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Design, Synthesis and Biological Evaluation of Caudatin Analogs as Potent Hepatitis B Virus Inhibitors

Li-Jun Wang[a], Hao Chen[a,b], Yun-Bao Ma[a], Xiao-Yan Huang[a], Chang-An Geng[a], Xue-Mei Zhang[a] and Ji-Jun Chen[a*]

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Abstract: Thirty-nine caudatin analogs were designed and synthesized. Their anti-hepatitis B virus (HBV) activities were evaluated *in vitro*. Among them, twenty-three compounds showed much better anti-HBV activity than caudatin, and eleven compounds significantly inhibited the HBV DNA replication with IC_{50} values < 10 μM. Interestingly, three compounds (**22**, **28**, **29**) exhibited excellent activity against the secretion of HBsAg (IC_{50} = 63.02 μM, 52.81 μM, 56.08 μM), HBeAg (IC_{50} = 204.80 μM, 173.51 μM, 70.39 μM), along with HBV DNA replication (IC_{50} = 24.55 μM, 5.69 μM, 8.23 μM) with lower cytotoxicity. The structure-activity relationships (SARs) of these caudatin analogs were also discussed.

Keywords: Anti-HBV activity, Caudatin analogs, Hepatitis B e Antigen (HBeAg), Hepatitis B Surface Antigen (HBsAg), HBV DNA replication, Structure-activity relationships(SARs), Synthesis.

1. INTRODUCTION

Hepatitis B virus (HBV) infection remains a global health problem, which can cause liver failure, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. Currently, therapies for HBV infection include immuno-modulators, interferons (interferon-alpha and pegylated interferon) and nucleosides/nucleotides analogues (lamivudine (3TC), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT) and tenofovir-DF (TDF)), but they are unsatisfactory. For example, vaccine failure will result that millions of the HBV patients will eventually succumb to the infection sequence; application of interferons is limited for low curing rate and serious side effects (influenza-like symptoms, fatigue, myalgia, nausea, headache, etc.); nucleoside analogues result in drug resistance and high recurrence for the single target on the viral DNA polymerase [2-5]. Thus, researchers are continuing to search for new anti-HBV agents.

Natural products and their derivatives by simple functional-group transformations offer many opportunities for finding novel anti-HBV agents [6-17]. As an ongoing search for potential anti-HBV inhibitors, our latest study revealed that caudatin (Fig. **1**) from *Cynanchum auriculatum* (Bai-Shou-Wu in Chinese) exhibited potent activity inhibiting the secretion of HBsAg and HBV DNA replication and 3-*O*-(3, 4, 5-trimethoxy) cinnamoyl caudatin (Fig. **1**) had the novel action mechanism inhibiting HBV by interfering HBV promoters and enhancers [6]. In additional, the anti-HBV activity largely depended on the size and character of the substituent for 3-*O*-substituted caudatin derivatives based on our previous report [8]. For example, introduction of nitrogen aromatic and cinnamoyl moiety at C-3 position could significantly enhance the anti-HBV activity, but substitution with diglycolyl, glutaryl, and phosphate esters led the contrary results, which indicated that the hydroxyl group at C-3 of caudatin was a good target for further chemical modification by introduction of the rational substitutions. Consequently, further modification on C-3 hydroxyl group of caudatin was conducted to gain more novel anti-HBV agents. Herein, we reported a series of caudatin derivatives modified on rings A and D at C-3, C-17, respectively. Structure-activity relationships (SARs) of these caudatin analogs with anti-HBV activity were also discussed.

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2. RESULTS AND DISCUSSION

2.1. Chemistry

The syntheses of caudatin analogs were summarized in Scheme **1**. Derivatives **1**-**31** were synthesized by the reaction of corresponding acids with caudatin in the presence of *N, N'*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). Compound **32** was obtained through caudatin reaction with gallic acid in the presence of diisopropyl azodicarboxylate (DIAD) and triphenylphosphine (TPP). As oxygen and sulfur heterocylic rings were effect functional groups to search potential anti-HBV activity according to previous investigation [18-22], some heterocylic rings containing oxygen and sulfur atoms were introduced into the caudatin in order to obtain the active analogs. In order to further evaluate the effect of hydroxyl groups at C-3 and C-17 for biological activity, the C-3, 17 diesters (**33**-**39**) of

1875-6638/15 $58.00+.00

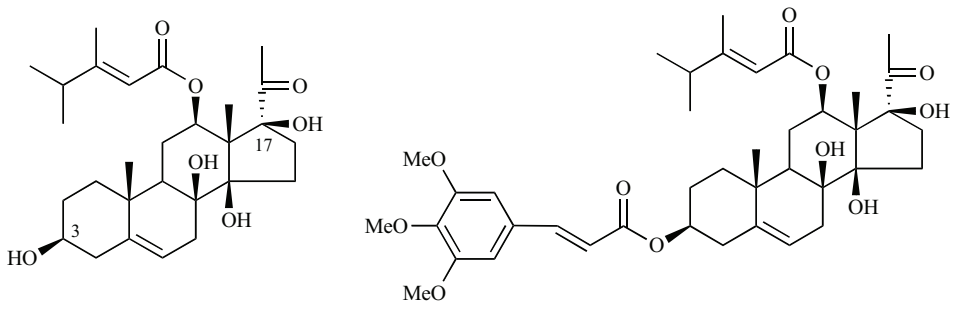
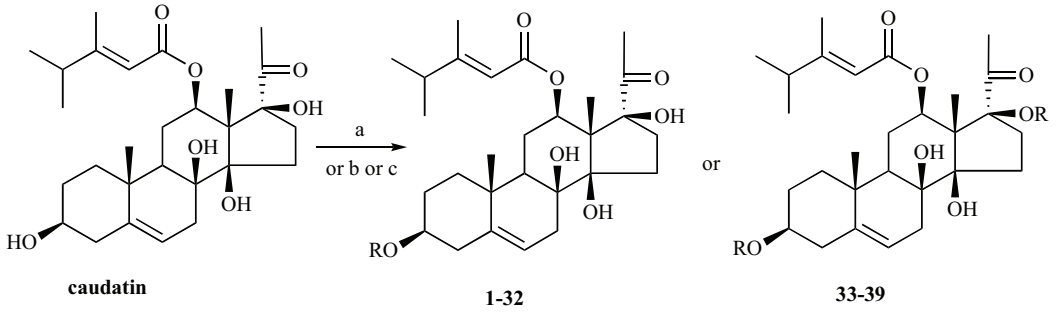


Fig. (1). Chemical structure of caudatin and 3-*O*-(3, 4, 5-trimethoxy) cinnamoyl caudatin.



Scheme 1. Reagents and conditions: (a) RCOOH, DMAP, DCC, CH_2Cl_2, rt; (b) gallic acid, DIAD, TPP, THF, rt; (c) Ac_2O, DMAP, Py, reflux.

caudatin were synthesized. All of these synthesized analogs were purified by column chromatography and their structures were characterized by spectroscopic means (^1H, ^{13}C NMR, MS and HRMS). For instance, compounds **33-39** were determined based on the expected down-field shift at C-17 (from 91.5 to 96.3-98.4) comparing to caudatin.

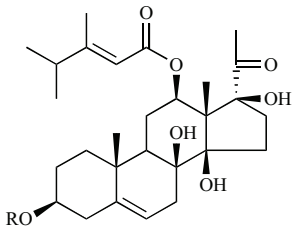
2.2. Biological Evaluation

These analogues were tested for their potential anti-HBV activities, including inhibiting the secretion of hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) and HBV DNA replication in HepG 2.2.15 cells using tenofovir as control, and the results of their anti-HBV activity and cytotoxicity were listed in Tables **1-3**.

As shown in Table **1**, acetyl derivative **1** showed inhibitory potency to the HBV DNA replication. Compound **2** showed potent activity inhibiting both the secretion of HBsAg (IC_{50}=8.25 μM), HBeAg (IC_{50}=7.85 μM), and HBV DNA replication (IC_{50}=5.95 μM). Compounds **3** and **4** exhibited inhibitory activity against the secretion of HBsAg and HBV DNA replication, instead of the secretion of HBeAg with the higher cytotoxicity than that of caudatin, which may be due to the unsaturated C≡C bond. The position of methyl group in compounds **5** and **6** could influence the activity based on the IC_{50} values of the secretion of HBsAg, HBeAg and HBV DNA replication. The anti-HBV activity and cytotoxicity would be enhanced (**7** *vs* **8**, **9** *vs* **10**) when the C=C double bond incorporated in caudatin derivatives. From the above results, it was suggested that the anti-HBV activity and cytotoxicity would be enhanced when the unsaturated C≡C and C=C double bond incorporation in caudatin derivatives. The anti-HBV activities of compounds **1-16** suggested that the chain length affect the anti-HBV activity of these analogs. The derivatives with eight carbons and less exhibited higher activity inhibiting the secretion of HBsAg and HBV DNA replication than caudatin, and their anti-HBV activities could be enhanced when the carbon atoms increased. Derivatives (**9-16**) with ten to eighteen carbon atoms showed lower anti-HBV activity and cell cytotoxicity than that of caudatin, except that compound **16** (with three C=C double bonds) was observed to possess activity against the HBV DNA replication with the IC_{50} value of 87.94 μM.

Based on previous investigations that the anti-HBV agents usually contain oxygen and sulfur heterocylic rings, caudatin analogs with diverse heterocylic rings containing the oxygen and sulfur atoms were synthesized to obtain more anti-HBV agents. Most of these analogs manifested potential anti-HBV activity (Table **2**). Compound **17** exhibited the potential activity that not only inhibited the secretion of HBsAg (IC_{50} = 71.19 μM) and HBeAg (IC_{50} = 52.68 μM), but also inhibited HBV DNA replication (IC_{50} = 4.73 μM). Compound **18** exhibited most activity against of HBV DNA replication with the IC_{50} value of 3.99 μM, but showed cytotoxicity (CC_{50} = 17.11 μM). The activities of compounds **19-21** with tetrahydrofuran-2-carbonyl and furan-2-carbonyl groups were weaker than that of caudatin. Compound **22**, 3-*O*-(benzofuran-2-carbonyl) caudatin, exhibited higher anti-HBV activity than that of caudatin. More interestingly compound **22** had low cytotoxicity (CC_{50} >1669.82 μM), resulting in the high selectivity index (SI_{HBsAg} > 26.5, SI_{HBeAg} >8.2, $SI_{HBV\ DNA}$ > 68.0). Compound **23** with 5-oxotetrahydrofuran-2-carbonyl group exhibited inhibitory activity on HBV DNA replication. Compounds **24-27** showed higher anti-HBV activities and cytotoxicity than that of caudatin. Compound **28** incorporated in caudatin derivatives with 4-oxo-

Table 1. Structure, anti-HBV activity and cytotoxicity of caudatin and its derivatives 1-16.[a]



1-16

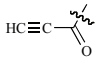
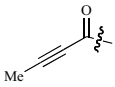
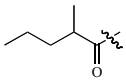
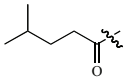
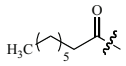
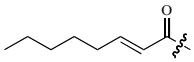
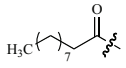
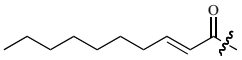
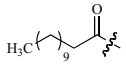
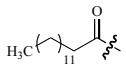
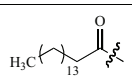
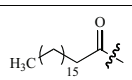
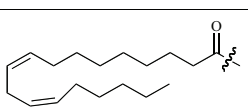
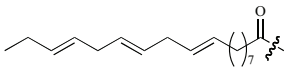
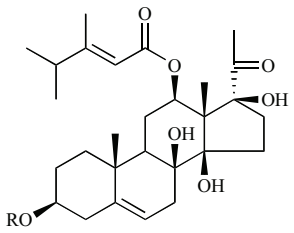
Compd	R	CC_{50}[b] (μM)	HBsAg[c]		HBeAg[d]		DNA Replication	
			IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]
Caudatin[i]		244.58	142.67	1.7	>183.44	<1.3	40.62	6.0
1		22.65	155.96	-	>1124.14	-[g]	23.91	-
2		8.39	8.25	1.0	7.85	1.1	5.95	1.4
3		55.32	84.82	-	>2120.60	-	10.38	5.3
4		<32.34	17.13	<1.9	>934.03	-	7.69	<4.2
5		32.10	48.76	-	35.69	-	7.04	4.6
6		70.50	62.00	1.1	73.66	-	50.75	1.4
7		86.91	82.22	1.1	85.80	1.0	26.77	3.2
8		21.53	281.04	-	>1436.12	-	10.02	2.1
9		619.96	>2053.90	-	>2053.90	-	66.57	9.3
10		367.39	>1161.90	-	>1161.90	-	25.09	14.6
11		>1676.51	>1676.51	-	>1676.51	-	>419.12	-
12		>596.66	>596.66	-	>596.66	-	>417.96	-
13		>394.65	>394.65	-	>394.65	-	207.93	>1.9
14		>251.12	>251.12	-	>251.12	-	>251.12	-
15		>299.00	251.64	>1.2	>299.00	-	>299.00	-

Table 1. contd….

Compd	R	CC_{50} [b] (μM)	HBsAg[c]		HBeAg[d]		DNA Replication	
			IC_{50} [e] (μM)	SI [f]	IC_{50} [e] (μM)	SI [f]	IC_{50} [e] (μM)	SI [f]
16		>373.08	>373.08	-	>373.08	-	87.94	>4.2
TF [h]		>1716.28	1389.42	>1.2	1237.86	>1.4	0.71	>2417.3

[a]Values are means of two independent experiments. [b]CC_{50} is 50% cytotoxicity concentration in HepG 2.2.15 cells. [c]HBsAg: hepatitis B surface antigen. [d]HBeAg: hepatitis B e antigen. [e]IC_{50} is 50% inhibitory concentration. [f]SI (selectivity index) = CC_{50}/IC_{50}. [g]No SI can be obtained. [h]Tenofovir as the positive control. [i]Data from Ref. 6.

Table 2. Structure, anti-HBV activity and cytotoxicity of caudatin and its derivatives 17-32.[a]



17-32

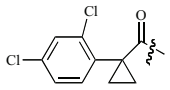
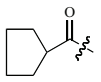
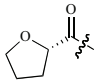
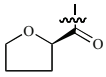
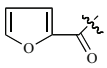
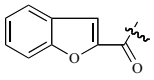
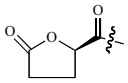
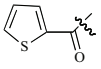
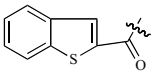
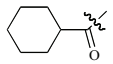
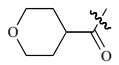
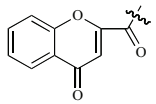
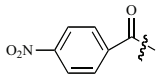
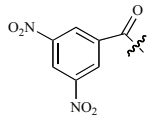
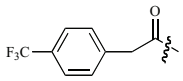
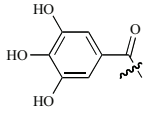
Compd	R	CC_{50} [b] (μM)	HBsAg[c]		HBeAg[d]		DNA Replication	
			IC_{50} [e] (μM)	SI [f]	IC_{50} [e] (μM)	SI [f]	IC_{50} [e] (μM)	SI [f]
Caudatin [i]		244.58	142.67	1.7	>183.44	<1.3	40.62	6.0
17		227.82	71.19	3.2	52.68	4.3	4.73	48.2
18		17.11	25.36	-	>1019.05	-[g]	3.99	4.3
19		44.20	57.79	-	>1189.87	-	>159.36	-
20		<67.95	<67.95	-	>1155.09	-	148.53	-
21		267.16	201.33	1.3	>684.11	-	87.58	3.1
22		>1669.82	63.02	>26.5	204.80	>8.2	24.55	>68.0
23		153.75	132.74	1.2	696.86	-	26.56	5.8
24		129.85	19.97	6.2	21.64	6.0	8.67	15.0
25		<8.19	165.03	-	522.88	-	4.00	<2.0

Table 2. contd….

Compd	R	CC_{50}[b] (μM)	HBsAg[c]		HBeAg[d]		DNA Replication	
			IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]
26		63.25	99.87	-	>169.77	-	8.01	7.9
27		48.91	36.88	1.3	>1041.76	-	26.23	1.9
28		>1689.80	52.81	>32.0	173.51	>9.7	5.69	>297.0
29		>461.44	56.08	>8.2	70.39	>6.6	8.23	>56.1
30		10.83	10.13	1.1	10.61	1.0	89.01	-
31		228.29	67.24	3.4	208.35	1.1	26.26	8.7
32		53.29	67.59	-	342.64	-	8.20	6.5
TF[h]		>1716.28	1389.42	>1.2	1237.86	>1.4	0.71	>2417.3

[a]Values are means of two independent experiments. [b]CC_{50} is 50% cytotoxicity concentration in HepG 2.2.15 cells. [c]HBsAg: hepatitis B surface antigen. [d]HBeAg: hepatitis B e antigen. [e]IC_{50} is 50% inhibitory concentration. [f]SI (selectivity index) = CC_{50}/IC_{50}. [g]No SI can be obtained. [h]Tenofovir as the positive control. [i]Data from Ref. 6.

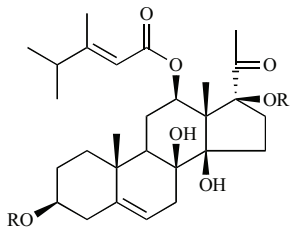
4H-chromene-2-carbonyl group showed potent anti-HBV activity by inhibiting the secretion of HBsAg (IC_{50} = 52.81 μM,), HBeAg (IC_{50} = 173.51 μM), and HBV DNA replication (IC_{50} = 5.69 μM). Based on these results, it could be concluded that introduction of heterocylic rings containing the oxygen and sulfur atoms could largely enhance the activity. Compound **29** with one nitro group exhibited the excellent activity against the secretion of HBsAg and HBeAg and HBV DNA replication with the IC_{50} value of 56.08 μM, 70.39 μM and 8.23 μM, respectively. The cytotoxicity of compound **30** increased when two nitro submitted in the benzoyl, which may be due to the influence of electron-withdrawing substituent on the aromatic moiety to have a higher activity while too much of these groups have a deleterious effect. Compound **31** showed better reducing the secretion of HBsAg and HBV DNA replication activity than that of caudatin, but its cytotoxicity was on the same level as caudatin. When the 3, 4, 5-trihydroxybenzoyl group was introduced into caudatin derivatives, the activity of compound **32** inhibiting HBV DNA replication increased 5 times, but the cytotoxicity was increased too. The HBsAg and HBeAg were illustrated that played the role in HBV infection and seroconversion was an important end point in the treatment of chronic hepatitis B [23-25]. The potential derivatives of caudatin inhibited not only the secretion of HBsAg and HBeAg but also the HBV DNA replication, even some of derivatives had greater activity against the secretion of HBsAg or HBeAg than that of the tenofovir (positive control, nucleotide drug), which suggested that they might had different mechanisms from the nucleoside analogs.

The effects of the functional group at C-17 for maintaining anti-HBV activity was further demonstrated by comparing the activities of 3-*O*-substituted derivatives with that of 3, 17-*O*-disubstitued derivatives. The decreased cytotoxicity of compounds (**34**-**37**, **39**) proved that the free hydroxyl groups could influence the activity and cytotoxicity as shown in the Table **3**. Among of the 3, 17-*O*-disubstitued derivatives, only compounds **33** and **38** showed higher anti-HBV activity than cuadatin, indicating that the type of substituent groups could also influence the activity and cytotoxicity.

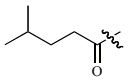
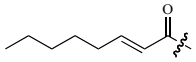
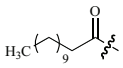
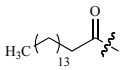
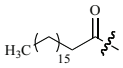
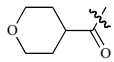
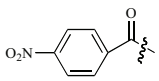
3. CONCLUSION

In summary, thirty-nine analogs of caudatin were synthesized, and evaluated their anti-HBV activity. Among them, twenty-three compounds showed greater anti-HBV activity than caudatin and eleven compounds exhibited significantly inhibitory activity of HBV DNA replication with IC_{50} values under 10 μM. Three compounds (**22**, **28**, **29**) exhibited potent

Table 3. Structure, anti-HBV activity and cytotoxicity of caudatin and its derivatives 33-39.[a]



33-39

Compd	R	CC_{50}[b] (μM)	HBsAg[c]		HBeAg[d]		DNA Replication	
			IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]
Caudatin[i]		244.58	142.67	1.7	>183.44	<1.3	40.62	6.0
33		35.78	62.22	-[g]	31.03	1.2	12.28	2.9
34		>165.88	>165.88	-	>165.88	-	>165.88	-
35		>201.85	>201.85	-	>201.85	-	>201.85	-
36		>346.5	>346.5	-	>346.5	-	142.19	2.4
37		>329.98	179.78	>1.8	>329.98	-	120.05	>2.8
38		536.12	78.39	6.8	117.58	4.6	27.58	19.3
39		>507.42	>507.42	-	>507.42	-	>126.86	-
TF[h]		>1716.28	1389.42	>1.2	1237.86	>1.4	0.71	>2417.3

[a]Values are means of two independent experiments. [b]CC_{50} is 50% cytotoxicity concentration in HepG 2.2.15 cells. [c]HBsAg: hepatitis B surface antigen. [d]HBeAg: hepatitis B e antigen. [e]IC_{50} is 50% inhibitory concentration. [f]SI (selectivity index) = CC_{50}/IC_{50}. [g]No SI can be obtained. [h]Tenofovir as the positive control. [i]Data from Ref. 6.

activity against the secretion of HBsAg (IC_{50} = 63.02 μM, 52.81 μM, 56.08 μM) and HBeAg (IC_{50} = 204.80 μM, 173.51 μM, 70.39 μM), along with HBV DNA replication (IC_{50} = 24.55 μM, 5.69 μM, 8.23 μM). These results demonstrated that the SARs of caudatin derivatives were summarized as (I) the anti-HBV activity and cytotoxicity would be enhanced when the unsaturated C≡C and C=C double bond incorporation in caudatin derivatives; (II) the activity would increase along with the increasing length of the introduced fatty chain till the carbon number of chain reached eight; (III) introduction of heterocylic rings containing the oxygen and sulfur atoms could largely enhance the activity; (IV) hydroxyl groups were important feature in maintaining antiviral activity; (V) hydroxyl group at C-3 of caudatin was a good target for further lead optimization by introduction of the rational substituent. This study provided valuable information for the designing, exploring, and developing novel anti-HBV agents to prevent HBV infection.

4. EXPERIMENTAL METHODS

4.1. Chemistry

^1H and ^{13}C NMR spectra were recorded on Bruker AM 400 MHz or Bruker DRX 500 MHz or Bruker Avance III 600 MHz spectrometers with tetramethylsilane (TMS) as the internal standard (Bruker, Bremerhaven, Germany). MS and HRMS spectra were determined on AutoSpec Premier P776 (VG, Manchester, UK) or API QSTAR Pulsar (AB, Foster City, USA) mass spectrometers. Column chromatography (CC): silica gel (200–300 mesh; Qingdao Makall Group CO., LTD; Qingdao; China). All reactions were monitored by thin-layer chromatography (TLC) on silica gel plates. Caudatin was isolated from *Cynanchum auriculatum* (Bai-Shou-Wu) and had the purity of > 95.0 %. Reagents were purchased from Alfa Aesar or J&K Scientific Ltd. Organic solvents were analytical reagent grade and purchased from Tianjin Chemical Reagent Co., Ltd.

4.2. Synthesis of Compound 3-*O*-Acetylcaudatin (1) [7]

A mixture of caudatin (0.2 mmol), acetic anhydride (1.2 equiv) and DMAP (0.2 equiv) was heated in anhydrous pyridine (5.0 mL) overnight under reflux until the starting material was not observed by TLC check. The reaction mixture was diluted with EtOAc (20 mL) and washed with 5% HCl solution (20 ×3 mL). The organic layer was dried using Na_2SO_4 and concentrated under reduced pressure. The residue was purified using a silica gel column to afford the pure target product. White amorphous powder, yield 86.3 % (after chromatography with petroleum ether/acetone, 85:15); ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.04 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.14 (3H, s, CH_3-19), 1.39 (3H, s, CH_3-18), 1.60 (1H, m, H-9), 1.78-1.97 (9H, overlap, H-1, 2, 11, 15, 16), 2.02 (3H, s, CH_3-7′), 2.11 (3H, s, CH_3-21), 2.15 (2H, s, H-7), 2.27 (3H, s, CH_3CO), 2.35-2.39 (3H, overlap, H-4′, 4), 2.83 (1H, m, H-16), 4.53-4.65 (2H, overlap, H-12, 3), 5.39 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 21.3 (C-2″), 24.1 (C-11), 26.8 (C-2), 27.2 (C-21), 31.7 (C-16), 33.2 (C-7), 34.2 (C-15), 36.9 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.5 (C-9), 57.9 (C-13), 71.5 (C-12), 73.6 (C-3), 74.1 (C-8), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 118.7 (C-6), 139.3 (C-5), 165.9 (C-3′), 167.0 (C-1′), 170.5 (C-1″), 208.9 (C-20); ESIMS: *m/z* 531 $[M-H]^-$, HRESIMS: calcd for $C_{30}H_{43}O_8$ $[M-H]^-$, 531.2957, found 531.2946.

4.3. General Procedure A for Preparation of Derivatives (2-31) [8]

The DCC (1.2 equiv) was added to the solution of caudatin (0.2 mmol), DMAP (0.2 equiv), and appropriate carboxylic acid (1.2 equiv) in anhydrous CH_2Cl_2 (8 mL) at 0 °C. The resulting mixture was stirred at room temperature until the starting material was not observed by TLC. The reaction mixture was filtered, and the residue was washed with CH_2Cl_2 (2×10 mL). Then, the CH_2Cl_2 solution was washed with 5% HCl (3×30 mL), saturated $NaHCO_3$ (3×30 mL) and saturated NaCl (3×30 mL), respectively. Subsequently, the organic layer was dried by Na_2SO_4 and concentrated to dryness under reduced pressure. At last, the residue was purified by column chromatography over the silica gel with petroleum ether/acetone (60:40 – 95:5) to yield the pure target compounds.

4.3.1. 3-O-Acryloylcaudatin (2)

White amorphous powder, yield 53.0 %; ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.17 (3H, s, CH_3-19), 1.40 (3H, s, CH_3-18), 1.62 (1H, m, H-9), 1.81-1.99 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.21 (2H, s, H-7), 2.32-2.44 (3H, overlap, H-4′, 4), 2.85 (1H, m, H-16), 4.56 (1H, dd, J = 10.2, 5.2 Hz, H-12), 4.72 (1H, m, H-3), 5.41 (1H, s, H-6), 5.52 (1H, s, H-2′), 5.80 (1H, d, J = 10.4 Hz, H-3″a), 6.09 (1H, dd, J = 17.5, 10.4 Hz, H-2″), 6.38 (1H, d, J = 17.5 Hz, H-3″b); ^{13}C NMR ($CDCl_3$, 125MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 26.9 (C-2), 27.1 (C-21), 31.8 (C-16), 33.2 (C-7), 34.2 (C-15), 36.9 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.6 (C-9), 57.9 (C-13), 71.6 (C-12), 73.8 (C-3), 74.2 (C-8), 88.0 (C-14), 91.4 (C-17), 112.9 (C-2′), 118.8 (C-6), 128.8 (C-2″), 130.5 (C-3″), 139.3 (C-5), 165.5 (C-3′), 165.9 (C-1″), 166.8 (C-1′), 208.8 (C-20); EIMS: *m/z* 544, HREIMS: calcd for $C_{31}H_{44}O_8$, 544.3036, found 544.3041.

4.3.2. 3-O-Propioloylcaudatin (3)

White amorphous powder, yield 46.4 %; ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.06 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.16 (3H, s, CH_3-19), 1.38 (3H, s, CH_3-18), 1.57 (1H, m, H-9), 1.82-2.00 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.15 (3H, s, CH_3-21), 2.21 (2H, s, H-7), 2.32-2.44 (3H, overlap, H-4′, 4), 2.82 (1H, m, H-16), 2.88 (1H, s, CH≡C), 4.57 (1H, dd, J = 10.5, 5.3 Hz, H-12), 4.74 (1H, m, H-3), 5.42 (1H, s, H-6), 5.52 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.3 (C-18), 16.5 (C-7′), 18.3 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 26.6 (C-2), 27.2 (C-21), 31.7 (C-16), 33.3 (C-7), 34.3 (C-15), 36.9 (C-10), 37.5 (C-1), 38.2 (C-4′), 38.3 (C-4), 43.5 (C-9), 58.0 (C-13), 71.6 (C-12), 74.1 (C-8), 74.4 (C-3″), 74.9 (C-2″), 76.0 (C-3), 87.8 (C-14), 91.4 (C-17), 112.9 (C-2′), 119.4 (C-6), 138.7 (C-5), 166.0 (C-3′), 152.0 (C-1″), 167.1 (C-1′), 208.8 (C-20); EIMS: *m/z* 542, HREIMS: calcd for $C_{31}H_{42}O_8$, 542.2880, found 542.2863.

4.3.3. 3-O-(But-2-ynoyl)caudatin (4)

White amorphous powder, yield 49.5 %; ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.03 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.12 (3H, s, CH_3-19), 1.37 (3H, s, CH_3-18), 1.52 (1H, m, H-9), 1.79-1.94 (9H, overlap, H-1, 2, 11, 15, 16), 1.96 (3H, s, CH_3-C≡C), 2.09 (3H, s, CH_3-7′), 2.13 (3H, s, CH_3-21), 2.17 (2H, s, H-7), 2.30-2.42 (3H, overlap, H-4′, 4), 2.82 (1H, m, H-16), 4.53 (1H, m, H-12), 4.67 (1H, m, H-3), 5.39 (1H, s, H-6), 5.49 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125MHz): δ 3.7 (C-4″), 9.3 (C-18), 16.4 (C-7′), 18.2 (C-19), 20.8 (2C-5′, 6′), 24.1 (C-11), 26.6 (C-2), 27.1 (C-21), 31.7 (C-16), 33.2 (C-7), 34.2 (C-15), 36.8 (C-10), 37.6 (C-1), 38.1 (C-4′), 38.3 (C-4), 43.5 (C-9), 57.8 (C-13), 71.5 (C-12), 72.5 (C-2″), 74.0 (C-8), 75.3 (C-3), 85.4 (C-3″), 87.9 (C-14), 91.4 (C-17), 112.8 (C-2′), 119.1 (C-6), 138.8 (C-5), 153.0 (C-1″), 165.9 (C-3′), 166.9 (C-1′), 208.8 (C-20); EIMS: *m/z* 556, HREIMS: calcd for $C_{32}H_{44}O_8$, 556.3036, found 556.2996.

4.3.4. 3-O-(2-Methylpentanoyl)caudatin (5)

White amorphous powder, yield 82.3 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 0.89 (3H, t, J = 7.2 Hz, CH_3-5″), 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.11 (3H, d, J = 6.9 Hz, CH_3-6″), 1.16 (3H, s, CH_3-19), 1.31 (2H, m, H-4″), 1.40 (3H, s, CH_3-18), 1.53-1.69 (2H, m, H-9, 3″), 1.82-2.00 (9H, overlap, H-1, 2, 11, 15, 16), 2.12 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.20 (2H, s, H-7), 2.32-2.42 (4H, overlap, H-4′, 4, 2″), 2.83 (1H, m, H-16), 4.54-4.64 (2H, overlap, H-12, 3), 5.40 (1H, s, H-6), 5.52 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 13.9 (C-5″), 16.5 (C-7′), 17.0 (C-6″), 18.5 (C-19), 20.4 (C-4″), 20.8 (C-5′), 20.9 (C-6′), 24.2 (C-11), 26.9 (C-2), 27.2 (C-21), 31.8 (C-16), 33.2 (C-7), 34.2 (C-15), 35.9 (C-3″), 37.0 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.4 (C-4), 39.4 (C-2″), 43.6 (C-9), 57.9 (C-13), 71.6 (C-12), 73.2 (C-3), 74.2 (C-8), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 118.6 (C-6), 139.5 (C-5), 166.0 (C-3′), 166.9 (C-1′), 176.3 (C-1″), 208.9 (C-20); ESIMS: *m/z* 587 $[M-H]^-$, HRESIMS: calcd for $C_{34}H_{51}O_8$ $[M-H]^-$, 587.3583, found 587.3584.

4.3.5. 3-O-(4-Methylpentanoyl)caudatin (6)

White amorphous powder, yield 80.1 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 0.88 (6H, d, J = 6.4 Hz, CH_3-5″, 6″), 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.15 (3H, s, CH_3-19), 1.39 (3H, s, CH_3-18), 1.47-1.69 (4H, m, H-9, 3″, 4″), 1.81-2.00 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.19 (2H, s, H-7), 2.26 (2H, t, J = 6.8 Hz, H-2″), 2.32-2.38 (3H, overlap, H-4′, 4), 2.84 (1H, m, H-16), 4.53-4.66 (2H, overlap, H-12, 3), 5.39 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.5 (C-19), 20.8 (C-5′), 20.9 (C-6′), 22.2 (2C-5″, 6″), 24.1 (C-11), 26.9 (C-2), 27.2 (C-21), 27.6 (C-4″),31.8 (C-16), 32.7 (C-2″), 33.2 (C-7), 33.7 (C-3″), 34.2 (C-15), 36.9 (C-10), 37.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.6 (C-9), 57.9 (C-13), 71.6 (C-12), 73.4 (C-3), 74.2 (C-8), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 118.7 (C-6), 139.4 (C-5), 166.0 (C-3′), 166.9 (C-1′), 173.5 (C-1″), 208.9 (C-20); E-SIMS: *m/z* 587 $[M-H]^-$, HRESIMS: calcd for $C_{34}H_{51}O_8$ $[M-H]^-$, 587.3583, found 587.3587.

4.3.6. 3-O-Octanoylcaudatin (7)

White amorphous powder, yield 78.6 %; ^{1}H NMR ($CDCl_3$, 600MHz): δ 0.82 (3H, t, J = 6.9 Hz, CH_3-8″), 1.02 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.12 (3H, s, CH_3-19), 1.19-1.25 (8H, overlap, 4″, 5″, 6″, 7″), 1.36 (3H, s, CH_3-18), 1.48-1.61 (3H, m, H-9, 3″), 1.82-1.96 (9H, overlap, H-1, 2, 11, 15, 16), 2.09 (3H, s, CH_3-7′), 2.15 (3H, s, CH_3-21), 2.17 (2H, s, H-7), 2.22 (2H, t, J = 6.2 Hz, H-2″), 2.30-2.36 (3H, overlap, H-4′, 4), 2.81 (1H, m, H-16), 4.51 (1H, dd, J = 10.9, 4.9 Hz, H-12), 4.58 (1H, m, H-3), 5.36 (1H, s, H-6), 5.48 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 150MHz): δ 9.6 (C-18), 14.4 (C-8″), 16.7 (C-7′), 18.7 (C-19), 21.1 (2C-5′, 6′), 22.8 (C-7″), 24.3 (C-11), 25.2 (C-3″), 27.1 (C-2), 27.4 (C-21), 29.1 (C-5″), 29.2 (C-4″), 31.8 (C-6″), 31.9 (C-16), 33.4 (C-7), 34.4 (C-15), 34.8 (C-2″), 37.1 (C-10), 38.0 (C-1), 38.3 (C-4′), 38.6 (C-4), 43.7 (C-9), 58.0 (C-13), 71.7 (C-12), 73.6 (C-3), 74.3 (C-8), 88.2 (C-14), 91.6 (C-17), 113.1 (C-2′), 118.9 (C-6), 139.5 (C-5), 166.1 (C-3′), 167.2 (C-1′), 173.5 (C-1″), 209.2 (C-20); ESIMS: *m/z* 651 $[M+Cl]^-$, HRESIMS: calcd for $C_{36}H_{56}O_8Cl$ $[M+Cl]^-$ 651.3663, found 651.3673.

4.3.7. 3-O-(Oct-2-enoyl)caudatin (8)

Wite amorphous powder, yield 89.5 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 0.88 (3H, t, J = 6.9 Hz, CH_3-8″), 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.16 (3H, s, CH_3-19), 1.24-1.31 (6H, overlap, H-5″, 6″, 7″), 1.40 (3H, s, CH_3-18), 1.57 (1H, m, H-9), 1.82-2.00 (9H, overlap, H-1, 2, 11, 15, 16), 2.12 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.19-2.21 (4H, overlap, H-7, 4″), 2.32-2.43 (3H, overlap, H-4′, 4), 2.85 (1H, m, H-16), 4.55 (1H, dd, J = 10.1, 5.9 Hz, H-12), 4.66 (1H, m, H-3), 5.40 (1H, s, H-6), 5.52 (1H, s, H-2′), 5.78 (1H, d, J = 15.6 Hz, H-2″), 6.95 (1H, m, H-3″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.4 (C-18), 13.9 (C-8″), 16.4 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 22.4 (C-7″), 24.1 (C-11), 26.9 (C-2), 27.1 (C-21), 27.6 (C-5″), 31.2 (C-6″), 31.7 (C-16), 32.1 (C-4″), 33.2 (C-7), 34.2 (C-15), 36.9 (C-10), 37.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.5 (C-9), 57.9 (C-13), 71.5 (C-12), 73.4 (C-3), 74.1 (C-8), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 118.7 (C-6), 121.3 (C-2″), 139.4 (C-5), 149.6 (C-3″), 165.9 (C-3′), 166.9 (C-1′), 166.1 (C-1″), 208.9 (C-20); ESIMS: *m/z* 649 $[M+Cl]^-$, HRESIMS: calcd for $C_{36}H_{54}O_8Cl$ $[M+Cl]^-$ 649.3507, found 649.3500.

4.3.8. 3-O-Decanoylcaudatin (9)

White amorphous powder, yield 84.8 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 0.86 (3H, t, J = 6.7 Hz, CH_3-10″), 1.04 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.14 (3H, s, CH_3-19), 1.24-1.27 (12H, overlap, 4″, 5″, 6″, 7″, 8″, 9″), 1.40 (3H, s, CH_3-18), 1.51-1.65 (3H, m, H-9, 3″), 1.80-1.98 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.15 (3H, s, CH_3-21), 2.19 (2H, s, H-7), 2.25 (2H, t, J = 7.5 Hz, H-2″), 2.31-2.38 (3H, overlap, H-4′, 4), 2.84 (1H, m, H-16), 4.55 (1H, dd, J = 10.4, 5.5 Hz, H-12), 4.61 (1H, m, H-3), 5.38 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 14.0 (C-10″), 16.4 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 22.6 (C-9″), 24.1 (C-11), 24.9 (C-3″), 26.9 (C-2), 27.1 (C-21), 29.0 (C-4″), 29.2 (2C-5″, 7″), 29.3 (C-6″), 31.7 (C-8″), 31.8 (C-16), 33.2 (C-7), 34.2 (C-2″), 34.6 (C-15), 36.9 (C-10), 37.8 (C-1), 38.0 (C-4′), 38.4 (C-4), 43.5 (C-9), 57.8 (C-13), 71.5 (C-12), 73.4 (C-3), 74.1 (C-8), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 118.7 (C-6), 139.3 (C-5), 165.9 (C-3′), 166.8 (C-1′), 173.3 (C-1″), 208.9 (C-20); ESIMS: *m/z* 679 $[M+Cl]^-$, HRESIMS: calcd for $C_{38}H_{60}O_8Cl$ $[M+Cl]^-$ 679.3976, found 679.3983.

4.3.9. 3-O-(Dec-2-enoyl)caudatin (10)

White amorphous powder, yield 77.3 %; ^{1}H NMR ($CDCl_3$, 600MHz): δ 0.83 (3H, t, J = 6.7 Hz, CH_3-10″), 1.02 (6H, d, J=6.8 Hz, CH_3-5′, 6′), 1.13 (3H, s, CH_3-19), 1.21-1.31 (10H, overlap, H-5″, 6″, 7″, 8″, 9″), 1.38 (3H, s, CH_3-18), 1.54 (1H, m, H-9), 1.78-1.97 (9H, overlap, H-1, 2, 11, 15, 16), 2.10 (3H, s, CH_3-7′), 2.14 (3H, s, CH_3-21), 2.16-2.19 (4H, overlap, H-7, 4″), 2.29-2.40 (3H, overlap, H-4′, 4), 2.82 (1H, m, H-16), 4.51 (1H, dd, J = 10.7, 4.5 Hz, H-12), 4.63 (1H, m, H-3), 5.36 (1H, s, H-6), 5.47 (1H, s, H-2′), 5.73 (1H, d, J = 15.0 Hz, H-2″), 6.91 (1H, m, H-3″); ^{13}C NMR ($CDCl_3$, 150MHz): δ 9.6 (C-18), 14.3 (C-10″), 16.7 (C-7′), 18.6 (C-19), 21.0 (C-5′), 21.1 (C-6′), 22.8 (C-9″), 24.3 (C-11), 26.9 (C-2), 27.1 (C-21), 28.1 (C-7″), 29.2 (C-5″), 29.4 (C-6″), 31.7 (C-16), 31.9 (C-8″), 32.4 (C-4″), 33.4 (C-7), 34.3 (C-15), 37.1 (C-10), 38.1 (C-1), 38.3 (C-4′), 38.6 (C-4), 43.7 (C-9), 58.0 (C-13), 71.7 (C-12), 73.6 (C-3), 74.3 (C-8), 88.2 (C-14), 91.6 (C-17), 113.0 (C-2′), 118.9 (C-6), 121.4 (C-2″), 139.4 (C-5), 149.9 (C-3″), 166.1 (C-3′), 166.2 (C-1″), 167.1 (C-1′), 209.2 (C-20); ESIMS: *m/z* 677 $[M+Cl]^-$, HRESIMS: calcd for $C_{38}H_{58}O_8Cl$ $[M+Cl]^-$ 677.3820, found 677.3830.

4.3.10. 3-O-Dodecanoylcaudatin (11)

White amorphous powder, yield 79.6 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 0.86 (3H, t, J = 6.7 Hz, CH_3-12″), 1.04 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.14 (3H, s, CH_3-19), 1.24-1.27 (16H, overlap, H-4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″), 1.40 (3H, s, CH_3-18), 1.51-1.65 (3H, m, H-9, 3″), 1.80-1.99 (9H, overlap, H-1, 2, 11, 15, 16), 2.09 (3H, s, CH_3-7′), 2.15 (3H, s, CH_3-21), 2.19 (2H, s, H-7), 2.25 (2H, t, J = 7.5 Hz, H-2″), 2.31-2.38 (3H, overlap, H-4′, 4), 2.85 (1H, m, H-16), 4.55 (1H, dd, J = 10.4, 5.5 Hz, H-12), 4.61 (1H, m, H-3), 5.38 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 14.0 (C-12″), 16.4 (C-7′), 18.3 (C-19), 20.7 (C-5′), 20.8 (C-6′), 22.6 (C-11″), 24.1 (C-11), 24.9 (C-3″), 26.8 (C-2), 27.1 (C-21), 29.0 (C-4″), 29.1 (C-9″), 29.2 (C-5″), 29.4

(C-7″), 29.5(2C-6″, 8″), 31.7 (C-10″), 31.8 (C-16), 33.1 (C-7), 34.1 (C-2″), 34.5 (C-15), 36.9 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.5 (C-9), 57.7 (C-13), 71.5 (C-12), 73.4 (C-3), 74.1 (C-8), 88.0 (C-14), 91.5 (C-17), 112.9 (C-2′), 118.7 (C-6), 139.2 (C-5), 165.9 (C-3′), 166.7 (C-1′), 173.3 (C-1″), 208.9 (C-20); ESIMS: *m/z* 707 $[M+Cl]^-$, HRESIMS: calcd for $C_{40}H_{64}O_8Cl$ $[M+Cl]^-$ 707.4289, found 707.4290.

4.3.11. 3-O-Tetradecanoylcaudatin (12)

White amorphous powder, yield 88.2 %; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.86 (3H, t, J = 6.7 Hz, CH_3-14″), 1.04 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.15 (3H, s, CH_3-19), 1.24-1.29 (20H, overlap, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″), 1.40 (3H, s, CH_3-18), 1.52-1.65 (3H, m, H-9, 3″), 1.81-1.98 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.19 (2H, s, H-7), 2.25 (2H, t, J = 7.5 Hz, H-2″), 2.32-2.38 (3H, overlap, H-4′, 4), 2.84 (1H, m, H-16), 4.55 (1H, dd, J = 10.5, 5.5 Hz, H-12), 4.62 (1H, m, H-3), 5.39 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 9.4 (C-18), 14.1 (C-14″), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 22.6 (C-13″), 24.1 (C-11), 25.0 (C-3″), 26.9 (C-2), 27.1 (C-21), 29.1 (C-4″), 29.2 (C-11″), 29.3 (C-5″), 29.4 (C-7″), 29.5 (C-9″), 29.6(3C-6″, 8″, 10″), 31.8 (C-12″), 31.9 (C-16), 33.2 (C-7), 34.2 (C-2″), 34.6 (C-15), 36.9 (C-10), 37.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.6 (C-9), 57.9 (C-13), 71.6 (C-12), 73.4 (C-3), 74.1 (C-8), 88.0 (C-14), 91.5 (C-17), 112.9 (C-2′), 118.7 (C-6), 139.4 (C-5), 165.9 (C-3′), 166.8 (C-1′), 173.3 (C-1″), 208.9 (C-20); E-SIMS: *m/z* 735 $[M+Cl]^-$, HRESIMS: calcd for $C_{42}H_{68}O_8Cl$ $[M+Cl]^-$ 735.4602, found 735.4606.

4.3.12. 3-O-Palmitoylcaudatin (13)

White amorphous powder, yield 72.5 %; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.88 (3H, t, J = 6.7 Hz, CH_3-16″), 1.06 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.17 (3H, s, CH_3-19), 1.26-1.29 (24H, overlap, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″), 1.47 (3H, s, CH_3-18), 1.53-1.68 (3H, m, H-9, 3″), 1.78-2.00 (9H, overlap, H-1, 2, 11, 15, 16), 2.13 (3H, s, CH_3-7′), 2.19 (2H, s, H-7), 2.20 (3H, s, CH_3-21), 2.27 (2H, t, J = 7.5 Hz, H-2″), 2.35-2.39 (3H, overlap, H-4′, 4), 2.89 (1H, m, H-16), 4.54 (1H, dd, J = 11.5, 4.1 Hz, H-12), 4.62 (1H, m, H-3), 5.40 (1H, s, H-6), 5.52 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.5 (C-18), 13.9 (C-16″), 16.4 (C-7′), 18.0 (C-19), 20.7 (C-5′), 20.7 (C-6′), 22.6 (C-15″), 24.1 (C-11), 24.9 (C-3″), 26.8 (C-2), 26.9 (C-21), 29.0 (C-4″), 29.1 (C-13″), 29.2 (C-5″), 29.3 (C-7″), 29.5 (6C-6″, 8″, 9″, 10″, 11″, 12″), 31.7 (C-14″), 31.8 (C-16), 33.0 (C-7), 34.1 (C-2″), 34.5 (C-15), 36.8 (C-10), 37.8 (C-1), 38.0 (C-4′), 38.3 (C-4), 43.5 (C-9), 57.4 (C-13), 71.4 (C-12), 73.6 (C-3), 73.8 (C-8), 88.2 (C-14), 91.5 (C-17), 112.8 (C-2′), 118.8 (C-6), 138.8 (C-5), 165.9 (C-3′), 166.6 (C-1′), 173.6 (C-1″), 209.6 (C-20); E-SIMS: *m/z* 735 $[M+Cl]^-$, HRESIMS: calcd for $C_{44}H_{72}O_8Cl$ $[M+Cl]^-$ 763.4915, found 763.4909.

4.3.13. 3-O-Stearoylcaudatin (14)

White amorphous powder, yield 50.2 %; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.88 (3H, t, J = 6.7 Hz, CH_3-18″), 1.06 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.17 (3H, s, CH_3-19), 1.25-1.28 (28H, overlap, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″, 16″, 17″), 1.47 (3H, s, CH_3-18), 1.53-1.68 (3H, m, H-9, 3″), 1.77-2.00 (9H, overlap, H-1, 2, 11, 15, 16), 2.13 (3H, s, CH_3-7′), 2.18 (2H, s, H-7), 2.19 (3H, s, CH_3-21), 2.27 (2H, t, J = 7.5 Hz, H-2″), 2.35-2.39 (3H, overlap, H-4′, 4), 2.89 (1H, m, H-16), 4.54 (1H, dd, J = 11.5, 4.2 Hz, H-12), 4.63 (1H, m, H-3), 5.40 (1H, s, H-6), 5.52 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.5 (C-18), 14.0 (C-18″), 16.4 (C-7′), 18.0 (C-19), 20.7 (C-5′), 20.8 (C-6′), 22.6 (C-17″), 24.1 (C-11), 24.9 (C-3″), 26.9 (C-2), 27.0 (C-21), 29.0 (C-4″), 29.1 (C-15″), 29.2 (C-5″), 29.3 (C-7″), 29.5 (C-9″), 29.6 (7C-6″, 8″, 10″, 11″, 12″, 13″, 14″), 31.7 (C-16″), 31.8 (C-16), 33.0 (C-7), 34.1 (C-2″), 34.5 (C-15), 36.8 (C-10), 37.8 (C-1), 38.0 (C-4′), 38.3 (C-4), 43.5 (C-9), 57.4 (C-13), 71.4 (C-12), 73.6 (C-3), 73.8 (C-8), 88.2 (C-14), 91.5 (C-17), 112.8 (C-2′), 118.8 (C-6), 138.8 (C-5), 165.9 (C-3′), 166.7 (C-1′), 173.6 (C-1″), 209.6 (C-20); ESIMS: *m/z* 735 $[M+Cl]^-$, HRESIMS: calcd for $C_{46}H_{76}O_8Cl$ $[M+Cl]^-$ 791.5228, found 791.5222.

4.3.14. 3-O-(Octadeca-9, 12-dienoyl)caudatin (15)

White amorphous powder, yield 69.6 %; ^{1}H NMR ($CDCl_3$, 500MHz): δ 0.83 (3H, t, J = 6.7 Hz, CH_3-18″), 1.02 (6H, d, J=6.8 Hz, CH_3-5′, 6′), 1.11 (3H, s, CH_3-19), 1.25-1.31 (14H, overlap, 4″, 5″, 6″, 7″, 15″, 16″, 17″), 1.37 (3H, s, CH_3-18), 1.48-1.64 (3H, overlap, H-9, 3″), 1.77-1.95 (9H, overlap, H-1, 2, 11, 15, 16), 1.98-2.03 (4H, overlap, 8″, 14″), 2.07 (3H, s, CH_3-7′), 2.11 (3H, s, CH_3-21), 2.15 (2H, s, H-7), 2.25 (2H, t, J = 7.5 Hz, H-2″), 2.28-2.38 (3H, overlap, H-4′, 4), 2.71 (2H, t, J = 6.5 Hz, 11″), 2.82 (1H, m, H-16), 4.50 (1H, dd, J = 11.0, 4.6 Hz, H-12), 4.57 (1H, m, H-3), 5.26-5.34 (5H, overlap, H-6, 9″, 10″, 12″, 13″), 5.47 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125MHz): δ 9.3 (C-18), 13.9 (C-18″), 16.4 (C-7′), 18.2 (C-19), 20.7 (C-5′), 20.8 (C-6′), 22.4 (C-17″), 24.0 (C-11), 24.8 (C-3″), 25.5 (C-11″), 26.8 (C-2), 27.0 (3C-21, 8″, 14″), 28.9 (2C-4″, 5″), 29.0 (C-15″), 29.2 (C-6″), 29.4 (C-7″), 31.3 (C-16″), 31.7 (C-16), 33.1 (C-7), 34.1 (C-15), 34.4 (C-2″), 36.8 (C-10), 37.8 (C-1), 38.0 (C-4′), 38.3 (C-4), 43.5 (C-9), 57.7 (C-13), 71.4 (C-12), 73.3 (C-3), 74.0 (C-8), 88.0 (C-14), 91.4 (C-17), 112.8 (C-2′), 118.6 (C-6), 127.7 (C-12″), 127.9 (C-10″), 129.8 (C-13″), 130.0 (C-9″), 139.1 (C-5), 165.8 (C-3′), 173.1 (C-1″), 166.5 (C-1′), 208.8 (C-20); ESIMS: *m/z* 787 $[M+Cl]^-$, HRESIMS: calcd for $C_{46}H_{72}O_8Cl$ $[M+Cl]^-$ 787.4915, found 787.4924.

4.3.15. 3-O-(Octadeca -9, 12, 15-trienoyl)caudatin (16)

White amorphous powder, yield 79.6 %; ^{1}H NMR ($CDCl_3$, 500MHz): δ 0.94 (3H, t, J = 7.5 Hz, CH_3-18″), 1.03 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.13 (3H, s, CH_3-19), 1.27-1.31 (8H, overlap, 4″, 5″, 6″, 7″), 1.38 (3H, s, CH_3-18), 1.50-1.63 (3H, overlap, H-9, 3″), 1.79-1.97 (9H, overlap, H-1, 2, 11, 15, 16), 2.01-2.05 (4H, overlap, 8″, 17″), 2.09 (3H, s, CH_3-7′), 2.13 (3H, s, CH_3-21), 2.17 (2H, s, H-7), 2.23 (2H, t, J = 7.5 Hz, H-2″), 2.31-2.36 (3H, overlap, H-4′, 4), 2.76-2.86 (5H, overlap, H-16, 11″, 14″), 4.52 (1H, dd, J = 10.7, 5.0 Hz, H-12), 4.60 (1H, m, H-3), 5.27-5.36 (7H, overlap, H-6, 9″, 10″, 12″, 13″, 15″, 16″), 5.49 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125MHz): δ 9.4 (C-18), 14.2 (C-18″), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.2 (C-11), 24.9 (C-3″), 25.6 (C-17″), 26.9 (C-2), 27.1 (C-21), 29.0 (3C-4″, 5″, 6″), 29.1 (C-7″), 29.5 (C-8″), 31.8 (C-16), 33.2 (C-7), 34.2 (C-15), 34.6 (C-2″), 36.9 (C-10), 37.9 (3C-1, 11″, 14″), 38.1 (C-4′), 38.4 (C-4), 43.6 (C-9), 57.8 (C-13), 71.6 (C-12), 73.4 (C-3), 74.1 (C-8), 88.0 (C-14), 91.5 (C-17), 112.9 (C-2′), 118.7 (C-6), 127.1 (C-10″), 127.7 (C-15″), 128.2 (2C-12″, 13″),

130.2 (C-9″), 131.9 (C-16″), 139.3 (C-5), 165.9 (C-3′), 173.2 (C-1″), 166.7 (C-1′), 208.8 (C-20); ESIMS: *m/z* 785 $[M+Cl]^-$, HRESIMS: calcd for $C_{46}H_{70}O_8Cl$ $[M+Cl]^-$ 785.4759, found 785.4746.

4.3.16. 3-O-[1-(2,4-dichlorophenyl)cyclopropanecarbonyl] caudatin (17)

White amorphous powder, yield 71.1 %; ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.06 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.11 (3H, s, CH_3-19), 1.48 (3H, s, CH_3-18), 1.56-1.77 (5H, overlap, H-9, 3″, 4″), 1.84-1.99 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.19 (3H, s, CH_3-21), 2.20 (2H, s, H-7), 2.31-2.39 (3H, overlap, H-4′, 4), 2.88 (1H, m, H-16), 4.52 (1H, dd, J = 9.2, 3.3 Hz, H-12), 4.59 (1H, m, H-3), 5.36 (1H, s, H-6), 5.52 (1H, s, H-2′), 7.40 (1H, s, H-7″), 7.20-7.25 (2H, overlap, H-9″, 10″); ^{13}C NMR ($CDCl_3$, 125MHz): δ 9.3 (C-18), 16.1 (C-7′), 17.2 (2C-3″, 4″), 17.8 (C-19), 20.4 (C-5′), 20.5 (C-6′), 23.8 (C-11), 26.4 (C-2), 26.7 (C-21), 27.3 (C-2″), 31.5 (C-16), 32.8 (C-7), 33.8 (C-15), 36.5 (C-10), 37.3 (C-1), 37.8 (C-4′), 38.0 (C-4), 43.3 (C-9), 57.1 (C-13), 71.2 (C-12), 73.5 (C-8), 74.6 (C-3), 88.1 (C-14), 91.4 (C-17), 112.6 (C-2′), 118.7 (C-6), 126.5 (C-9″), 128.8 (C-10″), 132.0 (C-7″), 133.3 (C-6″), 136.0 (C-8″), 137.2 (C-5″), 138.4 (C-5), 165.7 (C-3′), 166.3 (C-1′), 172.6 (C-1″), 209.5 (C-20); EIMS: *m/z* 702, HREIMS: calcd for $C_{38}H_{48}O_8Cl_2$, 702.2726, found 702.2680.

4.3.17. 3-O-(Cyclopentanecarbonyl)caudatin (18)

White amorphous powder, yield 69.2 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.06 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.15 (3H, s, CH_3-19), 1.40 (3H, s, CH_3-18), 1.54-1.78 (7H, overlap, H-9, 3″, 4″, 5″, 6″), 1.80-1.88 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.19 (2H, s, H-7), 2.32-2.37 (5H, overlap, H-4′, 4, 3″, 6″), 2.67 (1H, m, H-2″), 2.85 (1H, m, H-16), 4.54 (1H, dd, J = 10.4, 5.2 Hz, H-12), 4.61 (1H, m, H-3), 5.38 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 25.8 (2C-4″, 5″), 26.8 (C-2), 27.1 (C-21), 29.9 (2C-3″, 6″), 31.8 (C-16), 33.2 (C-7), 34.2 (C-15), 36.9 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.6 (C-9), 43.9 (C-2″), 57.8 (C-13), 71.5 (C-12), 73.3 (C-3), 74.1 (C-8), 88.0 (C-14), 91.5 (C-17), 112.9 (C-2′), 118.6 (C-6), 139.4 (C-5), 165.9 (C-3′), 166.8 (C-1′), 176.2 (C-1″), 208.9 (C-20); ESIMS: *m/z* 609 $[M+Na]^+$, HRESIMS: calcd for $C_{34}H_{50}O_8Na$ $[M+Na]^+$ 609.3403, found 609.3411.

4.3.18. 3-O-[(S)-tetrahydrofuran-2-carbonyl]caudatin (19)

White amorphous powder, yield 81.9 %; ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.00 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.09 (3H, s, CH_3-19), 1.36 (3H, s, CH_3-18), 1.57 (1H, m, H-9), 1.75-1.91 (11H, overlap, H-1, 2, 11, 15, 16, 4″), 2.05 (3H, s, CH_3-7′), 2.10 (3H, s, CH_3-21), 2.11 (2H, s, H-7), 2.12-2.34 (5H, overlap, H-4′, 4, 3″), 2.79 (1H, m, H-16), 3.83 (1H, m, H-5″α), 3.92 (1H, m, H-5″β), 4.34 (1H, m, H-2″), 4.48 (1H, dd, J = 11.2, 4.4 Hz, H-12), 4.61 (1H, m, H-3), 5.32 (1H, s, H-6), 5.45 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.3 (C-18), 16.3 (C-7′), 18.1 (C-19), 20.6 (C-5′), 20.7 (C-6′), 24.0 (C-11), 25.0 (C-4″), 26.6 (C-2), 27.0 (C-21), 30.0 (C-3″), 31.7 (C-16), 33.0 (C-7), 34.0 (C-15), 36.7 (C-10), 37.5 (C-1), 37.9 (C-4′), 38.2 (C-4), 43.4 (C-9), 57.5 (C-13), 69.1 (C-5″), 71.3 (C-12), 73.8 (C-3), 74.1 (C-8), 76.5 (C-2″), 88.0 (C-14), 91.4 (C-17), 112.7 (C-2′), 118.9 (C-6), 138.7 (C-5), 165.7 (C-3′), 166.5 (C-1′), 172.7 (C-1″), 208.9 (C-20); ESIMS: *m/z* 611 $[M+Na]^+$ HRESIMS: calcd for $C_{33}H_{48}O_9Na$ $[M+Na]^+$ 611.3196, found 611.3196.

4.3.19. 3-O-[(R)-tetrahydrofuran-2-carbonyl]caudatin (20)

White amorphous powder, yield 72.6 %; ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.00 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.10 (3H, s, CH_3-19), 1.37 (3H, s, CH_3-18), 1.57 (1H, m, H-9), 1.76-1.93 (11H, overlap, H-1, 2, 11, 15, 16, 4″), 2.06 (3H, s, CH_3-7′), 2.11 (3H, s, CH_3-21), 2.14 (2H, s, H-7), 2.16-2.36 (5H, overlap, H-4′, 4, 3″), 2.81 (1H, m, H-16), 3.85 (1H, m, H-5″β), 3.94 (1H, m, H-5″α), 4.35 (1H, m, H-2″), 4.49 (1H, dd, J = 11.2, 4.5 Hz, H-12), 4.61 (1H, m, H-3), 5.34 (1H, s, H-6), 5.46 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.3 (C-18), 16.3 (C-7′), 18.1 (C-19), 20.6 (C-5′), 20.7 (C-6′), 24.0 (C-11), 25.0 (C-4″), 26.6 (C-2), 27.0 (C-21), 30.0 (C-3″), 31.7 (C-16), 33.0 (C-7), 34.0 (C-15), 36.7 (C-10), 37.6 (C-1), 37.9 (C-4′), 38.2 (C-4), 43.4 (C-9), 57.6 (C-13), 69.1 (C-5″), 71.3 (C-12), 73.9 (C-3), 74.0 (C-8), 76.5 (C-2″), 87.9 (C-14), 91.4 (C-17), 112.7 (C-2′), 118.9 (C-6), 138.7 (C-5), 165.8 (C-3′), 166.6 (C-1′), 172.7 (C-1″), 208.9 (C-20); ESIMS: *m/z* 611 $[M+Na]^+$ HRESIMS: calcd for $C_{33}H_{48}O_9Na$ $[M+Na]^+$ 611.3196, found 611.3203.

4.3.20. 3-O-(Furan-2-carbonyl)caudatin (21)

White amorphous powder, yield 65.5 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.04 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.17 (3H, s, CH_3-19), 1.41 (3H, s, CH_3-18), 1.56 (1H, m, H-9), 1.79-1.98 (9H, overlap, H-1, 2, 11, 15, 16), 2.10 (3H, s, CH_3-7′), 2.15 (3H, s, CH_3-21), 2.20 (2H, s, H-7), 2.34 (1H, m, H-4′), 2.46 (1H, m, H-4), 2.84 (1H, m, H-16), 4.54 (1H, dd, J = 10.6, 5.1 Hz, H-12), 4.84 (1H, m, H-3), 5.41 (1H, s, H-6), 5.50 (1H, s, H-2′), 6.48 (1H, m, H-4″), 7.15 (1H, d, J = 3.5 Hz, H-3″), 7.55 (1H, s, H-5″); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.3 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 26.9 (C-2), 27.1 (C-21), 31.8 (C-16), 33.2 (C-7), 34.2 (C-15), 36.9 (C-10), 37.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.5 (C-9), 57.8 (C-13), 71.5 (C-12), 74.1 (C-8), 74.4 (C-3), 88.0 (C-14), 91.5 (C-17), 111.8 (C-4″), 112.9 (C-2′), 117.8 (C-3″), 119.1 (C-6), 139.0 (C-5), 144.8 (C-2″), 146.2 (C-5″), 165.9 (C-3′), 166.8 (C-1′), 158.1 (C-1″), 209.0 (C-20); ESIMS: *m/z* 607 $[M+Na]^+$ HRESIMS: calcd for $C_{33}H_{44}O_9Na$ $[M+Na]^+$ 607.2883, found 607.2889.

4.3.21. 3-O-(Benzofuran-2-carbonyl)caudatin (22)

White amorphous powder, yield 57.9 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.04 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.19 (3H, s, CH_3-19), 1.40 (3H, s, CH_3-18), 1.57 (1H, m, H-9), 1.82-2.01 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.20 (2H, s, H-7), 2.33 (1H, m, H-4′), 2.54 (1H, m, H-4), 2.85 (1H, m, H-16), 4.55 (1H, dd, J = 10.4, 4.0 Hz, H-12), 4.92 (1H, m, H-3), 5.43 (1H, s, H-6), 5.51 (1H, s, H-2′), 7.27 (1H, t, J = 7.8 Hz, H-6″), 7.41 (1H, t, J = 7.5 Hz, H-7″), 7.56 (1H, d, J = 8.4 Hz, H-8″), 7.64 (1H, d, J = 7.8 Hz, H-5″); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.2 (C-11), 26.9 (C-2), 27.2 (C-21), 31.8 (C-16), 33.2 (C-7), 34.3 (C-15), 36.9 (C-10), 37.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.6 (C-9), 57.9 (C-13), 71.6 (C-12), 74.1 (C-8), 75.0 (C-3),

88.0 (C-14), 91.5 (C-17), 112.3 (C-8″), 112.9 (C-2′), 113.9 (C-3″), 119.2 (C-6), 122.7 (C-5″), 123.7 (C-6″), 126.9 (C-7″), 127.5 (C-4″), 138.9 (C-5), 145.7 (C-2″), 155.6 (C-9″), 166.0 (C-3′), 166.9 (C-1′), 159.0 (C-1″), 209.0 (C-20); E-SIMS: *m/z* 657 $[M+Na]^+$ HRESIMS: calcd for $C_{37}H_{46}O_9Na$ $[M+Na]^+$ 657.3039, found 657.3029.

4.3.22. 3-O-(5-Oxotetrahydrofuran-2-carbonyl)caudatin (23)

White amorphous powder, yield 65.5 %; ^1H NMR ($CDCl_3$, 400MHz): δ 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.15 (3H, s, CH_3-19), 1.40 (3H, s, CH_3-18), 1.60 (1H, m, H-9), 1.81-1.99 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.27-2.62 (9H, overlap, H-4′, 4, 7, 3″, 4″), 2.84 (1H, m, H-16), 4.54 (1H, dd, J = 10.9, 4.7 Hz, H-12), 4.72 (1H, m, H-3), 4.90 (1H, m, H-2″), 5.41 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.3 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 25.8 (C-3″), 26.7 (2C-2, 4″), 27.1 (C-21), 31.8 (C-16), 33.2 (C-7), 34.2 (C-15), 36.8 (C-10), 37.6 (C-1), 38.1 (C-4′), 38.2 (C-4), 43.5 (C-9), 57.8 (C-13), 71.5 (C-12), 74.0 (C-8), 75.5 (C-3), 75.8 (C-2″), 88.0 (C-14), 91.4 (C-17), 112.9 (C-2′), 119.5 (C-6), 138.5 (C-5), 166.0 (C-3′), 166.9 (C-1′), 169.2 (C-1″), 176.1 (C-5″), 208.9 (C-20); ESIMS: *m/z* 625 $[M+Na]^+$ HRESIMS: calcd for $C_{33}H_{46}O_{10}Na$ $[M+Na]^+$ 625.2988, found 625.2996.

4.3.23. 3-O-(Thiophene-2-carbonyl)caudatin (24)

White amorphous powder, yield 72.5 %; ^1H NMR ($CDCl_3$, 500MHz): δ 1.06 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.20 (3H, s, CH_3-19), 1.41 (3H, s, CH_3-18), 1.60 (1H, m, H-9), 1.84-2.00 (9H, overlap, H-1, 2, 11, 15, 16), 2.13 (3H, s, CH_3-7′), 2.17 (3H, s, CH_3-21), 2.24 (2H, s, H-7), 2.35 (1H, m, H-4′), 2.49 (1H, m, H-4), 2.86 (1H, m, H-16), 4.59 (1H, m, H-12), 4.84 (1H, m, H-3), 5.45 (1H, s, H-6), 5.53 (1H, s, H-2′), 7.09 (1H, t, J = 4.3 Hz, H-4″), 7.54 (1H, d, J = 4.6 Hz, H-5″), 7.79 (1H, d, J = 3.2 Hz, H-3″); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.5 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.2 (C-11), 27.0 (C-2), 27.2 (C-21), 31.7 (C-16), 33.3 (C-7), 34.3 (C-15), 37.0 (C-10), 37.9 (C-1), 38.2 (C-4′), 38.4 (C-4), 43.6 (C-9), 58.0 (C-13), 71.6 (C-12), 74.2 (C-8), 74.5 (C-3), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 127.7 (C-4″), 132.2 (C-5″), 133.3 (2C-2″, 3″), 119.0 (C-6), 139.3 (C-5), 166.0 (C-3′), 167.0 (C-1′), 158.2 (C-1″), 208.9 (C-20); EIMS: *m/z* 600, HREIMS: calcd for $C_{33}H_{44}O_8S$ 600.2757, found 600.2748.

4.3.24. 3-O-[Benzo[b]thiophene-2-carbonyl]caudatin (25)

White amorphous powder, yield 86.2 %; ^1H NMR ($CDCl_3$, 400MHz): δ 0.95 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.10 (3H, s, CH_3-19), 1.40 (3H, s, CH_3-18), 1.55 (1H, m, H-9), 1.72-1.91 (9H, overlap, H-1, 2, 11, 15, 16), 2.01 (3H, s, CH_3-7′), 2.05 (3H, s, CH_3-21), 2.10 (2H, s, H-7), 2.25 (1H, m, H-4′), 2.43 (1H, m, H-4), 2.79 (1H, m, H-16), 4.42 (1H, dd, J = 11.5, 3.9 Hz, H-12), 4.74 (1H, m, H-3), 5.30 (1H, s, H-6), 5.42 (1H, s, H-2′), 7.25-7.34 (2H, overlap, H-6″, 7″), 7.72-7.75 (2H, overlap, H-8″, 5″), 7.92 (1H, s, H-3″); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.3 (C-18), 16.0 (C-7′), 17.6 (C-19), 20.3 (C-5′), 20.4 (C-6′), 23.9 (C-11), 26.6 (C-2), 26.7 (C-21), 31.6 (C-16), 32.8 (C-7), 33.3 (C-15), 36.6 (C-10), 37.6 (C-1), 37.8 (C-4′), 38.0 (C-4), 43.4 (C-9), 57.1 (C-13), 71.3 (C-12), 73.5 (C-8), 75.2 (C-3), 88.3 (C-14), 91.5 (C-17), 112.7 (C-2′), 122.3 (C-3″), 119.2 (C-6), 124.6 (C-8″),125.2 (C-5″), 126.7 (C-6″), 130.2 (C-7″), 133.5 (C-4″), 138.0 (C-2″), 138.4 (C-5), 141.2 (C-9″), 162.3 (C-1″), 165.8 (C-3′), 166.3 (C-1′), 209.9 (C-20); ESIMS: *m/z* 673 $[M+Na]^+$ HRESIMS: calcd for $C_{37}H_{46}O_8NaS$ $[M+Na]^+$ 673.2811, found 673.2825.

4.3.25. 3-O-(Cyclohexanecarbonyl)caudatin (26)

White amorphous powder, yield 83.3 %; ^1H NMR ($CDCl_3$, 400MHz): δ 1.07 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.17 (3H, s, CH_3-19), 1.42 (3H, s, CH_3-18), 1.55-2.03 (21H, overlap, H-1, 2, 9, 11, 15, 16, 3″, 4″, 5″, 6″, 7″), 2.14 (3H, s, CH_3-7′), 2.18 (3H, s, CH_3-21), 2.23-2.29 (2H, overlap, H-7, 2″), 2.34-2.40 (3H, overlap, H-4′, 4), 2.86 (1H, m, H-16), 4.56-4.67 (3H, overlap, H-12, 3), 5.41 (1H, s, H-6), 5.54 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.5 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.2 (C-11), 25.4 (2C-4″, 6″), 25.7 (C-5″), 26.9 (C-2), 27.1 (C-21), 29.0 (2C-3″, 7″), 31.8 (C-16), 33.2 (C-7), 34.2 (C-15), 37.0 (C-10), 37.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.3 (C-2″), 43.6 (C-9), 57.9 (C-13), 71.6 (C-12), 73.1 (C-3), 74.2 (C-8), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 118.6 (C-6), 139.5 (C-5), 166.0 (C-3′), 166.9 (C-1′), 175.5 (C-1″), 208.9 (C-20); E-SIMS: *m/z* 623 $[M+Na]^+$, HRESIMS: calcd for $C_{35}H_{52}O_8Na$ $[M+Na]^+$ 623.3559, found 623.3543.

4.3.26. 3-O-(Tetrahrdro-2H-pyran-4-carbonyl)caudatin (27)

White amorphous powder, yield 74.0 %; ^1H NMR ($CDCl_3$, 400MHz): δ 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.15 (3H, s, CH_3-19), 1.38 (3H, s, CH_3-18), 1.52-2.03 (15H, m, H-1, 2, 9, 11, 15, 16, 3″, 7″), 2.11 (3H, s, CH_3-7′), 2.15 (3H, s, CH_3-21), 2.20 (2H, s, H-7), 2.27-2.38 (3H, overlap, H-4′, 4), 2.49 (1H, m, H-2″), 2.82 (1H, m, H-16), 3.41 (2H, m, H-4″α, 6″α), 3.91 (2H, m, H-4″β, 6″β), 4.55 (1H, dd, J = 9.3, 5.9 Hz, H-12), 4.63 (1H, m, H-3), 5.39 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 26.8 (C-2), 27.1 (C-21), 28.6 (2C-3″, 7″), 31.7 (C-16), 33.3 (C-7), 34.2 (C-15), 36.9 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.4 (C-4), 40.2 (C-2″), 43.6 (C-9), 57.9 (C-13), 67.0 (2C-4″, 6″), 71.6 (C-12), 73.6 (C-3), 74.1 (C-8), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 118.9 (C-6), 139.2 (C-5), 166.0 (C-3′), 167.0 (C-1′), 173.9 (C-1″), 208.9 (C-20); EIMS: *m/z* 602, HREIMS: calcd for $C_{34}H_{50}O_9$ 602.3455, found 602.3468.

4.3.27. 3-O-(4-Oxo-4H-chromene-2-carbonyl)caudatin (28)

Light yellow amorphous powder, yield 75.8 %; ^1H NMR ($CDCl_3$, 400MHz): δ 1.07 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.23 (3H, s, CH_3-19), 1.42 (3H, s, CH_3-18), 1.60 (1H, m, H-9), 1.86-2.04 (9H, overlap, H-1, 2, 11, 15, 16), 2.13 (3H, s, CH_3-7′), 2.18 (3H, s, CH_3-21), 2.24 (2H, s, H-7), 2.37 (1H, m, H-4′), 2.55 (1H, m, H-4), 2.87 (1H, m, H-16), 4.59 (1H, dd, J = 10.4, 5.2 Hz, H-12), 4.93 (1H, m, H-3), 5.47 (1H, s, H-6), 5.54 (1H, s, H-2′), 7.11 (1H, s, H-3″), 7.45 (1H, m, H-7″), 7.62 (1H, d, J = 8.4 Hz, H-9″), 7.75 (1H, m, H-8″), 8.18 (1H, d, J = 8.0 Hz, H-6″); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 26.7 (C-2), 27.2 (C-21), 31.9 (C-16), 33.4 (C-7), 34.3 (C-15), 36.9 (C-10), 37.6 (C-1), 38.1 (C-4′), 38.3 (C-

4), 43.6 (C-9), 58.0 (C-13), 71.6 (C-12), 74.1 (C-8), 76.7 (C-3), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 114.7 (C-9″), 118.8 (C-6), 119.7 (C-3″), 124.3 (C-5″), 125.7 (C-7″), 125.9 (C-6″), 134.7 (C-8″), 138.5 (C-5), 152.3 (C-10″), 156.0 (C-2″), 159.8 (C-1″), 166.0 (C-3′), 167.0 (C-1′), 178.5 (C-4″), 208.9 (C-20); ESIMS: *m/z* 685 $[M+Na]^+$, HRESIMS: calcd for $C_{38}H_{46}O_{10}Na$ $[M+Na]^+$ 685.2988, found 685.2991.

4.3.28. 3-O-(4-Nitrobenzoyl)caudatin (29)

White amorphous powder, yield 78.6 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.08 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.24 (3H, s, CH_3-19), 1.42 (3H, s, CH_3-18), 1.61 (1H, m, H-9), 1.86-2.04 (9H, overlap, H-1, 2, 11, 15, 16), 2.14 (3H, s, CH_3-7′), 2.19 (3H, s, CH_3-21), 2.26 (2H, s, H-7), 2.37 (1H, m, H-4′), 2.56 (1H, m, H-4), 2.89 (1H, m, H-16), 4.60 (1H, dd, J = 10.0, 5.8 Hz, H-12), 4.90 (1H, m, H-3), 5.49 (1H, s, H-6), 5.55 (1H, s, H-2′), 8.21 (2H, d, J = 8.9 Hz, H-3″, 7″), 8.31 (1H, d, J = 8.9 Hz, H-4″, 6″); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 26.9 (C-2), 27.2 (C-21), 31.7 (C-16), 33.4 (C-7), 34.3 (C-15), 37.0 (C-10), 37.8 (C-1), 38.2 (C-4′), 38.4 (C-4), 43.6 (C-9), 58.0 (C-13), 71.6 (C-12), 74.1 (C-8), 75.4 (C-3), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 119.4 (C-6), 123.5 (2C-4″, 6″), 130.7 (2C-3″, 7″), 135.9 (C-2″), 138.8 (C-5), 150.4 (C-5″), 164.0 (C-1″), 166.0 (C-3′), 167.2 (C-1′), 208.9 (C-20); ESIMS: *m/z* 638 $[M-H]^-$, HRESIMS: calcd for $C_{35}H_{44}NO_{10}$ $[M-H]^-$ 638.2965, found 638.2950.

4.3.29. 3-O-(3, 5-Dinitrobenzoyl)caudatin (30)

White amorphous powder, yield 84.3 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.06 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.24 (3H, s, CH_3-19), 1.40 (3H, s, CH_3-18), 1.61 (1H, m, H-9), 1.86-2.03 (9H, overlap, H-1, 2, 11, 15, 16), 2.12 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.25 (2H, s, H-7), 2.37 (1H, m, H-4′), 2.56 (1H, m, H-4), 2.84 (1H, m, H-16), 4.58 (1H, dd, J = 10.2, 5.6 Hz, H-12), 4.98 (1H, m, H-3), 5.49 (1H, s, H-6), 5.53 (1H, s, H-2′), 9.14 (1H, s, H-5″), 9.22 (2H, s, H-3″, 7″); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.3 (C-18), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 26.9 (C-2), 27.2 (C-21), 31.6 (C-16), 33.4 (C-7), 34.3 (C-15), 36.9 (C-10), 37.8 (C-1), 38.2 (C-4′), 38.3 (C-4), 43.6 (C-9), 58.0 (C-13), 71.6 (C-12), 74.1 (C-8), 76.6 (C-3), 87.8 (C-14), 91.3 (C-17), 112.9 (C-2′), 119.8 (C-6), 122.3 (C-5″), 129.4 (2C-3″, 7″), 138.5 (C-5), 134.2 (C-2″), 148.6 (2C-4″, 6″), 161.8 (C-1″), 166.1 (C-3′), 167.2 (C-1′), 208.9 (C-20); ESIMS: *m/z* 719 $[M+Cl]^-$ HRESIMS: calcd for $C_{35}H_{44}N_2O_{12}Cl$ $[M+Cl]^-$ 719.2582, found 719.2582.

4.3.30. 3-O-[2-(4-Trifluromethylphenyl)acetyl]caudatin (31)

White amorphous powder, yield 82.2 %; ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.15 (3H, s, CH_3-19), 1.39 (3H, s, CH_3-18), 1.61 (1H, m, H-9), 1.82-2.03 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.19 (2H, s, H-7), 2.35-2.37 (2H, overlap, H-4′, 4), 2.83 (1H, m, H-16), 3.65 (1H, s, H-2″), 4.56 (1H, dd, J = 10.5, 4.2 Hz, H-12), 4.64 (1H, m, H-3), 5.39 (1H, s, H-6), 5.52 (1H, s, H-2′), 7.39 (2H, d, J = 6.5 Hz, H-4″, 8″), 7.57 (2H, d, J = 6.5 Hz, H-5″, 7″); ^{13}C NMR ($CDCl_3$, 100MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 26.8 (C-2), 27.2 (C-21), 31.7 (C-16), 33.3 (C-7), 34.2 (C-15), 36.9 (C-10), 37.7 (C-1), 38.1 (C-4′), 38.3 (C-4), 41.3 (C-2″), 43.5 (C-9), 57.9 (C-13), 71.6 (C-12), 74.1 (C-8), 74.5 (C-3), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 119.0 (C-6), 125.4 (C-8″), 129.6 (C-6″), 138.0 (C-3″), 125.4 (2C-5″, 7″), 139.1 (C-5), 129.6 (2C-4″, 8″), 170.1 (C-1″), 166.0 (C-3′), 167.0 (C-1′), 208.9 (C-20); ESIMS: *m/z* 699 $[M+Na]^+$ HRESIMS: calcd for $C_{37}H_{47}O_8F_3Na$ $[M+Na]^+$ 699.3120, found 699.3121.

4.4. Synthesis of Compound 3-*O*-(3, 4, 5-Trihydroxybenzoyl)caudatin (32) [6]

A solution of caudatin (0.2 mmol), the gallic acid (1.5 equiv) in dry THF were added TPP (1.5 equiv) at 0 °C. The reaction mixture was stirred at room temperature until the starting material disappeared by the TLC. Then the reaction was worked up by removal of the solvent and redissolved on EtOAc. The organic solution was washed with NaCl (3 × 30 mL), dried by Na_2SO_4, and concentrated to dryness under reduced pressure. The residue was chromatographed on a silica gel column with petroleum ether/acetone (70:30) to afford **32** (white amorphous powder, yield 20.6 %). ^{1}H NMR (CD_3OD, 600MHz): δ 1.05 (6H, d, J = 6.8 Hz, C<u>H</u>$_3$-5′, 6′), 1.20 (3H, s, C<u>H</u>$_3$-19), 1.46 (3H, s, CH_3-18), 1.61 (1H, m, H-9), 1.78-2.02 (9H, overlap, H-1, 2, 11, 15, 16), 2.12 (3H, s, CH_3-7′), 2.15 (3H, s, CH_3-21), 2.19 (2H, s, H-7), 2.35 (1H, m, H-4′), 2.47 (1H, m, H-4), 2.89 (1H, m, H-16), 4.52 (1H, dd, J = 11.7, 4.1 Hz, H-12), 4.72 (1H, m, H-3), 5.41 (1H, s, H-6), 5.53 (1H, s, H-2′), 7.05 (2H, s, H-3″, 7″); ^{13}C NMR (CD_3OD, 150MHz): δ 9.5 (C-18), 16.0 (C-7′), 17.6 (C-19), 20.3 (C-5′), 20.4 (C-6′), 23.3 (C-11), 26.7 (C-2), 27.1 (C-21), 32.0 (C-16), 33.1 (C-7), 34.1 (C-15), 36.9 (C-10), 38.0 (C-1), 38.2 (C-4′), 38.4 (C-4), 43.8 (C-9), 57.4 (C-13), 71.8 (C-12), 73.8 (C-8), 74.4 (C-3), 88.7 (C-14), 91.8 (C-17), 113.0 (C-2′), 119.3 (C-6), 120.7 (C-2″), 109.0 (2C-3″, 7″), 138.4 (2C-5, 6″), 145.1 (2C-4″, 6″), 163.5 (C-1″), 166.3 (C-3′), 166.9 (C-1′), 209.2 (C-20); EIMS: *m/z* 642, HREIMS: calcd for $C_{35}H_{46}O_{11}$ 642.3040, found 642.3003.

4.5. General Procedure for Preparation of Compounds (33-39)

The derivatives (**33**-**39**) were obtained by the reaction of caudatin (0.2 mmol) with an excess of acids (5 equiv) and DCC (5 equiv) in the presence of DMAP (0.8 equiv) was stirred in CH_2Cl_2 at room temperature, which had the similar treatment process to preparation of compounds **2**-**31**. The crude products were purified by chromatography with petroleum ether/acetone (8:1 – 20:1).

4.5.1. 3, 17-O-Di(4-methylpentanoyl)caudatin (33)

White amorphous powder, yield 45.6 %; ^{1}H NMR ($CDCl_3$, 600MHz): δ 0.87-0.93 (12H, overlap, 4×CH_3), 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.17 (3H, s, CH_3-19), 1.39 (3H, s, CH_3-18), 1.47-1.69 (7H, m, H-9, 3″, 4″), 1.81-2.03 (9H, overlap, H-1, 2, 11, 15, 16), 2.14 (3H, s, CH_3-21), 2.19 (3H, s, C<u>H</u>$_3$-7′), 2.20 (2H, s, H-7), 2.27-2.29 (4H, overlap, H-2″), 2.32-2.38 (3H, overlap, H-4′, 4), 2.40 (1H, m, H-16), 4.56 (1H, dd, J = 11.4, 3.7 Hz, H-12), 4.63 (1H, m, H-3), 5.41 (1H, s, H-6), 5.53 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 150MHz): δ 10.5 (C-18), 16.6 (C-7′), 18.2 (C-19), 20.8 (C-5′), 20.9 (C-6′), 22.1 (2C-5″, 6″), 22.2 (2C-5″, 6″), 23.9 (C-11), 26.9 (C-2), 27.6 (2C-4″, 21), 27.7 (C-4″), 29.7 (C-16),

32.7 (C-2″), 32.8 (C-2″), 33.4 (C-7), 33.7 (C-3″), 33.9 (C-3″), 34.6 (C-15), 36.9 (C-10), 37.9 (C-1), 38.2 (C-4′), 38.4 (C-4), 43.3 (C-9), 58.0 (C-13), 70.8 (C-12), 73.4 (C-3), 74.2 (C-8), 88.1 (C-14), 97.9 (C-17), 112.7 (C-2′), 119.0 (C-6), 138.8 (C-5), 165.3 (C-3′), 167.2 (C-1′), 172.5 (C-1″),173.5 (C-1″), 202.9 (C-20); EIMS: *m/z* 686, HREIMS: calcd for $C_{40}H_{62}O_9$, 686.4394, found 686.4363.

4.5.2. 3, 17-O-Di(oct-2-enoyl)caudatin (34)

White amorphous powder, yield 53.8 %; ^{1}H NMR ($CDCl_3$, 600MHz): δ 0.81-0.84 (6H, overlap, 2×CH_3-8″), 0.99 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.12 (3H, s, CH_3-19), 1.18-1.28 (12H, overlap, H-5″, 6″, 7″), 1.36 (3H, s, CH_3-18), 1.60 (1H, m, H-9), 1.82-2.00 (9H, overlap, H-1, 2, 11, 15, 16), 1.97 (3H, s, CH_3-21), 2.08 (3H, s, CH_3-7′), 2.11-2.37 (9H, overlap, H-7, 4″, 4′, 4, 16), 4.51 (1H, dd, J = 11.5, 4.0 Hz, H-12), 4.62 (1H, m, H-3), 5.35 (1H, s, H-6), 5.46 (1H, s, H-2′), 5.72 (1H, d, J = 15.6 Hz, H-2″), 5.79 (1H, d, J = 15.7 Hz, H-2″), 6.89 (1H, m, H-3″), 6.99 (1H, m, H-3″); ^{13}C NMR ($CDCl_3$, 150MHz): δ 10.6 (C-18), 14.1 (2C-8″), 16.6 (C-7′), 18.9 (C-19), 21.0 (C-5′), 21.1 (C-6′), 22.6 (2C-7″), 24.2 (C-11), 27.2 (C-2), 27.7 (C-5″), 27.9 (C-5″), 29.4 (C-21), 29.9 (C-16), 31.5 (C-6″), 31.6 (C-6″), 32.4 (C-4″), 32.6 (C-4″), 34.1 (C-7), 34.8 (C-15), 37.1 (C-10), 38.1 (C-1), 38.4 (C-4′), 38.7 (C-4), 43.5 (C-9), 58.3 (C-13), 71.0 (C-12), 73.6 (C-3), 74.5 (C-8), 88.4 (C-14), 96.3 (C-17), 113.0 (C-2′), 119.2 (C-6), 120.6 (C-2″),121.6 (C-2″), 139.1 (C-5), 149.9 (C-3″), 152.5 (C-3″), 165.4 (C-3′), 167.2 (C-1′), 166.4 (2C-1″), 203.2 (C-20); ESIMS: *m/z* 773 $[M+Cl]^-$, HRESIMS: calcd for $C_{44}H_{66}O_9Cl$ $[M+Cl]^-$ 773.4395, found 773.4373.

4.5.3. 3, 17-O-Didodecanoylcaudatin (35)

White amorphous powder, yield 45.9 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 0.88 (6H, t, J = 6.6 Hz, 2×CH_3-12″), 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.7 (3H, s, CH_3-19), 1.25 (32H, brs, 2×H-4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″), 1.53 (3H, s, CH_3-18), 1.57-1.68 (4H, m, H-9, 3″), 1.80-1.99 (9H, overlap, H-1, 2, 11, 15, 16), 2.03 (3H, s, CH_3-21), 2.14 (3H, s, CH_3-7′), 2.19 (2H, s, H-7), 2.25-2.39 (8H, overlap, H-2″, 4′, 4, 16), 4.55 (1H, dd, J = 11.3, 4.2 Hz, H-12), 4.62 (1H, m, H-3), 5.40 (1H, s, H-6), 5.53 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 10.3 (C-18), 14.1 (2C-12″), 16.6 (C-7′), 18.2 (C-19), 20.8 (C-5′), 20.9 (C-6′), 22.6 (2C-11″), 23.9 (C-11), 24.6 (C-3″), 24.7 (C-3″), 26.7 (C-21), 26.9 (C-2), 29.0 (2C-4″), 29.1 (2C-9″), 29.2 (C-5″), 29.3 (C-5″), 29.4 (2C-7″), 29.5 (4C-6″, 8″), 29.6 (C-16), 31.8 (2C-10″), 33.8 (C-7), 33.9 (C-2″), 34.6 (2C-15, 2″), 36.8 (C-10), 37.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.3 (C-9), 58.0 (C-13), 70.9 (C-12), 73.4 (C-3), 74.2 (C-8), 88.1 (C-14), 97.9 (C-17), 112.7 (C-2′), 118.9 (C-6), 138.8 (C-5), 165.3 (C-3′), 167.0 (C-1′), 172.3 (C-1″), 173.3 (C-1″), 202.7 (C-20); ESIMS: *m/z* 855 $[M+H]^+$, HRESIMS: calcd for $C_{52}H_{87}O_9$ $[M+H]^+$ 855.6350, found 855.6359.

4.5.4. 3, 17-O-Dipalmitoylcaudatin (36)

White amorphous powder, yield 55.6 %; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.86 (6H, t, J = 6.7 Hz, 2×CH_3-16″), 1.04 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.15 (3H, s, CH_3-19), 1.24 (48H, brs, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″), 1.52 (3H, s, CH_3-18), 1.57-1.67 (5H, m, H-9, 3″), 1.78-1.90 (9H, overlap, H-1, 2, 11, 15, 16), 2.01 (3H, s, CH_3-21), 2.12 (2H, s, H-7), 2.15 (3H, s, CH_3-7′), 2.23-2.38 (8H, overlap, H-2″, 4′, 4, 16), 4.54 (1H, dd, J = 11.5, 4.0 Hz, H-12), 4.62 (1H, m, H-3), 5.38 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 10.3 (C-18), 14.1 (2C-16″), 16.6 (C-7′), 18.2 (C-19), 20.8 (C-5′), 20.9 (C-6′), 22.6 (2C-15″), 23.9 (C-11), 24.7 (2C-3″), 27.0 (C-2), 27.9 (C-21), 29.1 (2C-4″), 29.2 (2C-13″), 29.3 (2C-5″), 29.4 (2C-7″), 29.6 (C-16), 29.7 (12C-6″, 8″, 9″, 10″, 11″, 12″), 31.9 (2C-14″), 33.9 (C-7), 34.6 (2C-2″), 34.8 (C-15), 36.9 (C-10), 37.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.4 (C-9), 58.0 (C-13), 70.9 (C-12), 73.4 (C-3), 74.2 (C-8), 88.1 (C-14), 98.0 (C-17), 112.8 (C-2′), 118.9 (C-6), 138.9 (C-5), 165.3 (C-3′), 167.0 (C-1′), 172.3 (C-1″), 173.3 (C-1″), 202.7 (C-20); ESIMS: *m/z* 1001 $[M+Cl]^-$, HRESIMS: calcd for $C_{60}H_{102}O_9Cl$ $[M+Cl]^-$ 1001.7212, found 1001.7207.

4.5.5. 3, 17-O-Distearoylcaudatin (37)

White amorphous powder, yield 51.3 %; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.85 (6H, t, J = 7.0 Hz, 2×CH_3-18″), 1.04 (6H, d, J = 7.0 Hz, CH_3-5′, 6′), 1.15 (3H, s, CH_3-19), 1.23-1.26 (56H, overlap, 4″, 5″, 6″, 7″, 8″, 9″, 10″, 11″, 12″, 13″, 14″, 15″, 16″, 17″), 1.51 (3H, s, CH_3-18), 1.55-1.65 (5H, m, H-9, 3″), 1.78-1.90 (9H, overlap, H-1, 2, 11, 15, 16), 2.00 (3H, s, CH_3-21), 2.11 (3H, s, CH_3-7′), 2.15 (2H, s, H-7), 2.22-2.37 (8H, overlap, H-2″, 4′, 4, 16), 4.54 (1H, dd, J = 11.4, 4.1 Hz, H-12), 4.61 (1H, m, H-3), 5.37 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 10.3 (C-18), 14.1 (2C-18″), 16.6 (C-7′), 18.2 (C-19), 20.8 (C-5′), 20.9 (C-6′), 22.7 (2C-17″), 23.9 (C-11), 24.7 (C-3″), 25.0 (C-3″), 27.0 (C-2), 29.1 (2C-4″), 29.2 (2C-15″), 29.3 (2C-5″), 29.4 (2C-7″), 29.5 (2C-9″), 29.6 (C-16), 29.7 (14C-6″, 8″, 10″, 11″, 12″, 13″, 14″), 30.8 (C-21), 31.9 (2C-16″), 33.9 (C-7), 34.6 (2C-2″), 34.7 (C-15), 36.9 (C-10), 37.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.4 (C-9), 58.0 (C-13), 70.9 (C-12), 73.4 (C-3), 74.2 (C-8), 88.1 (C-14), 98.0 (C-17), 112.8 (C-2′), 118.9 (C-6), 138.8 (C-5), 165.3 (C-3′), 166.9 (C-1′), 172.3 (C-1″), 173.2 (C-1″), 202.7 (C-20); ESIMS: *m/z* 1057 $[M+Cl]^-$, HRESIMS: calcd for $C_{64}H_{110}O_9Cl$ $[M+Cl]^-$ 1057.7838, found 1057.7847.

4.5.6. 3, 17-O-Di(tetrahrdro-2H-pyran-4-carbonyl)caudatin (38)

White amorphous powder, yield 40.4 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.16 (3H, s, CH_3-19), 1.52 (3H, s, CH_3-18), 1.62-1.91 (19H, m, H-1, 2, 9, 11, 15, 16, 3″, 7″), 2.02 (3H, s, CH_3-21), 2.13 (3H, s, CH_3-7′), 2.19 (2H, s, H-7), 2.33-2.63 (7H, overlap, H-4′, 4, 2″, 16), 3.42-47 (4H, overlap, 2×H-4″α, 6″α), 3.94-3.99 (4H, overlap, 2×H-4″β, 6″β), 4.55 (1H, dd, J = 11.2, 4.4 Hz, H-12), 4.63 (1H, m, H-3), 5.39 (1H, s, H-6), 5.52 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100MHz): δ 10.3 (C-18), 16.6 (C-7′), 18.3 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.0 (C-11), 26.9 (C-2), 28.2 (C-21), 28.6 (4C-3″, 7″), 30.8 (C-16), 33.9 (C-7), 34.6 (C-15), 36.9 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.4 (C-4), 40.2 (C-2″), 40.6 (C-2″), 43.4 (C-9), 58.2 (C-13), 66.9 (2C-4″, 6″), 67.0 (2C-4″, 6″), 70.9 (C-12), 73.6 (C-3), 74.1 (C-8), 87.8 (C-14), 98.0 (C-17), 112.7 (C-2′), 118.9 (C-6), 139.0 (C-5), 165.3 (C-3′), 167.2 (C-1′), 173.0 (C-1″), 173.8 (C-1″), 202.9 (C-20); EIMS: *m/z* 714, HREIMS: calcd for $C_{40}H_{58}O_{11}$ 714.3979, found 714.3968.

4.5.7. 3, 17-O-Di(4-nitrobenzoyl)caudatin (39)

White amorphous powder, yield 47.5 %; ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.24 (3H, s, CH_3-19), 1.65 (1H, m, H-9), 1.69 (3H, s, CH_3-18), 1.80-2.02 (9H, overlap, H-1, 2, 11, 15, 16), 2.09 (3H, s, CH_3-21), 2.16 (3H, s, CH_3-7′), 2.27-2.56 (5H, overlap, H-7, 4′, 4, 16), 4.64 (1H, dd, J = 11.0, 4.7 Hz, H-12), 4.91 (1H, m, H-3), 5.48 (1H, s, H-6), 5.55 (1H, s, H-2′), 8.20-8.24 (6H, overlap, H-3″, 4″, 6″, 7″); ^{13}C NMR ($CDCl_3$, 100MHz): δ 10.8 (C-18), 16.7 (C-7′), 18.6 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.0 (C-11), 26.9 (C-2), 27.0 (C-21), 30.6 (C-16), 33.6 (C-7), 34.5 (C-15), 37.0 (C-10), 37.8 (C-1), 38.2 (C-4′), 38.4 (C-4), 43.4 (C-9), 58.9 (C-13), 70.7 (C-12), 74.8 (C-8), 75.3 (C-3), 87.5 (C-14), 98.4 (C-17), 112.7 (C-2′), 119.1 (C-6), 123.5 (2C-4″, 6″), 123.7 (2C-4″, 6″), 130.7 (2C-3″, 7″), 130.9 (2C-3″, 7″), 135.5 (C-2″), 135.9 (C-2″), 139.2 (C-5), 150.4 (C-5″), 150.7 (C-5″), 164.0 (C-1″), 164.1 (C-1″), 166.0 (C-3′), 167.5 (C-1′), 203.1 (C-20); EIMS: *m/z* 788 HREIMS: calcd for $C_{42}H_{48}N_2O_{13}$ 788.3156, found 788.3178.

4.6. Biological Methods

4.6.1. In vitro anti-HBV Assay [26]

The assay was determined according to our previous description [6]. The anti-HBV activities and cytotoxicity of compounds **1**–**39** were evaluated on the Hep G 2.2.15 cell line.

The activities against of HBV antigen secretion were assayed using enzyme-linked immunosorbent assay (ELISA; Autobio Diagnostics Co., Ltd, China). The sub-toxic concentration of the identified compounds was measured with a serial dilution in 96-well microplates in which cells were seeded at a density of 3×10^4 cells/mL and cultured at 37 °C, 5% CO_2 for 12 days.

A real-time PCR assay was used for detection of HBV DNA. HepG 2.2.15 cells were seeded in 24-well culture plates at a density of 5×10^5 cells/mL. After 2 days, culture medium was replaced with fresh medium supplemented with (or without) the tested compounds; this was repeated every other day for an additional 5 days. Briefly, 10 μL of DNA sample was amplified in a 25 μL mixture containing 2×SYBR Green PCR Master Mix (Applied Biosystems) and 2 primers specific for HBV: a forward primer (HBV-t1: 5-CAA GGA ACC TCT ATG TAT CCC TCC-3) and reverse primer (HBV-t2: 5-TCC GTC CGA AGG TTT GGT AC-3) covering the 50–base pair insertion from 541 bp to 591 bp. Amplification and detection were performed in the Mastercycler Ep Realplex System (Eppendorf, Masteraycler Eprealplex, German) with incubation at 95 °C for 2 min and, subsequently, 40 three-step cycles (20 s at 95 °C; 15 s at 58 °C; 20 min at 72 °C) were performed.

Cytotoxicity of these compounds was tested by modified 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method (Gibco Invitrogen, Carlsbad, CA, USA). All the compounds were dissolved in DMSO (Gibco; solvent control) and the concentration was kept below 2.5 μL/mL in the culture. After Hep G 2.2.15 cells had been seeded in a 96-well microplate for 4 h, the samples (20 μL) were placed in each well and incubated for 3 days at 37 °C; then, 0.1 mL MTT was added for 4 h. After removal of MTT medium, DMSO (100 μL/well) was added into the microplate for 10 min. The formazan crystals were dissolved, and the absorbance was measured on a microplate reader at 490 nm. An antiviral agent, tenofovir (Jiangxi Chenyang Pharmaceutical Co. Ltd, China) was used as a control.

4.6.2. Cell Line and Cell Culture [6]

Hep G 2.2.15 cells were cultured in RPMI-1640 medium (Gibco), which were supplemented with 10% fetal calf serum (Gibco), 100 μg/mL G148 (Gibco), 100 IU/mL penicillin (Gibco), and 100 IU/mL streptomycin (Gibco). Then they were maintained at 37 °C in a moist atmosphere containing 5 % CO_2.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

HBV	=	Anti-hepatitis B Virus
HBsAg	=	Hepatitis B Surface Antigen
HBeAg	=	Hepatitis B e Antigen
HCC	=	Hepatocellular Carcinoma
DCC	=	*N′, N′*-Dicyclohexylcarbodiimide
DMAP	=	4-Dimethylaminopyridine
DIAD	=	Diisopropyl Azodicarboxylate
TPP	=	Triphenylphosphine
CC	=	Olumn Chromatography
TLC	=	Thin-Layer Chromatography
CC_{50}	=	Concentration of 50% cytotoxicity
IC_{50}	=	50% Inhibitory concentration
SI	=	Selectivity index

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