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ORIGINAL ARTICLE

Synthesis and biological activity of new 18β-glycyrrhetinic acid derivatives

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Abstract In an attempt to find out new, potent and safe anti-inflammatory molecules and as a contribution in the chemistry of triterpenes, a series of 18β-glycyrrhetinic acid (GTA) derivatives (**4a–j**, **5a–e**, **7–9**, **11–13**) were synthesized and evaluated as anti-inflammatory agents using carrageenan induced rat paw edema method. The synthesized derivatives proved superior anti-inflammatory activity to GTA. Moreover, some of the produced derivatives demonstrated higher effect than prednisolone and indomethacin. This remarkable anti-inflammatory effect was combined with no detrimental effect on the gastrointestinal tract (GIT) of the test rats. All of the synthesized compounds were characterized by NMR spectroscopy and high-resolution ESI mass spectrometry.

1. Introduction

Typically possessing low toxicity and a broad spectrum of biological activities, plant triterpenoids are valuable raw materials for the creation of new drugs (Schopke and Hiller, 1990; Tolstikov et al., 1998; Platonov et al., 1995; Shon et al., 1998). Glycyrrhizic (**GZA**) and glycyrrhetinic acid (**GTA**), as well as their derivatives, exhibit a various biological effects, including anti-inflammatory and anti-ulcer activity (Tolstikov et al., 1997, 1998; Platonov et al., 1995; Shon et al., 1998). Consequently, they serve as pronounced starting materials for effective anti-inflammatory, anti-allergic and antiulcer preparations (Finney and Tarnoky, 1960; Gheorghiu et al., 1971; Yano et al., 1989). **GZA**, **GTA** and their derivatives affect the arachidonic acid cycle similar to the non-steroidal anti-inflammatory agents (Inoue et al., 1988). *In vitro* research has also demonstrated that **GZA** inhibits cyclooxygenase activity and prostaglandin formation (specifically prostaglandin E_2) (Ohuchi et al., 1981; Okimasu et al., 1983; Ohuchi and Tsurufuji, 1982). Moreover, **GZA** and **GTA** are known to inhibit phospholipase A_2 activity, an enzyme critical to numerous inflammatory processes (Kase et al., 1998).

Recently, some derivatives of **GTA** have shown their inhibitory activity against interleukin-1β (IL-1β)-induced prostaglandin E_2 (PGE_2) (Tsukahara et al., 2005). Also, **GZA** inhibits reactive oxygen species (ROS) generation by neutrophils. **GZA** significantly decreases neutrophil-generated

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O_2, H_2O_2 and OH in a dose-dependent manner. It is thought that one of **GZA** anti-inflammatory effects is attributed to this effect (Akamatsu et al., 1991; Wang and Nixon, 2001).

Some of **GTA** derivatives inhibited nitric oxide (NO) generation and suppressed superoxide anion formation by rat neutrophils; they also inhibited xanthine oxidase activity. These actions may have value in the therapeutical treatment or prevention of certain central as well as peripheral inflammatory diseases associated with the increase of (NO) production (Maitraie et al., 2009). Although being anti-inflammatory molecule, **GTA** is characterized by being anti-ulcer agent at the same time. Its anti-ulcer effect is attributed to the sequential inhibition of 15-hydroxyprostaglandin dehydrogenase and Δ^{13}-ketoprostaglandin reductase; the two enzymes are involved in the gastric prostaglandin metabolism (Aly et al., 2005). Prostaglandins promote healing of ulcers by stimulating mucous secretion and cell proliferation in the stomach. Thus, the local increase of prostaglandin concentration by glycyrrhetinic-derived compounds, promotes healing of ulcers.

GTA produces an undesired aldosterone-like effect (Ulmann et al., 1975), potentiates the action of aldosterone (Ishikawa and Saito, 1980), and inhibits its metabolism. At the same time, **GTA** derivatives frequently exhibit a more pronounced therapeutic action than does the initial **GTA** and produce no aldosterone-like side effects Takahashi et al., 1980; Shibata et al., 1987). Hence, we aimed to make new derivatives that exceed **GTA** activity with a better safety profile.

2. Results and discussion

2.1. Chemistry

GTA was isolated from *Glycyrrhiza glabra* roots in our laboratory by an unpublished method. Its identity was confirmed by IR, m.p. [291–292 °C, lit. 292–294 (Abubakirov and Yatsyn, 1959)] and mixed m.p. The target derivatives were synthesized by modification of the two main active functional groups of **GTA** (20-COOH and 3-OH). For the synthesis of compounds **4** and **5** series, the hydroxyl group was protected by acetylation (Murav'ev and Savchenko, 1979) and the carboxylic group was activated by the preparation of acid chloride **(3)** using $SOCl_2$ (Adanin and Khaletskii, 1967) .The addition of few drops of dimethyl formamide (Ibrahim et al., 1995), to enhance the reaction led to decrease in the overall yield of the acid chloride. After purification, the acid chloride produced was reacted with some aryl, alicyclic, heterocyclic amines and aryl phenols to give the corresponding amides **(4a–j)** and esters **(5a–e)**, respectively (Scheme 1). The acid chloride method resulted in a high yield for the produced amides and esters. The mixed anhydride of **GTA** was prepared by reacting **GTA** with ethylchloroformate in presence of triethylamine in dichloromethane, but it resulted in a poor yield for the produced amides (~20%).

Compound **4g** was hydrolyzed by alcoholic KOH to produce compound **6**. The free –OH of the latter compound was esterified with each of succinic and phthalic anhydrides using excess amounts of both of them in presence of dry pyridine and molecular sieve 4A° to afford compounds **7** and **8**, respectively (Tsukahara et al., 2005). Compound **9** was prepared through further amidation of compound **4g** with p-toluidine *via* mixed anhydride formation (Scheme 2).

Methyl glycyrrhetate **(10)** was prepared by Fischer esterification of **GTA** with methanol; compounds **11–13** were prepared through the reaction of **(10)** with excess succinic, phthalic and maleic anhydrides, respectively in pyridine (Kondratenko et al., 2001) (Scheme 3). During the reaction with succinic and maleic anhydrides, reaction mixture got dark. It grows faster in case of maleic than succinic anhydride. Pyridine induces a chemical reaction of some violence which yields carbon dioxide and a black brittle residue (Rittenberg and Ponticorvo, 1960). Acetone was used during the working up to dissolve this black brittle residue.

2.2. Biological activity

In the present investigation, variable synthesized compounds were evaluated for their possible anti-inflammatory activity in a rat model of carrageenan-induced paw edema, which is a widely used animal model for determining the acute phase of inflammation. The anti-inflammatory potency of the tested compounds was compared with free **GTA** and two standard drugs, prednisolone and indomethacin. Figs. 1 and 2 demonstrate the potency of **GTA** and its synthesized derivatives versus prednisolone and indomethacin, respectively. It is noteworthy to mention that the derivatives **4g**, **4h**, **5a** and **11** showed greater anti-inflammatory potency than both prednisolone and indomethacin. However, compounds **4i**, **8** and **9** showed almost the same anti-inflammatory potency of the reference standards. On the other hand, free **GTA** showed the least potency as compared with the synthesized compounds.

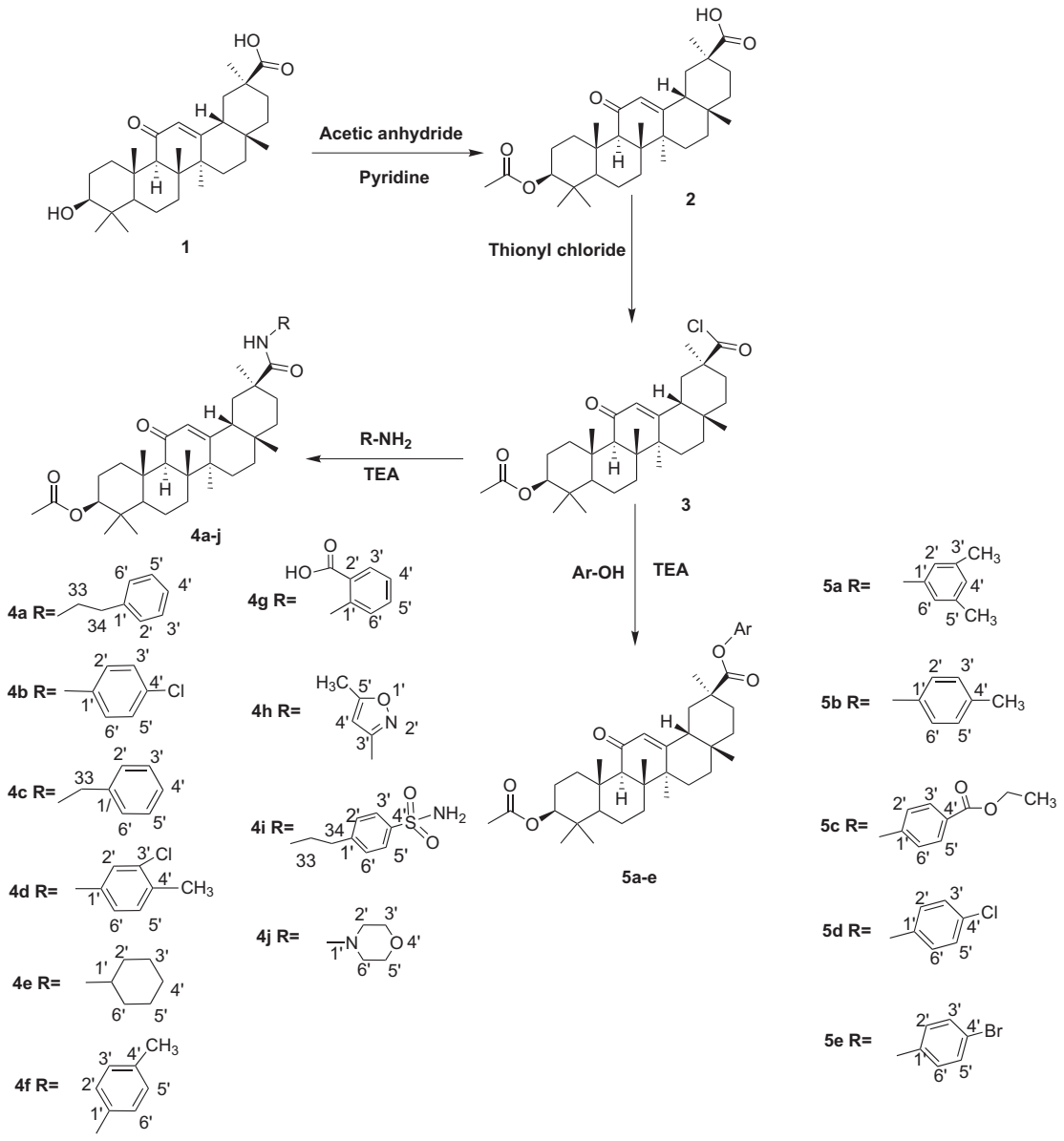
At the end of the experiment, rats were killed under light ether anesthesia by cervical dislocation; their stomachs were excised, opened at the greater curvature and examined for the presence of ulcers. All rat groups that received **GTA**, its synthesized derivatives and prednisolone showed very few gastric lesions or no ulcers at all, compared to rat groups that received indomethacin, which showed some gastric ulcers with considerable severity.

3. Materials and methods

Melting points were uncorrected and determined with electrotherma capillary melting point apparatus. The NMR spectra were measured on a Varian Unity 300 (300.145 MHz) and on Buker AM-200 (200 MHz) spectrometers. ESI mass spectra were recorded on a Finnigan LCQ spectrometer with quaternary pump Rheos 4000 (Flux Instrument). Flash chromatography was carried out on silica gel (230–400 mesh). R_f values were measured on Polygram SIL G/UV_{254} (Macherey–Nagel & Co.). Reaction progress was followed and monitored using thin layer chromatography (TLC, DF_{254}) and eluted with the following systems (a) benzene/ethyl acetate/AcOH (12:2:0.5) or (b) hexane/ethyl acetate (6:4). Visualization was carried out by either UV (254 nm, 365 nm) or spraying with methanol/sulfuric acid (5%) and then heated with air dryer.

3.1. Isolation of GTA from Glycyrrhiza glabra roots

The dry powdered root of *Glycyrrhiza glabra* (1 kg) was treated with 5 L of 5% sulfuric acid. The mixture was refluxed for 6 h and left to cool. The mixture was filtered off and the residue was carefully washed with water and dried. The residue



Scheme 1 Synthesis of amides and esters of GTA.

was extracted by dry benzene in a soxhlet. The pale yellow extract was treated with 200 ml of acetic anhydride and the mixture was refluxed for 1 h, then it was left for 24 h at room temperature. The reaction mixture was poured on crushed ice, while stirring and the produced precipitate was filtered off and washed with water. The produced glycyrrhetinic acid acetate **(2)** was crystallized several times from methanol to produce colorless crystals [m.p. 315–317 °C, lit. 317–318 °C (Murav'ev and Savchenko, 1979)]. Compound **2** was saponified by refluxing with 5% alcoholic NaOH for 3 h to liberate free **GTA** (**1**).

3.2. Synthesis of the target derivatives

3.2.1. Glycyrrhetinic acid acetate (2), acetyl glycyrrhetyl chloride (3) and methyl glycyrrhetate (10)

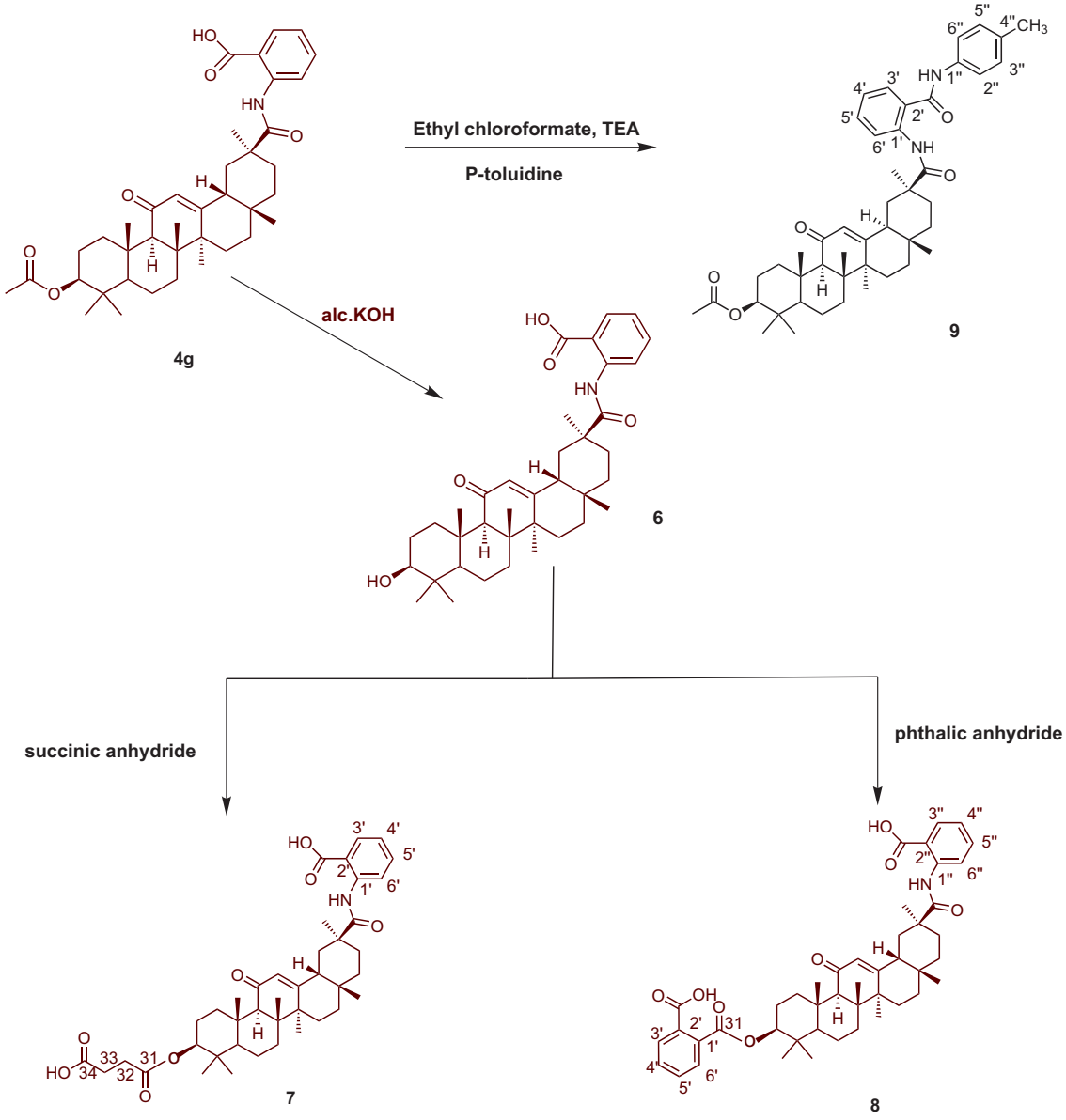
The three compounds were prepared according to published methods (Murav'ev and Savchenko, 1979; Baltina et al., 1997).

3.2.2. General procedures for the preparation of compounds 4a–j and 5a–e

A solution of 3 (100 mg, 0.19 mmol) in dry benzene was added drop wise to a stirred solution of the appropriate amine or phenol (0.19 mmol) and triethylamine (27 µl, 0.19 mmol) in dry benzene or THF. After complete addition, the mixture was heated under reflux for 3 h and the course of reaction was monitored using TLC. At the end of the reaction, water was added; the mixture was washed with 2 N HCl (compounds **4a**–**j**) or 2% NaOH (compounds **5a**–**e**); benzene layer was separated, dried over anhydrous sodium sulfate and distilled off under vacuum. The formed amide or ester was purified by flash column chromatography using the appropriate solvent system.

3.2.3. N-(phenethyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (4a)

M.p 121–123 °C; **yield** 68%; **(+)-ESI-MS:** *m/z* (%) 638 ($[M + Na]^+$), 1254 ($[2\,M + Na]^+$) 614 ($[M-H]^-$);

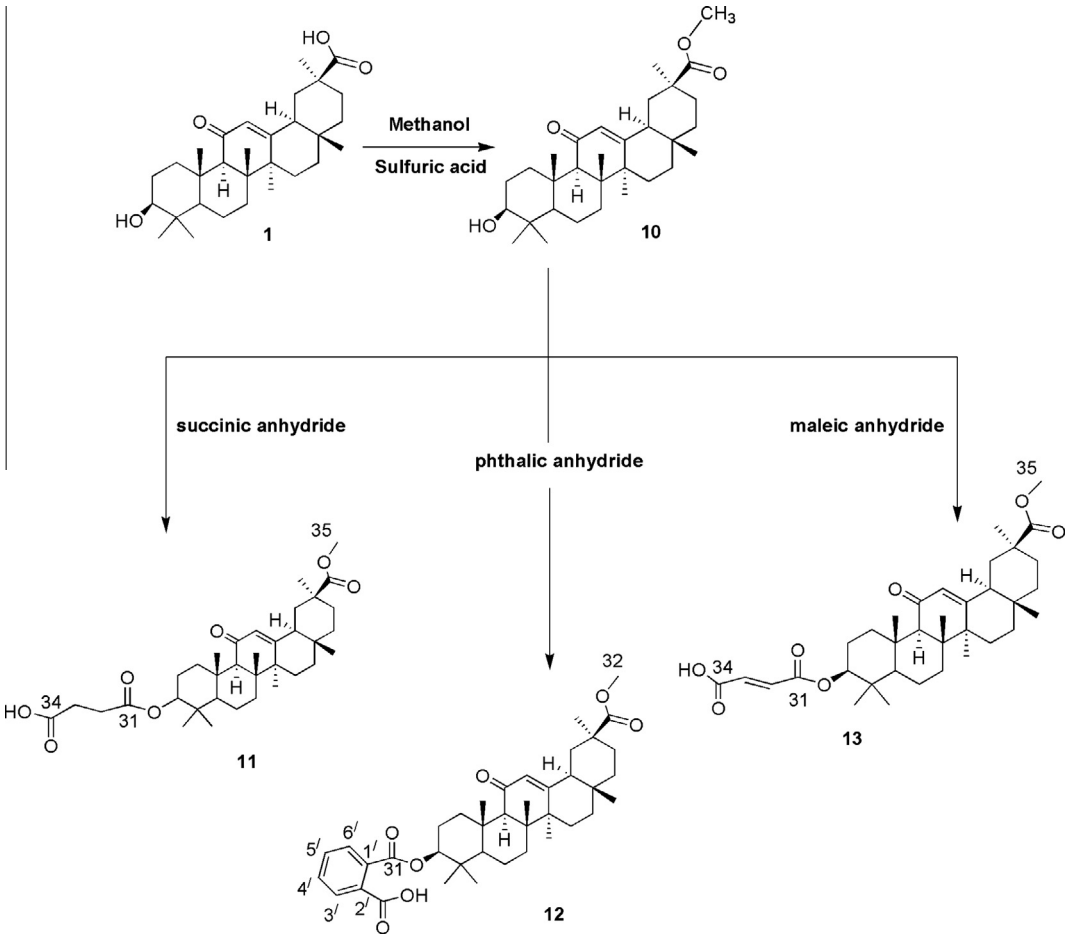


Scheme 2 Synthesis of compounds **7–9**.

(+)-HRESI-MS: *m/z* 638.41788 (calc: 638.41798 for $C_{40}H_{57}NO_4Na$); **^{1}H NMR:** δ at 0.82 (s, 3H, CH_3-28), 0.86 (s, 6H, CH_3-23, 24), 1.07 (s, 3H, CH_3-27), 1.12 (s, 3H, CH_3-25), 1.25 (s, 3H, CH_3-29), 1.34 (s, 3H, CH_3-26), 2.01 (s, 3H, CH_3-32), 2.29 (s, 1H, CH-9), 2.78 (d, 1H, CH-1 eq), 2.85 (m, 2H, CH_2-34), 3.52 (m, 2H, CH_2-33), 4.47 (dd, 1H, CH-3), 5.45 (s, 1H, CH-12), 5.75 (t, 1H, NH), 7.22 (m, 5H, CH-2′, 3′, 4′, 5′, 6′); **^{13}C NMR:** δ at 38.74 (C-1), 23.68 (C-2), 80.56 (C-3), 37.97 (C-4), 54.95 (C-5), 17.30 (C-6), 32.61 (C-7), 45.28 (C-8), 61.64 (C-9), 36.86 (C-10), 199.85 (C-11), 128.33 (C-12), 169.09 (C-13), 43.52 (C-14), 26.34 (C-15), 26.31 (C-16), 31.78 (C-17), 47.87 (C-18), 41.02 (C-19), 43.11 (C-20), 31.34 (C-21), 37.34 (C-22), 27.98 (C-23), 16.61 (C-24), 16. 34 (C-25), 18.59 (C-26), 23.48 (C-27), 28.36 (C-28), 29.95 (C-29), 175.66 (C-30), 179.96 (C-31), 21.23 (C-32), 43.11 (C-33), 40.38 (C-34), 138.69 (C-1′), 128.64 (C-2′), 128.67 (C-3′), 126.57 (C-4′), 128.67 (C-5′), 128.64 (C-6′).

*3.2.4. N-(4-chlorophenyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (**4b**)*

M.p 281–283 °C; yield 82%; **(+)-ESI-MS:** *m/z* (%) 645 ($[M + Na]^+$), 1267 ($[2\,M + Na]^+$), 621 ($[M-H]^-$); **(+)-HRESI-MS:** *m/z* 623.3663 (calc: 623.3658 for $C_{38}H_{53}NO_4Cl$); **^{1}H NMR:** δ at 0.83 (s, 3H, CH_3-28), 0.88 (s, 6H, CH_3-23, 24), 1.13 (s, 3H, CH_3-27), 1.14 (s, 3H, CH_3-25), 1.25 (s, 3H, CH_3-29), 1.39 (s, 3H, CH_3-26), 2.06 (s, 3H, CH_3-32), 2.37 (s, 1H, CH-9), 2.80 (d, 1H, CH-1 eq), 4.53 (dd, 1H, CH-3), 5.68 (1H, s, CH-12), 7.28 (d, 2H, CH-2', 6'), 7.45 (d, 2H, CH-3′, 5′), 3 J (2′, 3′) = 3 J (5′, 6′) = 9 Hz; **^{13}C NMR:** δ at 38.77 (C-1), 23.52 (C-2), 80.57 (C-3), 38.01 (C-4), 54.99 (C-5), 17.34 (C-6), 32.68 (C-7), 45.37 (C-8), 61.76 (C-9), 36.91 (C-10), 199.91 (C-11), 128.56 (C-12), 168.88 (C-13), 44.56 (C-14), 26.38 (C-15), 26.38 (C-16), 32.01 (C-17), 48.16 (C-18), 41.82 (C-19), 43.20 (C-20), 31.62 (C-21), 37.37 (C-22), 28.02 (C-23), 16.66 (C-24), 16. 38 (C-25), 18.64 (C-26),



Scheme 3 Synthesis of esters of GTA.

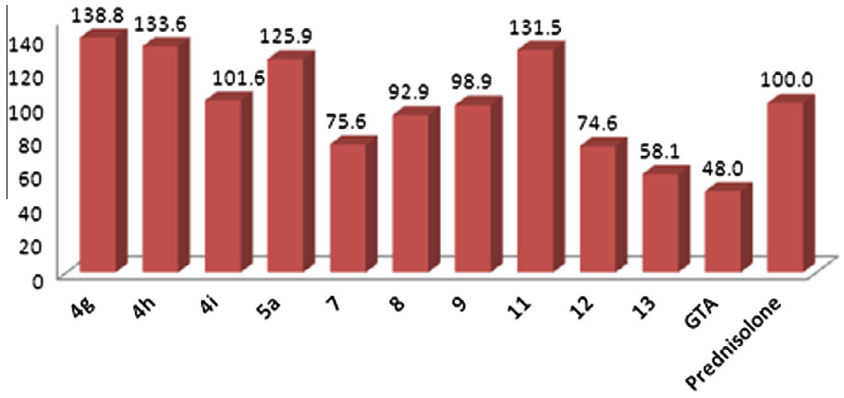


Figure 1 Anti-inflammatory potency of GTA and its derivatives as compared with prednisolone.

23.33 (C-27), 28.38 (C-28), 29.35 (C-29), 174.01 (C-30), 171.05 (C-31), 21.32 (C-32), 136.37 (C-1′), 121.34 (C-2′), 129.01 (C-3′), 128.77 (C-4′), 129.01 (C-5′), 121.34 (C-6′).

*3.2.5. N-(benzyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (**4c**)*

M.p 134–136 °C; yield 67%; **(+)-ESI-MS:** *m/z* (%) 624 ($[M + Na]^+$), 600 ($[M-H]^-$); **(+)-HRESI-MS:** *m/z* 602.4203 (calc: 602.4203 for $C_{39}H_{56}NO_4$); **^{1}H NMR:** δ at 0.76 (s, 3H, CH_3-28), 0.82 (s, 6H, CH_3-23, 24), 1.11 (s, 6H, CH_3-27, 25), 1.30 (s, 3H, CH_3-29), 1.36 (s, 3H, CH_3-26), 2.00 (s, 3H, CH_3-32), 2.28 (s, 1H, CH-9), 2.73 (d, 1H, CH-1 eq), 4.43 (s, 3H, CH_3 and CH_2-33), 5.51 (1H, s, CH-12), 5.91 (1H, s, NH), 7.24 (6H, m, CH-2′, 3′, 4′, 5′, 6′, $CDCl_3$); **^{13}C NMR:** δ at 38.74 (C-1), 23.48 (C-2), 80.56 (C-3), 37.99 (C-4), 54.95 (C-5), 17.32 (C-6), 32.56 (C-7), 45.31 (C-8), 61.66 (C-9), 36.86 (C-10), 199.95 (C-11), 128.37 (C-12), 169.07 (C-13), 43.60 (C-14), 26.34 (C-15), 26.34 (C-16), 31.89 (C-17), 48.12 (C-18), 41.81 (C-19), 43.15 (C-20), 31.43 (C-21), 37.40 (C-22), 28.00(C-23), 16.64 (C-24), 16. 35 (C-25), 18.63 (C-26),

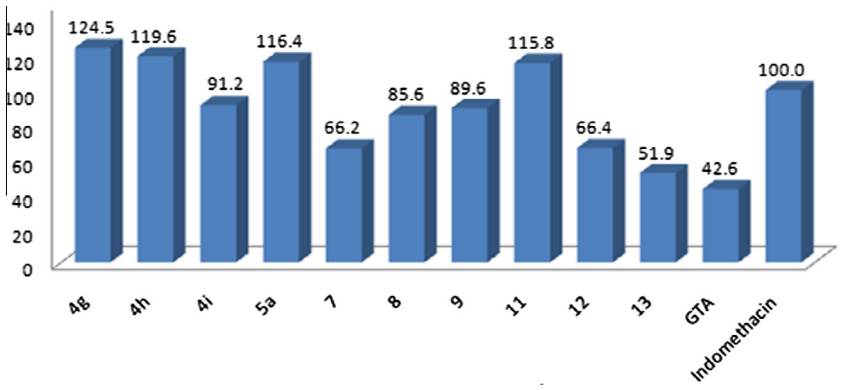


Figure 2 Anti-inflammatory potency of GTA and its derivatives as compared with indomethacin.

Table 1 Anti-inflammatory potency of glycyrrhetic acid and some of its derivatives in comparison to prednisolone.

Compound	Anti-inflammatory potency (%)				Average potency in 4 h (%)
	1 h	2 h	3 h	4 h	
4g	153.3	131.7	130.9	139.1	138.8
4h	149.9	121.6	123.6	139.1	133.6
4i	111.5	105.8	94.2	94.8	101.6
5a	102.0	135.4	131.7	134.5	125.9
7	101.2	71.6	64.3	65.3	75.6
8	78.7	84.9	106.9	101.2	92.9
9	100.0	98.1	99.4	98.1	98.9
11	168.9	125.7	111.7	119.5	131.5
12	89.0	75.4	66.2	68.0	74.6
13	66.3	55.8	54.1	56.1	58.1
GTA	58.4	46.5	43.6	43.5	48.0
Prednisolone	100.0	100.0	100.0	100.0	100.0

Table 2 Anti-inflammatory potency of glycyrrhetic acid and some of its derivatives in comparison to indomethacin.

Compound	Anti-inflammatory potency (%)				Average potency in 4 h (%)
	1 h	2 h	3 h	4 h	
4g	96.6	131.0	131.4	139.1	124.5
4h	94.5	121.0	124.1	139.1	119.6
4i	70.3	105.2	94.6	94.8	91.2
5a	64.3	134.7	132.2	134.5	116.4
7	63.8	71.2	64.5	65.3	66.2
8	49.6	84.4	107.3	101.2	85.6
9	63.0	97.6	99.8	98.1	89.6
11	106.5	125.0	112.1	119.5	115.8
12	56.1	75.0	66.5	68.0	66.4
13	41.8	55.5	54.3	56.1	51.9
GTA	36.8	46.2	43.7	43.5	42.6
Indomethacin	100.0	100.0	100.0	100.0	100.0

23.22 (C-27), 28.39 (C-28), 29.52 (C-29), 175.53 (C-30), 173.03 (C-31), 21.29 (C-32), 43.60 (C-33), 138.57 (C-1′), 127.71 (C-2′), 128.76 (C-3′), 127.56 (C-4′), 128.76 (C-5′), 127.71 (C-6′).

*3.2.6. N-(3-chloro-4-Methylphenyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (**4d**)*

M.p 167–169 °C; **yield** 73%; **(+)-ESI-MS:** *m/z* (%) 658 ($[M + Na]^+$), 634($[M-H]^-$); **(+)-HRESI-MS:** *m/z* 636.3800 (calc: 636.3814 for $C_{39}H_{55}NO_4Cl$), 658.3620 (calc: 658.3634 for $C_{39}H_{54}NO_4ClNa$); **^{1}H NMR:** δ at 0.81 (s, 3H, CH_3-28), 0.87 (s, 6H, CH_3-23, 24), 1.12 (s, 6H, CH_3-27, 25), 1.24 (s, 3H, CH_3-29), 1.37 (s, 3H, CH_3-26), 2.05 (s, 3H, CH_3-32), 2.29 (s, 3H, CH_3-Ar), 2.36 (s, 1H, CH-9), 2.78 (d, 1H, CH-1 eq), 4.52 (dd, 1H, CH-3), 5.69 (s, 1H, CH-12), 7.11 (m, 1H, CH-5′), 7.29 (m, 1H, CH-6′), 7.58 (s, 1H, CH-2′), 7.71 (s, 1H, NH); **^{13}C NMR:** δ at 38.72 (C-1), 23.44 (C-2), 80.54 (C-3), 37.96 (C-4), 54.91 (C-5), 17.29(C-6), 32.62 (C-7), 45.45 (C-8), 61.68 (C-9), 36.84 (C-10), 200.19 (C-11), 128.35

(C-12), 169.55 (C-13), 43.21 (C-14), 26.32 (C-15), 26.32 (C-16), 31.94 (C-17), 48.26 (C-18), 41.47 (C-19), 43.21 (C-20), 31.51 (C-21), 37.33 (C-22), 27.99 (C-23), 16.63 (C-24), 16.31 (C-25), 18.56 (C-26), 23.33 (C-27), 28.35 (C-28), 29.56 (C-29), 174.16 (C-30), 171.08 (C-31), 21.29 (C-32),136.83 (C-1′), 118.31 (C-2′), 134.35 (C-3′), 130.84 (C-4′), 131.68 (C-5′), 120.64 (C-6′), 20.22 (*C*H$_3$- Ar).

*3.2.7. N-(cyclohexyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (**4e**)*

M.p 201–203 °C; yield 77%; **(+)-ESI-MS:** *m/z* (%) 616 ([M + Na]$^+$), 1210 ([2 M + Na]$^+$); **(+)-HRESI-MS:** *m/z* 616.4333 (calc: 616.43362 for $C_{38}H_{59}NO_4Na$); **^{1}H NMR:** δ at 0.77 (s, 3H, CH$_3$-28), 0.84 (s, 6H, CH$_3$-23, 24), 1.09 (s, 3H, CH$_3$-27), 1.12 (s, 3H, CH$_3$-25), 1.22 (s, 3H, CH$_3$-29), 1.36 (s, 3H, CH$_3$-26), 1.65 (m, 2H, CH$_2$-d), 2.02 (s, 3H, CH$_3$-32), 2.21 (m, 2H, CH-3′, 5′), 2.32 (s, 1H, CH-9), 2.76 (d, 1H, CH-1 eq), 3.00 (m, 2H, CH-2′, 6′), 4.48 (dd, 1H, CH-3), 5.65 (s, 1H, CH-12), 3.78 (m, 1H, CH-1′); **^{13}C NMR:** δ at 38.58 (C-1), 23.49 (C-2), 80.55 (C-3), 37.84 (C-4), 54.82 (C-5), 17.35 (C-6), 32.52 (C-7), 45.32 (C-8), 61.70 (C-9), 36.75 (C-10), 199.86 (C-11), 128.32 (C-12), 169.25 (C-13), 43.14 (C-14), 26.28 (C-15), 26.28 (C-16), 31.72 (C-17), 48.08 (C-18), 41.83 (C-19), 43.14 (C-20), 31.35 (C-21), 37.26 (C-22), 27.85 (C-23), 16.45 (C-24), 16. 20 (C-25), 18.61 (C-26), 23.30 (C-27), 28.50 (C-28), 28.51 (C-29), 174.51(C-30), 170.85 (C-31), 21.22 (C-32), 51.10 (C-1′), 33.28 (C-2′), 22.73 (C-3′), 28.49 (C-4′), 22.73 (C-5′), 33.28 (C-6′).

*3.2.8. N-(4-Methylphenyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (**4f**)*

M.p 181–183 °C [lit. 180–182 °C 9 Dalimov et al., 2001)]; **yield** 69%; **(+)-ESI-MS:** *m/z* (%) 624 ([M + Na]$^+$), 600 ([M−H]$^-$); **(+)-HRESI-MS:** *m/z* 624.4021 (calc: 624.4023 for $C_{39}H_{55}NO_4$-Na); **^{1}H NMR:** δ at 0.81 (s, 3H, CH$_3$-28), 0.84 (s, 6H, CH$_3$-23, 24), 1.14 (s, 3H, CH$_3$-27), 1.16 (s, 3H, CH$_3$-25), 1.24 (s, 3H, CH$_3$-29), 1.38 (s, 3H, CH$_3$-26), 2.01 (s, 3H, CH$_3$-32), 2.31 (s, 3H, CH$_3$-Ar), 2.38 (s, 1H, CH-9), 2.79 (d, 1H, CH-1 eq), 4.52 (dd, 1H, CH-3), 5.64 (s, 1H, CH-12), 7.10 (d, 2H, CH-3′, 5′), 7.38 (d, 2H, CH-2′, 6′), $J_{2',3'} = J_{5',6'} = 6$ Hz, 7.43 (s, 1H, NH); **^{1}C NMR:** δ at 38.83 (C-1), 23.60 (C-2), 80.58 (C-3), 38.06 (C-4), 55.02 (C-5), 17.43 (C-6), 32.74 (C-7), 45.41 (C-8), 61.75 (C-9), 36.97 (C-10), 199.74 (C-11), 128.39 (C-12), 168.99 (C-13), 44.46 (C-14), 26.49 (C-15), 26.46 (C-16), 32.04 (C-17), 48.22 (C-18), 41.93 (C-19), 43.27 (C-20), 31.69 (C-21), 37.45 (C-22), 28.09 (C-23), 16.73 (C-24), 16. 43 (C-25), 18.73 (C-26), 23.39 (C-27), 28.44 (C-28), 29.42 (C-29), 173.72 (C-30), 170.86 (C-31), 21.36 (C-32), 135.19 (C-1′), 120.21 (C-2′), 129.36(C-3′), 133.87 (C-4′), 129.36 (C-5′), 120.21 (C-6′), 20.88 (*C*H$_3$- Ar).

*3.2.9. N-(2-carboxyphenyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (**4g**)*

M.p 174–176 °C; yield 75%; **(+)-ESI-MS:** *m/z* (%) 654 ([M + Na]$^+$), 630 ([M−H]$^-$); **(+)-HRESI-MS:** *m/z* 654.3609 (calc: 654.3609 for $C_{39}H_{53}NO_6Na$); **^{1}H NMR:** δ at 0.86 (s, 6H, CH$_3$-28, 24), 0.97 (s, 3H, CH$_3$-23), 1.18 (s, 3H, CH$_3$-27), 1.23 (s, 6H, CH$_3$-25, 29), 1.36 (s, 3H, CH$_3$-26), 2.04 (s, 3H, CH$_3$-32), 2.33 (s, 1H, CH-9), 2.65 (d, 1H, CH-1 eq), 4.49 (dd, 1H, CH-3), 5.94 (1H, s, CH-12), 6.60 (s, 1H, NH), 7.06 (t, 1H, CH-4′), 7.51(t, 1H, CH-5′), 8.03(d, 1H, CH-6′), 8.74 (d, 1H, CH-3′), 11.34 (br-s, 1H, Ar–COOH); **^{13}C NMR:** δ at 38.77 (C-1), 23.51 (C-2), 80.51 (C-3), 38.00 (C-4), 55.18 (C-5), 17.46 (C-6), 32.17 (C-7), 45.54 (C-8), 60.67 (C-9), 36.88 (C-10), 202.46 (C-11), 128.79 (C-12), 170.69 (C-13), 45.54 (C-14), 26.18 (C-15), 25.50 (C-16), 31.92 (C-17), 46.52 (C-18), 43.87 (C-19), 43.87 (C-20), 30.38 (C-21), 37.10 (C-22), 28.05 (C-23), 16.79 (C-24), 14. 11 (C-25), 18.48 (C-26), 22.68 (C-27), 29.35 (C-28), 29.69 (C-29), 175.97 (C-30), 170.99 (C-31), 21.30 (C-32), 141.82 (C-1′), 115.67 (C-2′), 131.23 (C-3′), 122.52 (C-4′), 134.48 (C-5′), 120.54 (C-6′), 164.54 (Ar-COOH).

*3.2.10. N-(5-Methyl isoxazol 3-yl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (**4h**)*

M.p 255–257 °C; yield 71%; **(+)-ESI-MS:** *m/z* (%) 615 ([M + Na]$^+$), 591([M−H]$^-$); **(+)-HRESI-MS:** *m/z* 615.3767 (calc: 615.3768 for $C_{36}H_{52}N_2O_5Na$); **^{1}H NMR:** δ at 0.77 (s, 3H, CH$_3$-28), 0.84 (s, 6H, CH$_3$-24, 23), 1.08 (s, 3H, CH$_3$-27), 1.12 (s, 3H, CH$_3$-25), 1.21 (s, 3H, CH$_3$-29), 1.35 (s, 3H, CH$_3$-26), 2.02 (s, 3H, CH$_3$-32), 2.32 (s, 1H, CH-9), 2,36 (s, 3H, CH$_3$-33), 2.78 (d, 1H, CH-1 eq), 4.48 (dd, 1H, CH-3), 5.68 (s, 1H, CH-12), 6.74 (s, 1H, CH-4'), 9.06 (s, 1H, NH); **^{13}C NMR:** δ at 38.77 (C-1), 23.52 (C-2), 80.59 (C-3), 38.00 (C-4), 55.00 (C-5), 17.34 (C-6), 32.68 (C-7), 45.32 (C-8), 61.67 (C-9), 36.88 (C-10), 199.75 (C-11), 128.65 (C-12), 169.70 (C-13), 44.58 (C-14), 26.38 (C-15), 26.35 (C-16), 31.68 (C-17), 47.66 (C-18), 41.06 (C-19), 43.13 (C-20), 32.23 (C-21), 37.44 (C-22), 28.01 (C-23), 16.64 (C-24), 16. 34 (C-25), 18.63 (C-26), 23.33 (C-27), 28.34 (C-28), 29.64 (C-29), 174.60 (C-30), 170.97 (C-31), 21.26 (C-32), 158.54 (C-3′), 96.92 (C-4′), 168.57 (C-5′), 12.57 (*C*H$_3$-isoxazole).

*3.2.11. N-[2-(4-sulfamoylphenyl)ethyl]-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (**4i**)*

M.p 153–155 °C; yield 78%; **(+)-ESI-MS:** *m/z* (%) 717 ([M + Na]$^+$), 1412 ([2 M + Na]$^+$); **(+)-HRESI-MS:** *m/z* 717.3913 (calc: 717.3908 for $C_{40}H_{58}N_2O_6SNa$); **^{1}H NMR:** δ at 0.81 (s, 3H, CH$_3$-28), 0.83 (s, 6H, CH$_3$-23, 24), 1.04 (s, 3H, CH$_3$-27), 1.09 (s, 3H, CH$_3$-25), 1.10 (s, 3H, CH$_3$-29), 1.27 (s, 3H, CH$_3$-26), 2.02 (s, 3H, CH$_3$-32), 2.26 (s, 1H, CH-9), 2.65 (d, 1H, CH-1 eq), 2.9 (m, 2H, CH$_2$-34), 3.76 (m, 2H, CH$_2$-33), 4.45 (dd, 1H, CH-3), 5.02 (s, 1H, CH-12), 5.82 (t, 1H, NH), 7.29 (d, 2H, CH-3′, 5′), 7.81 (d, 2H, CH-2′, 6′), $J_{2',3'} = J_{5',6'} = 6$ Hz; **^{13}C NMR:** δ at 38.57 (C-1), 23.46 (C-2), 80.52 (C-3), 37.98 (C-4), 54.95 (C-5), 17.27 (C-6), 32.60 (C-7), 45.49 (C-8), 61.77 (C-9), 36.94 (C-10), 201.24 (C-11), 127.99 (C-12), 170.55 (C-13), 43.76 (C-14), 26.33 (C-15), 26.23 (C-16), 31.90 (C-17), 48.09 (C-18), 41.53 (C-19), 43.27 (C-20), 31.55 (C-21), 37.52 (C-22), 27.97 (C-23), 16.61 (C-24), 16. 37 (C-25), 18.63 (C-26), 23.19 (C-27), 28.51 (C-28), 29.53 (C-29), 176.00 (C-30), 171.07 (C-31), 21.27 (C-32), 40.59 (C-33), 35.51 (C-34), 144.33 (C-1′), 129.50 (C-2′), 126.79 (C-3′), 140.94 (C-4′), 126.79 (C-5′), 129.50 (C-6′).

*3.2.12. N-(morpholyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (**4j**)*

M.p 169–171 °C; **yield** 75%; **(+)-ESI MS:** *m/z* (%) 604 ([M + Na]$^+$, 100), 1185 ([2 M + Na]$^+$, 40), 580 ([M−H]$^-$; **(+)-HRESI MS:** *m/z* 582.4151 (calc: 582.4153 for $C_{36}H_{56}NO_5$), 604.3973 (calc: 604.3972 for $C_{36}H_{55}NO_5Na$); **^{1}H NMR:** δ at 0.79 (s, 3H, CH$_3$-28), 0.82 (s, 6H, CH$_3$-23, 24), 1.07 (s, 3H, CH$_3$-27), 1.10 (s, 3H, CH$_3$-25), 1.19 (s, 3H,

CH_3-29), 1.31 (s, 3H, CH_3-26), 2.02 (s, 3H, CH_3-32), 2.17 (m, 4H, CH_2-2′, 6′), 2.31 (s, 1H, CH-9), 2.77 (d, 1H, CH-1 eq), 2.98 (m, 4H, CH_2-3′, 5′), 4.47 (dd, 1H, CH-3), 5.63 (s, 1H, CH-12); **^{13}C NMR:** δ at 38.45 (C-1), 23.23 (C-2), 80.25 (C-3), 37.69 (C-4), 54.63 (C-5), 17.04 (C-6), 32.70 (C-7), 45.64 (C-8), 61.34 (C-9), 36.61 (C-10), 199.64 (C-11), 128.10 (C-12), 169.42 (C-13), 44.96 (C-14), 26.37 (C-15), 26.07 (C-16), 32.38 (C-17), 47.81 (C-18), 42.98 (C-19), 43.48 (C-20), 31.43 (C-21), 37.38 (C-22), 26.59 (C-23), 16.73 (C-24), 16. 09 (C-25), 18.36 (C-26), 22.76 (C-27), 27.72 (C-28), 28.11 (C-29), 173.76 (C-30), 170.62 (C-31), 20.98 (C-32), 22.43 (C-2′), 50.83 (C-3′), 50.83 (C-5′), 22.43 (C-6′).

*3.2.13. (3,5-Dimethylphenyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-oate (**5a**)*

M.p 207–209 °C; yield 69%; **(+)-ESI-MS:** *m/z* (%) 617($[M + H]^+$), 639 ($[M + Na]^+$), 1455 ($[2\,M + Na]^+$); **(+)-HRESI-MS:** *m/z* 617.4171 (calc: 617.4201 for $C_{40}H_{57}O_5$); **^{1}H NMR:** δ at 0.87 (s, 9H, CH_3-28, 23, 24), 1.15 (s, 6H, CH_3-27, 25), 1.25 (s, 3H, CH_3-25), 1.33 (s, 3H, CH_3-29), 1.39 (s, 3H, CH_3-26), 2.05 (s, 3H, CH_3-32), 2.31 (s, 1H, CH-9), 2.32 (s, 6H, two CH_3–Ar), 2.79 (d, 1H, CH-1 eq), 4.52 (dd, 1H, CH-3), 5.69 (s, 1H, CH-12), 6.64 (s, 2H, CH-2', 6'), 6.86 (s, 1H, CH-4′); **^{13}C NMR:** δ at 39.81 (C-1), 23.99 (C-2), 81.05 (C-3), 38.48 (C-4), 55.44 (C-5), 17.81 (C-6), 33.14 (C-7), 45.85 (C-8), 62.16 (C-9), 37.34 (C-10), 199.92 (C-11), 128.00 (C-12), 169.51 (C-13), 44.66 (C-14), 26.86 (C-15), 26.86 (C-16), 31.58 (C-17), 48.85 (C-18), 41.49 (C-19), 43.29 (C-20), 30.61 (C-21), 38.17 (C-22), 28.48 (C-23), 17.12 (C-24), 16. 15 (C-25), 19.10 (C-26), 23.80 (C-27), 28.61 (C-28), 29.03 (C-29), 177.80 (C-30), 171.76 (C-31), 21.79 (C-32), 150.65 (C-33), 119.40 (C-34), 139.78 (C-1′), 129.07 (C-2′), 139.78 (C-3′), 119.40 (C-4′), 39.81 (C-5′), 32.99 (C-6′), 21.69 (Two *C*H_3–Ar).

*3.2.14. (4-methylphenyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-oate (**5b**)*

M.p 221–223 °C; **yield** 71%; **(+)-ESI-MS:** *m/z* (%) 625 ($[M + Na]^+$), 601 ($[M-H]^-$); **(+)-HRESI-MS:** *m/z* 603.4043 (calc: 603.4044 for $C_{39}H_{55}O_5$); **^{1}H NMR:** δ at 0.82 (s, 3H, CH_3-28), 0.85 (s, 6H, CH_3-23, 24), 1.14 (s, 3H, CH_3-27), 1.15 (s, 3H, CH_3-25), 1.33 (s, 3H, CH_3-29), 1.37 (s, 3H, CH_3-26), 2.02 (s, 3H, CH_3-32), 2.35 (s, 3H, CH_3-Ar), 2.37 (s, 1H, CH-9), 2.79 (d, 1H, CH-1 eq), 4.53 (dd, 1H, CH-3), 5.63 (s, 1H, CH-12), 6.9 (d, 2H, CH-3′, 5′), 7.17 (d, 2H, CH-2′, 6′), $J_{2',\,3'} = J_{5',\,6'} = 6$ Hz; **^{13}C NMR:** δ at 38.78 (C-1), 23.61 (C-2), 80.56 (C-3), 38.06 (C-4), 55.02 (C-5), 17.43 (C-6), 32.74 (C-7), 45.40 (C-8), 61.71 (C-9), 36.96 (C-10), 199.76 (C-11), 128.50 (C-12), 168.74 (C-13), 44.21 (C-14), 26.50 (C-15), 26.47 (C-16), 31.95 (C-17), 48.46 (C-18), 41.13 (C-19), 43.23 (C-20), 31.20 (C-21), 37.76 (C-22), 28.08 (C-23), 16.73 (C-24), 16. 44 (C-25), 18.74 (C-26), 23.39 (C-27), 28.14 (C-28), 29.62 (C-29), 175.03 (C-30), 170.71 (C-31), 21.34 (C-32), 148.39 (C-33), 120.98 (C-34), 129.81 (C-1′), 135.26 (C-2′), 129.81 (C-3′), 120.98 (C-4′), 38.78 (C-5′), 23.61 (C-6′), 20.90 ($\underline{C}H_3$- Ar).

*3.2.15. (4-ethyl carboxyphenyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-oate (**5c**)*

M.p 209–211 °C; **yield** 76%; **(+)-ESI-MS:** *m/z* (%) 683 ($[M + Na]^+$); **(+)-HRESI-MS:** *m/z* 683.3919 (calc: 683.3918 for $C_{41}H_{56}O_7Na$); **^{1}H NMR:** δ at 0.85 (s, 9H, CH_3-28, 23, 24), 1.12 (s, 6H, CH_3-27, 25), 1.36 (s, 9H, CH_3-26, 29), 1.42 (t, 3H, O–CH_2–CH_3), 2.02 (s, 3H, CH_3-32), 2.34 (s, 1H, CH-9), 2.76 (d, 1H, CH-1 eq), 4.35 (q, 2H, O═C–O–CH_2), 4.49 (dd, 1H, CH-3), 5.65 (s, 1H, CH-12), 7.08 (d, 2H, CH-3′, 5′), 8.06 (d, 2H, CH-2′, 6′), $J_{2',\,3'} = J_{5',\,6'} = 9$ Hz; **^{13}C NMR:** δ at 38.72 (C-1), 23.52 (C-2), 80.56 (C-3), 38.02 (C-4), 54.98 (C-5), 17.34 (C-6), 32.67 (C-7), 45.38 (C-8), 61.72 (C-9), 36.90 (C-10), 200.02 (C-11), 128.64 (C-12), 168.74 (C-13), 44.41 (C-14), 26.39 (C-15), 26.39 (C-16), 31.93 (C-17), 48.45 (C-18), 40.97 (C-19), 43.18 (C-20), 31.08 (C-21), 37.72 (C-22), 28.05 (C-23), 16.66 (C-24), 16. 38 (C-25), 18.64 (C-26), 23.36 (C-27), 28.05 (C-28), 28.57 (C-29), 174.61 (C-30), 171.04 (C-31), 21.32 (C-32), 154.40 (C-33), 121.43 (C-34), 131.14 (C-1′), 134.67 (C-2′), 131.14 (C-3′), 121.43 (C-4′), 38.72 (C-5′), 23.52 (C-6′), 167.90 (O═$\underline{C}$–Ar), 61.08 (O–CH_2), 14.31(CH_2–CH_3).

*3.2.16. (4-chlorophenyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-oate (**5d**)*

M.p 241–243 °C; **yield** 78%; **(+)-ESI-MS:** *m/z* (%) 645 ($[M + Na]^+$), 621 ($[M-H]^-$); **(+)-HRESI-MS:** *m/z* 623.3496 (calc: 623.3497 for $C_{38}H_{52}O_5Cl$); **^{1}H NMR:** δ at 0.87 (s, 9H, CH_3-28, 23, 24), 1.15 (s, 6H, CH_3-27, 25), 1.33 (s, 3H, CH_3-29), 1.38 (s, 3H, CH_3-26), 2.04 (s, 3H, CH_3-32), 2.36 (s, 1H, CH-9), 2.78 (d, 1H, CH-1 eq), 4.51 (dd, 1H, CH-3), 5.66 (s, 1H, CH-12), 7.02 (d, 2H, CH-2′, 6′), 7.34 (d, 2H, CH-3′, 5′),$J_{2',\,3'} = J_{5',\,6'} = 9$ Hz; **^{13}C NMR:** δ at 38.71 (C-1), 23.51 (C-2), 80.55 (C-3), 38.00 (C-4), 54.95 (C-5), 17.32 (C-6), 32.65 (C-7), 45.37 (C-8), 61.70 (C-9), 36.88 (C-10), 200.04 (C-11), 128.61 (C-12), 168.78 (C-13), 44.28 (C-14), 26.36 (C-15), 26.36 (C-16), 31.90 (C-17), 48.47 (C-18), 40.97 (C-19), 43.16 (C-20), 31.06 (C-21), 37.68 (C-22), 28.02 (C-23), 16.64 (C-24), 16. 37 (C-25), 18.62 (C-26), 23.33 (C-27), 28.02 (C-28), 28.55 (C-29), 174.88 (C-30), 171.03 (C-31), 21.30 (C-32), 149.50 (C-33), 122.81 (C-34), 129.49 (C-1′), 131.10 (C-2′), 129.49 (C-3′), 122.81 (C-4′), 38.71 (C-5′), 23.51 (C-6′).

*3.2.17. (4-bromophenyl)-3β-acetyl-11-oxoolean-12-en-18β-H-30-oate (**5e**)*

M.p 243–245 °C; **yield** 79%; **(+)-ESI-MS:** *m/z* (%) 667 ($[M + H]^+$); **(+)-HRESI-MS:** *m/z* 667.2989 (calc: 667.2992 for $C_{38}H_{52}O_5Br$).**^{1}H NMR:** δ at 0.87 (s, 9H, CH_3-28, 23, 24), 1.15 (s, 6H, CH_3-27, 25), 1.33 (s, 3H, CH_3-29), 1.36 (s, 3H, CH_3-26), 2.05 (s, 3H, CH_3-32), 2.36 (s, 1H, CH-9), 2.78 (d, 1H, CH-1 eq), 4.52 (dd, 1H, CH-3), 5.66 (s, 1H, CH-12), 6.93 (d, 2H, CH-2′, 6′), 7.49 (d, 2H, CH-3′, 5′), $J_{2',\,3'} = J_{5',\,6'} = 9$ Hz; **^{13}C NMR:** δ at 38.71 (C-1), 23.52 (C-2), 80.00 (C-3), 38.00 (C-4), 54.96 (C-5), 17.32 (C-6), 32.65 (C-7), 45.37 (C-8), 61.70 (C-9), 36.87 (C-10), 200.03 (C-11), 128.62 (C-12), 168.76 (C-13), 44.30 (C-14), 26.38 (C-15), 26.38 (C-16), 31.90 (C-17), 48.46 (C-18), 40.96 (C-19), 43.16 (C-20), 31.06 (C-21), 37.68 (C-22), 28.02 (C-23), 16.65 (C-24), 16. 37 (C-25), 18.63 (C-26), 23.33 (C-27), 28.02 (C-28), 28.55 (C-29), 174.62 (C-30), 171.03 (C-31), 21.31 (C-32), 149.78 (C-33), 123.25 (C-34), 132.47 (C-1′), 119.90 (C-2′), 132.47 (C-3′), 123.25 (C-4′), 38.71 (C-5′), 23.52 (C-6′).

*3.2.18. Preparation of N-(2-carboxyphenyl)-3β-hydroxy-11-ketoolean-12-en-18-βH-30-amide (**6**)*

To a solution of **4g** (500 mg, 0.75 mmol) in MeOH (50 ml) was added KOH (490 mg). After being refluxed for 3 h, the reaction mixture was neutralized with 2 N HCl, the formed precipitate was filtered under vacuum, washed with water and dried to give a yellowish residue**.** It was crystallized several times from methanol to give rise to 360 mg of compound **6**.

m.p 279–280 °C; **yield:** 77%; **^{1}H NMR:** δ at0.78 (s, 3H, CH_3-28), 0.86 (s, 3H, CH_3-24), 0.89 (s, 3H, CH_3-23), 1.08 (s, 3H, CH_3-27), 1.12 (s, 3H, CH_3-25), 1.25 (s, 3H, CH_3-29), 1.39 (s, 3H, CH_3-26), 2.40 (s, 1H, CH-9), 2.75 (d, 1H, CH-1 eq), 4.52 (dd, 1H, CH-3), 5.97 (s, 1H, CH-12), 7.09(t, 1H, CH-4′), 7.56(t, 1H, CH-5’), 8.10(d, 1H, CH-6′), 8.76 (d, 1H, CH-3′), 11.63 (br-s, 1H, Ar-COOH).

3.2.19. General procedures for the preparation of compounds 7 and 8

To a solution of **6** (150 mg, 0.26 mmol) in dry pyridine (10 ml), was added succinic anhydride (400 mg, 4 mmol) for the preparation of compound **7** or phthalic anhydride (600 mg, 4 mmol) for the preparation of compound **8**, in presence of 4A° molecular sieve. The mixture was refluxed for 8 h, neutralized by hydrochloric acid. The formed precipitate was filtered under vacuum, washed with water and dried. Each of the residues was purified on silica gel column chromatography.

3.2.20. N-(2-carboxyphenyl)-3β-O-carboxypropanoyloxy-11-oxoolean-18β-H-30-amide (7)

m.p 169–170 °C; **yield:**59%; **(+)-ESI-MS**: *m/z* (%) 688 ([M-H]$^-$, 100); **(+)-HRESI-MS**: *m/z* 688.3851 (calc: 688.3855 for $C_{41}H_{54}NO_8$). **^{1}H NMR:** δ at 0.77 (s, 3H, CH_3-28), 0.83 (s, 3H, CH_3-24), 0.86 (s, 3H, CH_3-23), 1.08 (s, 3H, CH_3-27), 1.11 (s, 3H, CH_3-25), 1.23 (s, 3H, CH_3-29), 1.39 (s, 3H, CH_3-26), 2.39 (s, 1H, CH-9), 2.66 (t, 4H, CH_2-32, 33),2.75 (d, 1H, CH-1 eq), 4.52 (dd, 1H, CH-3), 5.97 (s, 1H, CH-12), 7.08(t, 1H, CH-4′), 7.54(t, 1H, CH-5′), 8.09(d, 1H, CH-6′), 8.78 (d, 1H, CH-3’), 11.63 (br-s, 1H, Ar–COOH); **^{13}C NMR:** δ at 38.87 (C-1), 23.45 (C-2), 81.04 (C-3), 38.05 (C-4), 54.99 (C-5), 17.30 (C-6), 32.63 (C-7), 45.75 (C-8), 61.71 (C-9), 37.08 (C-10), 201.80 (C-11), 128.79 (C-12), 170.72 (C-13), 45.52 (C-14), 26.45 (C-15), 26.35 (C-16), 31.89 (C-17), 47.95 (C-18), 41.04 (C-19), 43.40 (C-20), 31.02 (C-21), 37.64 (C-22), 28.01 (C-23), 16.70 (C-24), 16. 43 (C-25), 18.72 (C-26), 23.35 (C-27), 28.50 (C-28), 29.03 (C-29), 177.12 (C-30), 175.25 (C-31), 29.69 (C-32), 29.69 (C-33), 172.14 (C-34), 142.23 (C-1′), 114.95 (C-2′), 131.54 (C-3′), 122.46 (C-4′), 134.90 (C-5′), 120.54 (C-6′), 171.82 (Ar–*C*OOH).

3.2.21. N-(2-carboxyphenyl)-3β-O-phthaloyl-11-oxoolean-18β-H-30-amide (8)

m.p 177–178 °C; **yield:**62%; **(+)-ESI-MS**: *m/z* (%) 760 ([M + Na]$^+$), 736 ([M−H]$^-$); **(+)-HRESI-MS**: *m/z* 760.3813 (calc: 760.3820 for $C_{45}H_{55}NO_8Na$). **^{1}H NMR:** δ at 0.77 (s, 3H, CH_3-28), 0.90 (s, 3H, CH_3-24), 0.96 (s, 3H, CH_3-23), 1.09 (s, 3H, CH_3-27), 1.10 (s, 3H, CH_3-25), 1.28 (s, 3H, CH_3-29), 1.41 (s, 3H, CH_3-26), 2.43 (s, 1H, CH-9), 2.78 (d, 1H, CH-1 eq), 4.79 (dd, 1H, CH-3), 6.13 (s, 1H, CH-12), 7.06 (t, 1H, CH-4″), 7.52 (m, 3H, CH-5′, 4′, 5′), 7.67 (m, 1H, CH-6′),7.86 (s, 1H,CH-3″), 8.03 (d, 1H, 6′), 8.76 (d, 1H, 3′).

3.2.22. Preparation of N-[2-(N-P-tolyl-benzamide)]-phenyl-3β-acetyl-11-oxoolean-12-en-18β-H-30-amide (9)

Compound **4g** (100 mg, 0.15 mmol) was dissolved in 10 ml dry dichloromethane and triethylamine (25 µl, 0.15 mmol) was added. After stirring for 5 min, 0.02 ml of ethylchloroformate was added, followed by stirring for one hour, then 17 mg (0.15 mmol) of p-toluidine was added. Stirring is continued over night. Water (10 ml) was added and the organic layer was separated. It was washed at first with 2 N HCl followed with water, dried over anhydrous sodium sulfate and the solvent distilled off. The residue was purified on silica gel column chromatography.

m.p 149–150 °C; **yield:** 67%; **(+)-ESI-MS**: *m/z* (%) 643 ([M + Na]$^+$, 100), 719 ([M−H]$^-$); **(+)-HRESI-MS**: *m/z* 743.4382 (calc: 743.4394 for $C_{46}H_{60}N_2O_5Na$). **^{1}H NMR:** δ at 0.77 (s, 3H, CH_3-28), 0.85 (s, 6H, CH_3-23, 24), 1.08 (s, 3H, CH_3-27), 1.13 (s, 3H, CH_3-25), 1.20 (s, 3H, CH_3-29), 1.37 (s, 3H, CH_3-26) 2.03 (s, 3H, CH_3-32), 2.29 (s, 3H, CH_3-Ar), 2.34 (s, 1H, CH-9), 2.76 (d, 1H, CH-1 eq), 4.50 (dd, 1H, CH-3), 5.79 (1H, s, CH-12), 7.07(t, 1H, CH-4′), 7.12 (d, 2H, CH-3″, 5″), 7.42 (d, 2H, CH-2″, 6″), 7.51 (t, 1H, CH-5′), 7.62 (d, 1H, CH-6′),8.15 (s, 1H, NH), 8.57 (d, 1H, CH-3′), 11.26 (s, 1H, NH).

3.2.23. General procedures for the preparation of compounds 11–13

A mixture of 100 mg (0.19 mmol) of compound **10** and 0.8 mmol of the appropriate acid anhydride were boiled for 10 h in 2 ml dry pyridine, in presence of 200 mg 4A° molecular sieve, without access of water. The reaction mixture was diluted with 2 ml of acetone, acidified with hydrochloric acid to pH ~ 3–4. The residue was filtered under vacuum, washed with hot water, dried over anhydrous sodium sulfate and the solvent distilled off. Each residue was purified on silica gel column chromatography. It was eluted with hexane–ethyl acetate system, (step gradient mode, 8:2–7:3 v/v).

3.2.24. 3-O-β-carboxypropionyl-11-oxoolean-12-en-18β-H-20β-O-methyl ester (11)

m.p 262–265 °C [lit. 262–264 °C (Kondratenko et al., 2001)]; **yield:** 68%;**(+)-ESI-MS**: *m/z* (%) 607 ([M + Na]$^+$), 583 ([M-H]$^-$); **(+)-HRESI-MS**: *m/z* 583.3646 (calc: 583.3640 for $C_{35}H_{51}O_7$).**^{1}H NMR:** δ at 0.78 (s, 3H, CH_3-28), 0.85 (s, 3H, CH_3-24, 23), 1.10 (s, 3H, CH_3-27), 1.12 (s, 3H, CH_3-25), 1.23 (s, 3H, CH_3-29), 1.34 (s, 3H, CH_3-26), 2.33 (s, 1H, CH-9), 2.63 (m, 4H, CH_2-32, 33), 2.77 (d, 1H, CH-1 eq), 3.66 (s, 3H, CH_3-35), 4.52 (dd, 1H, CH-3),5.64 (s, 1H, CH-12**)**.

3.2.25. 3-O-β-phthaloyl-11-oxoolean-12-en-18β-H-20β-O-methyl ester (12)

m.p 156–158 °C(lit. 154–157 °C (Kondratenko et al., 2001); **yield:** 71%;**(+)-ESI-MS**: *m/z* (%) 655 ([M + Na]$^+$), 631 ([M−H]$^-$); **(+)-HRESI-MS**: *m/z* 631.3642 (calc: 631.3640 for $C_{39}H_{51}O_7$). **^{1}H NMR:** δ at 0.78 (s, 3H, CH_3-28), 0.92 (s, 3H, CH_3-24), 0.96 (s, 3H, CH_3-23), 1.11 (s, 3H, CH_3-27), 1.13 (s, 3H, CH_3-25), 1.23 (s, 3H, CH_3-29), 1.36 (s, 3H, CH_3-26), 2.37 (s, 1H, CH-9), 2.85 (d, 1H, CH-1 eq), 3.67 (s, 3H, CH_3-32), 4.79 (dd, 1H, CH-3), 5.67 (s, 1H,

CH-12), 7.56 (m, 2H, CH-4′, 5′), 7.75 (m, 1H, CH-6′), 7.93 (m, 1H, CH-3′).

*3.2.26. 3-O-β-Carboxy-trans-propenoyl-11-oxoolean-12-en-18β-H-20β-O-methyl ester (**13**)*

m.p 209–211 °C; **yield:** 45%;**(+)-ESI-MS**: *m/z* (%) 605 ([M + Na]$^+$), 581 ([M-H]$^-$); **(+)-HRESI-MS**: *m/z* 581.3485 (calc: 581.3484 for $C_{35}H_{49}NO_7$). **^{1}H NMR:** δ at 0.78 (s, 3H, CH_3-28), 0.87 (s, 3H, CH_3-24), 0.90 (s, 3H, CH_3-23), 1.11 (s, 3H, CH_3-27), 1.13 (s, 3H, CH_3-25), 1.23 (s, 3H, CH_3-29), 1.35 (s, 3H, CH_3-26), 2.35 (s, 1H, CH-9), 2.80 (d, 1H, CH-1 eq), 3.67 (s, 3H, CH_3-35), 4.60 (dd, 1H, CH-3), 5.65 (s, 1H, CH-12), 6.82 (s, 2H, CH-32, 33).

3.3. Anti-inflammatory testing

Rats used in the biological testing of glycyrrhetinic acid and its derivatives were obtained from The Animal House Colony of the National Research Centre (NRC), Egypt. Seventy mature Wistar rats of both sexes, weighing 150–200 g were used. All animals were housed in hygienic cages in well ventilated rooms with exhaust fans; received standard pellet diet and water were provided *ad libitum*. The study was performed at the Pharmacology Research Unit- NRC and was approved by the Ethics Committee of The National Research Centre and in accordance with the recommendations of the proper care and use of laboratory animals (published by the National Academy of Science, National Academy Press, Washington, D.C.).

The anti-inflammatory test was performed according to the method of (Winter et al., 1962). Paw edema was induced in rats by subcutaneous (s.c.) injection of 0.1 ml of 1% (w/v) carageenan in distilled water in the sub-plantar region of their left hind paws. A group of rats was left without any treatment but were given a respective volume of the solvent (few drops of tween-80 in distilled water), and was kept as a control group. Drugs were administered orally at a dose of 100 mg/kg, one hour before carageenan injection. Oral prednisolone (Hostacortin-H®, 5 mg/kg) and indomethacin (Indocid®, 20 mg/kg) were administered to two groups of rats as reference drugs. The paw volume of each rat was measured using fluid displacement method, utilizing plethysmometer apparatus at 0–4 h of carageenan injection.

Edema rate and inhibition rate of each group were calculated at the previously mentioned time intervals as follows:

$$\text{Edema rate}(\%) = Vt - Vo/Vo\text{Inhibition rate}(\%) = Ec - Et/Ec \quad (1)$$

where:

Vo is the volume before carageenan injection (ml); Vt is the volume at t hour after carageenan injection (ml);

Ec is the edema rate of the control group; Et is the edema rate of treated group.

The anti-inflammatory potencies of glycyrrhetinic acid and its derivatives were calculated by comparing their inhibition rate at different time intervals; with those obtained from animals receiving either prednisolone or indomethacin, respectively, as standard anti-inflammatory drugs. Statistical analysis of the results, was done using analytical software named SPSS statistics 17.0, Release (Aug. 23, 2008), Chicago, USA. (See Tables 1 and 2).

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