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Research paper

Identification, structural modification, and dichotomous effects on human immunodeficiency virus type 1 (HIV-1) replication of ingenane esters from *Euphorbia kansui*

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ABSTRACT

Euphorbia kansui showed potent anti-HIV-1 activity during screening of a library composed of plant extracts from Euphorbiaceae and Thymelaeaceae families. Bioassay-guided isolation led to identification of ingenane esters as the active compounds. Further chemical modification resulted in 3-(2-naphthoyl) ingenol (**23**), which exhibited the most potent anti-HIV-1 activity. Compound **23** also acted as an HIV-1-latency-reversing agent on activation of HIV-1 replication in a latently infected U1 cell model and a T cell latent HIV-1 model JLat-A2.

1. Introduction

Since the first recognized cases emerged in 1981, acquired immunodeficiency syndrome (AIDS) has caused more than 35 million deaths and currently more than 37 million individuals are infected with human immunodeficiency virus (HIV) worldwide [1]. Combination antiretroviral therapy (cART) can effectively control plasma viremia in many patients, although the virus is suppressed rather than truly eradicated, and requires life-long administration to prevent relapse [2–4]. Additionally, cART can be compromised by the unwanted side effects of current medications and by emergence of drug-resistant viruses. Thus, a major goal of current HIV/AIDS therapy continues to be the development of new anti-HIV compounds as well as drug regimens for eradication of the HIV virus.

One of the current strategies for HIV-1 eradication requires pharmacological reactivation of latent viruses, which is believed to make the virus and infected cells susceptible to immune clearance and cytopathic effects of the virus [5]. Recently, diterpenoids from Euphorbiaceae and Thymelaeaceae families have attracted much interest as natural drug candidates for reactivation of a latent virus. Prostratin, a non-tumor promoting phorbol ester from Euphorbiaceae plants, can inhibit HIV-1 infection and induce HIV-1 reactivation in latent infection cell models [6]. As we previously reported, gnidimacrin, a daphnane diterpene from a Thymelaeaceae plant, exhibits an dichotomous activity by reactivating latent HIV-1 and inhibiting nascent HIV-1 infection through selective activation of protein kinase C βI and βII at low picomolar concentrations [7,8].

In continuation of our biological screening program on Euphorbiaceae and Thymelaeaceae plant extracts for discovery of

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anti-HIV natural products, methanol extracts from the two plant families were evaluated for selective inhibition of HIV replication. The methanolic extract of *Euphobia kansui* roots showed the strongest anti-HIV activity ($EC_{50} = 150$ ng/mL) compared with other species, such as *Daphne genkwa*, *Euphorbia fischeriana*, *Daphne giraldii*, *Wikstroemia indica*, *Euphorbia lathyris*, *Daphne odora* ($EC_{50} = 220–4500$ ng/mL), hence confirming the great potential of natural extracts as a source of anti-HIV agents. In the present study, we report the screening, bioassay-guided isolation, and semi-synthesis of ingenane esters as potent anti-HIV agents. The most promising ingenane ester derivative was selected to be further investigated for its potential as an anti-HIV latency drug candidate.

2. Results and discussion

2.1. Bioassay-guided isolation and identification of ingenane esters as potent anti-HIV agents

The HIV inhibitory MeOH extract of *E. kansui* roots was subjected to liquid–liquid partitioning to give an active EtOAc fraction with an EC_{50} value of 110 ng/mL. The EtOAc fraction was fractionated by Diaion HP-20 chromatography to afford four major subfractions (E1–E4), among which, the E3-fraction exhibited the lowest IC_{50} value (28 ng/mL). The E3-fraction was subsequently purified by RP-HPLC to afford five anti-HIV active ingenane diterpenes ($EC_{50} = 0.8–1076.9$ nM). Their chemical structures were identified as 5-*O*-benzoyl-20-deoxyingenol (**1**) [9], 3-*O*-benzoyl-20-deoxyingenol (**2**) [9], kansuiphorin C (**3**) [10], ingenol monoacetate (**4**) [11], and 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoylingenol (**5**) [12], by detailed spectroscopic analyses (Fig. 1). Meanwhile, three jatrophane diterpenoids, identified as kansuinin B (**6**) [13], kansuinin C (**7**) [13], and esulone A (**8**) [14], which were also isolated from the E3-fraction, showed no anti-HIV activity. Comparison of the anti-HIV data of **1**–**5** indicated that a long-chain ester unit at C-13 led to remarkably increased activity, and acylation at C-3 produced stronger anti-HIV activity than that at C-5 or C-3,5. Furthermore, deacylated compounds (**9** and **11**), which were obtained by removal of the C-3 ester groups of **1** and **5** by alkaline hydrolysis, showed dramatically weaker anti-HIV activities than **2** and **5**, suggesting that the 3-ester group was important for anti-HIV activity. Although **5** showed potent anti-HIV-1 activity [15], the structural similarity to phorbol 12-myristate 13-acetate (PMA), which is a potent tumor promoter and T-cell activator, reduced its impact for further anti-HIV drug development. Thus, **5** was excluded during further investigation.

2.2. Preparation of ingenane alcohols as starting materials for chemical synthesis of ingenane derivatives

To clarify the importance of the acyl group and discover more potent anti-HIV agents, a library of ingenane ester derivatives was synthesized from ingenane alcohols as starting materials. The total synthesis of ingenane alcohol has been achieved, but the process is complex (over 14 steps) and produces low yields (*ca.* 1%) [16–18]. In the present study, a simple and direct method was established to obtain ingenane alcohols from the *E. kansui* extract via a one-step deacylation as depicted in Scheme 1. Since the *E. kansui* extract contains numerous esters of dodecatrienoyl, benzoyl, and acetyl moieties, which are attached at polyhydroxyl groups on the ingenane skeleton, an LC-MS analysis was applied initially to monitor the simultaneous deacylation process. The ESI-MS fragmentation patterns of **1**–**5** indicated that 20-deoxyingenol, ingenol, and 13-oxyingenol esters readily produced fragmentation ions at *m/z* 297, 313, and 329, respectively, by the loss of organic acids (RCOOH) and/or one molecule of H_2O (Supplementary data). On the basis of the summarized characteristic fragmentation ions, an LC-MS analysis in positive and negative-full scan modes combined with SIM channels (*m/z* 297, 313 and 329) was then applied to monitor the deacylation process (Fig. 2). Consequently, methanolysis of *E. kansui* extract with K_2CO_3 in MeOH at room temperature for 4 h produced 20-deoxyingenol (**9**, 4%), ingenol (**10**, 2.5%), and 13-oxyingenol-13-dodecanoate (**11**, 0.8%) from the E3-fraction (see Scheme 1).

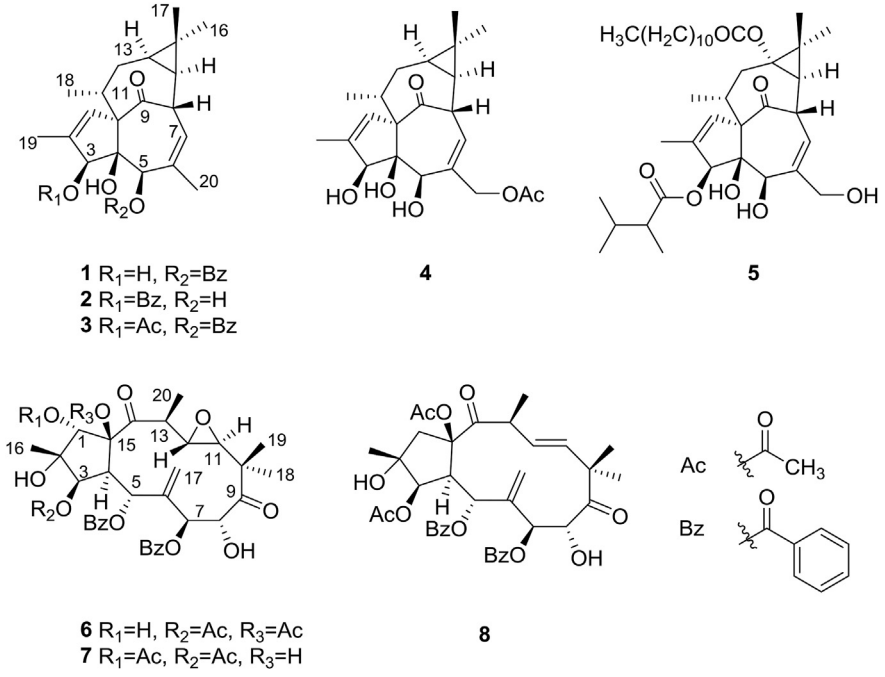

Fig. 1. Compounds obtained from E. kansui by bioactivity-guided isolation.