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Synthesis, structure–activity relationships and biological evaluation of caudatin derivatives as novel anti-hepatitis B virus agents

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ABSTRACT

A series of caudatin derivatives were synthesized, and their anti-hepatitis B virus (HBV) activity was evaluated in HepG 2.2.15 cells. Most of the 3-O-substituted caudatin derivatives showed effective anti-HBV activity. Among the tested compounds, six compounds (**2e–2h**, **2l**, **2r**) exhibited significantly inhibitory activity against HBV DNA replication with IC_{50} values in the range of 2.82–7.48 μM. Interestingly, two compounds (**2e**, **2f**) had potent activity inhibiting not only the secretion of HBsAg (IC_{50} = 18.68 μM, 21.71 μM), HBeAg (IC_{50} = 13.16 μM, 33.73 μM), but also HBV DNA replication (IC_{50} = 7.48 μM, 3.63 μM). The structure–activity relationships (SARs) of caudatin derivatives had been discussed, which were useful for caudatin derivatives to be explored and developed as novel anti-HBV agents.

1. Introduction

Hepatitis B virus (HBV) infection is a significant health problem throughout the world.[1] Despite the current therapies (immunomodulator, α-interferon, and nucleoside drugs) are effective for the treatment of HBV infection patients clinically, but still remain unsatisfactory,[2–5] which have been commented in detail in our previous articles.[6] Therefore, the search for anti-HBV agents with novel antiviral targets and mechanisms are urgently needed.[7] Currently, many potent non-nucleoside anti-HBV agents have been reported,[8–19] which would provide many clues to find the novel inhibitors.

Natural products and their derivatives possessing various skeletons are important sources for novel HBV inhibitors.[20–23] In our continuing research for active leads from traditional Chinese medicinal herbs, we investigated the anti-HBV constituents of *Cynanchum auriculatum* (baishouwu) which were traditionally used as a folk medicine for replenishing the liver and kidney, nourishing the blood and prolonging life. By the anti-HBV assay, caudatin was found to have inhibiting HBV DNA replication activity (IC_{50} = 40.62 μM, SI = 6.0). In view of the novel structural template, we synthesized analogues of caudatin to determine structure–activity relationships (SARs) and possibly develop more potent anti-HBV agents. In this article, we reported the synthesis, SARs, and anti-HBV activity of caudatin derivatives by modification of the A, B and D rings.

2. Results and discussion

2.1. Chemistry

We probed several structural changes based on the characteristics of caudatin, including acylation, oxidation, epoxidation, and hydrogenation, at positions C-3, C-5, C-6, C-7, C-8, C-14, C-17, and C-20.[10,24,25] The syntheses of caudatin derivatives were summarized in Scheme 1–3. Many potent anti-HBV inhibitors with nitrogen heterocylic ring were reported in previous investigation, such as quinolines,[6,8,12,13] tryptamines,[17] benzimidazoles,[26] indoles,[27] etc. Therefore, some nitrogen heterocylic rings were introduced into the caudatin in order to obtain the active derivatives. Derivatives **2a–2r** were synthesized by the reaction of corresponding acids with caudatin in the presence of *N*′,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). Derivative **2s** changed from compound **2r** was chromatographed using a silica gel column with petroleum ether and acetone. Compound **1** was treated with anhydride and a catalytic amount of DMAP in dry pyridine to afford the derivatives **2t** and **2u** as the major product, together with by-product **3** [3-*O*-(2-hydroxyacetyl)caudatin] from compound **2t** by cleavage one 2-hydroxyacetic acid molecular. The compounds **2v** and **2w** were obtained by caudatin with phosphoryl chloride in the mixture of pyridine and CH_2Cl_2.

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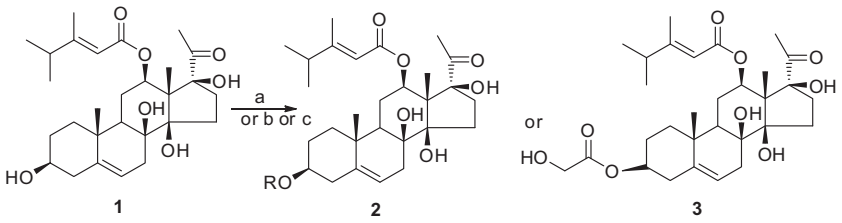
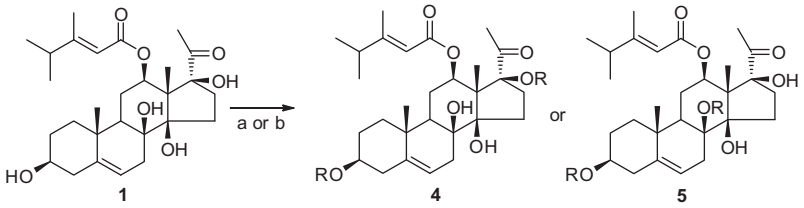
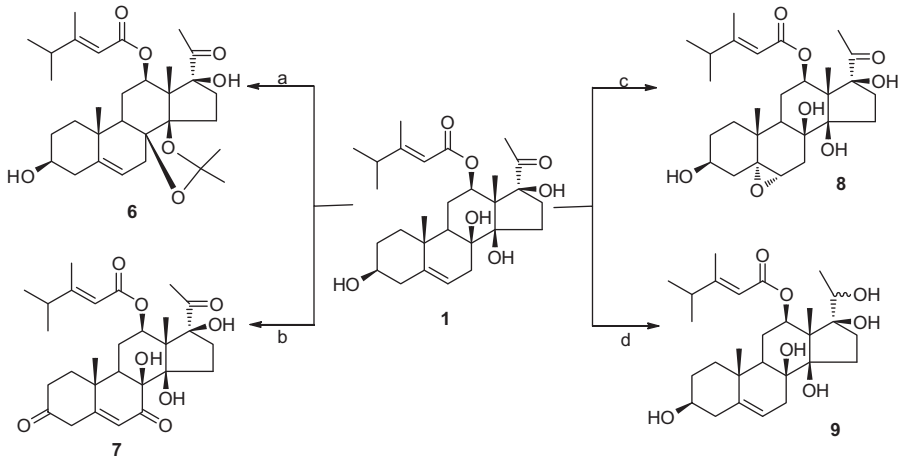


Scheme 1. Synthesis of compounds **2a**–**2w**, **3**. Reagents and conditions: (a) DMAP, DCC, CH_2Cl_2, rt; (b) $(RCO)_2O$, DMAP, anhydrous pyridine, reflux; (c) diphenylphosphoryl (dimethoxyphosphoryl) chloride, pyridine, CH_2Cl_2, rt.



Scheme 2. Synthesis of compounds **4a**–**4h**, **5**. Reagents and conditions: (a) DMAP, DCC, CH_2Cl_2, rt; (b) $(RCO)_2O$, DMAP, anhydrous pyridine, reflux.



Scheme 3. Synthesis of compounds **6**–**9**. Reagents and conditions: (a) $BF_3{\cdot}OEt_2$, acetone, rt; (b) PCC, CH_2Cl_2, rt; (c) *m*-CPBA, CH_2Cl_2, rt; (d) $NaBH_4$, EtOH, rt.

A solution of compound **1** with an excess of organic acids and DCC in the presence of DMAP was stirred in CH_2Cl_2 at room temperature to give the 3,17-O-disubsituted derivatives **4a**–**4f**, except for 3,8-O-dinicotinic acyl caudatin (**5**). Compound **1** with excess anhydride was refluxed in dry pyridine yielding the 3,17-O-disubsituted derivatives **4g**–**4h**. From those results, it suggested that the steric hindrance and rigid backbone of the caudatin influenced the acylation sequence of the hydroxyl groups as 3-OH > 17-OH > 8-OH, 14-OH.

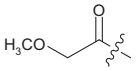
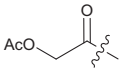
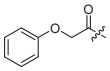
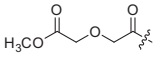
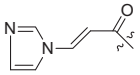
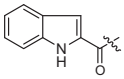
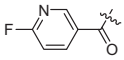
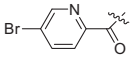
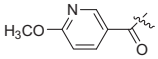
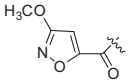
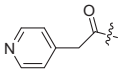
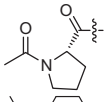
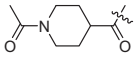
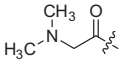
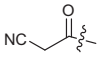
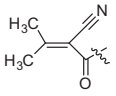
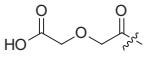
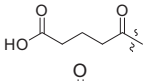
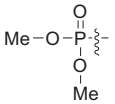
In order to evaluate the hydroxyl groups of C-8 and C-14 were the necessarily for anti-HBV activity, compound **6** was synthesized by compound **1** with $BF_3{\cdot}OEt_2$ as the catalyst in acetone. Oxidation derivative **7** was successfully obtained by treatment of compound **1** with pyridinium chlorochromate (PCC) in CH_2Cl_2. Epoxidation of compound **1** with *m*-chloroperoxybenzoic acid (*m*-CPBA) in CH_2Cl_2 at room temperature gave epoxide **8**. In the ^1H NMR spectrum of derivative **8**, the splitting pattern and the coupling constants of H-6 with Hα-7 (d, $J_{6\text{-H},7\text{-H}\alpha} = 4.0$ Hz), similar to that of reported 5α,6α-epoxysteroids and 5α,6α-epoxycaudatin,[28,29] thus the epoxide oxygen is α-oriented. Furthermore, as outlined in Scheme 3, C-20 keto of compound **1** was reduced to give derivative **9** with $NaBH_4$ for further evaluation that a carbonyl function at C-20 was necessary for biological activity. The structures of the synthesized derivatives were identified by comparing their spectroscopic data (^1H, ^{13}C NMR and MS) with those of caudatin. For instance, compounds **4a** and **5** were determined based on the obvious down-field shift of C-17 (from 91.5 to 98.6) and C-8 (from 74.3 to 91.3), respectively, compared to caudatin.

2.2. Anti-HBV activity

All the newly synthesized derivatives of caudatin were tested for their anti-HBV activity, namely inhibiting the secretion of HBsAg, HBeAg, and HBV DNA replication in HepG 2.2.15 cells using tenofovir as a positive control. The results of their anti-HBV activity and cytotoxicity were listed in Table 1.

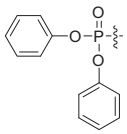
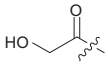
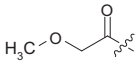
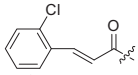
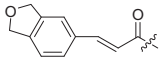
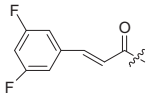
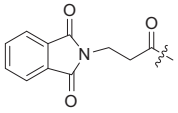
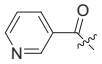
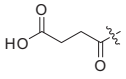
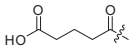
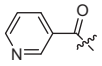
Most of the 3-O-acylated caudatin derivatives showed high-potency activity against of the secretion of HBsAg and HBV DNA replication. Derivatives (**2a–2d**) showed inhibitory potency to the secretion of HBsAg. Meanwhile, compounds **2c** and **2d** exhibited significant efficacy on suppressing the secretion of HBeAg. Among of the acetyl derivatives, only compound **2b** showed highly inhibitory activity on HBV DNA replication with the IC_{50} value of 13.82 μM, which indicated that the hydroxyl group at C-3 of

Table 1
Anti-HBV activity and cytotoxicity of the caudatin derivatives in vitro[a]

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]
1		244.58	142.67	1.7	>183.44	<1.3	40.62	6.0
2a		101.92	70.42	1.4	>835.85	—[g]	103.59	—
2b		115.6	68.20	1.7	>152.59	—	13.82	8.4
2c		319.11	76.66	4.2	96.11	3.3	40.97	7.8
2d		25.34	51.64	—	78.62	—	>105.00	—
2e		>1457.34	18.68	>78.0	13.16	>110.7	7.48	>194.8
2f		36.66	21.71	1.7	33.73	1.1	3.63	10.1
2g		50.42	20.84	2.4	>1038.05	—	4.05	12.4
2h		14.28	15.59	—	>687.36		4.97	2.9
2i		48.42	76.46	—	>626.11	—	12.49	3.9
2j		64.06	26.48	2.4	28.16	2.3	12.60	5.1
2k		61.34	95.52	—	<50.28	>1.2	47.92	1.3
2l		10.08	14.52	—	>720.99	—	2.82	3.6
2m		132.72	201.23	—	>1179.08	—	16.97	7.8
2n		39.91	19.68	—	>32.80	—	>414.10	—
2o		105.99	70.72	1.5	>638.23	—	65.72	1.6
2p		62.13	93.20	—	<62.13	>1.0	54.84	1.1
2q		50.48	87.21	—	>749.30		13.77	3.6
2r		122.02	100.48	1.2	>161.49	—	7.32	10.5
2s		452.23	136.14	3.3	221.14	2.0	30.55	14.8
2t		273.61	523.67	—	115.45	2.4	163.84	1.7
2u		309.96	>384.74	—	>384.74	—	288.71	1.1
2v		560.05	>1754.97	—	>1754.97	—	104.93	5.3

(*continued on next page*)

Table 1 (*continued*)

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]
2w		>1938.25	>1938.25	—	>1938.25	—	>484.56	—
3		135.97	192.07	—	146.84	—	219.23	—
4a		>1403.12	331.07	>4.2	>1403.12	—	288.40	>4.9
4b		>537.70	598.80	1.6	>256.63	—	>148.21	—
4c		>1018.70	575.26	>1.8	407.48	>2.5	>200.74	—
4d		>1580.93	>1580.93	—	>1580.93	—	182.67	>8.7
4e		>809.48	>809.48	—	>809.48	—	>202.37	—
4f		602.23	90.00	>6.7	134.54	>2.4	24.69	>24.4
4g		483.08	653.48	—	>965.75	—	>241.43	—
4h		>267.96	>267.96	—	>267.96	—	>267.96	—
5		349.87	198.81	1.8	203.66	1.7	97.00	3.6
6		511.52	168.06	3.0	>1716.00	—	218.32	2.3
7		154.53	205.43	—	>1632.49		112.78	1.4
8		226.15	189.12	1.2	1144.89	—	184.30	1.2
9		217.00	306.55	—	>1054.81	—	171.95	1.3
TF[h]		>1820.42	1446.35	>1.3	1236.61	>1.5	0.77	>2364.2

[a] Values are means determined from at least two experiments.
[b] CC_{50} is 50% cytotoxicity concentration in HepG2 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.

caudatin might be a good target for further lead optimization by introduction the rational substitutions. Based on many active anti-HBV agents with nitrogen heterocylic ring, caudatin derivatives with diverse heterocylic ring containing the nitrogen atoms were synthesized to obtain the significantly anti-HBV agents. The compounds **2e** and **2f** had the highest activity inhibiting not only the secretion of HBsAg (IC_{50} = 18.68 μM, 21.71 μM,) and HBeAg (IC_{50} = 13.16 μM, 33.73 μM), but also HBV DNA replication (IC_{50} = 7.48 μM, 3.63 μM). More the interesting was that compound **2e** had low cytotoxicity (CC_{50} >1457.34 μM), resulting in the high selectivity index (SI_{HBsAg} >78.0, SI_{HBeAg} >110.7, $SI_{HBV\ DNA}$ >194.8). The inhibiting HBV DNA replication of compounds **2g**–**2j** with one nicotinic acyl moiety was significantly enhanced with the IC_{50} value of 4.05 μM, 4.97 μM, 12.49 μM, 12.60 μM, respectively, along with better activity of against the secretion of HBsAg. Compound **2k** showed good activity inhibiting both of the secretion of HBsAg, HBeAg, and HBV DNA replication, but its concentration of activity and cytotoxicity was on the same level. Compounds with pyrazolyl or isioxazolyl moiety usually had good antiviral activity, so that the pyrazolyl and isioxazolyl groups were incorporated into caudatin. Compound **2l** [3-*O*-(1-methyl-1*H*-pyrazole-5-carbonyl)] caudatin, showed significantly activity inhibiting the replication of HBV DNA (IC_{50} = 2.82 μM) among the synthesized derivatives, but had the highest cytotoxicity with the CC_{50} value 10.08 μM. Compound **2m** with an isioxazolyl moiety only exhibited potent activity against HBV DNA replication with the IC_{50} value 16.97 μM. From the above results, it is indicated that the introduction of nitrogen aromatic moiety could enhance the anti-HBV activity. However, the anti-HBV activities of compounds **2o** and **2p** were at the same level as caudatin when the nitrogen non-aromatic rings were introduced. Derivative **2r** was more effective on inhibiting HBV DNA replication with the IC_{50} values of 7.32 μM (SI = 10.5) than that of caudatin. The anti-HBV activity and cytotoxicity of compound **2s** was weaker than that of compound **2r**, which suggested that double

bond affected the activity and cytotoxicity. Both of the mono-3-*O*-diglycolyl and glutaryl derivatives of caudatin (**2t**, **2u**) showed lower antiviral activity and cell cytotoxicity than that of caudatin. Compound **3** with the 2-hydroxyacetyl group, by-product of compound **2t**, showed weaker activity than that of caudatin. The anti-HBV activity of two phosphate esters (**2v, 2w**) also obviously decreased, along with the cytotoxicity. These results suggested that rational groups introduced at position C-3 could influence on the antiviral activities.

The 3,8-*O*-di and 3,17-*O*-diacyl derivatives of caudatin (**4a**–**4e**, **4g**–**4h**, **5**) lost suppressant properties on the HBV, suggesting that the C-8 and C-17 hydroxy groups were the important feature in the antiviral activity. However, there was an exception, 3,17-*O*-dinicotinic acyl derivative (**4f**) maintained the good activity inhibiting the secretion of HBsAg and HBV DNA replication with the IC_{50} values of 90.00 μM and 24.69 μM. The cytotoxicity of the 3,8-*O*-di and 3,17-*O*-diacyl derivatives reduced when the hydroxyl group was acylated, indicating that the free hydroxyl groups were not only the necessary for the activity, but also the factor of leading the cytotoxicity.

In an effort to gain more information of the SARs of caudatin derivatives, we studied additional structure changes. The weak anti-HBV activity was observed when compound **1** was converted to derivative **6** by treatment with $BF_3{\cdot}OEt_2$ in acetone. This result further demonstrated that the functionality at the position of C-8 and C-14 was important for potent anti-HBV activity. Modification of the C-3 and C-7 of caudatin produced the oxidative derivative **7** which exhibited low anti-HBV activity relative to that of caudatin. Compound **8** showed decreased inhibition to the secretion of HBsAg, HBeAg and HBV DNA replication compared to compound **1**, which revealed that epoxide group at C-5 (6) led to the decrease of suppressant property on HBV. Comparing caudatin with **9**, conversion of the C=O (C-20) to OH decreased activity lightly, postulated to possibly be due to the loss of hydrogen-bonding capacity, which proposed that the C-20 carbonyl was crucial for anti-HBV inhibition. The HBsAg and HBeAg, playing the role in HBV infection, seroconversion was suggested that was an important end point in the treatment of chronic hepatitis B.[30–32] Part of the derivatives had the higher activity against the secretion of HBsAg or HBeAg than that of the tenofovir (positive control, nucleoside drug), suggesting the mechanisms of caudatin derivatives might be different from the nucleoside analogs.

3. Conclusions

In conclusion, a series of caudatin derivatives were synthesized and examined for their anti-HBV activities and cytotoxicities in vitro. Ten tested compounds (**2a**–**2c**, **2e**–**2g**, **2j**, **2n**, **2o**, **4f**) with higher inhibitory activity against the secretion of HBsAg than caudatin were obtained. Compounds **2c**, **2e**, **2f**, **2j**, **4f** showed better activity inhibiting the secretion of HBeAg than that of caudatin. Six compounds (**2e**–**2h**, **2l**, **2r**) exhibited significant inhibition HBV DNA replication with IC_{50} values in the range of 2.82–7.48 μM, which were comparative to that of positive control tenofovir. Interestingly, compounds **2e** and **2f** had the potent activity inhibiting not only the secretion of HBsAg (IC_{50} = 18.68 μM, 21.71 μM), HBeAg (IC_{50} = 13.16 μM, 33.73 μM), but also HBV DNA replication (IC_{50} = 7.48 μM, 3.63 μM). Moreover, the derivative **2e** showed low cytotoxicity resulting in high selectivity index values. The active derivatives of caudatin might have the different anti-HBV mechanisms or unique antiviral targets from the nucleoside drugs.

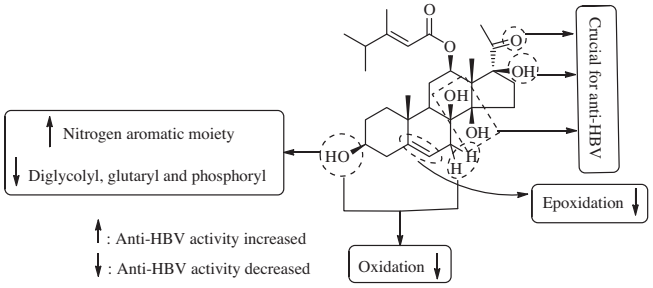


Figure 1. Structure–activity relationships of caudatin derivatives anti-HBV activity.

According to the above results, the SARs were summarized in Figure 1 and following conclusion could be drawn: (1) for 3-O-substituted caudatin derivatives, the anti-HBV activity largely depended on the size and character of the substituent. The introduction of nitrogen aromatic moiety could significantly enhance the anti-HBV activity of the caudatin derivatives. However, the antiviral activity decreased when the substitutions were diglycolyl, glutaryl, and phosphate esters. (2) Hydroxyl groups were an important feature in the anti-HBV activity of caudatin derivatives. (3) Oxidation of C-3 and C-7 to the carbonyl led to loss of anti-HBV activity. (4) Epoxide functionality at C-5(6) would cause the decrease of suppressant property on anti-HBV activity. (5) The present investigation indicated that the C-20 carbonyl was crucial for anti-HBV inhibition. This study provided valuable information for making those caudatin derivatives to be explored and developed as novel non-nucleoside anti-HBV agents.

4. Experimental

4.1. General experimental procedures

^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 using thin-layer chromatography (TLC) on silica gel plates. Caudatin was isolated from *Cynanchum auriculatum* (Bai-Shou-Wu) and had the purity of >95.0%. Reaction 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. Chemistry

4.2.1. General procedure for preparation of compounds 2a–2r

The DCC (1.2 equiv) was added to the solution of **1** (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 over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. At last, the residue was purified by column chromatography over the silica gel to yield the pure target compounds.

4.2.1.1. 3-*O*-(Methoxyacetyl)caudatin (2a). White amorphous power, yield 70.6% (after chromatography with petroleum ether/acetone, 85:15); ^1H NMR ($CDCl_3$, 500 MHz): δ 1.04 (6H, d, J = 6.8 Hz, C*H*$_3$-5′, 6′), 1.14 (3H, s, C*H*$_3$-19), 1.38 (3H, s, C*H*$_3$-18), 1.60 (1H, m, H-9), 1.80–1.97 (9H, overlap, H-1, 2, 11, 15, 16), 2.10 (3H, s, C*H*$_3$-7′), 2.14 (3H, s, C*H*$_3$-21), 2.18 (2H, s, H-7), 2.40

(3H, overlap, H-4′, 4), 2.83 (1H, m, H-16), 3.42 (3H, s, OCH_3), 3.98 (2H, s, CH_2CO), 4.53 (1H, dd, J = 5.0, 10.6 Hz, H-12), 4.71 (1H, m, H-3), 5.39 (1H, s, H-6), 5.50 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 9.4 (C-18), 16.4 (C-7′), 18.3 (C-19), 20.8 (2C-5′, 6′), 24.1 (C-11), 26.8 (C-2), 27.1 (C-21), 31.7 (C-16), 33.2 (C-7), 34.2 (C-15), 36.8 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.3 (C-4), 43.5 (C-9), 57.8 (C-13), 59.2 (C-3″), 69.9 (C-2″), 71.5 (C-12), 74.1 (C-3), 74.2 (C-8), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 119.0 (C-6), 138.9 (C-5), 165.9 (C-3′), 166.9 (C-1′), 169.6 (C-1″), 208.8 (C-20); ESIMS: m/z 585 $[M+Na]^+$ HRESIMS: calcd for $C_{31}H_{46}O_9Na$ $[M+Na]^+$ 585.3039, found 585.3040.

4.2.1.2. 3-*O*-(Ethoxyacetyl)caudatin (2b). White amorphous power, yield 81.7% (after chromatography with petroleum ether/acetone, 80:20); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.06 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.16 (3H, s, CH_3-19), 1.24 (3H, t, J = 7.0 Hz, CH_3CH$_2$O), 1.39 (3H, s, CH_3-18), 1.60 (1H, m, H-9), 1.82–1.97 (9H, overlap, H-1, 2, 11, 15, 16), 2.13 (3H, s, CH_3-7′), 2.16 (3H, s, CH_3-21), 2.23 (2H, s, H-7), 2.37 (1H, m, H-4′), 2.42 (2H, overlap, H-4), 2.84 (1H, m, H-16), 3.59 (2H, q, J = 7.0 Hz, CH$_3$CH_2O), 4.05 (2H, s, CH_2CO), 4.57 (1H, t, J = 6.8 Hz, H-12), 4.73 (1H, m, H-3), 5.42 (1H, s, H-6), 5.53 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.3 (C-18), 15.0 (C-4″), 16.5 (C-7′), 18.4 (C-19), 20.8 (C-6′), 20.9 (C-5′), 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), 37.8 (C-1), 38.2 (C-4′), 38.4 (C-4), 43.5 (C-9), 58.0 (C-13), 67.2 (C-3″), 68.2 (C-2″), 71.6 (C-12), 74.1 (C-3), 74.2 (C-8), 87.8 (C-14), 91.4 (C-17), 112.9 (C-2′), 119.0 (C-6), 139.1 (C-5), 166.0 (C-3′), 167.1 (C-1′), 169.9 (C-1″), 208.8 (C-20); EIMS: m/z 576, HREIMS: calcd for $C_{32}H_{48}O_9$ 576.3298, found 576.3283.

4.2.1.3. 3-*O*-(Phenoxyacetyl)caudatin (2c). White amorphous power, yield 70.9% (after chromatography with petroleum ether/acetone, 85:15); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.06 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.15 (3H, s, CH_3-19), 1.39 (3H, s, CH_3-18), 1.60 (1H, m, H-9), 1.82–1.96 (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), 2.30–2.43 (3H, overlap, H-4′, 4), 2.86 (1H, m, H-16), 4.60 (2H, s, CH_2CO–), 4.54 (1H, m, H-12), 4.76 (1H, m, H-3), 5.41 (1H, s, H-6), 5.52 (1H, s, H-2′), 6.89 (2H, d, J = 8.5 Hz, H-5″, 9″), 7.01 (1H, t, J = 10.0 Hz, H-7″), 7.29 (2H, m, H-6″, 8″); ^{13}C NMR ($CDCl_3$, 100 MHz): 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), 34.3 (C-15), 36.9 (C-10), 37.7 (C-1), 38.2 (C-4′), 38.3 (C-4), 43.5 (C-9), 57.9 (C-13), 65.5 (C-2″), 71.6 (C-12), 74.1 (C-8), 74.8 (C-3), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 114.6 (2C-5″, 9″), 119.2 (C-6), 121.7 (C-7″), 129.5 (2C-6″, 8″), 138.9 (C-5), 157.8 (C-4″), 166.0 (C-3′), 167.0 (C-1′), 168.4 (C-1″), 208.9 (C-20); ESIMS: m/z 623 $[M-H]^-$, HRESIMS: calcd for $C_{36}H_{47}O_9$ $[M-H]^-$ 623.3220, found 623.3206.

4.2.1.4. 3-*O*-[(2-Methoxy-2-oxoethoxy)acetyl]caudatin (2d). White amorphous power, yield 82.1% (after chromatography with petroleum ether/acetone, 70:30); ^{1}H NMR (CD_3COCD_3, 600 MHz): δ 1.06 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.19 (3H, s, CH_3-19), 1.52 (3H, s, CH_3-18), 1.62–1.94 (10H, overlap, H-1, 2, 9, 11, 15, 16), 2.11 (3H, s, CH_3-7′), 2.18 (3H, s, CH_3-21), 2.22 (2H, s, H-7), 2.364–2.41 (3H, overlap, H-4, 4′), 2.91 (1H, m, H-16), 3.69 (3H, s, CH_3-5″), 4.21 (2H, s, H-3″), 4.31 (2H, s, H-2″), 4.48 (1H, dd, J = 4.3, 11.6 Hz, H-12), 4.65 (1H, m, H-3), 5.37 (1H, s, H-6), 5.56 (1H, s, H-2′); ^{13}C NMR (CD_3COCD, 150 MHz): δ 10.4 (C-18), 16.4 (C-7′), 18.3 (C-19), 21.2 (C-5′), 21.3 (C-6′), 25.0 (C-11), 27.3 (C-21), 27.9 (C-2), 30.4 (C-16), 32.8 (C-7), 33.9 (C-15), 35.0 (C-10), 37.7 (C-4′), 38.7 (C-1), 38.9 (C-4), 44.4 (C-9), 51.9 (C-5″), 58.1 (C-13), 68.2 (C-2″), 68.4 (C-3″), 72.4 (C-12), 74.7 (C-8), 75.0 (C-3), 89.6 (C-14), 92.7 (C-17), 114.3 (C-2′), 120.5 (C-6), 138.9 (C-5), 165.7 (C-3′), 165.8 (C-1′), 169.9 (C-1″), 171.0 (C-4″), 208.8 (C-20); EIMS: m/z 620, HREIMS: calcd for $C_{33}H_{48}O_{11}$ 620.3197, found 620.3212.

4.2.1.5. 3-*O*-[(*E*)-3-(1*H*-Imidazol-1-yl)acryloyl]caudatin (2e). White amorphous power, yield 79.6% (after chromatography with petroleum ether/acetone, 70:30); ^{1}H NMR ($CDCl_3$, 400 MHz): 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), 1.58 (1H, m, H-9), 1.82–1.90 (9H,overlap, H-1, 2, 11, 15, 16), 2.10 (3H, s, CH_3-7′), 2.15 (3H, s, CH_3-21), 2.16 (2H, s, H-7), 2.37 (1H, m, H-4′), 2.42 (2H, m, H-4), 2.85 (1H, m, H-16), 4.41 (1H, m, H-12), 4.74 (1H, m, H-3), 5.41 (1H, s, H-6), 5.51 (1H, s, H-2′), 6.04 (1H, d, J = 14.2 Hz, H-2″), 7.13 (1H, d, J = 13.4 Hz, H-7″), 7.24 (1H, d, J = 13.4 Hz, H-8″), 7.81 (1H, s, H-5″), 7.89 (1H, d, J = 14.2 Hz, H-3″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.5 (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), 27.2 (C-21), 31.7 (C-16), 33.3 (C-7), 34.3 (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-3), 74.4 (C-8), 88.0 (C-14), 91.5 (C-17), 107.6 (C-2″), 112.9 (C-2′), 116.2 (C-8″), 119.2 (C-6), 131.3 (C-7″), 136.4 (C-5″), 137.7 (C-3″), 138.9 (C-5), 165.1 (C-3′), 165.9 (C-1″), 166.9 (C-1′), 209.0 (C-20); ESIMS: m/z 633 $[M+Na]^+$ HRESIMS: calcd for $C_{34}H_{46}N_2O_8Na$ $[M+Na]^+$ 633.3151, found 633.3160.

4.2.1.6. 3-*O*-(Indole-2-carbonyl)caudatin (2f). White amorphous power, yield 79.4% (after chromatography with petroleum ether/acetone, 80:20); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.07 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.22 (3H, s, CH_3-19), 1.44 (3H, s, CH_3-18), 1.66 (1H, m, H-9), 1.86–2.00 (9H, overlap, H-1, 2, 11, 15, 16), 2.14 (3H, s, CH_3-7′), 2.17 (3H, s, CH_3-21), 2.22 (2H, s, H-7), 2.37 (1H, m, H-4′), 2.55 (2H, overlap, H-4), 2.86 (1H, m, H-16), 4.66 (1H, dd, J = 5.8, 9.7 Hz, H-12), 4.92 (1H, m, H-3), 5.42 (1H, s, H-6), 5.56 (1H, s, H-2′), 7.14 (1H, t, J = 7.5 Hz, H-7″), 7.23 (1H, s, H-3″), 7.31 (1H, t, J = 7.6 Hz, H-6″), 7.45 (1H, d, J = 8.3 Hz, H-8″), 7.68 (1H, d, J = 8.0 Hz, H-5″), 9.47 (1H, s, 10″-NH); ^{13}C NMR ($CDCl_3$, 100 MHz): 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), 27.0 (C-2), 27.2 (C-21), 31.8 (C-16), 33.2 (C-7), 34.3 (C-15), 37.0 (C-10), 38.1 (C-1), 38.2 (C-4′), 38.4 (C-4), 43.5 (C-9), 57.9 (C-13), 71.6 (C-12), 74.2 (C-8), 74.5 (C-3), 88.1 (C-14), 91.5 (C-17), 108.6 (C-8″), 112.0 (C-3″), 112.9 (C-2′), 119.0 (C-6), 120.7 (C-6″), 122.5 (C-5″), 125.2 (C-7″), 127.4 (C-2″), 127.5 (C-4″), 137.0 (C-9″), 139.2 (C-5), 161.6 (C-1″), 166.1 (C-3′), 167.0 (C-1′), 209.0 (C-20); ESIMS: m/z 656 $[M+Na]^+$ HRESIMS: calcd for $C_{37}H_{47}NO_8Na$ $[M+Na]^+$ 656.3199, found 656.3235.

4.2.1.7. 3-*O*-(Nicotinyl)caudatin (2g). White amorphous power, yield 45.4% (after chromatography with petroleum ether/acetone, 85:15); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.02 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.19 (3H, s, CH_3-19), 1.40 (3H, s, CH_3-18), 1.56 (1H, m, H-9), 1.80–1.98 (9H, overlap, H-1, 2, 11, 15, 16), 2.09 (3H, s, CH_3-7′), 2.14 (3H, s, CH_3-21), 2.20 (2H, s, H-7), 2.32 (2H, m, 4′), 2.50 (2H, m, 4), 2.84 (1H, m, H-16), 4.54 (1H, dd, J = 4.9, 10.8 Hz, H-12), 4.87 (1H, m, H-3), 5.38 (1H, s, H-6), 5.49 (1H, s, H-2′), 7.36 (1H, dd, J = 7.2, 7.6 Hz, H-5″), 8.27 (1H, m, H-4″), 8.70 (1H, dd, J = 1.4, 4.7 Hz, H-6″), 9.13 (1H, s, H-2″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.4 (C-18), 16.4 (C-7′), 18.2 (C-19), 20.7 (C-5′), 20.8 (C-6′), 24.1 (C-11), 26.9 (C-2), 27.0 (C-21), 31.7 (C-16), 33.1 (C-7), 34.1 (C-15), 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), 74.0 (C-8), 74.8 (C-3), 87.9 (C-14), 91.4 (C-17), 112.8 (C-2′), 119.3 (C-6), 123.2 (C-5″), 126.3 (C-3″), 137.1 (C-4″), 138.7 (C-5), 150.5 (C-2″), 152.9 (C-6″), 164.4 (C-1″), 165.8 (C-3′), 166.8 (C-1′), 208.9 (C-20); EIMS: m/z 595, HREIMS: calcd for $C_{34}H_{45}NO_8$ 595.3145, found 595.3135.

4.2.1.8. 3-*O*-(6-Fluoronicotinyl)caudatin (2h). As white amorphous power, yield 60.7% (after chromatography with petroleum ether/acetone, 80:20); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.02 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.16 (3H, s, CH_3-19), 1.40 (3H, s, CH_3-18), 1.54 (1H, m, H-9), 1.82–1.95 (9H, overlap, H-1, 2, 11, 15, 16), 1.95 (3H, s, CH_3-7′), 2.14 (3H, s, CH_3-21), 2.19 (2H, s, H-7),

2.32 (1H, m, H-4′), 2.44 (2H, m, H-4), 2.82 (1H, m, H-16), 4.54 (1H, dd, J = 4.4, 10.9 Hz, H-12), 4.77 (1H, m, H-3), 5.39 (1H, s, H-6), 5.49 (1H, s, H-2′), 6.53 (1H, d, J = 9.5 Hz, H-6″), 7.97 (1H, dd, J = 1.9, 9.6 Hz, H-7″), 8.19 (1H, s, H-3″); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 9.4 (C-18), 16.4 (C-7′), 18.2 (C-19), 20.7 (C-5′), 20.8 (C-6′), 24.1 (C-11), 26.9 (C-2), 27.1 (C-21), 31.7 (C-16), 33.2 (C-7), 34.2 (C-15), 36.8 (C-10), 37.8 (C-1), 38.0 (C-4′), 38.3 (C-4), 43.5 (C-9), 57.8 (C-13), 71.5 (C-12), 74.0 (C-8), 74.5 (C-3), 88.0 (C-14), 91.4 (C-17), 111.4 (C-6″), 112.8 (C-2′), 119.1 (C-6), 119.3 (C-2″), 138.8 (C-5), 139.7 (C-7″), 141.1 (C-3″), 163.2 (C-1″), 165.2 (C-4″), 165.9 (C-3′), 166.8 (C-1′), 209.0 (C-20); EIMS: m/z 613, HREIMS: calcd for $C_{34}H_{44}NO_8F$ 613.3051, found 613.3130.

4.2.1.9. 3-*O*-(5-Bromo-pyridine-2-carbonyl)caudatin (2i). White amorphous power, yield 93.3% (after chromatography with petroleum ether/acetone, 85:15); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.00 (6H, d, J = 6.5 Hz, CH_3-5′, 6′), 1.14 (3H, s, CH_3-19), 1.38 (3H, s, CH_3-18), 1.54 (1H, m, H-9), 1.78–1.94 (9H, overlap, H-1, 2, 11, 15, 16), 2.06 (3H, s, CH_3-7′), 2.12 (3H, s, CH_3-21), 2.17 (2H, s, H-7), 2.30 (1H, m, H-4′), 2.46 (2H, m, H-4), 2.82 (1H, m, H-16), 4.51 (1H, dd, J = 4.7, 10.7 Hz, H-12), 4.89 (1H, m, H-3), 5.39 (1H, s, H-6), 5.47 (1H, s, H-2′), 7.91–7.96 (2H, overlap, H-3″, 4″), 8.73 (1H, s, H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.5 (C-18), 16.5 (C-7′), 18.3 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 26.8 (C-2), 27.1 (C-21), 31.8 (C-16), 33.2 (C-7), 34.2 (C-15), 36.9 (C-10), 37.7 (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), 75.6 (C-3), 88.1 (C-14), 91.5 (C-17), 112.9 (C-2′), 119.2 (C-6), 124.9 (C-5″), 126.3 (C-3″), 138.9 (C-5), 139.6 (C-4″), 146.6 (C-2″), 160.0 (C-6″), 163.9 (C-1″), 165.9 (C-3′), 166.8 (C-1′), 209.0 (C-20); EIMS: m/z 673, HREIMS: calcd for $C_{34}H_{44}BrNO_8$ 673.2847, found 673.2837.

4.2.1.10. 3-*O*-(2-Methoxynicotinyl)caudatin (2j). As white amorphous power, yield 80.8% (after chromatography with petroleum ether/acetone, 85:15); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.01 (6H, d, J = 6.8 Hz, CH_3-5′,6′), 1.16 (3H, s, CH_3-19), 1.39 (3H, s, CH_3-18), 1.55 (1H, m, H-9), 1.78–1.96 (9H, overlap, H-1, 2, 11, 15, 16), 2.08 (3H, s, CH_3-7′), 2.13 (3H, s, CH_3-21), 2.19 (2H, s, H-7), 2.33 (1H, m, H-4′), 2.47 (2H, m, H-4), 2.83 (1H, m, H-16), 3.94 (1H, s, CH_3-4″), 4.52 (1H, dd, J = 3.8, 8.7 Hz, H-12), 4.80 (1H, m, H-3), 5.40 (1H, s, H-6), 5.48 (1H, s, H-2′), 6.72 (1H, d, J = 6.9 Hz, H-6″), 8.09 (1H, dd, J = 1.7, 6.9 Hz, H-7″), 8.77 (1H, s, H-3″); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 9.4 (C-18), 16.4 (C-7′), 18.3 (C-19), 20.7 (C-5′), 20.8 (C-6′), 24.1 (C-11), 26.9 (C-2), 27.0 (C-21), 31.7 (C-16), 33.1 (C-7), 34.2 (C-15), 36.9 (C-10), 37.9 (C-1), 38.0 (C-4′), 38.3 (C-4), 43.5 (C-9), 53.9 (C-CH_3O), 57.7 (C-13), 71.5 (C-12), 74.0 (C-8), 74.2 (C-3), 88.0 (C-14), 91.4 (C-17), 110.4 (C-6″), 112.8 (C-2′), 118.9 (C-6), 119.8 (C-2″), 139.0 (C-5), 139.4 (C-7″), 149.8 (C-3″), 164.6 (C-1″), 165.8 (C-3′), 166.6 (C-1′), 166.7 (C-4″), 208.9 (C-20); EIMS: m/z 625, HREIMS: calcd for $C_{35}H_{47}NO_9$ 625.3251, found 625.3222.

4.2.1.11. 3-*O*-(2-Pyrazinecarbonyl)caudatin (2k). As light yellow amorphous power, yield 74.8% (after chromatography with petroleum ether/acetone, 80:20); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.05 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.17 (3H, s, CH_3-19), 1.38 (3H, s, CH_3-18), 1.55 (1H, m, H-9), 1.80–1.96 (9H, overlap, H-1, 2, 11, 15, 16), 2.12 (3H, s, CH_3-7′), 2.13 (3H, s, CH_3-21), 2.19 (2H, s, H-7), 2.31 (1H, m, H-4′), 2.55 (2H, m, H-4), 2.82 (1H, m, H-16), 4.52 (1H, dd, J = 5.0, 10.7 Hz, H-12), 4.96 (1H, m, H-3), 5.42 (1H, s, H-6), 5.48 (1H, s, H-2′), 8.68–8.72 (2H, overlap, H-3″, 4″), 9.25 (1H, s, H-6″); ^{13}C NMR ($CDCl_3$, 100 MHz): 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), 26.8 (C-2), 27.1 (C-21), 31.8 (C-16), 33.3 (C-7), 34.2 (C-15), 36.9 (C-10), 37.7 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.5 (C-9), 57.8 (C-13), 71.5 (C-12), 74.0 (C-8), 75.9 (C-3), 88.0 (C-14), 91.5 (C-17), 112.9 (C-2′), 119.2 (C-6), 138.7 (C-5), 143.6 (C-2″), 144.4 (C-5″), 146.2 (C-4″), 147.5 (C-3″), 163.2 (C-1″), 165.9 (C-3′), 166.9 (C-1′), 208.9 (C-20); ESIMS: m/z 619 $[M+Na]^+$ HRESIMS: calcd for $C_{33}H_{44}N_2O_8Na$ $[M+Na]^+$ 619.2995, found 619.3004.

4.2.1.12. 3-*O* -(1-Methyl-1*H*-pyrazole-5-carbonyl)caudatin (2l). As white amorphous power, yield 79.2% (after chromatography with petroleum ether/acetone, 80:20); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.00 (6H, d, J = 6.4 Hz, CH_3-5′, 6′), 1.02 (3H, s, CH_3-19), 1.39 (3H, s, CH_3-18), 1.54 (1H, m, H-9), 1.75–1.96 (9H, overlap, H-1, 2, 11, 15, 16), 2.08 (3H, s, CH_3-7′), 2.13 (3H, s, CH_3-21), 2.18 (2H, s, H-7), 2.31 (1H, m, H-4′), 2.47 (2H, m, H-4), 2.82 (1H, m, H-16), 4.12 (1H, s, NCH_3), 4.51 (1H, dd, J = 4.8, 10.9 Hz, H-12), 4.77 (1H, m, H-3), 5.39 (1H, s, H-6), 5.48 (1H, s, H-2′), 6.78 (1H, d, J = 5.2 Hz, H-3″), 7.39 (1H, d, J = 5.2 Hz, H-4″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.5 (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), 39.5 (C-NCH_3), 43.6 (C-9), 57.8 (C-13), 71.5 (C-12), 74.0 (C-8), 74.5 (C-3), 88.1 (C-14), 91.5 (C-17), 111.1 (C-3″), 112.9 (C-2′), 119.2 (C-6), 132.6 (C-2″), 137.6 (C-4″), 138.8 (C-5), 159.2 (C-1″), 165.9 (C-3′), 166.8 (C-1′), 209.0 (C-20); EIMS: m/z 598, HREIMS: calcd for $C_{33}H_{46}N_2O_8$ 598.3254, found 598.3234.

4.2.1.13. 3-*O*-(3-Methoxyisoxazole-5-carbonyl) caudatin (2m). As white amorphous power, yield 98.4% (after chromatography with petroleum ether/acetone, 85:15); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.00 (6H, d, J = 6.4 Hz, CH_3-5′, 6′), 1.02 (3H, s, CH_3-19), 1.38 (3H, s, CH_3-18), 1.53 (1H, m, H-9), 1.75–1.98 (9H,overlap, H-1, 2, 11, 15, 16), 2.07 (3H, s, CH_3-7′), 2.12 (3H, s, CH_3-21), 2.17 (2H, s, H-7), 2.31 (1H, m, H-4′), 2.43 (2H, m, H-4), 2.82 (1H, m, H-16), 3.96 (1H, s, OCH_3), 4.50 (1H, dd, J = 4.6, 11.0 Hz, H-12), 4.83 (1H, m, H-3), 5.39 (1H, s, H-6), 5.52 (1H, s, H-2′), 6.48 (1H, s, H-3″); ^{13}C NMR ($CDCl_3$, 100 MHz): 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), 26.7 (C-2), 27.1 (C-21), 31.8 (C-16), 33.2 (C-7), 34.2 (C-15), 36.9 (C-10), 37.6 (C-1), 38.1 (C-4′), 38.3 (C-4), 43.5 (C-9), 57.4 (C-OCH_3), 57.8 (C-13), 71.5 (C-12), 74.0 (C-8), 75.9 (C-3), 88.0 (C-14), 91.5 (C-17), 100.4 (C-3″), 112.9 (C-2′), 119.5 (C-6), 138.5 (C-5), 156.0 (C-4″), 160.7 (C-2″), 165.9 (C-3′), 166.8 (C-1′), 172.0 (C-1″), 209.0 (C-20); EIMS: m/z 615, HREIMS: calcd for $C_{33}H_{45}NO_{10}$ 615.3043, found 615.3050.

4.2.1.14. 3-*O*-(4-Pyridylacetyl) caudatin (2n). As yellow amorphous power, yield 69.4% (after chromatography with petroleum ether/acetone, 75:25); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.00 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.10 (3H, s, CH_3-19), 1.39 (3H, s, CH_3-18), 1.60 (1H, m, H-9), 1.75–1.92 (9H, overlap, H-1, 2, 11, 15, 16), 2.00 (3H, s, CH_3-7′), 2.12 (3H, s, CH_3-21), 2.13 (2H, s, H-7), 2.28–2.32 (3H, overlap, H-4, 4′), 2.82 (1H, m, H-16), 3.56 (1H, s, H-2″), 4.52 (1H, dd, J = 5.0, 10.7 Hz, H-12), 4.59 (1H, m, H-3), 5.32 (1H, s, H-6), 5.48 (1H, s, H-2′), 7.20 (2H, d, J = 4.5 Hz, H-4″, 8″), 8.45 (2H, d, J = 4.5 Hz, H-5″, 7″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.6 (C-18), 16.5 (C-7′), 18.2 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 26.8 (C-2), 27.1 (C-21), 31.8 (C-16), 33.3 (C-7), 34.3 (C-15), 36.8 (C-10), 37.7 (C-1), 38.1 (C-4′), 38.3 (C-4), 40.8 (C-2″), 43.5 (C-9), 57.6 (C-13), 71.5 (C-12), 73.9 (C-8), 74.8 (C-3), 88.2 (C-14), 91.5 (C-17), 112.9 (C-2′), 119.3 (C-6), 124.7 (2C-4″, 8″), 138.6 (C-5), 143.7 (C-3″), 149.1 (2C-5″, 7″), 165.9 (C-3′), 166.6 (C-1′), 169.2 (C-1″), 209.1 (C-20); ESIMS: m/z 610 $[M+H]^+$ HRESIMS: calcd for $C_{35}H_{48}NO_8$ $[M+H]^+$ 610.3379, found 610.3372.

4.2.1.15. 3-*O*-(*N* -Acetyl-L-prolinyl)caudatin (2o). As white amorphous power, yield 72.0% (after chromatography with petroleum ether/acetone, 80:20); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.01 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.11 (3H, s, CH_3-19), 1.38 (3H, s, CH_3-18), 1.50 (1H, m, H-9), 1.76–1.95 (11H, overlap, H-1, 2, 11,

15, 16, 4″), 2.08 (3H, s, C*H*$_3$-7′), 2.11–2.13 (8H, overlap, H-3″, C*H*$_3$-21, 7″), 2.22 (2H, s, H-7), 2.31–2.33 (3H, overlap, H-4, 4′), 2.82 (1H, m, H-16), 4.36 (1H, dd, *J* = 2.9, 6.9 Hz, H-2″), 4.49 (1H, dd, *J* = 3.5, 12.4 Hz, H-12), 4.62 (1H, m, H-3), 5.36 (1H, s, H-6), 5.47 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 9.4 (C-18), 16.4 (C-7′), 18.2 (C-19), 20.7 (C-5′), 20.8 (C-6′), 22.1 (C-7″), 24.0 (C-11), 24.6 (C-4″), 26.6 (C-2), 27.0 (C-21), 29.3 (C-3″), 31.7 (C-16), 33.1 (C-7), 34.2 (C-15), 36.8 (C-10), 37.6 (C-1), 38.0 (C-4′), 38.2 (C-4), 43.4 (C-9), 47.7 (C-5″), 57.6 (C-13), 58.7 (C-2″), 71.4 (C-12), 74.0 (C-8), 74.4 (C-3), 88.1 (C-14), 91.4 (C-17), 112.8 (C-2′), 118.9 (C-6), 138.9 (C-5), 165.8 (C-3′), 166.5 (C-1′), 169.5 (C-1″), 171.5 (C-6″), 208.9 (C-20); EIMS: *m/z* 629, HREIMS: calcd for $C_{35}H_{51}NO_9$ 629.3564, found 629.3546.

4.2.1.16. 3-*O*-(1-Acetyl-4-piperidinecarbonyl)caudatin (2p). As white amorphous power, yield 71.1% (after chromatography with petroleum ether/acetone, 70:30); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.00 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.11 (3H, s, C*H*$_3$-19), 1.38 (3H, s, C*H*$_3$-18), 1.60 (1H, m, H-9), 1.75–1.92 (13H, overlap, H-1, 2, 11, 15, 16, 3″), 2.04 (1H, s, H-6″), 2.07 (3H, s, C*H*$_3$-7′), 2.12 (3H, s, C*H*$_3$-21), 2.14 (2H, s, H-7), 2.30–2.32 (3H, overlap, H-4, 4′), 2.80–2.84 (2H, overlap, H-16, 2″), 3.98–4.02 (4H, overlap, H-4″), 4.50 (1H, dd, *J* = 4.5, 10.9 Hz, H-12), 4.58 (1H, m, H-3), 5.34 (1H, s, H-6), 5.47 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.5 (C-18), 16.5 (C-7′), 18.2 (C-19), 20.8 (C-5′), 20.9 (C-6′), 21.3 (C-6″), 24.1 (C-11), 26.8 (C-2), 27.1 (C-21), 29.2 (2C-3″), 31.8 (C-16), 33.3 (C-7), 34.2 (C-15), 36.9 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.3 (C-4), 40.9 (C-2″), 43.5 (C-9), 57.7 (C-13), 64.3 (2C-4″), 71.5 (C-12), 73.9 (C-8), 74.0 (C-3), 88.1 (C-14), 91.5 (C-17), 112.9 (C-2′), 119.1 (C-6), 138.8 (C-5), 165.9 (C-3′), 166.6 (C-1′), 169.0 (C-5″), 173.4 (C-1″), 209.0 (C-20); ESIMS: *m/z* 666 $[M+Na]^+$ HRESIMS: calcd for $C_{36}H_{53}NO_9Na$ $[M+Na]^+$ 666.3618, found 666.3617.

4.2.1.17. 3-*O*-(*N*,*N*-Dimethylglycinyl)caudatin (2q). As white amorphous power, yield 64.8% (after chromatography with petroleum ether/acetone, 80:20); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.05 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.15 (3H, s, C*H*$_3$-19), 1.40 (3H, s, C*H*$_3$-18), 1.54 (1H, m, H-9), 1.80–1.98 (9H, overlap, H-1, 2, 11, 15, 16), 2.12 (3H, s, C*H*$_3$-7′), 2.16 (3H, s, C*H*$_3$-21), 2.20 (2H, s, H-7), 2.33–2.44 (9H, overlap, H-4, 4′, 3″), 2.86 (1H, m, H-16), 3.13 (1H, s, H-2″), 4.55 (1H, dd, *J* = 5.2, 10.5 Hz, H-12), 4.71 (1H, m, H-3), 5.36 (1H, s, H-6), 5.52 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz): 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), 37.8 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.5 (C-9), 45.3 (2C-3″, 4″), 57.8 (C-13), 60.6 (C-2″), 71.5 (C-12), 73.9 (C-3), 74.1 (C-8), 88.0 (C-14), 91.4 (C-17), 112.9 (C-2′), 118.9 (C-6), 139.1 (C-5), 165.9 (C-3′), 166.8 (C-1′), 169.9 (C-1″), 208.9 (C-20); EIMS: *m/z* 575, HREIMS: calcd for $C_{32}H_{49}NO_8$ 575.3458, found 575.3455.

4.2.1.18. 3-*O*-(Cyanoacetyl)caudatin (2r). As white amorphous power, yield 74.9% (after chromatography with $CHCl_3$/CH_3OH, 100:1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.03 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.13 (3H, s, C*H*$_3$-19), 1.38 (3H, s, C*H*$_3$-18), 1.51 (1H, m, H-9), 1.78–1.96 (9H, overlap, H-1, 2, 11, 15, 16), 2.09 (3H, s, C*H*$_3$-7′), 2.13 (3H, s, C*H*$_3$-21), 2.17 (2H, s, H-7), 2.33 (2H, m, 4), 2.41 (2H, m, 4′), 2.84 (1H, m, H-16), 3.44 (2H, s, H-2″), 4.51 (1H, dd, 4.4, 11.2 Hz, H-12), 4.68 (1H, m, H-3), 5.39 (1H, s, H-6), 5.48 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 9.5 (C-18), 16.5 (C-7′), 18.2 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.1 (C-11), 25.0 (C-2″), 26.6 (C-2), 27.1 (C-21), 31.8 (C-16), 33.2 (C-7), 34.3 (C-15), 36.8 (C-10), 37.5 (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), 76.6 (C-3), 88.0 (C-14), 91.5 (C-17), 112.9 (C-2′), 113.2 (C-3″), 119.6 (C-6), 138.4 (C-5), 162.3 (C-1″), 165.9 (C-3′), 166.9 (C-1′), 209.0 (C-20); ESIMS: *m/z* 592 $[M+Cl]^-$, HRESIMS: calcd for $C_{31}H_{43}NO_8Cl$ $[M+Cl]^-$ 592.2677, found 592.2667.

4.2.1.19. 3-*O*-(2-Cyano-3-methylbut-2-enoyl)caudatin (2s). Derivatives **2s** was obtained from compound **2r** by chromatography using a silica gel column with petroleum ether and acetone, as white amorphous power, yield 86.3% (after chromatography with petroleum ether/acetone, 85:15); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.03 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.15 (3H, s, C*H*$_3$-19), 1.39 (3H, s, C*H*$_3$-18), 1.60 (1H, m, H-9), 1.79–1.96 (9H, overlap, H-1, 2, 11, 15, 16), 2.09 (3H, s, C*H*$_3$-7′), 2.14 (3H, s, C*H*$_3$-21), 2.18 (2H, s, H-7), 2.27 (3H, s, H-6″), 2.33 (2H, m, 4), 2.37 (3H, s, H-5″), 2.42 (2H, m, 4′), 2.83 (1H, m, H-16), 4.52 (1H, dd, *J* = 3.8, 8.7 Hz, H-12), 4.68 (1H, m, H-3), 5.39 (1H, s, H-6), 5.49 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 9.4 (C-18), 16.4 (C-7′), 18.3 (C-19), 20.7 (C-5′), 20.8 (C-6′), 22.7 (C-6″), 24.1 (C-11), 26.7 (C-2), 27.1 (C-21), 27.3 (C-5″), 31.7 (C-16), 33.2 (C-7), 34.2 (C-15), 36.9 (C-10), 37.6 (C-1), 38.1 (C-4′), 38.3 (C-4), 43.5 (C-9), 57.8 (C-13), 71.4 (C-12), 74.0 (C-8), 75.2 (C-3), 87.9 (C-14), 91.4 (C-17), 105.1 (C-2″), 112.8 (C-2′), 115.5 (C-3″), 119.0 (C-6), 138.9 (C-5), 161.1 (C-4″), 165.9 (C-3′), 166.8 (C-1′), 173.7 (C-1″), 208.8 (C-20); EIMS: *m/z* 597, HREIMS: calcd for $C_{34}H_{47}NO_8$ 597.3302, found 597.3297.

4.2.2. General procedure for preparation of compounds 2t, 2u and 3

A mixture of caudatin (0.2 mmol), appropriate 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 20 mL of EtOAc and washed three times with 60 mL of 5% HCl solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed using a silica gel column to afford the pure target products.

4.2.2.1. 3-*O*-Diglycolylcaudatin (2t). As white amorphous power, yield 64.3% (after chromatography with petroleum ether/acetone, 70:30); ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 1.06 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.19 (3H, s, C*H*$_3$-19), 1.42 (3H, s, C*H*$_3$-18), 1.60 (1H, m, H-9), 1.80–1.98 (9H, overlap, H-1, 2, 11, 15, 16), 2.13 (3H, s, C*H*$_3$-7′), 2.16 (3H, s, C*H*$_3$-21), 2.21 (2H, s, H-7), 2.44–2.49 (3H, overlap, H-4, 4′), 2.84 (1H, m, H-16), 4.31 (2H, s, H-3″), 4.32 (2H, s, H-2″), 4.45 (1H, dd, *J* = 4.1, 11.4 Hz, H-12), 4.76 (1H, m, H-3), 5.43 (1H, s, H-6), 5.52 (1H, s, H-2′); ^{13}C NMR (CD_3COCD, 100 MHz): δ 10.3 (C-18), 16.3 (C-7′), 18.3 (C-19), 21.1 (C-5′), 21.2 (C-6′), 24.9 (C-11), 27.2 (C-21), 27.7 (C-2), 30.4 (C-16), 32.7 (C-7), 33.8 (C-15), 34.9 (C-10), 37.5 (C-1), 38.5 (C-4′), 38.8 (C-4), 44.3 (C-9), 58.0 (C-13), 68.5 (2C-2″, 3″), 72.3 (C-12), 74.5 (C-8), 75.2 (C-3), 89.5 (C-14), 92.5 (C-17), 114.2(C-2′), 120.4 (C-6), 138.7 (C-5), 165.6 (C-3′), 165.8 (C-1′), 171.9 (C-1″), 170.4 (C-4″), 208.8 (C-20); EIMS: *m/z* 606, HREIMS: calcd for $C_{32}H_{46}O_{11}$ 606.3040, found 606.3019.

4.2.2.2. 3-*O*-Glutarylcaudatin (2u). As white amorphous power, yield 77.1% (after chromatography with petroleum ether/acetone, 70:30); ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 1.05 (6H, d, *J* = 6.4 Hz, C*H*$_3$-5′, 6′), 1.18 (3H, s, C*H*$_3$-19), 1.51 (3H, s, C*H*$_3$-18), 1.63–2.08 (12H, overlap, H-1, 2, 9, 11, 15, 16, 3″), 2.09 (3H, s, C*H*$_3$-7′), 2.15 (3H, s, C*H*$_3$-21), 2.21 (2H, s, H-7), 2.33–2.38 (7H, overlap, H-4, 4′, 2″, 4″), 2.87 (1H, m, H-16), 4.45 (1H, dd, *J* = 4.3, 11.4 Hz, H-12), 4.58 (1H, m, H-3), 5.35 (1H, s, H-6), 5.55 (1H, s, H-2′); ^{13}C NMR (CD_3COCD, 100 MHz): δ 10.3 (C-18), 16.3 (C-7′), 18.3 (C-19), 21.1 (C-5′), 21.2 (C-6′), 24.9 (C-11), 27.2 (C-2), 27.8 (C-21), 30.2 (C-16), 32.7 (C-7), 33.1 (C-3″), 33.8 (C-15), 33.9 (C-2″), 34.9 (C-10), 37.6 (C-1), 38.5 (C-4′), 38.7 (C-4), 39.0 (C-4″), 44.3 (C-9), 58.0 (C-13), 72.3 (C-12), 74.3 (C-8), 74.5 (C-3), 89.4 (C-14), 92.5 (C-17), 114.2(C-2′), 120.1 (C-6), 139.0 (C-5), 165.6 (C-3′), 165.7 (C-1′), 172.7 (C-1″), 174.1 (C-5″), 208.7 (C-20); EIMS: *m/z* 604, HREIMS: calcd for $C_{33}H_{48}O_{10}$ 604.3247, found 604.3238.

4.2.2.3. 3-*O*-(2-Hydroxuacetyl)caudatin (3). As white amorphous power, yield 2.6% (after chromatography with petroleum ether/acetone, 70:30); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.05 (6H, d, J = 6.8 Hz, C*H*$_3$-5′, 6′), 1.16 (3H, s, C*H*$_3$-19), 1.39 (3H, s, C*H*$_3$-18), 1.50–1.99 (10H,overlap, H-1, 2, 9, 11, 15, 16), 2.10 (3H, s, C*H*$_3$-7′), 2.14 (3H, s, C*H*$_3$-21), 2.20 (2H, s, H-7), 2.34–2.41 (3H, overlap, H-4, 4′), 2.83 (1H, m, H-16), 4.19 (2H, s, H-2″), 4.55 (1H, dd, J = 5.4, 10.4 Hz, H-12), 4.72 (1H, m, H-3), 5.40 (1H, s, H-6), 5.50 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 9.6 (C-18), 16.7 (C-7′), 18.7 (C-19), 21.1 (2C-5′, 6′), 24.3 (C-11), 27.1 (C-2), 27.5 (C-21), 31.9 (C-16), 33.5 (C-7), 34.5 (C-15), 37.1 (C-10), 38.0 (C-1), 38.4 (C-4′), 38.6 (C-4), 43.7 (C-9), 58.2 (C-13), 68.5 (C-2″), 71.8 (C-12), 74.3 (C-8), 74.8 (C-3), 88.0 (C-14), 91.6 (C-17), 113.1(C-2′), 119.4 (C-6), 139.2 (C-5), 166.3 (C-3′), 167.5 (C-1′), 169.4 (C-1″), 209.2 (C-20); ESIMS: m/z 571 $[M+Na]^+$ HRESIMS: calcd for $C_{30}H_{44}O_9Na$ $[M+Na]^+$ 571.2878, found 571.2807.

4.2.3. General procedure for preparation of compounds 2v and 2w

The diphenylphosphoryl (dimethoxyphosphoryl) chloride (1.5 equiv) was added the mixture of caudatin (0.2 mmol), pyridine (2 mL) in CH_2Cl_2 (5 mL). The resulting mixture was stirred at room temperature until the starting material was not observed by TLC. CH_2Cl_2 (20 mL) was added, and 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. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield the pure target compounds.

4.2.3.1. 3-*O*-(Diphenylphosphoryl)caudatin (2v). White amorphous power, yield 89.3% (after chromatography with petroleum ether/acetone, 70:30); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.05 (6H, d, J = 6.8 Hz, C*H*$_3$-5′, 6′), 1.14 (3H, s, C*H*$_3$-19), 1.39 (3H, s, C*H*$_3$-18), 1.50 (1H, m, H-9), 1.76–1.96 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, C*H*$_3$-7′), 2.16 (3H, s, C*H*$_3$-21), 2.18 (2H, s, H-7), 2.35 (1H, m, H-4′), 2.49 (2H, m, H-4), 2.84 (1H, m, H-16), 3.74 (6H, OC*H*$_3$), 4.19 (1H, m, H-3), 4.54 (1H, dd, J = 4.8, 10.9 Hz, H-12), 5.41 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.3 (C-19), 20.8 (C-6′), 20.9 (C-5′), 24.1 (C-11), 27.2 (C-21), 28.6 (C-2), 31.7 (C-16), 33.3 (C-7), 34.2 (C-15), 36.7 (C-10), 38.1 (C-1), 38.2 (C-4′), 39.6 (C-4), 43.5 (C-9), 54.2 (2C-O*C*H$_3$), 57.8 (C-13), 71.5 (C-12), 74.0 (C-8), 78.1 (C-3), 88.0 (C-14), 91.4 (C-17), 112.9 (C-2′), 119.4 (C-6), 138.7 (C-5), 166.0 (C-3′), 166.9 (C-1′), 208.9 (C-20); ESIMS: m/z 633 $[M+Cl]^-$, HRESIMS: calcd for $C_{30}H_{47}O_{10}PCl$ $[M+Cl]^-$ 633.2595, found 633.2596.

4.2.3.2. 3-*O*-(Dimethoxyphosphoryl)caudatin (2w). White amorphous power, yield 88.7% (after chromatography with petroleum ether/acetone, 70:30); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.06 (6H, d, J = 6.8 Hz, C*H*$_3$-5′, 6′), 1.13 (3H, s, C*H*$_3$-19), 1.38 (3H, s, C*H*$_3$-18), 1.50 (1H, m, H-9), 1.78–1.96 (9H, overlap, H-1, 2, 11, 15, 16), 2.11 (3H, s, C*H*$_3$-7′), 2.15 (3H, s, C*H*$_3$-21), 2.16 (2H, s, H-7), 2.34 (1H, m, H-4′), 2.47 (2H, m, H-4), 2.82 (1H, m, H-16), 4.42 (1H, m, H-3), 4.54 (1H, dd, J = 3.5, 8.8 Hz, H-12), 5.36 (1H, s, H-6), 5.51 (1H, s, H-2′), 7.17–7.21 (6H, overlap, H-2″, 4″, 6″), 7.33 (4H, m, H-3″, 5″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.3 (C-19), 20.8 (C-6′), 20.9 (C-5′), 24.1 (C-11), 27.2 (C-21), 28.6 (C-2), 31.7 (C-16), 33.3 (C-7), 34.3 (C-15), 36.7 (C-10), 38.1 (C-1), 38.2 (C-4′), 39.5 (C-4), 43.5 (C-9), 57.9 (C-13), 71.5 (C-12), 74.0 (C-8), 80.0 (C-3), 87.9 (C-14), 91.4 (C-17), 112.9 (C-2′), 119.6 (C-6), 120.0 (4C-2″, 6″), 125.3 (2C-4″), 129.7 (4C-3″, 5″), 138.5 (C-5), 150.5 (2C-1″), 166.0 (C-3′), 167.0 (C-1′), 208.9 (C-20); ESIMS: m/z 757 $[M+Cl]^-$, HRESIMS: calcd for $C_{40}H_{52}O_{10}PCl$ $[M+Cl]^-$ 757.2908, found 757.2919.

4.2.4. General procedure for preparation of compounds 4a–4f, and 5

The derivatives (**4a**–**4f**, **5**) 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 **2a**–**2r**.

4.2.4.1. 3,17-*O*-Di(methoxyacetyl)caudatin (4a). As white amorphous power, yield 36.6% (after chromatography with petroleum ether/acetone, 85:15); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.05 (6H, d, J = 6.8 Hz, C*H*$_3$-5′, 6′), 1.16 (3H, s, C*H*$_3$-19), 1.51 (3H, s, C*H*$_3$-18), 1.60 (1H, m, H-9), 1.81–1.92 (9H, overlap, H-1, 2, 11, 15, 16), 2.03 (3H, s, C*H*$_3$-7′), 2.12 (3H, s, C*H*$_3$-21), 2.17 (2H, s, H-7), 2.35–2.42 (3H, overlap, H-4′, 4), 3.06 (1H, m, H-16), 3.43 (3H, s, OC*H*$_3$), 3.47 (3H, s, OC*H*$_3$), 3.99 (2H, s, OC*H*$_2$CO), 4.06 (2H, s, OC*H*$_2$CO–), 4.55 (1H, dd, J = 4.0, 11.5 Hz, H-12), 4.74 (1H, m, H-3), 5.40 (1H, s, H-6), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 10.2 (C-18), 16.6 (C-7′), 18.2 (C-19), 20.8 (C-5′), 20.9 (C-6′), 23.8 (C-11), 26.9 (C-2), 29.3 (C-21), 30.3 (C-16), 33.8 (C-7), 34.6 (C-15), 36.8 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.3 (C-4), 43.4 (C-9), 58.2 (C-13), 59.3 (C-3″), 59.6 (C-3″), 70.0 (C-2″), 70.4 (C-2″), 71.0 (C-12), 74.2 (C-3), 74.3 (C-8), 87.8 (C-14), 98.6 (C-17), 112.7 (C-2′), 119.4 (C-6), 138.3 (C-5), 165.9 (C-3′), 166.8 (C-1′), 168.8 (C-1″), 169.6 (C-1″), 202.1 (C-20); EIMS: m/z 634, HREIMS: calcd for $C_{34}H_{50}O_{11}$ 634.3353, found 634.3322.

4.2.4.2. 3,17-*O*-Di(2-chlorocinnamoyl)caudatin (4b). As white amorphous power, yield 46.9% (after chromatography with petroleum ether / acetone, 85:15), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.05 (6H, d, J = 6.8 Hz, C*H*$_3$-5′, 6′), 1.21 (3H, s, C*H*$_3$-19), 1.34 (3H, s, C*H*$_3$-18), 1.86–2.61 (22H, overlap, H-1, 2, 4, 7, 9, 11, 15, 16, 4′, C*H*$_3$-7′, 21), 3.02 (1H, m, H-16), 4.65 (1H, dd, J = 4.8, 11.3 Hz, H-12), 4.77 (1H, m, H-3), 5.37 (1H, s, H-6), 5.52 (1H, s, H-2′), 6.38–6.42 (2H, m, 2″-H), 7.26–7.44 (6H, overlap, H- 7″, 8″, 9″), 7.60 (2H, m, H-6″), 8.07 (1H, d, J = 16.0 Hz, H-3″), 8.16 (1H, d, J = 16.0 Hz, H-3″); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 10.2 (C-18), 16.5 (C-7′), 18.5 (C-19), 20.8 (C-6′), 20.9 (C-5′), 24.5 (C-11), 27.0 (C-2), 27.3 (C-21), 27.9 (C-16), 33.3 (C-7), 36.6 (C-15), 37.0 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.6 (C-4), 45.9 (C-9), 57.5 (C-13), 70.8 (C-12), 73.8 (C-3), 89.9 (C-14), 90.5 (C-8), 91.4 (C-17), 112.8 (C-2′), 117.2 (C-6), 120.2 (C-2″), 121.1 (C-2″), 127.0 (C-8″), 127.1 (C-8″), 127.6 (C-9″), 127.8 (C-9″), 130.1 (C-6″), 130.2 (C-6″), 130.3 (C-7″), 130.9 (C-7″), 131.4 (C-5″), 131.6 (C-5″), 134.9 (C-4″), 135.3 (C-4″), 138.8 (C-5), 140.4 (C-3″), 142.6 (C-3″), 165.6 (C-1″), 165.7 (C-1″), 166.5 (C-3′), 169.3 (C-1′), 209.3 (C-20); EIMS: m/z 818, HREIMS: calcd for $C_{46}H_{52}O_9Cl_2$ 818.2988, found 818.2971.

4.2.4.3. 3,17-*O*-Di[3,4-(methylenedioxy)cinnamoyl]caudatin (4c). As white amorphous power, yield 40.3% (after chromatography with petroleum ether/acetone, 85:15), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.06 (6H, d, J = 6.8 Hz, C*H*$_3$-5′, 6′), 1.18 (3H, s, C*H*$_3$-19), 1.25 (3H, s, C*H*$_3$-18), 1.52–2.48 (21H, overlap, H-1, 2, 4, 7, 9, 11, 15, 16, 4′, C*H*$_3$-7′, 21), 3.17 (1H, m, H-16), 4.60 (1H, m, H-12), 4.76 (1H, m, H-3), 5.44 (1H, s, H-6), 5.44 (1H, s, H-2′), 6.00 (2H, s, OC*H*$_2$O), 6.03 (2H, s, OC*H*$_2$O), 6.21–6.31 (2H, overlap, 2″-H), 6.78–6.84 (2H, m, 8″-H), 6.99–7.05 (4H, overlap, H-5″, 9″), 7.56–7.65 (2H, m, 3″-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 10.6 (C-18), 16.6 (C-7′), 18.3 (C-19), 20.8 (C-6′), 20.9 (C-5′), 24.0 (C-11), 27.1 (C-2), 29.2 (C-21), 29.7 (C-16), 33.9 (C-7), 34.6 (C-15), 36.9 (C-10), 38.0 (C-1), 38.1 (C-4′), 38.5 (C-4), 43.4 (C-9), 58.1 (C-13), 70.9 (C-12), 73.7 (C-3), 74.3 (C-8), 88.2 (C-14), 97.9 (C-17), 101.5 (C-OC*H*$_2$O), 101.7 (C-OC*H*$_2$O), 106.4 (C-5″), 106.5 (C-5″), 108.5 (C-8″), 108.6 (C-8″), 112.8 (C-2′), 114.8 (C-2″), 116.4 (C-2″), 119.0 (C-6), 124.4 (C-9″), 125.1 (C-9″), 128.8 (C-4″), 128.9 (C-4″), 138.9 (C-5), 144.3 (C-3″), 146.5 (C-3″), 148.3 (C-7″), 148.5 (C-7″), 149.5 (C-6″), 150.2

(C-6″), 165.7 (C-3′), 166.5 (2C-1″), 166.9 (C-1′), 202.9 (C-20); EIMS: *m/z* 838, HREIMS: calcd for $C_{48}H_{54}O_{13}$ 838.3564, found 838.3589.

4.2.4.4. 3,17-*O*-Di(3,5-difluorocinnamoyl)caudatin (4d). As white amorphous power, yield 39.8% (after chromatography with petroleum ether/acetone, 85:15), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.05 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.21 (3H, s, C*H*$_3$-19), 1.62 (3H, s, C*H*$_3$-18), 1.72–2.49 (21H, overlap, H-1, 2, 4, 7, 9, 11, 15, 16, 4′, C*H*$_3$-7′, 21), 3.20 (1H, m, H-16), 4.61 (1H, dd, *J* = 4.3, 11.2 Hz, H-12), 4.76 (1H, m, H-3), 5.45 (1H, s, H-6), 5.55 (1H, s, H-2′), 6.38–6.49 (2H, overlap, H-2″), 6.86 (2H, m, H-7″), 7.02–7.07(4H, overlap, H-5″, 9″), 7.54–7.64 (2H, overlap, H-3″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 10.5 (C-18), 16.6 (C-7′), 18.3 (C-19), 20.8 (C-6′), 20.9 (C-5′), 24.0 (C-11), 26.9 (C-2), 30.1 (C-21), 32.2 (C-16), 33.8 (C-7), 34.6 (C-15), 37.0 (C-10), 37.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.4 (C-9), 58.3 (C-13), 70.8 (C-12), 74.2 (C-3), 74.4 (C-8), 87.9 (C-14), 98.1 (C-17), 105.3 (C-7″), 105.9 (C-7″), 110.5 (C-5″), 110.6 (C-5″), 110.7 (C-9″), 110.8 (C-9″), 112.8 (C-2′), 119.0 (C-6), 120.1 (C-2″), 121.2 (C-2″), 137.0 (C-4″), 137.6 (C-4″), 138.9 (C-5), 142.0 (C-3″), 143.9 (C-3″), 161.9 (C-8″), 162.0 (C-8″), 164.3 (C-6″), 164.4 (C-6″), 165.0 (C-1″), 165.5 (C-1″), 166.5 (C-3′), 167.1 (C-1′), 202.3 (C-20); ESIMS: *m/z* 857 $[M+Cl]^-$, HRESIMS: calcd for $C_{46}H_{50}O_9F_4Cl$ $[M+Cl]^-$ 857.3079, found 857.3074.

4.2.4.5. 3,17-*O*-Di(3-phthalimidopropianyl)caudatin (4e). As white amorphous power, yield 42.1% (after chromatography with petroleum ether/acetone, 70:30), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.03 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.11 (3H, s, C*H*$_3$-19), 1.49 (3H, s, C*H*$_3$-18), 1.77–1.86 (9H, overlap, H-1, 2, 11, 15, 16), 2.00 (3H, s, C*H*$_3$-7′), 2.11 (3H, s, C*H*$_3$-21), 2.15 (2H, s, H-7), 2.31–2.33 (3H, overlap, H-4′, 4), 2.68–2.71 (4H, m, H-2″), 3.05 (1H, m, H-16), 3.95–4.07 (4H, m, H-3″), 4.51 (1H, m, H-12), 4.60 (1H, m, H-3), 5.36 (1H, s, H-6), 5.50 (1H, s, H-2′), 7.70 (4H, m, H-6″, 9″), 7.82 (4H, m, H-7″, 8″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 10.5 (C-18), 16.6 (C-7′), 18.1 (C-19), 20.8 (C-6′), 20.9 (C-5′), 23.9 (C-11), 26.8 (C-2), 29.2 (C-21), 29.5 (C-16), 33.2 (C-7), 33.7 (C-2″), 33.8 (C-2″), 33.9 (C-3″), 34.2 (C-3″), 34.7 (C-15), 36.8 (C-10), 36.9 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.3 (C-9), 58.6 (C-13), 70.9 (C-12), 74.2 (C-3), 74.4 (C-8), 87.7 (C-14), 98.2 (C-17), 112.8 (C-2′), 119.1 (C-6), 123.3 (2C-6″, 9″), 123.5 (2C-6″, 9″), 131.7 (2C-5″, 10″), 132.0 (2C-5″, 10″), 134.0 (2C-7″, 8″), 134.3 (2C-7″, 8″), 168.0 (2C-4″, 11″), 168.1 (2C-4″, 11″), 138.6 (C-5), 165.3 (C-3′), 167.0 (C-1′), 169.7 (C-1″), 170.1 (C-1″), 203.0 (C-20); EIMS: *m/z* 892, HREIMS: calcd for $C_{50}H_{56}N_2O_{13}$ 892.3782, found 892.3784.

4.2.4.6. 3,17-*O*-Di(nicotinyl)caudatin (4f). As white amorphous power, yield 34.9% (after chromatography with petroleum ether/acetone, 85:15); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.04 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.23 (3H, s, C*H*$_3$-19), 1.68 (3H, s, C*H*$_3$-18), 1.86–2.61 (22H, overlap, H-1, 2, 4, 7, 9, 11, 15, 16, 4′, C*H*$_3$-7′, 21), 3.20 (1H, m, H-16), 4.63 (1H, dd, *J* = 4.5, 11.3 Hz, H-12), 4.90 (1H, m, H-3), 5.41 (1H, s, H-6), 5.54 (1H, s, H-2′), 7.37–7.43 (2H, m, H-5″), 8.28–8.34 (2H, m, H-4″), 8.73–8.79 (2H, m, H-6″), 9.18 (1H, s, H-2″), 9.30 (1H, s, H-2″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 10.7 (C-18), 16.6 (C-7′), 18.4 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.0 (C-11), 26.9 (C-2), 29.2 (C-21), 30.6 (C-16), 33.6 (C-7), 34.5 (C-15), 37.0 (C-10), 37.8 (C-1), 38.1 (C-4′), 38.4 (C-4), 43.5 (C-9), 58.9 (C-13), 70.9 (C-12), 74.6 (C-3), 74.9 (C-8), 87.5 (C-14), 98.1 (C-17), 112.8 (C-2′), 119.2 (C-6), 123.3 (C-5″), 123.5 (C-5″), 126.3 (C-3″), 126.4 (C-3″), 137.2 (C-4″), 137.3 (C-4″), 139.0 (C-5), 150.7 (C-2″), 150.9 (C-2″), 153.1 (C-6″), 153.7 (C-6″), 164.5 (2C-1″), 165.3 (C-3′), 167.2 (C-1′), 203.4 (C-20); EIMS: *m/z* 700, HREIMS: calcd for $C_{40}H_{48}N_2O_9$ 700.3360, found 700.3334.

4.2.4.7. 3,8-*O*-Di(nicotinyl)caudatin (5). As white amorphous power, yield 11.7% (after chromatography with petroleum ether/acetone, 85:15); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.07 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.20 (3H, s, C*H*$_3$-19), 1.32 (3H, s, C*H*$_3$-18), 1.85–2.47 (22H, overlap, H-1, 2, 4, 7, 9, 11, 15, 16, 4′, C*H*$_3$-7′, 21), 3.00 (1H, m, H-16), 4.69 (1H, dd, *J* = 4.5, 10.7 Hz, H-12), 4.92 (1H, m, H-3), 5.30 (1H, s, H-6), 5.60 (1H, s, H-2′), 7.50–7.56 (2H, m, H-5″), 8.32–8.47 (2H, m, H-4″), 8.83 (2H, m, H-6″), 9.22 (2H, s, H-2″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 10.5 (C-18), 16.6 (C-7′), 18.7 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.7 (C-11), 26.9 (C-2), 27.3 (C-21), 28.2 (C-16), 33.1 (C-7), 36.8 (C-15), 37.0 (C-10), 37.6 (C-1), 38.2 (C-4′), 38.3 (C-4), 46.2 (C-9), 57.6 (C-13), 70.6 (C-12), 75.5 (C-3), 90.4 (C-14), 91.1 (C-17), 91.3 (C-8), 112.6 (C-2′), 117.8 (C-6), 124.0 (C-5″), 124.7 (C-5″), 126.3 (2C-3″), 138.4 (C-4″), 138.6 (C-4″), 140.5 (C-5), 149.7 (2C-2″), 152.5 (2C-6″), 162.9 (C-1″), 167.4 (C-1″), 165.7 (C-3′), 166.9 (C-1′), 209.2 (C-20); EIMS: *m/z* 700, HREIMS: calcd for $C_{40}H_{48}N_2O_9$ 700.3360, found 700.3333.

4.2.5. General procedure for preparation of compounds 4g and 4h

Compound **1** (0.2 mmol) with excess anhydride (5 equiv) and DMAP (0.8 equiv) was refluxed in dry pyridine to obtain the 3,17-O-disubsituted derivatives **4g** and **4h**. The similar treatment process preparation of compounds **2t**, **2u** and **3** was used.

4.2.5.1. 3,17-*O*-Di(succinyl)caudatin (4g). As white amorphous power, yield 41.2% (after chromatography with petroleum ether/acetone, 70:30); ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 1.05 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.21 (3H, s, C*H*$_3$-19), 1.57 (3H, s, C*H*$_3$-18), 1.74–2.18 (19H,overlap, H-1, 2, 7, 9, 11, 15, 16, C*H*$_3$-7′, 21), 2.32–2.43 (3H, overlap, H-4, 4′), 2.55–2.58 (4H, m, H-2″), 2.67 (4H, m, H-3″), 2.96 (1H, m, H-16), 4.49 (1H, m, H-12), 4.57 (1H, m, H-3), 5.33 (1H, s, H-6), 5.58 (1H, s, H-2′); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 11.2 (C-18), 16.4 (C-7′), 18.3 (C-19), 21.0 (C-5′), 21.2 (C-6′), 24.7 (C-11), 27.3 (C-21), 27.8 (C-2), 29.0 (2C-3″), 29.1 (2C-2″), 31.8 (C-16), 34.4 (C-7), 35.4 (2C-10, 15), 37.6 (C-1), 38.6 (C-4′), 38.9 (C-4), 44.0 (C-9), 59.2 (C-13), 71.9 (C-12), 74.5 (C-3), 75.0 (C-8), 88.5 (C-14), 98.2 (C-17), 114.2 (C-2′), 120.3 (C-6), 138.7 (C-5), 165.4 (C-3′), 166.0 (C-1′), 172.2 (C-1″), 172.6 (C-1″), 173.6 (C-4″), 174.0 (C-4″), 204.1 (C-20); EIMS: *m/z* 690, HREIMS: calcd for $C_{36}H_{50}O_{13}$ 690.3251, found 690.3258.

4.2.5.2. 3,17-*O*-Di(glutaryl)caudatin (4h). As white amorphous power, yield 37.1% (after chromatography with petroleum ether/acetone, 70:30); ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 1.06 (6H, d, *J* = 6.8 Hz, C*H*$_3$-5′, 6′), 1.19 (3H, s, C*H*$_3$-19), 1.57 (3H, s, C*H*$_3$-18), 1.63–1.88 (11H, overlap, H-1, 2, 9, 11, 15, 16), 2.01 (3H, s, C*H*$_3$-7′), 2.06 (4H, m, H-3″), 2.09 (3H, s, C*H*$_3$-21), 2.18 (2H, s, H-7), 2.33–2.52 (7H, overlap, H-4, 4′, 2″, 4″), 2.98 (1H, m, H-16), 4.50 (1H, dd, *J* = 4.2, 11.4 Hz, H-12), 4.59 (1H, m, H-3), 5.34 (1H, s, H-6), 5.59 (1H, s, H-2′); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 11.2 (C-18), 16.4 (C-7′), 18.3 (C-19), 20.7 (2C-3″), 21.1 (C-5′), 21.2 (C-6′), 24.7 (C-11), 27.3 (C-21), 27.8 (C-2), 31.0 (C-16), 33.1 (C-7), 33.2 (C-15), 33.9(C-2″), 34.3 (C-2″), 34.5 (C-10), 35.4 (C-4″), 37.6 (C-1), 38.6 (C-4′), 38.7 (C-4), 38.9 (C-4″), 44.0 (C-9), 59.3 (C-13), 72.1 (C-12), 74.3 (C-8), 75.0 (C-3), 88.4 (C-14), 97.8 (C-17), 114.2 (C-2′), 120.3 (C-6), 138.9 (C-5), 165.5 (C-3′), 166.2 (C-1′), 172.7 (C-1″), 173.1 (C-1″), 174.3 (2C-5″), 204.0 (C-20); EIMS: *m/z* 718, HREIMS: calcd for $C_{38}H_{54}O_{13}$ 718.3564, found 718.3587.

4.2.6. 8,14-Dihydroxylisopropylidenylcaudatin (6)

To a solution of caudatin (0.4 mmol) in acetone (8 mL) was added $BF_3{\cdot}OEt_2$ (50 μL) at 0 °C. The resulting mixture was stirred at room temperature until the material was not determined, and then poured into aqueous saturated $NaHCO_3$. The solution was extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica

gel column chromatography (petroleum ether/acetone, 15:1) to afford compound **6.** Yield 59.6%; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 1.06 (6H, d, J = 6.4 Hz, CH_3-5′, 6′), 1.13 (3H, s, CH_3-19), 1.26 (6H, s, CH_3-2″), 1.41 (3H, s, CH_3-18), 1.51–2.01 (10H, overlap, H-1, 2, 9, 11, 15, 16), 2.13 (3H, s, CH_3-7′), 2.17 (3H, s, CH_3-21), 2.20 (2H, s, H-7), 2.28–2.38 (3H, overlap, H-4, 4′), 2.85 (1H, m, H-16), 3.49 (1H, m, H-12), 4.56 (1H, m, H-3), 5.37 (1H, s, H-6), 5.52 (1H, s, H-2′); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 9.4 (C-18), 16.5 (C-7′), 18.6 (C-19), 20.8 (C-5′), 20.9 (C-6′), 24.2 (C-11), 27.1 (2C-CH_3), 27.6 (C-21), 30.7 (C-2), 31.8 (C-16), 33.1 (C-7), 34.2 (C-15), 36.9 (C-10), 38.1 (C-4′), 38.7 (C-1), 41.9 (C-4), 43.6 (C-9), 57.9 (C-13), 71.5 (C-12), 71.8 (C-3), 74.3 (C-8), 88.0 (C-14), 91.5 (C-17), 112.9 (C-2′), 117.7 (C-6), 124.1 (C-1″), 140.6 (C-5), 165.9 (C-3′), 166.8 (C-1′), 208.9 (C-20); EIMS: m/z 530, HREIMS: calcd for $C_{31}H_{46}O_7$ 530.3244, found 530.3244.

4.2.7. Caudatin-3,7-dione (7)

A CH_2Cl_2 solution of caudatin (0.2 mmol) and pyridininm chlorochromate (1.2 mmol) was stirred for 3 h at room temperature. When TLC showed no start material, the mixture was poured into H_2O and extracted with chloroform. The organic layer was successively washed with H_2O, saturated aqueous $NaHCO_3$, once again with H_2O, and then dried over Na_2SO_4. The solution was evaporated under reduced pressure, loaded onto a silica gel column, and eluted with petroleum ether/acetone (70:30) to afford compound **7** as a white amorphous powder. Yield 47.1%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.06 (6H, d, J = 6.4 Hz, CH_3-5′, 6′), 1.30 (3H, s, CH_3-19), 1.36 (3H, s, CH_3-18), 1.97–2.03 (7H,overlap, H-1, 9, 11, 15), 2.11 (3H, s, CH_3-7′), 2.13 (3H, s, CH_3-21), 2.21–2.76 (7H, overlap, H-2, 12, 16, 4′), 3.04–3.24 (2H, s, H-4), 4.66 (1H, m, H-12), 5.55 (1H, s, H-2′), 6.12 (1H, s, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.3 (C-18), 16.5 (C-7′), 20.2 (C-19), 20.8 (2C-5′, 6′), 23.3 (C-11), 27.2 (C-21), 30.9 (C-16), 33.6 (C-15), 34.1 (C-1), 37.4 (C-2), 38.3 (C-4′), 40.1 (C-10), 44.6 (C-9), 49.3 (C-4), 58.4 (C-13), 71.8 (C-12), 77.3 (C-8), 86.1 (C-14), 90.8 (C-17), 112.6 (C-2′), 125.5 (C-6), 166.2 (C-3′), 161.4 (C-1′), 168.5 (C-5), 199.3 (C-7), 200.8 (C-3), 208.7 (C-20); ESIMS: m/z 537 $[M+Cl]^-$, HRESIMS: calcd for $C_{28}H_{38}O_8Cl$ $[M+Cl]^-$ 537.2255, found 537.2266.

4.2.8. 5α,6α-Epoxycaudatin (8)

To a solution of caudatin (0.4 mmol) in CH_2Cl_2 was added m-CPBA (1.2 equiv) at 0 °C. The resulting mixture was stirred at room temperature until the starting material was not observed by TLC. Then the saturated $NaHCO_3$ and $Na_2S_2O_3$ were added dropwise, and the mixture was extracted with EtOAc (2 × 30 mL). The EtOAc solution was washed with saturated NaCl (3 × 30 mL), dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column ($CHCl_3$:CH_3OH = 97:3) to yield compound **8**. Yield 78.0%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.07 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.12 (3H, s, CH_3-19), 1.43 (3H, s, CH_3-18), 1.47 (1H, m, H-9), 1.74–1.89 (14H, overlap, H-1, 2, 4, 7, 11, 15, 16), 1.89 (3H, s, CH_3-7′), 2.15 (3H, s, CH_3-21), 2.40 (1H, m, H-4′), 2.94 (1H, d, J = 4.0 Hz, H-6), 3.42 (1H, m, H-3), 4.44 (1H, m, H-12), 5.51 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.8 (C-18), 16.6 (C-7′), 17.4 (C-19), 20.8 (C-6′), 21.0 (C-5′), 24.8 (C-11), 27.1 (C-21), 30.2 (C-2), 30.6 (C-16), 31.6 (C-15), 32.6 (C-7), 35.7 (C-10), 38.1 (C-4′), 38.2 (C-1), 41.1 (C-4), 44.4 (C-9), 57.4 (C-13), 64.4 (C-5), 65.1 (C-6), 69.1 (C-12), 70.9 (C-3), 75.3 (C-8), 87.9 (C-14), 91.7 (C-17), 112.8 (C-2′), 165.8 (C-1′), 166.5 (C-3′), 209.1 (C-20); EIMS: m/z 506, HREIMS: calcd for $C_{28}H_{42}O_8$ 506.2880, found 506.2888.

4.2.9. 20-Hydroxylatecaudatin (9)

To a solution of caudatin (0.4 mmol) in EtOH was added $NaBH_4$ (2 equiv) at 0 °C. The resulting mixture was stirred at room temperature until the starting material was not observed by TLC. Then the 3% HCl was added dropwise, and the mixture was extracted with EtOAc (2 × 30 mL). The EtOAc solution was washed with saturated $NaHCO_3$ (3 × 30 mL) and saturated NaCl (3 × 30 mL), respectively. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a residue which was purified by silica gel column chromatography ($CHCl_3$/CH_3OH, 97:3) to afford compound **9**. Yield 72.0%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.09 (6H, d, J = 6.8 Hz, CH_3-5′, 6′), 1.11 (3H, s, CH_3-19), 1.18 (3H, s, CH_3-21),1.49 (3H, s, CH_3-18), 1.54 (1H, m, H-9), 1.76–1.96 (10H, overlap, H-1, 2, 11, 15, 16), 2.17 (3H, s, CH_3-7′), 2.33–2.42 (5H, overlap, H-4, 7, 4′), 3.50–3.62 (2H, overlap, H-3, 20), 4.65 (1H, dd, J = 3.8, 11.3 Hz, H-12), 5.37 (1H, s, H-6), 5.69 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.4 (C-18), 16.4 (C-7′), 17.0 (C-21), 18.1 (C-19), 20.7 (C-6′), 20.8 (C-5′), 24.6 (C-11), 30.8 (C-2), 31.6 (C-16), 33.4 (C-7), 34.5 (C-15), 36.7 (C-10), 38.3 (C-1), 38.7 (C-4′), 42.0 (C-4), 43.4 (C-9), 55.8 (C-13), 71.5 (C-20), 71.8 (C-3), 73.4 (C-12), 73.7 (C-8), 87.9 (2C-14, 17), 112.6 (C-2′), 118.4 (C-6), 139.3 (C-5), 166.0 (C-1′), 168.6 (C-3′); EIMS: m/z 492, HREIMS: calcd for $C_{28}H_{44}O_7$ 492.3087, found 492.3086.

4.3. In vitro anti-HBV assay

Inhibitory activity against HBV was determined according to our previous description.[6] The anti-HBV activities and cytotoxicity of compounds were evaluated on the Hep G 2.2.15 cell line. The anti-HBV antigen secretion activities were assayed by enzyme-linked immunosorbent assay (ELISA; Autobio Diagnostics Co., Ltd, China). A real-time PCR assay was used to detect the inhibiting HBV DNA replication of the derivatives. Cytotoxicity was assayed with a modified 3-(4,5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method (Gibco Invitrogen, Carlsbad, CA, USA).

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References and notes

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