RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Discover of 3-*O*-β-chacotriosyl oleanane-type triterpenes as H5N1 Entry Inhibitors

Gaopeng Song,*[a,f] Xintian Shen,[b,f] Sumei Li,[c] Hongzong Si,[d] Yibin Li,[a] Haiye Luan,[e] Jihong Fan,[a] Qianqian Liang,[a] and Shuwen Liu[b]*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A series of 3-*O*-β-chacotriosyl oleanane-type triterpenes have been designed, synthesized and evaluated as H5N1 entry inhibitors based on a small molecule inhibitor saponin **1** previously discovered by us. Detailed structure-activity relationships (SARs) studies on the aglycone of compound **1** indicated that oleanane-type triterpenes with conserved structural features as aglycon favored the antiviral activity. These results suggested that introduction of bulky groups into 28-COOH of OA was helpful to significantly improve the selective index while keeping their antiviral activities, which was opposite to GA analogs. Compound **5** was selected for further mechanistic study because of its distinguished inhibition activity and good selective index. Molecular simulation analysis confirmed that compound **5** stabilized HA2 subunit of hemagglutinin (HA) by binding with amino acid residues THR-189, LYS-156, ARG-133A, therefore may prevent HA from conformational rearranging, which is a critical step for viral entry.

1. Introduction

The H5N1 avian influenza A virus is a serious health threat and a future pandemic risk, which has caused acute upper respiratory tract infections with high morbidity and mortality.[1] Currently, two classes of anti-influenza drugs developed for interruption of specific processes in influenza infection are neuraminidase inhibitors, like zanamivir and oseltamivir,[2] or inhibitors of the viral M2 protein, such as amantadine and rimantadine.[3] However, the emergence of drug-resistant influenza viruses has limited the use of those drugs,[4,5] making the identification of novel anti-influenza drugs an urgent task.

Pentacyclic triterpenes are secondary plant metabolites found in different plant organs, which display inhibitory activity against various viruses in vitro and in vivo.[6-8] It is suggested the defense activities of pentacyclic triterpenes stem from their ability to prevent various pathogen and herbivore infections in the host.[9] For example, oleanolic acid (OA), an oleanane-type triterpene, and betulinic acid (BA), a lupane-type triterpene have been confirmed to display inhibitory activity against HIV entry.[10-12] Notably, certain triterpenoic glycosides exhibit good anti-influenza virus activity, of which biological effects are attributed to the presence of the aglycon as well as of the sugar moiety.[13-15] Glycyrrhizic acid (GL), the most intensively investigated bioactive compound of licorice roots, is a glycosyl triterpenoic acid whose aglycon, glycyrrhetinic acid (GA), can be obtained easily from the extract of liquorice roots.[16] GL is well known for its broad activity against several viruses *in vitro* and *in vivo* including IAV.[13] It was proved that inhibition of virus penetration was proposed as a mechanism of action of GL against various viruses infection, which can lead to an inhibition of fusion pore formation and hence reduce infection of various viruses.[13] In addition, compound Y3, an OA-acetyl galactose conjugate, is a member of triterpenoic glycosides that has displayed strong anti-influenza virus activity *in vitro*.[15]

Influenza virus infection started from the attachment of viral particles to the host cell. This process is mediated by hemagglutinin (HA), which is a kind of viral envelope glycoproteins and binds to sialic acid receptor on host cell, leading to viral endocytosis.[17] Influenza virus entry refers to a potential target for discovering novel anti-influenza drugs because blocking the first step of influenza virus infection could result in an efficient inhibition of virus propagation. In our previous study, we have used an efficient HIV-based pseudotyping system to screen a saponin library generated from semisynthesis, and discovered three small molecules H5N1 viral entry inhibitors **1**-**3** (Fig. 1A), which show good inhibitory activity against H5N1 entry with the IC_{50} values being 7.2-12.0 μM.[18] The trisaccharide moiety of these inhibitory molecules, namely chacotrioside, has been the subject of several SAR studies dealing with alkylations,[19] removal or position change of some of its α-L-rhamnosyl residues,[18,19] and conjugation to triterpene aglycones,[18] which indicates the chacotriosyl residue is essential for activity and the subtle modifications of aglycone may be tolerated without losing antiviral activity.

Based on the above results, given that GL shows good antiviral activity against IAV entry, we replaced chlorogenin moiety of active compound **1** with GA to attempt the synthesis of several 3-*O*-β-chacotriosyl GA analogs **4**-**8**. Structurally, OA belongs to oleanane-type triterpenes as do GA with the C-29 COOH group shifted to C-28. Compound **9** was synthesized to investigate the effect of subtle difference of oleanane-type triterpenes as the aglycone residue on the inhibitory activity. In addition, different hydrocarbyl oleanolate were selected as the aglycone substitutes to derive saponins **10**-**13** (Fig. 1B), which provides a means to judge the effects of esterification of the 28-COOH group of OA

on the inhibitory activity.

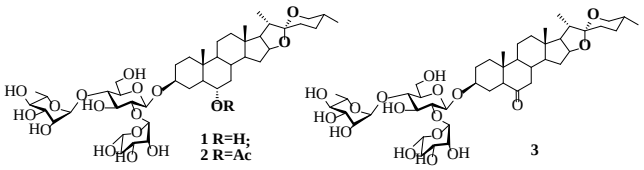


Fig. 1A Saponin inhibitors for H5N1 viral entry

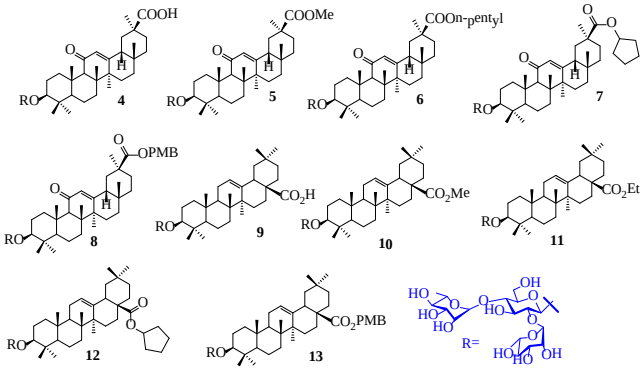
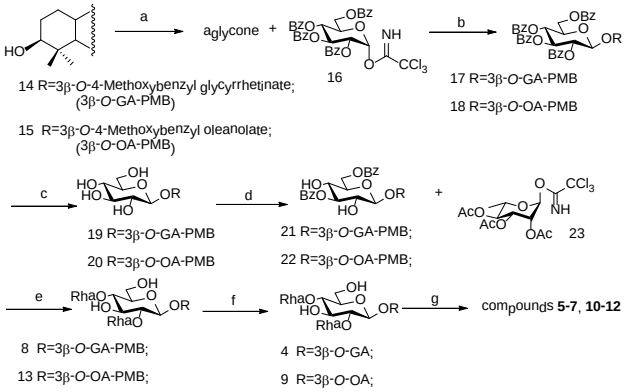


Fig. 1B Saponins designed for SARs studies

2. Results and discussion

2.1 Chemistry

As depicted in Scheme 1, treatment of glycyrrhetinic acid or oleanolic acid with 4-methoxybenzyl chloride in the presence of K_2CO_3 in DMF provided compound **14** or **15** in good yield, respectively. The activation of the anomeric center is mandatory for the glycoside synthesis. Ample examples show that the trichloroacetimidate group activated by the Lewis acid trifluoromethanesulfonate (TMSOTf) or boron fluoride ethyl ether ($BF_3 \cdot Et_2O$) becomes a suitable leaving group for the nucleophilic attack of the alcohol. Hence, 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranosyl trichloroacetimidate **16** was prepared via a three-step protocol.[18] Glycosylation of the intermediates **14**-**15** with trichloroacetimidate **16**, respectively, under the action of TMSOTf afforded the 3-*O*-β-glucopyranosides **17**-**18**, respectively. Removal of the benzoyl group was achieved using NaOMe in MeOH to yield compounds **19**-**20**, which were subject to 1-(benzoyloxy)-benzotriazole (1-BBTZ) to selective protection of the 3,6-OHs of the β-glucopyranosyl residues to obtain compounds **21**-**22**. Subsequent glycosylation of the 2, 4-OHs in **21**-**22** with 2,3,4-tri-*O*-acetyl-L-rhamnopyranosyl trichloroacetimidate **23**[18] under the "inverse addition conditions" with TMSOTf as the promoter, followed by deprotection of the acyl groups with MeONa in MeOH afforded saponins **8** and **13**, respectively. Debenzylation of compound **8** or **13** was carried out by hydrogenolysis using Pd/C to provide compound **4** or **9**. Finally, treatment of **4** or **9** with different halohydrocarbon in the presence of K_2CO_3 in DMF obtained the compounds **5**-**7** or **10**-**12**, respectively.



Scheme 1 Reagents and conditions: (a) PMBCl, K_2CO_3, DMF; (b) TMSOTf, CH_2Cl_2; (c) MeONa, CH_3OH; (d) 1-BBTZ, Et_3N, CH_2Cl_2; (e) (i) TMSOTf, CH_2Cl_2, (ii) MeONa, CH_3OH; (f) H_2, Pd/C, CH_3OH-CH_2Cl_2; (g) CH_3I, K_2CO_3, DMF or R-Br, K_2CO_3, DMF

2.2 Bioactivity

2.2.1 Inhibition of the infection of H5N1 pseudovirus

Previously, we have found that chlorogenin 3-*O*-β-chacotrioside (**1**) could inhibit the highly pathogenic H5N1 avian influenza A virus by blocking viral entry.[18] The inhibitory activity of compound **1** is specific to hemagglutinin, since it has no inhibitory activity toward VSV-G pseudovirus.[18]

Due to the safety concerns in studying viral H5N1 pathogens, the single-cycle pseudovirus was used instead of live H5N1 avian influenza virus to evaluate the inhibitory activities of compounds **4-13** against H5N1 entry. In this report, compounds **1-13** were evaluated for the inhibitory activity against the entry of H5N1 influenza virus based on an efficient HIV-based pseudotyping system with luciferase report element established by us,[20, 21] while the VSV-G/HIV pseudovirions were used as specificity controls. The obtained IC_{50} values are reported in Table 1 and indicate that all the compounds showed inhibitory activity against H5N1 pseudovirus with a potency ranging from moderate (IC_{50} >10 μM) to potent (IC_{50} <5 μM, whic h is comparable to the IC_{50} of a positive compound CL-385319 [21, 22]). Notably, the active compounds in series **5-6** and **10-13** display stronger inhibition activity than compound **1**, whereas weaker cytotoxicity against Madin Darby Canine Kidney (MDCK) cells than compound **1**.

Representative compounds **5** and **10** had no effect on VSV-G enveloped pseudovirus (Fig. 2A), which was similar to HA targeted compound CL-385319.[21, 22] These results demonstrate that these molecules did not inhibit HIV-backbone and luciferase reporting activity. In addition, compounds **5** and **10** at 20 μM did not inhibit neuraminidase (NA) (Fig. 2B), a glycoprotein enveloped in the pseudovirus system. Thus, these results revealed that compounds **4-13** might interfere with the entry of influenza virus by targeting hemagglutinin, the only other glycoprotein enveloped in the pseudovirus system.

Table 1. IC^{a}_{50} values (μM) of lead compounds **1-13** and **CL-385319** screened in MDCK cells infected with H5N1 pseudovirus and CC^{b}_{50} cytotoxicity values (μM) of lead compounds **1-13** against MDCK cells (data derived from the mean of three independent assays)

compound	IC^{a}_{50}	CC^{b}_{50}	SI[c]
1	7.72±0.11	12.02±0.32	1.7
2	9.16±0.23	16.85±0.24	1.8
3	11.62±0.55	17.22±0.30	1.5
4	22.08±0.25	35.80±0.53	1.6
5	3.35±0.44	33.83± 0.58	10.1
6	6.86±0.12	15.25±0.18	2.2
7	10.35±0.26	14.42±0.15	1.4
8	11.72±0.58	15.20±0.85	1.3
9	9.58±0.22	20.65±0.88	2.2
10	4.13±0.97	18.64±2.26	4.5
11	6.19±0.53	37.36±0.35	6.0
12	4.80±0.62	56.25±0.85	11.7
13	7.62±1.53	55.41±1.81	7.3
GA	>50	21.56±0.65	
Zanamivir	0.92±0.08	>100	>108.6
CL-385319	4.45±1.25	1480±10	332.3

[a] IC^{a}_{50} values (μM) of lead compounds **1-13** screened in MDCK cells infected with H5N1 pseudovirus (data derived from the mean of three independent assays);

CC^{b}_{50} cytotoxicity values (μM) of lead compounds **1-13** against MDCK cells (data derived from the mean of three independent assays)

[c] The selection index (SI^{c}) was CC^{b}_{50}/ IC^{a}_{50}

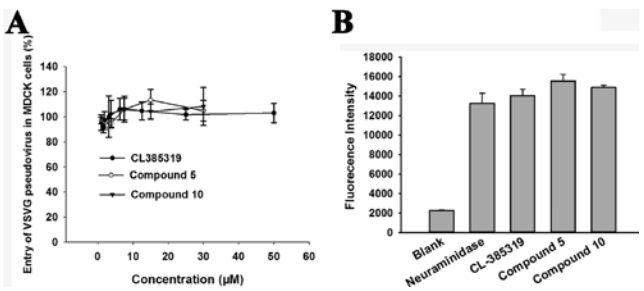


Fig.2 representative compounds did not inhibit VSVG pseudovirus and neuraminidase activity

2.2.2 Structure-Activity Relationship (SAR) analysis of compounds 4-13

As shown in Table 1, compounds **4-13** could inhibit infection by H5N1 influenza A virus in MDCK cells in varying extents (IC50 values 3.35-22.08 μM), some of which show inhibition activity *in vitro* comparable or higher than that of the positive compound CL-385319. Among them, compound **5** showed the most potent inhibition with an IC_{50} of 3.35 μM and a selection index (SI, CC_{50}/IC_{50}) of 10.1, respectively. Thus, compound **5** is exploitable as a new lead compound.

SAR exploration first focused on the oleanane-type triterpenes GA and OA as aglycone residue and their inhibition activities. Interestingly, compounds **5-6** and **10-12** showed stronger inhibitory activity than reported H5N1 entry inhibitor **1**, suggesting that replacement of the aglycone moiety of compound **1** to oleanane-type triterpenes with conserved structural features can enhance inhibition activity. Then we turned our attention to the influence of esterification of the COOH group with different carbon lengths and sizes on inhibition activity and cytotoxicity against MDCK cells. It was found that esterification of the COOH group of either GA or OA enhanced the inhibitory activity (**5** > **4**, **10** > **9**). We supposed that the hydroxyl group of COOH did not contribute to the interaction of aglycone residue with the receptor. The extent of the inhibitory activities of the compounds depending on the substitution of COOH group in the side chain can be ordered as follows: GA derivatives were in the order of **5** > **6** > **7** ≈ **8**; OA derivatives were in the order of **10** ≈ **12** > **11** > **13**. Replacement of the hydroxy at the 29-COOH position of GA with methyl group resulted in the most significant increases in potency while a dramatic loss of inhibition activity was observed when 29-COOH of GA was substituted by n-pentyl group or cyclopentyl group, indicating that the introduction of a bulky group maybe increase the steric hindrance and decrease binding with the receptor. However, when the 29-COOH of GA derivatives was substituted with a fatty alkyl group, the cytotoxicity against MDCK cells was significantly enhanced as the length of the carbon chain was increased (**8** ≈ **7** > **6** > **5**). Taken together these results suggested that when the 29-COOH group of GA was esterified, introduction of short straight alkyl groups was helpful to enhance inhibitory activity and decrease cytotoxicity, whereas introduction of bulky groups was not tolerated.

In contrast to GA derivatives, 28-COOH of OA was substituted by Et or PMB group (to give compound **11** or **12**) to lead to slightly reduced inhibition activity compared with compound **10**, suggesting that the inhibition activities of the OA derivatives were not affected significantly by the carbon length and size in the side chains of COOH group. This finding was different from modification of 29-COOH group in GA, which was presumably due to the obvious steric hindrance of 28-COOH group in OA. Compound **12**, an analog of compound **10**, had an improved selective index (4.5 to 11.7), indicating that introduction of a bulky group at the 28-COOH position of OA could significantly improve the safety of these compounds while keeping their antiviral activity.

2.2.3 Compound 5 does not block H5 HA adsorption to chicken RBCs

Compound **5** was selected for further evaluation and mechanistic study because of its distinguished inhibition activity and good selective index. The HA is able to absorb to chicken RBCs resulting in hemagglutination through the interaction of the receptor-binding domain in HA1 subunit with the sialic acid receptor in the RBC membrane. We investigated whether compound **5** can inhibit hemagglutination by interfering with the H5 HA adsorption to RBCs. It was found that compound **5** could not inhibit the adsorption of H5 HA to chicken RBCs even at the highest concentration of 20 μM, while the positive control antisera against H5 hemagglutinin (Anti-H5) could effectively inhibit H5 HA adsorption to chicken RBCs with a titer starting form 1:10 (Fig. 3). This result suggested that compound **5** may not target the receptor binding domain of the HA1.

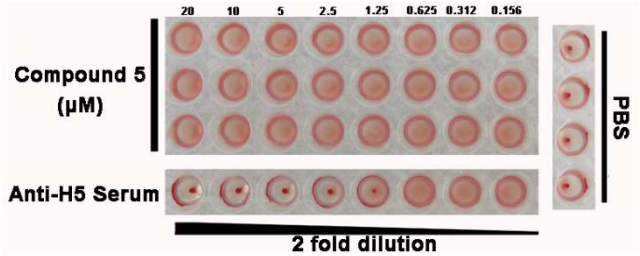


Fig. 3. Compound **5** did not inhibit hemagglutinination induced by H5 hemagglutinin

2.3 Molecular modeling for compound 5 binding to H5 hemagglutinin

We found that the HA2 sequence of A/Thailand/Kan353/2004 was 100% identical to that of A/Vietnam/1194/2004, of which the neutral-pH crystal structure of the HA has been well defined recently. On the basis of the results, we performed a docking study using Surflex-Dock program of SYBYL 7.3 in order to determine the mechanism and mode of interaction between compound **5** and the HA structure of A/Vietnam/ 1194/2004. The crystal structures of HA (1RVT) were extracted from Protein Data Bank. Compound **5** was docked within the active site of HA (1RVT) and the results showed that the compound **5** exhibited high binding affinity with the score of 4.267. Compound **5** could fit inside the same cavity in the stem region of the HA2 near the fusion peptide through hydrophobic contacts, Van der Waals interactions, and hydrogen bonding networks. As shown in Figure 4, at the top of cavity, compound **5** occupied the binding site with the C_2-OH of L-rhamnose moiety linked to C_2-OH of D-glucose forming stable hydrogen bonds with ARG-133A. Hydroxyl group at L-rhamnose moiety linked to C_4-OH of D-glucose and the carbonyl group at the C-29 position of GA also could form stable hydrogen bonds with LYS-156 and THR-189 at the bottom of cavity, respectively. As expected, the analysis of binding models also gave our preceding study support, suggesting that the chacotriosyl residue might play a very important role in the anti-H5N1 activity since altering the β-chacotriosyl moiety into α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl moiety or α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl moiety resulted in the loss of activity. [18] The results also show that compound **5** can be potential anti-H5N1 entry inhibitors, likely targeting to the HA2 protein, which is different from the other known entry inhibitors. [21, 22]

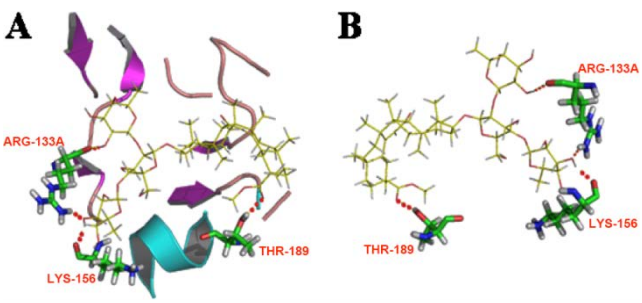


Fig. 4 Location of compound **5** docking site in the HA structure of the H5

2.4 Pharmacokinetic properties and bioavailability of compound 5

In this study, we have employed the ACD lab program to predict pharmacokinetic properties of compound **5**. As shown in Table 2, compound **5** showed no obvious carcinogenic toxicity and mutagenic toxicity. However, compound **5** exhibited poor gastrointestinal absorption and subsequent hepatic first-pass metabolism, which might be led to low oral bioavailability. The logarithm of a partition coefficient (log P) is a parameter which reflects a drug equilibrium partition ratio between polar (water) and non-polar (octanol) phases, and it has been proved to determine the absorption, the distribution, the biological availability, and pharmacological activity of drugs. Compound **5** exhibited high anhydrous solubility with the log P value being 3.32, suggesting that the water solubility of compound **5** should be further improved. In a recent study, Cao et al.[23] showed that propylene glycol-linked amino acid/dipeptide diester prodrugs of OA showed better stability, permeability, affinity, and bioavailability. In order to further increase bioavailability and optimize ADME properties of compound **5**, we next plan to design and synthesize a series of compound **5** analogs as prodrugs of 3-*O*-β-chacotriosyl GA, of which 29-COOH is modified with polar groups such as amino acid and sugars.

Table2 Predictive logP and toxicity of the lead compound **5**[a]

Compd	logP	Carcinogenic toxicity			Mutagenic toxicity		
		CP	CIP	Pred.	MP	MIP	Pred.
5	3.32	0.001	0.345	98%	0.01	0.39	98%

[a] LogP means octanol-water partition coefficient, which is equal to the logarithm of the ratio of concentrations of an unionized compound between octanol and water. For the carcinogenic toxicity, when the predicted value is more than 70%, and the value of CIP (Carcinogenic Impossibility) is more than 0.65, meanwhile its CIP value is greater than CP (Carcinogenic Possibility), the compound is considered as non-carcinogenic. Otherwise, they are considered to be carcinogenic. For the mutagenic toxicity, if the predictability value is more than 70% and its MIP (Mutagenic Impossibility) value is greater than the MP (Mutagenic Possibility), the compound is considered as nonmutagenic. Otherwise, they are considered to be mutagenic.

Conclusion

Based on our previously discovered small molecule inhibitor **1**, a series of 3-*O*-β-chacotriosyl oleanane-type triterpenes were designed and synthesized as H5N1 entry inhibitors, of which intensive SARs studies on the aglycone were conducted. Our results showed that oleanane-type triterpenes with conserved structural features as aglycon can improve inhibition activity. When the 29-COOH of GA was esterified, introduction of short straight saturated alkyl groups was helpful in enhancing inhibitory activity, but introduction of bulky groups should be avoided. Conversely, introduction of bulky groups into 28-COOH of OA kept antiviral activity and greatly decreased cytotoxicity against MDCK cells.

Molecular simulation analysis confirmed that compound **5** stabilized HA2 subunit of hemagglutinin (HA) by binding with amino acid residues THR-189, LYS-156, ARG-133A, therefore compound **5** may prevent HA from conformational rearranging

induced by acidification in cell endosome, which is a critical step for viral entry. Compound **5**, which can block entry of H5N1 avian influenza by interfering viral entry, can serve as a lead for optimization in order to design new compounds with improved potency and reduced toxicity.

3 Experimental protocols

3.1 General methods

Solvents were purified in a conventional manner. Thin layer chromatography (TLC) was performed on precoated E. Merck silica gel 60 F254 plates. Flash column chromatography was performed on silica gel (200-300 mesh, Qingdao, China). ^{1}H NMR and ^{13}C NMR spectra were taken on a JEOL JNM-ECP 600 spectrometer with tetramethylsilane as an internal standard, and chemical shifts are recorded in ppm values. Mass spectra were recorded on a Q-TOF Global mass spectrometer.

3.2 Synthesis

4-Methoxybenzyl 3β-hydroxy-11-oxo-olean-12-en-30-oate (14)

To a solution of GA (2.00 g, 4.25 mmol) in dry DMF (40 mL) was added potassium carbonate (1.18 g, 8.50 mmol). After 2 h of stirring at room temperature, 4-methoxybenzylchloride (0.41 mL, 6.57 mmol) was added and the mixture was stirred for an additional 3 h. The solvents were evaporated and the crude residue was dissolved in a mixture of CH_2Cl_2 (150 mL) and hydrochloric acid (60 mL, 1.0 M). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layer was washed with brine (200 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Recrystallization from EtOH provided the compound **14** (2.18 g, 90%) as a colourless solid; ^{1}H NMR ($CDCl_3$): δ 7.27 (d, 2H, J = 8.7 Hz, Ar-H), 6.87 (d, 2H, J = 8.6 Hz, Ar-H), 5.50 (s, 1H, H-12), 5.09 (d, 1H, J = 11.8 Hz, Ar-CH_2-1), 5.00 (d, 1H, J = 11.8 Hz, Ar-CH_2-2), 3.77 (s, 3H, OCH_3), 3.18 (dd, 1H, J = 11.5, 4.7 Hz, H-3), 2.74 (d, 1H, J = 11.6 Hz, H-1), 2.28 (s, 1H, H-9), 1.31, 1.07, 0.96, 0.77, 0.69 (each s, each 3H, CH_3), 1.10 (s, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$): δ 200.1 (C-11), 176.3 (C-30), 169.0 (C-13), 159.5, 130.0 (two), 128.4 (C-12), 128.3, 113.9 (two), 78.5, 65.9, 61.8, 55.3, 54.9, 48.1, 45.3, 43.9, 43.1, 41.0, 39.1, 37.6, 37.0, 32.7, 31.7, 31.1, 28.4, 28.2, 28.1, 27.2, 26.4 (two), 23.3, 18.6, 17.5, 16.4, 15.6; ESIMS calcd for $C_{38}H_{54}O_5Na$ 613.4; found 613.4.

4-Methoxybenzyl 3β-hydroxy-olean-12-en-28-oic acid (15)

Similarly, **15** was prepared as a white solid in 92% yield; ^{1}H NMR ($CDCl_3$): δ 7.28 (d, 2H, J = 8.3 Hz, Ar-H), 6.88 (d, 2H, J = 8.5 Hz, Ar-H), 5.28 (t, 1H, J = 3.7 Hz, H-12), 5.04 (d, 1H, J = 12.2 Hz, Ar-CH_2-1), 5.00 (d, 1H, J = 12.2 Hz, Ar-CH_2-2), 3.82 (s, 3H, OCH_3), 3.22 (dd, 1H, J = 11.0, 3.7 Hz, H-3), 2.90 (dd, 1H, J = 13.7, 3.7 Hz, H-18), 1.13, 0.99, 0.92, 0.90, 0.89, 0.79, 0.61 (each s, each 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 177.8 (C-28), 159.3, 143.7 (C-13), 129.8 (two), 128.4, 122.4 (C-12), 113.7 (two), 79.0, 65.8, 55.3, 55.2, 47.6, 46.7, 45.9, 41.7, 41.4, 39.3, 38.7, 38.5, 37.0, 33.9, 33.1, 32.7, 32.4, 30.7, 28.1, 27.6, 27.2, 25.8, 23.7, 23.4, 23.0, 18.3, 16.8, 15.6, 15.3; ESIMS calcd for $C_{38}H_{56}O_4Na$ 599.4; found 599.4.

4-Methoxybenzyl 3β-O-(2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl)-11-oxo-olean-12-en-30-oate (17)

To a solution of compound **14** (1.20 g, 2.03 mmol), 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranosyl trichloroacetimidate **16** (2.10 g, 2.85 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (50 mL) was added TMSOTf (36 μL, 0.20 mmol) at 0 °C under argon. The reaction mixture was stirred for 1 h and warmed to room temperature for 1 h. The reaction was quenched by Et_3N and concentrated. The residue was purified by silica gel column chromatography (petroleum ether-EtOAc-CH_2Cl_2, 6:1:1) to afford compound **17** (2.12 g, 89%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 6.91-8.03 (m, 24H, Ar-H), 5.92 (t, 1H, J = 9.6 Hz, H-3′), 5.60 (t, 1H, J = 9.5 Hz, H-4′), 5.58 (s, 1H, H-12), 5.57 (t-like, 1H, J = 9.5, 8.1 Hz, H-2′), 5.14 (d, 1H, J = 11.9 Hz, Ar-CH_2-1), 5.07 (d, 1H, J = 11.9 Hz, Ar-CH_2-2), 4.88 (d, 1H, J = 8.0 Hz, H-1′), 4.66 (dd, 1H, J = 11.9, 3.2 Hz, H-6′-1), 4.49 (dd, 1H, J = 11.9, 6.9 Hz, H-6′-2), 4.14-4.17 (m, 1H, H-5′), 3.83 (s, 3H, OCH_3), 3.15 (dd, 1H, J = 11.6, 4.7 Hz, H-3), 2.75 (dt, 1H, J = 13.4, 4.1 Hz, H-1), 2.22 (s, 1H, H-9), 1.31, 1.15, 1.10 1.08, 0.87, 0.73, 0.71 (each s, each 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 199.8 (C-11), 176.2 (C-30), 166.6, 165.9, 165.3, 165.0, 159.7, 133.4, 133.3, 133.2, 133.0, 130.1, 129.9, 129.8, 129.7, 129.6, 129.5, 128.9, 128.8, 128.5, 128.4 (two), 128.3 (two), 114.0, 103.1 (C-1′), 90.5, 73.0, 72.2, 72.0, 70.3, 66.0, 63.4, 61.7, 55.3, 55.2, 48.2, 45.3, 43.9, 43.1, 39.1, 39.0, 37.6, 36.8, 32.7, 31.7. 31.2, 28.4, 28.3, 27.6, 26.4 (two), 25.8, 23.3, 18.6, 17.3, 16.3, 16.2; ESIMS calcd for $C_{72}H_{80}O_{14}Na$ 1191.5; found 1191.5.

4-Methoxybenzyl 3β-O-(2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl)- olean-12-en-28-oic acid (18)

Similarly, **18** was prepared as a white solid in 90% yield; ^{1}H NMR ($CDCl_3$): δ 7.21-7.98 (m, 22H, Ar-H), 6.82 (d, 2H, J = 8.1 Hz, Ar-H), 5.87 (t, 1H, J = 9.4 Hz, H-3′), 5.52-5.54 (m, 2H, H-4′, H-2′), 5.23 (brs, 1H, H-12), 4.97 (d, 1H, J = 12.5 Hz, Ar-CH_2-1), 4.93 (d, 1H, J = 12.2 Hz, Ar-CH_2-2), 4.83 (d, 1H, J = 7.7 Hz, H-1′), 4.51-4.53 (m, 2H, H-6′), 4.10-4.13 (m, 1H, H-5′), 3.76 (s, 3H, OCH_3), 3.07 (dd, 1H, J = 11.0, 3.4 Hz, H-3), 2.84 (d, 1H, J = 13.7 Hz, H-18), 1.04, 0.88, 0.87, 0.76, 0.64, 0.58, 0.50 (each s, each 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 177.3 (C-28), 165.9, 165.8, 165.2, 165.0, 159.4, 143.7 (C-13), 133.5, 133.1, 129.8, 129.7, 129.6, 128.4, 128.3, 128.2, 122.3 (C-12), 113.7 (two), 103.2 (C-1′), 90.7, 73.0, 72.1, 71.9, 70.2, 65.7, 63.3, 55.4, 55.2, 47.5, 46.6, 45.9, 41.6, 41.3, 39.2, 38.7, 38.2, 36.6, 33.8, 33.1, 32.6, 32.3, 30.7, 29.6, 27.6, 27.5, 25.8, 23.6, 23.3, 22.9, 18.0, 16.8, 16.1, 15.1; ESIMS calcd for $C_{72}H_{82}O_{13}Na$ 1177.6; found 1177.6.

4-Methoxybenzyl 3β-O-D-glucopyranosyl-11-oxo-olean-12-en-30-oate (19)

Compound **17** (1.88 g, 1.63 mmol) was dissolved in CH_2Cl_2 and CH_3OH (V:V = 1:1) and then NaOMe was added until pH = 10. After stirred at r.t. for 6 h, the solution was neutralized with Dowex 50 × 8 (H^+) resin until pH = 7, filtered and concentrated. Then the residue was purified by silica gel column chromatography (CH_2Cl_2-MeOH, 10:1) to provide compound **19** (1.11 g, 93%); ^{1}H NMR ($CDCl_3$): δ 7.31 (d, 2H, J = 8.7 Hz, Ar-H), 6.90 (d, 2H, J = 8.7 Hz, Ar-H), 5.56 (s, 1H, H-12), 5.13 (d, 1H, J = 11.8 Hz, Ar-CH_2-1), 5.03 (d, 1H, J = 11.8 Hz, Ar-CH_2-2), 4.38 (d, 1H, J = 7.1 Hz, H-1′), 3.82-3.85 (m, 2H, H-6′), 3.81 (s, 3H, OCH_3), 3.63 (t, 1H, J = 9.3 Hz, H-4′), 3.55 (t, 1H, J = 9.0 Hz, H-3′), 3.43-3.45 (m, 1H, H-5′), 3.31 (d, 1H, J = 10.5 Hz, H-3), 3.19 (t-like, 1H, J = 8.8, 7.8 Hz, H-2′), 2.77-2.80 (m, 1H, H-1),

2.31 (s, 1H, H-9), 1.34, 1.15, 1.14, 1.05, 0.86, 0.73 (each s, each 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 200.0 (C-11), 176.2 (C-30), 169.0, 159.6, 130.0 (two), 128.5, 128.4, 114.1 (two), 105.1 (C-1′), 89.8, 76.4, 75.3, 73.9, 69.7, 66.0 (two), 61.8, 60.4, 55.3, 48.1, 45.4, 44.0, 43.1, 41.1, 39.5, 39.2, 37.6, 36.8, 32.8, 31.7, 31.1, 28.4, 28.2, 28.0, 26.5, 26.1, 23.4, 18.7, 17.4, 16.7, 16.7, 16.4; ESIMS calcd for $C_{44}H_{64}O_{10}Na$ 775.4; found 775.4.

4-Methoxybenzyl 3β-O-D-glucopyranosyl-olean-12-en-28-oic acid (20)

Similarly, **20** was prepared as a white solid in 92% yield; ^1H NMR ($CDCl_3$): δ 7.28 (d, 2H, J = 8.6 Hz, Ar-H), 6.88 (d, 2H, J = 8.7 Hz, Ar-H), 5.29 (brs, 1H, H-12), 5.05 (d, 1H, J = 12.1 Hz, Ar-CH_2-1), 4.98 (d, 1H, J = 12.1 Hz, Ar-CH_2-2), 4.38 (d, 1H, J = 7.0 Hz, H-1′), 4.19 (brs, 4H, 4 × OH), 4.12-4.15 (m, 1H, H-6′-1), 3.89-3.91 (m, 1H, H-6′-2), 3.82 (s, 3H, OCH_3), 3.65-3.72 (m, 2H, H-3′, H-4′), 3.44-3.48 (m, 1H, H-5′), 3.34 (brs, 1H, H-2′), 3.16 (d, 1H, J = 10.5 Hz, H-3), 2.90 (d, 1H, J = 16.6 Hz, H-18), 1.13, 0.99, 0.93, 0.91, 0.89, 0.81, 0.62 (each s, each 3H, CH_3);^{13}C NMR ($CDCl_3$): δ 177.4 (C-28), 159.4, 143.6 (C-13), 129.9 (two), 128.5, 122.4 (C-12), 113.8 (two), 104.7 (C-1′), 90.3, 76.1, 74.9, 73.9, 65.7, 61.5, 55.5, 55.2 (two), 47.6, 46.7, 45.9, 41.7, 41.4, 39.3, 38.5, 36.7, 33.9, 33.1, 32.8, 32.3, 30.7, 28.1, 27.6, 26.1, 25.9, 23.7, 23.5, 23.1, 18.2, 16.9, 16.7, 15.4; ESIMS calcd for $C_{44}H_{66}O_9Na$ 761.5; found 761.5.

4-Methoxybenzyl 3β-O-(3,6-Di-O-benzoyl-β-D-glucopyranosyl)-11-oxo-olean-12-en-30-oate (21)

To a mixture of **19** (1.08 g, 1.44 mmol) and 1-BBTZ (1.03 g, 4.31 mmol) in dried CH_2Cl_2 (100 mL) was added Et_3N (0.80 mL, 5.76 mmol). After stirred at r.t. for 24 h, the mixture was concentrated and purified by silica gel column chromatography (EtOAc/petroleum ether/CH_2Cl_2, 1:8:2) to provide **21** (1.01 g, 70%) as a white solid; ^1H NMR ($CDCl_3$): δ 8.07-8.11 (m, 4H, Ar-H), 7.60 (t, 2H, J = 8.2 Hz, Ar-H), 7.45-7.50 (m, 4H, Ar-H), 7.33 (d, 2H, J = 8.6 Hz, Ar-H), 6.93 (d, 2H, J = 8.6 Hz, Ar-H), 5.60 (s, 1H, H-12), 5.23 (t, 1H, J = 9.1 Hz, H-3′), 5.15 (d, 1H, J = 11.9 Hz, Ar-CH_2-1), 5.07 (d, 1H, J = 11.9 Hz, Ar-CH_2-2), 4.72 (dd, 1H, J = 11.2, 3.2 Hz, H-6′-1), 4.62 (dd, 1H, J = 11.2, 5.6 Hz, H-6′-2), 4.52 (d, 1H, J = 7.8 Hz, H-1′), 3.83 (s, 3H, OCH_3), 3.74-3.76 (m, 3H, H-4′, H-5′, H-2′), 3.22 (d, 1H, J = 11.8, 4.6 Hz, H-3), 2.75 (dt, 1H, J = 13.4, 3.1 Hz, H-3), 2.27 (s, 1H, H-9), 1.34, 1.16, 1.14, 1.11, 1.03, 0.86, 0.74 (each s, each 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 199.9 (C-11), 176.3 (C-30), 169.0, 167.7, 166.7, 159.5, 133.5, 133.3, 130.1 (two), 130.0 (two), 129.8 (two), 129.7, 129.4, 128.5 (two), 128.4 (two), 128.3, 114.0 (two), 104.8 (C-1′), 90.1, 78.6, 74.2, 72.8, 70.0, 66.0, 64.0, 61.7, 55.3, 55.2, 48.2, 45.3, 44.0, 43.1, 41.1, 39.3, 39.0, 37.6, 36.8, 32.7, 31.8, 31.2, 28.4, 28.3, 28.2, 26.5, 26.4, 25.9, 23.3, 18.7, 17.3, 16.6, 16.3; ESIMS calcd for $C_{58}H_{72}O_{12}Na$ 983.5; found 983.5.

4-Methoxybenzyl 3β-O-(3,6-Di-O-benzoyl-β-D-glucopyranosyl)-olean-12-en-28-oic acid (22)

Similarly, **22** was prepared as a white solid in 73% yield; ^1H NMR ($CDCl_3$): δ 8.08-8.11 (m, 4H, Ar-H), 7.60 (t, 1H, J = 7.3 Hz, Ar-H),7.59 (t, 1H, J = 7.1 Hz, Ar-H), 7.45-7.48 (m, 4H, Ar-H), 7.28 (d, 2H, J = 7.2 Hz, Ar-H), 6.88 (d, 2H, J = 8.6 Hz, Ar-H), 5.30 (t, 1H, J = 3.4 Hz, H-12), 5.22 (t, 1H, J = 9.1 Hz, H-3′), 5.04 (d, 1H, J = 12.2 Hz, Ar-CH_2-1), 4.99 (d, 1H, J = 12.2 Hz, Ar-CH_2-2), 4.68 (brs, 1H, H-6-1′), 4.67 (brs, 1H, H-6-2′), 4.50 (d, 1H, J = 7.7 Hz, H-1′), 3.82 (s, 3H, OCH_3), 3.75-3.78 (m, 3H, H-5′, H-4′, H-2′), 3.17 (dd, 1H, J = 11.8, 4.4 Hz, H-3), 2.91 (dd, 1H, J = 13.7, 4.1 Hz, H-18), 1.12, 1.00, 0.94, 0.92, 0.86, 0.82, 0.59 (each s, each 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 177.5 (C-28), 167.8, 166.7, 159.4, 143.8 (C-13), 133.5, 133.2, 130.0, 129.9, 129.8 (two), 129.3, 128.6, 128.5, 128.4, 122.4 (C-12), 113.8, 104.9 (C-1′), 90.3, 78.7, 74.2, 72.7, 70.1, 65.7, 63.9, 55.5, 55.3, 47.6, 46.7, 45.9, 41.7, 41.4, 39.3, 38.9, 38.3, 36.6, 33.9, 33.1, 32.7, 32.3, 30.7, 28.2, 27.6, 25.9, 25.8, 23.7, 23.4, 23.0, 18.1, 16.9, 16.6, 15.2; ESIMS calcd for $C_{58}H_{74}O_{11}Na$ 969.5; found 969.5.

4-Methoxybenzyl 3β-O-[2,4-Di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-11-oxo-olean-12-en-30-oate (8)

To a mixture of **21** (0.48 g, 0.51 mmol) and 4 Å molecular sieves in dried CH_2Cl_2 (20 mL) at - 30 °C under argon was added TMSOTf (18 μL, 0.10 mmol), followed by a solution of **23** (0.88 g, 2.04 mmol) in CH_2Cl_2 (5 mL). After stirring at - 30 °C for 1 h and then at r.t. for 1 h, the reaction was quenched with Et3N. The solid was filtered, and the filtrate was concentrated under vacuum to give yellow oil. The oil was subjected to column chromatography on silica gel (EtOAc-petroleum ether, 1:3) to give the desired crude trisaccharide. The above trisaccharide mixture was dissolved in CH_2Cl_2 and CH_3OH (V:V = 1:1) and then NaOMe was added until pH = 10. After stirred at r.t. for 12 h, the solution was neutralized with Dowex 50 × 8 (H^+) resin until pH = 7, filtered and concentrated. Then the residue was purified by silica gel column chromatography (CH_2Cl_2-MeOH, 6:1) to provide compound **8** (0.36 g, 68%) as a white solid; ^1H NMR (CD_3OD): δ 7.60 (d, 2H, J = 8.6 Hz, Ar-H), 6.94 (d, 2H, J = 8.6 Hz, Ar-H), 5.44 (s, 1H, H-12), 5.38 (s, 1H, Rha-1-H), 5.20 (d, 1H, J = 11.8 Hz, Ar-CH_2-1), 5.03 (d, 1H, J = 11.8 Hz, Ar-CH_2-2), 4.59 (s, 1H, Rha-1-H), 4.44 (d, 1H, J = 7.7 Hz, H-1′), 3.98-4.00 (m, 2H), 3.91-3.92 (m, 1H), 3.85 (dd, 1H, J = 3.0, 1.8 Hz, Rha-H-2), 3.81 (dd, 1H, J = 12.0, 1.8 Hz, H-6′-1), 3.81 (s, 3H, OCH_3), 3.75 (dd, 1H, J = 9.5, 3.2 Hz, Rha-H-3), 3.68 (dd, 1H, J = 12.0, 4.0 Hz, H-6′-2), 3.63 (dd, 1H, J = 9.4, 3.3 Hz, Rha-H-3), 3.60 (t, 1H, J = 8.8 Hz), 3.55 (t, 1H, J = 9.2 Hz), 3.46 (t, 1H, J = 8.3 Hz), 3.36-3.45 (m, 3H), 3.20 (dd, 1H, J = 11.8, 4.3 Hz, H-3), 2.71 (td, 1H, J = 13.3, 4.1 Hz, H-1), 2.42 (s, 1H, H-9), 2.11 (td, 1H, J = 13.6, 4.1 Hz, H-18), 1.39, 1.15, 1.14, 1.13, 1.08, 0.89, 0.71 (each s, each 3H, CH_3), 1.28 (d, 3H, J = 6.2 Hz, Rha-H-6), 1.23 (d, 3H, J = 6.2 Hz, Rha-H-6); ^{13}C NMR (CD_3OD): δ 201.1 (C-11), 176.5 (C-30), 171.0, 159.9, 130.1 (two), 128.4, 127.5, 113.6 (two), 104.1 (C-1′), 101.7 (Rha-C-1), 100.6 (Rha-C-1), 88.7, 79.1, 78.1, 77.9, 76.8, 75.1, 72.6, 72.3, 71.0, 70.7, 70.6, 69.4, 68.6, 65.7, 61.8, 60.6, 55.3, 54.4, 48.2, 45.3, 43.7, 43.2, 41.0, 39.2 (two), 37.4, 36.6, 32.4, 31.4, 30.7, 27.6, 27.0 (two), 26.1, 25.9, 25.8, 22.4, 17.9, 17.1, 17.0, 16.6, 16.5, 15.8, 15.7; HRESIMS calcd for $C_{56}H_{84}O_{18}Na$ 1067.5555; found 1067.5551.

4-Methoxybenzyl 3β-O-[2,4-Di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl] -olean-12-en-28-oic acid (13)

Similarly, **13** was prepared as a white solid in 56% yield; ^1H NMR (CD_3OD): δ 7.28 (d, 2H, J = 8.6 Hz, Ar-H), 6.90 (d, 2H, J = 8.6 Hz, Ar-H), 5.37 (d, 1H, J = 1.1 Hz, Rha-H-1), 5.18 (t, 1H, J = 3.0 Hz, H-12), 5.03 (d, 1H, J = 11.9 Hz, Ar-CH_2-1), 4.92 (d, 1H, J = 11.8 Hz, Ar-CH_2-2), 4.85 (d, 1H, J = 1.1 Hz, Rha-H-1),

4.44 (d, 1H, *J* = 7.7 Hz, H-1′), 3.98-4.00 (m, 1H), 3.98 (dd, 1H, *J* = 3.4, 1.9 Hz, Rha-H-2), 3.88-3.92 (m, 1H), 3.84 (dd, 1H, *J* = 3.0, 1.9 Hz, Rha-H-2), 3.82 (dd, 1H, *J* = 11.9, 1.9 Hz, H-6-1′), 3.80 (s, 3H, OCH_3), 3.76 (dd, 1H, *J* = 9.5, 3.2 Hz, Rha-H-3), 3.67 (dd, 1H, *J* = 11.9, 4.0 Hz, H-6-2′), 3.64 (dd, 1H, *J* = 9.5, 3.3 Hz, Rha-H-3), 3.60 (t, 1H, *J* = 8.6 Hz), 3.55 (t, 1H, *J* = 9.2 Hz), 3.46 (t, 1H, *J* = 8.1 Hz), 3.42 (t, 1H, *J* = 9.5 Hz), 3.40 (t, 1H, *J* = 9.5 Hz), 3.17 (dd, 1H, *J* = 11.5, 4.3 Hz, H-3), 2.24 (d, 1H, *J* = 11.3 Hz), 1.28 (d, 3H, *J* = 6.2 Hz, Rha-H-6), 1.23 (d, 3H, *J* = 6.2 Hz, Rha-H-6), 1.09, 1.05, 0.97, 0.93, 0.88, 0.87, 0.57 (each s, each 3H, CH_3); ^{13}C NMR (CD_3OD): δ 177.8 (C-28), 159.8, 143.4 (C-13), 129.9 (two), 128.3, 122.5 (C-12), 113.5 (two), 104.1 (C-1′), 101.7 (Rha-1-C), 100.6 (Rha-1-C), 89.0, 79.1, 78.0, 77.8, 76.8, 75.0, 72.6, 72.3, 71.0, 70.7, 70.6, 69.4, 68.6, 65.6, 60.6, 55.9, 54.3, 48.2, 46.6, 45.7, 41.4 (two), 39.2, 38.8, 38.7, 36.4, 33.4, 32.6, 32.2, 32.1, 30.2, 27.2, 27.1, 25.8, 24.9, 23.1, 22.6, 22.5, 17.9, 16.6, 16.5, 16.3, 15.8, 14.6; HRESIMS calcd for $C_{56}H_{86}O_{17}Na$ 1053.5758; found 1053.5757.

3β-O-[2,4-Di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-11-oxo-olean-12-en-30-oic acid (4)

Compound **8** (200.8 mg, 0.19 mol) was dissolved in CH_2Cl_2 (5 mL) and MeOH (5 mL) and then 10%-Pd/C (80 mg) was added. After stirred for 8 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 (CH_2Cl_2-MeOH, 5:1) to give **4** (168.5 mg, 95%) as a white solid; ^{1}H NMR (DMSO-d6): δ 5.39 (s, 1H, H-12), 5.16 (s, 1H, Rha-1-H), 4.65 (s, 1H, Rha-1-H), 4.26 (d, 1H, *J* = 7.6 Hz, H-1′), 3.75-3.79 (m, 2H), 3.74 (dd, 1H, *J* = 3.1, 1.8 Hz, Rha-H-2), 3.62 (dd, 1H, *J* = 3.2, 1.8 Hz, Rha-H-2), 3.56 (dd, 1H, *J* = 11.1, 1.7 Hz, H-6′-1), 3.48 (dd, 1H, *J* = 9.4, 3.3 Hz, Rha-H-3), 3.42 (dd, 1H, *J* = 9.2, 3.2 Hz, Rha-H-3), 3.33-3.40 (m, 4H), 3.26 (t-like, *J* = 8.1, 7.8 Hz), 3.20 (t, 1H, *J* = 9.3 Hz), 3.19 (t, 1H, *J* = 9.3 Hz), 3.14-3.15 (m, 1H, H-3), 3.02-3.05 (m, 1H, H-1), 2.29 (s, 1H, H-9), 1.39, 1.05, 0.98, 0.97, 0.92, 0.71, 0.70 (each s, each 3H, CH_3), 1.09 (d, 3H, *J* = 6.2 Hz, Rha-H-6), 1.04 (d, 3H, *J* = 6.2 Hz, Rha-H-6); ^{13}C NMR (DMSO-d6): δ 177.1 (C-30), 143.4 (C-13), 122.5 (C-12), 104.1 (C-1′), 100.9 (Rha-C-1), 100.5 (Rha-C-1), 88.5, 77.6, 77.5, 76.6, 75.5, 72.3, 72.2, 70.9, 70.7, 70.6, 70.5, 69.2, 68.6, 65.7, 61.8, 60.6, 55.7, 47.5, 46.5, 41.6, 41.4, 36.6, 33.1, 30.7, 27.8, 26.0, 23.7, 23.3, 23.0, 18.2 (two), 17.0, 16.6, 15.6, 15.5; HRESIMS calcd for $C_{48}H_{76}O_{17}Na$ 947.4975; found 947.4978.

3β-O-[2,4-Di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-olean-12-en-28-oic acid (9)

Similarly, **9** was prepared as a white solid in 46% yield; ^{1}H NMR (CD_3OD): δ 5.38 (s, 1H, Rha-H-1), 5.25 (t, 1H, *J* = 3.0 Hz, H-12), 4.86 (s, 1H, Rha-H-1), 4.44 (d, 1H, *J* = 7.7 Hz, H-1′), 3.97-4.01 (m, 1H), 3.98 (dd, 1H, *J* = 3.2, 1.8 Hz, Rha-H-2), 3.91-3.93 (m, 1H), 3.85 (dd, 1H, *J* = 3.2, 1.9 Hz, Rha-H-2), 3.81 (d, 1H, *J* = 10.8 Hz, H-6-1′), 3.76 (dd, 1H, *J* = 9.5, 3.3 Hz, Rha-H-3), 3.68 (dd, 1H, *J* = 12.0, 4.0 Hz, H-6-2′), 3.64 (dd, 1H, *J* = 9.4, 3.2 Hz, Rha-H-3), 3.60 (t, 1H, *J* = 8.7 Hz), 3.55 (t, 1H, *J* = 8.9 Hz), 3.46 (t, 1H, *J* = 8.2 Hz), 3.42 (t, 1H, *J* = 9.4 Hz), 3.39 (t, 1H, *J* = 9.5 Hz), 3.18 (dd, 1H, *J* = 11.6, 4.1 Hz, H-3), 2.86 (dd, 1H, *J* = 13.9, 3.7 Hz), 1.28 (d, 3H, *J* = 6.2 Hz, Rha-H-6), 1.22 (d, 3H, *J* = 6.2 Hz, Rha-H-6), 1.17, 1.06, 0.96, 0.95, 0.92, 0.87, 0.83 (each s, each 3H, CH_3); ^{13}C NMR (CD_3OD): δ 180.8 (C-28), 143.8 (C-13), 122.2 (C-12), 104.0 (C-1′), 101.6 (Rha-1-C), 100.6 (Rha-1-C), 89.0, 79.0, 77.8, 76.7, 75.0, 72.5, 72.3, 71.0, 70.7, 70.6, 69.4, 68.6, 60.6, 55.9, 46.3, 45.9, 41.5, 41.3, 39.2, 38.8, 38.7, 36.5, 33.5, 32.6, 32.4, 32.2, 30.2, 27.4, 27.1, 25.8, 25.0, 23.1, 22.7, 22.6, 17.9, 16.6, 16.5, 16.3, 15.8, 14.6; HRESIMS calcd for $C_{48}H_{78}O_{16}Na$ 933.5182; found 933.5184.

General procedure for the preparation of 5-7 and 10 -12

A mixture of compound **4** or **9** (1 mmol) and K_2CO_3 (2 mmol) in DMF (10 mL) was stirred at room temperature for 4 h. The iodomethane (6 mmol) or bromoalkane (3 mmol) was then dripped slowly into the mixture. After being stirred for another 10 h, the reaction mixture was poured into the 40 mL distilled water and partitioned with butyl alcohol (3 × 80 mL). The organic layer was washed with saturated sodium chloride, dried over Na_2SO_4, and purified via silica gel column chromatography (MeOH/CH_2Cl_2, 1:5) to provide **5-7** and **10 -12** as white solids, respectively.

Methyl 3β-O-[2,4-Di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-11-oxo-olean-12-en-30-oate (5)

Compound **5** was synthesized as a white solid in 95% yield; ^{1}H NMR (CD_3OD): δ 5.58 (s, 1H, H-12), 5.38 (d, 1H, *J* = 1.3 Hz, Rha-H-1), 4.60 (brs, 1H, Rha-H-1), 4.44 (d, 1H, *J* = 7.7 Hz, H-1′), 3.97-4.00 (m, 2H), 3.91-3.93 (m, 1H), 3.85 (dd, 1H, *J* = 3.2, 1.7 Hz, Rha-H-2), 3.81 (dd, 1H, *J* = 12.0, 1.5 Hz, H-6′-1), 3.76 (dd, 1H, *J* = 9.5, 3.3 Hz, Rha-H-3), 3.67 (dd, 1H, *J* = 12.1, 4.1 Hz, H-6′-2), 3.63 (dd, 1H, *J* = 9.4, 3.2 Hz, Rha-H-3), 3.61 (s, 3H, OCH_3), 3.60 (t, 1H, *J* = 8.9 Hz), 3.55 (t, 1H, *J* = 8.9 Hz), 3.38-3.48 (m, 2H), 3.21 (dd, 1H, *J* = 11.8, 4.3 Hz, H-3), 3.09-3.13 (m, 1H, H-5′), 2.72 (dt, 1H, *J* = 13.5, 2.8 Hz, H-1), 2.46 (s, 1H, H-19), 1.28 (d, 3H, *J* = 6.2 Hz, Rha-H-6), 1.25 (d, 3H, *J* = 6.2 Hz, Rha-H-6), 1.43, 1.16, 1.15, 1.14, 1.08, 0.90, 0.83 (each s, each 3H, CH_3), 0.99 (d, 3H, *J* = 6.4 Hz, CH_3), 0.91 (d, 3H, *J* = 6.4 Hz, CH_3); ^{13}C NMR (CD_3OD): δ 201.2 (C-11), 171.2 (C-30), 171.1 (C-13), 121.5 (C-12), 104.1 (C-1′), 101.7 (Rha-C-1), 100.6 (Rha-C-1), 88.1, 79.1, 78.1, 77.9, 76.8, 75.1, 72.6, 72.3, 71.0, 70.7, 70.6, 69.4, 68.7, 61.8, 60.6, 55.3, 50.9, 48.5, 45.3, 43.9, 43.2, 41.0, 39.2, 39.1, 37.6, 36.7, 32.4, 31.5, 30.6, 27.7, 27.1, 27.0, 26.1, 25.9, 25.8, 22.4, 17.9, 17.0, 16.6, 16.5, 15.7, 15.6; HRESIMS calcd for $C_{49}H_{78}O_{17}Na$ 961.5131; found 961.5138.

Pentyl 3β-O-[2,4-Di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-11-oxo-olean-12-en-30-oate (6)

Compound **6** was synthesized as a white solid in 94% yield; ^{1}H NMR (CD_3OD): δ 5.38 (s, 1H, H-12), 5.14 (s, 1H, Rha-H-1), 4.66 (s, 1H, Rha-H-1), 4.25 (d, 1H, *J* = 7.9 Hz, H-1′), 3.79-3.96 (m, 2H), 3.75 (dd, 1H, *J* = 3.3, 1.7 Hz, Rha-H-2), 3.62 (dd, 1H, *J* = 3.2, 1.8 Hz, Rha-H-2), 3.57 (dd, 1H, *J* = 11.0, 5.2 Hz, H-6′-1), 3.41-3.48 (m, 3H), 3.35-3.38 (m, 2H), 3.25 (t, 1H, *J* = 8.9 Hz), 3.17-3.21 (m, 3H), 3.14-3.15 (m, 1H, H-3), 3.01-3.03 (m, 1H, H-1), 2.26 (s, 1H, H-9), 1.28, 1.25, 1.04, 0.97, 0.91, 0.71, 0.68 (each s, each 3H, CH_3), 1.09 (d, 3H, *J* = 6.2 Hz, Rha-H-6), 1.05 (d, 3H, *J* = 6.2 Hz, Rha-H-6), 0.81 (t, 3H, *J* = 6.5 Hz, C*H*$_2$CH$_3$); ^{13}C NMR (CD_3OD): δ 199.9 (C-11), 176.6 (C-30), 167.0 (C-13), 127.6 (C-12), 104.0 (C-1′), 100.9 (Rha-C-1), 100.5 (Rha-C-1), 88.4, 77.8, 77.5, 76.4, 75.3, 72.1 (two), 70.9, 70.8, 70.6, 70.5, 70.4, 69.2, 68.7, 61.6, 60.4, 55.0, 48.5, 45.3, 44.0, 43.3, 36.7, 31.8, 28.6, 28.3, 28.2, 28.1, 27.6, 23.3, 22.1, 18.7, 18.0 (two), 16.6, 16.5, 14.2; HRESIMS calcd for $C_{53}H_{86}O_{17}Na$ 1017.5757;

found 1017.5754.

Cyclopentyl 3β-O-[2,4-Di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-11-oxo-olean-12-en-30-oate (7)

Compound **7** was synthesized as a white solid in 91% yield; ^{1}H NMR (CD_3OD): δ 5.56 (s, 1H, Rha-H-1), 5.38 (s, 1H, Rha-H-1), 5.20 (s, 1H, H-12), 4.45 (d, 1H, J = 7.8 Hz, H-1′), 3.97-4.00 (m, 2H), 3.91-3.93 (m, 1H), 3.85 (dd, 1H, J = 3.1, 1.8 Hz, Rha-H-2), 3.81 (dd, 1H, J = 12.0, 1.8 Hz, H-6′-1), 3.76 (dd, 1H, J = 9.3, 3.2 Hz, Rha-H-3), 3.68-3.71 (m, 1H, OCH), 3.66-3.68 (m, 1H, H-6′-2), 3.64 (dd, 1H, J = 9.4, 3.2 Hz, Rha-H-3), 3.52-3.60 (m, 2H), 3.46 (t, 1H, J = 7.9 Hz), 3.39-3.45 (m, 2H), 3.23 (dd, 1H, J = 14.6, 7.4 Hz, H-3), 2.72 (d, 1H, J = 14.0 Hz, H-1), 2.46 (s, 1H, H-9), 2.34 (d, 1H, J = 13.6 Hz, H-18), 1.40, 1.16, 1.15, 1.14, 1.08, 0.90, 0.84 (each s, each 3H, CH_3), 1.28 (d, 3H, J = 6.2 Hz, Rha-H-6), 1.23 (d, 3H, J = 6.2 Hz, Rha-H-6); ^{13}C NMR (CD_3OD): δ 209.1 (C-11), 176.5 (C-30), 152.0 (C-13), 127.5 (C-12), 103.9 (C-1′), 101.7 (Rha-C-1), 100.6 (Rha-C-1), 88.6, 79.0, 77.9, 77.2, 76.8, 75.1, 72.5, 72.4, 72.3, 71.0, 70.7, 70.6, 69.4, 68.6, 63.0, 61.8, 60.6, 55.3, 48.6, 46.5, 45.4, 43.6, 43.2, 40.9, 39.2, 36.7, 32.3, 32.2, 31.7, 31.5, 29.4, 29.3, 28.7, 28.8, 28.4, 27.8, 27.1, 27.0, 26.1, 25.9, 25.8, 22.4, 22.3, 17.9, 16.6, 16.5, 15.7, 15.6; HRESIMS calcd for $C_{53}H_{84}O_{17}Na$ 1015.5601; found 1015.5600.

Methyl 3β-O-[2,4-Di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-olean-12-en-28-oic acid (10)

Compound **10** [18] was synthesized as a white solid in 92% yield; ^{1}H NMR (CD_3OD): δ 5.36 (d, 1H, J = 1.9 Hz, Rha-H-1), 5.25 (t, 1H, J = 3.2 Hz, H-12), 4.84 (d, 1H, J = 1.8 Hz, Rha-H-1), 4.42 (d, 1H, J = 7.8 Hz, H-1′), 3.96-3.98 (m, 2H), 3.89-3.92 (m, 1H), 3.82 (dd, 1H, J = 3.3, 1.9 Hz, Rha-H-2), 3.79 (dd, 1H, J = 11.9, 1.9 Hz, H-6′-1), 3.75 (dd, 1H, J = 9.5, 3.3 Hz, Rha-H-3), 3.66 (dd, 1H, J = 11.9, 4.1 Hz, H-6′-2), 3.62 (dd, 1H, J = 9.5, 3.3 Hz, Rha-H-3), 3.60 (s, 3H, CH_3), 3.58 (t, 1H, J = 8.7 Hz), 3.54 (t, 1H, J = 9.7 Hz), 3.44 (t, 1H, J = 7.7 Hz), 3.40 (t, 1H, J = 9.7 Hz), 3.38 (t, 1H, J = 9.6 Hz), 3.16 (dd, 1H, J = 11.9, 4.1 Hz, H-3), 2.86-2.88 (m, 1H), 1.26 (d, 3H, J = 6.4 Hz, CH_3), 1.20 (d, 3H, J = 6.4 Hz, CH_3), 1.15, 1.05, 0.95, 0.93, 0.85, 0.74 (each s, each 3H, CH_3); HRESIMS calcd for $C_{49}H_{80}O_{16}Na$ 947.5344; found 947.5381.

Ethyl 3β-O-[2,4-Di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-olean-12-en-28-oic acid (11)

Compound **11** was synthesized as a white solid in 93% yield; ^{1}H NMR (DMSO-d6): δ 5.20 (s, 1H, Rha-H-1), 5.18 (brs, 1H, H-12), 4.72 (s, 1H, Rha-H-1), 4.29 (d, 1H, J = 7.9 Hz, H-1′), 4.00-4.04 (m, 2H), 3.84-3.93 (m, 3H), 3.79 (dd, 1H, J = 3.2, 1.8 Hz, Rha-H-2), 3.66 (dd, 1H, J = 3.3, 1.7 Hz, Rha-H-2), 3.61 (d, 1H, J = 11.6 Hz, H-6′-1), 3.53 (dd, 1H, J = 9.5, 3.4 Hz, Rha-H-3), 3.46 (dd, 1H, J = 12.0, 4.1 Hz, H-6′-2), 3.44 (dd, 1H, J = 9.4, 2.9 Hz, Rha-H-3), 3.41 (t, 1H, J = 8.7 Hz), 3.30 (t, 1H, J = 7.9 Hz), 3.23 (t, 1H, J = 9.4 Hz), 3.22 (t, 1H, J = 9.2 Hz), 3.16-3.17 (m, 1H), 3.02 (d, 1H, J = 10.8 Hz, H-3), 2.77 (dd, 1H, J = 13.3, 7.6 Hz, H-18), 1.15 (q, 2H, J = 7.1 Hz, CH_2CH$_3$), 1.14 (d, 3H, J = 6.2 Hz, Rha-H-6), 1.08 (d, 3H, J = 6.2 Hz, Rha-H-6), 1.09, 0.95, 0.89, 0.88, 0.87, 0.75, 0.66 (each s, each 3H, CH_3); ^{13}C NMR (DMSO-d6): δ 177.4 (C-28), 143.9 (C-13), 122.2 (C-12), 104.2 (C-1′), 100.9 (Rha-C-1), 100.6 (Rha-C-1), 88.7, 77.7, 77.3, 76.5, 75.4, 72.2, 72.1, 70.9, 70.6 (two), 70.4, 69.2, 68.7, 60.3, 55.8, 47.5, 46.9, 46.0, 41.6, 41.3, 36.7, 36.6, 33.6, 33.1, 32.8, 32.4, 30.7, 27.8, 27.5, 26.1, 23.7, 23.3, 22.9, 18.1, 18.0, 17.1, 16.6, 15.5, 14.5; HRESIMS calcd for $C_{50}H_{82}O_{16}Na$ 961.5495; found 961.5496

Cyclopentyl 3β-O-[2,4-Di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-olean-12-en-28-oic acid (12)

Compound **12** was synthesized as a white solid in 92% yield; ^{1}H NMR (CD_3OD): δ 5.37 (d, 1H, J = 1.4 Hz, Rha-H-1), 5.24 (t, 1H, J = 3.8 Hz, H-12), 4.87 (d, 1H, J = 1.6 Hz, Rha-H-1), 4.44 (d, 1H, J = 7.8 Hz, H-1′), 3.98-4.00 (m, 2H), 3.91-3.93 (m, 1H), 3.85 (dd, 1H, J = 3.2, 1.9 Hz, Rha-H-2), 3.81 (dd, 1H, J = 11.9, 1.7 Hz, H-6′-1), 3.75 (dd, 1H, J = 9.5, 3.4 Hz, Rha-H-3), 3.67 (dd, 1H, J = 12.1, 4.0 Hz, H-6′-2), 3.63 (dd, 1H, J = 9.4, 3.3 Hz, Rha-H-3), 3.60 (t, 1H, J = 8.7 Hz), 3.55 (t, 1H, J = 8.9 Hz), 3.45 (t, 1H, J = 8.3 Hz), 3.42 (t, 1H, J = 9.5 Hz), 3.40 (t, 1H, J = 9.6 Hz), 3.31-3.34 (m, 1H), 3.18 (dd, 1H, J = 11.8, 4.2 Hz, H-3), 2.21 (d, 1H, J = 11.2 Hz, H-18), 1.28 (d, 3H, J = 6.4 Hz, Rha-H-6), 1.22 (d, 3H, J = 6.3 Hz, Rha-H-6), 1.13, 1.07, 0.98, 0.98, 0.88, 0.82, 0.74 (each s, each 3H, CH_3); ^{13}C NMR (CD_3OD): δ 178.7 (C-28), 143.7 (C-13), 122.6 (C-12), 104.2 (C-1′), 101.8 (Rha-C-1), 100.7 (Rha-C-1), 89.1, 79.1, 77.9, 76.9, 76.8, 75.2, 72.4, 72.3, 71.1, 70.8, 70.7, 69.4, 68.7, 60.7, 56.0, 51.0, 46.8, 45.8, 41.5, 39.3, 38.9, 36.6, 36.4, 33.3, 32.6, 30.2, 27.5, 27.2, 25.8, 25.0, 23.2, 22.7, 22.5, 20.1, 18.0, 16.7, 16.6, 16.3, 15.9, 14.7, 7.9; HRESIMS calcd for $C_{53}H_{86}O_{16}Na$ 1001.5808; found 1001.5810.

3.3 Measurement of the inhibitory activity against H5N1pseudovirus

MDCK cells and 293T cells were obtained from the American Type Culture Collection (ATCC). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Gibco) containing glutamine, supplemented with 10% fetal calf serum (FCS). The H5N1 pseudoviruses were prepared by transfecting HA plasmid from the H5 subtype strain A/Thailand/ Kan353/2004 (H5N1) strain and the NA plasmid from the N1 subtype strain A/Thailand/Kan353/2004. Briefly, 293T cells (70-80% confluent) were co-transfected with 1 μg HA plasmid, 1 μg NA plasmid and 3 μg HIV backbone plasmid (pNL4-3.luc.R_E_) into six-well plate with polyethylenimine (PEI). 20 Forty-eight hours after transfection, the culture supernatants were harvested and centrifuged at 2000 g for 10 min. Aliquots were stored at -80 °C. For measuring the inhibitory activities of test compounds, MDCK cells (104/well) were seeded in 96-well plates and grown overnight. Tested compounds at indicated concentrations were incubated with pseudotyped particles for 30 min at 37 °C. Subsequently, the viruse-compound mixture was transferred to the cells and incubated for an additional 48 h. Cells were washed with phosphate buffer saline (PBS) and lysed with luciferase cell culture lysis reagent (Promega, Madison, WI). Aliquots of cell lysates were transferred to 96-well flat bottom luminometer plates (Costar), followed by the addition of luciferase assay substrate (Promega). The luciferase activity was measured in a microplate luminometer (Genios Pro, Tecan, US). As a negative control, VSV-G pseudotyped particles were incubated with the tested compound instead of H5N1 pseudovirus.

3.4 Neuraminidase activity assay

The neuraminidase activity was measured by a fluorescence based assay using a Neuraminidase Inhibitors Screen Kit by following the manufacturer's instruction (Beyotime Institute of Biotechnology, China). Briefly, 10 μL of purified N1-typed neuraminidase was added to 70 μL of detection buffer, followed by adding 10 μL of a test compound and 10 μL of neuraminidase

substrate sequentially. After incubation at 37 °C for 30 min, the fluorescence intensity was measured at an excitation wavelength of 340 nm and an emission wavelength of 535 nm using a microplate reader (Genios Pro, Tecan, US).

3.5 hemagglutination inhibition assay.

The inhibitory activity of the compound on HA-mediated hemagglutination of avian RBCs was tested by hemagglutination inhibition assay. Briefly, 25 μL of H5 standard antigen (4 hemagglutination units) was incubated with 25 μL H5 standard antiserum (both H5 standard antigen and antiserum were provided by Haerbin Veterinary Research Institute, China) or a test compound at indicated concentration for 1 h at room temperature. Then, 50 μL chicken RBCs (0.5%) in saline solution were added to each well and incubated at room temperature for 45 min. The hemagglutination was recorded.

3.6 Molecular modeling assay

The recently published X-ray structure of H5 hemagglutinin trimeric protein (A/Vietnam/1194/2004 PDB: 2IBX) was used for molecular docking of compound **5**.[21] The structure of compound **5** was drawn using SYBYL 7.3 sketch molecule program. The molecular structure optimization and conformation energy minimization are performed using Concord clean-up command. The H5N1 hemagglutinin protein is prepared using SYBYL7.3 protein preparation tool, removing water, adding polar hydrogen atoms and fixing incomplete side chain. GRID was used to identify potential binding site since HA is in unliganded state. The HA2 stem region is selected for a probable binding site for docking. Docking calculation of compound **5** was performed using Surflex-Dock module of SYBYL 7.3 with default docking parameters.

Acknowledgements

This project was supported by the National Science Foundation for Young Scientists of China (No. 21202047), Foundation for Distinguished Young Talents in Higher Education of Guangdong, China (No. LYM10037), The PhD Start-up Fund of Natural Science Foundation of Guangdong Province, China (S2012040007711) and the Jiangsu Agriculture Science and Technology Innovation Fund (No. SCX(14)5076).

Notes and references

[a]*College of Resources and Environment, South China Agricultural University, 510642, Guangzhou, China, Fax: + 86-20-85280292; Tel: + 86-20-85280293; E-mail: vinsin1021@126.com*

[b]*School of Pharmaceutical Sciences, Southern Medical University, 510515, Guangzhou, China Fax: +86 -20 -6164 8655; Tel: +86- 20-6164 8538;E-mail: liusw@smu.edu.cn*

[c]*Department of Human anatomy, School of Medicine, 510632, Jinan University, Guangzhou, China*

[d]*Institute for Computational Science and Engineering, Qingdao University, 266071, Qingdao, China*

[e]*Institute of Agricultural Science in Coastal Area of Jiangsu Province, 224002, Yancheng, China*

[f]*These authors contributed equally to this work*

†Electronic Supplementary Information (ESI) available: [scan spectral data of the new compounds.]. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

1 D. Schade, J.Kotthaus, L. Riebling, J. Kotthaus, H. Müller-Fielitz, W. Raasch, O. Koch, N. Seidel, M. Schmidtke, and B. Clement, *J. Med. Chem*., 2014, **57**, 759.

2 M. von Itzstein, W. Y. Wu, G. B. Kok, M. S. Pegg, J. C. Dyason, B. Jin, T. Van Phan, M. L. Smythe, H. F. White, S. W. Oliver, and A. Et, *Nature*, 1993, **363**, 418.

3 H. P. Hsieh, and J. T. Hsu, Curr. Pharm. Des., 2007, 13, 3531-3542.

4 A. Moscona, *N. Engl. J. Med*. 2009, **360**, 953.

5 Y. C. Xie, D. Q. Xu, B. Huang, X. L. Ma, W. B. Qi, F. Y. Shi, X. Y. Liu, Y. J. Zhang, and W. F. Xu, *J. Med. Chem*., 2014, **57**, 8445.

6 V. V. Grishko, N. V. Galaiko, I. A. Tolmacheva, I. I. Kucherov, V. F. Eremin, E. I. Boreko, O. V. Savinova, and P. A. Slepukhin, *Eur. J. Med. Chem*., 2014, **83**, 601.

7 F. Yu, Q. Wang, Z. Zhang, Y. Peng, Y. Qiu, Y. Shi, Y. Zheng, S. Xiao, H. Wang, X. Huang, L. Zhu, K. Chen, C. Zhao, C. Zhang, M. Yu, D. Sun, L. Zhang, and D. Zhou, *J. Med. Chem*., 2013, **56**, 4300.

8 T. Ikeda, K. Yokomizo, M. Okawa, R. Tsuchihashi, J. Kinjo, T. Nohara, and M. Uyeda, *Biol. Pharm. Bull*., 2005, **28**, 1779.

9 H. Wang, Q. Wang, S. L. Xiao, F. Yu, M. Ye, Y. X. Zheng, C. K. Zhao, D. A. Sun, L. H. Zhang, D. M. Zhou, *Eur. J. Med. Chem*., 2013, **64**, 160.

10 K. Qian, R. Y. Kuo, C. H. Chen, L. Huang, S. L. Morris-Natschke, and K. H. Lee, *J. Med. Chem*., 2010, **53**, 3133.

11 P. Wang, J. Wang, T. T. Guo, and Y. X. Li, *Carbohydr. Res*., 2010, **345**, 607.

12 B. K. Cassels, and M. Asencio, Phytochem. Rev., 2011, **10**, 545-564.

13 A. Wolkerstorfer, H. Kurz, N. Bachhofner, and O. H. J. Szolar, *Antivir. Res*., 2009, **83**, 171.

14 M. Takei, M. Kobayashi, X. D. Li, R. B. Pollard, and F. Suzuki, Pathobiology., 2005, **72**, 117-123.

15 M. R. Yu, L. L. Si, Y. F. Wang, Y. M. Wu, F. Yu, P. X. Jiao, Y. Y. Shi, H. Wang, S. L. Xiao, G. Fu, K. Tian, Y. T. Wang, Z. H. Guo, X. S. Ye, L. H. Zhang, and D. M. Zhou, *J. Med. Chem*., 2014, **57**, 10058.

16 L. A. Baltina, *Curr. Med. Chem*., 2003, **10**, 155.

17. E. Vanderlinden, and L. Naesens, *Med. Res. Rev*., 2014, **34**, 301.

18 G. P. Song, S. Yang, W. Zhang, Y. L. Cao, P. Wang, N. Ding, Z. H. Zhang, Y. Guo, and Y. X. Li, J. Med. Chem., 2009, **52**, 7368-7371.

19 N. Ding, Q. Chen, W. Zhang, S. M. Ren, Y. Guo, and Y. X. Li, *J. Med. Chem*., 2012, **53**, 316.

20 Z. B. Zhu, R. M. Li, G. K. Xiao, Z. P. Chen, J. Yang, Q. H. Zhu, and S.W. Liu, *Eur. J. Med. Chem*., 2012, **57**, 211.

21 S. W. Liu, R. M. Li, R. T. Zhang, C. C. S. Chan, B. M. Xi, Z. B. Zhu , J. Yang, V. K. M. Poon, J. Zhou, M. Chen, J. Münch , F. Kirchhoff, S. Pleschka, T. Haarmann, U. Dietrich, C. G. Pan, L.Y. Du, S. B. Jiang, and B. J. Zheng, *Eur. J. Pharmacol*., 2011, **660**, 460.

22 S. J. Plotch, B. O'Hara, J. Morin, O. Palant, J. LaRocque, J. D. Bloom Jr., S. A. Lang, M. J. DiGrandi, M. Bradley, R. Nilakantan, and Y. Gluzman, *J.Virol*., 1999, **73**, 140.

23 F. Cao, Y. Gao, M. Wang, L. Fang, Q. Ping, *Mol. Pharm*., 2013, **10**, 1378.