DOCTORAL THESIS

Identification and structural determination of anti-HIV chemical constituents from justicia genus

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ABSTRACT

Until now, emerging viral diseases have been posing ongoing threats to the global public health. Among the notorious viruses, the HIV that causes the AIDS has been spreading continuously since it was first identified in 1981 and is the most quickly spreading disease of the century. Although considerable advance has been made by drug discovery groups, the therapeutic management is still challenged by the rapid mutations of the virus to yield resistant strains, so as the emergence of side effects. Therefore, the development of novel potent anti-HIV agents is urgently sought. Owing to the chemical diversity, we believe that natural products may serve as potential "lead" compounds for discovery of anti-HIV drugs.

In order to search for novel naturally occurring compounds with potent inhibitory effects against HIV, we began with isolation of natural products from two medicinal plants of *Justicia* by means of silica gel column chromatography, and preparative HPLC, namely, *J. gendarussa* that displayed potent anti-HIV activity in our initial screening, and *J. procumbens*, and their chemical structures and determinated by spectroscopic and chemical methods such as IR, UV, HRESIMS, ^{1}H NMR and ^{13}C NMR spectrometry (including DEPT, ^{1}H-^{1}H COSY, and HMBC techniques). Upon the complete identification of compounds, we focused on the synthesis of one potential lead compound isolated from *J. gendarussa*, patentiflorin A (**3**). Nevertheless, we evaluated all the isolated natural compounds and synthetic **3** via bioactivity screening for anti-HIV activity.

In the phytochemical investigation of *J. gendarussa*, a rare, shade-loving, quick-growing, evergreen scented shrub collected in Vietnam, the bioassay-directed fractionation of the methanol extract of the roots and stems of the plant led to the

isolation two new arylnaphthalide lignan glycosides, named justiprocumins A and B (**1–2**), together with a known one, patentiflorin A (**3**). On the other hand, the phytochemical investigation of the methanol extract of the aerial parts of *J. procumbens* resulted in the isolation of four novel arylnaphthalide lignans, procumbenosides G (**4**), H (**5**), I (**6**) and J (**7**), along with 23 known compounds, namely, tuberculatin (**8**), procumbenoside B (**9**), procumbenoside E (**10**), ciliatoside B (**11**), ciliatoside A (**12**), 5-methoxy-4, 4′-di-*O*-methylsecolariciresinol (**13**), secoisolariciresinol dimethyl ether (**14**), 2, 3-bis(3, 4-dimethoxybenzyl)-4-hydroxybutyl acetate (**15**), secoisolariciresinol (**16**), hemiariensin (**17**), ariensin (**18**), secoisolariciresinol dimethyl ether diacetate (**19**), hinokinin (**20**), justicidin E (**21**), justicidin D (**22**), justicidin C (**23**), cilinaphthalide A (**24**), 5′-methoxy-4′-*O*-methyllariciresinol (**25**), 3, 5, 7, 3′, 4′, 5′-hexamethoxyflavone (**26**), 3, 5, 7, 8, 3′, 4′-heptamethoxyflavone (**27**), 3, 5, 7, 8, 3′, 4′, 5′-heptamethoxyflavone (**28**), methyl ferulate (**29**) and loliolide (**30**). In addition, the compound **3** was totally synthesized with a yield of 68.3%.

In the anti-HIV evaluation for all the isolated compounds using the defective HIV-based pseudotyped assay, patentiflorin A (**3**) was found to have anti-HIV activity with an IC_{50} value of 26.9 nM, while justicin E (**21**) showed 65.4 % inhibitory effect against HIV replication at 2.5 μg/mL. In the evaluation for the broadness of the spectrum of anti-HIV activity using a standardized human PBMC assay, **2** gave IC_{50} values of 14-21, and **3** gave IC_{50} values 24-37 nM in inhibiting the particle production of all the four HIV-1 isolates [BAL and SF162 (both are M-tropic), LAV0.04 (T-tropic), and 89.6 (dual tropic)], while the synthetic **3** showed quite similar activity as that of natural **3**. In the test of cytotoxicity, natural **3** exhibited no apparent cytotoxicity at 19.0 μM in A549 and Hela cells, and the synthetic **3** displayed much

lower cytotoxicity (CC_{50}: 75.5 μM) than that of the natural **3** (CC_{50}: 18.4 μM) in PBMC cells. That means **2** and **3** have great potentials as anti-HIV lead compounds for further drug development.

In conclusion, natural compounds isolated from medicinal plants serves as one of the most important sources of potentially anti-HIV compounds, which can be employed as “lead” compounds to develop novel therapeutic drugs against HIV.

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