

2017

Potent inhibitor of drug-resistant HIV-1 strains identified from the medicinal plant *Justicia gendarussa*

Hong-Jie Zhang

Hong Kong Baptist University, zhanghj@hkbu.edu.hk

Emily Rumschlag-Booms

University of Illinois at Chicago

Yi-Fu Guan

Hong Kong Baptist University

Dong-Ying Wang

Hong Kong Baptist University

Kang-Lun Liu

Hong Kong Baptist University

See next page for additional authors

This document is the authors' final version of the published article.

Link to published article: <http://dx.doi.org/10.1021/acs.jnatprod.7b00004>

APA Citation

Zhang, H., Rumschlag-Booms, E., Guan, Y., Wang, D., Liu, K., Li, W., Nguyen, V., Cuong, N., Soejarto, D., Fong, H., & Rong, L. (2017). Potent inhibitor of drug-resistant HIV-1 strains identified from the medicinal plant *Justicia gendarussa*. *Journal of Natural Products*, 80 (6), 1798-1807. <https://doi.org/10.1021/acs.jnatprod.7b00004>

Authors

Hong-Jie Zhang, Emily Rumschlag-Booms, Yi-Fu Guan, Dong-Ying Wang, Kang-Lun Liu, Wan-Fei Li, Van H. Nguyen, Nguyen M. Cuong, Djaja D. Soejarto, Harry H. S. Fong, and Lijun Rong

Potent Inhibitor of Drug-Resistant HIV-1 Strains Identified from the Medicinal Plant *Justicia gendarussa*

Hong-Jie Zhang,^{*,†,○} Emily Rumschlag-Booms,^{‡,▽,○} Yi-Fu Guan,[†] Dong-Ying Wang,[†] Kang-Lun Liu,[†] Wan-Fei Li,[†] Van H. Nguyen,[§] Nguyen M. Cuong,[⊥] Djaja D. Soejarto,^{||} Harry H. S. Fong,^{||} and Lijun Rong^{*,‡}

[†]School of Chinese Medicine, Hong Kong Baptist University, Kowloon, Hong Kong SAR, People's Republic of China

[‡]Department of Microbiology and Immunology, College of Medicine, University of Illinois at Chicago, 835 South Wolcott Avenue, Chicago, Illinois 60612, United States

[§]Institute of Marine Biochemistry of the Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet road, Cau Giay, Hanoi, Vietnam

[⊥]Cuc Phuong National Park, Nho Quan, Ninh Binh, Vietnam

^{||}Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, United States

[▽]Present address: Northeastern Illinois University, 5500 N St Louis Ave, Chicago, Illinois 60625, United States

ABSTRACT: *Justicia gendarussa* (Acanthaceae), a medicinal plant collected in Vietnam, was identified as a potent anti-HIV-1 active lead from the evaluation of over 4,500 plant extracts. Bioassay-guided separation of the extracts of the stems and roots of this plant led to the isolation of an anti-HIV aryl-naphthalene lignan (ANL) glycoside, patentiflorin A (**1**). Evaluation of the compound against both the M- and T-tropic HIV-1 isolates showed it to possess a significantly higher inhibition effect than the clinically used anti-HIV drug AZT. Patentiflorin A and two congeners were synthesized, de novo, as an efficient strategy for re-supply as well as for further structural modification of the anti-HIV ANL glycosides in the search for drug leads. Subsequently, it was determined that the presence of a quinovopyranosyloxy group in the structure is likely essential to retain the high degree of anti-HIV activity of this type of compounds. Patentiflorin A was further investigated against the HIV-1 gene expression of the R/U5 and U5/gag transcripts, and the data showed that the compound acts as a potential inhibitor of HIV-1 reverse transcription. Importantly, the compound displayed potent inhibitory activity against drug-resistant HIV-1 isolates of both the nucleotide analogue (AZT) and non-nucleotide analogue (nevirapine). Thus, the ANL glycosides have the potential to be developed as novel anti-HIV drugs.

AIDS (acquired immunodeficiency syndrome) has become a worldwide epidemic since its first report in 1981 in the United States.^{1,2} About 78 million people have been infected with the human immunodeficiency virus (HIV), and nearly half of them have died since. There were roughly 2.1 million people newly infected with HIV in 2015.³⁻⁵ AIDS is presently the leading cause of death in Africa, and reportedly caused the death of 1.1 million people globally in 2015.⁶

The first anti-HIV drug, zidovudine (AZT) was developed and approved in 1987. Today, more than 40 FDA-approved drugs, including a number of combination cocktails, are available on the market for the treatment of HIV infected patients. These drugs are classified as non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), entry and/or fusion inhibitors, and integrase inhibitors.⁷ Highly Active Antiretroviral Therapy (HAART) combining three or more anti-HIV medications in a daily regimen is the recommended treatment for those infected with HIV. Although these drugs have significantly extended the life span of HIV-positive people, especially those in economically wealthy countries, they are unable to eliminate the virus from the infected patients; have potential side effects; and show diminishing effectiveness on chronic use due to the development of drug-resistance HIV isolates.⁸⁻¹⁰ In addition, AIDS patients in developing countries in Sub-Saharan Africa and other regions of the world with the greatest prevalence of HIV infections, often cannot afford these medications, or simply do not have access to them. Thus, it is imperative to continuously push forward in the discovery and development of novel, more effective, more accessible, and affordable anti-HIV therapeutics.

Natural products, especially the plant natural products, have played the most important role in the history of drug discovery.¹¹ Many compounds have also been reported from higher plants to exhibit anti-HIV activity in the past two decades. Examples of important higher plant-derived anti-HIV agents or lead compounds include betulinic acid and its derivatives,^{12,13} calanolide coumarins,^{14,15} and ingenol

diterpenoids.¹⁶⁻¹⁸ Thus, higher plants are clearly an excellent source for discovering new antiviral agents.

The current study reports the identification of an aryl-naphthalene lignan glycoside possessing specific and potent anti-HIV-1 activity from the plant, *Justicia gendarussa*, and of its synthesis and that of two congeners. These compounds have the potential to be developed as novel anti-HIV therapeutic agents, capable of being added to the current HAART drugs.

RESULTS AND DISCUSSION

Through the collaborations among several institutes in Vietnam, Laos, and the United States, a program was established for the search for anti-HIV, antimalarial, anti-TB, and anticancer agents from higher plants.¹⁹ More than 4,500 plant samples were collected in Vietnam and Laos, and subjected to subsequent extract preparation and evaluation for bioactivities against these disease targets. Interestingly, one of the CH₂Cl₂ plant extracts (SV5614) displayed complete inhibition against HIV replication at a concentration of 20 µg/mL.²⁰ This bioactivity was confirmed by the evaluation of the MeOH extract (SVA5614), prepared from a recollected sample of the same plant, with HIV-1 replication inhibition at an IC₅₀ value of 40 ng/mL. These results indicate that *J. gendarussa* is a promising lead plant in the search for anti-HIV agents.

As noted above, the extracts SV5614 and SVA5614 were prepared with CH₂Cl₂ and MeOH, respectively, from two separate field collected plant materials. SV5614 was prepared from the initial collection of the stems and roots of the plant *J. gendarussa* Brum. f. (synonym: *Gendarussa vulgaris* Nees) (Acanthaceae family), with SVA5614 being derived from a larger recollection of the plant materials.

J. gendarussa is a bush of up to 0.3 m in height, and is found in Vietnam, China, Indonesia, Thailand, The Philippines, India, and Burma. The plant leaves and roots have been used as an herbal

medicine for the treatment of fall injury, rheumatism, pain, swelling, bone fracture, as well as thermogenic and diaphoretic agents in these countries.^{21,22}

Bioassay-directed separation of SVA5614 led to the isolation of patentiflorin A (**1**) (Figures 1A-1C).²³ The compound showed anti-HIV-1 activity with an IC₅₀ value of 26.9 nM in the defective HIV-based pseudotyped assay, which is also known as the “One-Stone-Two-Birds” protocol (Figure 1D and Figure S1, Supporting Information).²⁰

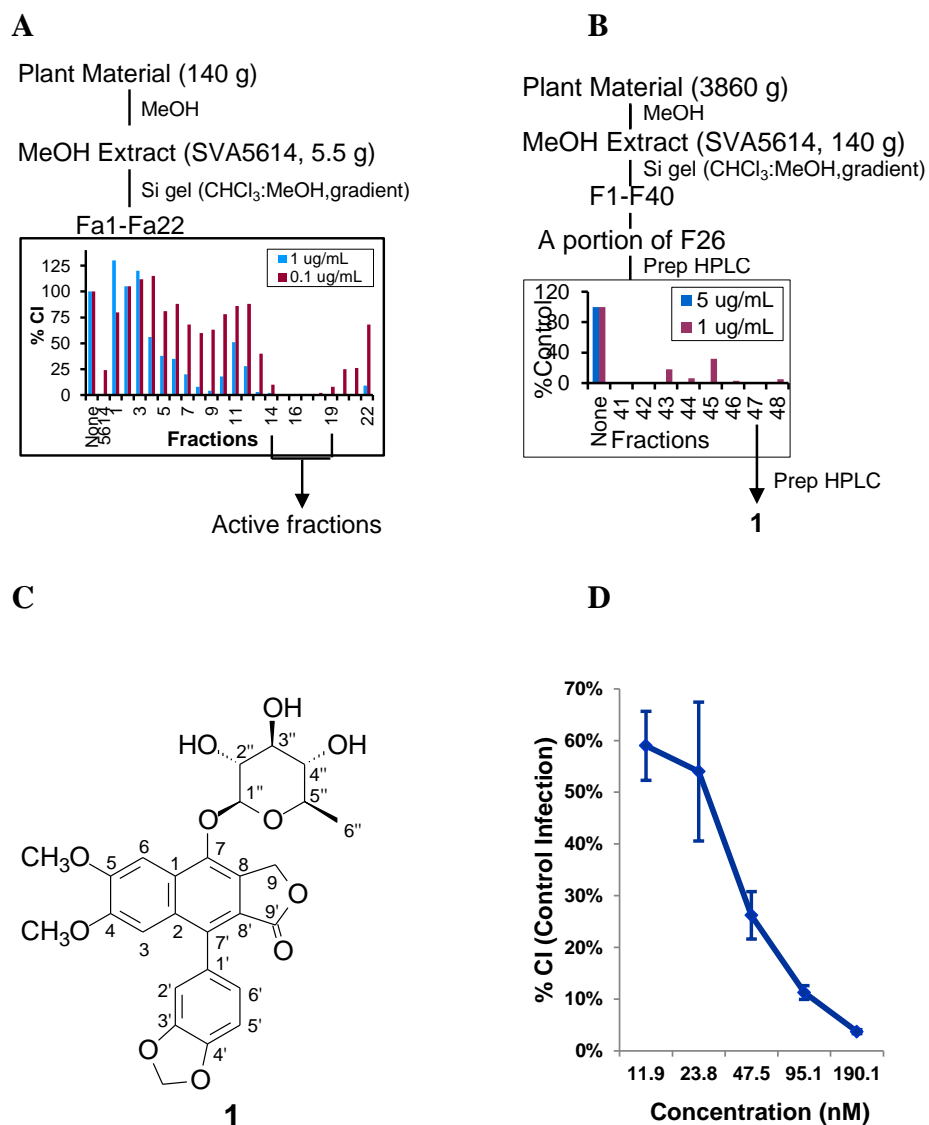


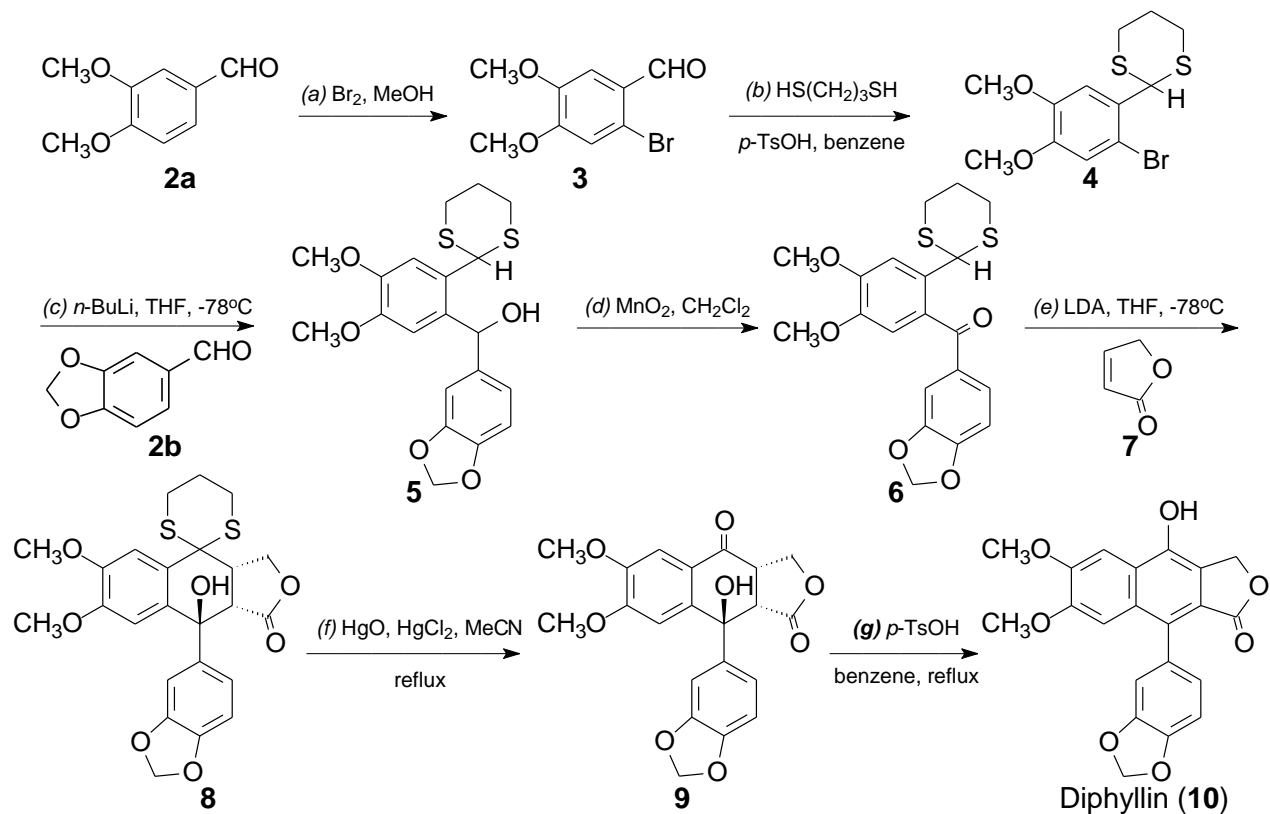
Figure 1. Confirmation, separation, and chemical structures of the anti-HIV compound. (A) Confirmation of anti-HIV activity of the plant: A small portion (5.52 g) of SVA5614 was rapidly fractionated into 22 fractions (Fa1-Fa22) by a silica gel column in one hour. Fa14-F18 obtained from the eluents of CHCl₃/MeOH 85:15 to 8:2 showed almost complete inhibition of HIV replication at the concentrations of 1.0 and 0.1 μg/mL. (B) Isolation of the anti-HIV compound: Scale-up separation of SVA5614 by silica gel chromatography yielded 40 fractions (F1-F40). Fraction F26, showing a similar HPLC profile as that of the active fraction Fa16, was further subjected to preparative HPLC separation to yield the active compound patentiflorin A (**1**). (C) Chemical structure of patentiflorin A (**1**). (D) Titration of patentiflorin A (**1**): Patentiflorin A (**1**) was shown to inhibit HIV replication with an IC₅₀ value of 26.9 nM in the “One-Stone-Two-Birds” assay. The bioassay experiments (repeated three times each) were performed in triplicate. The pseudoviral evaluation system is described in Figure S1, Supporting Information.

Patentiflorin A (**1**), [α]_D²⁰ -23.5 (*c* 1.20, MeOH), was determined to be an arylnaphthalene lignan glycoside containing a methylenedioxy and two methoxy groups by comparison of their ¹H and ¹³C NMR data to those of known arylnaphthalene lignans (Table S1, Supporting Information).^{24,25} The γ -lactone carbonyl carbon was assigned at C-9' rather than at C-9 due to the presence of the HMBC cross-peaks of H₂-9 to C-7. Analysis of 1D (¹H, ¹³C and DEPT) and 2D (¹H-¹H COSY, HMQC and HMBC) NMR data positioned the two methoxy groups at C-4 and C-5, and the methylenedioxy group at C-3'/C-4' to form a piperonyl (1, 3-benzodioxole) group.

Compound **1** was identified as the known compound patentiflorin A by analysis of its spectroscopic data (Table S1, and Figures S2-S7, Supporting Information), and a comparison with

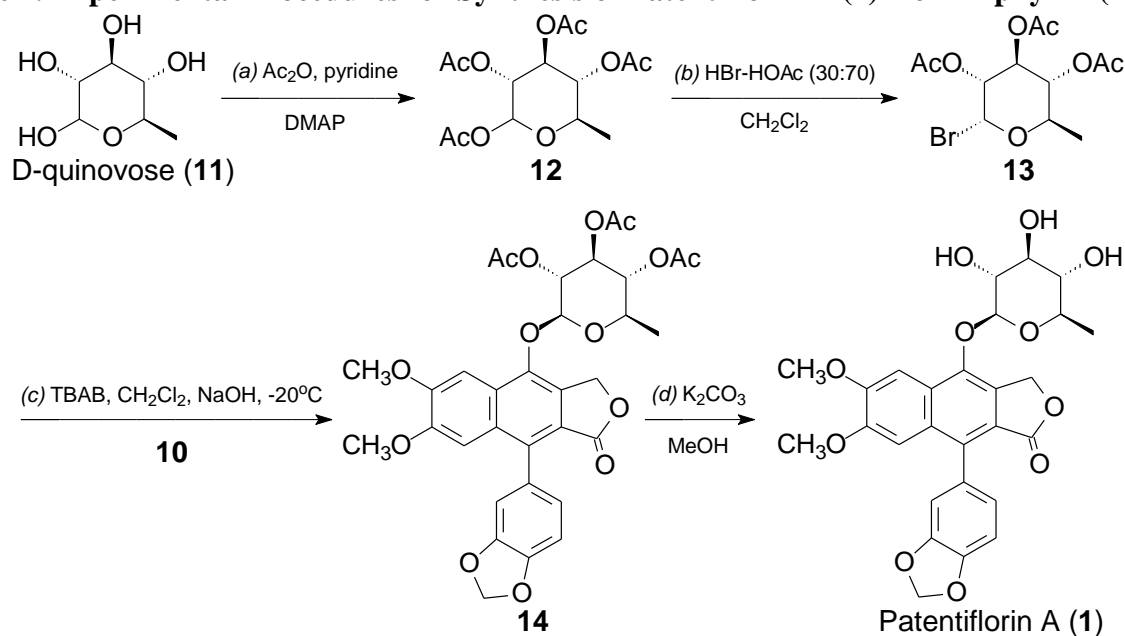
literature data.²³ We further confirmed the identity by a *de novo* total synthesis (Schemes 1 and 2) of the compound. The synthesized patentiflorin A (**1**) gave the same ¹H and ¹³C NMR spectroscopic and specific rotation data as those of the natural isolate (Figure S26, Supporting Information), thus providing additional confirming evidence of chemical identification.

Scheme 1. Experimental Procedures for Synthesis of Diphyllin (**10**)^a



^aConditions: (a) **2a** (63.3 mmol), Br₂ (69.1 mmol), MeOH, rt, 6 h, 86%; (b) **3** (50.2 mmol), HS(CH₂)₂SH (50.2 mmol), *p*-TsOH (2.79 mmol), benzene, reflux, 10 h, 93%; (c) **4** (46.6 mmol), **2b** (55.9 mmol), *n*-BuLi (69.9 mmol), THF, -78 °C to r.t., 2 h, 50 %; (d) **5** (20.7 mmol), MnO₂ (345 mmol), CH₂Cl₂, rt, 16 h, 98 %; (e) **6** (14.3 mmol), **7** (17.3 mmol), LDA (45 mmol), THF, -78 °C to rt, 1 h, 57 %; (f) **8** (0.65 mmol), HgO (0.71 mmol), HgCl₂ (1.43 mmol), MeCN, reflux, 3 h, 52 %; (g) **9** (0.34 mmol), *p*-TsOH (0.22 mmol), benzene, reflux, 16 h, 85 %.

Scheme 2. Experimental Procedures for Synthesis of Patentiflorin A (1) from Diphyllin (10)^a



^aConditions: (a) **11** (0.61 mmol), DMAP (0.06 mmol), Ac₂O (1.5 mL), pyridine, rt, overnight; (b) **12**, HBr-HOAc (33 % HBr, 1.5 mL), CH₂Cl₂, rt, 15 min, 99%; (c) **12** (0.95 mmol), **13** (0.61 mmol), TBAB (0.95 mmol), NaOH (20 mL, 0.1 M), CHCl₃, 40 °C, 6 h; (d) **14**, K₂CO₃ (1.0 mmol), MeOH, rt, 1 h, 38 %.

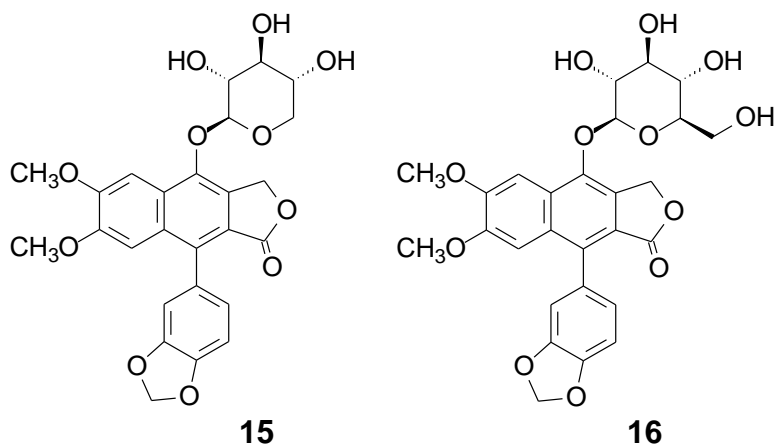


Figure 2. The chemical structures of the synthesized congeners 7β-D-xyloxyloxydiphyllin (**15**) and 7β-D-glucopyranosyloxydiphyllin (**16**).

In addition to patentiflorin A (**1**), two structural congeners (**15** and **16**) (Figure 2) were also synthesized and evaluated for potential HIV replication inhibition activities. The synthesis routes for these compounds are summarized in Schemes 1 and 2.

The aglycone diphyllin (**10**) was determined to be the key intermediate for the synthesis of patentiflorin A (**1**). The successful synthesis of this intermediate enabled us not only to obtain patentiflorin A but also to afford a synthesis route to other related aryl-naphthalene lignan derivatives. Scheme 1 illustrates our successful synthesis to diphyllin (**10**) through the key feature of an intermolecular Michael addition reaction of intermediate **6** with furan-2(5*H*)-one (**7**) to produce **8**.

Glycosylation of diphyllin (**10**) at C-7 with D-quinovose (**11**) led to patentiflorin A (**1**) (Scheme 2) (Figures S8-27, Supporting Information). The MS and NMR data of the synthesized compound being the same as those of the natural isolate confirmed that the two compounds are identical (Figure S26, Supporting Information).

The same strategy for the synthesis of patentiflorin A (**1**) was employed to synthesize two congeners (**15** and **16**). By replacing D-quinovose (**11**) with D-xylose and D-glucose in Scheme 2, the congeners, 7 β -D-xyloxyoxydiphyllin (**15**) and 7 β -D-glucopyranosyloxydiphyllin (**16**), were obtained (Figure 2 and Figures S28-31, Supporting Information), respectively. 7 β -D-xyloxyoxydiphyllin (**15**, diphyllinin) and 7 β -D-glucopyranosyloxydiphyllin (**16**, cleistanthin B) are two natural products that were originally isolated from the aerial parts of *Haplophyllum hispanicum*²⁶ and the bark of *Cleistanthus collinus*,²⁷ respectively.

The biological activities of the natural and synthesized patentiflorin A (**1**), diphyllin (**10**) and compounds **15** and **16** were evaluated and compared (Figure 3). As expected, the synthesized patentiflorin A (**1**) showed similar anti-HIV activity as that of the natural one (Figure 3A). However,

compounds **15** and **16** showed much lower anti-HIV activity (Figure 3D) than patentiflorin A (**1**), although these compounds differ from one another only by the sugar units. Although the aglycone diphyllin (**10**) displayed some level of activity (Figure 3A), it is clear that the presence of a sugar unit is required for effective inhibitory action against HIV-1.

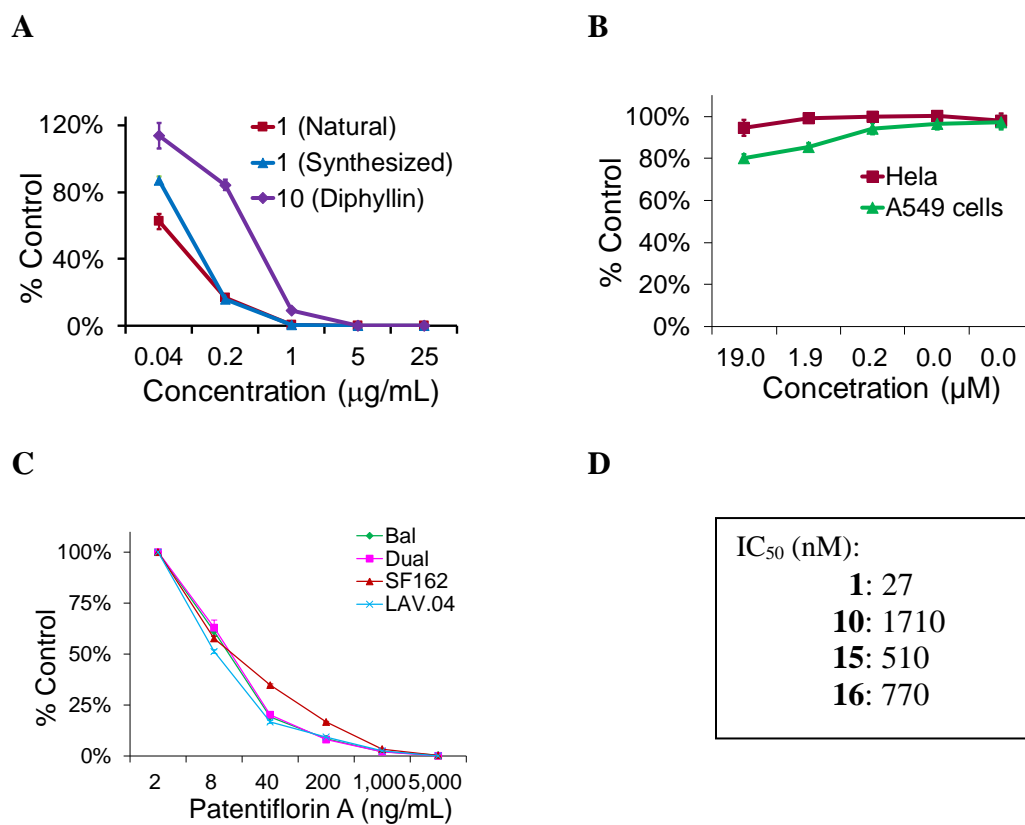


Figure 3. The anti-HIV activity and cytotoxicity of the anti-HIV compounds. (A) Comparison of the anti-HIV activity of the natural and synthesized patentiflorin A (**1**) and diphyllin (**10**) in the defective HIV-1 pseudoviral assay. (B) Cytotoxicity of the natural patentiflorin A (**1**) against A549 and HeLa cells. (C) Inhibition of patentiflorin A (**1**) against four HIV viral strains (Bal:M-Tropic, 89.6: Dual Tropic, SF162:M-Tropic and LAV.04:T-Tropic): patentiflorin A (**1**, natural) displayed a dose dependent effect against the four strains with IC₅₀ values in the range of 24-37 nM. (D) The IC₅₀ values of the anti-HIV

activity of the compounds **1**, **10** and **15-16** were obtained using the “One-Stone-Two-Birds” evaluation system, with the data obtained from three experiments, each performed in triplicate.

The cytotoxicity of the natural patentiflorin A (**1**) was initially evaluated in A549 (human alveolar adenocarcinoma) and Hela (human epithelial cervical adenocarcinoma) cell lines. As shown in Figure 3B, at a concentration of 19 μ M, the natural patentiflorin A exhibited no apparent cytotoxicity to these cells. When the natural and synthesized patentiflorin A (**1**) were evaluated in PBMC (human peripheral blood mononuclear cell), both displayed the same level of cytotoxicity (CC_{50} : 75 μ M).

Table 1. Inhibition Activity of Patentiflorin A (1, Natural and Synthesized) against HIV Clinical Strains and Resistant Strains

Compound	Strains (IC_{50} , nM)						
	Bal: M-Tropic	89.6: Dual-Tropic	SF162: M-Tropic	Lav.04: T-Tropic	HIV-1 _{LAV}	HIV-1 ₁₆₁₇₋ 1	HIV-1 _{N119}
1 (Natural)	30	32	37	24	–	–	–
1 (Synthesized)	32	31	30	32	108	61	47
AZT	77	95	85	79	72	3508	5.2
Nevaripine	–	–	–	–	532	156	>100000 ^a

^aThe datum is taken from the literature.^{29,30}

To determine the anti-HIV-1 activity spectrum of the natural and synthesized patentiflorin A (**1**), both were tested against four HIV-1 clinical isolates: BAL and SF162 (both M-tropic), LAV0.04 (T-tropic), and 89.6 (dual tropic) using a standardized human peripheral blood mononuclear cell culture (PBMC) assay.²⁸

The results clearly showed that compound **1** is a broad spectrum inhibitor. Like AZT, it inhibited the particle production of all four HIV-1 isolates effectively in a dose-dependent manner (Figure 3C). Compound **1** gave an IC₅₀ value of 24-37 nM, compared to 77-95 nM for AZT, depending on the isolates (Table 1). As expected, the synthesized patentiflorin A (**1**) had a similar anti-HIV-1 activity profile compared to that of the natural compound (Table 1). This finding is important because it validates that both the isolated and synthesized patentiflorin A (**1**) are active anti-HIV compounds with higher degrees of potency than AZT. Most significantly, the ability to chemically synthesize a potential drug lead such as patentiflorin A (**1**), would eliminate the need of a dependence for resupply on the phytochemical isolation of the compound from the source plant matrix for drug development efforts, or for commercial production, should it become an approved drug.

The emergence of the drug-resistant HIV isolates is one of the major problems in the long-term treatment of the HIV/AIDS patients. Thus, one critical parameter in the search for novel anti-HIV therapeutics is that a new candidate should have a good therapeutic efficacy against the drug-resistant HIV isolates. Thus, patentiflorin A (**1**) was investigated to determine whether it was effective against an NRTI (nucleoside reverse transcriptase inhibitor)-resistant isolate (HIV-1₁₆₁₇₋₁) and an NNRTI (non-nucleoside reverse transcriptase inhibitor)-resistant isolate (HIV-1_{N119}) using the PBMC assay as described above.²⁸

As shown in Table 1, patentiflorin A (**1**) effectively inhibited all three of the HIV-1 isolates tested, LAV, HIV-1₁₆₁₇₋₁ and HIV-1_{N119}. In contrast, AZT, as an NRTI, was effective against LAV, HIV-1_{N119}, but not HIV-1₁₆₁₇₋₁, while nevirapine, which is an NNRTI, was effective against LAV, HIV-1₁₆₁₇₋₁, but not against HIV-1_{N119}.^{29,30} These results clearly demonstrate that patentiflorin A (**1**) is a potent inhibitor against both NRTI- and NNRTI-resistant HIV-1 isolates.

Because of the nature of the initial anti-HIV evaluation protocol employed, it was apparent that patentiflorin A (**1**) would most likely target a post-entry step in the HIV-1 replication cycle such as

reverse transcription, integration, transcription, or protein expression. To further delineate the mechanism of action of **1**, the inhibition profiles of the natural isolate and AZT (control) against HIV gene expression were investigated and compared (Figure 4). HIV gene expression of the R/U5 and U5/gag transcripts was measured using TaqMan-based real time PCR.^{31,32} In this assay, A549 target cells were challenged with a VSV-G/HIV pseudovirus in the presence or absence of the test compound. Hence, patentiflorin A (**1**), and AZT were added simultaneously with the virus. AZT is a powerful NRTI of HIV that blocks the conversion of viral RNA into DNA. In this assay, AZT blocked R/U5 and U5/gag transcription in a dose-dependent manner with more potent inhibition against the late transcript. In contrast, the test compound (**1**) was shown to inhibit both the early and late transcripts at levels greater than AZT. The inhibition by **1** is most notable at 0.005 $\mu\text{g}/\text{mL}$, with gene expression being decreased by more than 75% as compared to 40% for AZT. These data suggest that patentiflorin A (**1**) is a potential inhibitor of HIV reverse transcription (RT). Further research is needed to determine the anti-HIV-1 mechanism of action and target of compound **1**.

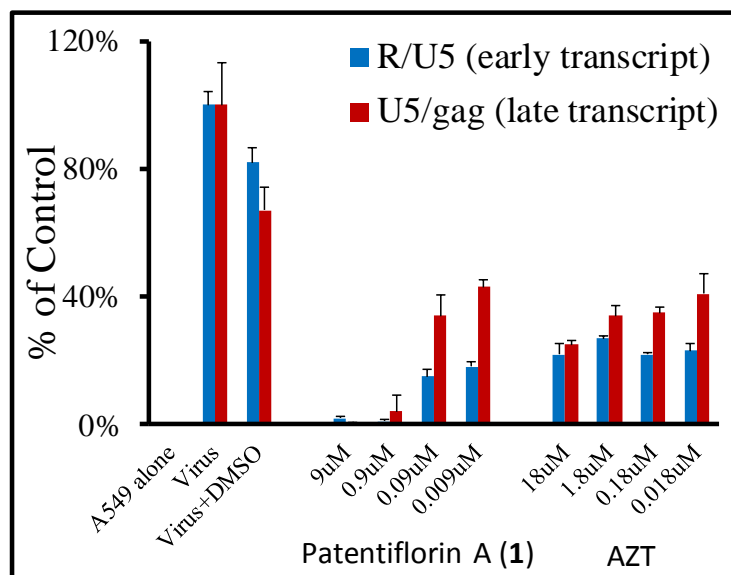


Figure 4. Real time PCR data of patentiflorin A (**1**) and AZT against HIV gene expression. A549 target cells were challenged with VSV-G/HIV pseudotyped virus while concurrently being treated with either purified compound **1** or AZT (10-fold dilutions beginning with 5 µg/mL). Forty-eight hours post-infection/treatment, cells were prepared for RNA isolation, reverse transcription PCR, and subsequent TaqMan-based real-time PCR. Detection of the HIV R/U5 region serves as a measure of early transcription product while the HIV U5/gag region serves as an measure of late transcription product.

Patentiflorin A (**1**) was isolated and identified as a potent anti-HIV lead compound from the medicinal plant *Justicia gendarussa*. It displayed broad spectrum activity against both M-tropic and T-tropic HIV-1 isolates with IC₅₀s lower than that of zidovudine (AZT), the first anti-HIV drug developed and still used in the treatment of HIV/AIDS. The *de novo* total synthesis of the aryl-naphthalene lignin (ANL) glycoside was subsequently carried out. We demonstrated that the newly discovered inhibitor targets reverse transcription in the HIV-1 life cycle, and it is highly potent against NRTI- and NNRTI-resistant HIV-1 variants. Therefore, the new inhibitor and its derivatives have potential to be developed as novel anti-HIV therapeutic agents.

A variety of biological activities including antibacterial, antitumor, and anti-HIV activities have been reported for ANL compounds.³³⁻³⁷ However, no reported ANL compounds showed the same level of anti-HIV potency as that of patentiflorin A (**1**). Our data showed that the glycoside patentiflorin A (**1**) was at least 50-fold more potent than its parent aglycone, diphyllin (**10**), in inhibiting HIV-1 replication (Figure 3).

Patentiflorin A (**1**) was first isolated from the plant *J. patentiflora*, and was shown to be cytotoxic in several cancer cell lines with CC₅₀ values in the range of 3-40 nM.²³ However, in our study, the compound showed cytotoxicity at much lower concentrations in several human cell lines. To further characterize **1**, the compound was synthesized in order to verify not only its chemical structure but also

its biological activities, as well as being a means of re-supply for future uses. The synthesized patentiflorin A showed similar anti-HIV activity profiles as those of the natural patentiflorin A in all the HIV strains tested, and it demonstrated low toxicity to human PMBC with a CC_{50} value at 75.5 μ M (Table 1).

To further determine the anti-HIV potential and structure relationships among ANL glycosides, two congeners [7 β -D-xylosyloxydiphyllin (**15**) and 7 β -D-glucopyranosyloxydiphyllin (**16**)] of patentiflorin A (**1**) were synthesized. Compounds **1**, **15** and **16** all contain a sugar unit at C-7. The sugar units differ from one another only by the C-5'' substituent, with a methyl group for **1**, a hydroxymethyl group for **16** and no substituent at C-5'' for **15**. It is most interesting to note that with only a slight chemical structural difference at C-5'', the three ANL glycosides exhibited such differences in their anti-HIV activity, with **1** being *ca.* 20-30 times more potent than **15** and **16** (Figure 3D). Compound **1** contains a quinovopyranosyloxy sugar unit while **15** and **16** are glycosylated with xylose and glucose, respectively, suggesting that the presence of a quinovopyranosyloxy sugar moiety may be essential to maintain the anti-HIV potency among these three ANL compounds. To fully elucidate the anti-HIV SAR of ANL glycosides, it will be essential to synthesize and evaluate a full complement of other glycosides.

Several aspects of patentiflorin A (**1**) render it an attractive candidate as a lead compound for anti-HIV-1 drug development. It is a highly potent molecule and has a broad activity spectrum against both M- and T-tropic HIV-1 isolates. The compound was also shown to be more effective than AZT in inhibiting four different HIV-1 isolates, either M- or T-tropic, in human PBMCs with IC_{50} s in the range of 14-32 nM (Table 1). Further, it demonstrated an excellent selectivity index of 2500. This is the first report that this natural product displayed not only comparable, but higher anti-HIV activity than that of the clinically used drug AZT.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. IR spectra were recorded on a Jasco FT/IR-410 spectrometer, equipped with a Specac Silver Gate ATR system by applying a film on a Germanium plate (Jasco, Maryland). 1D and 2D NMR spectra were recorded on a Bruker DRX-500 MHz, a Bruker DPX-400 MHz, or a Bruker DPX-360 MHz spectrometer (Rheinstetten, Germany). Chemical shifts (δ) were expressed in ppm with reference to the solvent signals (CDCl_3 : ^1H : 7.24 ppm, ^{13}C : 77.00 ppm; methanol- d_4 : ^1H : 3.30 ppm, ^{13}C : 49.00 ppm; DMSO- d_6 : ^1H : 2.50 ppm, ^{13}C : 39.50 ppm), and coupling constants (J) are reported in Hz. All NMR experiments were obtained by using standard pulse sequences supplied by the vendor. Column chromatography was carried out on silica gel (230–400 mesh, Natland International Corporation, Research Triangle Park, NC). Reversed-phase flash chromatography was accomplished with RP-18 silica gel (40–63 μm , EM Science, Gibbstown, NJ), and reversed-phase preparative HPLC was carried out on a Waters 600E Delivery System pump, equipped with a Waters 996 photodiode detector (Milford, MA), and a Watrex GROM-Saphir 110 C_{18} column (120 \AA , 12 μm , 300 \times 40 mm^2 , Rottenburg-Hailfingen, Switzerland) or a Phenomenex LUNA-C-18 column (120 \AA , 12 μm , 250 \times 50 mm^2 , Torrance, CA). Thin-layer chromatography was performed on EMD glass-backed plates coated with 0.25 mm layers of silica gel 60 F₂₅₄ (Kassel, Germany). HRTOFMS spectra were recorded on a Micromass QTOF-2 (Milford, MA) or an Agilent 6540 Q-TOF (Santa Clara, CA) or an Agilent 6460 Triple Quadrupole (Santa Clara, CA) or a Bruker Q-TOF mass spectrometer (Bremen, Germany).

Plant Materials. The initial collection of root and stem samples of *Justicia gendarussa* Burm. f. (Acanthaceae) was made in 2006 in the “Cave of Prehistoric Man” area, close to hotspot 33 of Cuc Phuong National Park, with geographic coordinate readings of 20° 21' 633" N 105° 38' 257" E. A larger amount of the plant sample for the current isolation work, consisting of the same plant part (bark

and stem) (4.0 kg), was subsequently recollected from plants located in the same area. The recollection of root and stem samples of *J. gendarussa* was made on July 15, 2008 at geographic coordinate readings of 20° 17' 250" N 105° 40' 235" E. Voucher specimens (*M.V. Xinh* #742 for the initial collection and *N.M. Cuong* #2162 for the recollection) were collected and are in deposit at the Herbarium of Cuc Phuong National Park.

Extraction and Isolation. The dried, milled plant material (4.0 kg) was extracted with MeOH (3×10 L) to afford 155 g of extract (SVA5614) following evaporation of the solvent. A small portion of the MeOH extract (5.5 g) was rapidly fractionated to 22 fractions (Fa1-Fa22) in a 1 h period over a silica gel (10 g) column eluting with gradient CHCl₃, CHCl₃/acetone, and CHCl₃/MeOH solutions. Anti-HIV evaluation of the fractions determined that the fractions Fa14-Fa18 inhibited HIV replication by more than 95 % at a concentration of 0.1 µg/mL without apparent cytotoxicity at 20 µg/mL. Scale-up fractionation of SVA5614 was carried out to isolate the anti-HIV active compound(s) in the extract. The MeOH extract (140 g) was absorbed onto 214 g silica gel, and chromatographed over a silica gel column (1545 g). The column was developed by gradient elution with CHCl₃, CHCl₃/acetone, and CHCl₃/acetone/MeOH solutions to afford 40 fractions (F1-F40). Fraction F26 showed similar TLC and HPLC profiles to those of Fa16. A portion (120 mg) of F26 was thus subjected to preparative HPLC separation on a Phenomenex LUNA-C-18 column (solvent system: MeOH/H₂O 65:35) to yield eight fractions (F41-F48). Further bioassaying showed that fraction F47 inhibited HIV replication by more than 90 % at a concentration of 0.2 µg/mL. The remaining portion of fraction F26 (2.93 g) was chromatographed on a pre-packed RP-18 silica gel (40 µm, 275 Å, J. T. Baker) (7.3 g) flash column, eluting with a gradient solvent mixture of MeOH/H₂O to afford fractions F50-F54. Fractions F51 (670 mg) and F52 (890 mg) were pooled and subjected to a preparative HPLC separation (Phenomenex LUNA-C-18 column; solvent system: MeOH/H₂O 65:35), which resulted in 12 fractions (F55-F66).

Fraction F62 showed similar TLC and HPLC profiles as that of F47. Further HPLC separation (Phenomenex LUNA-C-18 column; solvent system: MeCN/H₂O 35:65) of F62 (139 mg) led to the isolation of patentiflorin A (**1**) (29.6 mg): white powder, $[\alpha]_{\text{D}}^{20} = -51.2$ (*c* 0.6, MeOH); ¹H and ¹³C NMR data, see Table S1, Supporting Information.

Production of HIV Pseudovirions. HIV/VSV-G or HIV/HA virions were produced, respectively, by co-transfecting with either 0.5 μg VSV-G envelope expression plasmid or 0.5 μg hemagglutinin (HA) envelope expression plasmid with 0.5 μg neuraminidase (NA) expression plasmid and 2 μg replication-defective HIV vector (pNL4-3-Luc-RE) into human embryonic kidney 293T cells (90% confluent) in six-well plates via PEI (Invitrogen), as previously described with a modified procedure.²⁰ The HIV vector pNL4-3.Luc. R.E. was obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH).^{38,39} Sixteen hours post-transfection, all media were replaced with fresh, complete DMEM. Eight hours post-transfection, all media were replaced with fresh, complete DMEM. Forty-eight hours post-transfection, the supernatants were collected and filtered through a 0.45-μm-pore size filter (Millipore) and the pseudovirions were directly used for infection.

“One-Stone-Two-Birds” Anti-HIV Evaluation Assay. This protocol is designed to identify potential inhibitors for HIV replication (post-entry steps) (Figure S1, Supporting Information). In this system, the HIV vector pNL4-3.Luc. R.E. was co-transfected with the VSV-G to generate HIV/VSV-G virions, and the same HIV vector was co-transfected with the H5N1 HA and NA constructs to generate HIV virions with bird flu HA on the viral surface (HIV/HA). This pNL4-3 was derived from an infectious molecular clone of a SI, T-tropic virus,⁴⁰ which is replication deficient since the HIV is Env⁻ and Vpr⁻. In addition, the luciferase gene (luc) carried by this recombinant HIV vector served as the reporter for HIV replication (reverse transcription, integration and HIV gene expression). The infection

level was measured as relative light units (RLUs) in the infected cells. The luciferase activities of the 293T cells infected with the HIV vector pNL4-3.Luc. R.E. reached the range of 10^5 - 10^6 RLUs, approximately 100-fold higher than the background levels when measured using the HIV virions without VSV-G. The evaluation principle is that the level of the luciferase activity in the cells should be proportional to the level of viral entry and replication. If a compound (or fraction) can interfere with HIV replication/or HA-mediated viral entry, the level of the luciferase activity in the infected cells will be reduced. Thus, using this protocol, we were able to identify fractions or compounds capable of inhibiting HIV replication. The test fractions or compounds were evaluated as follows. Target A549 human lung cells were seeded at 0.5×10^5 cells per well (24-well plate) in complete DMEM. The lung cell line was used since it is susceptible to HA-mediated viral entry. The stock HIV/VSV-G or HIV/HA virions (approximately 2×10^6 relative light units, or RLUs, on the target cells) were mixed with the individual sample first, and the mixture was incubated with the A549 target cells for 24 hours. Ten microliter of each sample in varying concentrations and 190 μ L of the pseudovirus were incubated with target cells. Twenty-four (24) hours post-infection, all media containing sample and virus was removed from target cells and replaced with fresh and complete DMEM. Forty-eight (48) hours post-infection, the target cells were lysed and the luciferase activity was determined.

Cytotoxicity Assay. Approximately 5000 cells seeded in a 96-well tissue culture plate in DMEM supplemented with FBS and penicillin/streptomycin. DMSO alone or compounds in 100% DMSO were added on the following day at appropriate concentrations and incubated with cells at 37 °C in 5% CO₂ for 24 h. After 24 h, all media were removed and replaced with 100 μ L fresh complete DMEM. After 48 h post initial addition of DMSO or compound, 20 μ L of CellTiter 96 Aqueous One Solution was added per well. After gentle mixing, plates were incubated at 37 °C in 5% CO₂ for 4 h. Twenty-

five μL of a 10% SDS solution was added per well and plates were stored at room temperature for approximately 12 h. Absorbance was measured at 450 nm using a plate reader.

HIV Gene Expression Assay of the R/U5 and U5/gag Transcripts. A549 target cells were challenged with VSV- G/HIV pseudovirus while concurrently being treated with either purified patentiflorin A (**1**) or AZT (10-fold dilutions beginning with 5 $\mu\text{g}/\text{mL}$) in triplicate. Forty-eight hours post-infection/treatment, cells were prepared for real-time PCR according to Power SYBR Green Cells-to- C_T kit (Applied Biosystems). Transcript products were detected with the following probes: 5'GGCTAACTAGGGAACCCACTGC, 5'CAACAGACGGGCACACACTACT, 5' AGATCCCTCAGACCCTTTTAGTCA, 5' CTTTCGCTTTCAAGTCCGTGTT. GAPDH and 18S served as internal controls.

Anti-HIV Assay against Broad Spectrum HIV Isolates, Normal, and Resistant Strains. Four HIV1 clinical strains, BAL and SF162 (M-tropic), BAL (T-tropic), and 89.6 (a dual tropic strain), as well as HIV-1_{LAV} (wild type), NRTI (nucleoside reverse transcriptase inhibitor)-resistant isolate (HIV-1₁₆₁₇₋₁) (AZT resistant strain from AIDS repository) and NNRTI (non-nucleoside reverse transcriptase inhibitor)-resistant isolate (HIV-1_{N119}) (nevirapine resistant strain from AIDS repository) were used in the current study. A standardized human peripheral blood mononuclear cell culture (PBMC) assay was used to determine the compound susceptibility of these HIV-1 strains.²⁸ AZT, an anti-HIV drug in clinical use, was used as a positive control. All data were generated from three independent experiments, each performed in triplicate. Prior to HIV-1 infection, human PBMCs were used in each experiment. Briefly, donor PBMCs were suspended in R-3 medium [RPMI 1640 medium supplemented with 15-20% FBS (fetal bovine serum), 5% IL-2 (human interleukin-2), 250 U of penicillin per mL, 250 μg of streptomycin per mL and 2 mM L-glutamine] was stimulated with PHA (phytohaemagglutinin, 2-3 $\mu\text{g}/\text{mL}$) for seven days. The preparations (compound or fraction) were

added to the cultured cells, and the different HIV-1 strains were used to challenge the cultured cells in 96-well plates [1×10^5 cells per well with 1000 TCID₅₀ (virus 50% tissue culture infectious doses) of HIV strain]. After seven days of incubation, the supernatants were collected and the HIV p24 levels of the infected cells were determined using a p24 antigen ELISA. To measure IC₅₀ values, each drug was tested using seven concentrations (5, 1, 0.2, 0.04, 0.008, 0.016 and 0 μ g/mL). The IC₅₀s were calculated by comparing p24 antigen values for the compounds (fraction)-containing wells with those for no drug control wells. For the p24 assay, the maximum cutoff should be around 120-150 pg/mL.

Chemical Synthesis of Patentiflorin A (1) and Its Glycoside Congeners (15 and 16). A schematic illustration for the synthesis of patentiflorin A (**1**) is summarized in Schemes 1 and 2. The same synthesis approach was used to synthesize two patentiflorin A congener diphyllin glycosides: 7 β -D-xyloxyloxydiphyllin (**15**) and 7 β -D-glucopyranosyloxydiphyllin (**16**) by replacing D-quinovose with D-xylose and D-glucose in Scheme 2, respectively. All starting materials and reagents are commercially available. Intermediates and final products were purified by silica gel column chromatography, and analyzed via NMR and MS data. The aglycone diphyllin (**10**) is the key intermediate to the synthesis of patentiflorin A and its congeners.

2-Bromo-4, 5-dimethoxybenzaldehyde (3). 3, 4-Dimethoxybenzaldehyde (veratral, **2a**) (10.5 g, 63.3 mmol) was dissolved in dry, degassed MeOH (250 mL) and stirred under argon. To this solution was added Br₂ (3.56 mL, 69.1 mmol), and the reaction mixture was stirred for 6 h at rt. After the completion of the reaction, as indicated by TLC, solvent was removed through evaporation and the resulting mixture was partitioned between CH₂Cl₂ (250 mL) and a saturated solution of Na₂S₂O₃ (250 mL). The aqueous layer was extracted with CH₂Cl₂ (2 \times 150 mL), and the combined organic layers were washed with water (250 mL), brine (250 mL), and dried over anhydrous Na₂SO₄. After the solvent was removed, the residue was purified by silica gel chromatography, eluting with 20% EtOAc

in *n*-hexane, to afford 2-bromo-4, 5-dimethoxybenzaldehyde (**3**) as a white foam (13.4 g, 86%): ¹H NMR (CDCl₃, 500 MHz) δ 10.18 (1H, s, H-7), 7.41 (1H, s, H-6), 7.06 (1H, s, H-3), 3.97 (3H, s, OCH₃), 3.92 (3H, s, OCH₃); ¹³C NMR (CDCl₃, 125 MHz) δ 190.9 (CH, C-7), 154.6 (C, C-4), 149.0 (C, C-5), 126.6 (C, C-1), 120.5 (C, C-2), 115.5 (CH, C-6), 110.5 (CH, C-3), 56.6 (CH₃, OCH₃), 56.3 (CH₃, OCH₃).

2-(2-Bromo-4, 5-dimethoxyphenyl)-[1, 3]dithiane (**4**). To a flask containing dry benzene (300 mL), the bromoaldehyde (**3**) (12.3 g, 50.2 mmol), 1, 3-propanedithiol [HS(CH₂)₂SH, 5.04 mL, 50.2 mmol], and *p*-toluenesulfonic acid (0.48 g, 2.79 mmol) were added. The flask was connected to a Dean-Stark trap and refluxed for 10 h. The solvent was removed *in vacuo*, and the residue was partitioned between Et₂O (150 mL) and 1 M NaOH (150 mL). The organic layer was washed with 1 M NaOH (150 mL), H₂O (250 mL), brine (250 mL), and dried over anhydrous Na₂SO₄. After the solvent was removed, the residue was purified by silica gel chromatography, eluting with 20% EtOAc in *n*-hexane to yield 2-(2-bromo-4, 5-dimethoxyphenyl)-[1, 3]dithiane (**4**) (15.6 g, 93%) as a white foam: ¹H NMR (CDCl₃, 500 MHz) δ 7.16 (1H, s, H-3), 6.99 (1H, s, H-6), 5.53 (1H, s, H-7), 3.90 (3H, s, OCH₃), 3.85 (3H, s, OCH₃), 3.12 (2H, brt, *J* = 13.0 Hz, H-S1a and H-S3a), 2.90 (2H, brd, *J* = 11.5 Hz, H-S1b and H-S3b), 2.18 (1H, brdt, *J* = 12.0, 2.0 Hz, H-S2a), 1.93 (1H, brqd, *J* = 11.5, 3.0 Hz, H-S2b); ¹³C NMR (CDCl₃, 125 MHz): δ 149.5 (C, C-5), 149.0 (C, C-4), 130.3 (C, C-1), 115.3 (CH, C-3), 113.0 (C, C-2), 111.9 (CH, C-6), 56.29 (CH₃, OCH₃), 56.27 (CH₃, OCH₃), 50.6 (CH, C-7), 32.4 (2C, CH₂, C-S1 and C-S3), 25.1 (CH₂, C-S2).

Benzo[1, 3]dioxol-5-yl-(2-[1, 3]dithian-2-yl-4, 5-dimethoxyphenyl)methanol (**5**). To a solution of the dithiane (**4**) (15.6 g, 46.6 mmol) in dry THF (150 mL) was added *n*-BuLi (43.7 mL, 1.6 M solution in *n*-hexane, 69.9 mmol) at -78 °C under argon, and the mixture was stirred for 1 h. A solution of piperonal (**2b**, 8.39 g, 55.9 mmol) in dry THF (30 mL) was added at -78 °C. The mixture was stirred

for 2 h and allowed to warm up to rt over 3 h. The reaction was quenched with a saturated solution of NH_4Cl (100 mL), and the mixture was extracted with Et_2O (3×80 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 , and concentrated. The residue was purified by silica gel chromatography, eluting with 20-40% EtOAc in *n*-hexane to afford the diphenyl alcohol **5** as a white foam (9.5 g, 50 %): ^1H NMR (CDCl_3 , 400 MHz) δ 7.12 (1H, s, H-3), 6.86 (1H, brd, $J = 7.6$ Hz, H-6'), 6.85 (1H, s, H-6), 6.83 (1H, s, H-2'), 6.12 (1H, brs, H-7'), 5.94 (2H, s, $-\text{OCH}_2\text{O}-$), 5.39 (1H, s, H-7), 3.90 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 2.96 (2H, brt, $J = 12.8$ Hz, H-S1a and H-S3a), 2.80 (2H, brd, $J = 13.6$ Hz, H-S1b and H-S3b), 2.14 (1H, brd, $J = 14.4$ Hz, H-S2a), 1.89 (1H, brq, $J = 14.0$ Hz, H-S2b); ^{13}C NMR (CDCl_3 , 100 MHz) δ 148.9 (C, C-3'), 148.8 (C, C-4'), 147.7 (C, C-4), 146.8 (C, C-5), 137.2 (C, C-1'), 133.4 (C, C-2), 128.9 (C, C-1), 119.9 (CH, C-6'), 111.4 (CH, C-6), 110.9 (CH, C-3), 108.1 (CH, C-5'), 107.4 (CH, C-2'), 101.1 (CH_2 , $-\text{OCH}_2\text{O}-$), 72.0 (CH, C-7'), 56.1 (CH_3 , OCH_3), 56.0 (CH_3 , OCH_3), 47.9 (CH, C-7), 32.6 (CH_2 , C-S1 or C-S3), 32.5 (CH_2 , C-S3 or C-S1), 25.1 (CH_2 , C-S2).

Benzo[1,3]dioxol-5-yl-(2-[1,3]dithian-2-yl-4,5-dimethoxyphenyl)methanone (6). The diphenyl alcohol **5** (8.4 g, 20.7 mmol) was stirred with activated MnO_2 (30.0 g, 345 mmol) in CH_2Cl_2 (200 mL) at rt under nitrogen for 16 h. The solution was filtered through a plug of celite, and washed with CHCl_3 (300 mL). The filtrate was subsequently evaporated to dryness to afford the diphenyl ketone **6** as a white foam (8.2 g, 98%): ^1H NMR (CDCl_3 , 400 MHz) δ 7.39 (1H, brd, $J = 1.2$ Hz, H-2'), 7.35 (1H, brdd, $J = 8.0, 1.6$ Hz, H-6'), 7.31 (1H, s, H-3), 6.84 (1H, d, $J = 8.0$ Hz, H-5'), 6.80 (1H, s, H-6), 6.07 (2H, s, $-\text{OCH}_2\text{O}-$), 5.45 (1H, s, H-7), 3.99 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 2.94 (2H, brtd, $J = 12.4, 1.5$ Hz, H-S1a and H-S3a), 2.82 (2H, brdt, $J = 14.4, 3.6$ Hz, H-S1b and H-S3b), 2.10 (1H, brdq, $J = 14.0, 1.6$ Hz, H-S2a), 1.88 (1H, brqt, $J = 12.4, 2.8$ Hz, H-S2b); ^{13}C NMR (CDCl_3 , 100 MHz) δ 195.0 (C, C-7'), 152.2 (C, C-4'), 151.1 (C, C-5), 148.2 (C, C-3'), 147.8 (C, C-4), 132.7 (C, C-1), 131.7 (C, C-1'), 129.9 (C, C-2), 127.6 (CH, C-2'), 112.1 (CH, C-6'), 111.9 (CH, C-3), 109.6 (CH, C-6), 107.9

(CH, C-5'), 102.1 (CH₂, -OCH₂O-), 56.3 (CH₃, OCH₃), 56.2 (CH₃, OCH₃), 47.7 (CH, C-7), 32.4 (2C, CH₂, C-S1 and C-S3), 25.1 (CH₂, C-S2).

9-Benzo[1, 3]dioxol-5-yl-9-hydroxy-4-([1, 3]-dithian-2-yl)-6, 7-dimethoxy-3a, 4, 9, 9a-tetrahydro-3H-naphtho[2, 3-c]furan-1-one (8). *n*-Butyllithium (1.6 M solution in hexanes, 62.5 mL, 100 mmol) was added dropwise over 5 min to a cooled (-78 °C) solution of diisopropylamine (14.1 mL, 100 mmol) in THF (43 mL) under argon. The solution was warmed to ambient temperature over 30 min to afford lithium diisopropylamide (LDA). The freshly prepared solution (25.7 mL) was added, dropwise via syringe over 3 min to a cooled (-78 °C) THF solution (60 mL) of the diphenyl ketone **6** (5.78 g, 14.3 mmol). After 40 min, 2(*5H*)-furanone (**7**) (1.44 g, 17.3 mmol) in THF (10 mL) was added to the solution over 1 min. The reaction mixture was warmed to rt for 1 h, and quenched with H₂O (2 mL). The solvent was removed by evaporation under vacuum. The residue was purified by silica gel chromatography by eluting with 10 % MeOH in CH₂Cl₂ to yield the lactone **8** (3.95 g, 57% yield) as a white powder: ¹H NMR (CDCl₃, 500 MHz) δ 7.58 (1H, s, H-3), 6.93 (1H, dd, *J* = 8.0, 1.5 Hz, H-6'), 6.87 (1H, d, *J* = 1.5 Hz, H-2'), 6.77 (1H, d, *J* = 8.0 Hz, H-5'), 6.40 (1H, s, H-6), 5.95 (2H, s, -OCH₂O-), 4.41 (1H, brt, *J* = 8.0 Hz, H-9a), 4.20 (1H, dd, *J* = 12.0, 9.0 Hz, H-9b), 3.96 (3H, s, 5-OCH₃), 3.87 (1H, dt, *J* = 12.0, 7.5 Hz, H-8), 3.67 (3H, s, 4-OCH₃), 3.40 (1H, d, *J* = 6.0 Hz, H-8'), 3.22 (1H, brtd, *J* = 12.5, 2.5 Hz, H-S1a or H-S3a), 3.14 (1H, brtd, *J* = 12.5, 2.0 Hz, H-S3a or H-S1a), 2.90 (1H, brdt, *J* = 11.5, 3.0 Hz, H-S1b or H-S3b), 2.78 (1H, brdt, *J* = 11.5, 3.0 Hz, H-S3b or H-S1b), 2.34 (1H, brs, 7'-OH), 2.23 (1H, brd, *J* = 14.0 Hz, H-S2a), 2.01 (1H, brq, *J* = 13.0 Hz, H-S2b); ¹H NMR (DMSO-d₆, 400 MHz) δ 7.44 (1H, s, H-3), 6.85 (1H, d, *J* = 8.0 Hz, H-5'), 6.81 (1H, d, *J* = 1.2 Hz, H-2'), 6.75 (1H, dd, *J* = 8.0, 1.6 Hz, H-5'), 6.41 (1H, s, H-6), 6.04 (1H, brs, 7'-OH), 6.00 (2H, s, -OCH₂O-), 4.30 (1H, m, H-8), 4.02 (2H, m, H₂-9), 3.78 (3H, s, 5-OCH₃), 3.52 (3H, s, 4-OCH₃), 3.43 (1H, brtd, *J* = 12.8, 2.0 Hz, H-S1a or H-S3a), 3.27 (1H, brt, *J* = 12.8 Hz, H-S3a or H-S1a), 3.09 (1H, brd, *J* = 6.0 Hz, H-8'),

2.87 (1H, brd, $J = 14.4$ Hz, H-S1b or H-S3b), 2.72 (1H, brd, $J = 14.8$ Hz, H-S3b or H-S1b), 2.14 (1H, brd, $J = 14.0$ Hz, H-S2a), 1.76 (1H, brq, $J = 13.2$ Hz, H-S2b); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 174.2 (C, C-9'), 148.8 (C, C-4'), 148.6 (C, C-3'), 146.7 (C, C-4), 145.7 (C, C-5), 143.6 (C, C-1'), 133.8 (C, C-2), 126.4 (C, C-1), 119.3 (CH, C-6'), 112.6 (CH, C-6), 109.5 (CH, C-3), 107.4 (CH, C-5'), 106.8 (CH, C-2'), 101.0 (CH₂, -OCH₂O-), 70.0 (C, C-7'), 68.3 (CH₂, C-9), 55.5 (CH₃, OCH₃), 55.4 (CH₃, OCH₃), 51.0 (CH, C-8'), 49.0 (C, C-7), 41.1 (CH, C-8), 28.2 (CH₂, C-S1 or C-S3), 26.2 (CH₂, C-S3 or C-S1), 23.7 (CH₂, C-S2).

9-Benzo[1, 3]dioxol-5-yl-9-hydroxy-6, 7-dimethoxy-3, 3a, 9, 9a-tetrahydronaphtho[2, 3-c]furan-1, 4-dione (9). A solution of the lactone **8** (315 mg, 0.65 mmol), HgO (153 mg, 0.71 mmol) and HgCl₂ (388 mg, 1.43mmol) in aqueous MeCN (85%, 30 mL) was refluxed for 3 h. The reaction solution was then partitioned between CHCl₃ and saturated (NH₄)₂CO₃. The organic layer was washed with brine, dried over MgSO₄, concentrated, followed by silica gel column chromatography, eluting with 10 % MeOH in CH₂Cl₂ to yield the benzofuranone **9** as a yellowish powder (134 mg, 52%): ^1H NMR (CDCl₃, 400 MHz) δ 7.46 (1H, s, H-6), 7.23 (1H, s, H-3), 6.80 (1H, d, $J = 1.9$ Hz, H-2'), 6.62 (1H, d, $J = 8.2$ Hz, H-5'), 6.38 (1H, dd, $J = 8.2, 2.0$ Hz, H-6'), 5.91 (2H, s, -OCH₂O-), 5.72 (1H, brs, 7'-OH), 4.70 (1H, d, $J = 9.2$ Hz, H-9a), 4.27 (1H, dd, $J = 9.2, 5.8$ Hz, H-9b), 3.94 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 3.42 (1H, d, $J = 7.4$ Hz, H-8'), 3.06 (1H, dd, $J = 7.4, 5.6$ Hz, H-8); ^{13}C NMR (CDCl₃, 100 MHz) δ 192.9 (C, C-7), 176.8 (C, C-9'), 155.7 (C, C-4), 149.5 (C, C-5), 148.1 (C, C-4'), 147.5 (C, C-3'), 141.9 (C, C-1'), 138.3 (C, C-1), 125.4 (C, C-2), 120.0 (CH, C-6'), 108.7 (CH, C-3), 108.0 (CH, C-6), 107.8 (CH, C-5'), 106.8 (CH, C-2'), 101.3 (CH₂, -OCH₂O-), 72.8 (C, C-7'), 70.7 (CH₂, C-9), 56.4 (CH₃, OCH₃), 56.1 (CH₃, OCH₃), 50.3 (CH, C-8'), 46.0 (CH, C-8).

Diphyllin (10). The benzofuranone **9** (134 mg, 0.34 mmol) and *p*-toluenesulfonic acid (37 mg, 0.22 mmol) were refluxed in benzene (15 mL) for 16 h. The solvent was removed by evaporation

under vacuum. The residue was purified by silica gel column chromatography, eluting with 50% EtOAc in *n*-hexane to yield diphyllin (**10**) as a yellowish powder (106 mg, 82%).⁴¹

Tetra-O-acetyl-D-quinovose (12). To a solution of D-quinovose (**11**, 100 mg, 0.61 mmol) in pyridine (5 mL) was added DMAP (7.5 mg, 0.06 mmol) and Ac₂O (1.5 mL) at rt. The reaction mixture was stirred overnight, and quenched with MeOH (1 mL). Evaporation of the solvent to dryness afforded the tetraacetate **12** as a white powder (203 mg, 100 %): ¹H NMR (CDCl₃, 400 MHz) δ 6.27 (1H, d, *J* = 4.0 Hz, H-1''), 5.43 (1H, t, *J* = 10.0 Hz, H-2''), 5.06 (1H, dd, *J* = 10.4, 3.6 Hz, H-3''), 4.86 (1H, t, *J* = 10.0 Hz, H-4''), 4.02 (1H, m, H-5''), 2.17 (3H, s, COCH₃), 2.06 (3H, s, COCH₃), 2.03 (3H, s, COCH₃), 2.01 (3H, s, COCH₃), 1.20 (3H, d, *J* = 6.0, 6''-CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 170.3 (C, COCH₃), 169.8 (C, COCH₃), 169.7 (C, COCH₃), 169.1 (C, COCH₃), 91.7 (CH, C-1''), 73.3 (CH, C-5''), 69.9 (CH, C-3''), 69.6 (CH, C-4''), 67.9 (CH, C-2''), 21.0 (CH₃, COCH₃), 20.8 (CH₃, COCH₃), 20.7 (CH₃, COCH₃), 20.5 (CH₃, COCH₃), 17.4 (CH₃, 6''-CH₃).

Tri-O-acetyl-D-quinovosyl bromide (13). The tetraacetate **12** (203 mg) was dissolved in glacial HOAc (1 mL) to which 1.5 mL of 33% HBr-HOAc was added slowly at rt with stirring. After stirring for 15 min, the solution was dissolved in CH₂Cl₂ (20 mL) and extracted with H₂O and 1 M aqueous NaHCO₃. The CH₂Cl₂ layer was dried over Na₂SO₄ and concentrated *in vacuo* to give the brominated quinovose **13** (215 mg, 99% yield from D-quinovose): ¹H NMR (CDCl₃, 400 MHz) δ 6.58 (1H, d, *J* = 4.0 Hz, H-1''), 5.52 (1H, t, *J* = 9.6 Hz, H-2''), 4.89 (1H, t, *J* = 9.6 Hz, H-4''), 4.80 (1H, dd, *J* = 10.0, 4.0 Hz, H-3''), 4.19 (1H, m, H-5''), 2.10 (3H, s, COCH₃), 2.07 (3H, s, COCH₃), 2.03 (3H, s, COCH₃), 1.26 (3H, d, *J* = 6.4, 6''-CH₃).

Tri-O-acetylpatentiflorin A (14). To a solution of diphyllin (**10**) (360 mg, 0.95 mmol) and tetrabutylammonium bromide (TBAB) (306 mg, 0.95 mmol) in CHCl₃ (15 mL) was added 20 mL of aqueous 0.1 M NaOH. After stirring for 10 min at 40 °C, the quinovosyl bromide (**13**) (215 mg, 0.61

mmol) was added. After the two-phase reaction mixture was stirred for 6 h at 40 °C, CHCl₃ (20 mL) was added. The organic phase was washed with H₂O, dried over Na₂SO₄, and evaporated under reduced pressure. The residue was purified by flash silica gel chromatography, eluting with EtOAc-petroleum ether (1:1) to afford **14** as a white powder (247 mg, 62%): ¹H NMR (CDCl₃, 400 MHz) δ 7.543/7.539 (1H, s, H-6), 7.08 (1H, s, H-3), 6.96 (1H, d, *J* = 8.0, H-5'), 6.82 (1H, overlap, H-6'), 6.79 (1H, overlap, H-2'), 6.10/6.05 (2H, s, -OCH₂O-), 5.48 (1H, d, *J* = 8.0 Hz, H-1''), 5.46 (1H, d, *J* = 14.0, H-9a), 5.43/5.42 (1H, d, *J* = 14.4, H-9b), 5.28 (1H, t, *J* = 9.6 Hz, H-2''), 5.13 (1H, dd, *J* = 8.0, 0.8 Hz, H-3''), 5.00 (1H, t, *J* = 9.6 Hz, H-4''), 4.07 (3H, s, 5-OCH₃), 3.81 (3H, s, 4-OCH₃), 3.70 (1H, m, H-5''), 2.12 (3H, s, COCH₃), 2.07 (3H, s, COCH₃), 2.06 (3H, s, COCH₃), 1.27 (3H, d, *J* = 6.8, 6''-CH₃).

Patentiflorin A (1).²³ To a MeOH solution (10 mL) of compound **14** (247 mg), K₂CO₃ (138 mg, 1.0 mmol) was added. After stirring for 60 min at rt, the reaction solution was neutralized with 1 M HCl and concentrated under reduced pressure to afford a solid residue that was dissolved in CHCl₃ (10 mL). The resulting CHCl₃ was washed with H₂O and dried over Na₂SO₄. After evaporation of the solvent, the residue was purified by silica gel flash chromatography, eluting with 5% MeOH in CH₂Cl₂ to afford patentiflorin A (**1**) as a yellowish powder (122 mg, 38 % yield from **10**): [α]_D²⁰ -53.2 (*c* 0.7, MeOH); ¹H NMR (methanol-*d*₄, 360 MHz) δ 8.049/8.039 (1H, s, H-6), 6.968/6.945 (1H, s, H-3), 6.89 (1H, d, *J* = 8.1 Hz, H-5'), 6.725/6.648 (1H, d, *J* = 2.6 Hz, H-2'), 6.677/6.655 (1H, dd, *J* = 8.0, 1.7, H-6'), 6.027/6.016 (2H, m, -OCH₂O-), 5.536/5.533 (1H, d, *J* = 15.2, H-9a), 5.409/5.404 (1H, d, *J* = 15.2, H-9b), 4.761/4.759 (1H, d, *J* = 7.8 Hz, H-1''), 3.972/3.968 (3H, s, 5-OCH₃), 3.668/6.662 (3H, s, 4-OCH₃), 3.640/3.636 (1H, dd, *J* = 9.3, 8.0 Hz, H-2''), 3.409 (1H, t, *J* = 9.2 Hz, H-3''), 3.270 (1H, m, H-5''), 3.137 (1H, t, *J* = 9.2 Hz, H-4''), 1.303/1.301 (3H, d, *J* = 6.1, 6''-CH₃); ¹³C NMR (methanol-*d*₄, 90 MHz) δ 172.14 (C, C-9'), 153.25/153.22 (C, C-5), 151.63/151.60 (C, C-4), 148.94 (C, C-4'), 148.94 (C,

C-3'), 146.34 (C, C-7), 137.48 (C, C-7'), 132.20 (C, C-8), 131.79 (C, C-2), 129.89/129.86 (C, C-1'), 128.84 (C, C-1), 124.76/124.71 (CH, C-6), 119.89/119.83 (C, C-8'), 111.78/111.76 (C, C-2'), 108.94/108.89 (CH, C-5'), 106.83 (CH, C-3), 106.66 (CH, C-1''), 102.68/102.60 (CH₂, -OCH₂O-), 77.81 (CH, C-3''), 76.70 (CH, C-4''), 75.70 (CH, C-2''), 73.64 (CH, C-5''), 69.10 (CH₂, C-9), 56.69 (CH₃, 5-OCH₃), 55.98 (CH₃, 4-OCH₃), 18.20 CH₃, C-6''); positive ESIMS m/z 527.02 [M + H]⁺ (calcd for C₂₇H₂₇O₁₁, 527.16).

7β-D-xylosyloxydiphyllin (15, diphyllinin):²⁶ A yellowish powder (9.6 mg); ¹H NMR (CDCl₃, 400 MHz) δ 7.86 (1H, s, H-6), 7.06 (1H, brs, H-5'), 6.93 (1H, s, H-3), 6.73-6.85 (2H, m, H-2' and H-6'), 6.070/6.024 (2H, m, -OCH₂O-), 5.44 (1H, brd, J = 15.8, H-9a), 5.42 (1H, d, J = 15.7, H-9b), 4.81 (1H, brs, H-1''), 4.02 (3H, s, 5-OCH₃), 3.83 (1H, m, H-5''a), 3.78 (3H, s, 4-OCH₃), 3.52-3.65 (2H, m, H-2'' and H-4''), 3.14-3.25 (2H, m, H-3'' and H-5''); positive HRESIMS m/z 513.1386 [M + H]⁺ (calcd for C₂₆H₂₅O₁₁, 513.1397).

7β-D-glucopyranosyloxydiphyllin (16, cleristanthin B):²⁷ A yellowish powder (11.2 mg); ¹H NMR (methanol-*d*₄, 400 MHz) δ 8.16 (1H, s, H-6), 7.05 (1H, brs, H-5'), 6.95 (1H, s, H-3), 6.68-6.88 (2H, m, H-2' and H-6'), 6.03 (2H, s, -OCH₂O-), 5.77 (1H, brd, J = 15.4, H-9a), 5.47 (1H, d, J = 15.4, H-9b), 4.83 (1H, brs, H-1''), 4.01 (3H, s, 5-OCH₃), 3.90 (1H, m, H-6''a), 3.72 (3H, s, 4-OCH₃), 3.58-3.70 (2H, m, H-2'' and H-6''b), 3.36-3.53 (2H, m, H-3'' and H-5''), 3.26 (1H, m, H-5''); positive HRESIMS m/z 543.1503 [M + H]⁺ (calcd for C₂₇H₂₇O₁₂, 543.1397).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: xx.xxxx/acs.jnat-prod.xxxxxxx.

¹H and ¹³C NMR data of **1**, “One-Stone-Two-Birds” anti-HIV evaluation system, 1D and 2D NMR, IR, MS spectra of **1**, and NMR and MS spectra of the synthetic compounds (**3-6**, **8-10** and **13-16**) (PDF)

AUTHOR INFORMATION

Corresponding Authors

*Tel: +852-34112956. Fax: +852-34112461. E-mail: zhanghj@hkbu.edu.hk (H.-J. Zhang).

*Tel: 312-3550203. E-mail: lijun@uic.edu (L. Rong).

Author Contributions

^oH.-J. Zhang and E. Rumschlag-Booms contributed equally to this work

ORCID

Hong-Jie Zhang: 0000-0002-7175-5166

Harry H. S. Fong: 0000-0002-0615-6335

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This project was supported by the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. HKBU 262912), the Health and Medical Research Fund (12132161) of the Food and Health Bureau, Hong Kong SAR, the Hong Kong Baptist University (HKBU) Interdisciplinary Research Matching Scheme (RC-IRMS/15-16/02), Faculty Research Grants, Hong Kong Baptist University (FRG2/11-12/134 and FRG2/14-15/047), NIH Grants 3U01TW001015-10S1 and 2U01TW001015-11A1, administered by the Fogarty International Center as part of an International Cooperative Biodiversity Groups (ICBG) program, through funds from NIH, NSF, and

Foreign Agricultural Service of the USDA, and Mr. Kwok Yat Wai and Madam Kwok Chung Bo Fun Graduate School Development Fund. The authors are grateful to the Molecular Infectious Disease Core facility at Rush University Medical Center, Chicago, Illinois, USA for conducting the anti-HIV assays against the wild type and resistant isolates in the PBMC.

REFERENCES

- (1) CDC (Centers for Disease Control and Prevention, USA), *Morbidity and Mortality Weekly Report (MMWR)* **1981**, 30, 250.
- (2) Barré-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vézinet-Brun, F.; Rouzioux, C.; Rozenbaum, L.; Montagnier, W. *Science* **1983**, 220, 868–871.
- (3) UNAIDS. Fact Sheet 2016; <http://www.unaids.org/en/resources/campaigns/HowAIDSchangedeverything/factsheet> (accessed March 31, 2017).
- (4) WHO (World Health Organization). Global Health Observatory (GHO) data - HIV/AIDS; <http://www.who.int/gho/hiv/en/> (accessed December 28, 2016).
- (5) UNAIDS. Global Aids Update 2016; http://www.unaids.org/sites/default/files/media_asset/global-AIDS-update-2016_en.pdf (accessed March 31, 2017).
- (6) WHO (World Health Organization). Global Health Observatory (GHO) data - the top 10 causes of death; <http://www.who.int/mediacentre/factsheets/fs310/en/> (accessed March 31, 2017).
- (7) FDA (U.S. Food and Drug Administration). Drugs used in the treatment of HIV infection; <http://www.fda.gov/forpatients/illness/hivaids/ucm118915.htm> (accessed March 31, 2017).
- (8) Thayer, A. M. *C&EN* **2008**, Sep 22, 29–36.

- (9) WHO (World Health Organization). WHO HIV drug resistance report 2012; http://apps.who.int/iris/bitstream/10665/75183/1/9789241503938_eng.pdf (accessed March 31, 2017).
- (10) Cos, P.; Maes, L.; Berghe, D. V.; Hermans, N.; Pieters, L.; Vlietinck, A. *J. Nat. Prod.* **2004**, *67*, 284–293.
- (11) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2016**, *79*, 629–661.
- (12) Kashiwada, Y.; Hashimoto, F.; Cosentino, L. M.; Chen, C. H.; Garrett, P. E.; Lee, K. H. *J. Med. Chem.* **1996**, *39*, 1016–1017.
- (13) Smith, P. F.; Ogundele, A.; Forrest, A.; Wilton, J.; Salzwedel, K.; Doto, J.; Allaway, G. P.; Martin, D. E. *Antimicrobial Agents Chemother.* **2007**, *51*, 3574–3581.
- (14) Kashman, Y.; Gustafson, K. R.; Fuller, R. W.; Cardellinall, J. H.; McMahon, J. B.; Currens, M. J.; Buckheit Jr., R. W.; Hughes, S. H.; Cragg, G. M.; Boyd, M. R. *J. Med. Chem.* **1992**, *35*, 2735–2743.
- (15) Ma, T.; Liu, L.; Xue, H.; Li, L.; Han, C. Y.; Wang, L.; Chen, Z. W.; Liu, G. *J. Med. Chem.* **2008**, *51*, 1432–1446.
- (16) Zechmeister, K.; Brandl, F.; Hoppe, W.; Hecker, E.; Opferkuch, H. J.; Adolf, W. *Tetrahedron Lett.* **1970**, *11*, 4075–4078.
- (17) Fujiwara, M.; Ijichi, K.; Tokuhisa, K.; Katsuura, K.; Shigeta, S.; Konno, K.; Wang, G. Y.; Uemura, D.; Yokota, T.; Baba, M. *Antimicrobial Agents and Chemother.* **1996**, *40*, 271–273.
- (18) Fujiwara, M.; Ijichi, K.; Tokuhisa, K.; Katsuura, K.; Wang, G. Y. S.; Uemura, D.; Shigeta, S.; Konno, K.; Yokota, T.; Baba, M. *Antiviral Chem. Chemother.* **1996**, *7*, 230–236.
- (19) Soejarto, D. D.; Zhang, H. J.; Fong, H. H. S.; Tan, G. T.; Ma, C. M.; Gyllenhaal, C.; Riley, M. C.; Kadushin, M. R.; Franzblau, S. G.; Bich, T. Q.; Cuong, N. M.; Hiep, N. T.; Loc, P.

K.; Xuan, L. T.; Hai, N. V.; Hung, N. V.; Chien, N. Q.; Vu, B. M.; Ly, H. M.; Southavong, B.; Sydara, K.; Bouamanivong, S.; Pezzuto, J. M.; Rose, W.; Dietzman, G.; Miller, B.; Thuy, T. V. *J. Nat. Prod.* **2006**, *69*, 473–481.

(20) Rumschlag-Booms, E.; Zhang, H. J.; Soejarto, D. D.; Fong, H. H. S.; Rong, L. J. *J. Antivir. Antiretrovir.* **2011**, *3*, 8–10.

(21) Jiangsu College of New Medicine. *Dictionary of Chinese Traditional Medicine*; Shanghai Science and Technology Press: Shanghai, 1986; Item 2471.

(22) Kavitha, K.; Sridevisangeetha, K. S.; Sujatha, K.; Umamaheswari. *J. Pharm. Res.* **2014**, *8*, 990–997.

(23) Susplugas, S.; Hung, N. V.; Bignon, J.; Thoison, O.; Kruczynski, A.; Sévenet, T.; Guéritte, F. *J. Nat. Prod.* **2005**, *68*, 734–738.

(24) Yang, M. H.; Wu, J.; Fan, C.; Zhou, Y. *Mag. Res. Chem.* **2006**, *44*, 727–730.

(25) Liu, G. R.; Wu, J.; Si, J. Y.; Wang, J. M.; Yang, M. H. *Mag. Res. Chem.* **2008**, *46*, 283–286.

(26) Gonzalez, A. G.; Moreno, R. O.; Rodrigues, F. L. *Anales de Quimica (1968-1979)* **1974**, *70*, 234–238.

(27) Lakshmi, Mrs. T. G.; Scrimannaryana, G.; Rao, N. V. Subba. *Curr. Sci.* **1970**, *39*, 395–396.

(28) Japour, A. J.; Mayers, D. L.; Johnson, V. A.; Kuritzkes, D. R.; Beckett, L. A.; Arduino, J. M.; Lane, J.; Black, R. J.; Reichelderfer, P. S.; D'Aquila, R. T. *Antimicrob. Agents Chemother.* **1993**, *37*, 1095–1101.

(29) Fujihashi, T.; Hara, H.; Sakata, T.; Mori, K.; Higuchi, H.; Tanaka, A.; Kaji, H.; Kaji, A. *Antimicrob. Agents Chemother.* **1995**, *39*, 2000–2007.

- (30) Richman, D.; Shih, C. K.; Lowy, I.; Rose, J.; Prodanovich, S. G.; Griffin, J. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 11241–11245.
- (31) Greene, W. C. *Ann. Rev. Immun.* **1990**, *8*, 453–475.
- (32) Knipe, D. M.; Howley, P. M. *Fields Virology: 5th Edition*, 2 Vols.; Wolters Kluwer Health/Lippincott Williams & Wilkins: Philadelphia, 2007.
- (33) Zhang, Y. L.; Bao, F. K.; Hu, J. J.; Liang, S. W.; Zhang, Y.; Du, G. H.; Zhang, C. J.; Cheng, Y. X. *Planta Med.* **2007**, *73*, 1596–1599.
- (34) Lee, J. C.; Lee, C. H.; Su, C. L.; Huang, C. W.; Liu, H. S.; Lin, C. N.; Won, S. J. *Carcinogenesis* **2005**, *26*, 1716–1730.
- (35) Tuchinda, P.; Kornsakulkarn, J.; Pohmakotr, M.; Kongsaree, P.; Prabpai, S.; Yoosook, C.; Kasisit, C.; Sophasan, S.; Reutrakul, V. *J. Nat. Prod.* **2008**, *71*, 655–663.
- (36) Chang, C. W.; Lin, M. T.; Lee, S. S.; Liu, K. C.; Hsu, F. L.; Lin, J. Y. *Antivir. Res.* **1995**, *27*, 367–374.
- (37) Ren, Y. L.; Lantvit, D. D.; Deng, Y.; Kanaqasabai, R.; Gallucci, J. C.; Ninh, T. N.; Chai, H. B.; Soejarto, D. D.; Fuchs, J. R.; Yalowich, J. C.; Yu, J.; Swanson, S. M.; Kinghorn, A. D. *J. Nat. Prod.* **2014**, *77*, 1494–1504.
- (38) Connor, R. I.; Chen, B. K.; Choe, S.; Landau, N. R. *Viol.* **1995**, *206*, 935–944.
- (39) He, J.; Choe, S.; Walker, R.; Di Marzio, P.; Morgan, D. O.; Landau, N. R. *J. Virol.* **1995**, *69*, 6705–6711.
- (40) Michael, N. L.; Nelson, J. A.; KewalRamani, V. N.; Chang, G.; O'Brien, S. J.; Mascola, J. R.; Volsky, B.; Loulde, M.; White, G. C. *J. Virol.* **1998**, *72*, 6040–6047.
- (41) Innocenti, G.; Puricelli, L.; Piacente, S.; Caniato, R.; Filippini, R.; Cappelletti, E. M. *Chem. Pharm. Bull.* **2002**, *50*, 844–846.

Table of Contents (TOC)

A Potent Inhibitor to Drug-Resistant HIV-1 Strains Identified from the Medicinal Plant *Justicia gendarussa*

Hong-Jie Zhang,* Emily Rumschlag-Booms, Yi-Fu Guan, Dong-Ying Wang, Kang-Lun Liu, Wan-Fei Li, Van Hung Nguyen, Nguyen Manh Cuong, Djaja Doel Soejarto, Harry H. S. Fong, and Lijun Rong*

