Regular Article

Design, Preparation and Studies Regarding Cytotoxic Properties of Glycyrrhetinic Acid Derivatives

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Glycyrrhetinic acid (GA) is a natural product with certain antitumor activity. In order to enhance the cytotoxicity, a total of eighteen derivatives of GA were designed and synthesized. Their cytotoxicity against MDA-MB-231cells (human breast cancer cells) and HeLa cells (human cervical cancer cells), were evaluated by the MTT method (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). The results indicated that these target compounds have a wide molar activity range and some of them show better activity than the commercial drugs gefitinib and doxorubicin. Compound 6g induces apoptosis of 7, 10 and 44% of MDA-MB-231 cells at 5, 10, and 20 μM, respectively.

Key words glycyrrhetinic acid; cytotoxicity; MDA-MB-231; apoptosis

INTRODUCTION

Cancer, a type of diseases caused by abnormal cell growth, has been the major reason of death around the world. According to data from WHO, there are about 9.6 million cancer deaths, accounting for 1/6 of the global death toll, and most cancer deaths come from low- or middle-income countries in 2018.[1)] Due to the very high mortality, researchers are paying increasing attention to the development of new drugs which have a better selectivity and activity. Compared with the construction of new skeletons of compounds, modification of natural compounds may be seen as a more efficient and rapid way to reach these goals.[2)]

Glycyrrhetinic acid (GA, see Fig. 1), which is isolated from *Glycyrrhiza uralensis* Fisch, *Glycyrrhiza inflata* Batalin and *G. glabra* L. and is the main biologically active component of these plants, and GA derivatives show a variety of pharmaceutical effects, for example, anticancer,[3–7)] anti-oxidation,[8)] anti-inflammatory,[9,10)] antiviral[11–14)] and anti-ulcer[15)] properties, *etc.* In our previous work, we have reported on antitumor activity of derivatives of GA, and some of these compounds showed better anti-tumor activity than GA itself and Gefitinib.[16–18)]

However, one of the limiting factors for the application of glycyrrhetinic acid is that its biological activity is too low. It has been reported[19)] that the esterification of C30 carboxyl groups could increase the antitumor activity of glycyrrhetinic acid. Benzyl glycyrrhetinate, which was obtained from GA and benzyl bromide, has a better activity than ethyl glycyrrhetinate, which was synthesized by GA and ethyl bromide. Hence, we would like to enhance the lipophilicity of GA by introduction 4-(arylmethyl)benzyloxy glycyrrhetinates and expect that the activity would be increased. Compounds **6a**–**6g** were designed and synthesized according to this idea. To study the effect of the C3 hydroxyl groups on the antitumor activity of glycyrrhetinic acid, we designed 3-oxo-(substituted)benzyl glycyrrhetinates, **8a**–**8c** and 3-fluorobenzoyl benzyl glycyrrhetinates, **9a**–**9e**. We tested the cytotoxicity against human breast cancer, MDA-MB-231 cell lines and human cervical cancer HeLa cell lines of all synthetic compounds, **6a**–**6g**, **7a**–**7c**, **8a**–**8c** and **9a**–**9e**, Charts 1, 2 and 3, respectively, by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), method, with gefitinib and doxorubicin as standards.

RESULTS AND DISCUSSION

Chemistry: Design and Synthesis In this work, a total of eighteen derivatives of GA were designed and synthesized. The overall route of synthesis of targeted compounds is shown in Charts 1–3, respectively. The target compounds, **6a**–**6g** and intermediates, **7a**–**7c**, were synthesized starting from GA and the corresponding benzyl halides using K_2CO_3 base in *N*,*N*-dimethylformamide (DMF).

4-Benzyloxy benzyl bromides, the benzyl halides for the preparation of compounds **6a**–**6g**, were synthesized following three steps, including Williamson etherification using K_2CO_3 in DMF,[20)] reduction of aldehydes with $NaBH_4$ in methanol[21)] (MeOH) and bromination of alcohols using PBr_3 in ethyl ether[22,23)] (Et_2O). In the Williamson reactions, an excess of hydroxy benzaldehyde, is used involving easy purification by removal of the excess with base, such as K_2CO_3. Similarly, a slight excess of sodium borohydride and phosphorus tribromide allows complete conversion of the substrate for the reduction and bromination reactions, respectively, without column chromatography. We used dichloromethane (DCM) and diethyl ether as solvents for the bromination reactions respectively and crude product could be used directly in the next step when Et_2O was used as solent. Compound **8** was prepared from compound **7** under the action of Dess–Martin Periodinane in DCM. Acylation reactions of compound **7** were performed using 4-dimethylaminopyridine (DMAP) base in DCM at room temperature,[24)] overnight. Another method for synthesis of compound **9** was to use pyridine as both solvent

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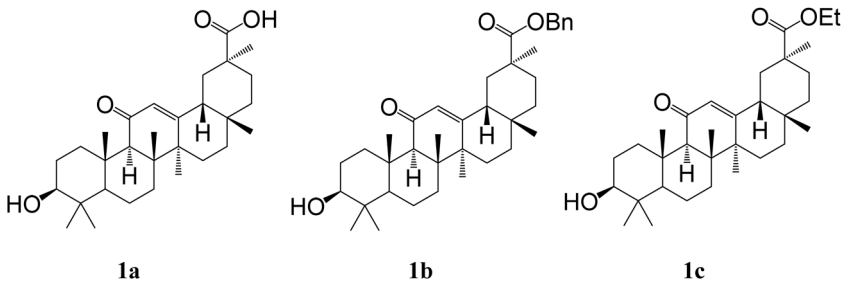
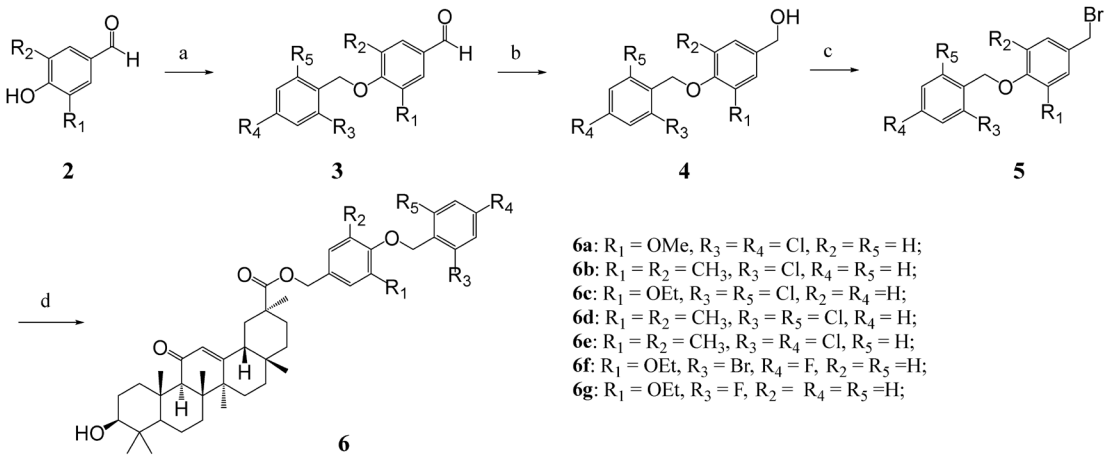
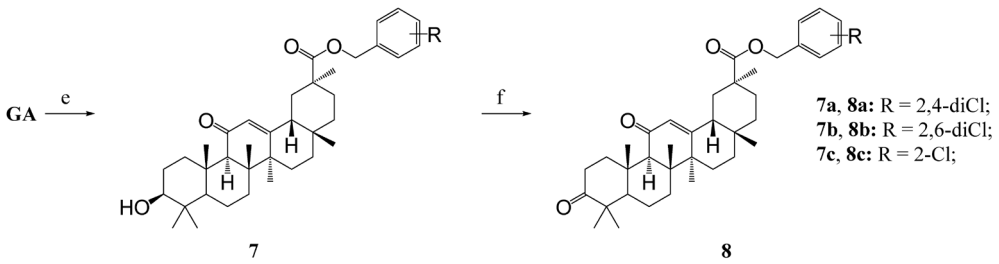


Fig. 1. The Structure of 18β-GA, **1a**, Benzyl Glycyrrhetinate **1b**, Ethyl Glycyrrhetinate, **1c**



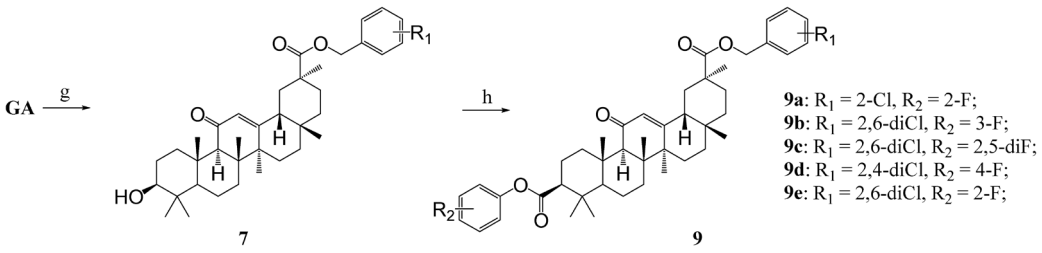
Reagents and conditions: a) appropriate halogenated hydrocarbons, K_2CO_3, DMF, room temperature (r.t.), overnight; b) $NaBH_4$, MeOH, 0–5°C for 30 min and r.t. for 3–4 h; c) PBr_3, Et_2O, 0–5°C for 30 min and r.t. for 3–4 h, d) GA, K_2CO_3, DMF, r.t., overnight.

Chart 1. The Synthetic Route of 4-(Arylmethyl)benzyloxy Glycyrrhetinates



Reagents and conditions: e) appropriate halogenated hydrocarbons, K_2CO_3, DMF, r.t., overnight; f) Dess-Martin peroxidation, DCM, r.t, 3–4 h.

Chart 2. The Synthetic Route of 3-Oxo-(substituted)benzyl Glycyrrhetinate



Reagents and conditions: g) appreciate halogenated hydrocarbons, K_2CO_3, DMF, r.t., overnight; h) appropriate benzoyl chloride, 4-dimethylaminopyridine, DCM, 0–5°C for 30 min and r.t. overnight.

Chart 3. The Synthetic Route of 3-Fluorobenzoyl Benzyl Glycyrrhetinate

and base.[25]

Antitumor Activity The IC_{50} values of the compounds against two cancer cell lines, human breast cancer cells, MDA-MB-231, and human cervical cancer cells, HeLa, are shown in Table 1. All data were determined *in vitro* with the MTT method. All target compounds had a cytotoxicity with a wide molar range. 2″-Fluorobenzyloxy-3′-ethoxybenzyl glycyrrhetinate (**6g**) and 3-oxo-2′,6′-dichlorobenzyl glycyrrhetinate (**8b**) have a better activity against HeLa cells, with IC_{50} 1.14 and 2.04 μM, respectively, than doxorubicin and gefitinib.

Table 1. Inhibitory Effects of Target Compounds on the Growth of HeLa Cells and MDA-MB-231 Cells

Compound	IC_{50} (μM)[a)]	
	MDA-MB-231	HeLa
6a	>100	>100
6b	>100	>100
6c	>100	>100
6d	57.35	30.75
6e	52.64	>100
6f	51.99	>100
6g	17.01	1.14
7a	9.56	22.16
7b	>100	10.24
7c	52.64	17.53
8a	>100	>100
8b	>100	2.04
8c	>100	>100
9a	>100	>100
9b	60.54	63.92
9c	56.82	58.46
9d	>100	>100
9e	17.03	34.48
GA[26,27)]	>100	>100
Gefitinib	10.38	6.78
Doxorubicin	4.78	2.40

[a)] IC_{50} is 50% inhibitory concentration.

Compounds **6d**, **7a**, **7b**, **7c**, **9b**, **9c** and **9e** have a moderate activity against HeLa cells, with values of IC_{50} from 22.16 to 63.92 μM. For MDA-MB-231 cells, only compound **7a** has a better activity than gefitinib, but the activity is lower than doxorubicin. Compounds **6d**, **6e**, **6f**, **6g**, **7c**, **9b**, **9c** and **9e** have a moderate activity, with values of IC_{50} from 17.01 to 60.54 μM.

Apoptosis Induced by Compound 6g Apoptosis is one of the mechanisms of cell death and is a common response to anticancer agents. For a more in-depth study of whether the anti MDA-MB-231 activity of compound **6g** is related to apoptosis, we detected the apoptosis rate by the Annexin V/7-AAD (7-aminoactinomycin D, a kind of dyeing agent used for apoptosis detection) double staining method (Fig. 2). The result indicated that compound **6g**, induces apoptosis in 7, 10, and 44% of MDA-MB-231 cells at 5, 10, and 20 μM, respectively, after treatment of different concentrations (0 μM as control, 5, 10 and 20 μM).

Structure–Activity Relationship According to this study, an introduction of halogenated benzyl, compounds **7a** and **7c**, led an increasing of cytotoxicity for both MDA-MB-231 cells and HeLa cells. Compound **7b**, which has a 2,4-dichlorobenzyl fragment, shows a good cytotoxicity and selectivity over HeLa cells. The results illustrated that halogenated benzyl glycyrrhetinates show a good cytotoxicity to MDA-MB-231 cells and HeLa cells. As we assumed, the newly synthesized 4-(arylmethyl)benzyloxy glycyrrhetinates, compounds **6d**, **6e**, **6f** and **6g**, will also show good cytotoxicity than glycyrrhetinic acid. Among them, compound **6g** shows the best cytotoxicity to both MDA-MB-231 cells and HeLa cells and selectivity over HeLa cells. The oxidation of the C3 hydroxyl groups, compounds **8a** and **8c**, reduces the antitumor activity of glycyrrhetinic acid except for the inhibition of HeLa cells by compound **8b**. Compared to benzyl glycyrrhetinates, compounds **7a**, **7b** and **7c**, benzyl fluorobenzoyl glycyrrhetinates, compounds **9a**, **9b**, **9c**, **9d** and **9e**, have a decreasing activity against both MDA-MB-231 cells and HeLa cells. This result demonstrated that the hydroxy, and perhaps the active hydrogen, at C3 position is a key group or fragment.

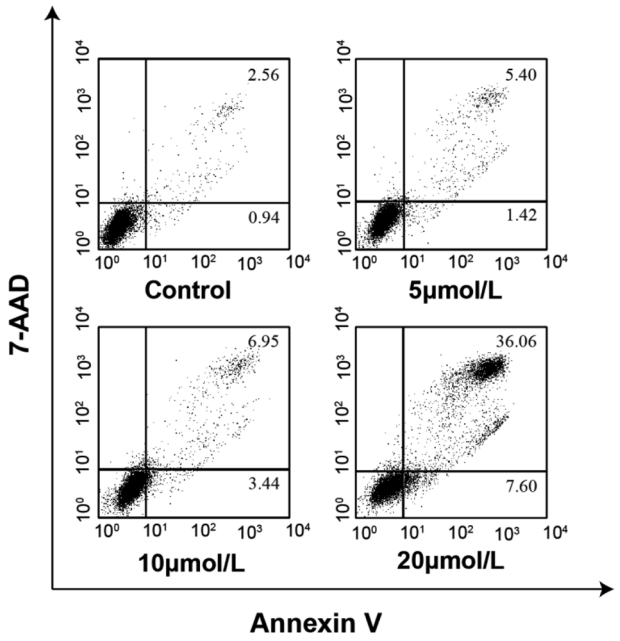


Fig. 2. Apoptosis of MDA-MB-231 Cells Was Detected by Annexin V/7-AAD Assay after Coincubation with Various Concentrations of **6g** (Drug Concentrations Were 0, 5, 10 and 20 μM) for 24 h

CONCLUSION

In this work, a total of eighteen derivatives of GA were designed and synthesized and the cytotoxicity against human breast cancer cells, MDA-MB-231, and human cervical cancer cells, HeLa, was detected by the MTT method. The result indicated that 2″-fluorobenzyloxy-3′ ethoxybenzyl glycyrrhetinate (**6g**) and 3-oxo-2′,6′-dichlorobenzyl glycyrrhetinate (**8b**) have a better activity against HeLa cells, with IC_{50} 1.14 and 2.04 μM, respectively, than doxorubicin and gefitinib. Compound **7a** has a better activity than gefitinib, but less than doxorubicin. Half of these compounds can be studied as lead compounds for each cell line. Compound **6g** induces apoptosis of MDA-MB-231 in 7, 10, and 44% of MDA-MB-231 cells at 5, 10, and 20 μM, respectively.

MATERIALS AND METHODS

Chemistry

General Information

All organic reagents were purchased from Aladdin (Shanghai, China), Energy-e (Shanghai, China), Macklin (Shanghai, China) and Ehsy (Shanghai, China) and used without further purification. All of the inorganic reagents were bought from Xinyue Chemical and Glass Co., Ltd. (Weihai, China). DCM, Et_2O, *n*-hexane and MeOH were obtained from Fuyu Chemical Co., Ltd. (Tianjin, China) *via* dealer Haituo Chemical Co., Ltd. (Weihai, China). Silica gel (200–300 mesh) for column chromatography and silica gel plates (GF254) were purchased

from Hailang Chemical Co., Ltd. (Qingdao, China). All the reactions were detected on silica gel plates (GF254) under UV light, 254 nm. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (101 MHz) spectra were recorded on a Bruker-400 instrument (Bruker; Fallanden, Switzerland) at 25°C with tetramethylsilane (TMS) as an internal standard and $CDCl_3$ as solvents. Mass spectra were measured on an LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, U.S.A.).

General Method for the Synthesis of **3**

A mixture of **2** (10 mmol), the corresponding halogenated hydrocarbons (8 mmol), K_2CO_3 (2.07 g, 15 mmol) in DMF (5 mL) was stirred in a 50 mL round bottom flask at 25°C for 8–12 h. Saturated brine was added when the reaction was over, and the mixture was extracted with DCM (25 mL × 2). The combined organic layers were washed with a saturated solution of potassium carbonate, until the spot of **2** was invisible on the TLC plate, and with water, and dried with anhydrous sodium sulfate. Solvent was removed *in vacuo* to furnish a white solid and the product can be used for the next step without further purification.

General Method for the Synthesis of **4**

A mixture of **3** (8 mmol) in MeOH (35 mL) was cooled down to 0°C and then $NaBH_4$ (0.45 g, 12 mmol) was added in ten portions. The mixture was stirred at 0°C for 30 min and at room temperature for 3–4 h. An equal volume of saturated brine was added into the mixture when the reaction was over. MeOH was removed *in vacuo* and the resulting mixture was extracted by EtOAc (25 mL × 2). The combined organic layers were washed with dilute hydrochloric acid (30 mL × 2) and water (30 mL × 2), dried with anhydrous sodium sulfate and the solvent removed *in vacuo* to give a white solid.

General Method for the Synthesis of **5**

A solution of **4** (8 mmol) in Et_2O (30 mL) was cooled to 0°C and then a solution of PBr_3 (1.13 mL, 12 mmol) in Et_2O (10 mL) was added slowly in a dropwise manner, about one drop per second. The mixture was stirred at 0°C for 3–4 h and the reaction was monitored by TLC. After the reaction was completed, ice saturated sodium bicarbonate was added in a dropwise manner, and the organic layer was extracted from the system and washed with saturated sodium bicarbonate and water/ice, dried with anhydrous sodium sulfate and the solvent was removed *in vacuo* to give a white or yellow solid.

General Method for the Synthesis of **8**

A mixture of **7** (1 mmol) and Dess–Martin reagent (1.5 mmol) in DCM (25 mL) was stirred at 25°C for about 30 min. Then, the mixture was washed with a saturated solution of sodium bicarbonate (25 mL × 2) and water (25 mL × 2). The organic layer was dried with anhydrous sodium sulfate and the solvent was removed *in vacuo* to give the compound. Crude products were purified bycolumn chromatography, with silica gel as the stationary phase and eluting with *n*-hexane : EtOAc, 1 : 1, v/v.

General Method for the Synthesis of **6** and **7**

A mixture of GA (1 mmol), **5** or appropriate halogenated hydrocarbon (1 mmol), K_2CO_3 (0.21 g, 1.5 mmol) in DMF (5 mL) was stirred in a 50 mL round bottom flask at 25°C for 8–12 h. Saturated brine was added when the reaction was over, and the mixture was extracted with DCM (25 mL × 2). The combined organic layers were washed with water and dried with anhydrous sodium sulfate. The solvent was removed *in vacuo* to furnish a white solid and the product was purified by column chromatography, with silica gel as the stationary phase and eluting with *n*-hexane : EtOAc, 1 : 1, v/v.

General Method for the Synthesis of **9**

A solution of **7** (1 mmol), DMAP (0.18g, 1.5 mmol) and the corresponding benzoyl chloride (1 mmol) in dried DCM (20 mL) was stirred in a 50 mL round bottom flask at 30°C overnight. Saturated brine (20 mL) was added after the reaction was finished and the organic layer was washed with a solution of sodium bicarbonate (5% (w/w) 30 mL × 2). Then the solvent was removed *in vacuo* to furnish target compound **9** and the crude products were purified by column chromatography, with *n*-hexane : EtOAc, from 8 : 1 to 4 : 1 (v/v).

Characterization of Target Compounds

4-((2,4-Dichlorobenzyl)oxy)-3-methoxybenzyl Glycyrrhetinate (**6a**)

White solid. Yield (Y) = 68%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.51 (d, J = 8.3 Hz, 1H), 7.40 (d, J = 2.1 Hz, 1H), 7.25 (dd, J = 8.4, 2.1 Hz, 1H), 6.93 (d, J = 1.8 Hz, 1H), 6.89 (dd, J = 8.2, 1.8 Hz, 1H), 6.84 (d, J = 8.2 Hz, 1H), 5.56 (s, 1H), 5.20 (s, 2H), 5.08 (dd, J = 26.3, 12.0 Hz, 2H), 3.91 (s, 3H), 3.22 (dd, J = 10.6, 5.6 Hz, 1H), 2.78 (dt, J = 13.2, 3.3 Hz, 1H), 2.32 (s, 1H), 2.06–1.94 (m, 3H), 1.92 (dd, J = 13.5, 2.7 Hz, 1H), 1.80 (td, J = 13.5, 4.2 Hz, 1H), 1.71–1.54 (m, 5H), 1.51 (s, 2H), 1.43 (d, J = 13.0 Hz, 2H), 1.38 (d, J = 4.3 Hz, 1H), 1.34 (s, 3H), 1.27 (ddd, J = 8.7, 5.9, 2.0 Hz, 2H), 1.20 (d, J = 12.5 Hz, 1H), 1.14 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.00 (s, 3H), 0.95 (dd, J = 12.8, 4.5 Hz, 1H), 0.80 (s, 3H), 0.71 (s, 3H), 0.68 (s, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 200.07, 176.26, 169.08, 149.65, 147.67, 133.95, 133.47, 132.89, 129.95, 129.52, 129.10, 128.48, 127.34, 121.26, 113.96, 112.38, 78.76, 77.24, 67.68, 66.11, 61.81, 56.09, 54.94, 48.21, 45.33, 43.98, 43.17, 41.11, 39.14, 37.66, 37.08, 32.75, 31.75, 31.20, 28.47, 28.24, 28.10, 27.31, 26.46, 26.40, 23.38, 18.68, 17.49, 16.37, 15.59. High resolution (HR) MS electrospray ionization (ESI^+): Calculated for $C_{45}H_{59}Cl_2O_6$ $(M+H)^+$: 765.3689, found: 765.3707.

4-((2-Chlorobenzyl)oxy)-3,5-dimethylbenzyl Glycyrrhetinate (**6b**)

White solid. Y = 72%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.72 (dd, J = 7.5, 1.5 Hz, 1H), 7.39 (dd, J = 7.8, 1.4 Hz, 1H), 7.33 (tt, J = 3.9, 1.9 Hz, 1H), 7.29 (dd, J = 7.6, 1.8 Hz, 1H), 7.05 (s, 2H), 5.51 (s, 1H), 5.19 (d, J = 11.9 Hz, 1H), 4.94 (d, J = 11.9 Hz, 1H), 4.89 (s, 2H), 3.22 (dd, J = 10.6, 5.6 Hz, 1H), 2.78 (dt, J = 13.4, 3.3 Hz, 1H), 2.32 (s, 7H), 2.07–1.88 (m, 4H), 1.80 (td, J = 13.6, 4.4 Hz, 1H), 1.68–1.54 (m, 5H), 1.52–1.36 (m, 5H), 1.34 (s, 3H), 1.29 (t, J = 7.4 Hz, 2H), 1.16 (s, 3H), 1.12 (s, 3H), 1.09 (s, 3H), 1.00 (s, 3H), 0.97–0.93 (m, 1H), 0.80 (s, 3H), 0.73 (s, 3H), 0.69 (d, J = 10.4 Hz, 1H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 200.00, 176.30, 169.04, 155.67, 135.49, 132.59, 131.90, 131.48, 129.26, 129.17, 129.09, 128.92, 128.46, 126.97, 78.78, 77.24, 70.75, 65.97, 61.78, 54.94, 48.15, 45.29, 43.94, 43.17, 41.15, 39.14, 37.64, 37.06, 32.74, 31.74, 31.25, 28.42, 28.24, 28.10, 27.32, 26.48, 26.41, 23.35, 18.69, 17.48, 16.38, 16.36, 15.59. HRMS (ESI^+): Calculated for $C_{46}H_{62}ClO_5$ $(M+H)^+$: 729.4286, found: 729.4307.

4-((2,4-Dichlorobenzyl)oxy)-3-ethoxybenzyl Glycyrrhetinate (**6c**)

White solid. Y = 81%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.33 (d, J = 7.9 Hz, 2H), 7.21 (dd, J = 8.5, 7.5 Hz, 1H), 7.05 (d, J = 8.5 Hz, 1H), 6.94–6.88 (m, 2H), 5.61 (s, 1H), 5.32 (s, 2H), 5.13–5.03 (m, 2H), 4.10 (dq, J = 17.5, 7.1 Hz, 3H), 3.22 (dd, J = 10.7, 5.6 Hz, 1H), 2.79 (dt, J = 13.3, 3.2 Hz, 1H), 2.30

(d, J = 15.0 Hz, 1H), 2.07–1.96 (m, 4H), 1.91 (dd, J = 13.2, 10.4 Hz, 1H), 1.80 (td, J = 13.5, 4.3 Hz, 1H), 1.63 (qd, J = 13.3, 7.3 Hz, 5H), 1.52–1.43 (m, 2H), 1.40 (t, J = 7.0 Hz, 4H), 1.35 (s, 3H), 1.34–1.29 (m, 1H), 1.29–1.22 (m, 2H), 1.19 (t, J = 7.9 Hz, 1H), 1.14 (d, J = 4.6 Hz, 6H), 1.11 (s, 3H), 1.00 (s, 3H), 0.96 (dd, J = 12.4, 4.3 Hz, 1H), 0.80 (s, 3H), 0.72 (s, 3H), 0.66 (d, J = 13.4 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 200.04, 176.27, 169.10, 150.13, 148.71, 137.21, 132.53, 130.77, 130.24, 128.55, 128.39, 121.45, 117.37, 114.83, 78.77, 77.24, 67.31, 66.12, 64.99, 61.80, 54.95, 48.13, 45.34, 43.98, 43.18, 41.13, 39.14, 37.67, 37.09, 32.76, 31.75, 31.22, 28.46, 28.25, 28.11, 27.32, 26.46, 26.42, 23.39, 18.67, 17.49, 16.37, 15.59, 14.90. HRMS (ESI$^+$): Calculated for $C_{46}H_{61}Cl_2O_6$ $(M+H)^+$: 779.3845, found: 779.3865.

4-((2,6-Dichlorobenzyl)oxy)-3,5-dimethylbenzyl Glycyrrhetinate (**6d**)

White solid. Y = 75%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.36 (d, J = 8.0 Hz, 2H), 7.24 (dd, J = 8.6, 7.4 Hz, 1H), 7.02 (s, 2H), 5.56 (s, 1H), 5.19–5.12 (m, 3H), 4.97 (d, J = 11.9 Hz, 1H), 3.23 (dd, J = 10.7, 5.6 Hz, 1H), 2.80 (dt, J = 13.3, 3.3 Hz, 1H), 2.34 (s, 6H), 2.03 (dd, J = 20.3, 7.4 Hz, 3H), 1.97–1.90 (m, 1H), 1.81 (td, J = 13.6, 4.3 Hz, 1H), 1.64 (dt, J = 19.6, 8.5 Hz, 5H), 1.52 (s, 1H), 1.48–1.39 (m, 3H), 1.36 (s, 3H), 1.33–1.24 (m, 3H), 1.17 (s, 3H), 1.16 (s, 1H), 1.13 (s, 3H), 1.11 (s, 3H), 1.01 (s, 4H), 0.99–0.95 (m, 1H), 0.81 (s, 3H), 0.74 (s, 3H), 0.70 (d, J = 10.5 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 199.99, 176.30, 169.07, 155.53, 137.09, 132.96, 131.80, 131.67, 130.30, 129.15, 128.59, 128.50, 78.76, 77.37, 77.26, 77.05, 76.74, 68.63, 65.94, 61.78, 54.94, 48.10, 45.30, 43.93, 43.17, 41.12, 39.14, 37.64, 37.07, 32.75, 31.73, 31.25, 28.45, 28.25, 28.11, 27.32, 26.47, 26.42, 23.37, 18.69, 17.49, 16.78, 16.36, 15.60. HRMS (ESI$^+$): Calculated for $C_{46}H_{61}Cl_2O_5$ $(M+H)^+$: 763.3896, found: 763.3907.

4-((2,4-Dichlorobenzyl)oxy)-3,5-dimethylbenzyl Glycyrrhetinate (**6e**)

White solid. Y = 71%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.67 (d, J = 8.4 Hz, 1H), 7.41 (d, J = 2 Hz, 1H), 7.32 (dd, J = 2 Hz, 8.4 Hz, 1H),7.05 (s, 2H), 5.48 (s, 1H), 5.30 (s, 1H), 5.20 (d, J = 12 Hz, 1H), 4.92 (d, J = 12 Hz, 1H), 4.84 (s, 2H), 3.22 (q, J = 5.6 Hz, 10.8 Hz, 1H), 2.80–2.75 (m, 1H), 2.29 (s, 6H), 2.05–1.89 (m, 5H), 1.83–1.76 (m, 1H), 1.66–1.52 (m, 7H), 1.52–1.36 (m, 3H), 1.34 (s, 3H), 1,32–1.24 (m, 3H), 1.16 (s, 3H), 1.11 (s, 3H), 1.09 (s, 3H), 1.00 (s, 3H), 0.80 (s, 3H), 0.73 (s, 3H), 0.68 (d, J = 10.4 Hz, 1H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 200.03, 176.29, 169.08, 155.49, 134.19, 134.00, 133.11, 132.05, 131.38, 129.84, 129.21, 129.06, 128.41, 127.29, 78.76, 77.25, 70.14, 65.92, 61.78, 54.92, 48.18, 45.29, 43.94, 43.16, 41.14, 39.13, 39.11, 37.63, 37.05, 32.73, 31.74, 31.24, 28.42, 28.23, 28.10, 27.30, 26.47, 26.40, 23.35, 18.67, 17.48, 16.37, 15.59. HRMS (ESI$^+$): Calculated for $C_{46}H_{61}Cl_2O_5$ $(M+H)^+$: 763.3896, found: 763.3911.

4-((4-Bromo-2-fluorobenzyl)oxy)-3-ethoxybenzyl Glycyrrhetinate (**6f**)

White solid. Y = 75%. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.74 (t, J = 8.4 Hz, 1H), 7.61–7.55 (m, 3H), 7.20 (s, 2H), 5.88 (s, 1H), 5.46 (s, 2H), 5.38 (q, J = 24 Hz, 12 Hz, 2H), 4.40 (q, J = 12 Hz, 8 Hz, 2H), 3.56–3.51 (m, 1H), 3.12–3.07 (m, 1H), 2.63 (s, 1H), 2.36–2.28 (m, 3H), 2.25–2.20 (m, 1H), 2.14–2.07 (m, 1H), 2.02–1.87 (m, 6H), 1.77–1.72 (m, 5H), 1.70–1.68 (m, 1H), 1.66 (s, 3H), 1.64–1.61 (m, 1H), 1.59–1.57 (m, 2H), 1.55–1.49 (m, 1H), 1.44 (d, J = 4 Hz, 6H), 1.41 (s, 3H), 1.31 (s, 3H), 1.28–1.24 (m, 1H), 1.12 (s, 3H), 1.02 (s, 3H), 0.99 (s, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 200.07, 176.27, 169.12, 149.26, 147.99, 130.66, 130.62, 130.17, 128.50, 127.60, 127.56, 121.27, 118.99, 118.74, 115.02, 114.00, 78.77, 77.25, 66.10, 64.70, 64.66, 64.62, 61.80, 54.93, 48.17, 45.34, 43.98, 43.17, 41.10, 39.14, 37.65, 37.08, 32.74, 31.74, 31.20, 28.46, 28.24, 28.10, 27.31, 26.45, 26.39, 23.39, 18.66, 17.48, 16.37, 15.59. HRMS (ESI$^+$): Calculated for $C_{46}H_{61}BrFO_5$ $(M+H)^+$: 807.3630, found: 809.3735 $(M+2+H)^+$.

4-((2-Fluorobenzyl)oxy)-3-methoxybenzyl Glycyrrhetinate (**6g**)

White solid. Y = 85%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.54 (dt, J = 7.5, 3.8 Hz, 1H), 7.32–7.24 (m, 1H), 7.13 (t, J = 7.2 Hz, 1H), 7.10–7.03 (m, 1H), 6.92 (dd, J = 4.9, 3.2 Hz, 2H), 6.88 (dd, J = 8.2, 1.7 Hz, 1H), 5.59 (s, 1H), 5.20 (s, 2H), 5.10–5.02 (m, 2H), 4.10 (q, J = 7.0 Hz, 2H), 3.22 (dd, J = 10.7, 5.6 Hz, 1H), 2.79 (dt, J = 13.3, 3.3 Hz, 1H), 2.32 (s, 1H), 2.08–1.96 (m, 3H), 1.91 (dt, J = 20.6, 10.9 Hz, 1H), 1.80 (td, J = 13.5, 4.2 Hz, 1H), 1.72–1.54 (m, 7H), 1.50–1.37 (m, 5H), 1.35 (s, 3H), 1.31 (dd, J = 8.1, 5.1 Hz, 1H), 1.28–1.22 (m, 2H), 1.18 (t, J = 8.3 Hz, 1H), 1.13 (d, J = 1.1 Hz, 6H), 1.10 (s, 3H), 1.00 (s, 3H), 0.95 (dd, J = 12.4, 4.2 Hz, 1H), 0.80 (s, 3H), 0.71 (s, 3H), 0.68 (s, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 200.07, 176.29, 169.14, 149.23, 148.32, 129.86, 129.54, 129.50, 129.44, 129.36, 128.52, 124.20, 124.16, 121.33, 115.26, 115.05, 114.86, 114.10, 78.76, 77.26, 66.15, 64.69, 61.80, 54.93, 48.14, 45.34, 43.98, 43.17, 41.10, 39.14, 37.67, 37.08, 32.75, 31.74, 31.20, 28.46, 28.25, 28.11, 27.31, 26.45, 26.40, 23.39, 18.66, 17.49, 16.37, 15.60. HRMS (ESI$^+$): Calculated for $C_{46}H_{62}FO_6$ $(M+H)^+$: 729.4530, found: 729.4551.

2′,4′-Dichlorobenzyl Glycyrrhetinate (**7a**)

White solid. Y = 90%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.42 (d, J = 2.0 Hz, 1H), 7.38 (d, J = 8.3 Hz, 1H), 7.28 (dd, J = 8.3, 2.0 Hz, 1H), 5.60 (s, 1H), 5.19 (s, 2H), 4.09 (dd, J = 19.4, 12.3 Hz, 1H), 3.21 (dt, J = 24.7, 12.4 Hz, 1H), 2.83–2.70 (m, 1H), 2.33 (s, 1H), 2.12–1.88 (m, 7H), 1.81 (tt, J = 17.5, 8.8 Hz, 1H), 1.71–1.53 (m, 5H), 1.43 (dd, J = 23.7, 11.8 Hz, 3H), 1.36 (s, 3H), 1.31 (t, J = 5.2 Hz, 1H), 1.28–1.23 (m, 3H), 1.18 (s, 3H), 1.12 (d, J = 6.3 Hz, 6H), 1.00 (s, 3H), 0.95 (dd, J = 13.0, 4.1 Hz, 1H), 0.81 (s, 3H), 0.77 (s, 3H), 0.70 (d, J = 11.3 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 200.10, 175.94, 168.94, 134.78, 134.43, 132.27, 130.92, 129.45, 128.53, 127.25, 78.53, 63.09, 61.76, 60.36, 54.88, 48.15, 45.32, 44.12, 43.14, 40.95, 39.12, 37.65, 37.02, 32.71, 31.76, 31.08, 28.44, 28.32, 28.10, 27.21, 26.39, 26.34, 23.36, 21.02, 18.63, 17.45, 15.63. HRMS (ESI$^+$): Calculated for $C_{37}H_{50}Cl_2O_6$ $(M+H)^+$: 629.3164, found: 629.3173.

2′,6′-Dichlorobenzyl Glycyrrhetinate (**7b**)

White solid. Y = 88%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.38 (d, J = 8.2 Hz, 2H), 7.30–7.26 (m, 1H), 5.48–5.41 (m, 2H), 5.34 (d, J = 11.6 Hz, 1H), 5.30 (s, 1H), 3.19 (dt, J = 35.7, 17.9 Hz, 1H), 2.76 (dt, J = 13.3, 3.3 Hz, 1H), 2.29 (s, 1H), 2.20–2.07 (m, 1H), 2.06–1.94 (m, 3H), 1.92–1.75 (m, 2H), 1.66–1.55 (m, 6H), 1.38 (dd, J = 11.7, 9.3 Hz, 4H), 1.32 (s, 3H), 1.26 (t, J = 7.1 Hz, 1H), 1.17 (s, 3H), 1.12 (d, J = 5.9 Hz, 6H), 0.99 (s, 3H), 0.93 (dd, J = 13.1, 4.6 Hz, 1H), 0.80 (s, 3H), 0.67 (t, J = 9.2 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 200.16, 176.11, 169.02, 136.85, 131.47, 130.73, 128.52, 128.47, 78.73, 61.76, 61.37, 60.41, 54.90, 48.25, 45.33, 44.31, 43.13, 40.94, 39.13, 37.66, 37.04, 32.75, 31.81, 31.14, 28.44, 28.38, 28.10, 27.28, 26.48, 26.38, 23.30, 18.67, 17.48, 16.38, 15.59. HRMS (ESI$^+$): Calculated for

$C_{37}H_{50}Cl_2O_6$ $(M+H)^+$: 629.3164, found: 629.3180.

2′-Chlorobenzyl Glycyrrhetinate (**7c**)

White solid. Y = 75%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.48–7.37 (m, 2H), 7.32–7.29 (m, 1H), 7.26 (d, J = 5.4 Hz, 2H), 5.53 (d, J = 14.9 Hz, 1H), 5.34–5.13 (m, 2H), 3.22 (dd, J = 10.7, 5.6 Hz, 1H), 2.78 (dt, J = 13.4, 3.4 Hz, 1H), 2.30 (d, J = 16.3 Hz, 1H), 2.13–2.01 (m, 3H), 1.99–1.90 (m, 1H), 1.81 (td, J = 13.6, 4.4 Hz, 1H), 1.71–1.51 (m, 6H), 1.50–1.36 (m, 3H), 1.35 (s, 3H), 1.35–1.22 (m, 3H), 1.19 (s, 3H), 1.13 (s, 3H), 1.11 (s, 3H), 1.00 (s, 3H), 0.98–0.89 (m, 1H), 0.80 (s, 3H), 0.76 (s, 3H), 0.69 (d, J = 10.3 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 200.18, 176.09, 169.01, 133.84, 133.69, 130.19, 129.75, 129.69, 128.56, 126.98, 78.77, 77.24, 63.80, 61.79, 54.92, 48.19, 45.36, 44.17, 43.17, 41.03, 39.14, 37.67, 37.06, 32.76, 31.81, 31.17, 28.44, 28.41, 28.10, 27.30, 26.47, 26.40, 23.37, 18.67, 17.49, 16.39, 15.59. HRMS (ESI^+): Calculated for $C_{37}H_{52}ClO_4$ $(M+H)^+$: 595.3554, found: 595.3560.

3-Oxo-2′,4′-dichlorobenzyl Glycyrrhetinate (**8a**)

White solid. Y = 70%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.44 (d, J = 2.0 Hz, 1H), 7.37 (d, J = 8.3 Hz, 1H), 7.27 (s, 1H), 5.64 (s, 1H), 5.19 (s, 2H), 2.96 (ddd, J = 13.5, 7.0, 4.0 Hz, 1H), 2.64 (ddd, J = 15.8, 11.2, 7.1 Hz, 1H), 2.44–2.31 (m, 2H), 2.12 (dd, J = 13.4, 3.3 Hz, 1H), 2.08–1.90 (m, 3H), 1.84 (td, J = 13.6, 4.3 Hz, 1H), 1.74–1.50 (m, 5H), 1.49–1.39 (m, 3H), 1.36 (s, 3H), 1.31 (d, J = 6.5 Hz, 2H), 1.27 (s, 3H), 1.21 (d, J = 8.6 Hz, 1H), 1.18 (s, 3H), 1.16 (s, 3H), 1.09 (s, 3H), 1.06 (s, 3H), 1.05 (s, 1H), 0.79 (s, 3H), ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 217.22, 199.38, 175.99, 169.43, 134.93, 134.60, 132.33, 131.04, 129.57, 128.53, 127.28, 77.24, 63.20, 61.07, 55.43, 48.23, 47.79, 45.21, 44.18, 43.31, 41.06, 39.79, 37.68, 36.70, 34.23, 32.13, 31.83, 31.13, 28.52, 28.36, 26.49, 26.39, 23.33, 21.43, 18.80, 18.53, 15.68. HRMS (ESI^+): Calculated for $C_{37}H_{49}Cl_2O_4$ $(M+H)^+$: 627.3008, found: 627.3026.

3-Oxo-2′,6′-dichlorobenzyl Glycyrrhetinate (**8b**)

White solid. Y = 75%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.41–7.36 (m, 2H), 7.30–7.28 (m, 1H), 5.45 (d, J = 11.7 Hz, 1H), 5.34 (d, J = 11.6 Hz, 1H), 2.93 (ddd, J = 13.5, 7.0, 4.0 Hz, 1H), 2.62 (ddd, J = 15.9, 11.1, 7.1 Hz, 1H), 2.40–2.30 (m, 2H), 2.15 (dd, J = 13.3, 3.2 Hz, 1H), 2.07–1.96 (m, 2H), 1.92–1.77 (m, 2H), 1.74–1.48 (m, 5H), 1.49–1.35 (m, 4H), 1.33 (s, 3H), 1.32–1.28 (m, 2H), 1.26 (s, 3H), 1.21 (s, 1H), 1.17 (d, J = 8.6 Hz, 6H), 1.09 (s, 3H), 1.06 (s, 3H), 1.03–0.99 (m, 1H), 0.82 (s, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 217.26, 199.40, 176.07, 169.53, 136.87, 131.47, 130.74, 128.52, 128.38, 77.25, 61.40, 61.01, 55.40, 48.30, 47.77, 45.17, 44.31, 43.26, 41.00, 39.77, 37.65, 36.66, 34.21, 32.12, 31.83, 31.13, 28.48, 28.36, 26.54, 26.41, 23.23, 21.41, 18.80, 18.53, 15.69. HRMS (ESI^+): Calculated for $C_{37}H_{49}Cl_2O_4$ $(M+H)^+$: 627.3008, found: 627.3020.

3-Oxo-2′-chlorobenzyl Glycyrrhetinate (**8c**)

White solid. Y = 72%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.46–7.39 (m, 2H), 7.33–7.28 (m, 2H), 7.27 (s, 1H), 5.59 (s, 1H), 5.24 (dd, J = 46.4, 12.8 Hz, 2H), 2.95 (ddd, J = 13.4, 7.0, 4.0 Hz, 1H), 2.63 (ddd, J = 15.9, 11.2, 7.1 Hz, 1H), 2.42 (s, 1H), 2.36 (ddd, J = 15.8, 6.4, 4.1 Hz, 1H), 2.12 (dd, J = 13.4, 3.6 Hz, 1H), 2.08–1.91 (m, 3H), 1.83 (td, J = 13.6, 4.2 Hz, 1H), 1.73–1.50 (m, 4H), 1.43 (ddd, J = 17.6, 9.6, 4.8 Hz, 2H), 1.36 (s, 3H), 1.35–1.28 (m, 3H), 1.27 (s, 3H), 1.25–1.20 (m, 1H), 1.20 (s, 3H), 1.15 (s, 3H), 1.09 (d, J = 7.6 Hz, 3H), 1.07 (s, 3H), 1.03 (dd, J = 13.7, 2.0 Hz, 1H), 0.78 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 217.25, 199.41, 176.04, 169.51, 133.86, 133.67, 130.22, 129.78, 129.70, 128.46, 126.98, 77.25, 63.82, 61.03, 55.41, 48.21, 47.78, 45.19, 44.17, 43.29, 41.08, 39.78, 37.66, 36.68, 34.23, 32.12, 31.82, 31.16, 28.48, 28.38, 26.52, 26.41, 23.30, 21.43, 18.80, 18.52, 15.68. HRMS (ESI^+): Calculated for $C_{37}H_{50}ClO_4$ $(M+H)^+$: 593.3398, found: 593.3408.

3-(2′-Fluorobenzoyl)-2″-chlorobenzyl Glycyrrhetinate (**9a**)

White solid. Y = 34%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.94 (t, J = 7.0 Hz, 1H), 7.54–7.46 (m, 1H), 7.46–7.38 (m, 2H), 7.33–7.28 (m, 2H), 7.20 (t, J = 7.6 Hz, 1H), 7.16–7.09 (m, 1H), 5.56 (s, 1H), 5.33–5.14 (m, 2H), 2.85 (d, J = 13.7 Hz, 1H), 2.38 (s, 1H), 2.17–1.92 (m, 4H), 1.82 (dd, J = 15.5, 8.3 Hz, 3H), 1.72–1.57 (m, 3H), 1.45 (dt, J = 27.9, 8.4 Hz, 3H), 1.37 (s, 3H), 1.36–1.23 (m, 5H), 1.20 (s, 6H), 1.13 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H), 0.87 (d, J = 11.0 Hz, 2H), 0.77 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 199.96, 176.02, 169.02, 133.78, 133.65, 132.06, 130.14, 129.70, 129.64, 128.48, 126.94, 123.86, 123.83, 119.29, 117.04, 116.82, 81.99, 63.75, 61.65, 55.01, 48.18, 45.36, 44.14, 43.16, 40.98, 38.79, 38.26, 37.63, 36.93, 32.67, 31.78, 31.14, 29.67, 28.41, 28.37, 28.10, 26.43, 26.37, 23.51, 23.32, 18.66, 17.39, 16.83, 16.43. HRMS (ESI^+): Calculated for $C_{44}H_{55}ClFO_5$ $(M+H)^+$: 717.3717, found: 717.3731.

3-(3′-Fluorobenzoyl)-2″,6″-dichlorobenzyl Glycyrrhetinate (**9b**)

White solid. Y = 40%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.83 (d, J = 7.7 Hz, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.45–7.34 (m, 3H), 7.28 (d, J = 11.1 Hz, 1H), 7.23 (s, 1H), 5.48–5.42 (m, 2H), 5.34 (d, J = 11.6 Hz, 1H), 4.75 (dd, J = 11.5, 5.0 Hz, 1H), 2.83 (d, J = 13.4 Hz, 1H), 2.35 (s, 1H), 2.13 (d, J = 9.6 Hz, 1H), 2.01 (d, J = 13.2 Hz, 2H), 1.91–1.65 (m, 5H), 1.62 (d, J = 13.5 Hz, 2H), 1.55 (s, 2H), 1.45 (dd, J = 27.2, 15.3 Hz, 4H), 1.34 (s, 3H), 1.25 (s, 1H), 1.19 (d, J = 7.7 Hz, 6H), 1.14 (s, 3H), 1.03 (s, 3H), 0.97 (d, J = 5.6 Hz, 2H), 0.94 (s, 3H), 0.86 (d, J = 11.6 Hz, 1H), 0.81 (s, 3H). ^{13}C-NMR (101 MHz, cdcl3) δ: 199.92, 176.07, 169.08, 136.83, 131.44, 130.68, 129.95, 129.87, 128.48, 128.39, 125.21, 119.87, 116.48, 116.25, 81.76, 77.19, 61.60, 61.35, 55.01, 48.26, 45.33, 44.29, 43.13, 40.90, 38.74, 38.38, 37.63, 36.89, 32.65, 31.79, 31.12, 28.42, 28.34, 28.18, 26.46, 26.35, 23.53, 23.24, 18.66, 17.37, 16.94, 16.41. HRMS (ESI^+): Calculated for $C_{44}H_{54}Cl_2FO_5$ $(M+H)^+$: 751.3327, found: 751.3340.

3-(2′,5′-Difluorobenzoyl)-2″,6″-dichlorobenzyl Glycyrrhetinate (**9c**)

White solid. Y = 40%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.60 (dd, J = 10.1, 6.8 Hz, 1H), 7.38 (d, J = 8.0 Hz, 2H), 7.28 (d, J = 8.4 Hz, 1H), 7.19 (dd, J = 10.0, 6.4 Hz, 1H), 7.10 (td, J = 9.3, 4.1 Hz, 1H), 5.48–5.41 (m, 2H), 5.34 (d, J = 11.7 Hz, 1H), 4.76 (dd, J = 10.7, 5.6 Hz, 1H), 2.83 (d, J = 13.6 Hz, 1H), 2.34 (s, 1H), 2.13 (d, J = 10.3 Hz, 1H), 2.01 (d, J = 13.0 Hz, 2H), 1.84 (dd, J = 36.5, 10.0 Hz, 4H), 1.72–1.51 (m, 5H), 1.50–1.38 (m, 3H), 1.34 (s, 3H), 1.25 (t, J = 16.3 Hz, 2H), 1.19 (d, J = 3.8 Hz, 6H), 1.13 (s, 3H), 1.07 (d, J = 18.1 Hz, 1H), 1.00 (s, 3H), 0.96 (s, 3H), 0.85 (d, J = 11.5 Hz, 1H), 0.81 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 199.93, 176.07, 169.09, 163.15, 159.18, 156.73, 156.65, 136.82, 131.43, 130.69, 128.48, 128.39, 82.55, 77.19, 61.60, 61.35, 54.97, 48.26, 45.32, 44.29, 43.12, 40.89, 38.74, 38.24, 37.63, 36.88, 32.65, 31.79, 31.11, 29.68, 28.42, 28.35, 28.09, 26.46, 26.35, 23.45, 23.25, 18.66, 17.38, 16.81, 16.43. HRMS (ESI^+): Calculated for $C_{44}H_{53}Cl_2F_2O_5$ $(M+H)^+$: 769.3233, found: 769.3232.

3-(4′-Fluorobenzoyl)-2″,4″-dichlorobenzyl Glycyrrhetinate (**9d**)

White solid. Y = 45%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.05 (dd, J = 8.5, 5.6 Hz, 2H), 7.44 (d, J = 1.7 Hz, 1H), 7.37 (d,

$J = 8.3$ Hz, 1H), 7.29 (d, $J = 1.7$ Hz, 1H), 7.11 (t, $J = 8.6$ Hz, 2H), 5.62 (s, 1H), 5.19 (s, 2H), 4.75 (dd, $J = 11.3$, 4.7 Hz, 1H), 2.85 (d, $J = 13.7$ Hz, 1H), 2.39 (s, 1H), 2.16–1.91 (m, 4H), 1.81 (dd, $J = 24.3$, 8.4 Hz, 4H), 1.62 (dd, $J = 32.1$, 18.4 Hz, 3H), 1.52–1.40 (m, 3H), 1.38 (s, 3H), 1.30 (dd, $J = 27.3$, 12.7 Hz, 3H), 1.20 (d, $J = 9.9$ Hz, 6H), 1.14 (s, 3H), 1.03 (s, 3H), 1.02–0.98 (m, 1H), 0.94 (s, 3H), 0.88 (d, $J = 11.3$ Hz, 1H), 0.78 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 199.93, 175.98, 168.98, 165.29, 134.86, 132.31, 132.04, 131.95, 130.95, 129.52, 128.55, 127.24, 115.51, 115.30, 81.41, 77.19, 63.14, 61.67, 55.04, 48.21, 45.36, 44.16, 43.18, 40.95, 38.77, 38.39, 37.65, 36.93, 32.66, 31.79, 31.11, 28.45, 28.34, 28.19, 26.41, 26.36, 23.60, 23.34, 18.66, 17.37, 16.96, 16.41. HRMS (ESI$^+$): Calculated for $C_{44}H_{54}Cl_2FO_5$ $(M + H)^+$: 751.3327, found: 751.3341.

3-(2′-Fluorobenzoyl)-2″,6″-Dichlorobenzyl Glycyrrhetinate (**9e**)

White solid. Y = 35%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.93 (t, $J = 7.3$ Hz, 1H), 7.50 (dd, $J = 12.6$, 7.0 Hz, 1H), 7.38 (d, $J = 8.0$ Hz, 2H), 7.28 (d, $J = 8.4$ Hz, 1H), 7.20 (t, $J = 7.6$ Hz, 1H), 7.15–7.09 (m, 1H), 5.50–5.38 (m, 2H), 5.34 (d, $J = 11.6$ Hz, 1H), 4.76 (dd, $J = 10.0$, 6.4 Hz, 1H), 2.82 (d, $J = 13.6$ Hz, 1H), 2.35 (s, 1H), 2.13 (d, $J = 10.1$ Hz, 1H), 2.01 (d, $J = 12.9$ Hz, 2H), 1.93–1.76 (m, 4H), 1.72–1.54 (m, 4H), 1.44 (dt, $J = 26.5$, 11.0 Hz, 4H), 1.34 (s, 3H), 1.31–1.22 (m, 2H), 1.19 (d, $J = 4.6$ Hz, 6H), 1.13 (s, 3H), 1.06 (d, $J = 5.1$ Hz, 1H), 0.99 (d, $J = 14.8$ Hz, 6H), 0.85 (d, $J = 11.3$ Hz, 1H), 0.81 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 199.96, 176.06, 169.04, 136.81, 134.12, 132.05, 131.43, 130.68, 128.48, 128.39, 123.86, 123.82, 117.04, 116.82, 81.99, 77.21, 61.62, 61.34, 54.99, 48.26, 45.33, 44.29, 43.12, 40.89, 38.78, 38.25, 37.63, 36.90, 32.67, 31.79, 31.11, 28.41, 28.34, 28.09, 26.45, 26.35, 23.49, 23.25, 18.66, 17.38, 16.83, 16.43. HRMS (ESI$^+$): Calculated for $C_{44}H_{54}Cl_2FO_5$ $(M + H)^+$: 751.3327, found: 751.3337.

In-Vitro **Antitumor Assays** MDA-MB-231 cells and HeLa cells in logarithmic growth phase were inoculated in 96-well plates, with a cell density of $3–4 \times 10^3$/well. The cells were incubated in a 5% CO_2, 37°C cell culture. When the cells had adhered, the test compound was added at the specified concentration. The same concentration of dimethyl sulfoxide, DMSO, was used as the negative control. After 48 h of incubation, 20 μL MTT (5 mg/mL) was added to each well, and the culture was continued for 4 h. After aspirating the supernatant with a pump, 150 μL of DMSO was added and the OD of each well was measured at 570 nm using a microplate reader. IC_{50} values were calculated using IC_{50} software (Prism 5.0).

Apoptosis Test The apoptosis rate of MDA-MB-231 cells was examined by double staining with Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI). Cells were seeded at a density of 5×10^4 cells/mL in 4-well plates (Nest, Wuxi, China). After 24 h incubation, the cells were treated with 250 μM PQ for 24 h, and pre-treated with VE and various concentrations (0, 5, 10, 20 μM) of **6g** for 3 h. After treatment with the indicated drugs, cells were washed twice with cold PBS and resuspended in 4°C binding buffer (Annexin V-FITC kit) containing 5 μL Annexin V-FITC stock for 15 min in the dark at 4°C; then, 10 μL PI was added to each tube for another 5 min, and then analysed using flow cytometry. The Annexin V + cells were considered apoptotic cells, the percentage of which was calculated by WinMDI 2.8 (J. Trotter, 1993–1998).

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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