Lamiridosins, Hepatitis C Virus Entry Inhibitors from *Lamium album*

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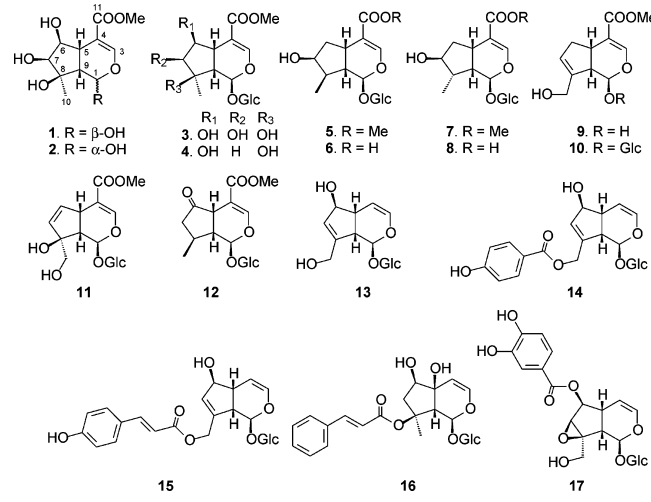
Phytochemical study of the aqueous extract of the flowering tops of *Lamium album* led to identification of the antiviral iridoid isomers lamiridosins A and B (**1**, **2**). These compounds were found to significantly inhibit hepatitis C virus entry (IC_{50} 2.31 μM) in vitro. Studies of 14 iridoid analogues showed that, while the parent iridoid glucosides demonstrated no anti-HCV entry activity, the aglycones of shanzhiside methyl ester (**4**), loganin (**5**), loganic acid (**6**), geniposide (**10**), verbenalin (**12**), eurostoside (**15**), and picroside II (**17**) exhibited significant anti-HCV entry and anti-infectivity activities.

Hepatitis C virus (HCV) is one of five currently identified hepatitis viruses (A, B, C, D, and E). It is a small, enveloped, single-stranded positive RNA virus belonging to the *Hepacivirus* genus (Flaviviridae), and it mainly targets hepatocytes.[1−3] To enter the cells, the virus must pass through their plasma membrane. This step in infection is mediated by interactions between viral glycoproteins E1 and E2 and several host factors. While the exact entry mechanism has yet to be elucidated, several host cell factors involved have been identified. These include the tetraspanin CD81,[4−7] the human scavenger receptor class B type I (SR-BI),[8−10] the tight-junction proteins claudin-1, -6, and -9, and the low-density lipoprotein receptor,[11] possibly some glycosaminoglycans.[12,13] Inhibitors that can efficiently block HCV entry may be ideal therapeutic agents against HCV infection.

HCV is a leading cause of liver problems such as cirrhosis and cancer in the United States. Approximately 3 million Americans and 170 million people worldwide are infected with HCV.[14,15] There is currently no vaccine to prevent HCV infection. The current treatment for HCV relies heavily on administration of interferon alone or in combination with ribavirin.[16,17] Interferon-based therapy shows a sustained response in 40−50% of HCV-infected patients and is often accompanied by side effects such as depression, psychoses, and extreme fatigue.[18,19] Furthermore, the most common HCV found in the United States is genotype 1, which responds less effectively to interferon therapy than do other strains. Even the use of pegylated interferon in combination with ribavirin gave favorable responses in only 45−55% of patients infected with genotype 1 HCV.[16,17] Few options exist for patients who either do not respond to interferon therapy or respond and later relapse. Although two recent clinical trials have shown improved rates of sustained virologic response to as high as 65% when telaprevirin, a specific inhibitor of the HCV protease, was added to the combination of peginterferon and ribavirin, telaprevir increased the rate of anemia, nausea, diarrhea, pruritus, and rash.[20,21] Hence, it is imperative to search for alternative therapies, including natural products, for treatment of HCV-infected patients.

According to an open label clinical observation study conducted in the Dominican Republic, a product prepared from the extract of a mixture of herbs was reported to be effective in the treatment of HCV infections. In that study, the levels of the hepatic enzymes ALT and AST were normalized after 45 days of oral treatment, and viral loads were decreased significantly in patients who did not respond to standard interferon treatment.[22] The formulation used in that study contained aqueous extracts from three herbs including that of the flowering tops of *Lamium album* L. (Lamiaceae). In our laboratories, evaluation of a commercially prepared aqueous extract of this plant material showed that it was active against HCV in an HCVpp (HCV pseudoparticles) entry assay. Subsequent bioactivity-guided phytochemical studies of a methanol extract prepared from the dried flowering tops of *L. album* resulted in the isolation of an iridoid glucoside, lamalbid (lamiridoside) (**3**). Although this iridoid glucoside was inactive against HCVpp, the corresponding aglycone (nonsugar portion) and its inseparable epimer, which we named lamiridosins A/B (**1/2**), were found to be significantly active against HCVpp. Further study of analogues showed that these compounds represent a group of specific anti-HCVpp agents that target viral entry into host cells.

To assess the anti-HCV potential of structurally related compounds, and to determine structure−activity relationships, a series of commercially available iridoids were acquired and evaluated for their potency in disrupting HCVpp infectivity and host-cell entry. The current work reports the discovery of entry inhibitors of HCV activity of iridoid constituents from *L. album* and of some structurally related analogues.



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Results and Discussion

An aqueous extract of *L. album*, a component of a commercial preparation used in an open label clinical observation study conducted in the Dominican Republic, showed anti-HCV entry activity of 50% inhibition compared to a negative control against the HCVpp infection at a concentration of 100 μg/mL. To determine this, HCVpp or VSVGpp were preincubated with 100 μg/mL of *L. album* extract or DMSO. Huh7 cells were incubated with this cocktail, and 72 h later luciferase levels were assessed as a measure of entry. Workup of a methanol extract prepared from the corresponding plant material led to the isolation of lamalbid (**3**) as a major component of the flowering tops of *L. album* in ca. 0.4% yield. In contrast, lamiridosins A/B (**1/2**) were found as major constituents in the extract of the commercial aqueous sample, as well as an aqueous extract of *L. album* prepared in our laboratory.

Compound **3**, a white powder, was determined to have a sugar unit. Anomeric signals of the sugar unit were observed in the ^{1}H and ^{13}C NMR spectra [δ 4.60 (1H, d, J = 7.91 Hz) and δ 99.72 (d)], as were NMR signals of the sugar unit corresponding to those of a β-D-glucopyranosyl group. The aglycone of **3** was determined to be an iridoid monoterpene by comparison of its NMR data with those of known iridoids.[23] Comparison with published data indicated that the compound was lamalbid (**3**), previously reported by Eigtved et al. from the same plant species.[24]

Enzyme hydrolysis of **3** by incubation with β-glucosidase led to pair of epimers that we were unable to separate and which we named lamiridosins A/B (**1/2**). Although an aglycone (**1**) had been reported as an enzyme hydrolysis product of **3**,[24] the compound was not obtained in purified form. The presence of **1** was inferred by a reported characterization of a triacetate derivative without supporting physical and spectroscopic data being presented. In our study, we were not able to obtain the single product **1** deduced by Eigved et al. Our enzymatic hydrolytic experiments employing β-glucosidase under the same condition reported by Eigved et al. resulted in a pair of epimers (**1/2**, named lamiridosins A/B) in a 1:1 ratio. The epimers were not separable by HPLC systems using a number of different solvent systems and conditions. The NMR data of the epimers were clearly distinguishable and assignable to each epimer through analysis of ^{1}H−^{1}H COSY, HMQC, and HMBC data (Table 1). The OH group at C-1 of lamiridosin A (**1**) was assigned as β-oriented due to the presence of ROESY cross-peaks between H-1 and H-10. No cross-peak was observed between H-1 and H-10 of lamiridosin B (**2**) in the ROESY spectrum, which confirmed the OH group of C-1 in lamiridosin B (**2**) as being α-oriented. Since lamiridosins A/B (**1/2**) have not been previously reported as an isolate, or as chemically or enzymatically prepared entities, they can be considered as novel compounds.

Interestingly, lamiridosins A/B were not found in a MeOH extract of *L. album*. However, they were found in a proprietary prepared aqueous extract, as well as in an aqueous extract prepared in our lab by maceration of dried plant material with deionized water overnight (24 h), as major constituents. On the other hand, we found that lamalbid (**3**) was the major compound in the MeOH extract of *L. album*, but not in either of the aqueous extracts. This phenomenon is probably due to the glucoside (**3**) being subjected to enzymatic hydrolysis to the aglycone epimers (**1/2**) by naturally existing enzymes in the plant matrix during the maceration/extraction process using water as the solvent. MeOH, on the other hand, inactivated the associated hydrolytic enzyme in the plant matrix, resulting in **3** remaining an intact molecule.

Evaluation of **3** showed it to be inactive in the anti-HCV entry (100 μg/mL) assay. However, iridoids, including aucubin (**13**), have been reported to possess antihepatitis or liver cirrhosis mitigating effects.[25−28] Aucubin was found to protect against chemically and biologically induced liver damage,[25] and its aglycone, aucubigenin, suppressed hepatitis B virus DNA replication in vitro.[27] The structurally related iridoids, geniposide (**10**), catalpol, rehmannioside D, harpagoside (**16**), and harpagide, following incubation with β-glucosidase, were found to suppress hepatitis B virus DNA replication in vitro.[26] These findings suggest that the aglycones of these compounds are responsible for their antihepatitis activity. Thus, it is possible that **13** and its related iridoid glucosides act like precursor agents (pro-drugs), which are inactive in their native forms, but once in the gastrointestinal tract or in other appropriate media the sugar moiety is removed, and the aglycone of the compound acts to prevent viral replication. By the same token, the inactivity of lamalbid (**3**) in the anti-HCVpp assay may also be due to the presence of a glucoside moiety in the molecule. Thus, the evaluation of lamalbid-derived aglycones for anti-HCV activity appeared to be prudent.

Biological evaluation of lamiridosins A/B (**1/2**) showed that they did exhibit significant inhibition against HCV entry (Figure 1A) with an IC_{50} value of 2.31 μM (Figure 1B) and that they were noncytotoxic to the Hep G2 2.2.[15] cells at a concentration of 50 μg/mL. The presence of lamiridosins A/B (**1/2**) in the proprietary aqueous extract of *L. album* indicates that they are at least partly responsible for the anti-HCV entry activity of the aqueous extract (ca. 50% inhibition against HCVpp infection at a concentration 100 μg/mL).

To determine the specificity of lamiridosins A/B toward HCV, their ability to block entry of the VSVG/HIV (vesicular stomtatis virus G protein) pseudovirions into HUH7 cells was tested. The VSVG virus has a broad host range and can infect multiple cell types. The results showed that lamiridosins A/B do not block VSVG viral entry, but that they do block HCV viral entry, which indicates that this epimeric mixture is a selective antiviral agent against the HCV (Figure 1A).

Table 1. ^{1}H, ^{13}C, DEPT, ^{1}H−^{1}H COSY[a], HMQC[b], HMBC, and ROESY[c] Data in CD_3OD for Compounds **1** and **2**

	1			**2**			**3**	
no.	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC	δ_H[d]	δ_C[e]
1	5.30 d (3.1)	93.78 d	C-3, C-5	5.41 d (3.3)	92.10 d	C-3, C-5	5.61 d (1.8)	94.81 d
3	7.41 brd (0.9)	153.76 d	C-1, C-4, C-5, C-11	7.39 d (1.4)	152.90 d	C-1, C-4, C-5, C-11	7.40 dd (1.2, 0.4)	152.81 d
4		110.28 s			110.13 s			111.68 s
5	2.93 ddd (10.4, 3.7, 1.2)	38.41 d	C-3, C-4, C-7, C-8	2.98 ddd (8.4, 5.4, 1.3)	41.24 d	C-1, C-3, C-4, C-7, C-9	2.92 ddd (10.8, 3.5, 1.2)	37.55 d
6	3.90 brt (4.3)	77.97 d	C-4, C-8	3.95 overlap	76.78 d	C-4, C-8	3.94 brt (3.9)	77.88 d
7	3.53 d (4.8)	78.63 d	C-5, C-9, C-10	3.95 overlap	77.31 d	C-10	3.54 d (4.4)	78.26 d
8		78.80 s			79.82 s			78.59 s
9	2.62 dd (10.3, 3.0)	50.36 d	C-1, C-4, C-5, C-8	2.17 dd (8.5, 3.3)	49.00 d	C-7, C-8	2.80 dd (10.8, 1.7)	49.23 d
10	1.19 s	22.19 q	C-7, C-8, C-9	1.34 s	21.74 q	C-7, C-8, C-9	1.19 s	169.5 s
11		169.74 s			170.65 s			
OMe	3.72 s	51.85 q	C-11	3.74 s	51.88 q	C-11	3.72 s	51.93 q

[a] All ^3J bond H−H correlations in the structures were observed in the ^{1}H−^{1}H COSY spectrum, and the ^4J bond H−H correlations between H-3 and H-5 were also observed. [b] All ^1J bond C−H correlations in the structures were observed in the HMQC spectrum. [c] The cross-peaks observed for compound **1** in the ROESY spectrum: H-1 and H-10, and H-6 and H-10. [d] ^{1}H NMR data of the sugar unit of compound **3**: 4.60 (1H, d, J = 7.9 Hz), 3.88 (1H, dd, J = 11.9, 2.0 Hz), 3.65 (1H, dd, J = 11.9, 5.8 Hz), 3.35 (1H, brt, J = 8.7 Hz), 3.29 (1H, m), 3.26 (1H, brt, J = 9.5 Hz), 3.16 (1H, dd, J = 9.0, 7.9 Hz). [e] ^{13}C NMR data of the sugar unit of compound **3**: 99.72 (d), 78.97 (d), 77.88 (d), 74.53 (d), 71.52 (d), and 62.76 (t).

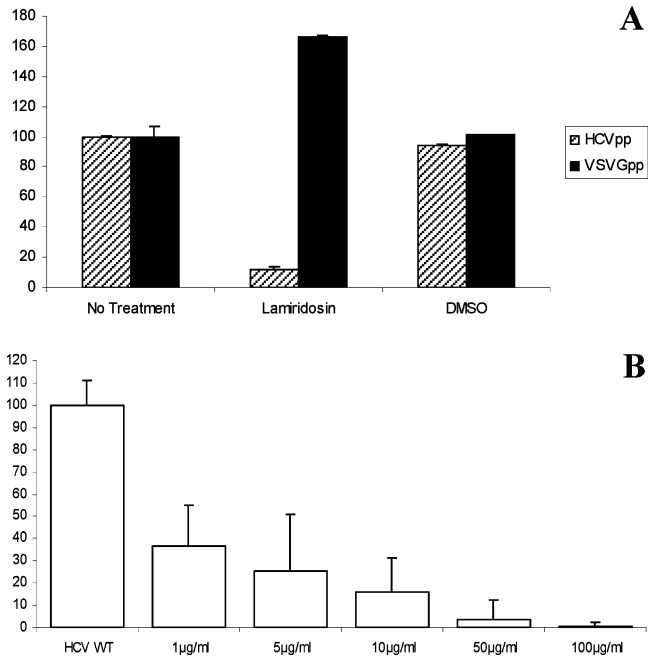

Figure 1. Determination of lamiridosins A/B (**1/2**) inhibition of HCVpp specific entry. (A) HCVpp or VSVGpp was preincubated at rt for 20 min with 100 μg/mL of lamiridosins A/B 1:1 epimer (**1/2**) or DMSO. The pseudovirus and lamiridosins A/B 1:1 epimer (**1/2**) or DMSO cocktail was incubated with Huh7 cells for 6 h, then aspirated off, and DMEM media was added. Luciferase levels were assessed 72 h postinfection and used as a measure of infectivity. The *y*-axis is expressed as a percentage of luciferase level of the no-drug treatment sample (no treatment). (B) Lamiridosins A/B 1:1 epimer (**1/2**) was assayed at different concentrations to determine the IC_{50}. The y-axis is expressed as a percentage of luciferase level of the no-drug treatment sample (HCV WT), and the *x*-axis is expressed as concentration (μg/mL) of lamiridosins A/B.

To further evaluate lamiridosins A/B (**1/2**) and their structural congeners/derivatives for anti-HCV host-cell entry activity, 13 commercially available iridoids, 12 glucosides and one aglycone (genipin, **9**), were acquired to evaluate their anti-HCV potentials. None of the tested iridoid glucosides showed anti-HCV entry activity at a concentration of 20 μg/mL. On the other hand, the lone free aglycone (genipin, **9**) in the 14 acquired iridoids showed significant inhibition against HCVpp infection at a concentration of 20 μg/mL (Figure 2), which provided further evidence that the direct antihepatitis viral activity is mitigated by the iridoid aglycones rather than the parent glucosides. As a further substantiation of this hypothesis and to determine their antiviral activity profiles, the 13 commercially obtained iridoid glucosides were subjected to enzymatic hydrolysis by incubation with β-glucosidase for 0.5 h prior to anti-HCV entry assay. As expected, the resulting hydrolytic products (aglycones) of shanzhiside methyl ester (**4**), loganin (**5**), lognic acid (**6**), verbenalin (**12**), eurostoside (**15**), and picroside II (**17**) gave 49.0, 24.8, 39.4, 21.9, 46.6, and 31.6% inhibition against HCVpp infection at a concentration of 20 μg/mL, respectively (Table 2). Their inhibition activity against HCVpp infection was further improved to 61.2, 41.4, 52.5, 43.4, 56.6, and 59.4%, respectively, after a 4 h enzyme incubation process (Table 2 and Figure 2). Interestingly, while the 4 h enzyme-hydrolyzed products of loganin (**5**) and loganic acid (**6**) showed more than 40% inhibition against HCVpp infection, the enzyme hydrolytic products of their epimers (**7** and **8**) showed no or very little inhibition against HCVpp infection. Thus, further study of the structure and activity relationships are needed to maximize the anti-HCV activity of natural iridoids.

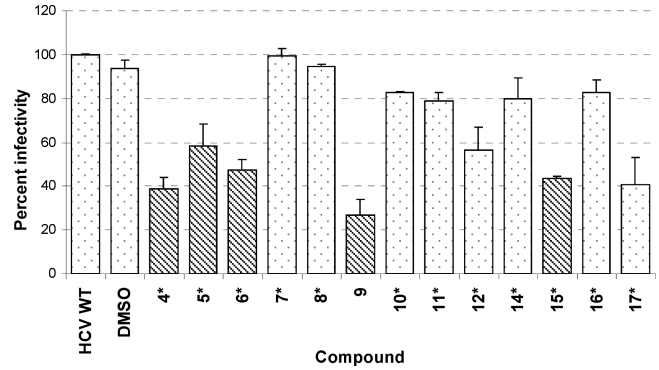

Figure 2. Inhibition of HCVpp specific entry by lamiridosins A/B analogues. The analogues of lamiridosins A/B (**1/2**) were assayed for HCVpp inhibition at 20 μg/mL. Compound numbers marked with an "*" indicate products of enzyme hydrolysis (4 h). Hatched bars represent compounds structurally related to lamiridosins that were further evaluated in the HCVcc system.

Table 2. Anti-HCVpp Entry Activity of Enzyme Hydrolysis Products (Iridoids **4**−**12** and **14**−**17**)

compound	time (h)[a]	inhibition (%)[b]	compound	time (h)	inhibition (%)
4	4.0	61.2	**10**	4.0	17.1
4	0.5	49.0	**11**	4.0	21.1
4	none[c]	−13.1	**11**	0.5	−3.4
5	4.0	41.4	**12**	4.0	43.4
5	0.5	24.8	**12**	0.5	21.9
6	4.0	52.5	**14**	4.0	20.3
6	0.5	39.4	**14**	0.5	12.9
7	4.0	0.4	**15**	4.0	56.6
7	0.5	−12.7	**15**	0.5	46.6
8	4.0	5.3	**16**	4.0	17.1
8	0.5	−21.6	**16**	0.5	−41.7
9	none	73.2	**16**	none	2.1
10	none	1.1	**17**	4.0	59.4
10	0.5	9.8	**17**	0.5	31.6

[a] Processing time of enzyme hydrolysis. [b] HCV inhibition is expressed as percentage of luciferase level of the no-drug treatment sample (HCV WT in Figure 2) at 20 μg/mL. [c] No enzyme processing.

Lamiridosins A/B (**1/2**), genipin (**9**), and the enzyme hydrolytic products of loganin (**5**), loganic acid (**6**), shanzhiside methyl ester (**4**), and eurostoside (**15**) were further evaluated in the HCVcc foci system.[29−31] They exhibited inhibition against HCVcc infection with IC_{50} values of 15, 20, 16, 11, 18, and 20 μg/mL, respectively. The anti-HCV action of these compounds in the current study represents the first report of such biological activity for the iridoid compounds.

CD81 is a co-receptor for HCV entry. To investigate whether the reduction in HCVpp entry in the presence of these compounds was due to disruption of HCV E2 binding to CD81, the binding of E1E2 to recombinant soluble CD81-GST in the presence of these compounds was assayed.[32] As negative controls, GST protein without soluble CD81 and soluble CD81-GST with DMSO were used to pull down HCV E1E2. None of the compounds evaluated interfered with CD81 and HCV E2 glycoprotein binding (Figure 3). These results indicate that these compounds did not block CD81/E2 interaction.

In summary, the present study succeeded in the identification, for the first time, of the anti-HCV activity of iridoids. The presence of the anti-HCV iridoid aglycone epimers, lamiridosins A/B (**1/2**), in the proprietary prepared aqueous extract of *Lamium album*, a component herb in a commercial liver health herbal formula, may explain in part the purported hepatitis C viral load reduction reported in observational case studies.[22]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra were run on a Jasco

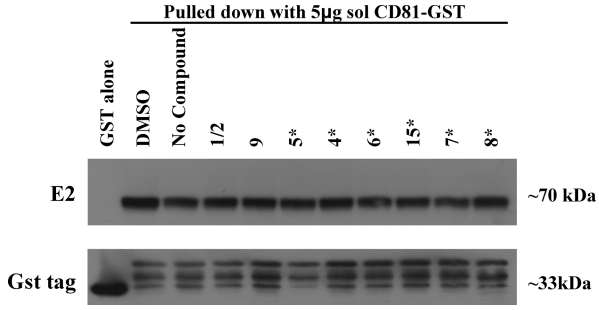

Figure 3. Binding of HCV E1E2 glycoproteins to soluble CD81 in the presence of inhibitors. 293T cells transfected with HCV wt glycoproteins were lysed 24 h post-transfection. Cleared cell lysate was incubated with 50 μg/mL of compound and 5 μg of soluble CD81-GST fusion protein. Binding was determined by western blotting for E2 and the GST tag. As negative controls, GST protein without soluble CD81 and soluble CD81-GST with DMSO were used to pull down HCV E1E2. Compounds marked with an "*" indicate products of enzyme hydrolysis.

FT/IR-410 spectrometer, equipped with a Specac Silver Gate ATR system by applying a film on a germanium plate. NMR spectra were recorded on a Bruker Avance-360 MHz spectrometer. Chemical shifts (δ) for both ^{1}H (360 MHz) and ^{13}C NMR (90 MHz) are expressed in ppm with reference to the solvent signals (CD_3OD; ^{1}H: 3.30 ppm; ^{13}C: 49.00 ppm), and coupling constants (J) are reported in Hz. All NMR data 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), and reversed-phase flash chromatography was accomplished with RP-18 silica gel (40−63 μm, EM Science). RP- HPLC was carried out on a Waters 600E Delivery System pump, equipped with a Waters 996 photodiode detector. An Alltima C18 5 μm column (60 Å, 5 μm, 250 × 10 mm) was used for HPLC analytical analysis, whereas a Phenomenex Luna 10 μm C18(2) column (120 Å, 10 μm, 250 × 50 mm) was used for preparative HPLC separations. UV spectra of purified compounds were extracted from the HPLC analysis data. TLC was performed on Whatman glass-backed plates coated with 0.25 mm layers of silica gel 60. HRTOFMS were recorded on a ThermoFinnigan LTQFT spectrometer. Loganin (**5**, ASB-00012351-010), lognic acid (**6**, ASB-00012341-005), geniposide (**10**, ASB-0007070-005), gardenoside (**11**, ASB-00007055-005), verbenalin (**12**, ASB-00003780-005), aucubin (**13**, ASB-00011086-005), agnuside (**14**, ASB-00011150-005), harpagoside (**16**, ASB-00008055-010), and picroside II (**17**, ASB-00016820-010) were purchased from ChromaDex, Inc., Santa Ana, CA. Genipin (**9**, 078-03021) was purchased from Wako Pure Chemicals USA, Inc., Richmond, VA. Shanzhiside methyl ester (**4**, 020214S) was purchased from Indofine Chemical Company, Inc., Hillsborough, NJ. 8-Epiloganin (**7**), 8-epiloganic acid (**8**), and eurostoside (**15**) were purchased from CHD Biotechnology Ltd., Shenzhen, China.

Plant Materials. Dried flowering tops of *Lamium album* L. (Lamiaceae) (1.0 kg, Lot No. 2006/13051) were obtained from Amorós Nature S.L., Can Batalló, s/n, 17450 Hostalric, Girona, Spain, from cultivated plants harvested in Europe in 2006. Reference samples of these herbs have been retained at Exxentia Groupo Phytotherapicos, SA, Madrid, Spain.

Extraction and Fractionation. The milled *L. album* plant material (85.1 g) was extracted with MeOH (0.5 L × 5) at room temperature to afford the corresponding MeOH extract (13.5 g). A sample of the MeOH extract (11.7 g) was absorbed on 17.4 g of silica gel and chromatographed over a Si gel (112.8 g) column, eluting with $CHCl_3$/Me_2CO/MeOH in various ratios to afford 23 fractions [$CHCl_3$ (eluate F1: 500 mL), $CHCl_3$/Me_2CO, 9:1 (eluate F2: 240 mL; eluate F3: 500 mL; eluate F4: 615 mL), $CHCl_3$/Me_2CO, 8:2 (eluate F5: 630 mL), $CHCl_3$/Me_2CO/MeOH, 77.6:19.4:3.0 (eluate F6: 300 mL; eluate F7: 410 mL; eluate F8: 500 mL; eluate F9: 475 mL; eluate F10: 480 mL; eluate F11: 425 mL), $CHCl_3$/Me_2CO/MeOH, 74.4:18.6:7.0 (eluate F12: 1020 mL), $CHCl_3$/Me_2CO/MeOH, 70.4:17.6:12.0 (eluate F13: 400 mL; eluate F14: 490 mL; eluate F15: 500 mL), $CHCl_3$/Me_2CO/MeOH, 64.0:16.0:20.0 (eluate F16: 150 mL; eluate F17: 430 mL; eluate F18: 350 mL; eluate F19: 480 mL; eluate F20: 480 mL; eluate F21: 520 mL), $CHCl_3$/Me_2CO/MeOH, 56.0:14.0:30.0 (eluate F22: 650 mL), and MeOH (eluate *F*23: 430 mL), respectively]. Fraction F19 (325.0 mg) was subjected to preparative HPLC separation, eluting with MeOH/H_2O, 3:7, with a flow rate of 12 mL/min to afford lamalbid (**3**, 97.2 mg). Lamalbid (**3**) was also detected in fractions F18 and F19−F21 by HPLC analysis.

Preparation of Lamiridosins A/B (1/2). Lamalbid (**3**) (18.7 mg) was dissolved in H_2O (2 mL) containing β-glucosidase (25.2 mg) and allowed to react at 37 °C for 24 h. The solution was then evaporated to dryness to yield a mixture, which was extracted with MeOH. The MeOH extract was subjected to preparative HPLC, eluting with MeOH/H_2O, 3:7, with a flow rate of 12 mL/min to afford the inseparable 1:1 mixture of epimers lamiridosins A/B (**1/2**), 9.21 mg: colorless gum; $[\alpha]^{20}_D$ +4.68 (*c* 0.36, MeOH); UV λ_{max} [AU (absorbance units)] = 239.1 (2.469) nm; IR (film) ν_{max} 3365, 2960, 2922, 2847, 1687, 1635, 1541, 1441, 1378, 1302, 1165, 1109, 1082, 1020, 864, 814, 773; ^{1}H NMR (360 MHz, CD_3OD) and ^{13}C NMR (90 MHz, CD_3OD): see Table 1; negative HRESIMS *m/z* 259.08219 (calcd for $C_{11}H_{15}O_7$, 259.08178).

Anti-HCV Entry Assay. 1. Pseudotyping. Pseudotyped viruses were produced by cotransfecting DNA encoding HCV E1E2 or vesicular stromatis G protein (VSVG) glycoprotein expression plasmid with the envelope and Vpr deficient HIV vector carrying a luciferase reporter gene inserted into the Nef position (pNL4-3-Luc-R$^-$-E$^-$) in 293T producer cells. One microgram of HCV E1E2 or VSVG expression plasmid and 3 μg of pNL4-3-Luc-R$^-$-E$^-$ were used to transfect 293T cells (90% confluent) in six-well plates with polyethylenimine (PEI). The DNA cocktail was added to 200 μL of Opti-MEM media, and PEI was added at 2× the volume of DNA. The mixture was incubated at room temperature for 15 min. The 293T producer cells were rinsed with PBS (no Ca^{2+}/no Mg^{2+}). Opti-MEM (800 μL) was added to each well, and the PEI/DNA mixture was added. After 5−6 h incubation at 37 °C, the DNA cocktail was aspirated off, and 3 mL cell culture media was added per well. The supernatants containing the pseudotyped viruses were collected 48 h post-transfection and filtered through a 0.45 μm pore size filter (Nalgene). Huh7 cells were seeded in 12-well plates at a density of 8×10^4 per well one day prior to infection. Each compound at the indicated concentrations was mixed at room temperature with HCVpp or VSVGpp, and 500 μL of this cocktail was added to the corresponding well of Huh7 cells. Cells were incubated with compound and pseudovirus for 6 h; then the cocktail was removed and cell growth media was added. The cells were lysed in 200 μL of cell culture lysis reagent (Promega) at 72 h postinfection. The luciferase activity was measured with a luciferase assay kit (Promega) and a FB12 luminometer (Berthold detection system) according to the supplier's protocol.[33] Each sample was done in duplicate, and experiments were repeated at least three times.

2. HCVcc Assay. HCVcc JFH-1 was generated as described by Zhong et al.[29] The effect of each compound on HCVcc infection was determined by a viral foci-reduction assay in Huh7 cells plated in 96-well plates. Each compound at the indicated concentrations was mixed at room temperature with 100 foci forming units of HCVcc. Culture media was removed from the Huh7 cells, and 100 μL of fresh media containing the compound and HCVcc cocktail was added to the corresponding wells. Plates were incubated at 37 °C for 24 h before 100 μL of 0.5% methyl cellulose overlay was added to each well. Forty-eight hours postinfection cells were fixed and HCV-positive foci were visualized by staining for NS5A.

CD81 Binding Assay. The CD81 clone used was kindly provided by Shoshana Levy, Ph.D. (Stanford University). A glutathione S-transferase (GST) fusion protein containing the large extracellular loop of human CD81 was generated as previously described.[32] 293T producer cells were transfected with 1 μg of HCV E1E2 DNA using PEI. After 48 h, cells were lysed in 0.5% Triton X-100 lysis buffer with protease inhibitor on ice for 30 min. Cell lysates were clarified by centrifuging at 20 000 rpm for 30 min at 4 °C. Two-hundred microliters of clarified lysates from these cells was incubated with 50 μg/mL of compound (or DMSO alone) and 5 μg of CD81-GST fusion protein or GST protein alone with gentle rocking at 4 °C for 16 h. Fifty microliters of glutathione sepharose 4B (GSH) beads (GE Healthcare) rinsed three times with PBS (140 mM NaCl, 27 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) was added and incubated at 4 °C for 1 h. The slurry was spun down for 1 min at 14 000 rpm, and GSH beads were rinsed two times with 0.5% Triton X-100 lysis buffer. SDS-PAGE loading dye was added to the beads, and samples were boiled at 95 °C for 5

min. Slurry was spun down again, and supernatant was collected for SDS-PAGE and western blot analysis.

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Supporting Information Available: Spectroscopic data (UV, IR, MS, and 1D and 2D NMR spectra) of lamiridosins A/B (**1/2**) as well as HPLC analysis of the leaves and stems of *L. album* are available free of charge via the Internet at http://pubs.acs.org.

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