity was exhibited by the oleanolic acid. Among the compounds tested, those having sulfur and chlorine atoms were found to be antibacterial (Chouaïb et al., 2015).

On the other hand, isoxazoles have attracted an increasing research interest and potential pharmacophores endowed with anticancer (Kumbhare et al., 2012), neuroprotective (Koufaki et al., 2011), anti-obesity (Jadhav et al., 2012), antidepressant (Yu et al., 2012), insecticidal (Silva-Alves et al., 2013), antidiabetic (Konya et al., 2012) and anti-inflammatory (Kankala et al., 2013) activities.

Generally, in designing new bioactive agents for various pharmaceutical areas, besides the development of completely new agents, there is another approach involving the synthesis of hybrid molecules. Indeed, hybrid constructs from the entities of known

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http://dx.doi.org/10.1016/j.indcrop.2016.03.024
0926-6690/

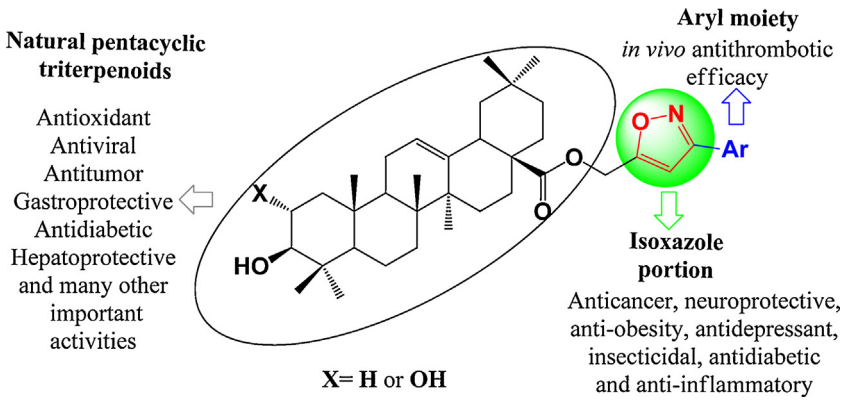

Fig. 1. Intrinsic biological properties of the building blocks.

biological activity could be another important source for molecular diversity. This is a very promising approach in the development of leads for medicinal chemistry applications, which benefits from the intrinsic activity of all or part of the components of the hybrid (Tietze et al., 2003). As part of our interest in the application of 'click' reactions to biologically interesting molecules, we envisaged that the 1,3-dipolar cycloaddition could be useful for the conjugation of arylnitrile oxides to of natural pentacyclic triterpenoids-alkynes derivatives resulting in the isoxazole compounds through methyl ester linker bearing several points of diversity as shown in Fig. 1.

The development of new methods for the synthesis of five membered heterocyclic compounds is an ever-expanding area in medicinal chemistry. Specifically, those containing the isoxazole ring have been widely used as key building blocks for drugs.

The major synthetic strategies to construct this heterocycle are either the condensation of a 1,3-dicarbonyl compound with hydroxylamine or intramolecular cyclization of amino acids (Kondrat'eva et al., 1987). These procedures have often exhibited quite low yields, side reactions result in impurities, and both regioisomers are often obtained. In particular, nitrile oxides which undergo efficient [3 + 2] cycloaddition with terminal alkynes can be a convenient protocol to the synthesis of isoxazoles. However, a survey of the literature showed that 1,3-dipolar cycloadditions reaction between dipolarophiles derived from activated alkynes and nitrile oxides generally resulted in moderate yields and low regioselectivity. Further, the use of catalysts in 1,3-dipolar cycloaddition of nitrile oxides and alkynes permitted significant improvements, especially concerning yields and regioselectivity (Himo et al., 2005). Indeed, several groups (Heaney, 2012; McIntosh et al., 2012; Dadiboyena and Nefzi, 2012; Kumar et al., 2012) have accessed to isoxasoles through a metal-free cycloaddition of alkynes with nitrile oxides, usually in modest yields or long reaction times. Use of organocatalysts (Tietze et al., 2003) or hypervalent iodine (Yoshimura et al., 2013) reagents improved the yield and the regioselectivity of the reaction. In general, the 3,5-regioisomer was favored under Cu-catalyzed conditions (Bharate et al., 2013; Hansen et al., 2005).

In order to use maslinic acid **1** and oleanolic acid **2** (Fig. 2) as starting materials in such 1,3-dipolar cycloaddition reactions to access to new isoxazole derivatives, we have isolated them from pomace olive (*Olea europaea* L.) *cultivar*: Chemlali, under ultrasonication conditions with high quantities (17 g (8.5 mg/g DW) and 6.8 g (3.4 mg/g DW) respectively) (Chouaïb et al., 2015).

On the other hand, we have prepared pentacyclic triterpenoid-alkynes derivatives to be used as dipolarophiles for the synthesis of 3,5-disubstituted isoxazoles via 1,3-dipolar cycloaddition using various aromatic hydroximyl chlorides. A regiospecific, simple and versatile copper(I)-catalyzed, microwave-assisted procedure for preparation of a series of 3,5-disubstituted isoxazoles was developed. The anti-inflammatory and anti-proliferative activities of most of the prepared compounds were evaluated and discussed.

2. Experimental

2.1. General experimental procedures

Solvents were purified and dried using standard methods. Melting points were determined on a Büchi 510 apparatus using capillary tubes. Commercial TLC plates (Silica gel 60, F254, sds) were used to monitor the progress of the reaction. Column chromatography was performed with silica gel 60 (particle size 40–63 μm, sds). HRMS spectra were acquired with an ESI-TOF spectrometer (LCT Premier XE, Waters) using the reflectron mode. Leucine-enkephaline peptide was employed as a lock mass for the LockSpray. ^{1}H (300 MHz, 16–32 scans) and BB-decoupled ^{13}C (75 MHz, 256–2048 scans) NMR spectra were recorded at room temperature (rt) on a Bruker AM-300 Fourier Transform spectrometer equipped with a 10 mm probe in deuterated chloroform, acetone and pyridine with all chemical shifts (δ), reported in ppm, refereed to residual non deuterated solvent. Coupling constants were measured in Hz. and signals are using the following abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet, etc.

2.2. Chemistry

2.2.1. General procedure for the synthesis of maslinic acid-alkyne derivatives ***4–8***

To a solution of **1** (3 g, 6.3 mmol) in dry DMF, sodium hydride (302 mg, 12.6 mmol) and propargyl bromide (18.9 mmol) were added and the reaction mixture was stirred at room temperature for 2 h. Reaction was monitored by TLC. After reaction completion the residue was diluted with water (300 mL). The mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel and eluted with ethyl acetate:petroleum ether (2:8 then 3:7) to obtain the alkyl derivatives **4–8** in 44, 5, 15, 11 and 7% yield respectively.

*2.2.1.1. Propargyl-(2α,3β)-2,3-dihydroxyolean-12-en-28-oate (**4**).* White solid; mp: 297–299 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.23 (1H, t, *J* = 3.6 Hz), 4.61 (1H, dd, *J* = 15.6; 2.4 Hz), 4.50 (1H, dd, *J* = 15.6; 2.7 Hz), 3.60 (1H, td, *J* = 9.3; 2.7 Hz), 2.93 (1H, d, *J* = 9.6 Hz), 2.80 (1H, dd, *J* = 13.8; 3.9 Hz), 2.34 (1H, t, *J* = 2.7 Hz), 1.99 (4H, m), 1.93–1.83 (4H, m), 1.62–1.46 (6H, m), 1.45–1.21 (6H, m), 1.06 (3H, s), 0.96 (3H, s), 0.91 (3H, s), 0.86 (3H, s), 0.83 (3H, s), 0.75 (3H, s), 0.67 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.3, 142.9, 121.9, 83.4, 77.6,