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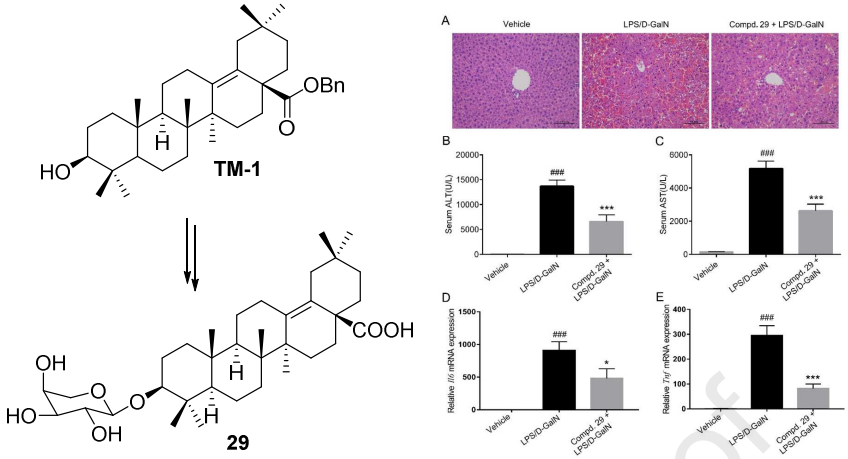
Graphical abstract



Synthesis and anti-inflammatory activity of saponin derivatives of δ-oleanolic acid

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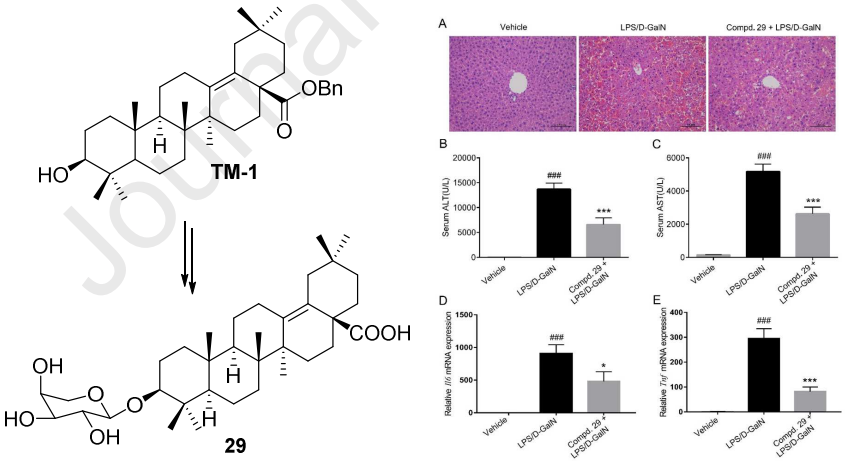
Graphical abstract



Keywords

Pentacyclic triterpenes; δ-oleanolic acid; saponins; AMPK activator; anti-inflammation

ABSTRACT

Pentacyclic triterpenes (PTs) are the active ingredients of many medicinal herbs and pharmaceutical formulations, and are well-known for their anti-inflammatory activity. On the other hand, anti-inflammatory effects of AMP-activated protein kinase

(AMPK) have recently drawn much attention. In this study, we found that a variety of naturally occurring PTs sapogenins and saponins could stimulate the phosphorylation of AMPK, and identified δ-oleanolic acid (**10**) as a potent AMPK activator. Based on these findings, 23 saponin derivatives of δ-oleanolic acid were synthesized in order to find more potent anti-inflammatory agents with improved pharmacokinetic properties. The results of cellular assays showed that saponin **29** significantly inhibited LPS-induced secretion of pro-inflammatory factors TNF-α and IL-6 in THP1-derived macrophages. Preliminary mechanistic studies showed that **29** stimulated the phosphorylation of AMPK and acetyl-CoA carboxylase (ACC). The bioavailability of **29** was significantly improved in comparison with its aglycon. More importantly, **29** showed significant anti-inflammatory and liver-protective effects in LPS/D-GalN-induced fulminant hepatic failure mice. Taken together, PTs saponins hold promise as therapeutic agents for inflammatory diseases.

1. Introduction

Inflammation is a host defense response to danger signals from pathogens, damaged cells, and irradiation, etc [1, 2]. The characteristic signs of acute inflammation include redness, swelling, warmth, and pain [3], which occur during a variety of acute infection or tissue damage. On the other hand, there are no classic signs of acute inflammation in the context of chronic and low-grade inflammation, which plays a key role in the initiation and progression of aging and many chronic diseases, such as metabolic disease, cardiovascular disease, neurodegenerative diseases, respiratory diseases, autoimmune disease, and cancer, etc [3, 4, 5]. Although many anti-inflammatory agents are available in clinic, there are still significant unmet medical needs for safer and effective treatment of inflammatory diseases.

Pentacyclic triterpenes (PTs), the major active ingredients of many Traditional Chinese Medicines (TCMs), are well-known for their anti-inflammatory activities [6]. For example, oleanolic acid (**1**) and glycyrrhizic acid (**16**) (Figure 1) have a long history of clinical utility in China for treating hepatitis and liver-protection [7]. Hederacoside C (**15**) is the major component of ivy leaf preparations (e. g. Prospan)

which is used worldwide for treating respiratory diseases [8]. Madecassic acid (**8**) exhibited promising anti-colitis effect [9]. PTs present in apple peel, including oleanolic acid (**1**), ursolic acid (**5**), corosolic acid (**6**), and betulinic acid (**9**), exhibited therapeutic potential against inflammatory bowel disease [10]. Asiatic acid (**7**) had anti-inflammatory and antioxidant activities against lipopolysaccharide/D-GalN-induced fulminant liver failure [11]. Nevertheless, the mechanisms of action of PTs having anti-inflammatory effects are still not fully understood.

AMPK, which exists in the form of a heterotrimeric complex (αβγ), is a key kinase that regulates the body's energy metabolism and stress response [12]. It is well-known that activation of AMPK protects the body against tissue damage caused by acute and chronic inflammation [13]. Importantly, the existing AMPK activators could exert anti-inflammatory effects in a variety of inflammation models [14-18]. However, anti-inflammatory mechanisms of AMPK are still unclear [19]. Notably, some naturally occurring PTs such as oleanolic acid (**1**) [20-23], hederagenin (**3**) [24], ursolic acid (**5**) [25,26], corosolic acid (**6**) [27], asiatic acid (**7**) [28], madecassic acid (**8**) [9], betulinic acid (**9**) [29, 30], glycyrrhizic acid (**16**) [31] and α-hederin [32] have been reported to be AMPK activators, which might have a high relevance with their anti-inflammatory effects.

In view of the clinical significance of PTs in the management of various chronic diseases, our laboratory has been working in this field for more than 15 years [7, 33-36]. Our recent efforts in PTs program are focused on searching for novel PTs related compounds with anti-inflammatory effects against acute and chronic inflammatory diseases. In this study, we found that most of the tested PTs sapogenins (**1~10**) and saponins (**11~20**) had the capability to stimulate AMPK phosphorylation. Notably, δ-oleanolic acid (δ-OA, **10**) was proved to be a very potent AMPK activator. We then designed and synthesized 23 novel saponin derivatives of δ-OA, most of which exhibited significant anti-inflammatory activity. The result of preliminary mechanistic studies indicated that the δ-OA saponins might exert anti-inflammatory effects, at least in part, through AMPK activation.

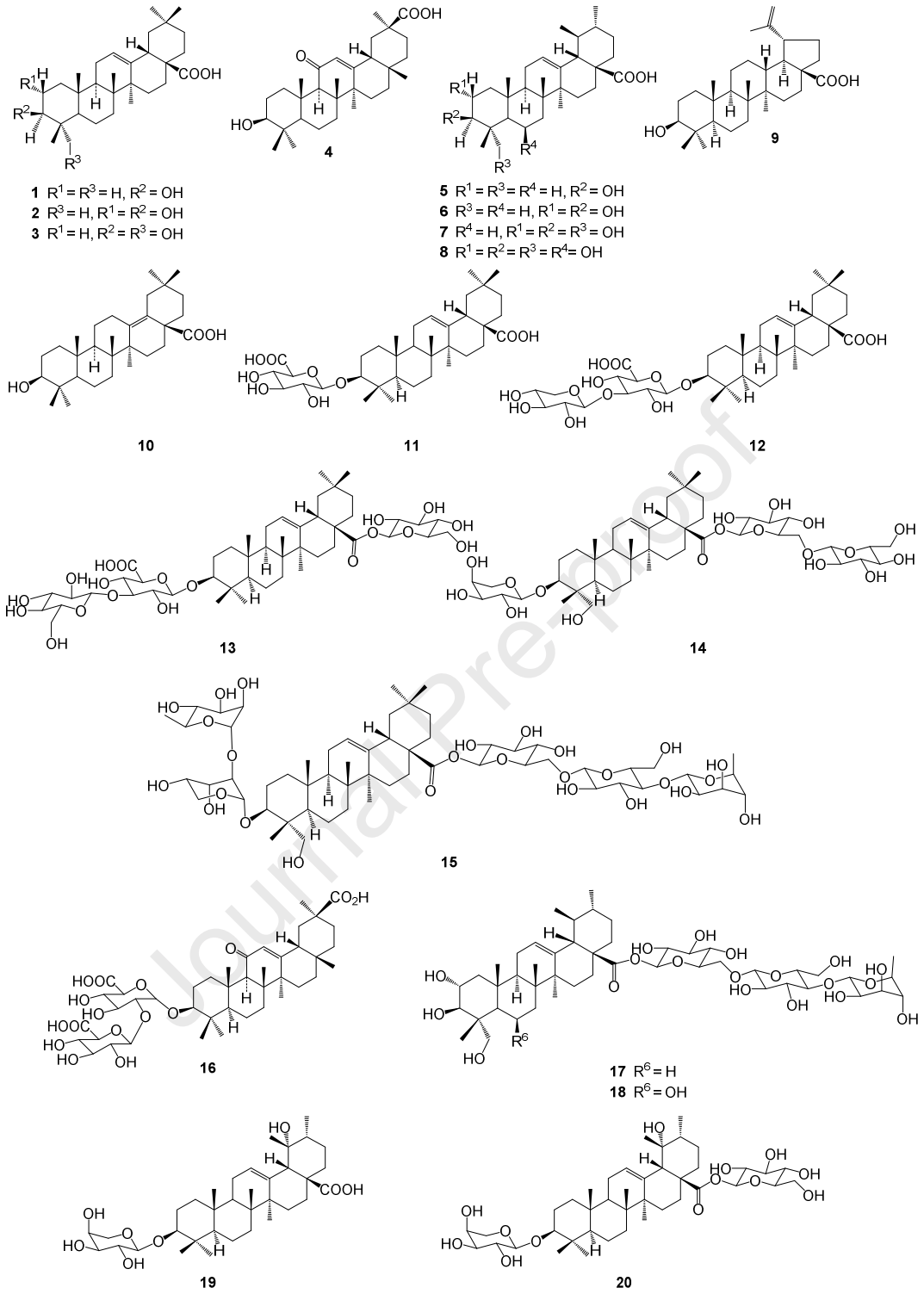


Figure 1. Structures of naturally occurring PTs sapogenins **1~10** and saponins **11~20**.

2. Results and discussion

2.1. Naturally occurring PTs increased AMPK phosphorylation

We examined the effect of some representative PTs sapogenins (**1~10**) and

saponins (**11~20**) on AMPK phosphorylation in hepatocyte Huh7 cells. It was found that oleanolic acid (**1**), betulinic acid (**9**) and δ-oleanolic acid (**10**) significantly stimulated Thr172-AMPKα phosphorylation at 10 μM, and were more potent than the well-known AMPK activator AICAR (Figures 2A). Other sapogenins such as masilinic acid (**2**), hederagenin (**3**), glycyrrhetinic acid (**4**), ursolic acid (**5**), corosolic acid (**6**), asiatic acid (**7**) and madecassic acid (**8**) could also increase AMPK phosphorylation in Huh7 cells. Calenduloside E (**11**), momordin Ic (**12**), ginsenoside Ro (**13**), asperosaponin VI (**14**), hederacoside C (**15**), glycyrrhizic acid (**16**), asiaticoside (**17**), madecassoside (**18**), ziyuglycoside II (**19**) and ziyuglycoside I (**20**) are the major active PTs saponins of some TCMs. We found that **13**, **14**, **17**, **18** and **20** significantly increased AMPK phosphorylation in Huh7 cells (Figures 2B). Other tested saponins could also stimulated AMPK phosphorylation. Taken together, a variety of naturally occurring PTs sapogenins and saponins could enhance AMPK phosphorylation in Huh7 cells. In particular, δ-oleanolic acid (δ-OA, **10**) is one of the most potent PTs.

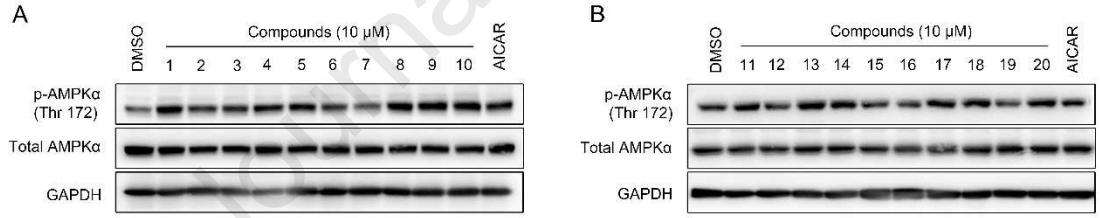


Figure 2. PTs sapogenins and saponins stimulated the phosphorylation of AMPK (pThr172) in Huh7 cells. A, Effect of PTs sapogenins **1~10** on the phosphorylation of AMPK. B: Effect of PTs saponins **11~20** on the phosphorylation of AMPK. Huh7 cells were incubated with 10 μM of PTs sapogenins or saponins, and AICAR (0.2 mM) was used as a positive control. After 12 h of incubation, the p-AMPK/AMPK levels were evaluated using the Western blot assay.

2.2. δ-Oleanolic acid stimulated the phosphorylation of AMPK and ACC in hepatocytes

To further confirm the effect of δ-OA on AMPK signaling, western-blot analysis of a time course assay was conducted in Huh7 and HepG2 cells. The results showed that δ-OA increased AMPK phosphorylation in a time-dependent manner (Figures 3A, 3B). We next examined the dose-dependent effect of δ-OA on AMPK phosphorylation, and found that δ-OA dose-dependently stimulated AMPK phosphorylation (Figures

3C, 3D). As expected, δ-OA time- and dose-dependently increased phosphorylation of acetyl-CoA carboxylase (ACC), a downstream substrate of AMPK (Figure 3). Together, δ-OA was proved to be a potent AMPK activator.

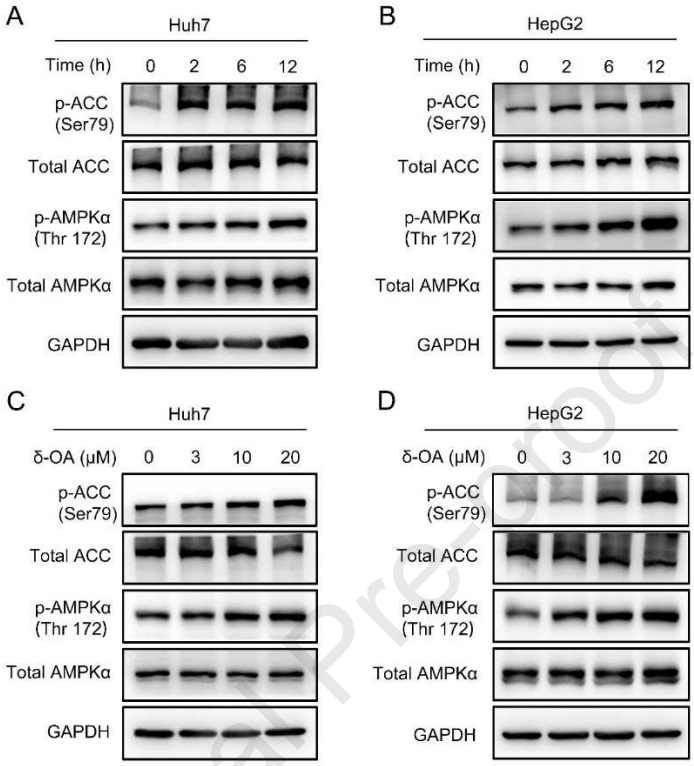


Figure 3. δ-Oleanolic acid (δ-OA) stimulates the phosphorylation of AMPK (pThr172) and ACC (pSer79) in a time- and dose-dependent manner. A/B, δ-OA (10 μM) induced phosphorylation of AMPK (pThr172) and ACC (pSer79) in a time-dependent manner in Huh7 and HepG2 cells. C/D, δ-OA induced phosphorylation of AMPK (pThr172) and ACC (pSer79) in a dose-dependent manner in Huh7 and HepG2 cells. After cells were treated with δ-OA, protein levels of p-AMPKα, AMPKα, p-ACC, ACC and GAPDH were evaluated using the Western blot assay.

2.3. Synthesis of δ-OA saponins

δ-OA, which is a very rare pentacyclic triterpene acid in plants, has been reported to have anti-inflammatory activity [37]. Given our findings that δ-OA was a potent AMPK activator, and PTs saponins significantly stimulated AMPK phosphorylation, we became interested in δ-OA saponins as potential AMPK activators. Moreover, δ-OA saponins should have better bioavailability than δ-OA which has very poor water solubility. To the best of our knowledge, there is only one δ-OA saponin reported by far [38]. Therefore, we decided to synthesize a series of new δ-OA saponins (Figure 4: **21~43**).

Sugar donors **S1~S12** are shown in **Figure 5.** Sugar bromides **S1~S6** and glycosyl trichloroacetimidates **S7~S12** were prepared according to the literature methods [39-42].

The synthesis of δ-OA 28-glycosides **21~26** is depicted in **Scheme 1**. We recently developed protonated montmorillonite-mediated highly specific isomerization of oleanolic acid benzyl ester into δ-OA benzyl ester **TM-1**, allowing for a practical preparation of δ-OA (**10**) [43]. Therefore, catalytic hydrogenolysis of **TM-1** gave **10** in 95% yield [43]. Glycosidation of **10** with sugar bromides **S1~S6** under phase-transfer-catalyzed conditions (K_2CO_3, Bu_4NBr, CH_2Cl_2-H_2O, 50°C) afforded benzoyl- or acetyl-protected glycosides **21a~26a** in 41-94% yields. Removal of the protecting groups of **21a~26a** by sodium methoxide in MeOH or sodium hydroxide (aq.) in THF gave the corresponding δ-OA 28-glycosides **21~26** in 64-93% yields.

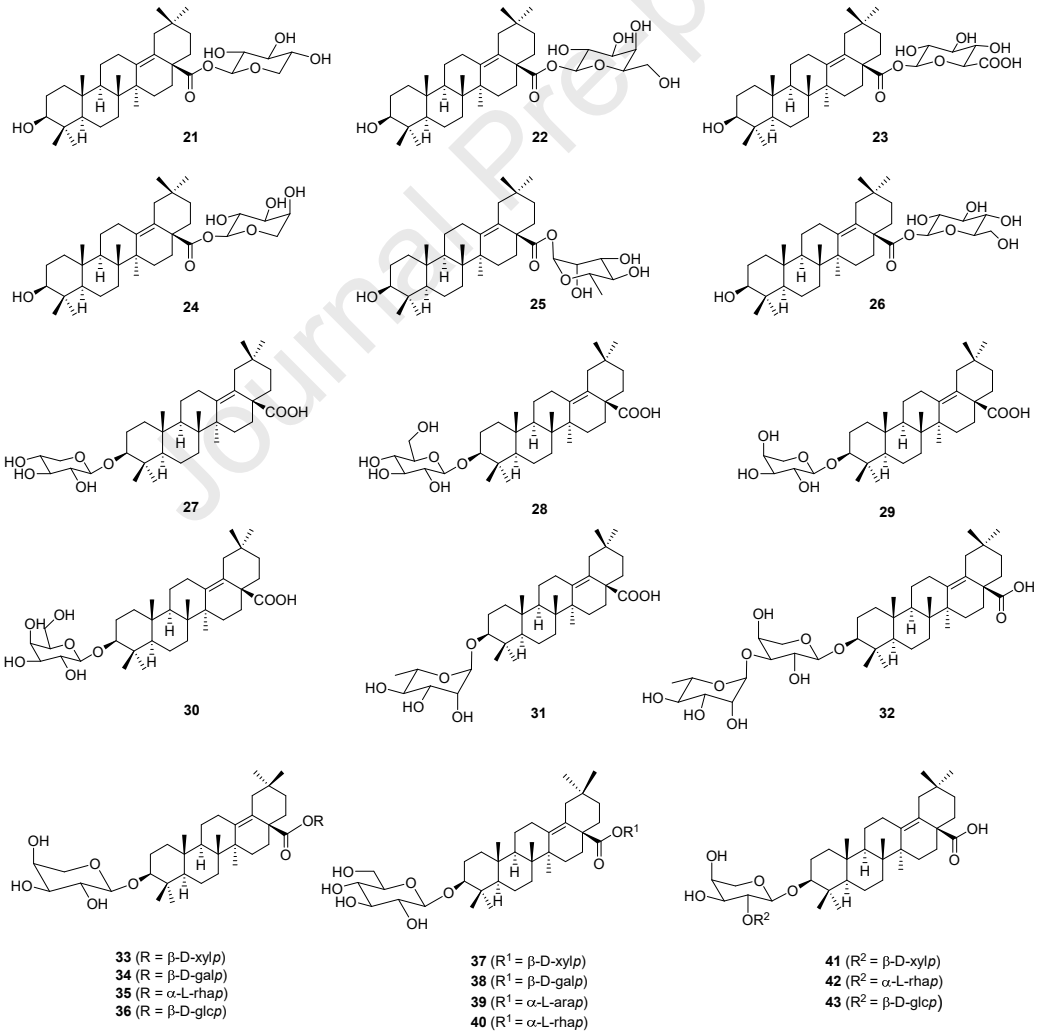


Figure 4. Structures of δ-OA saponins **21~43.**

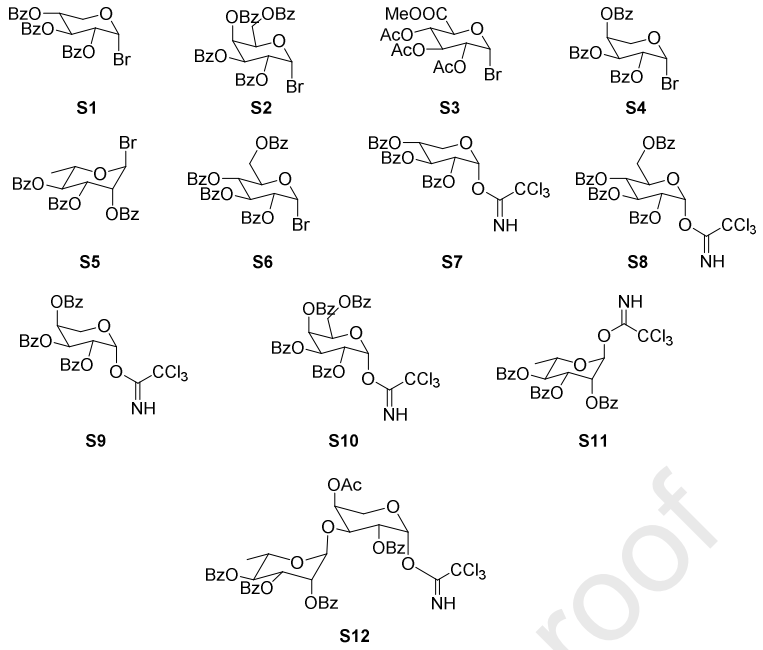


Figure 5. Sugar donors **S1~S12**.

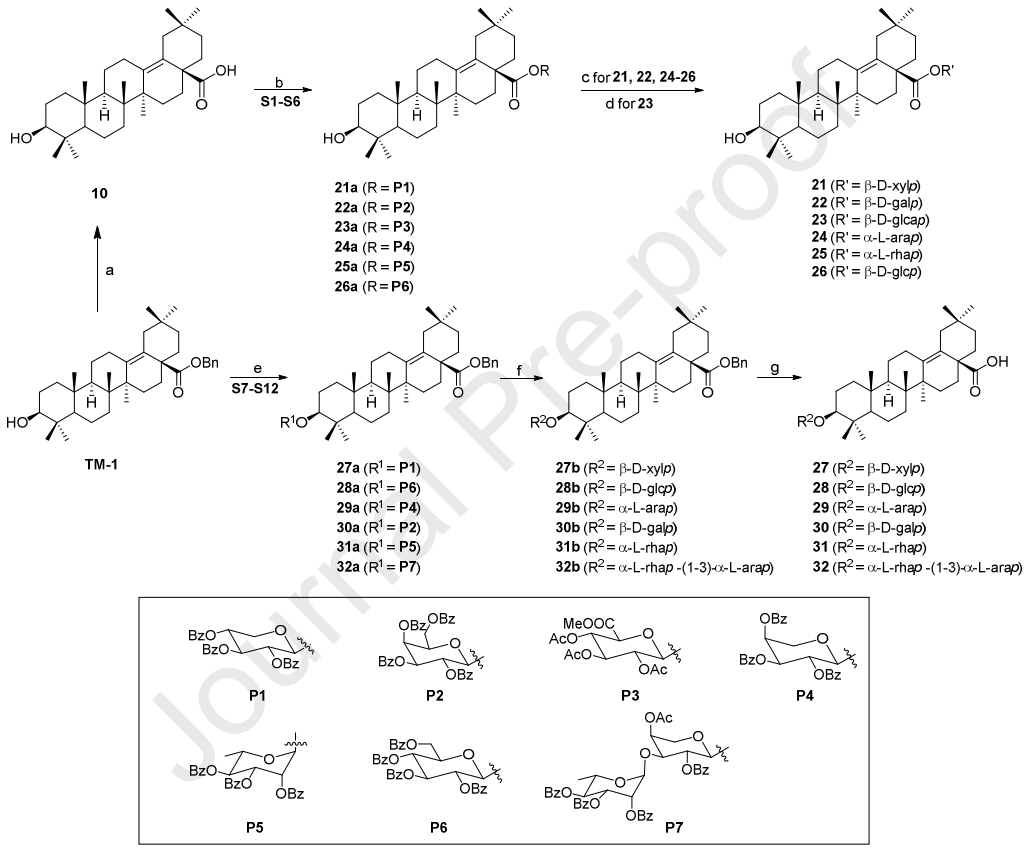
The synthetic route to δ-OA 3-glycosides **27~32** is shown in **Scheme 1**. Reaction of **TM-1** with glycosyl trichloroacetimidates **S7~S12** in the presence of trimethylsilyl trifluoromethanesulfinate (TMSOTf) afforded glycosides **27a~32a** in 71-95% yields. Removal of the benzoyl-protecting groups of **27a~32a** by sodium methoxide in MeOH gave δ-OA benzyl ester 3-glycosides **27b~32b** in 63-83% yields. Catalytic hydrogenolysis of **27b~32b** furnished δ-OA 3-glycosides **27~32** in 63-88% yields.

The synthetic route to δ-OA 3,28-diglycosides is showed in **Schemes 2** and **3**. Catalytic hydrogenolysis of **28a** and **29a** furnished **28c** and **29c** in 72% and 91% yields, respectively. Glycosidation of **28c** and **29c** with sugar bromides **S1~S2** and **S4~S6** under phase-transfer-catalyzed conditions (K_2CO_3, n-Bu_4NBr, CH_2Cl_2-H_2O, 50 ℃) afforded benzoyl protected glycosides **33a~36a** and **37a~40a** in 80-85% and 56-95% yields, respectively. Removal of the protecting groups of **33a~36a** and **37a~40a** by sodium methoxide in MeOH gave the corresponding δ-OA 3,28-diglycosides **33~36** and **37~40** in 44-60% and 45-56% yields, respectively.

The synthesis of δ-OA 3-diglycosides **41~43** is depicted in **Scheme 4**. Debenzoylation of **29a** using methanolic MeONa in CH_2Cl_2 followed by isopropylidene formation with 2,2-dimethoxypropane mediated by *p*-toluenesulfonic acid hydrate (*p*-TsOH·H_2O) to furnish saponin **TM-2** in 85% yields. Coupling of

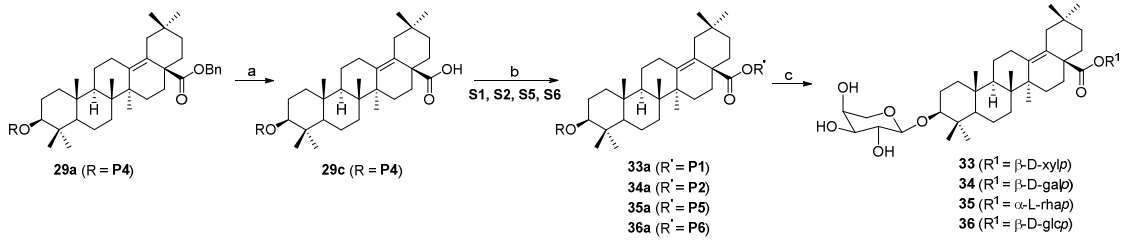
TM-2 with **S7**, **S8** or **S11** was carried out in the presence of catalytic amount of $BF_3 \cdot Et_2O$ to generate **41a~43a** in 59-74% yields. Compounds **41a~43a** were treated with methanol/*p*-TsOH·H_2O to give **41b~43b** in 72-87% yields. Removal of the benzoyl-protecting groups of **41b~43b** by sodium methoxide in MeOH and then catalytic hydrogenolysis furnished δ-OA 3-diglycosides **41~43** in 37-63% yields.

Scheme 1. Synthesis of δ-OA 28-glycosides and 3-glycosides



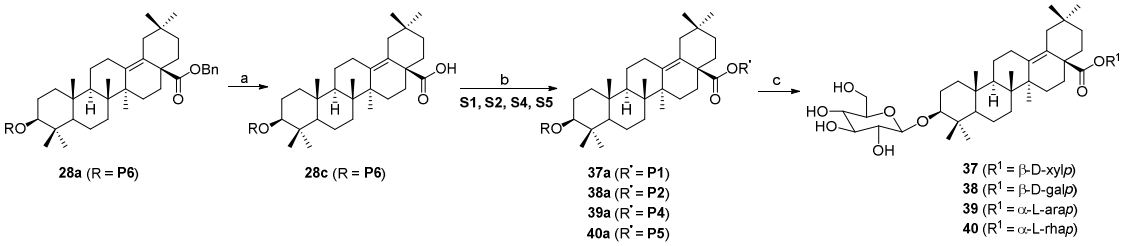
Reagents and conditions: (a) H_2, Pd/C, THF, rt, 95%; (b) K_2CO_3, CH_2Cl_2-H_2O, n-Bu_4NBr, 50 °C, 41-94%; (c) MeONa, CH_2Cl_2-MeOH, rt, 64-80%; (d) NaOH (aq.), THF, rt, 93%.(e) TMSOTf, 4Å MS, CH_2Cl_2, 0 °C~rt, 71-95%; (f) NaOMe, CH_2Cl_2-MeOH, rt, 63-83%; (g) H_2, Pd/C, THF, 63-88%.

Scheme 2. Synthesis of δ-OA 3,28-diglycosides (3-O-α-L-arabinopyranosyl)



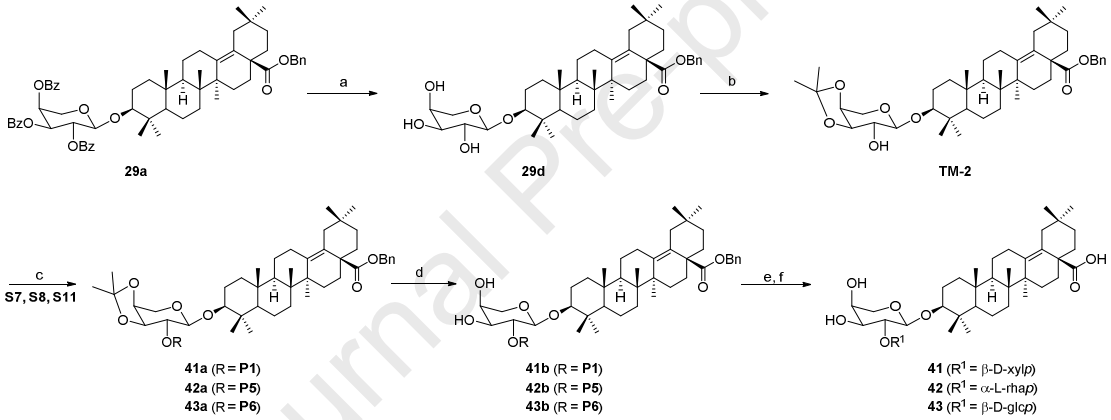
Reagents and conditions: (a) H_2, Pd/C, 91%, THF; (b) K_2CO_3, n-Bu_4NBr, CH_2Cl_2-H_2O, 50 °C, 80-85%; (c) MeONa, CH_2Cl_2-MeOH, rt, 44-60%.

Scheme 3. Synthesis of δ-OA 3,28-diglycosides (3-O-β-D-glucopyranosyl)



Reagents and conditions: (a) H_2, Pd/C, THF, 72%; (b) K_2CO_3, n-Bu_4NBr, CH_2Cl_2-H_2O, 50 °C, 56-95%; (c) MeONa, CH_2Cl_2-MeOH, rt, 45-56%.

Scheme 4. Synthesis of δ-OA 3-diglycosides



Reagents and conditions: (a) MeONa, CH_3OH/CH_2Cl_2; (b) $(CH_3)_2C(OCH_3)_2$, *p*-TsOH·H_2O, dry-DMF, 85%; (c) $BF_3 \cdot Et_2O$, 4Å MS, dry-CH_2Cl_2, 59-74%; (d) *p*-TsOH·H_2O, CH_2Cl_2/CH_3OH, 72-87%; (e) CH_3ONa, CH_3OH/CH_2Cl_2; (f) H_2, Pd/C, CH_3OH, 37-63%.

2.4. In vitro anti-inflammatory activity of δ-OA saponins

We examined *in vitro* anti-inflammatory activity of the synthesized δ-OA saponins (10 μM) in THP1-derived macrophages. AMPK activator AICAR (0.2 mM) was used as a positive control, and it showed significant inhibitory effect on secretion of pro-inflammatory factors. As expected, most of the saponins significantly inhibited LPS-induced secretion of pro-inflammatory factors tumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) in THP1-derived macrophages (Figures 6A, 6B), and were more potent than AICAR. Among them, Saponins **29**, **33** and **43** were the most potent ones. We next examined anti-inflammatory activity and possible cytotoxicity of δ-OA,

29 and **33** at different concentrations. The results showed that δ-OA dose-dependently inhibited LPS-induced secretion of IL-6 in THP1-derived macrophages (Figure S1B), while it had very weak inhibitory effect on the secretion of TNF-α (Figure S1A). By contrast, saponins **29** and **33** significantly inhibited LPS-induced secretion of both TNF-α and IL-6 in THP1-derived macrophages (Figure S1). Notably, δ-OA, **29** and **33** did not show significant cytotoxic effects on THP1-derived macrophages within 1~10 μM concentration range (Figure S2), indicating that their *in vitro* anti-inflammatory effects were not correlated with cytotoxicity.

Preliminary analysis of structure-activity relationship (SAR) shows that δ-OA 3-glycosides (e. g. **27**, **28**, **29**) are generally more potent than δ-OA 28-glycosides (e. g. **23**, **24**, **25**). Saponins bearing an arabinoside moiety at 3-position of δ-OA display prominent potency (e. g. **29**, **33**, **43**). Saponin **29** showed the best potency on inhibition of LPS-induced TNF-α secretion in THP1-derived macrophages. When adding β-D-xylopyranosyl group, β-D-galactopyranosyl group, α-L-rhamnopyranosyl or β-D-glucopyranosyl group at 28-O position of **29** to result in saponins **33**, **34**, **35** or **36**, respectively, their inhibitory effect on LPS-induced TNF-α secretion was decreased with **33** still remaining significant potency. When adding β-D-glucopyranosyl-(1→2) group at α-L-arabinopyranoside group of **29** to result in saponin **43**, the potency still remained significant, while adding β-D-xylopyranosyl-(1→2) group at α-L-arabinopyranoside group of **29** led to almost loss of the potency (**41**). On the other hand, in the inhibitory assay of LPS-induced IL-6 secretion in THP1-derived macrophages, **29** was again the most potent saponin, and **33** and **43** also showed significant inhibitory effect. Together, saponins **29**, **33** and **43** exhibited significant *in vitro* anti-inflammatory effects, and were more potent than their aglycon δ-OA.

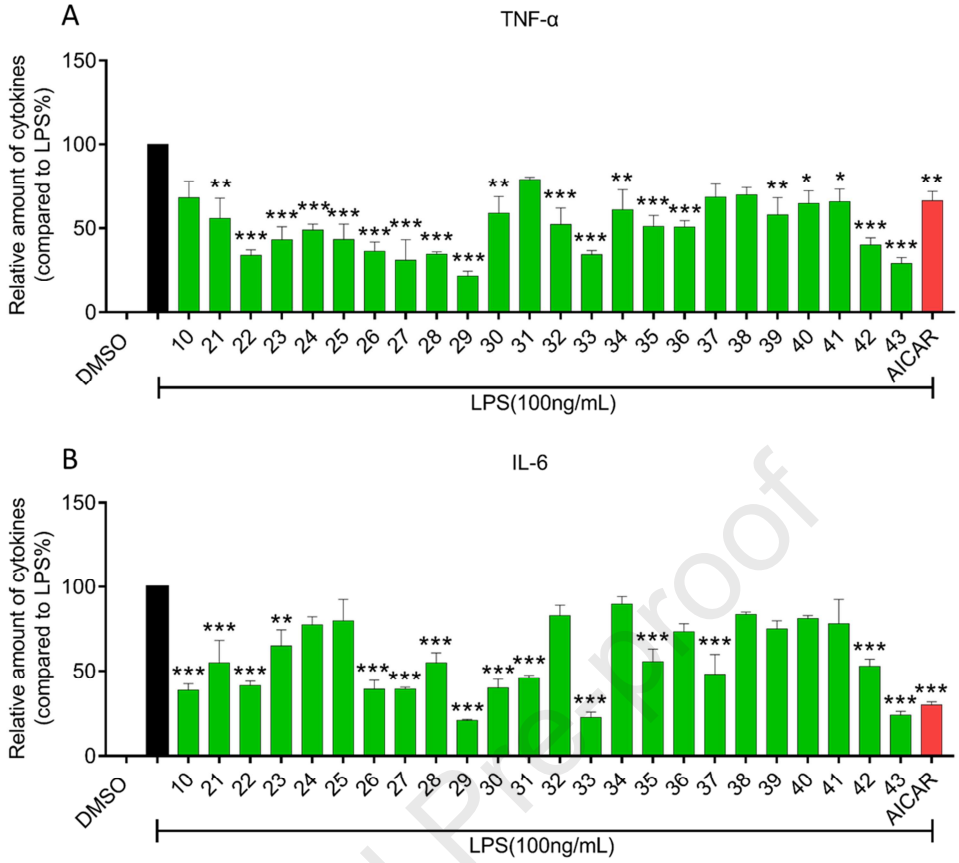


Figure 6. Inhibitory effects of δ-oleanolic acid (**10**) and its saponins **21~43** on LPS-induced inflammatory response in THP1-derived macrophages. A, Effect of the compounds on LPS-induced secretion of TNF-α. B, Effect of the compounds on LPS-induced secretion of IL-6. Compounds concentration: 10 μM; LPS: lipopolysaccharide, 100 ng/mL; AICAR (0.2 mM) was used as the positive control. The results were expressed as the means ± SEM (n=3). **p* < 0.05, ***p* < 0.01, ****p* < 0.001 compared to the LPS group.

2.5. In vitro metabolic stability of saponins 29, 33 and 41 in liver microsomes

We examined the metabolic stability of saponins **29**, **33** and **41** in human and mouse liver microsomes. The results (Table 1) showed that saponins **29**, **33** and **41** had good metabolic stability in mouse liver microsomes ($T_{1/2}$>120 min). In human liver microsomes, saponins **29** and **41** ($T_{1/2}$ >120 min) showed better metabolic stability than saponin **33** ($T_{1/2}$= 45.09 min).

Table 1 Metabolic stability of saponins **29**, **33** and **41** in liver microsomes

Compd.	Species	$T_{1/2}$ (min)	Cl_{int} (mL/min/kg)
29	Human	>120	0.00
	Mouse	>120	0.00

33	Human	45.09	38.56
	Mouse	>120	0.00
41	Human	>120	3.25
	Mouse	>120	2.34
Ketanserin*	Human	42.40	41.00
	Mouse	19.30	282.75

Ketanserin* was used as the positive control.

2.6. Pharmacokinetic studies of saponins 29, 33 and 41

Saponins **29**, **33** and **41** were subjected to pharmacokinetic (PK) studies in Balb/C mice. The pharmacokinetic data in mice showed that the oral bioavailability of **29** (24.66%) and **33** (18.64%) was much better than that of oleanolic acid (**1**), ursolic acid (**5**) and betulinic acid (**9**), whose peak plasma concentrations never exceed 1% of the oral dose [44]. The oral bioavailability of **41** (1.13%) was much lower than that of **29** and **33**. The half-lives of **29**, **33** and **41** were 0.35 h, 1.14 h, and 1.26 h, respectively. Notably, after oral dosing of **29** (10 mg/kg) in mice, only trace amount of δ-OA was detected as a metabolite (data not shown), indicating that saponin **29** was seldom metabolically converted to its aglycon δ-OA. Therefore, it is speculated that saponin **29** may take effects *in vivo* through its original form. Moreover, we found that after oral administration of saponin **33** in mice, it could not be metabolically converted to either **29** or δ-OA (data not shown). Together, saponins **29** and **33** showed significantly improved oral bioavailability, and they took effects in mice through their original forms. Therefore, we chose saponin **29** for further studies.

Table 2 Pharmacokinetic studies of saponins **29**, **33** and **41** in Balb/C mice.

Compd.	Admin.	Dose	T_{max}	C_{max}	AUC(0-t)	AUC(0-∞)	$T_{1/2}$	F
		mg/kg	h	ng/mL	h*ng/mL	h*ng/mL	h	%
29	p.o.	10	0.42	1838.00	1669.63	1688.31	0.35	24.66
	i.v.	2	0.08	4413.83	1354.08	1362.44	0.35	--
33	p.o.	10	2.67	728.21	3002.66	3082.96	1.14	18.64
	i.v.	2	0.08	3500.40	3222.20	3295.92	1.74	--
41	p.o.	10	0.75	54.21	145.43	150.87	1.26	1.13
	i.v.	2	0.08	8823.73	2573.79	2589.03	2.25	--

2.7. Saponin 29 increased the phosphorylation of AMPK and ACC

We investigated the AMPK agonistic effect of saponin **29** on Huh7 and HepG2 cells. The results (Figures S3) showed that **29** time- and dose-dependently stimulated the phosphorylation of AMPK and ACC in Huh7 and HepG2 cells. To preliminarily probe the mechanism for **29** to stimulate AMPK phosphorylation, we examined whether **29** had direct interaction with two major AMPK isoforms, AMPK α1β1γ1 and AMPK α2β2γ1. The results (Figures S4) of the protein assays showed that **29** had no influence on the enzymatic activity of recombinant AMPK α1β1γ1 and α2β2γ1 heterotrimers, indicating that **29** probably increased AMPK phosphorylation in an indirect manner.

2.8. Saponin 29 showed anti-inflammatory and liver-protective effects in LPS/D-GalN-induced fulminant hepatic failure mice

Fulminant hepatic failure (FHF) is a life-threatening and fatal clinical syndrome with poor prognosis and high mortality. It is well-known that lipopolysaccharide (LPS)/D-galactosamine (D-GalN)-induced FHF is closely correlated with macrophage-derived inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α[11]. To determine the *in vivo* anti-inflammatory effects of **29**, we evaluated this compound with using LPS/D-GalN-induced FHF mouse model. As illustrated in Figure 7A, histological analysis of the mouse liver sections in the LPS/D-GalN group showed obviously disturbed architecture, such as hepatocyte necrosis, hemorrhage, and neutrophil infiltration. Not surprisingly, the LPS/D-GalN-induced liver alterations were effectively relieved by the pretreatment with **29**. Moreover, the serum ALT and AST levels were significantly increased by LPS/D-GalN administration, while this increase was markedly inhibited by **29** (Figures 7B, 7C). Furthermore, we examined the mRNA expression levels of IL-6 and TNF-α in the liver. The results showed that LPS/D-GalN significantly increased the mRNA expression of IL-6 and TNF-α in the liver, while **29** significantly inhibited the expression of the inflammatory cytokines (Figures 7D and 7E). Together, saponin **29** exhibited significant anti-inflammatory and liver-protective effects against LPS/D-GalN-induced fulminant hepatic failure.

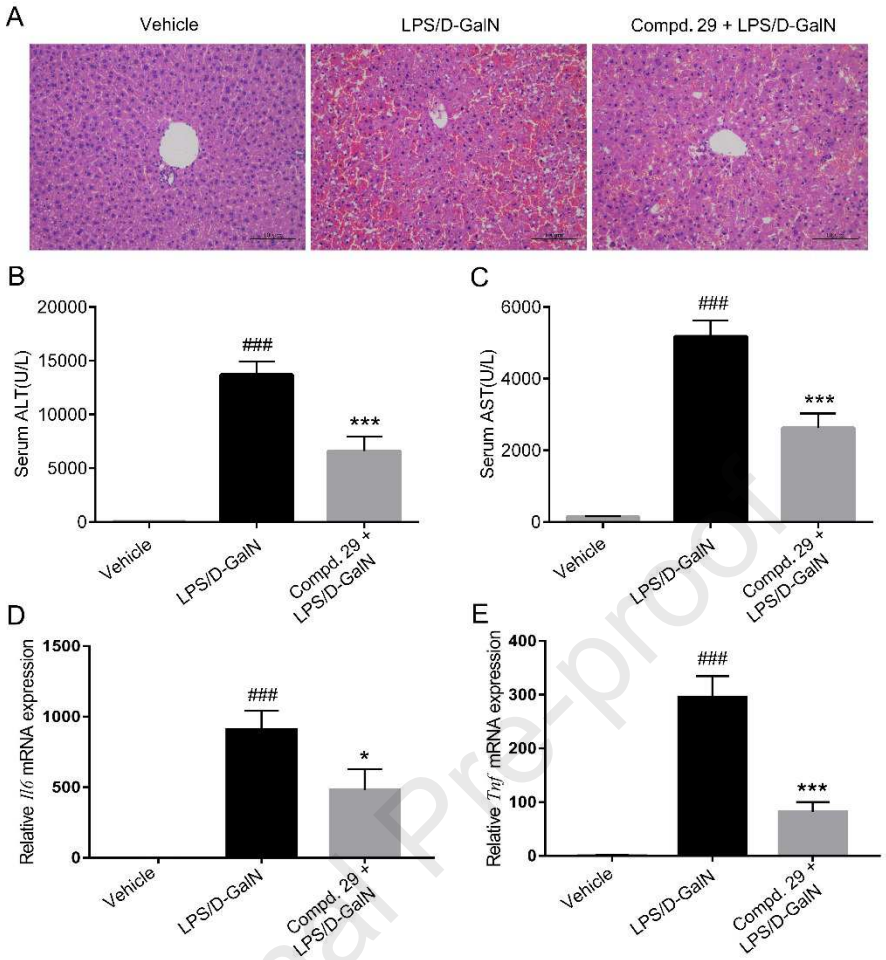


Figure 7. Liver-protective effects of saponin **29** (Compd. **29**) on LPS/D-GalN induced FHF mice. A, Representative histological sections of the livers were stained with hematoxylin and eosin (H&E, magnification 200×). B/C, Effects of Compd. **29** on the serum ALT and AST levels. D/E, Effect of Compd. **29** on hepatic inflammation related *Il6* and *Tnf* mRNA levels in liver. All data were presented as means ± SEM (n = 8-14 per group). ###$p < 0.001$ compared to Vehicle group; *$p < 0.05$ and ***$p < 0.001$ compared to LPS/D-GalN group.

3. Conclusions

In summary, the present study showed that a variety of naturally occurring pentacyclic triterpenes stimulated the phosphorylation of AMPK. Among them, δ-oleanolic acid (**10**), a very rare pentacyclic triterpene acid, was proved to be a very potent AMPK activator. With **10** as a lead compound, 23 new saponin derivatives of δ-oleanolic acid were synthesized in order to find more potent anti-inflammatory agents with better pharmacokinetic properties. Among the synthesized saponins, **29** could activate AMPK in a time- and dose-dependent manner, and inhibited LPS-induced secretion of pro-inflammatory factors TNF-α and IL-6 in THP1-derived

macrophages. **29** had significantly improved oral bioavailability, and took effects mainly through its original form. More importantly, **29** showed significant anti-inflammatory and liver-protective effects in LPS/D-GalN-induced fulminant hepatic failure mice. Taken together, pentacyclic triterpene saponins hold promise as anti-inflammatory agents.

4. Experimental section

4.1. Chemistry

4.1.1. Materials

Naturally occurring pentacyclic triterpenes used in this study were commercially purchased products with purity ≥95%, including oleanolic acid, maslinic acid, glycyrrhetinic acid, corosolic acid, asiatic acid, madecassic acid, betulinic acid and glycyrrhizic acid from Yuanye Bio-Technology Co., Ltd. (Shanghai, China); hederagenin from Herbpurify Co., Ltd. (Chengdu, China); ursolic acid from Boliante Chem Co., Ltd. (Xi 'an, China); calenduloside E, momordin Ic, asiaticoside, madecassoside, ziyuglycoside II and ziyuglycoside I from Push Bio-Technology Co., Ltd. (Chengdu, China); ginsenoside Ro from Biopurify Phytochemicals Ltd. (Chengdu, China); asperosaponin VI and hederacoside C from Meilunbio. Co., Ltd. (Dalian, China).

4.1.2. General information

Commercially available reagents and solvents were used without further purification. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F254 plates (Qingdao Ocean Chemical Company, China). Column chromatography was carried out on silica gel (200-300 mesh, Qingdao Ocean Chemical Company, China). ^{1}H and ^{13}C NMR spectra were recorded on an ACF*300Q Bruker spectrometer with Me_4Si as the internal reference. Proton coupling patterns were described as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), triplet of doublets (td), quartet (q), multiplet (m), and broad (br). Low and high resolution mass spectra (LRMS and HRMS) were given with electron impact mode. The mass analyzer type used for the

HRMS measurements was TOF.

*4.1.3. Synthesis of δ-OA 28-glycosides **21~26***

δ-Oleanolic acid (**10**)

A mixture of δ-OA benzyl ester **TM-1** (1.0 g, 1.829 mmol) and 10% Pd/C (100 mg) in THF (40 mL) was stirred overnight at room temperature under H_2 atmosphere. The mixture was filtered through celite, and the filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (petroleum ether/EtOAc, 2:1) to afford δ-oleanolic acid (**10**) (793 mg, 95%) as a white powder.IR (KBr)ν_{max} 3525, 3435, 2940, 2603, 1699, 1453, 1386, 1029 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.23 (dd, *J* = 10.6, 5.6 Hz, 1H, H-C(3)), 2.81-2.69 (m, 1H,H-C(12)), 2.44 (d, *J* = 14.1 Hz, 1H, H-C(12)), 2.23-2.14 (m, 1H, H-C(19)), 1.17 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.76 (s, 3H, CH_3), 0.74 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 181.94 (C-28), 139.09 (C-13), 127.64 (C-18), 79.23, 55.52, 50.90, 48.36, 44.54, 41.54, 41.18, 39.04, 38.98, 37.51, 36.93, 35.85, 35.06, 33.16, 32.87, 32.23, 28.24, 27.49, 27.29, 25.37, 24.33, 21.76, 21.28, 18.51, 17.72, 16.51, 15.65. HRMS (ESI): *m/z* calcd for $[M-H]^-$ $C_{30}H_{47}O_3$: 455.3531; found: 455.3519.

***β-D-xylopyranosyl δ-oleanolate* (21)**

To a solution of δ-oleanolic acid (**10**, 300 mg, 0.657 mmol) and benzoylbromoglycoside **S1** (449 mg, 0.855 mmol) in CH_2Cl_2/H_2O (30 mL/3 mL) was added K_2CO_3 (182 mg, 1.317 mmol) and n-Bu_4NBr (85 mg, 0.264 mmol). The mixture was refluxed under nitrogen atmosphere. After completion of the reaction, the mixture was diluted with CH_2Cl_2 (10 mL), and the organic layer was washed with water (20 mL×2), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was subjected to was subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 100:1) to afford **21a** (528 mg, 89%). To a solution of **21a** (520 mg, 0.577 mmol) in MeOH-CH_2Cl_2 (16 mL/8 mL) was added MeONa (94 mg, 1.74 mmol), and the mixture was stirred at room temperature for 5 h. The

mixture was neutralized with DOWEX50WX2-100 ion-exchange resin (H^+), and then filtered and concentrated. The residue was subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 15:1) to afford **21** (273 mg, 80%) as a white powder. Mp 240-242 ℃; IR (KBr) ν_{max} 3361, 2967, 2937, 2906, 2865, 1744, 1702, 1460, 1029 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 5.30 (d, J = 7.7 Hz, 1H, H-C(1′)), 5.13 (d, J = 6.0 Hz, 1H), 5.07 (d, J = 4.7 Hz, 1H), 5.02 (d, J = 4.9 Hz, 1H), 4.29 (d, J = 5.0 Hz, 1H), 3.77 (dd, J = 11.2, 4.9 Hz, 1H), 3.32-2.99 (m, 5H), 2.74 (d, J = 14.0 Hz, 1H, H-C(12)), 2.40 (d, J = 14.2 Hz, 1H, H-C(12)), 2.19-2.10 (m, 1H, H-C(19)), 1.15 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.74 (s, 3H, CH_3), 0.70 (s, 3H,CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.53 (C-28), 137.80 (C-13), 127.13 (C-18), 94.98, 76.79, 76.44, 72.04, 69.28, 66.25, 54.85, 50.05, 47.85, 43.79, 40.87, 40.46, 38.40, 36.82, 36.26, 35.05, 34.47, 32.19, 31.88, 28.15, 27.06, 26.37, 24.68, 23.85, 21.16, 20.68, 17.96, 17.22, 16.14, 15.86. HRMS (ESI): *m/z* calcd for $[M+Na]^+$ $C_{35}H_{56}O_7Na$: 611.3918; found: 611.3918.

δ-OA saponins **22**, **24**, **25** and **26** were synthesized according to the synthetic route shown in **Scheme 1** and the procedure described for preparation of **21.**

***β-D-galactopyranosyl δ-oleanolate* (22)**

White powder. Mp 244-247 ℃; IR (KBr) ν_{max} 3538, 3396, 2947, 2874, 1708, 1450, 1450, 1062 cm^{-1};^1H NMR (300 MHz, DMSO-d_6) δ 5.31 (d, J = 7.3 Hz, 1H, H-C(1′)), 4.89 (d, J = 5.3 Hz, 1H), 4.78 (d, J = 4.9 Hz, 1H), 4.61-4.54 (m, 1H), 4.48 (d, J = 3.9 Hz, 1H), 4.29 (d, J = 4.9 Hz, 1H), 3.73-3.66 (m, 1H), 3.59-3.51 (m, 1H), 3.50-3.36 (m, 4H), 3.07-2.97 (m, 1H, H-C(3)), 2.77-2.66 (m, 1H, H-C(12)), 2.38 (d, J = 13.9 Hz, 1H, H-C(12)), 2.17-2.07 (m, 1H,H-C(19)), 1.13 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.72 (s, 3H, CH_3), 0.67 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.32 (C-28), 137.77 (C-13), 127.31 (C-18), 94.93, 76.79, 75.96, 73.43, 69.55, 67.81, 60.03, 54.85, 50.08, 47.86, 43.82, 40.82, 38.41, 36.82, 36.21, 34.93, 34.48, 32.19, 31.98, 31.88, 28.16, 27.06, 26.48, 24.67, 23.96, 21.20, 20.73, 18.00, 17.23, 16.15, 15.86. HRMS (ESI): *m/z* calcd for $[M+Na]^+$ $C_{36}H_{58}O_8Na$: 641.4024; found: 641.4030.

***α-L-arabopyransoyl δ-oleanolate* (24)**

White powder. Mp 247-249 ℃; IR (KBr) ν_{max} 3490, 2991, 2954, 2937, 2894, 2869, 1707, 1450, 1208, 922 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 5.34 (d, J = 5.3 Hz, 1H, H-C(1′)), 5.06 (d, J = 3.7 Hz, 1H), 4.75 (s, 1H), 4.63 (d, J = 3.7 Hz, 1H), 4.29 (d, J = 4.9 Hz, 1H), 3.80-3.67 (m, 2H), 3.55-3.42 (m, 3H), 3.04 (dd, J = 13.1, 7.6 Hz, 1H, H-C(3)), 2.73 (d, J = 12.8 Hz, 1H, H-C(12)), 2.39 (d, J = 14.0 Hz, 1H, H-C(12)), 2.20-2.12 (m, 1H, H-C(19)), 1.15 (s, 3H,CH_3), 0.92 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.73 (s, 3H, CH_3), 0.68 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.33 (C-28), 137.76 (C-13), 127.29 (C-18), 94.75, 76.79, 71.96, 69.49, 66.54, 65.07, 54.84, 50.08, 47.96, 43.84, 40.81, 38.40, 36.82, 36.28, 35.03, 34.47, 32.18, 31.99, 31.88, 28.15, 27.05, 26.51, 24.67, 23.92, 21.20, 20.70, 17.97, 17.25, 16.13, 15.85. HRMS (ESI): *m/z* calcd for $[M+Na]^+$ $C_{35}H_{56}O_7Na$: 611.3924; found: 611.39198.

***α-L-rhamnopyranosyl δ-oleanolate* (25)**

White powder. Mp 235-237 ℃; IR (KBr) ν_{max} 3554, 3512, 3346,2990, 2939, 2866, 1723, 1455, 1139, 969 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 5.81 (s, 1H, H-C(1′)), 5.14 (d, J = 4.1 Hz, 1H), 4.97 (d, J = 5.2 Hz, 1H), 4.77 (d, J = 5.7 Hz, 1H), 4.29 (d, J = 5.0 Hz, 1H), 3.63 (s, 1H), 3.56-3.42 (m, 2H), 3.31-3.22 (m, 1H), 3.09-2.98 (m, 1H, H-C(3)), 2.74 (d, J = 13.4 Hz, 1H, H-C(12)), 2.45 (d, J = 14.2 Hz, 1H, H-C(12)), 2.06 (d, J = 13.6 Hz, 1H, H-C(19)), 1.16 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 0.92 (s, 6H, 2×CH_3), 0.84 (s, 6H, 2×CH_3), 0.75 (s, 3H, CH_3), 0.69 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.99 (C-28), 138.25 (C-13), 126.89 (C-18), 94.66, 76.76, 71.33, 71.22, 70.61, 69.45, 54.81, 50.02, 47.98, 43.97, 40.65, 38.39, 36.81, 36.18, 35.42, 34.40, 32.20, 31.96, 28.14, 27.05, 26.67, 24.72, 23.92, 21.22, 20.81, 17.97, 17.66, 17.39, 16.09, 15.84. HRMS (ESI): *m/z* calcd for $[M+Na]^+$ $C_{36}H_{58}O_7Na$: 625.4075; found: 625.4072.

***β-D-glucopyranosyl δ-oleanolate* (26)**

White powder. Mp 263-265 ℃; IR (KBr) ν_{max} 3503, 3347, 2941, 2871, 1720,1449, 1207, 1250, 1085, 1057 cm^{-1};^1H NMR (300 MHz, DMSO-d_6) δ 5.35 (d, J = 8.1 Hz, 1H, H-C(1′)), 5.09 (d, J = 6.0 Hz, 1H), 5.04 (s, 1H), 4.98 (d, J = 5.0 Hz, 1H), 4.51-4.41 (m, 1H), 4.29 (d, J = 4.6 Hz, 1H), 3.74-3.63 (m, 1H), 3.52-3.41 (m, 1H),

3.29-3.19 (m, 2H), 3.15-2.98 (m, 3H), 2.73 (d, *J* = 12.5 Hz, 1H, H-C(12)), 2.39 (d, *J* = 13.9 Hz, 1H, H-C(12)), 2.18-2.09 (m, 1H, H-C(19)), 1.14 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.73 (s, 3H, CH_3), 0.68 (s, 3H, CH_3).^{13}C NMR (75 MHz, DMSO-d_6) δ 174.30 (C-28), 137.76 (C-13), 127.26 (C-18), 94.36, 77.76, 76.79, 76.68, 72.51, 69.76, 60.82, 54.85, 50.08, 47.83, 43.80, 40.86, 38.40, 36.82, 36.24, 34.97, 34.96, 34.47, 32.21, 32.10, 31.87, 28.16, 27.06, 26.44, 24.68, 23.92, 21.19, 20.73, 17.98, 17.21, 16.15, 15.86. HRMS (ESI): *m/z* calcd for $[M+Na]^+$ $C_{36}H_{58}O_8Na$:641.4024; found: 641.4017.

***δ-Oleanolic acid 28-O- [β-D-glycuronic acid] ester* (23)**

To a solution of δ-oleanolic acid (**10**, 200 mg, 0.438 mmol) and 1-Bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucuronic acid methyl ester **S3** (261 mg, 0.657 mmol) in CH_2Cl_2/H_2O (8 mL/5 mL) was added K_2CO_3 (151 mg,1.093 mmol) and Bu_4NBr (56 mg,0.174 mmol). The mixture was refluxed under nitrogen atmosphere. After completion of the reaction, the mixture was diluted with CH_2Cl_2 (10 mL), and the organic layer was washed with water (20 mL×2), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (petroleum ether/EtOAc, 3:1) to afford **23a** (308 mg, 91%).

To a solution of **23a** (308 mg, 0.398 mmol) in THF (8 mL) was added 3N sodium hydroxide (2 mL), and the mixture was stirred at room temperature for 8 h. The mixture was neutralized with HCl (1 M), and then ethyl acetate (8 mL) was added. The mixture was stirred at room temperature for 0.5 h, and the resulting suspension was filtered. The filter cake was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 10:1) to afford **23** (234 mg, 93%) as a white powder. Mp 280 ℃ (decomposed); IR (KBr) ν_{max} 3487, 2941, 2872, 1725, 1450, 1093 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.37 (d, *J* = 8.1 Hz, 1H, H-C(1′)), 5.24 (d, *J* = 5.9 Hz, 1H), 5.20 (s, 1H), 4.30 (d, *J* = 4.7 Hz, 1H), 3.71 (d, *J* = 9.1 Hz, 1H), 3.30 (s, 2H), 3.13 (d, *J* = 6.4 Hz, 1H), 3.02 (s, 1H), 2.71 (d, *J* = 14.1 Hz, 1H, H-C(12)), 2.38 (d, *J* = 14.2 Hz, 1H, H-C(12)), 2.11 (d, *J* = 11.6 Hz, 1H, H-C(19)), 1.13 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.71 (s, 3H, CH_3), 0.67 (s, 3H,

CH_3). ^{13}C NMR (75 MHz, DMSO- d_6) δ 174.43 (C-28), 169.85, 137.95 (C-13), 127.07 (C-18), 94.26, 76.79, 76.10, 76.01, 72.12, 71.34, 54.85, 50.06, 47.85, 43.83, 40.86, 38.41, 36.82, 36.21, 34.95, 34.46, 32.18, 31.86, 28.16, 27.07, 26.38, 24.68, 23.90, 21.18, 20.72, 17.99, 17.21, 16.15, 15.86.HRMS(ESI): *m/z* calcd for $[M-H]^-$ $C_{36}H_{55}O_9$:631.3852; found: 631.3841.

4.1.4. Synthesis of δ-OA 3-glycosides ***27~32***

δ-Oleanolic acid 3-O-β-D-glucopyranoside **(28)**

A suspension of δ-O benzyl ester **TM-1** (550 mg, 1.01 mmol), trichloroacetimidate **S8** (969 mg, 1.30 mmol) and powdered 4 Å molecular sieves (171 mg) was stirred for 30 min at 0°C in dry CH_2Cl_2 (15 mL), and then TMSOTf (55 μL, 0.3 mmol) was added. The mixture was stirred at room temperature for 3 h before the reaction was quenched by Et_3N (0.1 mL). The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (petroleum ether/ EtOAc, 6:1) to afford **28a** (779 mg, 69 %).

To a solution of **28a** (779 mg, 0.693 mmol) in MeOH-CH_2Cl_2 (10 mL/10 mL) was added $NaOCH_3$ (187 mg, 3.46 mmol), and the mixture was stirred at room temperature for 5 h. The mixture was neutralized with DOWEX50WX2-100 ion-exchange resin (H^+), and then filtered. The filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 10:1) to afford **28b** (330 mg, 67%).

A mixture of **28b** (330 mg, 0.467 mmol) and 10% Pd/C (33 mg) was stirred overnight at room temperature under H_2 at atmospheric pressure. The mixture was filtered through celite, and the filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 10:1) to afford **28** (145 mg, 50%) as a white powder. Mp 235-237 °C; IR (KBr) ν_{max} 3457, 3393, 2938, 2874, 1701, 1450, 1082, 1028 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 12.00 (s, 1H, COOH), 4.89-4.76 (m, 3H), 4.33 (t, *J* = 5.5 Hz, 1H), 4.15 (d, *J* = 7.7 Hz, 1H), 3.72-3.58 (m, 1H), 3.47-3.36 (m, 1H), 3.17-2.91 (m, 5H), 2.70 (d, *J* = 14.7 Hz, 1H, H-C(12)), 2.37 (d, *J* = 13.7 Hz, 1H, H-C(12)), 2.08-2.00 (m, 1H, H-C(19)), 1.12

(s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.83 (s, 6H, 2×CH_3), 0.74 (s, 3H, CH_3), 0.70 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.31 (C-28), 137.02 (C-13), 127.99 (C-18), 105.36, 87.90, 76.88, 76.56, 73.94, 70.19, 61.22, 55.09, 50.15, 47.51, 43.90, 40.83, 40.62, 38.50, 36.54, 36.48, 35.30, 34.59, 32.45, 32.27, 31.99, 27.56, 26.69, 25.65, 24.70, 23.92, 21.29, 20.77, 17.79, 17.47, 16.33, 16.17. HRMS(ESI): *m/z* calcd for $[M+Na]^+$ $C_{36}H_{58}O_8Na$:641.4024; found: 641.4026.

δ-OA saponins **27**, **29**, **30~32** were synthesized according to the synthetic route shown in **Scheme 1** and the procedure described for preparation of **28**.

***δ-Oleanolic acid 3-O-β-D-xylopyranoside* (27)**

White powder. Mp 202-205 ℃; IR (KBr) ν_{max} 3548, 3454, 3414, 2946, 1679, 1227, 1041 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.02 (s, 1H, COOH), 4.89 (s, 3H), 4.15 (d, *J* = 7.4 Hz, 1H), 3.68 (dd, *J* = 11.0, 4.9 Hz, 1H), 3.15-2.93 (m, 4H), 2.72 (d, *J* = 15.7 Hz, 1H, H-C(12)), 2.39 (d, *J* = 13.8 Hz, 1H, H-C(12)), 2.12-2.00 (m, 1H,H-C(19)), 1.15 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.86 (s, 6H, 2×CH_3), 0.76 (s, 3H, CH_3), 0.73 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.29 (C-28), 137.00 (C-13), 127.99 (C-18), 106.13, 87.69, 76.72, 73.75, 69.60, 65.56, 55.02, 50.12, 47.50, 43.89, 40.82, 40.61, 38.70, 38.39, 36.55, 36.47, 35.30, 34.56, 32.44, 32.27, 31.98, 27.48, 26.69, 25.84, 24.70, 23.90, 21.27, 20.77, 17.76, 17.46, 16.28, 16.15. HRMS(ESI): *m/z* calcd for $[M+Na]^+$ $C_{35}H_{56}O_7Na$:611.3918; found: 611.3909.

***δ-Oleanolic acid 3-O-α-L-arabinopyranoside* (29)**

White powder. Mp 245-247 ℃; IR (KBr) ν_{max} 3436, 2943, 2875, 1701, 1456, 1088; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.03 (s, 1H, COOH), 4.80 (s, 1H), 4.59-4.38 (m, 2H), 4.13 (d, *J* = 5.0 Hz, 1H), 3.71-3.56 (m, 2H), 3.37 (s, 3H), 3.08-2.97 (m, 1H, H-C(3)), 2.70 (d, *J* = 14.6 Hz, 1H, H-C(12)), 2.37 (d, *J* = 13.8 Hz, 1H, H-C(12)), 2.11-2.00 (m, 1H, H-C(19)), 1.12 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.83 (s, 6H, 2×CH_3), 0.74 (s, 3H, CH_3), 0.71 (s, 3H, CH_3).^{13}C NMR (75 MHz, DMSO-d_6) δ 177.31 (C-28), 137.01 (C-13), 128.02 (C-18), 109.51, 105.78, 87.72, 72.67, 70.99, 67.56, 65.00, 55.03, 50.13, 47.52, 43.91, 40.84, 40.63, 38.68, 38.40, 36.58, 36.49, 35.32, 34.58, 32.46, 32.29, 32.00, 27.60, 26.70, 25.77, 24.72,

23.93, 21.29, 20.80, 17.81, 17.47, 16.32, 16.16. HRMS(ESI): *m/z* calcd for $[M+Na]^+$ $C_{35}H_{56}O_7Na$:611.3918; found: 611.3912.

***δ*-Oleanolic acid 3-O-*β*-D-galactopyranoside (30)**

White powder. Mp 270-272 ℃; IR (KBr) ν_{max} 3480, 3421, 2943, 2874, 1695, 1453, 1071 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 12.03 (s, 1H, COOH), 4.70 (s, 1H), 4.59 (s, 1H), 4.52-4.42 (m, 1H), 4.29 (d, *J* = 3.3 Hz, 1H), 4.13 (d, *J* = 6.2 Hz, 1H), 3.64 (s, 1H), 3.59-3.39 (m, 2H), 3.29 (s, 3H), 3.11-3.00 (m, 1H, H-C(3)), 2.71 (d, *J* = 13.0 Hz, 1H, H-C(12)), 2.39 (d, *J* = 13.8 Hz, 1H, H-C(12)), 2.12-2.00 (m, 1H, H-C(19)), 1.14 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.85 (s, 6H, 2×CH_3), 0.76 (s, 3H, CH_3), 0.73 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.28 (C-28), 137.02 (C-13), 127.98 (C-18), 105.97, 87.89, 74.83, 73.55, 71.11, 68.03, 60.33, 55.11, 50.13, 47.50, 43.89, 40.83, 40.60, 38.47, 36.54, 36.47, 35.29, 34.57, 32.44, 32.26, 31.98, 27.59, 26.69, 25.76, 24.69, 23.91, 21.28, 20.77, 17.78, 17.46, 16.33, 16.14.HRMS(ESI): *m/z* calcd for $[M+Na]^+$ $C_{36}H_{58}O_8Na$:641.4024; found: 641.4018.

***δ*-Oleanolic acid 3-O-*α*-L-rhamnopyranoside (31)**

White powder. Mp 275-277 ℃; IR (KBr) ν_{max} 3586, 3348, 3247, 2937, 1669, 1454, 1047, 840, 808 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 12.03 (s, 1H, COOH), 4.73-4.65 (m, 2H), 4.61 (s, 1H), 4.48 (d, *J* = 5.7 Hz, 1H), 3.65 (s, 1H), 3.60-3.49 (m, 1H), 3.49-3.39 (m, 1H), 3.27-3.14 (m, 1H), 3.10-3.00 (m, 1H, H-C(3)), 2.72 (d, *J* = 14.8 Hz, 1H, H-C(12)), 2.40 (d, *J* = 13.3 Hz, 1H, H-C(12)), 2.12-2.03 (m, 1H, H-C(19)), 1.17-1.09 (m, 6H, 2×CH_3), 0.91 (s, 6H, 2×CH_3), 0.86 (s, 6H, 2×CH_3), 0.73 (s, 6H, 2×CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 177.38 (C-28), 137.03 (C-13), 128.03 (C-18), 102.87, 87.46, 72.09, 70.80, 70.72, 68.47, 54.67, 50.07, 47.56, 43.95, 40.83, 40.65, 38.58, 38.25, 36.59, 36.51, 35.32, 34.54, 32.47, 32.32, 32.03, 27.87, 26.69, 25.04, 24.69, 23.93, 21.32, 20.82, 17.90, 17.75, 17.50, 16.30, 16.16. HRMS(ESI) : *m/z* calcd for $[M+Na]^+$ $C_{36}H_{62}O_7N$:620.4521; found: 620.4516.

***δ*-oleanolic acid 3-O-*α*-L-rhamnopyranosyl -(1→3)- *α*-L-arabinopyranoside (32)**

White powder. Mp 238-241 ℃; IR (KBr) ν_{max} 3443, 2941, 1703, 1453, 1142, 1053, 980 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 12.02 (s, 1H, COOH), 5.02 (d, *J* =

5.5 Hz, 1H, H-C(1′)), 4.82 (s, 1H, H-C(1″)), 4.60 (dd, *J* = 10.7, 4.7 Hz, 2H), 4.49 (d, *J* = 4.4 Hz, 1H), 4.43 (d, *J* = 5.9 Hz, 1H), 4.18 (d, *J* = 6.6 Hz, 1H), 3.79-3.64 (m, 4H), 3.63-3.35 (m, 6H), 3.30-3.11 (m, 2H), 3.10-3.00 (m, 1H, H-C(3)), 2.71 (d, *J* = 13.5 Hz, 1H, H-C(12)), 2.38 (d, *J* = 13.4 Hz, 1H, H-C(12)), 2.11-2.01 (m, 1H, H-C(19)), 1.13 (m, 3H, CH_3), 1.11 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.85 (s, 6H, 2×CH_3), 0.76 (s, 3H, CH_3), 0.72 (s, 3H, CH_3).^{13}C NMR (126 MHz, DMSO-d_6) δ 177.43 (C-28), 137.10 (C-13), 128.04 (C-18), 105.96, 101.75, 87.90, 72.16, 70.50, 70.45, 69.74, 68.34, 67.55, 55.06, 50.15, 47.59, 43.97, 40.88, 40.69, 38.87, 38.42, 36.63, 36.54, 35.36, 34.63, 32.51, 32.36, 32.07, 27.63, 26.74, 25.87, 24.77, 23.97, 21.36, 21.35, 20.85, 17.86, 17.57, 16.38, 16.24. HRMS(ESI):*m/z* calcd for $[M+Na]^+$ $C_{41}H_{66}O_{11}Na$:757.4497; found:757.4492.

4.1.5 Synthesis of δ-oleanolic 3,28-diglycosides ***33~40***

3-O-α-L-arabinopyranosyl-28-O-β-D-xylopyranosyl δ-oleanolate **(33)**

A solution of **29a** (1.2 g, 1.211 mmol) and 10% Pd/C (120 mg) in THF (15 mL) was stirred overnight at room temperature under H_2 at atmospheric pressure. The mixture was filtered through celite, and filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (petroleum ether/EtOAc,4:1) to afford **29c** (1 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22-7.84 (m, 6H), 7.62 -7.28 (m, 9H), 5.81-5.72 (m, 1H), 5.67 (s, 1H), 5.60 (dd, *J* = 8.7, 3.3 Hz, 1H), 4.79 (d, *J* = 6.2 Hz, 1H), 4.33 (dd, *J* = 12.8, 3.7 Hz, 1H), 3.87 (d, *J* = 12.3 Hz, 1H), 3.17 (dd, *J* = 10.7, 5.1 Hz, 1H), 2.74 (d, *J* = 14.3 Hz, 1H), 2.44 (d, *J* = 14.0 Hz, 1H), 2.22-2.15 (m, 1H), 1.14 (s, 3H), 0.92 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H), 0.78 (s, 3H), 0.74 (s, 3H), 0.64 (s, 3H).

To a solution of **29c** (150 mg, 0.167mmol) and benzoylbromoglycoside **S1** (114 mg, 0.217 mmol) in CH_2Cl_2/H_2O (10 mL/1 mL) was added K_2CO_3 (46 mg, 0.333mmol) and Bu_4NBr (22 mg, 0.068 mmol). The mixture was refluxed under nitrogen atmosphere. After completion of the reaction, the mixture was diluted with CH_2Cl_2 (10 mL), and the organic layer was washed with water (20 mL×2), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was

subjected to silica gel column chromatography (petroleum ether/EtOAc,5:1) to afford **33a**. To a solution of **33a** (198 mg, 0.147 mmol) in MeOH-CH_2Cl_2 (16 mL/8 mL) was added $NaOCH_3$ (40 mg, 0.740 mmol), and the mixture was stirred at room temperature for 10 h. The mixture was neutralized with DOWEX50WX2-100 ion-exchange resin (H^+), and then filtered and concentrated. The residue was subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 15:1) to afford **33** (47 mg, two steps: 44%) as a white powder. Mp 224-226 ℃; IR (KBr) ν_{max} 3427, 2940, 2872, 1716, 1653, 1451, 1362, 1090, 996 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 5.26 (d, J = 7.6 Hz, 1H, H-C(1′)), 5.11 (d, J = 6.0 Hz, 1H, H-C(1″)), 5.06 (d, J = 4.4 Hz, 1H), 5.00 (d, J = 4.8 Hz, 1H), 4.77 (d, J = 3.4 Hz, 1H), 4.50-4.40 (m, 2H), 4.13 (d, J = 5.6 Hz, 1H), 3.77-3.57 (m, 3H), 3.37 (s, 3H), 3.27-2.96 (m, 5H), 2.76-2.63 (m, 1H, H-C(12)), 2.37 (d, J = 13.7 Hz, 1H, H-C(12)), 2.15-2.05 (m, 1H, H-C(19)), 1.12 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.84 (s, 6H, 2×CH_3), 0.74 (s, 3H, CH_3), 0.70 (s, 3H,CH_3).^{13}C NMR (75 MHz, DMSO-d_6) δ 174.52 (C-28), 137.76 (C-13), 127.12 (C-18), 105.67, 94.98, 87.68, 76.40, 72.64, 72.03, 70.96, 69.27, 67.48, 66.22, 64.89, 54.98, 49.99, 47.82, 43.77, 40.86, 38.69, 38.37, 36.53, 36.23, 35.05, 34.40, 32.17, 31.84, 27.57, 26.36, 25.70, 24.68, 23.83, 21.13, 20.65, 17.75, 17.73, 17.19, 16.25, 16.12. HRMS(ESI):*m/z* calcd for $[M+Na]^+$ $C_{40}H_{64}O_{11}Na$:743.4341; found:743.4334.

δ-OA saponins **34~36** were synthesized according to the synthetic route shown in **Scheme 2** and the procedure described for preparation of **33**.

3-O-α-L-arabinopyranosyl-28-O-β-D-galactopyranosyl δ-oleanolate (**34**)

White powder. Mp 229-231 ℃; IR (KBr) ν_{max} 3415, 2944, 2869, 1731, 1452, 1066 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 5.30 (d, J = 7.4 Hz, 1H, H-C(1′)), 4.86 (d, J = 5.5 Hz, 1H, H-C(1″)), 4.80-4.72 (m, 2H), 4.60-4.52 (m, 1H), 4.49-4.39 (m, 3H), 4.13 (d, J = 6.0 Hz, 1H), 3.74-3.50 (m, 4H), 3.48-3.32 (m, 7H), 3.06-2.98 (m, 1H, H-C(3)), 2.75-2.65 (m, 1H, H-C(12)), 2.37 (d, J = 13.5 Hz, 1H, H-C(12)), 2.16-2.07 (m, 1H, H-C(19)), 1.12 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.83 (s, 6H, 2×CH_3), 0.75 (s, 3H, CH_3), 0.71 (s, 3H, CH_3).^{13}C NMR (75 MHz, DMSO-d_6) δ 174.32 (C-28), 137.73 (C-13), 127.31 (C-18), 105.73, 94.93, 87.70, 75.96, 73.45,

72.66, 70.97, 69.56, 67.79, 67.52, 64.94, 60.02, 55.00, 50.03, 47.83, 43.81, 40.82, 38.78, 38.37, 36.55, 36.23, 34.94, 34.45, 32.19, 31.96, 31.86, 27.58, 26.48, 25.73, 24.70, 23.95, 20.72, 17.79, 17.23, 16.27, 16.16. HRMS(ESI):*m/z* calcd for $[M+Na]^+$ $C_{41}H_{66}O_{12}Na$:773.4447; found:773.4438.

3-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl δ-oleanolate (**35**)

White powder. Mp 252-254 ℃; IR (KBr) ν_{max} 3429, 2939, 1740, 1720, 1451, 1140, 1065, 967 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 5.80 (s, 1H, H-C(1′)), 5.13 (d, *J* = 4.1 Hz, 1H), 4.95 (d, *J* = 5.3 Hz, 1H), 4.77 (dd, *J* = 10.2, 5.0 Hz, 2H), 4.46 (t, *J* = 5.4 Hz, 2H), 4.14 (d, *J* = 5.9 Hz, 1H), 3.74-3.58 (m, 3H), 3.56-3.34 (m, 5H), 3.32-3.20 (m, 2H), 3.08-2.97 (m, 1H, H-C(3)), 2.73 (d, *J* = 12.6 Hz, 1H, H-C(12)), 2.44 (d, *J* = 14.1 Hz, 1H, H-C(12)), 2.10-2.01 (m, 1H, H-C(19)), 1.14 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.76 (s, 3H, CH_3), 0.73 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.99 (C-28), 138.22 (C-13), 126.89 (C-18), 105.70, 94.67, 87.64, 72.63, 71.32, 71.22, 70.95, 70.59, 69.44, 67.49, 64.91, 54.93, 49.96, 47.96, 43.94, 40.64, 39.52, 38.76, 38.33, 36.53, 36.19, 35.41, 34.33, 32.19, 31.94, 27.56, 26.66, 25.72, 24.73, 23.91, 21.18, 20.79, 17.74, 17.64, 17.37, 16.25, 16.08. HRMS(ESI):*m/z* calcd for $[M+Na]^+$ $C_{41}H_{66}O_{12}Na$:757.4497; found:757.4488.

3-O-α-L-arabinopyranosyl-28-O-β-D-glucopyranosyl δ-oleanolate (**36**)

White powder. Mp 225-228 ℃; IR (KBr) ν_{max} 3406, 2941, 2874, 1747, 1645, 1452, 1070 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 5.35 (d, *J* = 8.0 Hz, 1H, H-C(1′)), 5.08 (d, *J* = 6.1 Hz, 1H, H-C(1″)), 5.02 (d, *J* = 4.6 Hz, 1H), 4.96 (d, *J* = 5.2 Hz, 1H), 4.78 (s, 1H), 4.46 (d, *J* = 3.3 Hz, 3H), 4.15 (d, *J* = 5.4 Hz, 1H), 3.77-3.57 (m, 3H), 3.52-3.35 (m, 3H), 3.30-2.98 (m, 6H), 2.78-2.68 (m, 1H, H-C(12)), 2.39 (d, *J* = 14.1 Hz, 1H, H-C(12)), 2.20-2.08 (m, 1H, H-C(19)), 1.15 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.86 (s, 6H, 2×CH_3), 0.77 (s, 3H, CH_3), 0.73 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.31 (C-28), 137.74 (C-13), 127.27 (C-18), 105.74, 94.37, 87.71, 77.76, 76.69, 72.65, 72.51, 70.97, 69.78, 67.53, 64.95, 60.85, 55.01, 50.04, 47.82, 43.79, 40.87, 38.78, 38.40, 36.56, 36.24, 34.98, 34.44, 32.22, 31.86,

27.59, 26.44, 25.76, 24.72, 23.92, 21.18, 20.72, 17.80, 17.21, 16.28, 16.17. HRMS(ESI): *m/z* calcd for $[M+Na]^+$ $C_{41}H_{66}O_{12}Na$:773.4447; found:773.4446.

***3-O-β-D-glucopyranosyl-28-O-β-D-xylopyranosyl δ-oleanolate*（37）**

A solution of **28a** (2.0 g, 1.780 mmol) and 10% Pd/C (200 mg) in THF (20 mL) was stirred overnight at room temperature under H_2 at atmospheric pressure. The mixture was filtered through celite, and filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (petroleum ether/EtOAc, 2:1) to afford **28c** (1.32 g, 72%).^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10-7.75 (m, 8H), 7.62-7.27 (m, 12H), 5.91 (t, *J* = 9.6 Hz, 1H), 5.66-5.49 (m, 2H), 4.86 (d, *J* = 7.9 Hz, 1H), 4.64-4.48 (m, 2H), 4.22-4.07 (m, 1H), 3.10 (dd, *J* = 11.5, 4.6 Hz, 1H), 2.76 (d, *J* = 14.8 Hz, 1H), 2.45 (d, *J* = 14.1 Hz, 1H), 2.21-2.12 (m, 1H), 1.13 (s, 3H), 0.94 (s, 3H), 0.85 (s, 3H), 0.79 (s, 3H), 0.76 (s, 3H), 0.68 (s, 3H), 0.60 (s, 3H).

To a solution of **28c** (200 mg, 0.193 mmol) and benzoylbromoglycoside **S1** (132 mg, 0.251 mmol) in CH_2Cl_2/H_2O (15 mL/1.5 mL) was added K_2CO_3 (67 mg, 0.485 mmol) and Bu_4NBr (25 mg, 0.078 mmol). The mixture was refluxed under nitrogen atmosphere. After completion of the reaction, the mixture was diluted with CH_2Cl_2 (10 mL), and the organic layer was washed with water (20 mL×2), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was subjected to was subjected to silica gel column chromatography (petroleum ether/EtOAc,7:1) to afford **37a**. To a solution of **37a** (285 mg, 0.193 mmol) in MeOH-CH_2Cl_2 (8 mL/4 mL) was added $NaOCH_3$ (52 mg, 0.963 mmol), and the mixture was stirred at room temperature for 10 h. The mixture was neutralized with DOWEX50WX2-100 ion-exchange resin (H^+), and then filtered and concentrated. The residue was subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 15:1) to afford **37** (74 mg, two steps:51%) as a white powder. Mp 215-217 ℃; IR (KBr) ν_{max} 3405, 2942, 2874, 1732, 1643, 1454, 1077 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.29 (d, *J* = 7.7 Hz, 1H, H-C(1′)), 5.13 (d, *J* = 6.1 Hz, 1H, H-C(1″)), 5.07 (d, *J* = 4.7 Hz, 1H), 5.02 (d, *J* = 4.9 Hz, 1H), 4.92-4.80 (m, 3H), 4.35 (t, *J* = 5.6 Hz, 1H), 4.17 (d, *J* = 7.6 Hz, 1H), 3.76 (dd, *J* = 11.0, 4.8 Hz, 1H), 3.68 (dd, *J* = 11.0, 5.1 Hz, 1H),

3.52-3.39 (m, 1H), 3.31-3.03 (m, 8H), 3.04-2.94 (m, 1H, H-C(3)), 2.73 (d, *J* = 13.2 Hz, 1H, H-C(12)), 2.40 (d, *J* = 13.8 Hz, 1H, H-C(12)), 2.18-2.08 (m, 1H, H-C(19)), 1.15 (s, 3H,CH_3), 1.00 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.87 (s, 6H, 2×CH_3), 0.77 (s, 3H, CH_3), 0.73 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.66 (C-28), 137.87 (C-13), 127.16 (C-18), 105.43, 95.02, 87.94, 76.90, 76.62, 76.51, 73.96, 72.10, 70.21, 69.33, 66.32, 61.24, 55.11, 50.09, 47.90, 43.84, 40.92, 40.55, 38.78, 38.56, 36.57, 36.32, 35.10, 34.52, 32.26, 31.96, 27.59, 26.39, 25.70, 24.76, 23.89, 21.23, 20.69, 17.80, 17.29, 16.38, 16.26. HRMS(ESI):*m/z* calcd for $[M+Na]^+$ $C_{41}H_{66}O_{12}Na$:773.4447; found:773.4446.

δ-OA saponins **38~40** were synthesized according to the synthetic route shown in **Scheme 3** and the procedure described for preparation of **37**.

***3-O-β-D-glucopyranosyl-28-O-β-D-galactopyranosyl δ-oleanolate* (38)**

White powder. Mp 225-227 ℃; IR (KBr) ν_{max} 3589, 3547, 3357, 2942, 1742, 1649, 1450, 1079 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.32 (d, *J* = 7.4 Hz, 1H, H-C(1′)), 4.98-4.65 (m, 5H), 4.57 (s, 1H), 4.47 (d, *J* = 3.5 Hz, 1H), 4.34 (s, 1H), 4.17 (d, *J* = 7.7 Hz, 1H), 3.76-3.37 (m, 8H), 3.19-3.02 (m, 4H), 3.02-2.92 (m, 1H, H-C(3)), 2.80-2.65 (m, 1H, H-C(12)), 2.39 (d, *J* = 13.9 Hz, 1H, H-C(12)), 2.17-2.06 (m, 1H, H-C(19)), 1.14 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.85 (s, 6H, CH_3), 0.76 (s, 3H, CH_3), 0.73 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.88 (C-28), 138.17 (C-13), 127.58 (C-18), 105.58, 95.22, 88.32, 77.08, 76.76, 76.23, 74.22, 73.70, 70.45, 69.83, 68.13, 61.47, 60.39, 55.39, 50.38, 48.19, 44.13, 41.14, 40.74, 39.52, 38.81, 36.83, 36.52, 35.25, 34.79, 32.53, 32.31, 32.20, 27.87, 26.77, 25.96, 25.15, 25.03, 24.26, 21.53, 20.99, 18.09, 17.57, 16.64, 16.52.HRMS(ESI):*m/z* calcd for $[M+Na]^+$ $C_{42}H_{68}O_{13}Na$:803.4552; found：803.4551.

***3-O-β-D-glucopyranosyl-28- O-α-L-arabinopyranosyl δ-oleanolate* (39)**

White powder. Mp 203-206 ℃; IR (KBr) ν_{max} 3386, 2940, 2874, 1745, 1644, 1450, 1077, 974 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.34 (d, *J* = 5.8 Hz, 1H, H-C(1′)), 5.05 (d, *J* = 4.9 Hz, 1H, H-C(1″)), 4.94-4.78 (m, 3H), 4.73 (d, *J* = 4.4 Hz, 1H), 4.62 (d, *J* = 4.1 Hz, 1H), 4.34 (t, *J* = 5.7 Hz, 1H), 4.17 (d, *J* = 7.7 Hz, 1H),

3.83-3.60 (m, 3H), 3.56-3.37 (m, 4H), 3.29-2.92 (m, 5H), 2.73 (d, *J* = 13.3 Hz, 1H, H-C(12)), 2.39 (d, *J* = 13.9 Hz, 1H, H-C(12)), 2.21-2.10 (m, 1H, H-C(19)), 1.15 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.86 (s, 6H, 2×CH_3), 0.75 (d, *J* = 9.5 Hz, 6H, 2×CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.45 (C-28), 137.82 (C-13), 127.33 (C-18), 105.43, 94.82, 87.94, 76.90, 76.62, 73.96, 72.05, 70.21, 69.52, 66.67, 65.22, 61.24, 55.11, 50.11, 48.01, 43.89, 40.86, 38.79, 38.55, 36.57, 36.35, 35.09, 34.52, 32.26, 32.05, 31.96, 27.60, 26.54, 25.70, 24.75, 23.96, 21.26, 20.71, 17.81, 17.33, 16.37, 16.26. HRMS(ESI):*m/z* calcd for $[M+Na]^+$ $C_{44}H_{66}O_{12}Na$:773.4447; found：773.4455.

***3-O-β-D-glucopyranosyl-28- O-α-L-rhamnopyranosyl δ-oleanolate* (40)**

White powder. Mp 254-256 ℃; IR (KBr) ν_{max} 3396, 2940, 2874, 1741, 1643, 1451, 1079, 1026 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.80 (s, 1H, H-C(1′)), 5.12 (d, *J* = 4.2 Hz, 1H, H-C(1″)), 4.95 (d, *J* = 5.3 Hz, 1H), 4.84 (t, *J* = 5.2 Hz, 3H), 4.75 (d, *J* = 5.7 Hz, 1H), 4.34 (t, *J* = 5.7 Hz, 1H), 4.16 (d, *J* = 7.7 Hz, 1H), 3.74-3.57 (m, 2H), 3.55-3.41 (m, 3H), 3.29-3.21 (m, 1H), 3.18-2.92 (m, 5H), 2.73 (d, *J* = 12.9 Hz, 1H, H-C(12)), 2.44 (d, *J* = 13.8 Hz, 1H, H-C(12)), 2.10-2.00 (m, 1H, H-C(19)), 1.14 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.76 (s, 3H, CH_3), 0.74 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.13 (C-28), 138.35 (C-13), 126.96 (C-18), 105.43, 94.73, 87.95, 76.92, 76.64, 73.99, 71.44, 71.27, 70.64, 70.24, 69.53, 61.27, 55.09, 50.08, 48.07, 44.04, 40.73, 40.46, 38.80, 38.53, 36.59, 36.28, 35.51, 34.47, 32.30, 32.07, 27.62, 26.73, 25.71, 24.83, 24.00, 21.31, 20.85, 17.83, 17.76, 17.49, 16.40, 16.24. HRMS (ESI): *m/z* calcd for $[M+Na]^+$ $C_{42}H_{68}O_{12}Na$:787.4603; found：787.4596.

4.1.6 Synthesis of δ-OA 3-diglycosides ***41~43***

***δ-oleanolic acid 3-O-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranoside*(41)**

TO a solution of **29a** (1.29 g, 1.301 mmol) in CH_2Cl_2/CH_3OH (5 mL/10 mL) was added CH_3ONa (211 mg, 3.906 mmol). The mixture was stirred at room temperature for 6 h. After completion of the reaction, the mixture was neutralized with DOWEX50WX2-100 ion-exchange resin (H^+), and then was filtered and concentrated.

The residue was subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 10:1) to afford **29d** (797 mg, 90%) as a white powder. To a solution of **29d** (797 mg,1.174 mmol) in dry DMF (15 mL) was added *p*-TsOH·H_2O (33 mg, 0.173 mmol) and $Me_2C(OMe)_2$ (433 μL, 3.552 mmol) at 0 ℃. The mixture was then allowed to warm up to room temperature and stirred for 4 h before Et_3N (0.1 mL) was added. The solution was concentrated and purified through silica gel column chromatography (petroleum ether/EtOAc,4:1) to afford **TM-2** (480 mg, 57%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43-7.27 (m, 5H), 5.11 (dd, *J* = 12.3 Hz, 2H), 4.25-4.16 (m, 3H), 4.06 (dd, *J* = 7.4, 5.8 Hz, 1H), 3.75 (dd, *J* = 13.9, 3.6 Hz, 1H), 3.68-3.59 (m, 1H), 3.13 (dd, *J* = 11.4, 4.7 Hz, 1H), 2.79-2.67 (m, 1H), 2.40 (d, *J* = 14.3 Hz, 1H), 2.25 (d, *J* = 2.4 Hz, 1H), 2.23-2.14 (m, 1H), 1.37 (s, 3H), 1.34 (s, 3H), 1.13 (s, 3H), 0.98 (s, 3H), 0.86 (s, 3H), 0.86 (s, 3H), 0.80 (s, 3H), 0.78 (s, 3H), 0.72 (s, 3H).ESI-MS: *m/z* 741.6 $[M+23]^+$.

A suspension of **TM-2** (150 mg, 0.209 mmol), **S7** (165 mg,0.271 mmol) and powdered 4A molecular sieves (225mg) in dry CH_2Cl_2 (15 mL) was stirred for 40 min and then cooled to -78℃ BF_3·Et_2O (19 μL, 0.146 mmol) was added and the mixture was stirred at -78 ℃ for 2 h before the reaction was quenched by Et_3N (0.1 mL).The suspension was then filtered and the filtrate was concentrated and subjected to a silica gel chromatography (petroleum ether/EtOAc,10:1) to afford **41a.** *p*-TsOH·H_2O (24 mg) was added to a solution of **41a** (144 mg, 0.124 mmol) in CH_2Cl_2-CH_3OH (4 mL/8 mL) and the solution was stirred at room temperature for 5 h. When TLC showed that deprotection had completed, Et_3N (0.1 mL) was added and the mixture was concentrated and purified through a silica gel column chromatography (petroleum ether/EtOAc,2:1) to afford **41b** (100 mg, 72%) as a white powder.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (t, *J* = 8.2 Hz, 6H), 7.59-7.27 (m, 14H), 5.75 (t, *J* = 7.0 Hz, 1H), 5.41-5.33 (m, 1H), 5.28 (dd, *J* = 10.9, 6.6 Hz, 1H), 5.11 (d, *J* = 6.9 Hz, 2H), 5.08-5.01 (m, 1H), 4.90 (s, 1H), 4.46 (dd, *J* = 12.1, 4.0 Hz, 1H), 3.97 (s, 1H), 3.91-3.63 (m, 4H), 3.57 (dd, *J* = 11.1, 5.0 Hz, 1H), 3.38 (d, *J* = 9.7 Hz, 1H), 3.24-3.14 (m, 1H), 2.74 (d, *J* = 12.5 Hz, 1H), 2.40 (d, *J* = 14.2 Hz, 1H), 2.26-2.09 (m, 2H), 1.13 (s, 3H), 0.98 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.79 (s, 6H), 0.72 (s, 3H).

A suspension of **41b** (280 mg,0.189 mmol) and CH_3ONa (51 mg,0.944 mmol) in CH_2Cl_2/CH_3OH (5 mL/10 mL) was stirred at room temperature for 5h and then neutralized with DOWEX50WX2-100 ion-exchange resin (H^+). The mixture was filtered and filtrate was concentrated. The residue was dissolved in CH_3OH (10 mL), and Pd/C (10 mg) was added. The mixture was stirred overnight at room temperature under H_2 at atmospheric pressure. The mixture was filtered through celite, and filtrate was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (CH_2Cl_2/CH_3OH,10:1) to afford **41** (38 mg, two steps 62%).Mp 235-237 ℃; IR (KBr) ν_{max} 3398, 2940, 2864, 1733, 1645, 1455, 1361, 1047, 990 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 12.02 (s, 1H, COOH), 5.33 (d, *J* = 4.0 Hz, 1H, H-C(1′)), 5.00-4.87 (m, 3H), 4.61 (d, *J* = 4.3 Hz, 1H), 4.33 (d, *J* = 7.2 Hz, 2H), 3.74-3.54 (m, 5H), 3.47-3.36 (m, 1H), 3.29-3.18 (m, 1H), 3.17-3.09 (m, 1H), 3.07-2.97 (m, 3H), 2.79-2.66 (m, 1H, H-C(12)), 2.40 (d, *J* = 14.0 Hz, 1H, H-C(12)), 2.12-2.00 (m, 1H, H-C(19)), 1.15 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.86 (s, 6H, 2×CH_3), 0.76 (s, 3H, CH_3), 0.73 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.24 (C-28), 136.99 (C-13), 127.97 (C-18), 104.62, 103.54, 87.92, 79.13, 76.28, 74.23, 71.59, 69.52, 66.71, 65.79, 63.90, 55.00, 50.09, 47.48, 43.88, 40.81, 40.58, 38.77, 38.38, 36.54, 36.44, 35.27, 34.54, 32.42, 32.24, 31.95, 27.24, 26.67, 25.71, 24.68, 23.90, 21.24, 20.77, 17.75, 17.42, 16.11, 15.71.HRMS(ESI):*m/z* calcd for $[M+Na]^+$ $C_{40}H_{64}O_{11}Na$:743.4341; found：743.4340.

δ-OA saponins **41~43** were synthesized according to the synthetic route shown in **Scheme 4** and the procedure described for preparation of **41**.

δ-oleanolic acid 3-O-α-L-rhamnopyranosyl -(1→2)- α-L-arabinopyranoside（**42**）

White powder. Mp 267-269 ℃; IR (KBr) ν_{max} 3537, 3443, 2941, 2870, 1688, 1448, 1059, 988 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 12.02 (s, 1H, COOH), 5.06 (s, 1H, H-C(1′)), 4.68 -4.53 (m, 4H), 4.44 (s, 1H), 4.34 (d, *J* = 5.1 Hz, 1H), 3.76-3.66 (m, 3H), 3.60 (d, *J* = 5.4 Hz, 3H), 3.53-3.44 (m, 1H), 3.43-3.35 (m, 1H), 3.27-3.15 (m, 1H), 3.09-2.99 (m, 1H, H-C(3)), 2.72 (d, *J* = 14.1 Hz, 1H, H-C(12)), 2.40 (d, *J* = 13.8 Hz, 1H, H-C(12)), 2.12-2.02 (m, 1H, H-C(19)), 1.15 (s, 3H, CH_3), 1.10 (d, *J* = 6.1 Hz, 3H, CH_3), 0.96 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.86 (s, 6H, 2×CH_3), 0.76 (s, 3H,

CH_3), 0.73 (s, 3H, CH_3).^{13}C NMR (126 MHz, DMSO-d_6) δ 177.38 (C-28), 137.05 (C-13), 128.01 (C-18), 103.75, 100.01, 87.81, 74.49, 72.29, 71.98, 70.41, 68.29, 67.41, 63.71, 55.14, 50.14, 47.55, 43.94, 40.84, 40.66, 40.11, 38.73, 38.59, 36.60, 36.51, 35.34, 34.60, 32.49, 32.32, 32.03, 27.39, 26.68, 25.80, 24.74, 23.94, 21.30, 20.81, 17.80, 17.51, 16.28, 16.14.HRMS(ESI):*m/z* calcd for $[M+Na]^+$ $C_{41}H_{66}O_{11}Na$:757.4497; found:757.4498.

***δ-oleanolic acid 3-O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside* (43)**

White powder. Mp 230-232 ℃; IR (KBr) ν_{max} 3458, 3336, 2945, 1695, 1453, 1078, 1022, 989 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.02 (s, 1H, COOH), 5.29 (d, *J* = 4.2 Hz, 1H, H-C(1′)), 4.92 (d, *J* = 3.8 Hz, 1H), 4.86 (d, *J* = 4.4 Hz, 2H), 4.59 (d, *J* = 4.6 Hz, 1H), 4.45 (d, *J* = 4.7 Hz, 1H), 4.37 (d, *J* = 7.6 Hz, 1H), 4.17 (t, *J* = 5.4 Hz, 1H), 3.78-3.57 (m, 5H), 3.56-3.45 (m, 1H), 3.37 (s, 1H), 3.22-2.94 (m, 5H), 2.72 (d, *J* = 13.4 Hz, 1H, H-C(12)), 2.39 (d, *J* = 13.9 Hz, 1H, H-C(12)), 2.11-2.00 (m, 1H, H-C(19)), 1.15 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.86 (s, 6H, 2×CH_3), 0.76 (s, 3H, CH_3), 0.73 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 177.41 (C-28), 137.07 (C-13), 128.04 (C-18), 103.78, 103.27, 87.97, 78.53, 76.75, 76.39, 74.50, 71.18, 69.88, 66.29, 63.06, 60.85, 55.00, 50.14, 47.58, 43.95, 40.86, 40.68, 38.77, 38.38, 36.60, 36.53, 35.35, 34.61, 32.50, 32.35, 32.05, 27.62, 26.72, 25.68, 24.74, 23.95, 21.57, 21.34, 20.84, 17.86, 17.54, 16.21, 16.09.HRMS(ESI):*m/z* calcd for $[M+Na]^+$ $C_{41}H_{66}O_{12}Na$:773.4447; found:773.4443.

4.2. Biology

4.2.1. Materials

Dulbecco's modified Eagle medium (DMEM), Roswell Park Memorial Institute-1640 (RPMI 1640) medium, Fetal Bovine Serum, and Penicillin-Streptomycin Solution were purchased from Biological Industries (Israel). Gluta MAX^{TM}, Sodium Pyruvate Solution, and MEM Non-Essential Amino Acids Solution were purchased from Gibco (USA). Minimum Eagle medium were purchased from HyClone (USA). All cell lines were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). BCA protein quantitative kit

was obtained from Beyotime Biotechnology (Shanghai, China). Anti-pAMPKα (Thr172), Anti-AMPKα, Anti-pACC (Ser79), Anti-ACC were purchased from Cell Signaling Technology (USA). Anti-GAPDH were purchased from arigo Biolaboratories Corp (Shanghai, China). HRP-labeled goat anti-mouse IgG secondary antibody was purchased from Fcmacs Biotech Co., Ltd. (Nanjing, China) and HRP-labeled goat anti-rabbit IgG secondary antibody was purchased from CoWin Biosciences (Beijing, China). Western-blot detected kit (Tanon^{TM}High-sig ECL Western Blotting Luminol/Enhancer Solution and Tanon^{TM}High-sig ECL Western Blotting Peroxide Buffer was purchased from Tanon Science & Technology Co., Ltd (shanghai, China). Interleukin-6 (IL-6) and Tumor necrosis factor-α (TNF-α) ELISA kits were purchased from Biolegend (USA). Phorbol 12-myristate 13-acetate (PMA) was obtained from MedChemExpress (USA). DMSO and LPS were purchased from Sigma (USA). D/GalN was purchased from Sangon Biotech (Shanghai, China). Trizol, PimeScript™ RT Master Mix (Perfect Real Time) and SYBR® Premix Ex Taq were purchased from Vazyme Biotech Co., Ltd. (Nanjing, China).

4.2.2. Cell cultures

Huh7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and Penicillin-Streptomycin Solution. HepG2 cells were cultured in MEM medium, supplemented with 10% fetal bovine serum (FBS), Penicillin-Streptomycin Solution，Gluta MAX^{TM}，Sodium Pyruvate Solution, and MEM non-essential amino acid. THP1 cells were cultured in RPMI-1640, supplemented with 10% heat-inactivated FBS, and Penicillin-Streptomycin Solution. All cells were cultured routinely at 37 °C in an atmosphere of 5% CO_2 until 85−90% confluent and then treated with preparations for experiments.

4.2.3. Western blot analysis of AMPK and ACC phosphorylation

Huh7 or HepG2 cells were seeded at 3×10^5/well in 12-well plates, then incubated for 24 h. For the Western blot analysis, the cells were exposed to tested compounds

for 12 h. Then, the cells were lysed using a lysis buffer [120 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl (pH 7.6)], and proteins were obtained after centrifugation. The concentrations of proteins were determined using a BCA protein assay kit. The equal amounts of proteins were then electrophoresed on 10% or 6% SDS polyacrylamide gels. After transferring to PVDF membranes, the membranes were blocked with a 5% BSA solution for 1h and incubated overnight with primary antibodies for p-AMPKα (Thr172), AMPKα, p-ACC (Ser79), ACC or GAPDH. Then membranes were incubated continually with secondary antibodies, and the bands were detected using a Western blot detection kit.

4.2.4. Measurement of cytokine secretion

THP1-derived macrophages (4×10^5 cells/well in 12-well plates) were treated with LPS (100 ng/mL, sigma) and compounds simultaneously for 12 h. Then, culture supernatant fractions were collected and assayed for TNF-α and IL-6 production with the ELISA kit, following the manufacturer's instructions. Cells were lysed to quantify total cell proteins.

4.2.5. In vitro metabolic stability in liver microsomes

Preheat 0.1 M potassium phosphate buffer (K-buffer), pH 7.4. Spiking solutions of test and reference compounds 500 µM spiking solution: Add 5 µL of 10 mM stock solution into 95 µL of ACN. 1.5 µM spiking solution in microsomes (0.75 mg/mL): add 1.5 µL of 500 µM spiking solution and 18.75 µLof 20 mg/mL liver microsomes into 479.75µL of K-Buffer. 3 × NADPH stock solution (6 mM, 5 mg/mL) is prepared by dissolving NADPH into buffer. Dispense 30 µL of 1.5 µM spiking solution containing 0.75 mg/mL microsomes solution to the assay plates designated for different time points (0-,15-,30-,45-,60-min). For 0-min, add 150 µL of ACN containing IS to the wells of 0-min plate and then add 15 µL of NADPH stock solution (6 mM) . Pre-incubate all other plate at 37 °C for 5 minutes. Add 15 µL of NADPH stock solution to the plates to start the reaction and timing. At 15-min, 30-min, 45-min and 60-min add 150 µL of ACN containing IS to the wells of

corresponding plates respectively to stop the reaction. After quenching, shake the plates at the vibrator for 10 min (600 rpm/min) and then centrifuge at 6000rmp for 15 min. Transfer 80 μL of the supernatant from each well into a 96-well sample plate containing 140 μL of water for LC/MS analysis.

4.2.6. In vivo pharmacokinetic parameters of compounds

Compounds **29**, **33** and **41** (5% DMSO + 10% solutol + 85% saline) were subjected to pharmacokinetic studies in Balb/C mice (3 animals per group). Test compounds **29**, **33**, and **41** were administered orally at a dose of 10 mg/kg. Blood samples were collected 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, and 24 h after oral administration.

On the other hand, compounds **29**, **33** and **41** were administered intravenously at 2 mg/kg, and blood samples were collected at 0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, and 24 h after intravenous administration. The blood samples are collected on ice and centrifuged to separate plasma within 1 hour (centrifugation conditions: 6800g, 6 minutes, 2-8ºC). The plasma samples are stored in a -80ºC refrigerator before analysis. LC-MS/MS was used to determine the concentration of the original compound and possible metabolites in the supernatant.

4.2.7. Animal study

Male BALB/c mice (8-9 weeks), weighing approximately 21–24 g, were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animals were housed in a room with temperature at 24 ± 1°C, a 12 h light-dark cycle. Animals were allowed free access to tap water and normal food, and were randomly assigned to independent groups: Vehicle group, LPS/D-GalN group, Compd. **29** + LPS/D-GalN group. Vehicle group, LPS/D-GalN group was intragastric administration 0.5% CMCNa once a day for 3 days, and Compd. **29** + LPS/D-GalN group was intragastric administration **29** at 200 mg/kg once a day for 3 days. On the fourth day, after last intragastric administration and a single intraperitoneal injection of LPS/D-GalN (10 μg/700 mg/kg) or solvent for 5.5 h, blood was collected and serum was obtained by centrifugation at 3000 rpm, 4°C for 15 min. After sacrificed,

part of the mouse liver was fixed in 4% paraformaldehyde for hematoxylin and eosin (H&E) staining, while the rest was frozen to liquid nitrogen for mRNA detection. All experimental procedures have been reviewed and approved by the Animal Health Committee of China Pharmaceutical University.

4.2.8. Histopathology

Liver tissue samples were fixed in 4% paraformaldehyde, subjected to standard histological processing, and embedded in paraffin. The liver tissue was cut to 5 μm thick, stained with hematoxylin and eosin, and the histopathological lesions were randomly evaluated at 200-fold magnification through the selected histological field.

4.2.9. Biochemical indexes assay

ALT and AST levels in serum were analyzed and evaluated with an automatic biochemical analyzer.

4.2.10. Measurement of cytokine mRNA levels by real-time PCR

Approximate 10 mg of liver was homogenized in 500 μL Trizol. Total mRNA was extracted according to the manufacturer's instructions. The total mRNA was reversed to cDNA using HiScript II Q RT SuperMix kit (Vazyme) according to the manufacturer's protocol. The Power SYBR Green Master Mix was used for real-time PCR analysis. Differences in gene expression between groups were calculated using cycle threshold (Ct) values. Relative expression of genes was calculated by the $2^{-\Delta\Delta Ct}$ method. The necessary primers were purchased from Sangon Biotech. The following primer pairs were used: 5'-AACGATGATGCACTTGCAGA-3' and 5'-GGTACTCCAGAAGACCAG-AGGA-3' for *Il6*, 5'-CCACGTCGTAGCAAACCAC-3' and 5'-TGGGTGAGGAGCACGTAGT-3' for *Tnf*, 5'-GGCATTGTGGAAGGGCTCAT-3' and 5'-GGCAGCACCAGTGGATGCAG-3' for *Gapdh*. Gene expression values were normalized to the expression levels of *Gapdh.*

4.2.11. Statistical analysis

All data are presented as mean ± SEM, and analyses were performed with Graphpad Prism 7 software. Comparisons between experimental groups were conducted using One-way ANOVA, Values of $p< 0.05$ were considered significant.

Declaration of competing interest

The authors declare no competing financial interest.

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Supplementary data

Figure S1: The dose-dependent relationship of compounds **10**, **29** and **33** in inhibiting the secretion of inflammatory factors on LPS-induced inflammatory response in THP1-derived macrophages. Figure S2: The cytotoxic evaluation in THP1-derived macrophages analyzed by MTT assay. Figure S3: Saponin **29** stimulated the phosphorylation of AMPK (pT172) and ACC (pS79) in Huh7 and HepG2 cells. Figure S4: Effects of saponin **29** on AMPK heterotrimers *in vitro*. Copies of ^{1}H NMR and ^{13}C NMR spectra of saponins (Figures S5~55). Supplementary data related to this article can be found at http://

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Highlights

- δ-Oleanolic acid was identified as a potent AMPK activator.
- 23 new saponin derivatives of δ-oleanolic acid were synthesized and biologically evaluated.
- The bioavailability of saponins **29** and **33** was much better than their aglycon.
- Saponin **29** exhibited significant *in vitro* and *in vivo* anti-inflammatory effects.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: