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Original article

Structure–activity relationships of saponin derivatives: A series of entry inhibitors for highly pathogenic H5N1 influenza virus

Ning Ding [a,1], Qing Chen [b,1], Wei Zhang [a], Sumei Ren [a], Ying Guo [b,**], Yingxia Li [a,*]

[a] Department of Medicinal Chemistry, School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai 201203, China
[b] Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

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ABSTRACT

The occurrence of highly pathogenic avian influenza virus H5N1 highlights the urgent need for new classes of antiviral drugs. Theoretically, each of steps in influenza viral life cycle can be a target of antiviral therapeutics. However, up to date, no licenced entry inhibitor drug is available for H5N1 or any other influenza viruses. Our strategy for developing new anti-influenza therapeutics is to target the interaction between HA and sialic acid which is influenza viral receptor presented on host cell surface. Here, based on our previously discovered small molecule inhibitor saponin **1**, intensive SAR studies around the sugar chain and aglycone were conducted. The results showed that both the chacotriosyl residue and the chlorogenin moiety of active compound **1** are important for the antiviral activity, although several subtle modifications can be made on particular positions.

1. Introduction

Highly pathogenic H5N1 virus can possibly be the most severe pandemic threat in near future. Till 2010, 59% of the 508 confirmed human cases infected by H5N1 have proved mortal [1]. Two classes of antiviral drugs—ion channel inhibitors and neuraminidase inhibitors—are at present licensed for use against influenza A viruses [2]. Adamantanes block the ion channel formed by the M2 protein, which is critical in the release of viral ribonucleoprotein complexes (vRNPs) into the cytoplasm. Although ion channel inhibitors can be effective against influenza virus A infection, they have been reported to cause CNS side effects [3,4], and have given rise to the rapid emergence of drug-resistant viral strains [5]. Thus they are not recommended for a general and uncontrolled use [6]. Two neuraminidase inhibitors, oseltamivir and zanamivir, were both approved in 1999 for treatment and preventive use for acute uncomplicated flu caused by influenza A and B [7]. Neuraminidase inhibitors interfere with the enzymatic activity of the NA protein, which is critical for the efficient release of newly synthesized viruses from infected cells. However, resistance of H5N1 to oseltamivir has also been observed recently [8]. Therefore, there is an urgent need for new classes of agents to combat avian H5N1 variants that are resistant to treatment by targeting at other potential viral factors.

The earliest stage of influenza virus infection is viral entry, which is mediated by interaction of viral envelope protein hemagglutinin (HA) and its receptor on host cell surface, sialic acid sugars [9]. Therefore, to identify and develop potent entry inhibitors against H5N1 virus can be a potential antiviral treatment. In our previous work [10], we used an efficient HIV-based pseudotyping system [11–14] to screen a saponin library generated from semisynthesis, and discovered the first three small molecule H5N1 viral entry inhibitors (Fig. 1). These inhibitory molecules contained a 2,4-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranose trisaccharide known as a chacotriose. Chlorogenin 3-*O*-β-chacotrioside (**1**), chlorogenin 6-α-*O*-acetyl-3-*O*-β-chacotrioside (**2**) and methyl ursolate 3-*O*-β-chacotrioside (**3**) displayed strong inhibitory activity against H5N1 entry with IC_{50} of 6.00–9.25 μM [10]. The preliminary structure–activity relations (SARs) study [10] showed that the 3-*O*-β-chacotriosyl residue was critical for the activity, since substituted the β-chacotriosyl moiety of compound **1** by α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl or α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl moiety leaded inactive. In addition, compounds with replacement of the aglycone moiety of compound **1** to dihydrochlorogenin, dehydroisoandrosterone or stigmasterine were all found to be inactive except for methyl ursolate (compound **3**).

* Corresponding author. Tel.: +86 21 51980120; fax: +86 21 51980127.
** Corresponding author. Tel./fax: +86 010 63165176.
E-mail addresses: yingguo6@yahoo.com (Y. Guo), liyx417@fudan.edu.cn (Y. Li).
[1] These authors contributed equally to this work.

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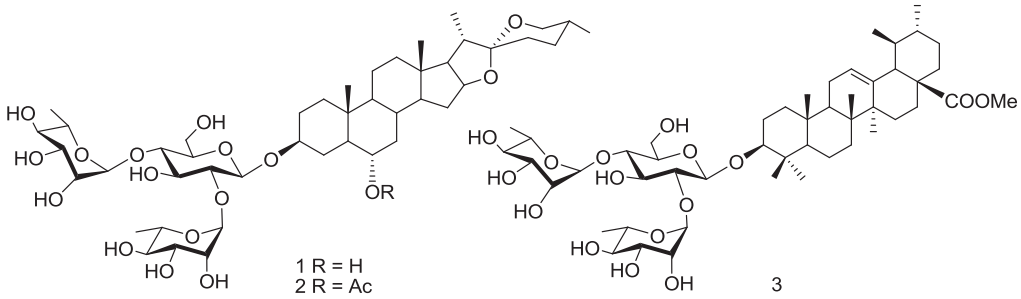


Fig. 1. Three saponin inhibitors for H5N1 viral entry.

Based on the above results, in this report, compound **1** was chosen as the lead compound to design and synthesize a series of analogs to further investigate the SARs of saponins with different sugar chains and aglycones.

For understanding the function of sugar residue, we designed and synthesized compounds **4**–**11** (Fig. 2). Chacotriose (compound **4**) was synthesized and tested to investigate the sugar chain of compound **1** alone on the viral entry inhibitory activity. In order to mimic the β-chacotriosyl linkage in active compounds **1**, ethyl 1-thio-β-chacotrioside **5** was synthesized as well. Compound **6**, with two L-arabinosyl residues instead of the two L-rhamnosyl residues, and compounds **7**–**11**, on which one or two sugar hydroxyls were blocked by methyl groups, were designed and synthesized in order to further investigate the effect of sugar moiety on the activity.

The preliminary SAR study also showed that reductive ring opening of spirostan saponin **1** led to the disappearance of inhibitory activity [10]. However, 6α-OH acetylated compound **2** showed similar inhibitory activity as compound **1**, indicating the 6-position of the aglycone might be further optimized. Therefore, compound **12** with a carbonyl group on C6, compound **13** with a β-OH on C6, and compound **14** with a L-rhamnosyl residue attached to 6-α-OH were designed and synthesized (Fig. 3).

2. Chemistry

The preparation of target compounds **4** and **5** was shown in Scheme 1. The known building block **15** [10] was subjected to 1-BBTZ [1-(benzoyloxy)-benzotriazole] in the presence of Et_3N to protect selectively the 3,6-OHs, leading to intermediate **16** [10]. Glycosylation of the 2,4-OHs in **16** with 2,3,4-tri-*O*-acetyl-L-rhamnopyranosyl trichloroacetimidate **17** [10] with TMSOTf as the catalyst led to the trisaccharide **18**. Removal of the benzoyl groups with MeONa in MeOH gave desired compound **5**. Treatment of **5** with NBS in water/acetone mixture provided compound **4**.

The synthesis of target saponins **6**–**9** and **12**–**14** was done by the similar route as that for compounds **1** and **2** reported previously by us [10,15]. As shown in Scheme 2, glycosylation of saponin derivatives **20a**–**d** with 3,6-di-*O*-benzoyl-2,4-di-*O*-levulinoyl-D-glucopyranosyl trichloroacetimidate **19** [10], respectively, under the action of TMSOTf afforded the 3-*O*-β-glucopyranosides **21a**–**d** [10]. Removal of the Lev (levulinoyl) groups with AcOH–NH_2NH_2 gave **22a**–**d**. Subsequent glycosylation of the 2,4-OHs in **22a**–**d** with L-arabinosyl trichloroacetimidate **23** [16] and L-rhamnopyranosyl trichloroacetimidates **24** [17], **25** [18], **26** [19] and **17** under the "inverse addition conditions" [20] with TMSOTf as the catalyst led to the intermediates **27**–**30** and **31b**–**d**. Final treatment with

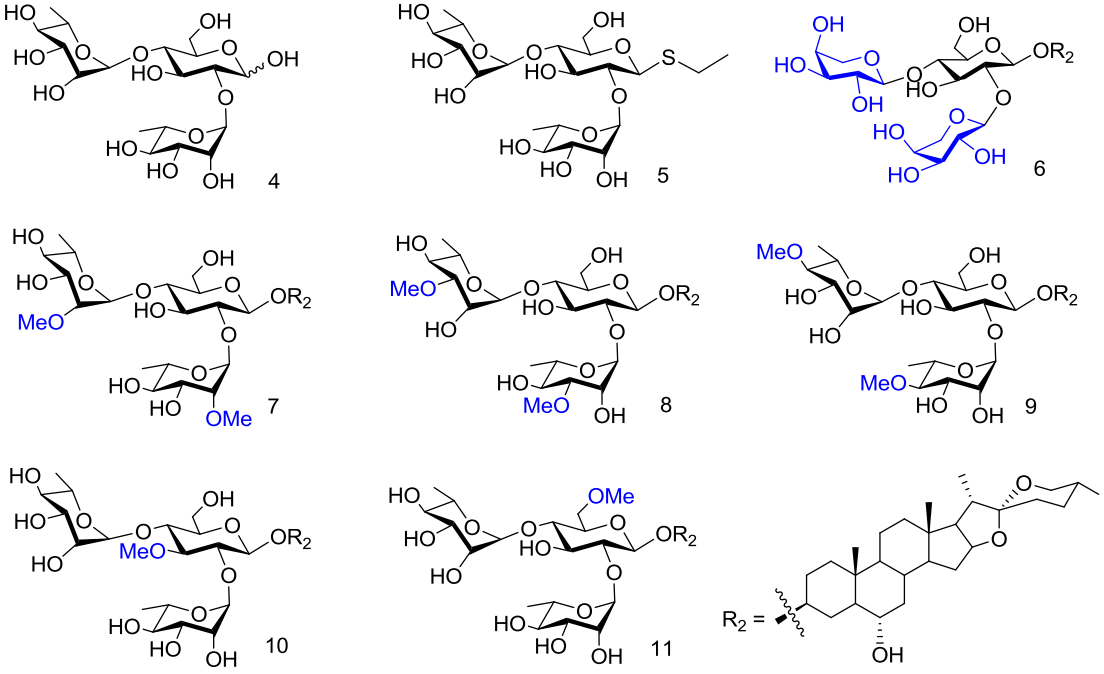


Fig. 2. Saponins designed for SAR studies on the sugar chain.

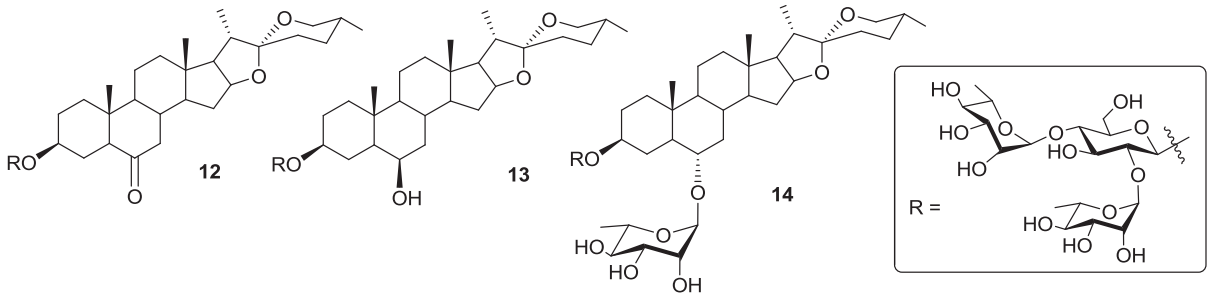


Fig. 3. Saponins designed for SAR studies on the aglycone.

MeONa in MeOH afforded target saponins **6**–**9** and **12**–**14** smoothly.

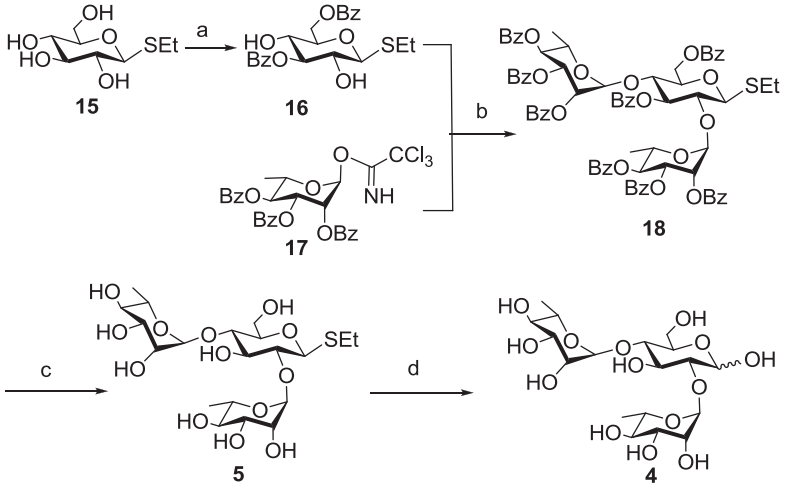
The stereochemistry of the glycosidic bond formed, was confirmed by NMR spectroscopy. In the ^{1}H NMR spectrum of compound **6**, the presence of three anomeric protons (δ 4.62, 4.60 and 4.31) as doublets with J = 6.0, 7.8, 6.8 Hz, respectively, supported the presence of three β-glycosidic linkages. All structures containing the chacotriosyl residues have alpha stereochemistry for the rhamnose moieties. For instance, ^{1}H NMR of **7** showed two anomeric protons at δ 5.30 and 5.03 as a singlet and a doublet with J = 1.4 Hz, respectively, supported the presence of two α-rhamnosyl glycosidic bonds. And the signal of an anomeric proton at δ 4.53 as a doublet with J = 7.8 Hz is consistent with the β-configuration of the glucosidic linkage.

The key building block **20a** was synthesized according to our previous work [10]. **20b**–**d** were synthesized as shown in Scheme 3. Oxidation of the compound **32** [10] with Jones reagent at 0 °C in 20 min provided compound **33** [21] smoothly. Removal of the 3-*O*-benzyl group by hydrogenation afforded the key building block **20b** [21]. Stereoselective reduction of the carbonyl group at C6 of compound **33** to a β-OH was achieved by using $NaBH_4$ as the reagent (led to **34** [22]), on which a benzoyl protecting group was introduced to give compound **35**. Then removal of the 3-*O*-benzyl group led to the key building block **20c**. Glycosylation of the 6-OH in **32** with L-rhamnopyranosyl trichloroacetimidate **17** with TMSOTf as the catalyst led to the intermediate **36**, which was then subjected to Pd/C–H_2, affording **20d**.

The synthetic route toward target saponins **10** and **11** is depicted in Scheme 4. The trichloroacetimidates **37** and **38** with methylated 3 or 6-OH were employed as donors to carry out the glycosylation with acceptor **20a**, giving the glycosides **39** and **40**, respectively. Removal of the benzoyl groups from **39** and **40** led to compounds **41** and **42**. Selectively protection of the 3-OH in **42** by using 1-BBTz in the presence of Et_3N afforded **43**. The important intermediates **41** and **43** were subjected to glycosylation with perbenzoylated L-rhamnopyranosyl trichloroacetimidate **17**, giving **44** and **45**. After global deprotection the target saponins **10** and **11** were provided.

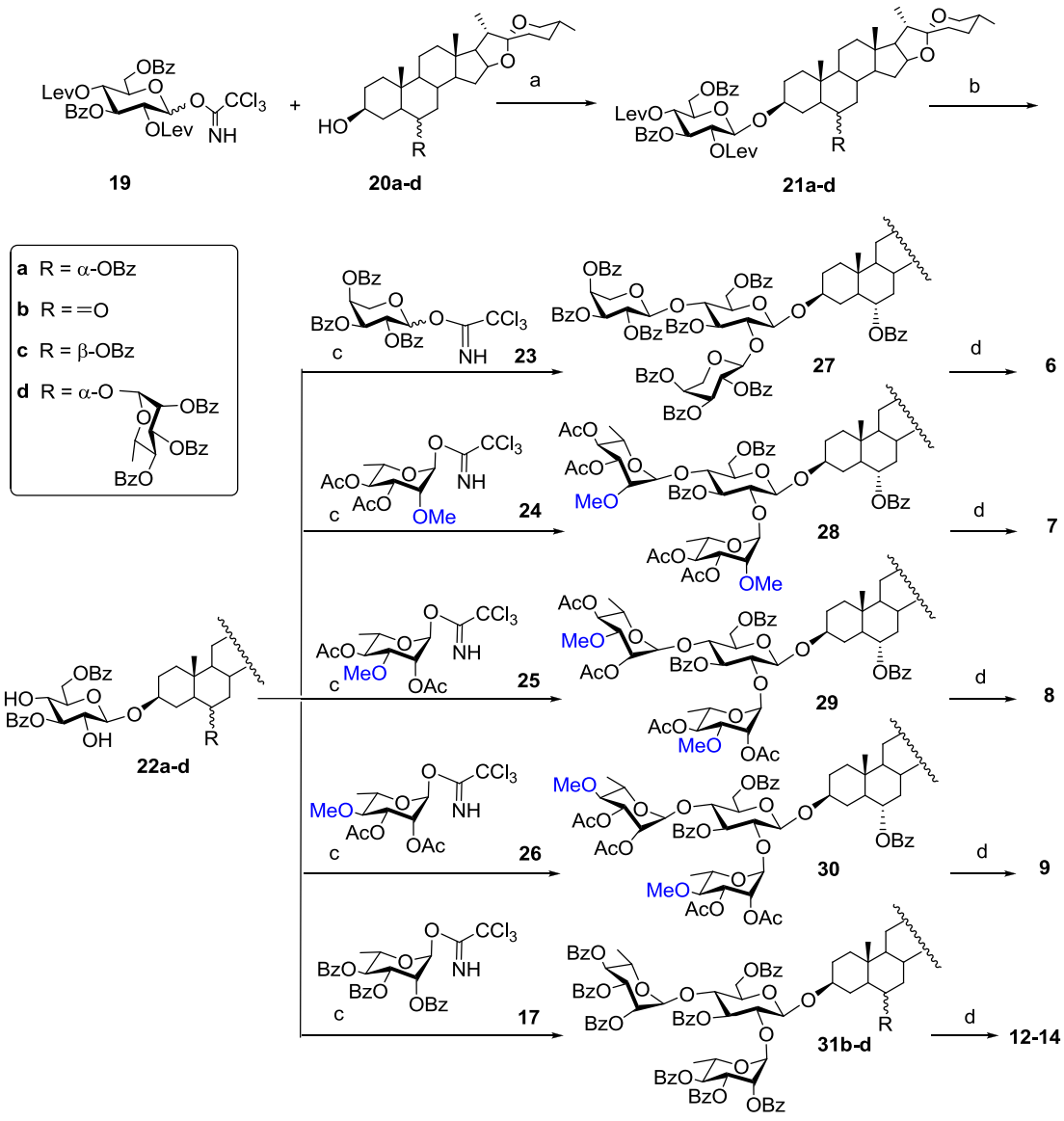
3. Pharmacology

Influenza viral entry is mediated by HA, which is responsible for viral attachment and membrane fusion. An HA/HIV pseudotyped virus model was performed to test saponins on their viral entry inhibitory effects. Two HAs we used, with sequence alignments 97% similarity, were from highly pathogenic H5N1 viruses which isolated from human and avian. Vesicular stomatitis virus glycoprotein (VSV-G) had a broad host range, so that VSVG/HIV pseudoviral transduction was used as a specificity control to exclude inhibitory effect on post-entry for HIV infection. Only a compound exhibited inhibitory activity on HA/HIV transduction, but not on VSVG/HIV, can be considered as an inhibitor of influenza viral entry. All compounds listed in Table 1 had no effects on VSVG/HIV viral infection (data not shown). It should be noted that no cytotoxicity was detected for all the designed compounds under the



Conditions: (a) BBtz, Et_3N, CH_2Cl_2, 60%; (b) TMSOTf, CH_2Cl_2, -40° C, 31%; (c) CH_3Na/CH_3OH, 45%; (d) (i) NBS, acetone/H_2O; (ii) Ac_2O, pyridine; (iii) CH_3ONa, CH_3OH, 55% for 3 steps.

Scheme 1. Synthesis of compounds **4** and **5**.



Conditions: (a) TMSOTf, CH_2Cl_2; 40% for **21b**, 49% for **21c**, 67% for **21d**; (b) AcOH-NH_2NH_2, 84% for **22b**, 73% for **22c**, 94% **22d**; (c) TMSOTf, CH_2Cl_2, 75% for **28**, 30% for **31b**, 28% for **32c**, 55% for **31d**; (d) CH_3Na/CH_3OH, 21% for **6** (2 steps), 80% for **7**, 41% for **8** (2 steps), 66% for **9** (2 steps), 34% for **12**, 65% for **13**, 40% for **14**.

Scheme 2. Synthesis of compounds **6**–**9** and **12**–**14**.

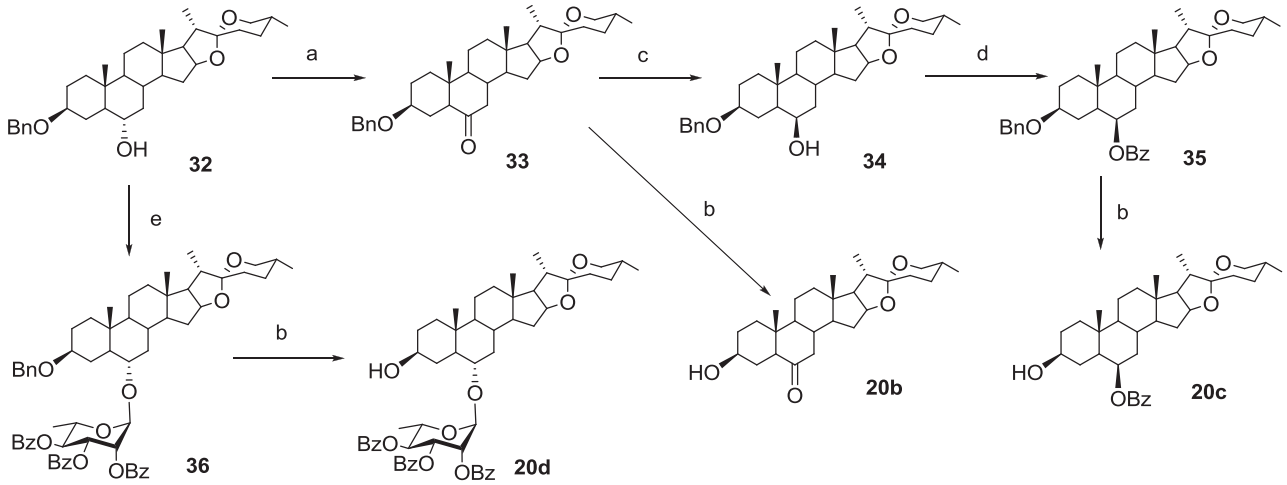
concentrations for the antiviral activity screening. The effects of compounds on HA mediated viral entry were summarized in Table 1.

4. Result and discussion

The activity of compounds **1**–**3** was reported by us previously [10]. First, chlorogenin, chacotriose **4** and ethyl 1-thio-β-chacotrioside **5** were tested but showed no H5N1 entry inhibitory activity, which indicated that the active saponin **1** acted as an integral structure but not the sugar chain or the aglycone alone.

To further understand the role of the chacotriosyl residue, compound **6** in which the two L-rhamnosyl residues were replaced by two L-arabinosyls was tested. It's interesting that the changes led to the total loss of activity, suggesting that the 2,4-L-rhamnosyls of chacotriosyl residue is essential for the activity. Whereas, blocks one or two hydroxyls by methyls in the chacotriosyl residue (compounds **7**–**11**) did not affect the activity significantly, and only slightly reduced activity was observed. We suppose that during the interaction of chacotriosyl residue with the receptor the hydroxyls contributed as composition of forces. Thus methylations on one or two hydroxyls were not able to induce the total loss but only the slight drop of activity. The supposition can be further supported by the results that the monomethylated compounds (**10** and **11**) both had a slightly better antiviral activity than those dimethylated (**7**–**9**).

For the saponins with the same 3-*O*-β-chacotriose residue (**1**, **2** and **12**–**14**), all of them except for **14** exhibited excellent inhibitory



Conditions. (a) Jones reagent, acetone, 0° C, 91%; (b) Pd/C-H_2, EtOAc/CH_3OH, 60° C, 83% for **20b**, 95% for **20c**, 87% for **20d**; (c) $NaBH_4$, CH_3OH/1,4-dioxane, 0° C, 92%; (d) BzCl, DMAP, CH_2Cl_2, 92%; (e) 4Å MS, TMSOTf (0.2 eq), CH_2Cl_2, 96%.

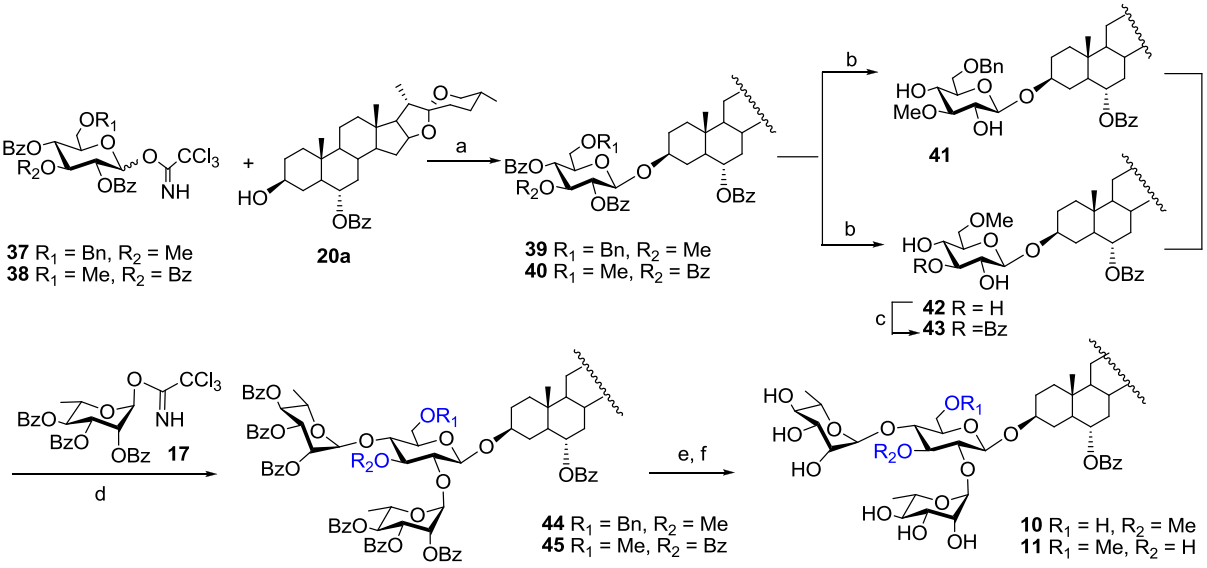
Scheme 3. Synthesis of compounds **20b**–**d**.

activity in the IC_{50} range of 6.0–20 μM. We also noticed that compounds **1** and **2** with the same 6α-substitution both showed slightly better activity than compounds **12** or **13**, which has a carbonyl group or a β-OH on C6, respectively. However, when a rhamnosyl residue was introduced to the 6α-OH of compound **1**, compound **14** lost the inhibitory activity. These results suggested that subtle modifications of aglycone on C6 had no important effect on the antiviral activity but introduction of bulk groups should be avoided.

In addition, compounds **1** and **2** showed a slight selectivity toward HA(QH)/HIV-luc strains, because HA of each H5N1 virus has its own special chemical structure, whereas all the methylated compounds **7**–**11** inhibited HA(viet)/HIV-luc strains more effectively. The reverse of selectivity suggested that the chacotriosyl residue might has a more affinity toward the target on HA(QH)/HIV-luc strains than that on HA(viet)/HIV-luc strains, thus methylations on chacotriosyl residue induced the drop of inhibitory activity more rapidly on HA(QH)/HIV-luc strains. Similarly, subtle modifications of aglycone on C6 could also induce the change of selectivity, since both compounds **12** and **13** inhibited HA(viet)/HIV-luc strains more effectively than HA(QH)/HIV-luc strains.

5. Conclusion

In conclusion, based on our previously discovered small molecule inhibitor saponin **1**, intensive SAR studies around the sugar chain and aglycone were conducted. The results showed that both



Conditions: (a) 4Å MS, TMSOTf (0.2 eq), CH_2Cl_2, 86% for **39**; (b) NaOMe, CH_2Cl_2-MeOH, 40% for **41**, 70% for **42** over 2 steps; (c) 1-BBTz, TEA, CH_2Cl_2, 53%; (d) 4Å MS, TMSOTf (0.2 eq), CH_2Cl_2; (e) **44**, Pd-C, H_2, EtOH-EtOAc; (f) NaOMe, CH_2Cl_2-MeOH, 46% for **10** over 3 steps, 44% for **11** over 2 steps.

Scheme 4. Synthesis of compounds **10** and **11**.

Table 1
Effect of compounds on HA mediated viral entry (IC_{50}, μM).

Compounds	HA(QH)/HIV-luc[a]	HA(viet)/HIV-luc[b]
1	7.2	7.8
2	7.6	9.3
3	6.0	8.5
Chlorogenin	>50	>50
4	>50	>50
5	>50	>50
6	>50	>50
7	31	15
8	28	16
9	N	24
10	25	13
11	12	9
12	20	12
13	18	11
14	>50	>50

[a] HA(QH): HA, H5N1(Goose/Qinghai/59/05).
[b] HA(Viet): HA, H5N1(A/Viet Nam/1203/2004).

the chacotriosyl residue and the chlorogenin moiety of active compound **1** are important for the antiviral activity, although several subtle modifications can be made on particular positions. Such efforts are of vital importance for the further development of novel and effective small molecule inhibitors.

6. Experimental protocols

6.1. General methods

All chemical reagents were used as supplied unless indicated. Solvents used in organic reactions were distilled under an inert atmosphere. Unless otherwise noted, all reactions were carried out at room temperature and were performed under a positive pressure of argon. Crushed 4 Å molecular sieves were activated by thorough flame-drying and cooled in vacuo prior to use. Flash column chromatography was performed on silica gel (200–300 mesh, Qingdao, China). Amberlite 15 (H^+-form) was used where acidic ion-exchange resin is indicated. Analytical thin layer chromatography (TLC) was performed on glass plates pre-coated with a 0.25 mm thickness of silica gel. ^1H NMR and ^{13}C NMR spectra were taken on a Jeol JNM-ECP 600 or a Bruker Avance III 400 spectrometer at r.t. Chemical shifts of the ^1H NMR spectra are expressed in ppm relative to the solvent residual signal 7.26 in $CDCl_3$ or to tetramethylsilane ($\delta = 0.00$). Chemical shifts of the ^{13}C NMR spectra are expressed in ppm relative to the solvent signal 77.00 in $CDCl_3$ or to tetramethylsilane ($\delta = 0.00$) unless otherwise noted. COSY, HMQC, and HMBC were routinely used to definitively assign the signals of ^1H NMR and ^{13}C NMR spectra. Electrospray (ESI) mass spectra were recorded on a Global Q-TOF mass spectrometer.

6.2. Synthesis of 2,4-di-O-(α-L-rhamnopyranosyl)-α/β-D-glucopyranose (chacotriose, **4**)

To a stirred solution of compound **5** (41.0 mg, 0.079 mmol) in acetone and H_2O (5 mL, v/v = 9:1) at −20 °C was added NBS (28.1 mg, 0.16 mmol). After 20 min, the reaction was quenched with saturated aqueous $NaHCO_3$ and concentrated. The residue was dissolved in pyridine (5 mL) and Ac_2O (2 mL). After stirred overnight, the reaction mixture was quenched by addition of CH_3OH (5 mL) and concentrated. The residue was diluted with $CHCl_3$ (20 mL) and washed with 1 mmol/L HCl aqueous solution, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography to afford peracetylated chacotriose. The physical data are identical with those provided in the literature: Morillo, M.; Lequart, V.; Grand, E.; Goethals, G. Usubillaga, A.; Villa, P.; Martin, P. *Carbohydr. Res.* 2001, *334*, 281–287. The above peracetylated chacotriose was dissolved in CH_3OH/CH_2Cl_2 (20 mL, v/v = 1:1), and then NaOMe was added until pH = 8.5. After stirred at 35 °C for 4 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by Sephadex LH-20 column chromatography (65% methanol in H_2O) to afford **4** (*α/β mixture*) as a white powder (20.5 mg, 55%): $R_f = 0.13$ (CH_2Cl_2/CH_3OH, 3:1); ^1H NMR (CD_3OD, 400 MHz): δ 5.36 (s, 1H), 5.19 (s-like, 0.27H), 5.16 (d, 0.25H, $J = 3.1$ Hz), 4.87 (s, 0.34H), 4.53 (d, 0.38H, $J = 9.8$ Hz), 4.14 (d, 1H, $J = 9.8$ Hz), 3.98–3.93 (m, 3H), 3.92–3.77 (m, 6H), 3.76–3.58 (m, 8H), 3.58–3.49 (m, 2H), 3.45–3.32 (m), 3.21–3.14 (m, 1.72H), 2.90–2.73 (m, 1.79H), 1.40–1.15 (m); ^{13}C NMR (CD_3OD, 100 MHz): δ 103.2, 103.0, 88.6, 81.1, 78.6, 78.3, 74.9, 73.7, 72.5–72.1, 70.9, 70.7, 61.6, 41.9, 17.9; ESI-HRMS calcd for $C_{18}H_{32}NaO_{14}$ $[M + Na]^+$ 495.1684, found 495.1684.

6.3. Synthesis ethyl 1-thio-β-chacotrioside (**5**)

To a mixture of acceptor **16** (518.4 mg, 1.20 mmol), imidate **17** (990.5 mg, 1.60 mmol) and powdered 4 Å molecular sieves in dried CH_2Cl_2 (30 mL) at −40 °C was added TMSOTf (53.3 mg, 0.2 mmol). After stirred at −40 °C for 0.5 h and then at r.t. for 2 h, the reaction was quenched with Et_3N. The solid was filtered off. The filtrate was concentrated and purified by column chromatography (EtOAc/petroleum ether, 1:3) to afford compound **18** as an amorphous solid (506.2 mg, 31%).

Compound **18** (506.2 mg, 0.38 mmol) was dissolved in CH_3OH/CH_2Cl_2 (20 mL, v/v = 1:1), and then NaOMe was added until pH = 8.5. After stirred at 35 °C for 4 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH, 3:1) to afford compound **5** as a yellow syrup (87.2 mg, 45%): $R_f = 0.20$ (CH_2Cl_2/CH_3OH, 3:1); ^1H NMR (CD_3OD, 400 MHz): δ 5.27 (s, 1H, H-1″), 4.92 (s, 1H), 4.42 (d, 1H, $J = 9.8$ Hz), 4.18–4.16 (m, 1H), 3.95–3.94 (m, 2H), 3.82–3.80 (m, 2H), 3.66–3.52 (m, 5H), 3.48–3.38 (m, 3H), 2.82–2.67 (m, 2H), 1.25–1.21 (m, 9H); ^{13}C NMR (CD_3OD, 100 MHz): δ 103.1, 84.7, 80,8, 80.0, 79.9, 79.5, 73.9, 73.7, 72.5, 72.4, 72.3, 72.2, 70.7, 70.6, 62.1, 24.6, 17.9, 17.8, 15.1; ESI-HRMS calcd for $C_{20}H_{36}NaO_{13}S$ $[M + Na]^+$ 539.1769, found 539.1769.

6.4. Synthesis of chlorogenin 3β-O-[2,4-di-O-(α-L-arabinopyranosyl)-β-D-glucopranoside] (**6**)

To a mixture of **22a** (50.0 mg, 0.06 mmol) and 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (2.0 μL, 0.01 mmol). After stirred at −20 °C for 5 min donor **23** (133.1 mg, 0.22 mmol) was added. The mixture was stirred at −20 °C for 20 min and then at r.t. for 8 h. The reaction was quenched with Et_3N. The solid was then filtered off.

The residue was dissolved in CH_3OH/CH_2Cl_2 (20 mL, v:v = 1:1), and then NaOMe was added until pH = 8.5. After stirred at 35 °C for 4 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 10:1–4:1) to afford **6** as a white syrup (10.1 mg, 21% for two steps): $R_f = 0.52$ ($CHCl_3$/MeOH, 3:1); ^1H NMR (CD_3OD, 600 MHz): δ 4.62 (d, 1H, $J = 6.0$ Hz, Ara-H-1), 4.60 (d, 1H, $J = 7.8$ Hz, H-1′), 4.39 (q, 1H, $J = 7.8$ Hz, H-16), 4.31 (d, 1H, $J = 6.8$ Hz, Ara-H-1), 3.98 (dd, 1H, $J = 12.4$, 4.1 Hz, Ara-H-4), 3.93 (dd, 1H, $J = 12.8$, 2.3 Hz, Ara-H-4), 3.85–3.83 (m, 2H, 2× Ara-H-3), 3.71 (t, 1H, $J = 9.2$ Hz, H-3′), 3.69–3.53 (m, 10H, 2× Ara-H-2, H-6′, H-5′, 2× Ara-H-5, H-4′), 3.46–3.32 (m, 4H, H-2′, H-3, H-26), 2.33 (brd, 1H, $J = 10.1$ Hz), 0.95 (d, 3H, $J = 6.9$ Hz, Me), 0.86 (s, 3H, Me), 0.79 (d, 3H, $J = 6.0$ Hz, Me), 0.79 (s, 3H, Me); ^{13}C NMR (CD_3OD,

150 MHz): δ 110.7, 105.4, 101.0, 82.3, 81.1, 79.9, 76.3, 74.1, 73.9, 73.2, 72.6, 70.1, 68.0, 67.9, 63.9, 61.9, 57.5, 55.4, 52.9, 43.1, 42.8, 41.9, 41.2, 38.7, 37.7, 35.4, 33.2, 32.8, 32.6, 31.6, 30.9–30.0, 29.5, 26.2, 23.9, 22.3, 17.7, 17.1, 15.0, 14.6, 14.0; ESI-HRMS calcd for $C_{43}H_{70}NaO_{17}$ $[M + Na]^+$ 881.4505, found 881.4505.

6.5. Synthesis of chlorogenin 3β-O-[2,4-di-O-(2-O-methyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside] (**7**)

To a mixture of **22a** (50.0 mg, 0.10 mmol) and 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (2.0 μL, 0.02 mmol). After stirred at −20 °C for 5 min donor **24** (147.0 mg, 0.50 mmol) was added. The mixture was stirred at −20 °C for 20 min and then at r.t. for 8 h. The reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 1:3) to give **28** as an amorphous solid (57.4 mg, 75%).

Compound **28** (57.4 mg, 0.04 mmol) was dissolved in CH_3OH/CH_2Cl_2 (20 mL, v:v = 1:1), and then NaOMe was added until pH = 8.5. After stirred at 35 °C for 4 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 10:1–4:1) to afford **7** as a white amorphous solid (30.4 mg, 80%): R_f = 0.54 ($CHCl_3$/MeOH, 8:1); ^1H NMR (CD_3OD, 600 MHz): δ 5.30 (s, 1H, Rha-H-1), 5.03 (d, 1H, J = 1.4 Hz, Rha-H-1), 4.53 (d, 1H, J = 7.8 Hz, H-1′), 4.39 (q, 1H, J = 7.8 Hz, H-16), 4.13–4.08 (m, 1H, Rha-H-5), 3.95–3.90 (m, 1H, Rha-H-5), 3.82 (dd, 1H, J = 11.9, 1.9 Hz, H-6a′), 3.68–3.65 (m, 4H, H-6b′, Rha-H-3), 3.61–3.57 (m, 2H, Rha-H-3), 3.54 (dd, 1H, J = 3.7, 1.9 Hz, Rha-H-2), 3.45 (s, 3H, OMe), 3.44 (s, 3H, OMe), 3.46–3.44 (m, 1H, Rha-H-2), 3.40–3.29 (m, H-2′, H-3′, H-4′, H-5′, Rha-H-4, H-26), 2.32 (d, 1H, J = 12.4 Hz), 1.25 (d, 3H, J = 6.0 Hz, Rha-6-Me), 1.22 (d, 3H, J = 6.0 Hz, Rha-6-Me), 0.96 (d, 3H, J = 7.3 Hz, Me), 0.86 (s, 3H, Me), 0.79 (d, 3H, J = 6.4 Hz, Me), 0.78 (s, 3H, Me), 0.70 (td, 1H, J = 11.0, 3.7 Hz); ^{13}C NMR (CD_3OD, 150 MHz): δ 110.5, 100.2, 99.2, 82.5, 82.1, 79.8, 79.3, 78.7, 77.9, 76.7, 74.3, 74.1, 72.1, 72.0, 70.5, 69.9, 69.7, 67.9, 63.8, 59.2, 57.3, 55.3, 52.8, 47.9, 42.9, 42.7, 41.7, 41.0, 38.7, 37.6, 35.3, 32.7, 32.4, 31.5, 30.4, 29.9, 29.3, 22.1, 18.0, 17.9, 17.5, 16.9, 14.9, 13.9, 9.2; ESI-HRMS calcd for $C_{47}H_{78}NaO_{17}$ $[M + Na]^+$ 937.5131, found 937.5134.

6.6. Synthesis of chlorogenin 3β-O-[2,4-di-O-(3-O-methyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside] (**8**)

To a mixture of **22a** (50.0 mg, 0.10 mmol) and 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (4.0 μL, 0.02 mmol). After stirred at −20 °C for 5 min donor **25** (147.0 mg, 0.50 mmol) was added. The mixture was stirred at −20 °C for 20 min and then at r.t. for 8 h. Then the reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 1:4–1:2) to give **29** as an amorphous solid.

Compound **29** was dissolved in CH_3OH/CH_2Cl_2 (20 mL, v:v = 1:1), and then NaOMe was added until pH = 8.5. After stirred at 35 °C for 4 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 10:1–4:1) to afford **8** as a white amorphous solid (24.5 mg, 49% for two steps): R_f = 0.45 (CH_2Cl_2/MeOH, 5:1); ^1H NMR (CD_3OD, 600 MHz): δ 5.21 (s, 1H, Rha-1-H), 4.89 (s, 1H, Rha-H-1), 4.54 (d, 1H, J = 7.7 Hz, H-1′), 4.39 (q, 1H, J = 7.4 Hz, H-16), 4.17–4.15 (m, 2H, Rha-H-5, Rha-H-2), 4.04 (d, 1H, J = 2.8 Hz, Rha-H-2), 3.98–3.96 (m, 1H, Rha-H-5), 3.81–3.79 (m, 1H, H-6a′), 3.70–3.65 (m, 1H, H-6b′), 3.58 (t, 1H, J = 9.2 Hz, H-4′), 3.54 (t, 1H, J = 9.2 Hz, H-3′), 3.48–3.44 (m, 2H, Rha-H-4, Rha-H-4), 3.44 (s, 3H, OMe), 3.43 (s, 3H, OMe), 3.39 (t, 1H, J = 8.3 Hz, H-2′), 3.38–3.27 (m, 4H, H-2′, H-5′, Rha-H-3, Rha-H-3), 2.33 (d, 1H, J = 12.8 Hz), 1.25 (d, 3H, J = 6.4 Hz, Rha-6-Me), 0.96 (d, 3H, J = 7.3 Hz, Me), 0.86 (s, 3H, Me), 0.79 (d, 3H, J = 5.5 Hz, Me), 0.79 (s, 3H, Me); ^{13}C NMR (CD_3OD, 150 MHz): δ 110.6, 102.8, 102.4, 100.3, 82.2–82.0, 79.6, 78.7, 76.7, 72.8, 72.6, 70.6, 69.9, 69.7, 68.4, 67.9, 63.9, 62.0, 57.4, 57.2, 55.4, 52.8, 48.0, 43.0, 42.7, 41.8, 41.1, 38.7, 37.7, 35.3, 32.7, 32.5, 31.5, 30.4, 29.9, 29.4, 22.1, 18.1, 17.9, 17.5, 17.0, 14.9, 13.9, 9.3; ESI-HRMS calcd for $C_{47}H_{79}O_{17}$ $[M + H]^+$ 915.5311, found 915.5311.

6.7. Synthesis of chlorogenin 3β-O-[2,4-di-O-(4-O-methyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside] (**9**)

To a mixture of **22a** (50.0 mg, 0.06 mmol) and 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (2.0 μL, 0.01 mmol). After stirred at −20 °C for 5 min and then donor **26** (147.0 mg, 0.50 mmol) was added. The mixture was stirred at −20 °C for 20 min and then at r.t. for 8 h. Then the reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 1:4–1:2) to give **30** as an amorphous solid.

Compound **30** was dissolved in CH_3OH/CH_2Cl_2 (10 mL, v:v = 1:1), and then NaOMe was added until pH = 8.5. After stirred at 35 °C for 4 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 10:1–4:1) to afford **9** as a white amorphous solid (54.2 mg, 66% for two steps): R_f = 0.55 ($CHCl_3$/MeOH, 8:1); ^1H NMR (CD_3OD, 600 MHz): δ 5.9 (d, 1H, J = 1.7 Hz, Rha-H-1), 4.81 (d, 1H, J = 1.6 Hz, Rha-H-1), 4.53 (d, 1H, J = 8.2 Hz, H-1′), 4.40 (q, 1H, J = 7.7 Hz, H-16), 4.22–4.19 (m, 1H, Rha-H-5), 3.95–3.91 (m, 1H, Rha-H-5), 3.88 (dd, 1H, J = 3.3, 1.6 Hz, Rha-H-2), 3.80 (dd, 1H, J = 3.3, 2.2 Hz, Rha-H-2), 3.79 (m, 2H, Rha-H-3), 3.75 (dd, 1H, J = 9.9, 3.3 Hz, Rha-H-3), 3.70 (dd, 1H, J = 9.9, 3.3 Hz, Rha-H-3), 3.64 (dd, 1H, J = 12.1, 4.4 Hz, H-6b′), 3.68–3.66 (m, 1H, H-26a), 3.56 (t, 1H, J = 8.8 Hz, H-3′), 3.55 (s, 3H, OMe), 3.54 (s, 3H, OMe), 3.51 (t, 1H, J = 8.8 Hz, H-4′), 3.39 (t-like, 1H, J = 8.8, 8.2 Hz, H-2′), 3.29–3.27 (m, 1H, H-5), 3.10 (t-like, J = 9.9, 9.4 Hz, Rha-H-4), 3.06 (t-like, 1H, J = 10.4, 9.4 Hz, Rha-H-4), 2.34 (d, 1H, J = 12.6 Hz), 1.26 (d, 3H, J = 6.1 Hz, Rha-Me), 1.24 (d, 3H, J = 6.6 Hz, Rha-Me), 0.96 (d, 3H, J = 7.1 Hz, Me), 0.86 (s, 3H, Me), 0.79 (d, 3H, J = 6.1 Hz, Me), 0.79 (s, 3H, Me), 0.70 (td, 1H, J = 11.0, 3.8 Hz); ^{13}C NMR (CD_3OD, 150 MHz): δ 110.6, 102.8, 99.8, 84.5, 84.2, 82.2, 79.7, 78.7, 76.7, 72.5, 72.2, 69.9, 69.6, 68.7, 67.9, 63.8, 6.1, 57.3, 55.3, 52.6, 47.9, 42.9, 42.8, 41.7, 38.6, 37.7, 32.7, 32.4, 31.4, 30.8, 30.2, 29.1, 22.1, 18.2, 18.1, 17.5, 17.0, 14.9, 9.3; ESI-HRMS calcd for $C_{47}H_{79}O_{17}$ $[M + H]^+$ 915.5311, found 915.5311.

6.8. Synthesis of chlorogenin 3β-O-[3-O-methyl-2,4-di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside] (**10**)

To a mixture of **41** (25.0 mg, 0.03 mmol) and 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (1.0 μL, 0.01 mmol). After stirred at −20 °C for 5 min **17** (92.3 mg, 0.15 mmol) was added. The mixture was stirred at −20 °C for 20 min and at r.t. for 8 h. The reaction was quenched with Et_3N and the solid was filtered off.

The residue was dissolved in MeOH (5 mL) and 10%-Pd/C (500.0 mg) was added. After stirred for 24 h at room temperature under an atmosphere of hydrogen gas, the mixture was filtered through Celite pad, and the filtrate was concentrated.

The residue was dissolved in CH_3OH/CH_2Cl_2 (20 mL, v:v = 1:1), and NaOMe was added until pH = 8.5. After stirred at 35 °C for 4 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 10:1–4:1) to afford **10** as syrup (13.1 mg, 46% for three steps): R_f = 0.65 ($CHCl_3$/MeOH, 3:1);

^{1}H NMR (DMSO-d_6, 600 MHz): δ 4.83 (s, 1H, Rha-H-1), 4.80 (t, 1H, J = 6.0 Hz, OH), 4.76–4.74 (m, 4H, Rha-H-1, H-1′and 2× OH), 4.70 (d, 1H, J = 4.6 Hz, OH), 4.55 (t, 1H, J = 6.4 Hz, OH), 4.48 (d, 1H, J = 6.8 Hz, OH), 4.36 (d, 1H, J = 5.5 Hz, OH), 4.26 (q, 1H, J = 7.3 Hz, H-16), 3.98–3.95 (m, 1H, Rha-H-5), 3.69–3.67 (m, 2H, Rha-H-5 and H-2′), 3.60–3.47 (m, 6H), 3.40 (s, 3H, OMe), 3.27–3.13 (m, 6H), 1.10 (d, 3H, J = 6.4 Hz, Rha-Me), 1.08 (d, 3H, J = 6.0 Hz, Rha-Me), 0.90 (d, 3H, J = 6.9 Hz, Me), 0.74 (s, 3H, Me), 0.72–0.70 (m, 6H), 0.60 (td, 1H, J = 6.0, 4.0 Hz); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 108.3, 100.3, 97.0, 85.5, 80.1, 75.8, 75.3, 74.9, 73.4, 71.8, 71.7, 70.5, 68.7, 68.3, 67.2, 65.8, 61.8, 61.5, 55.4, 53.3, 50.7, 45.4, 41.9, 41.0, 37.0, 35.9, 33.3, 31.4, 31.2, 30.8, 29.7, 28.9–28.4, 27.0, 22.0, 17.9, 17.8, 16.1, 14.6, 13.9, 13.0; ESI-HRMS calcd for $C_{46}H_{76}NaO_{17}$ $[M + Na]^+$ 923.4975, found 923.4975.

6.9. Synthesis of chlorogenin 3β-O-[6-O-methyl-2,4-di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside] (**11**)

To a mixture of **43** (100.0 mg, 0.10 mmol) and 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (4.0 μL, 0.02 mmol). After stirred at −20 °C for 5 min and then **17** (340.0 mg, 0.50 mmol) was added. The mixture was stirred at −20 °C for 20 min and then at r.t. for 8 h. Then the reaction was quenched with Et_3N and the solid was filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 1:3) to give an amorphous solid.

The solid was dissolved in CH_3OH/CH_2Cl_2 (20 mL, v:v = 1:1), and NaOMe was added until pH = 8.5. After stirred at 35 °C for 4 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 8:1) to afford **11** as a white amorphous solid (48.7 mg, 44% for two steps): R_f = 0.65 ($CHCl_3$/MeOH, 3:1); ^{1}H NMR (CD_3OD, 600 MHz): δ 5.19 (d, 1H, J = 1.3 Hz, Rha-H-1), 4.77 (d, 1H, J = 1.9 Hz, Rha-H-1), 4.50 (d, 1H, J = 7.7 Hz, H-1′), 4.40 (q, 1H, J = 7.3 Hz, H-16), 4.16–4.11 (m, 1H, Rha-H-5), 3.97–3.92 (m, 1H, Rha-H-5), 3.92 (dd, 1H, J = 3.2, 1.9 Hz, Rha-H-2), 3.82 (dd, 1H, J = 3.7, 1.8 Hz, Rha-H-2), 3.67 (dd, 1H, J = 9.6, 3.7 Hz, Rha-H-3), 3.68–3.30 (m, 7H, H-6′, H-5′, H-4′, H-3′, H-2′, H-26), 2.31 (brd, 1H, J = 12.4 Hz), 1.25 (d, 3H, J = 6.4 Hz, Rha-Me), 1.24 (d, 3H, J = 6.4 Hz, Rha-Me), 0.96 (d, 3H, J = 6.9 Hz, Me), 0.86 (s, 3H, Me), 0.80 (d, 3H, J = 5.5 Hz, Me), 0.79 (s, 3H, Me), 0.70 (td, 1H, J = 10.5, 4.4 Hz); ^{13}C NMR (CD_3OD, 150 MHz): δ 110.7, 102.5, 100.6, 82.3, 79.9, 79.5, 79.0, 78.1, 75.7, 74.1, 73.9, 72.6, 72.3, 70.7, 70.1, 69.8, 68.0, 64.0, 57.5, 55.5, 52.9, 43.1, 42.8, 41.9, 41.2, 38.8, 37.8, 35.4, 32.8, 32.6, 31.6, 30.5, 30.0, 29.5, 22.2, 18.1, 18.0, 17.7, 17.0, 16.0, 14.0; ESI-HRMS calcd for $C_{46}H_{76}NaO_{17}$ $[M + Na]^+$ 923.4975, found 923.4975.

6.10. Synthesis of laxogenin 3β-O-[2,4-di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside] (**12**)

To a solution of **31b** (22.0 mg, 0.013 mmol) in dry CH_2Cl_2/MeOH (10 mL, v:v = 1:1) was added NaOMe to make the pH = 10. The mixture was stirred at r.t. for 24 h at 35 °C. The solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by flash chromatography (CH_2Cl_2/CH_3OH, 3:1) to give **12** as a white amorphous solid (4.0 mg, 34%): R_f = 0.30 (CH_2Cl_2/CH_3OH, 3:1); ^{1}H NMR (CD_3OD, 400 MHz): δ 5.19 (s, 1H, H-1″), 4.83 (s, 1H, H-1‴), 4.53–4.51 (d, 1H, J = 7.4 Hz, H-1′), 4.44–4.37 (m, 1H), 4.13–4.06 (m, 1H), 3.95–3.90 (m, 2H), 3.84–3.33 (m, 16H), 1.04 (s, 3H), 0.98–0.94 (d, 4H, J = 6.7 Hz), 0.81–0.76 (d, 9H, J = 11.7 Hz), 2.35–0.84 (m); ^{13}C NMR (CD_3OD, 100 MHz): δ 213.4, 110.6, 103.0, 102.4, 99.8, 81.9, 80.0, 79.5, 78.0, 77.5, 76.6, 74.0, 72.5, 72.3, 72.2, 72.1, 70.7, 69.9, 67.9, 63.7, 62.0, 57.5, 57.4, 54.9, 47.5, 42.9, 42.2, 42.1, 40.6, 38.8, 37.7, 32.5, 32.4, 31.4, 30.7, 29.9, 27.0, 22.4, 18.0, 17.9, 17.5, 16.8, 14.8, 13.5; ESI-HRMS calcd for $C_{45}H_{72}NaO_{17}$ $[M + Na]^+$ 907.4662, found 907.4662.

6.11. Synthesis of 6β-OH chlorogenin 3β-O-[2,4-di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside] (**13**)

To a solution of **31c** (35 mg, 0.019 mmol) in dry CH_2Cl_2/MeOH (10 mL, v:v = 1:1) was added NaOMe to make the pH value of the mixture at 10. After stirred at r.t. for 24 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 3:1) to give **13** as a white amorphous solid (11.0 mg, 65%). R_f = 0.25 (CH_2Cl_2/MeOH, 4:1); ^{1}H NMR (CD_3OD, 400 MHz): δ 5.19 (s, 1H, H-1″), 4.83 (s, 1H, H-1‴), 4.53–4.51 (d, 1H, J = 7.4 Hz, H-1′), 4.41–4.36 (m, 1H), 4.18–4.13 (m, 1H), 3.94–3.89 (m, 2H), 3.84–3.76 (m, 4H), 3.71–3.47 (m, 6H), 3.46–3.33 (m, 5H), 1.04 (s, 3H), 0.98–0.94 (d, 4H, J = 7.0 Hz), 0.83 (s, 3H), 0.81–0.78 (d, 3H, J = 6.3 Hz), 2.02–0.68 (m); ^{13}C NMR (CD_3OD, 100 MHz): δ 110.6, 103.0, 102.4, 82.2, 80.0, 79. 7, 79.0, 78.1, 76.6, 74.1, 73.7, 72.5, 72.4, 72.2, 70.7, 69.7, 67.9, 63.9, 62.0, 57.3, 55.8, 42.9, 41.8, 41.1, 40.7, 39.9, 36.9, 32.7, 32.4, 31.4, 30.8, 30.6, 29.9, 22.0, 18.0, 17.9, 17.5, 17.0, 14.9; ESI-HRMS calcd for $C_{45}H_{74}NaO_{17}$ $[M + Na]^+$ 909.4818, found 909.4818.

6.12. Synthesis of 6-O-α-L-rhamnopyranosyl-chlorogenin 3β-O-[2,4-di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside] (**14**)

To a mixture of **22d** (100.0 mg, 0.04 mmol) and 4 Å molecular sieves in dried CH_2Cl_2 (4 mL) at −20 °C was added TMSOTf (2.0 μL, 0.01 mmol). After stirred at −40 °C for 5 min **17** (244.1 mg, 0.20 mmol) was added. The mixture was stirred at −20 °C for 20 min and then at r.t. for 8 h. The reaction was quenched with Et_3N and the solid was filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 8:1) to give **31d** as a white solid (95.4 mg, 55%).

Compound **31d** (95.4 mg, 0.04 mmol) was dissolved in CH_3OH/CH_2Cl_2 (10 mL, v:v = 1:1), and then NaOMe was added until pH = 8.5. After stirring at 35 °C for 4 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 10:1–4:1) to afford **14** as a white amorphous solid (18.1 mg, 40%): R_f = 0.29 (CH_2Cl_2/MeOH, 4:1); ^{1}H NMR (CD_3OD, 600 MHz): δ 5.26 (s, 1H, Rha-H-1), 4.85 (s, 1H, Rha-H-1), 4.75 (s, 1H, Rha-H-1), 4.50 (d, 1H, J = 8.2 Hz, H-1′), 4.43 (q, 1H, J = 6.8 Hz, H-16), 4.20–4.15 (m, 1H, Rha-H-5), 3.94–3.90 (m, 3H, 2× Rha-H-2, H-5′), 3.84 (dd, 1H, J = 3.2, 1.8 Hz, Rha-H-2), 3.79 (brd, 1H, J = 11.0 Hz, H-6a′), 3.70–3.52 (m, 6H, 2× Rha-H-5, 2× Rha-H-3, H-6b′, H-26, Rha-H-4), 3.50–3.46 (m, 1H, H-3), 3.43–3.29 (m, 6H, 2× Rha-H-4, H-2′, H-3′, H-4′, Rha-H-3), 2.16 (brd, 1H, J = 11.9 Hz), 2.09 (dt, 1H, J = 11.9, 4.1 Hz), 1.26 (d, 3H, J = 6.4 Hz, Rha-Me), 1.25 (d, 3H, J = 6.0 Hz, Rha-Me), 1.24 (d, 3H, J = 6.0 Hz, Rha-Me), 0.96 (d, 3H, J = 6.9 Hz, Me), 0.88 (s, 3H, Me), 0.81 (d, 3H, J = 6.4 Hz, Me), 0.80 (s, 3H, Me), 0.72 (td, 1H, J = 11.9, 4.1 Hz); ^{13}C NMR(CD_3OD, 150 MHz): δ 110.7, 104.0, 103.1, 102.1, 82.1, 80.7, 78.6, 78.5, 76.6, 74.1, 73.8, 72.6, 72.3, 70.8, 70.1, 69.8, 68.0, 63.9, 57.5, 55.2, 51.8, 43.1, 41.9, 41.5, 41.2, 38.7, 37.8, 35.4, 32.8, 32.5, 31.7, 30.8, 30.6, 30.0, 22.2, 18.0, 17.9, 17.6, 17.1, 15.0, 14.0; ESI-HRMS calcd for $C_{51}H_{84}NaO_{21}$ $[M + Na]^+$ 1055.5397, found 1055.5398.

6.13. Synthesis of 6β-O-benzoyl chlorogenin (**20c**)

Compound **34** (741.8 mg, 1.42 mmol) was dissolved in pyridine (10 mL), and benzoyl chloride (0.33 mL) was added dropwise at 0 °C. The solution was stirred for 1 h at 0 °C. The reaction mixture was diluted with EtOAc and washed consecutively with water, 1 mol/L HCl aqueous solution, saturated $NaHCO_3$ solution and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 1:20) to provide **35** as white solid (818.4 mg, 92%): R_f = 0.15 (EtOAc/petroleum ether, 1:20); ^{1}H NMR

($CDCl_3$, 400 MHz): δ 8.03 (d, 2H, J = 7.0 Hz), 7.56–7.54 (m, 1H), 7.46–7.44 (m, 2H), 7.32–7.30 (m, 4H), 7.26–7.24 (m, 1H), 5.21 (d, 1H, J = 2.7 Hz), 4.53 (d, 1H, J = 11.7 Hz), 4.51 (d, 1H, J = 11.7 Hz), 4.38 (q, 1H, J = 7.8 Hz), 3.48–3.35 (m, 3H), 1.21 (s, 3H), 0.98 (d, 3H, J = 6.04 Hz), 0.90–0.88 (m, 7H).

Compound **35** (751.7 mg, 1.20 mmol) was dissolved in CH_2Cl_2/EtOH (20 mL, v:v = 1:1) containing 2 drops of Et_3N. 10%-Pd/C (200.0 mg) was added. After stirred for 12 h at room temperature under an atmosphere of hydrogen gas, the mixture was filtered through Celite pad and the filtrate was concentrated. The residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 1:2) to give **20c** as a white solid (610.1 mg, 95%): R_f = 0.28 (EtOAc/petroleum ether, 1:3); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.03 (d, 2H, J = 7.0 Hz), 7.58–7.56 (m, 1H), 7.46–7.44 (m, 2H), 5.21 (d, 1H, J = 2.7 Hz), 4.38 (q, 1H, J = 7.4 Hz), 3.66–3.64 (m, 1H), 3.47–3.45 (m, 1H), 3.37 (t, 1H, J = 11.0 Hz), 1.21 (s, 3H), 0.98 (d, 3H, J = 7.0 Hz), 0.90–0.88(m, 7H). ESIMS: calcd for $[M + H]^+$ m/z 537.4; found: 537.4.

6.14. Synthesis of chlorogenin 6-O-(2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyide) (**20d**)

To a mixture of **32** (100.0 mg, 0.20 mmol), imidate **17** (154.0 mg, 0.30 mmol) and powdered 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (6.8 μL, 0.04 mmol). After stirred at −20 °C for 0.5 h, the reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 1:20–1:10) to give **36** as a white solid (180.1 mg, 96%).

Compound **36** (180.1 mg, 0.18 mol) was dissolved in EtOAc (5 mL) and EtOH (5 mL) and then 10%-Pd/C (50 mg) was added. After stirred for 24 h at room temperature under an atmosphere of hydrogen gas, the mixture was filtered through Celite pad, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 1:2) to give **20d** as a white solid (142.7 mg, 87%): R_f = 0.45 (EtOAc/petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.09 (d, 2H, J = 7.7 Hz, PhH), 7.98 (d, 2H, J = 6.0 Hz, PhH), 7.82 (d, 2H, J = 7.7 Hz, PhH), 7.61 (t, 1H, J = 7.1 Hz, PhH), 7.53–7.25 (m, 8H, PhH), 5.80 (d, 1H, J = 7.1 Hz, H-3′), 5.66 (t, 1H, J = 9.4 Hz, H-4′), 5.62 (brs, 1H, H-2′), 5.01 (s, 1H, H-1′), 4.41 (q, 1H, J = 7.1 Hz, H-16), 4.29–4.23 (m, 1H, H-5′), 3.69 (brs, 1H, OH), 3.47–3.41 (m, 2H, H-26a, H-3), 3.35 (t, 1H, J = 11.0 Hz, H-26b), 1.31 (d, 3H, J = 6.1 Hz, 6′-Me), 0.97 (d, 3H, J = 7.1 Hz, Me), 0.86 (s, 3H, Me), 0.77 (d, 3H, J = 5.0 Hz, Me), 0.71 (s, 3H, Me); ESIMS: calcd for $[M + H]^+$ m/z 891.5; found: 891.9.

6.15. Synthesis of laxogenin 3β-O-(3,6-di-O-benzoyl-2,4-di-O-levulinoyl-β-D-glucopyranoside) (**21b**)

To a mixture of **20b** (60.6 mg, 0.14 mmol), imidate **19** (153.8 mg, 0.21 mmol) and powdered 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (6.8 μL, 0.04 mmol). After stirred at −20 °C for 0.5 h, the reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 2:3) to give **21b** as a white solid (56.1 mg, 40%): R_f = 0.15 (EtOAc/petroleum ether, 2:3); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.07 (d, 2H, J = 7.4 Hz), 7.97 (d, 2H, J = 7.0 Hz), 7.56 (m, 2H), 7.44 (m, 4H), 5.50 (t, 1H, J = 9.4 Hz), 5.31 (t, 1H, J = 10.2 Hz), 5.14 (t, 1H, J = 9.4 Hz), 4.73 (d, 1H, J = 7.8 Hz), 4.60–4.55 (m, 1H), 4.49–4.39 (m, 2H), 3.95–3.92 (m, 1H), 3.59–3.50 (m, 2H), 3.36 (t, 1H, J = 11.4 Hz), 2.78 (s, 1H), 2.04 (s, 3H), 1.98 (s, 3H), 0.97 (d, 3H, J = 6.7 Hz), 0.80 (d, 3H, J = 5.9 Hz), 0.78 (s, 3H), 0.73 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 210.1, 205.8, 175.5–165.8, 133.4–128.4, 109.3, 99.2, 80.4, 78.4, 73.2, 71.9, 71.6, 69.1, 66.9, 62.8, 62.0, 56.5, 53.8, 46.7, 41.6, 40.9, 39.5, 37.9, 37.8, 37.2, 36.4, 31.6, 31.3, 30.3–27.8, 26.2, 21.3, 17.1, 16.4, 14.5, 13.1; ESI-HRMS calcd for $C_{57}H_{72}NaO_{15}$ $[M + Na]^+$ 1019.4763, found 1019.4763.

6.16. Synthesis of 6β-O-benzoyl chlorogenin 3β-O-(3,6-di-O-benzoyl-2,4-di-O-levulinoyl-β-D-glucopyranoside) (**21c**)

To a mixture of **20c** (75.1 mg, 0.14 mmol), imidate **19** (153.8 mg, 0.21 mmol) and powdered 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (6.8 μL, 0.04 mmol). After stirred at −20 °C for 0.5 h, the reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 1:3) to give **21c** as a white solid (75.6 mg, 49%): R_f = 0.3 (EtOAc/petroleum ether, 1:3); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.08–7.92 (m, 6H), 7.55–7.53 (m, 3H), 7.44–7.39 (m, 6H), 5.49 (t, 1H, J = 9.8 Hz), 5.28 (t, 1H, J = 9.4 Hz), 5.19–5.18 (m, 1H), 5.08 (t, 1H, J = 9.3 Hz), 4.70 (d, 1H, J = 7.8 Hz), 4.55–4.53 (m, 1H), 4.46–4.35 (m, 2H), 3.95–3.93 (m, 1H), 3.61–3.59 (m, 1H), 3.46–3.40 (m, 1H), 3.37–3.35 (m, 1H), 2.78 (s, 6H), 1.98 (s, 3H), 1.89 (s, 3H), 0.96 (d, 3H, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 205.8, 205.4, 176.8–165.8, 133.3–128.4, 109.2, 99.8, 83.5, 80.7, 79.9, 74.1, 73.1, 71.9, 71.6, 69.1, 66.8, 62.8, 62.1, 55.9, 53.9, 46.4, 42.5, 41.6, 40.6, 39.9, 39.5, 38.1, 37.8, 36.5, 35.6, 31.7–27.8, 23.9, 20.8, 19.3, 17.1, 16.6, 15.9, 14.5; ESI-HRMS calcd for $C_{64}H_{78}NaO_{16}$ $[M + Na]^+$ 1125.5182, found 1125.5182.

6.17. Synthesis of laxogenin 3β-O-(3,6-di-O-benzoyl-β-D-glucopyranoside) (**22b**)

Compound **21b** (76.1 mg, 0.076 mmol) was dissolved in dry CH_2Cl_2/MeOH (5 mL, v:v = 4:1), and $AcOH{\cdot}NH_2NH_2$ (140.0 mg, 1.52 mmol) was added. The solution was stirred at r.t. for 3 h. The mixture was diluted with CH_2Cl_2 and washed consecutively with water, 1 mol/L HCl aqueous solution, saturated $NaHCO_3$ solution and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 2:3) to provide **22b** as syrup (51.1 mg, 84%): R_f = 0.2 (EtOAc/petroleum ether, 2:3); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12–8.06 (m, 4H), 7.62–7.56 (m, 2H), 7.48–7.40 (m, 4H), 5.22–5.19 (t, 1H, J = 3.0 Hz), 4.72–4.62 (m, 2H), 4.61–4.56 (d, 1H, J = 7.8 Hz, H-1′), 4.45–4.36 (m, 1H), 3.77–3.73 (s, 2H), 3.72–3.61 (m, 2H), 3.50–3.43 (m, 1H), 3.40–3.32 (m, 2H), 3.00–2.83 (d, 1H, J = 13.7 Hz), 2.47 (s, 1H), 1.98 (s, 3H), 1.72 (s, 3H), 1.25 (s, 6H), 0.98 (s, 3H), 0.96 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 210.2, 167.9, 166.7, 133.6, 133.2, 130.1, 129.9, 128.6, 128.5, 128.4, 109.3, 101.3, 80.4, 74.4, 72.1, 69.9, 66.9, 63.8, 62.1, 55.6, 56.5, 53.8, 46.7, 41.6, 40.9, 39.5, 37.3, 36.5, 31.9–28.7, 26.4, 22.7, 21.3, 17.1, 16.4, 14.5, 14.1, 13.2; ESIMS: calcd for $[M + H]^+$ m/z 801.4; found: 801.2.

6.18. Synthesis of 6β-O-benzoyl chlorogenin 3β-O-(3,6-di-O-benzoyl-β-D-glucopyranoside) (**22c**)

Compound **21b** (104.1.1 mg, 0.094 mmol) was dissolved in dry CH_2Cl_2/MeOH (5 mL, v:v = 4:1), and $AcOH{\cdot}NH_2NH_2$ (176.7 mg, 1.92 mmol) was added. The solution was stirred at r.t. for 3 h. The mixture was diluted with CH_2Cl_2 (100 mL) and washed consecutively with water (10 mL), 1 mmol/L HCl aqueous (3 × 10 mL), saturated $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 1:2) to provide **22b** as syrup (62.5 mg, 73%): R_f = 0.2 (EtOAc/petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10–8.03 (m, 4H), 8.03–7.98 (d, 2H, J = 7.0 Hz), 7.61–7.53 (m, 3H), 7.48–7.40 (m, 6H), 5.19–5.13 (s, 2H), 4.72–4.62 (m, 2H), 4.55–4.50 (d, 1H, J = 7.8 Hz, H-1′), 4.42–4.36 (m, 1H), 3.78–3.67 (m, 2H), 3.67–3.59 (m, 2H), 3.48–3.43 (m, 1H), 3.40–3.37 (m, 2H), 2.47 (s,

1H), 1.17 (s, 3H), 0.80 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz): 167.9, 166.7, 166.3, 133.5, 133.3, 133.2, 132.9, 130.5, 130.0, 129.8, 129.6, 129.3, 128.4, 109.2, 101.3, 80.6, 79.2, 78.7, 78.5, 74.3, 74.0, 72.1, 69.8, 66.8, 63.8, 62.1, 55.9, 53.9, 46.4, 41.6, 40.6, 39.9, 38.1, 36.5, 35.6, 31.6, 31.4, 31.4, 30.7, 30.3, 29.4, 28.7, 20.8, 17.1, 16.6, 15.9, 14.5. ESI-HRMS calcd for $C_{54}H_{66}NaO_{12}$ $[M + Na]^+$ 929.4447, found 929.4446.

6.19. Synthesis of laxogenin 3β-O-[3,6-di-O-benzoyl-2,4-di-O-(2,3,4-tri-O-benzoyl -α-L-rhamnopyranosyl)-β-D-glucopyranoside] (**31b**)

To a mixture of **22b** (80.0 mg, 0.10 mmol) and 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (4.0 μL, 0.04 mmol). After stirred at −20 °C for 5 min donor **17** (309.5 mg, 0.5 mmol) was added. The mixture was stirred at −20 °C for 20 min and then at r.t. for 8 h. The reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 1:4) to give **31b** as an amorphous solid (51.5 mg, 30%): $R_f = 0.45$ (EtOAc/petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$ + D_2O, 400 MHz): δ 8.16–7.12 (m, 40H), 5.93–5.75 (m, 2H), 5.72–5.60 (m, 2H), 5.58–5.38 (m, 5H), 5.20 (m, 1H), 5.08 (m, 1H), 5.03–4.92 (m, 3H), 4.52–3.30 (m, 7H), 2.35–0.64 (m); ESI-HRMS calcd for $C_{101}H_{104}NaO_{25}$ $[M + Na]^+$ 1739.6759, found 1739.6759.

6.20. Synthesis of 6β-O-benzoyl chlorogenin 3β-O-[3,6-di-O-benzoyl-2,4-di-O-(2,3,4-tri-O-benzoyl -α-L-rhamnopyranosyl)-β-D-glucopyranoside] (**31c**)

To a mixture of **22b** (90.6 mg, 0.10 mmol) and 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at −20 °C was added TMSOTf (4.0 μL, 0.04 mmol). After stirred at −20 °C for 5 min donor **17** (309.5 mg, 0.5 mmol) was added. The mixture was stirred at −20 °C for 20 min and then at r.t. for 8 h. The reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 1:5) to give **31b** as an amorphous solid (51.0 mg, 28%): $R_f = 0.55$ (EtOAc/petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12–8.06 (m, 4H), 7.94–7.80 (m, 10H), 7.77–7.73 (m, 2H), 7.68–7.65 (m, 2H), 7.56–7.48 (m, 6H), 7.44–7.34 (m, 14H), 7.24–7.10 (m, 7H), 5.80–5.74 (m, 1H), 5.66 (t, 2H, J = 11.4 Hz), 5.56–5.40 (m, 3H), 5.35 (s, 1H), 5.18 (s, 1H), 5.05 (d like, 2H, J = 9.8 Hz), 4.99–4.95 (m, 1H), 4.82 (d, 1H, J = 7.4 Hz), 4.72–4.69 (m, 1H), 4.49–4.43 (m, 1H), 4.43–4.35 (m, 1H), 4.16–4.10 (m, 1H), 4.07–4.01(m, 2H), 3.94–3.88 (m, 1H), 3.82–3.76 (m, 1H), 3.49–3.44 (m, 1H), 3.39 (d, 1H, J = 11.7 Hz), 2.04–2.02 (m, 1H), 1.14 (s, 3H), 0.79 (s, 3H), 2.05–0.71 (m); ESI-HRMS calcd for $C_{108}H_{110}NaO_{26}$ $[M + Na]^+$ 1845.7178, found 1845.7178.

6.21. Synthesis of 6α-O-benzoyl chlorogenin 3β-O-(3-O-methyl-6-O-benzyl-β-D-glucopyranoside) (**41**)

To a mixture of **20a** (100.0 mg, 0.18 mmol), imidate **38** (120.0 mg, 0.22 mmol) and powdered 4 Å molecular sieves in dried CH_2Cl_2 (10 mL) at 0 °C was added TMSOTf (5 μL, 0.02 mmol). After stirred at 0 °C for 0.5 h and then at r.t. for 0.5 h, the reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether, 1:10) to give **39** as a white solid (162.0 mg, 86%).

Compound **39** was dissolved in $CH_3OH/CHCl_3$ (20 mL, v:v = 1:1), and then NaOMe was added until pH = 8.5. After stirred at r.t. for 4 h, the solution was neutralized with ion-exchange resin (H^+) and filtered and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 30:1) to afford **41** as a white amorphous solid (51.0 mg, 40%): $R_f = 0.48$ ($CHCl_3$/MeOH, 10:1); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.03 (d, 2H, J = 8.3 Hz, Ph), 7.56 (t, 1H, J = 7.7 Hz, Ph), 7.44 (t, 2H, J = 7.7 Hz, Ph), 7.35–7.28 (m, 5H, Ph), 4.96 (td, 1H, J = 11.0, 5.0 Hz, H-3), 4.60 (d, 1H, J = 12.1 Hz, PhCH*H*), 4.56 (d, 1H, J = 11.5 Hz, PhCH*H*), 4.37 (q, 1H, J = 7.1 Hz, H-16), 4.31 (d, 1H, J = 7.7 Hz, H-1′), 3.76 (dd, 1H, J = 9.9, 3.8 Hz, H-6a′), 3.76 (dd, 1H, J = 10.4, 5.5 Hz, H-6b′), 3.60 (s, 3H, OMe), 3.51 (t, 1H, J = 9.4 Hz, H-4′), 3.48–3.42 (m, 2H, H-26 and H-5′), 3.36 (td, 1H, J = 8.8, 2.8 Hz, H-2′), 3.11 (t, 1H, J = 8.8 Hz, H-3′), 0.96 (d, 3H, J = 7.1 Hz, 21-Me), 0.94 (s, 3H, 18-Me), 0.78 (s, 3H, 19-Me), 0.78 (d, 3H, J = 6.0 Hz, 27-Me); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.2, 137.8, 132.9, 130.5–127.7, 109.3, 109.2, 108.6, 101.3, 85.2, 80.6, 78.5, 74.0, 73.9, 73.6, 72.8, 71.5, 70.5, 66.8, 62.0, 60.5, 56.0, 53.7, 48.7, 41.5, 40.5, 39.9, 39.8, 39.5, 37.9, 37.1, 36.8, 33.7, 31.6, 31.3, 30.2, 29.2, 28.8, 20.9, 17.1, 16.4, 14.5, 13.4; ESIMS: calcd for $[M + H]^+$ m/z 803.5; found: 803.4.

6.22. Synthesis of 6α-O-benzoyl chlorogenin 3β-O-(6-O-methyl-β-D-glucopyranoside) (**42**)

To a mixture of **20a** (573.6 mg, 1.0 mmol), imidate **39** (832.9 mg, 1.28 mmol) and powdered 4 Å molecular sieves in dried CH_2Cl_2 (20 mL) at 0 °C was added TMSOTf (20.5 μL, 0.21 mmol). After stirring at 0 °C for 0.5 h and then at r.t. for 0.5 h, the reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated. The residue was dissolved in $CH_3OH/CHCl_3$ (60 mL, v:v = 1:1), and then NaOMe was added until pH = 8.5. After stirred at r.t. for 4 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 30:1) to afford **42** as a white amorphous solid (529.8 mg, 70% for two steps): $R_f = 0.47$ (CH_2Cl_2/MeOH = 15:1); ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.96 (d, 2H, J = 7.3 Hz, Ph), 7.66 (t, 1H, J = 6.9 Hz, Ph), 7.52 (t, 1H, J = 7.3 Hz, Ph), 4.97–4.83 (m, 3H, OH × 3), 4.29–4.28 (m, 1H), 4.15 (d, 1H, J = 7.3 Hz, H-1′), 3.55–3.32 (m, 6H), 3.23 (s, 3H, OMe), 3.02 (t, 1H, J = 8.8 Hz, H-4′), 2.95 (t, 1H, J = 9.2 Hz, H-3′), 2.84 (t, 1H, J = 7.8 Hz, H-2′), 1.99 (t, 1H, J = 6.4 Hz), 0.90 (d, 3H, J = 6.9 Hz, 21-Me), 0.88 (s, 3H, 18-Me), 0.74 (s, 3H, 19-Me), 0.70 (d, 3H, J = 6.4 Hz, 27-Me); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 165.5, 133.3, 130.1, 129.3, 129.0, 108.6, 101.5, 80.3, 77.1, 76.8, 75.5, 73.5, 72.7, 72.1, 70.1, 66.1, 62.0, 58.7, 55.4, 53.1, 48.2, 41.3, 40.4, 37.6, 36.7, 36.6, 35.5, 31.4, 31.0, 30.0, 29.7, 28.8, 28.7, 20.7, 17.2, 16.4, 14.8, 13.2; ESIMS: calcd for $[M + Na]^+$ m/z 735.4; found: 735.4.

6.23. Synthesis of 6α-O-benzoyl chlorogenin 3β-O-(3-O-benzoyl-6-O-methyl-β-D-glucopyranoside) (**43**)

To a mixture of **42** (466.0 mg, 0.70 mmol) and 1-BBTZ (470.8 mg, 2.0 mmol) in dried CH_2Cl_2 (20 mL) was added Et_3N (0.25 mL, 1.8 mmol). After stirred at r.t. overnight, the mixture was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether/$CHCl_3$, 1:8:2) to give **43** as a white solid (281.8 mg, 53%): R_f = 0.42 (EtOAc/petroleum ether, 1:2); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.06–7.55 (m, 10H, Ar–H), 5.11 (t, 1H, J = 9.2 Hz, H-3′), 4.96 (td, 1H, J = 11.0, 4.6 Hz, H-6), 4.45 (d, 1H, J = 7.7 Hz, H-1′), 4.37 (d, 1H, J = 6.8 Hz, H-16), 3.77 (t, 1H, J = 9.2 Hz, H-4′), 3.71–3.64 (m, 3H, H-26, H-6a′), 3.58 (dd, 1H, J = 9.1, 7.3 Hz, H-2′), 3.51–3.44 (m, 2H, H-5′, H-6b′), 3.36 (t, 1H, J = 10.5 Hz), 0.96 (d, 3H, J = 6.2 Hz, Me), 0.94 (s, 3H, Me), 0.77 (s, 3H, Me), 0.78 (d, 3H, J = 7.7 Hz, Me); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.3, 133.5, 132.9, 130.4, 130.1, 129.6, 128.4, 109.3, 100.7, 80.6, 78.4, 76.0, 74.0, 73.3, 72.9, 72.3, 70.9, 66.8, 62.0, 59.6, 56.0, 53.7, 48.7, 41.6, 40.6, 39.8, 37.9, 36.8, 33.7, 31.6, 31.3, 30.3, 29.0, 28.7, 20.9, 17.1, 16.4, 14.5, 13.4; ESIMS: calcd for $[M + Na]^+$ m/z 839.4; found: 839.6.

6.24. Plasmids and cell lines

HA (QH) gene is from a high pathogenic H5N1 influenza virus in goose (Goose/Qinghai/59/05). HA (QH) was cloned into pcDNA3 and

sequences were confirmed. A codon-optimized HA gene is from A/Viet Nam/1203/2004 (H5N1) and was cloned into pcDNA3. NA A/PR/8/34 influenza virus (H1N1) in the vector pEF6/V5-His-TOPO was kindly provided by John C Olsen (University of North Carolina, Chapel Hill).

A549 was purchased from ATCC, and 293T was from Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union College. Both cell lines were maintained in medium according to the protocols supplemented with 10% FBS and penicillin/streptomycin (25 units/ml).

6.25. Production of HA/HIV and VSVG/HIV pseudovirions

Human embryonic kidney 293T cells were transiently co-transfected with 8 μg hemagglutinin envelope expression plasmid and 5 μg NA or 3 μg VSVG envelope expression plasmid with 10 μg Env-deficient HIV vector (pNL4-3-Luc-R^-E^-) in 100-mm plates by a standard $Ca_3(PO_4)_2$ protocol. Sixteen hours post-transfection, cells were washed by PBS w/o Ca^{2+}, Mg^{2+}, then added 10 mL fresh medium into each plate. Forty-eight hours post-transfection, the supernatants were collected and filtered through a 0.45-micron pore size filter (Nalgene) and the pseudovirions were directly used for infection.

6.26. Infection assay of HIV pseudovirions

To investigate the effect of the test compounds, A549 cells were seeded into 24-well plates at 5×10^4/well and tested compounds were added to each well incubating for 15 min followed by adding HA/HIV or VSVG/HIV pseudovirions to each well and incubating at 37 °C. Cells were lysed in 50 μL of cell culture lysis reagent (Promega) 48 h post-infection. The luciferase activity was measured with a luciferase assay kit (Promega) and an FB15 luminometer (Berthold detection system) according to the supplier's protocols.

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