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Chinese Journal of Natural Medicines 2018, **16**(3): 0231–0240
Chinese Journal of Natural Medicines

Discovery of novel limonin derivatives as potent anti-inflammatory and analgesic agents

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Available online 20 Mar., 2018

[ABSTRACT] Novel series of limonin derivatives (**V-A-1**–**V-A-8**, **V-B-1**–**V-B-8**) were synthesized by adding various tertiary amines onto the C (7)-position of limonin. The synthesized compounds possessed favorable physicochemical property, and the intrinsic solubility of the novel compounds were significantly improved, compared with limonin. Different pharmacological models were used to evaluate the analgesic and anti-inflammatory activities of the target compounds. Compound **V-A-8** exhibited the strongest *in vivo* activity among the novel limonin analogs; its analgesic activity was more potent than aspirin and its anti-inflammatory activity was stronger than naproxen under our testing conditions.

[KEY WORDS] Limonin derivatives; Analgesic; Anti-inflammatory

[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2018)03-0231-10

Introduction

Limonoids are highly oxygenated triterpenoids and mainly found in plants belonging to the Rutaceae and Meliaceae family. Over 300 limonin analogs have been isolated from natural resource [1]. Limonin (**I-A**), nomilin (**2**) and obacunone (**3**) (Fig. 1) are the most abundant limonoids existing in citrus species' leaves and fruits and rhizomes [2]. Because limonoids possess various biological effects, such as anti-inflammatory [3-4], analgesic [3-4], antitumor [5-8], anti-microbial [9] and anti-feedant [10] activities, the research of these natural products has attracted great interests in medicinal chemistry [11-15].

Limonin (**I-A**) is known as the most common limonoid in the natural environment, which is isolated from navel orange. Since limonin was first identified as analgesic and anti-inflammatory agent by Matsuda [16-17], various efforts have been put to study its possible clinical application. However, the rigid structure and poor solubility in water (< 0.005 mg·mL^{-1}) of limonin result in low oral bioavailability, discouraging its further pharmacological research [18]. Therefore, structural modification to produce limonin derivatives with favorable physicochemical property has become one of the key research topics.

In order to increase their water solubility, various tertiary amine moieties were introduced onto C(7)-position of limonin or deoxylimonin to afford 16 amide derivatives (**V-A-1**–**V-A-8**, **V-B-1**–**V-B-8**) in the present study. The physiochemical properties of the target compounds were studied. The *in vivo* analgesic activities were evaluated by acetic acid induced writhing and tail-immersion in mice, and the *in vivo* anti-inflammatory effects were determined by xylene-induced ear swelling in mice.

Results and Discussion

Chemistry

The synthetic routes for target compounds are depicted in

[Received on] 20-Jun.-2017
[Research funding] This work was supported by the National Natural Science Foundation of China (Grant No. 21472242), the National Science and Technology Major Project for "Significant New Drugs Creation" of China (Grant No. 2015ZX09102001), and State Key Laboratory of Natural Medicines, China Pharmaceutical University (No.SKLNMZZCX201406).
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These authors have no conflict of interest to declare.

Scheme 1. Treatment of limonin (**I-A**) or desoxylimonin (**I-B**) [19] with hydroxylamine hydrochloride yielded exclusively limonin-7-oxime (**II-A**) [20] or desoxylimonin 7-oxime (**II-B**). Intermediate **II-A** was reduced to **III-A** by $NaBH_3CN$. Compound **III-A** was condensed with chloroacetyl chloride or 3-chloropropionyl chloride to give **IV-A-1** or **IV-A-2**, which were reacted with the corresponding amine (R^1R^2NH) to render the target compounds **V-A-1**–**V-A-8**. Compounds **V-B-1**–**V-B-8** were prepared according to the procedure described for **V-A-1**–**V-A-8** with good yields. All the target compounds were characterized by $^1H/^{13}C$ NMR and HRMS spectroscopy.

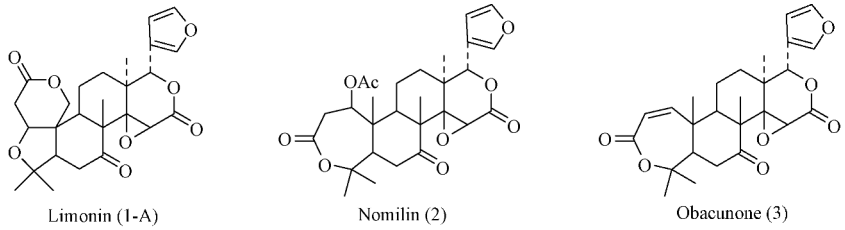
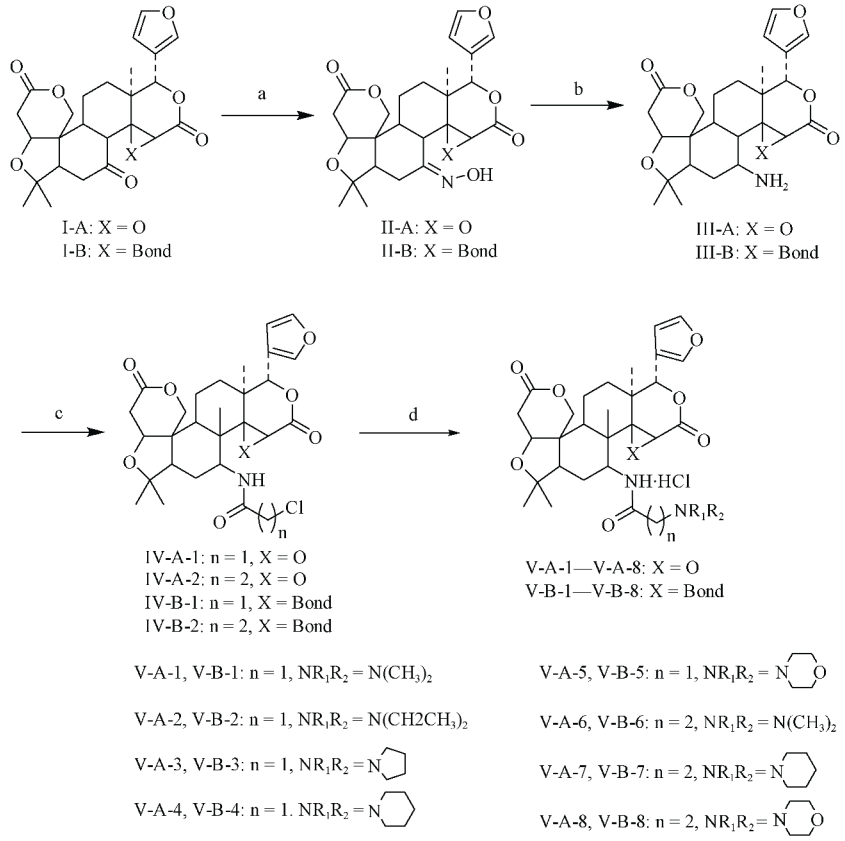


Fig. 1 Structures of Limonin (I-A), Nomilin (2) and Obacunone (3)



Scheme 1 Reagents and conditions: (a) hydroxylamine hydrochloride, pyridine, anhydrous ethanol, reflux; (b) $NaBH_3CN$, ammonium acetate, titanium trichloride, methyl alcohol, 0 °C to room temperature; (c) chloroacetyl chloride or 3-chloropropionyl chloride, DMAP, dichloromethane, 0 °C to room temperature; (d) i: corresponding amine (R^1R^2NH), potassium carbonate, acetone, 50 °C; ii: hydrogen chloride, anhydrous ether, anhydrous dichloromethane

Physicochemical properties of the target compounds

The physiochemical properties of the target compounds, including pKa (ionization constants), log$D_{7.4}$ (partition coefficient at pH 7.4), and aqueous solubility, were studied according to the method of Avdeef and Tsinman on a Gemini Profiler instrument (pION) by the 'gold standard' Av-deef-Bucher potentiometric titration method [21]. The results are summarized in Table 1. A balance between hydrophilicity and lipophilicity is very important for oral absorption. In general, log $D_{7.4}$ values from –0.5 to 2.0 are considered to be optimal for oral absorption of compounds [22]. The log $D_{7.4}$ values for **V-A** series compounds, possessing oxygen bridge between C(14) and C(15), were in the range from –0.42 to 1.45, which were more favorable than **V-B** series compounds (log $D_{7.4}$: from –0.90 to 1.10). In terms of pKa, the un-ionized form of a drug is better absorbed than its ionized counterpart. Since the pH