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Semi-synthetic studies of α-onocerin derivatives for cytotoxicity

Pornkanok Pongpamorn[a], Sakuna Wan-erlor[b], Somsak Ruchirawat[a,b,c], Nopporn Thasana[a,b,c,*]

[a] Chemical Biology Program, Chulabhorn Graduate Institute, Laksi, Bangkok 10210, Thailand
[b] Laboratory of Medicinal Chemistry, Chulabhorn Research Institute, Laksi, Bangkok 10210, Thailand
[c] Center of Excellence on Environmental Health and Toxicology, Commission on Higher Education, Ministry of Education, Bangkok, Thailand

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ABSTRACT

Up to 0.064% of α-onocerin (**1**) was isolated from *Lycopodium clavatum*. Twenty-one of its derivatives, 18 being new, were semi-synthesized through acylation, reduction, oxidation, and various other reactions. Their molecular structures were confirmed by means of NMR spectroscopy and mass spectrometry. The derivatives were evaluated for their inhibitory activities against acetylcholinesterase (AChE) and four cancer cell lines: HuCCA-1 (human cholangiocarcinoma), A-549 (lung carcinoma), HepG2 (hepatocarcinoma), and MOLT-3 (acute lymphoblastic leukemia). Introduction of a hydroxyl group at C-2/C-20 on α-onocerin (**1**) enhanced the cytotoxic activity. Most notably, the α-onocerin oxime derivative (**22**) selectively and significantly exerted cytotoxic activity against only the HepG2 cancer cell line.

1. Introduction

The compound α-onocerin (**1**) is a triterpenoid originally isolated from the *Ononis spinosa* (Hlasiwetz, 1855), and subsequently from several species of *Lycopodium* plants, including *L. clavatum* (Ageta et al., 1962; Trofimova et al., 1996), *L. obscurum* (Cai et al., 1989), and *L. japonicum* (Yan et al., 2005). Acetylcholinesterase (AChE) inhibitors are indicated for the treatment of Alzheimer's disease (Galimberti and Scarpini, 2016), and α-onocerin possessed AChE inhibitory properties (Yan et al., 2005). The IC_{50} value of **1** for *Ee*AChE (*Electrophorus electricus* AChE) was determined to be 5.2 μM (Orhan et al., 2003). Serratene-triterpenoids are biogenetically derived (Zhang et al., 2014) from α-onocerin (**1**), and some of them, such as lycoclavanol (Fig. 1) also exhibited AChE inhibitory properties (Nguyen et al., 2015; Yan et al., 2005). There have been no reports on the chemopreventive activity of α-onocerin; only its natural product derivatives and some of the serratene-triterpenoids manifested chemotherapeutic properties (Ham et al., 2012; Wittayalai et al., 2012; Yan et al., 2005, 2012; Zhang et al., 2014). In this study, various moieties on α-onocerin (**1**) were modified to obtain 21 derivatives, comprising of 3 known and 18 new derivatives. They were evaluated for their biological activities to develop subsequently a preliminary Structure Activity Relationship (SAR) profile.

2. Results and discussion

2.1. Chemistry of α-onocerin derivatives

Following the isolation of α-onocerin from the club moss *Lycopodium clavatum* in large quantity, chemical modifications were performed on and around its two exocyclic double bonds and hydroxyl groups. Preparation methodologies for the 11 acylated derivatives of α-onocerin (**1**) are illustrated in Scheme 1.

Acetylation of the hydroxyl groups and isomerization of the exocyclic double bonds occurred in a one-pot reaction when α-onocerin was stirred under acetic acid and 65% nitric acid for 5 days in room temperature to afford the derivative **2** (Condition a, Scheme 1).

Ten *O*-acylated derivatives (**3–12**), both linear (**3–9**) and benzoyl products (**10–12**), were semi-synthesized in moderate to good yields by acylation (Soldi et al., 2008) of the hydroxyl groups on α-onocerin. Derivatives **3** (80%), **6** (79%), **7** (84%), and **8** (50%) were prepared using the appropriate acid anhydrides in the presence of 4-dimethylaminopyridine (Condition b, Scheme 1). The spectroscopic data of derivative **3** were in accordance with the compound previously described by (Cai et al., 1989). Appropriate acid chlorides were used for the semi-synthesis of derivatives **4** (30%), **5** (73%), **9** (50%), **10** (48%), **11** (86%), and **12** (84%) (Condition b, Scheme 1). A deshielding effect at

* Corresponding author at: Chemical Biology Program, Chulabhorn Graduate Institute, and Laboratory of Medicinal Chemistry, Chulabhorn Research Institute, Laksi, Bangkok 10210, Thailand.
E-mail address: nopporn@cri.or.th (N. Thasana).

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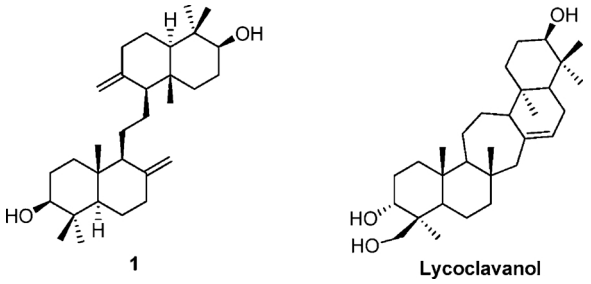


Fig. 1. Structures of α-onocerin (**1**) and lycoclavanol.

H-3/H-21 was observed in the ^{1}H NMR spectra for all of the esterification products.

Hydrogenation, ozonolysis (Biedermann et al., 2010), and oxidation (Dess and Martin, 1991) reactions were also implemented to assess the importance of exocyclic double bonds and hydroxyl groups of **1** (Scheme 2). Hydrogenation of **1** under Pd/C, in the solvent EtOAc, at room temperature gave compound **13** at 77% yield as an inseparable isomeric mixture. Ozonolysis of the exocyclic double bonds of **1**, similarly to the work of (Cai et al., 1989), generated the diketone **14** in moderate yield (62% yield). Oxidation of the two hydroxy groups of **1** using DMP, in dichloromethane, afforded diketone **15** in a very good yield (88% yield). The structure of **15** was consistent with the compound previously reported by (Barton and Overton, 1955).

The α-hydroxy ketone derivative **18** was accomplished over three steps beginning with derivative **15** (Scheme 3). Treatment of **15** with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf) and triethylamine (Et_3N) gave the silyl enol ether **16** (Condition a, Scheme 3). Rubottom oxidation (Abad et al., 2007) was performed to attain **18** from **16**, after hydrolysis (Marcos et al., 2005) of the TBDMS group on **17** by using 1.0 M tetrabutylammonium fluoride (TBAF) solution in tetrahydrofuran (THF) (Scheme 3). The C-25/C-28 methyl of **16** pointing in the β-face forced epoxidation to occur on the more accessible α-face of the olefin. Increasing the equivalent of TBAF and extending the duration of reaction furnished diosphenol derivative **19** instead (Scheme 3), which could have arisen from the basic environment (Wen et al., 2008). The H-1/H-19 at δ6.33 ppm is a characteristic peak in an α,β-unsaturated ketone system of **19**. The anisotropy effect, from the carbonyl group at C-3/C-21, caused the sharp singlet peak (δ6.03 ppm) of the hydroxyl group's proton to be deshielded.

Diosphenol **19** underwent reduction (Sommerwerk et al., 2015; Wen et al., 2008) using sodium borohydride ($NaBH_4$). The hydride entered at the less hindered α-face, which forced the hydroxyl groups at C-2/C-20 and C-3/C21 to orient in the β-face, obtaining enantiomerically pure diol **20** (Scheme 3). The NOESY cross-peaks, observed between H-2/H-20 and H-3/H21 (δ4.13 and δ3.23 ppm) with the H-5/H-17 at δ1.15 ppm (Fig. S77), confirmed the stereochemistry of both hydroxyl groups to be β-oriented. Derivative **20** demonstrated cytotoxic activity (Table S1).

Alpha-hydroxylation (Urban et al., 2004) to acquire **20** was also achieved over two steps (Scheme 4). Oxygen gas was introduced into a solution of **15** in *tert*-butanol (*t*-BuOH), with the presence of potassium *tert*-butoxide, for 66 h at room temperature. After purification using column chromatography (SiO_2), an inseparable mixture of compound **19** and some other side products was obtained. Subsequent treatment of this mixture with $NaBH_4$ provided pure diol **20** at 12% yield (Scheme 4).

When the solvent was changed to tetrahydrofuran (THF) and the reaction time was reduced, derivative **21** resulted instead in a yield of 27% (Scheme 4). Formation of lactol **21** was based on the mechanism of formation of another related compound (Alvarez et al., 1986), which involved an elimination reaction of C-2/C-20. A NOESY correlation observed between H-1/H19 (δ5.46 ppm) and H_3-24/H_3-30 (δ1.18 ppm) implied the hydroxyl group at C-1/C-19 oriented in the α-face (Fig. S82). Derivative **15** was converted to oxime **22**, through a reaction with hydroxylamine hydrochloride (Scheme 4) (Heller et al., 2015).

2.2. Cytotoxic activities of α-onocerin (1) and its derivatives

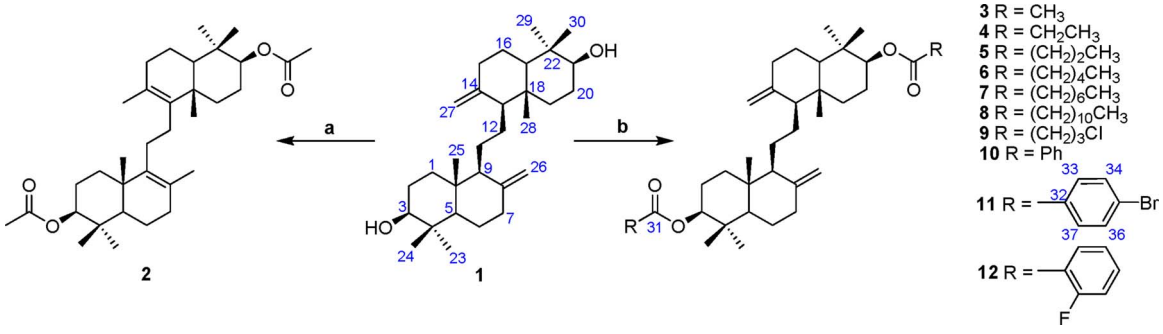
The cytotoxic activities of the compounds against the four cancer cell lines, HuCCA-1, A-549, HepG2, and MOLT-3, were demonstrated in Table S1. α-Onocerin (**1**) and its derivative **2** did not possess any inhibitory activities against any cancer cell lines.

In the case of the ester derivatives, masking their hydroxyl groups proved not to be beneficial in inhibiting cancer cells; except for derivative **9**, which had very weak and modest inhibition against A549 (lung carcinoma) IC_{50} = 46.58 ± 0.82 μg/mL and MOLT-3 (acute lymphoblastic leukemia) IC_{50} = 7.01 ± 0.62 μg/mL, respectively. Derivative **11** which weakly inhibited HuCCA-1 (human cholangiocarcinoma) cells with IC_{50} = 22.95 ± 3.78 μg/mL.

Derivative **14** had weak activity against MOLT-3 cells, with an IC_{50} value of 26.47 ± 10.05 μg/mL, indicating that polar groups or hydrogen bond acceptors in the area of C-8/C-14 are favorable for MOLT-3 inhibition.

Derivative **15** did not inhibit MOLT-3 cells or other cancer cells at 50 μg/mL. Nevertheless, derivatives **18**, **19**, and **21** inhibited MOLT-3 cells with IC_{50} values of 11.84 ± 1.72, 15.12 ± 0.84, 18.19 ± 0.50 μg/mL. This meant that the carbonyl groups at C-3/C-21 did not contribute to the inhibitory activity, but instead were the hydroxyl groups at C-2/C-20 or C-1/C-19. This was further demonstrated in derivative **20**, which had cytotoxic activity against MOLT-3 cells plus the other three cancer cell lines.

Lastly, oxime **22** showed inhibitory effect specifically towards HepG2 cancer cells; having an IC_{50} that was the lowest among all



Scheme 1. Synthesis of 11 acylated α-onocerin derivatives (**2**–**12**).
Reagents and conditions: (a) 65% HNO_3, AcOH, 0 °C-rt, 5 days, 71%; (b) acid anhydride, DMAP, EtOAc, reflux, 1.5–2.0 h: **3** (80%), **6** (79%), **7** (84%), and **8** (50%); acyl chloride, pyridine, reflux, 1.5–3.0 h: **4** (30%), **5** (73%), **10** (48%), **11** (86%), and **12** (84%); 4-chlorobutyryl chloride, DMF, CH_2Cl_2, reflux, 18 h, 9 (50%).