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SYNTHESIS AND ANTITUMOR ACTIVITY OF 1-ACETYL-3-(4-PHENYL)-4,5-DIHYDRO-2-PYRAZOLINE-5-PHENYLURSOLATE AND 4-CHALCONE URSOLATE DERIVATIVES

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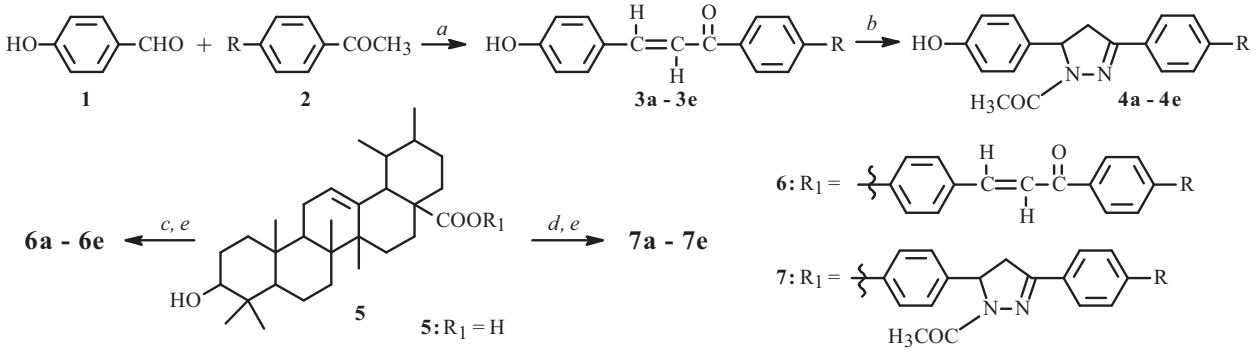
New 4-chalcone ursolate and 1-acetyl-3-(4-phenyl)-4,5-dihydro-2-pyrazoline-5-phenyl ursolate derivatives were synthesized by esterification of UA and chalcone or pyrazoline. The compounds were structurally confirmed by IR, ^1H NMR, ^{13}C NMR, and HR-MS spectroscopy. The cytotoxicity of ten derivatives was evaluated against A549, SKOV3, and HepG2 cell lines by MTT assay. The result showed that several compounds were more potent than UA against A549 and SKOV3 cells; however, none of them were more potent than UA against HepG2.

Keywords: ursolic acid, chalcone, pyrazoline, MTT assay, esterification.

Ursolic acid (UA, 3β-hydroxy-urs-12-en-28-oic acid, **5**), a pentacyclic triterpene, has been isolated from a large variety of vegetarian foods and many traditional medicinal plants. In past decades, UA has attracted considerable interest owing to its significant biological activities and promising clinical application as a chemotherapeutic and chemopreventive agent. Indeed, UA exhibits attractive pharmacological properties, including anti-inflammatory activity [1, 2], anti-HIV [3], anti-malarial [4], anti-microbial [5], anti-melanoma [6], and antitumor [7–11].

The conjugation of two bioactive compounds is now accepted as an effective strategy for designing ligands, inhibitors, and other drugs [8]. Chalcones, which belong to the flavonoid compounds, exhibit antioxidant and anti-inflammatory properties and have recently attracted attention also for their antitumor activity in preclinical models [12, 13]. The 2-pyrazoline ring system has attracted significant interest in organic and medicinal chemistry over the past several decades. Scaffolds containing the 2-pyrazoline (4,5-dihydropyrazole) heterocycle have demonstrated a wide range of biological activity, including anticancer activity [14]. In order to explore biologically more active derivatives of this naturally occurring triterpene, two series of ursolic acid derivatives were synthesized with chalcone and pyrazoline structures through reactions as shown in Scheme 1.



2, **3a**, **4a**, **6a**, **7a**: R = H; **2**, **3b**,**4b**,**6b**,**7b**: R = CH_3; **2**, **3c**,**4c**,**6c**,**7c**: R = OCH_3; **2**, **3d**,**4d**,**6d**,**7d**: R = Cl; **2**, **3e**,**4e**,**6e**,**7e**: R = F

a. NaOH, CH_3OH, stir, 0–5°C for 0.5 h then room temperature for 18 h; *b*. $N_2H_4 \cdot H_2O$, CH_3COOH, reflux, 2 h; *c*. $(CF_3CO)_2O$, toluene, stir for 0.5 h, then **3a–3e**, reflux, 8 h; *d*. $(CF_3CO)_2O$, toluene, stir for 0.5 h, then **4a–4e**, reflux, 8 h; *e*. 10% NaOH, acetone, stir, 22 h.

Scheme 1

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TABLE 1. Cytotoxic Acitivities on A549, SKOV3, HepG2* Cells by MTT Assay

Compound	IC_{50} μg/mL		Compound	IC_{50} μg/mL	
	A549	SKOV3		A549	SKOV3
5	16.4	21.3	**7a**	21.5	20.8
6a	13.6	14.6	**7b**	27.1	29.4
6b	19.8	18.4	**7c**	21.7	26.5
6c	24.6	22.2	**7d**	25.3	27.8
6d	13.2	15.2	**7e**	18.4	18.9
6e	21.2	23.1			

*HepG2 – none of them were more potent than UA, HepG2 for compound **5** – 6.3 μg/mL.

The cytotoxicity of the derivatives was evaluated against HepG2, A549, and SKOV3 cells by MTT assay.

In summary, a concise and effective procedure was successfully developed to synthesize UA derivatives containing chalcone and pyrazoline structures. C-28 chalcone ursolates with an electron-withdrawing group might be important for improving tumor cell growth inhibitory activity. Compounds **6a**, **6d** with an electron-withdrawing group showed potent cytotoxic activity against SKOV3, A549 cell lines. Compounds **7a–7e** with the C-28 pyrazoline ursolate structure showed weak inhibitory activity, suggesting that UA-flavonoid compounds conjugated at C-28 could influence antiproliferative activity against SKOV3, A549 cell lines.The present investigation should be valuable for further study.

EXPERIMENTAL

Materials. All reagents were purchased from commercial suppliers. Ursolic acid was purchased from Sichuan Chemical Company of China. The human HepG2, A549, and SKOV3 cell lines were purchased from the American Tissue Culture Collection (ATCC, Rockville, MD, USA). The culture medium was a mixture of DEME medium (Gibco, USA) and 10% fetal calf serum (National Hyclone Bio-Engineering Company, Lanzhou, China). Cells were grown as monolayers in a controlled atmosphere (37°C, 5% CO_2) in DEME medium supplemented with 10% fetal calf serum [15].

Melting points were determined on an XT-4 microscope melting point apparatus and were uncorrected. NMR spectra were measured with a Bruker AV II-400 MHz spectrometer. Infrared (IR) spectra were measured with a Perkin–Elmer 16PC-FT infrared spectrometer. Chemical shifts refer to tetramethylsilane, which was used as an internal reference. HR-MS spectra were measured with a Bruker Daltonics ESI-Bio TOF-Q mass spectrometer. Thin-layer chromatography was accomplished using silica gel GF254, and detection of compounds was achieved by spraying with a solution of vanillin–ethanol (0.50 g/10 mL) or by irradiation with a UV lamp. Column chromatography was carried out using silica gel (300–400 mesh) with an appropriate solvent.

General Procedure for 4-Hydroxy-4′-(un)substituted Chalcone [16]. Acetophenone **2** (10.0 mmol) and sodium hydroxide (10.0 mmol) were dissolved in dry ethanol. To this solution was added 4-hydroxybenzaldehyde (**1**) (10.0 mmol) dropwise. The mixture was stirred for 0.5 h at 0–5°C and continued for 18 h at room temperature. The solution was poured into ice water and neutralized with dilute HCl. The products were filtered as yellow precipitates and recrystallized from ethanol to afford **3a–3e**.

4-Hydroxychalcone (3a). Yield 89%, yellow powder, mp 180–182°C [17].

4-Hydroxy-4′-methylchalcone (3b). Yield 90%, yellow powder, mp 156–158°C. IR (KBr, ν, cm^{-1}): 3217, 3027, 2813, 2590, 1648, 1557, 1278, 1172, 1037, 979, 815. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.40 (3H, s, CH_3), 6.82–8.05 (8H, m, PhH), 7.71 (2H, m, CH=CH), 10.08 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 21.09 (CH_3), 115.82, 127.98, 128.41, 129.23, 130.43, 135.43, 143.09, 160.04 (Ar), 125.82, 144.09 (olefinic-C), 188.52 (C=O). HR-MS-ESI *m/z* 261.3845, $C_{15}H_{12}O_2Na$ $[M + Na]^+$, calcd 261.3819.

4-Hydroxy-4′-methoxychalcone (3c). Yield 77%, yellow powder, mp 178–180°C [18].

4-Hydroxy-4′-chlorochalcone (3d). Yield 94%, yellow powder, mp 174–176°C [17].

4-Hydroxyl-4′-fluorochalcone (3e). Yield 81%, yellow powder, mp 184–186°C. IR (KBr, ν, cm^{-1}): 3340, 1655, 1600, 1561, 1509, 1340, 1286, 1167, 1037, 979, 817. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 6.83–8.24 (9H, m, PhH), 7.73

(2H, m, CH=CH), 10.12 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 120.79, 121.05, 130.96, 136.45, 136.54, 139.82, 165.43, 171.33 (Ar), 123.44, 149.92 (olefinic-C), 192.75 (C=O). HR-MS (ESI) *m/z* 265.3426, $C_{15}H_{11}O_2FNa$ $[M + Na]^+$, calcd 265.3395.

General Procedure for 4-[1-Acetyl-3-4′-(un)substituted-phenyl-2-pyrazolin-5-yl]phenol (4a–4e) [13, 19]. A solution of **3a–3e** (5 mmol) and hydrazine hydrate (1 mL) in acetic acid (15 mL) was refluxed at 120° for 2 h. The reaction mixture was cooled at room temperature and then poured into ice-cold water (200 mL). The product was isolated, filtered, and washed with water and dichloromethane to give **4a–4e**.

4-(1-Acetyl-3-phenyl-2-pyrazolin-5-yl)phenol (4a). Yield 83%, white powder, mp 212–214°C. IR (KBr, ν, cm^{-1}): 3259, 3056, 3021, 2958, 1641, 1594, 1513, 1456, 1418, 1362, 1224, 1163, 1037, 963, 828. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.28 (3H, s, $COCH_3$), 3.09 (2H, d, J = 18.4, CH_2C=N), 3.80 (1H, t, J = 12.0, PhCHN), 6.68–7.79 (9H, m, PhH), 9.35 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 21.73 (keto-CH_3), 42.02 (CH_2), 58.93 (CH-N), 115.21, 126.54, 126.70, 128.73, 130.19, 131.17, 132.76, 154.09 (Ar), 156.49 (C=N), 167.21 (C=O). HR-MS (ESI) *m/z* 303.4315, $C_{17}H_{16}O_2N_2Na$ $[M + Na]^+$, calcd 303.4282.

4-(1-Acetyl-3-4′-methylphenyl-2-pyrazolin-5-yl)phenol (4b). Yield 83%, white powder, mp 238–240°C. IR (KBr, ν, cm^{-1}): 3304, 3015, 2950, 2590, 1873, 1646, 1596, 1454, 1361, 1224, 1165, 1034, 960, 818. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.26 (3H, s, $COCH_3$), 2.34 (3H, s, $PhCH_3$), 3.18 (2H, d, J = 4.0, H_2C=N), 3.74 (1H, t, J = 7.4, PhCHN), 6.68–7.68 (8H, m, PhH), 9.35 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 20.98 (CH_3), 21.09 (keto-CH_3), 42.06 (CH_2), 58.83 (CH-N), 115.82, 126.52, 126.67, 128.45, 129.30, 132.81, 140.03, 154.09 (Ar), 156.48 (C=N), 167.09 (C=O). HR-MS-ESI *m/z* 317.4689, $C_{18}H_{18}O_2N_2Na$ $[M + Na]^+$, calcd 317.4611.

4-(1-Acetyl-3-4′-methoxyphenyl-2-pyrazolin-5-yl)phenol (4c). Yield 89%, yellow crystal, mp 202–204°C. IR (KBr, ν, cm^{-1}): 3205, 2962, 2933, 2835, 2596, 2362, 1882, 1641, 1514, 1454, 1366, 1252, 1173, 1036, 861. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.26 (3H, s, $COCH_3$), 3.06 (2H, d, J = 17.6, CH_2C=N), 3.75 (1H, t, J = 12.0, PhCHN), 3.80 (3H, s, OCH_3), 5.41 (1H, dd, J = 8.0, PhCHN), 6.68–7.73 (8H, m, PhH), 9.34 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 42.12 (CH_2), 21.72 (keto-CH_3), 55.28 (CH-N), 58.77 (OCH_3), 114.16, 115.20, 123.70, 126.67, 128.21, 132.86, 153.88, 160.85 (Ar), 156.45 (C=N), 166.96 (C=O). HR-MS-ESI *m/z* 333.4674, $C_{18}H_{18}O_3N_2Na$ $[M + Na]^+$, calcd 333.4605.

4-(1-Acetyl-3-4′-chlorophenyl-2-pyrazolin-5-yl)phenol (4d). Yield 84%, white powder, mp 240–242°C. IR (KBr, ν, cm^{-1}): 3258, 3019, 2957, 1878, 1644, 1594, 1513, 1453, 1362, 1225, 1140, 1088, 962, 826. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.27 (3H, s, $COCH_3$), 3.15 (2H, d, J = 14.0, CH_2C=N), 3.79 (1H, t, J = 12.0, PhCHN), 6.68–7.80 (8H, m, PhH), 9.36 (1H, s, OH). ^{13}C NMR (400 MHz, DMSO-d_6, δ): 21.73 (keto-CH_3), 41.91 (CH_2), 59.14 (CH-N), 115.20, 115.30, 126.73, 127.03, 128.27, 128.39, 128.81, 130.08, 132.63, 153.10 (Ar), 156.51 (C=N), 167.28 (C=O). HR-MS-ESI *m/z* 337.8576, $C_{17}H_{15}O_2N_2ClNa$ $[M + Na]^+$, calcd 337.8688.

4-(1-Acetyl-3-4′-fluorophenyl-2-pyrazolin-5-yl)phenol (4e). Yield 86%, white powder, mp 234–236°C. IR (KBr, ν, cm^{-1}): 3250, 3022, 2960, 2814, 1889, 1643, 1604, 1513, 1457, 1332, 1226, 1102, 963, 832. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.27 (3H, s, $COCH_3$), 3.17 (2H, d, J = 17.6, CH_2C=N), 3.79 (1H, t, J = 16.0, PhCHN), 6.68–7.85 (8H, m, PhH), 9.35 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 21.71 (keto-CH_3), 42.08 (CH_2), 59.05 (CH-N), 115.20, 115.71, 126.71, 127.82, 128.86, 128.92, 132.70, 153.50 (Ar), 156.50 (C=N), 167.20 (C=O). HR-MS-ESI *m/z* 321.4237, $C_{17}H_{15}O_2N_2FNa$ $[M + Na]^+$, calcd 321.4142.

General Procedure for 4′-(Un)substituted-4-chalcone Ursolate and 1-Acetyl-3-[4-(un)substituted phenyl]-4,5-dihydro-2-pyrazoline-5-phenyl Ursolate [8]. The toluene (10 mL) solution of UA (**5**, 2.5 mmol) and TFAA (1.4 mL) was stirred at room temperature for 0.5 h, **3a–3e** and **4a–4e** (3 mmol) were added, and the reaction mixture was refluxed at 92°C for 8 h and concentrated in vacuo to obtain the product as a solid after the mixture was cooled. The solid was dissolved in acetone and solution of 10% NaOH was added to adjust the pH to 8–9. The mixture solution was stirred at room temperature for 22 h. The crude product was extracted with ethyl acetate, and the organic phase was washed with water and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo to obtain the product as a solid. The crude solid was purified by silica gel column chromatography using petroleum ether–acetone (6:1) to give **6a–6e** and **7a–7e**.

4-Chalcone Ursolate (6a). Yield 62%, yellow powder, mp 128–130°C. IR (KBr, ν, cm^{-1}): 3441, 2925, 2869, 1748, 1700, 1663, 1601, 1506, 1453, 1210, 1162, 1090, 1024, 976, 690. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, CH_3), 0.79 (3H, s, CH_3), 0.88 (3H, s, CH_3), 0.89 (3H, d, J = 6.4, CH_3), 0.94 (3H, s, CH_3), 0.99 (3H, d, J = 6.0, CH_3), 1.14 (3H, s, CH_3), 1.35 (2H, t, J = 6.4, 4.2, CH_2), 1.50 (2H, t, J = 8.4, 7.6, CH_2), 1.91 (2H, t, J = 4.4, CH_2), 2.35 (1H, d, J = 11.2, H-18), 5.34 (1H, s, OH), 6.98–7.97 (9H, m, PhH), 7.45 (1H, d, J = 12.0, α-H), 7.79 (1H, d, J = 12.5, β-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 190.42, 175.71, 152.91, 143.91, 138.18, 137.75, 132.82, 132.25, 129.53, 128.64, 128.50, 126.13, 122.23, 121.93,

78.99, 55.23, 52.97, 48.66, 47.56, 42.24, 39.74, 39.14, 38.86, 38.76, 38.67, 36.98, 36.58, 33.18, 30.94, 30.66, 28.16, 28.14, 27.22, 24.30, 23.47, 23.37, 21.17, 18.32. HR-MS-ESI *m/z* 68.4854, $C_{45}H_{58}O_4Na$ $[M + Na]^+$, calcd 685.4256.

4′-Methyl-4-chalcone Ursolate (6b). Yield 72%, yellow powder, mp 132–134°C. IR (KBr, ν, cm^{-1}): 3441, 2925, 2869, 1748, 1700, 1663, 1601, 1506, 1453, 1210, 1162, 1090, 1024, 976, 690. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.79 (3H, s, CH_3), 0.89 (3H, d, J = 6.4, CH_3), 0.90 (3H, d, J = 6.0, CH_3), 0.94 (3H, s, CH_3), 0.99 (3H, d, J = 6.0, CH_3), 1.14 (3H, s, CH_3), 1.35 (2H, t, J = 6.4, 4.2, CH_2), 1.50 (2H, t, J = 8.4, 7.6, CH_2), 1.91 (2H, t, J = 4.4, CH_2), 2.17 (3H, s, CH_3), 2.35 (1H, d, J = 11.2, H-18), 5.34 (1H, s, OH), 6.98–7.97 (8H, m, PhH), 7.45 (1H, d, J = 12.0, *α*-H), 7.79 (1H, d, J = 12.5, *β*-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 184.62, 170.45, 147.56, 138.42, 138.21, 132.51, 130.36, 127.13, 125.43, 124.22, 124.09, 123.86, 123.40, 120.89, 116.70, 73.73, 50.00, 47.73, 47.67, 43.41, 43.29, 42.32, 37.00, 36.95, 34.51, 34.47, 33.90, 33.62, 33.51, 31.74, 31.34, 27.94, 25.42, 24.03, 22.93, 21.98, 19.06, 18.13, 18.13, 16.44, 15.92, 13.08, 12.38, 11.75, 10.28. HR-MS-ESI *m/z* 699.4403, $C_{46}H_{60}O_4Na$ $[M + Na]^+$, calcd 699.4384.

4′-Methoxy-4-chalcone Ursolate (6c). Yield 74%, yellow powder, mp 120–122°C. IR (KBr, ν, cm^{-1}): 3446, 2925, 2869, 1747, 1658, 1601, 1505, 1457, 1212, 1164, 1025, 974, 833. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, CH_3), 0.79 (3H, s, CH_3), 0.88 (3H, s, CH_3), 0.91 (3H, d, J = 6.4, CH_3), 0.94 (3H, s, CH_3), 0.99 (3H, d, J = 6.0, CH_3), 1.00 (3H, s, CH_3), 1.33 (2H, t, J = 6.4, 4.2, CH_2), 1.52 (2H, t, J = 8.4, 7.6, CH_2), 1.91 (2H, t, J = 4.4, CH_2), 2.34 (1H, d, J = 11.2, H-18), 3.90 (3H, s, OCH_3), 5.34 (1H, s, OH), 6.98–8.02 (8H, m, PhH), 7.51 (1H, d, J = 12.0, *α*-H), 7.79 (1H, d, J = 12.0, *β*-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 188.57, 175.71, 163.75, 163.46, 152.73, 143.03, 137.76, 137.34, 132.46, 131.37, 131.05, 130.82, 130.58, 130.05, 129.42, 126.13, 122.17, 121.74, 121.41, 113.86, 78.96, 69.55, 55.50, 55.25, 53.83, 52.96, 48.65, 48.53, 47.57, 42.24, 39.75, 39.13, 38.76, 36.98, 36.58, 33.18, 31.74, 30.67, 29.27, 28.17, 27.22, 24.29, 23.47, 21.17, 18.33, 17.63, 17.00, 15.68, 15.52. HR-MS-ESI *m/z* 715.4491, $C_{46}H_{60}O_5Na$ $[M + Na]^+$, calcd 715.4416.

4′-Chloro-4-chalcone Ursolate (6d). Yield 67%, yellow powder, mp 138–140°C. IR (KBr, ν, cm^{-1}): 3446, 2925, 2869, 1747, 1664, 1596, 1505, 1454, 1210, 1163, 1090, 1003, 975, 827. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, CH_3), 0.79 (3H, s, CH_3), 0.88 (3H, s, CH_3), 0.89 (3H, d, J = 6.4, CH_3), 0.94 (3H, s, CH_3), 0.99 (3H, d, J = 6.0, CH_3), 1.14 (3H, s, CH_3), 1.33 (2H, t, J = 6.4, 4.2, CH_2), 1.52 (2H, t, J = 8.4, 7.6, CH_2), 1.91 (2H, t, J = 4.4, CH_2), 2.35 (1H, d, J = 11.2, H-18), 5.32 (1H, s, OH), 6.98–7.97 (8H, m, PhH), 7.43 (1H, d, J = 12.0, *α*-H), 7.79 (1H, d, J = 12.0, *β*-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 189.02, 175.69, 153.06, 144.40, 139.28, 139.24, 137.75, 136.47, 132.06, 130.35, 129.91, 129.61, 128.96, 126.14, 122.29, 121.50, 121.32, 78.98, 55.23, 52.97, 52.91, 48.67, 48.55, 47.55, 42.24, 42.19, 39.74, 39.71, 39.13, 38.86, 38.84, 38.76, 38.67, 36.98, 36.58, 33.17, 30.65, 28.16, 27.22, 24.30, 23.47, 23.37, 21.16, 18.31, 17.62, 17.56, 16.99, 15.66, 15.52. HR-MS-ESI *m/z* 719.3891, $C_{45}H_{57}O_4ClNa$ $[M + Na]^+$, calcd 719.3916.

4′-Fluoro-4-chalcone Ursolate (6e). Yield 66%, yellow powder, mp 124–126°C. IR (KBr, ν, cm^{-1}) : 3442, 2926, 2870, 1749, 1701, 1664, 1601, 1505, 1455, 1209, 1159, 1090, 1027, 976, 836. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.72 (3H, s, CH_3), 0.79 (3H, s, CH_3), 0.89 (3H, s, CH_3), 0.94 (3H, d, J = 6.4, CH_3), 0.99 (3H, s, CH_3), 1.00 (3H, d, J = 6.0, CH_3), 1.14 (3H, s, CH_3), 1.33 (2H, t, J = 6.4, 4.2, CH_2), 1.56 (2H, t, J = 8.4, 7.6, CH_2), 1.89 (2H, t, J = 4.4, CH_2), 2.38 (1H, d, J = 11.2, H-18), 5.34 (1H, s, OH), 6.79–7.80 (8H, m, PhH), 7.43 (1H, d, J = 12.0, *α*-H), 7.75 (1H, d, J = 12.0, *β*-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 188.67, 175.69, 166.45, 164.77, 152.99, 144.10, 137.75, 134.49, 132.12, 131.12, 131.06, 130.69, 129.56, 126.13, 122.26, 121.47, 121.40, 115.83, 115.68, 78.93, 55.23, 52.97, 48.66, 47.55, 42.24, 42.19, 39.74, 39.13, 38.86, 38.76, 38.67, 36.98, 36.58, 33.17, 30.93, 30.65, 29.27, 28.16, 28.13, 27.22, 24.29, 23.47, 23.37, 21.16, 18.31, 17.62, 17.04, 16.99, 15.66, 15.52. HR-MS-ESI *m/z* 703.4411, $C_{45}H_{57}O_4FNa$ $[M + Na]^+$, calcd 703.4316.

1-Acetyl-3-phenyl-4,5-dihydro-2-pyrazoline-5-phenyl Ursolate (7a). Yield 48%, white powder, mp 166–168°C. IR (KBr, ν, cm^{-1}): 3455, 2926, 2869, 1746, 1668, 1507, 1448, 1203, 1165, 1028, 853. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.74 (3H, s, CH_3), 0.77 (3H, s, CH_3), 0.83 (3H, d, J = 2.4, CH_3), 0.86 (3H, d, J = 1.2, CH_3), 0.91 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.35 (2H, t, J = 6.4, J = 4.2, CH_2), 1.50 (2H, t, J = 8.4, 7.6, CH_2), 1.91 (2H, t, J = 4.4, CH_2), 2.31 (1H, d, J = 11.2, H-18), 2.41 (3H, s, $COCH_3$), 3.20 (2H, t, J = 4.0, $CH_2C{=}N$), 3.80 (1H, t, J = 8.0, PhCHN), 5.28 (1H, s, OH), 6.95–7.74 (9H, m, PhH). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 175.02, 167.36, 154.07, 149.64, 139.69, 137.55, 131.01, 130.27, 128.72, 126.69, 126.59, 125.30, 121.62, 76.76, 58.86, 54.71, 52.52, 47.42, 46.94, 41.97, 41.74, 38.33, 38.28, 38.22, 36.45, 36.07, 32.72, 30.65, 29.96, 29.56, 28.45, 28.20, 27.51, 26.94, 23.75, 23.10, 22.86, 21.67, 20.88, 17.92, 17.26, 16.83, 16.03, 15.21. HR-MS-ESI *m/z* 719.4780, $C_{47}H_{62}O_4N_2$ $[M]^+$, calcd 719.4788.

1-Acetyl-3-(4-methylphenyl)-4,5-dihydro-2-pyrazoline-5-phenyl Ursolate (7b). Yield 48%, white powder, mp 172–174°C. IR (KBr, ν, cm^{-1}): 3443, 2925, 2868, 1746, 1666, 1508, 1450, 1204, 1164, 1096, 813. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.75 (3H, s, CH_3), 0.77 (3H, s, CH_3), 0.84 (3H, d, J = 2.4, CH_3), 0.86 (3H, s, CH_3), 0.92 (3H, d, J = 1.2, CH_3), 0.99 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.36 (2H, t, J = 6.4, CH_2), 1.44 (3H, d, J = 3.2, CH_3), 1.51 (2H, t, J = 8.4, J = 7.6, CH_2),

1.91 (2H, t, J = 4.4, CH_2), 2.18 (3H, s, CH_3), 2.31 (1H, d, J = 11.2, H-18), 2.41 (3H, s, $COCH_3$), 3.18 (2H, d, J = 6.0, CH_2C=N), 3.74 (1H, t, J = 7.2, PhCHN), 5.29 (1H, s, OH), 6.94–7.63 (8H, m, PhH). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 174.99, 167.22, 154.03, 149.63, 140.09, 139.73, 137.54, 129.28, 126.55, 125.31, 121.59, 76.76, 58.76, 54.73, 52.52, 47.81, 46.96, 42.01, 41.74, 38.88, 38.33, 36.45, 36.07, 32.74, 29.98, 29.56, 28.20, 27.52, 26.96, 23.77, 23.10, 22.87, 21.65, 20.99, 20.87, 17.93, 17.25, 16.81, 16.03, 15.21. HR-MS-ESI *m/z* 733.4948, $C_{48}H_{64}O_4N_2$ $[M]^+$, calcd 733.4923.

1-Acetyl-3-(4-methoxyphenyl)-4,5-dihydro-2-pyrazoline-5-phenyl Ursolate (7c). Yield 52%, white powder, mp 176–178°C. IR (KBr, ν, cm^{-1}): 3429, 2927, 2870, 1699, 1607, 1512, 1457, 1383, 1203, 1167, 1037, 837. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.74 (3H, s, CH_3), 0.77 (3H, s, CH_3), 0.83 (3H, d, J = 2.4, CH_3), 0.86 (3H, s, CH_3), 0.92 (3H, d, J = 1.2, CH_3), 0.99 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.36 (2H, t, J = 6.4, CH_2), 1.44 (3H, d, J = 3.2, CH_3), 1.51 (2H, t, J = 8.4, 7.6, CH_2), 1.91 (2H, t, J = 4.4, CH_2), 2.31 (1H, d, J = 11.2, H-18), 2.41 (3H, s, $COCH_3$), 3.21 (2H, d, J = 8.0, CH_2C=N), 3.68 (1H, t, J = 12.0, PhCHN), 3.83 (3H, s, OCH_3), 5.29 (1H, s, OH), 6.98–7.68 (8H, m, PhH). ^{13}C NMR (100 MHz, DMSO-d_6, δ):179.76, 171.84, 165.67, 158.58, 154.36, 144.55, 142.30, 139.00, 133.00, 131.36, 126.33, 118.89, 67.67, 63.46, 60.55, 60.04, 57.27, 52.57, 51.72, 46.83, 46.49, 41.20, 34.73, 34.31, 32.95, 27.85, 26.39, 25.62, 22.00, 21.57, 20.78, 19.96. HR-MS-ESI *m/z* 749.4903, $C_{48}H_{64}O_5N_2$ $[M]^+$, calcd 749.4893.

1-Acetyl-3-(4-chlorophenyl)-4,5-dihydro-2-pyrazoline-5-phenyl Ursolate (7d). Yield 52%, white powder, mp 154–156°C. IR (KBr, ν, cm^{-1}): 3441, 2925, 2870, 1745, 1667, 1597, 1506, 1446, 1204, 1164, 1028, 829. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.71 (3H, s, CH_3), 0.77 (3H, s, CH_3), 0.84 (3H, d, J = 2.0, CH_3), 0.94 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.35 (2H, t, J = 6.8, 5.6, CH_2), 1.44 (3H, d, J = 3.2, CH_3), 1.91 (2H, t, J = 4.4, CH_2), 2.31 (1H, d, J = 11.2, H-18), 2.41 (3H, s, $COCH_3$), 3.21 (2H, d, J = 12.0, CH_2C=N), 3.68 (1H, t, J = 8.0, PhCHN), 5.29 (1H, s, OH), 6.95–7.67 (8H, m, PhH). ^{13}C NMR (400 MHz, DMSO-d_6, δ): 175.00, 167.42, 153.07, 149.66, 139.57, 137.54, 134.85, 129.93, 128.80, 128.31, 126.70, 125.30, 121.61, 76.75, 68.46, 59.06, 55.79, 54.71, 52.51, 47.81, 46.94, 41.86, 41.73, 38.32, 36.44, 36.07, 32.72, 32.06, 30.64, 29.97, 29.55, 28.20, 27.50, 26.94, 23.75, 23.09, 22.85, 21.65, 20.87, 17.92, 17.25, 16.82, 16.02, 15.20. HR-MS-ESI *m/z* 753.4372, $C_{47}H_{61}O_4N_2Cl$ $[M]^+$, calcd 753.4398.

1-Acetyl-3-(4-fluorophenyl)-4,5-dihydro-2-pyrazoline-5-phenyl Ursolate (7e). Yield 49%, white powder, mp 150–152°C. IR (KBr, ν, cm^{-1}): 3444, 2926, 2869, 1743, 1666, 1605, 1510, 1448, 1234, 1163, 1029, 838, 537. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.74 (3H, s, CH_3), 0.78 (3H, s, CH_3), 0.84 (3H, d, J = 1.6, CH_3), 0.86 (3H, s, CH_3), 0.91 (3H, d, J = 4.0, CH_3), 0.99 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.25 (2H, t, J = 2.8, CH_2), 1.52 (3H, s, CH_3), 1.91 (2H, t, J = 4.0, CH_2), 2.31 (1H, d, J = 11.2, H-18), 2.40 (3H, s, $COCH_3$), 3.19 (1H, d, J = 16.6, CH_2C=N), 3.72 (1H, t, J = 12.0, PhCHN), 5.28 (1H, s, OH), 6.95–7.74 (8H, m, PhH). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 175.04, 167.38, 164.04, 162.39, 153.22, 149.65, 139.63, 137.55, 128.99, 128.94, 127.65, 126.70, 125.29, 121.62, 115.87, 115.73, 76.76, 58.97, 54.70, 52.51, 47.81, 46.94, 42.03, 41.74, 38.32, 38.22, 36.44, 36.06, 32.71, 30.64, 29.96, 28.19, 27.50, 26.93, 23.74, 23.09, 22.86, 21.65, 20.88, 17.92, 17.26, 16.82, 16.03, 15.20. HR-MS-ESI *m/z* 737.4677, $C_{47}H_{61}O_4N_2$ F$[M]^+$, calcd 737.4734.

Biological Activity Tests. HepG2, A549, and SKOV3 cells (2×10^4 per well) were seeded in 96-well culture plates and cultured for one day before treatment. The compounds were dissolved immediately in DMSO, then diluted in serum-free DEME medium. A final concentration of the compounds in DMSO that did not exceed 1% did not affect cell survival. Cells were treated for 4 h with triterpenes alone. After treatment, cells were washed twice with DEME medium and cultured again for 2 days in drug-free culture medium. Cell survival was measured by the tetrazolium dye assay. All test compounds were solubilized in dimethylsulfoxide (DMSO) and were tested three times at different concentrations.

The results are shown in Table 1. As shown in Table 1, esterification of the 28-COOH of ursolic acid and pyrazoline to yield compounds **7a**, **7e** resulted in increasing inhibitory activity against SKOV3 cell lines (IC_{50} values of **7a**, **7e**, 20.8, 18.9 μg/mL). Significant further improvement of cell growth inhibition in SKOV3 cell lines was achieved when the selected chalcone was coupled to the C-28. Compounds **6a**, **6b**, **6d** (IC_{50} values of **6a**, **6b**, **6d**, 14.6, 18.4, 15.2 μg/mL) presented strong inhibition in SKOV3 cell lines. Additionally, compounds **6a–6e** and **7a–7e** were also evaluated for cytotoxic activity in A549 and HepG2 cell lines. Compounds **6a**, **6d** show more potent activity (IC_{50} values of **6a**, **6d**, 13.6, 13.2 μg/mL) than that of UA; however, none of them displayed more potent cytotoxic activity against HepG2 (IC_{50} values of UA, 6.3 μg/mL). UA derivatives do not seem to exert its effects through this mechanism in HepG2 cells. The results show that UA-chalcone conjugates have more potent activity than UA-pyrazoline conjugates. They may act through the large conjugate system by inducing apoptosis and blocking the cell cycle in the S phase. Compounds with an electron-withdrawing group display more potent activity than those with an electron-releasing group. Currently, further evaluation is in progress.

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