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Synthesis and antitumor activity of *N*-sulfonyl-3,7-dioxo-5β-cholan-24-amides, ursodeoxycholic acid derivatives

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ABSTRACT

A series of *N*-sulfonyl-3,7-dioxo-5β-cholan-24-amides, ursodeoxycholic acid derivatives, have been designed and synthesized in nine steps starting from ursodeoxycholic acid. The *in vitro* antitumor activity of the target compounds has been evaluated against HCT-116, MCF-7, K562, and SGC-7901 cell lines. The pharmacological results showed that most of the prepared compounds display excellent selective cytotoxicity toward HCT-116, MCF-7, and K562 cell lines. Particularly, compounds **10c**, **10f** and **10g** show high inhibitory activity on these human cancer cell lines (IC50: 2.39–9.34 μM). Conversely, all compounds are generally inactive against SGC-7901, with only **10b** having IC_{50} below 50 μM.

1. Introduction

Steroids are an important class of natural products which have high ability to penetrate cells and bind to nucleus and membrane receptors. They include great variations in structure and play a very important role in life [1,2], such as cholesterol, bile acids, sex hormones, vitamin D, corticoid hormones, cardiac aglycones, antibiotics, and insect molting hormones. A variety of steroids with unusual and interesting structures have been synthesized and evaluated for their anti-tumor activity [3–8]. As a relatively inexpensive bile acid and a traditional medicine, ursodeoxycholic acid has been used to synthesize some new derivatives with biological activity [9–11].

Sulfonamides are also an important group of drugs. They exhibit a broad spectrum of antibacterial activities. Recently, some sulfonamide derivatives have been reported to have potential antitumor effects [12–19]. However there are few literature references available for steroid sulfonamide derivatives. As part of our current efforts in understanding various steroid sulfonamide analogs as antitumor agents, this paper presents a series of *N*-sulfonyl-3,7-dioxo-5β-cholan-24-amides, an ursodeoxycholic acid derivatives, designed and synthesized by us. Their antitumor activity against different cancer cell lines *in vitro* has been investigated in order to screen potent and selective chemotherapy agents.

2. Experimental section

2.1. Chemistry

2.1.1. General methods

All compounds were fully characterized by spectroscopic techniques. The NMR spectra were recorded on a Bruker DRX500 (^{1}H: 500 MHz, ^{13}C: 125 MHz), chemical shifts (δ) are expressed in ppm, and *J* values are given in Hz. Deuterated $CDCl_3$-d_6 was used as a solvent. IR spectra were recorded on a FT-IR Thermo Nicolet Avatar 360 using a KBr pellet. The reactions were monitored by thin layer chromatography (TLC) using silica gel GF_{254}. The melting points were determined on an XT-4A melting point apparatus and are uncorrected. HRMS was performed on an Agilent LC-MSD TOF instrument.

All chemicals and solvents were used as received without further purification unless otherwise stated. Column chromatography was performed on silica gel (200–300 mesh).

Ursodeoxycholic acid and all of sulfonyl chlorides were purchased from Adrich Corporation Limited.

2.1.2. 3α,7β-Dihydroxy-methylcholanat **2**

Ursodeoxycholic acid (**1**) (5 g, 12.76 mmol) was dissolved in cold methanol (50 mL) containing anhydrous HCl (generated in situ by the dropwise addition of $SOCl_2$ into methanol). After stirring for 0.5 h at room temperature, the reagent was refluxed for 3 h. When the reaction was finished, the volatiles were removed under reduced pressure. The crude product was column purified on silica gel using 30% ethyl acetate in *n*-hexane to afford **2** in 95% yield as colorless crystals (4.92 g)[1]. mp 210–212 °C. ^{1}H NMR

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(500 MHz, $CDCl_3$-*d*) δ 0.64 (3H, s, 18-CH_3), 0.89 (3H, d, J = 6.5 Hz, 21-CH_3), 0.91 (3H, s, 19-CH_3), 1.04–1.97 (m, 24H), 2.18 (1H, m, 23-H1), 2.32 (1H, m, 23-H2), 3.51(1H, m, 3β-H), 3.53(1H, m, 7α-H), 3.62(3H, s, -OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.5, 18.8, 21.6, 23.8, 27.3, 29.0, 30.6, 31.4, 31.5, 34.4, 35.4, 35.7, 37.5, 37.7, 39.7, 40.6, 42.9, 44.0, 44.1, 51.9, 55.4, 56.3, 71.5, 175.1.

2.1.3. 3α,7β,24-Trihydroxy-5β-cholan ***3***

A solution of ester **2** (4.92 g, 12.12 mmol) in dry ethanol (100 mL) was stirred rapidly as sodium borohydride (1.2 eq., 550 mg) was added carefully in small portions. The reaction was continued overnight at room temperature before the suspension was poured carefully onto cooled ethyl acetate (20 mL). The suspension was filtered, and the filtrate was dried under reduced pressure. The residual solids were extracted with water (300 mL) and ethyl acetate (300 mL) for three times. The combined organic layers were dried under reduced pressure. The crude product was column purified on silica gel using 50% ethyl acetate in *n*-hexane to afford **3** in 92% yield as colorless needles (4.21 g)[2]. mp 150–152 °C. ^{1}H NMR (500 MHz, CD_3OD-d_4) δ 0.74 (3H, s, 18-CH_3), 0.99 (3H, br.s., 21-CH_3), 0.99 (3H, s, 19-CH_3), 1.06–2.08 (m, 26H), 3.37 (2H, m, 24-H), 3.53 (2H, m, 3β-H and 7α-H). ^{13}C NMR (125 MHz, CD_3OD-d_4) δ 13.11, 19.8, 22.8, 24.4, 28.4, 30.2, 30.8, 31.5, 33.7, 35.6, 36.6, 37.4, 38.5, 39.1, 41.2, 42.1, 44.5, 45.0, 45.2, 57.2, 58.0, 64.0, 72.4, 72.6.

2.1.4. 3α,7β-Dihydroxy-24-triphenylmethoxy-5β-cholan 4

A solution of alcohol **3** (4.21 g, 11.14 mmol) in dry pyridine (100 mL) was stirred rapidly under nitrogen as triphenylmethyl chloride (1.5 eq., 4.64 g) was added in one portion. The reaction was continued overnight at 90 °C before the solvent was removed in vacuo. The resultant oily solid was purified by column chromatography using 30% ethyl acetate in *n*-hexane to afford **4** in 85% yield as colorless solid (5.87 g)[2]. mp 85–87 °C. ^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ 0.65 (3H, s, 18-CH_3), 0.91 (3H, d, J = 6.5 Hz, 21-CH_3), 0.95 (3H, s, 19-CH_3), 0.98–2.01 (m, 26H), 3.02 (2H, m, 24-H), 3.59 (2H, m, 3β-H and 7α-H), 7.20–7.45 (m, 15H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.6, 19.2, 21.6, 23.8, 27.0, 27.3, 29.1, 30.1, 30.8, 32.8, 34.5, 35.4, 35.8, 37.2, 37.8, 39.6, 40.6, 42.9, 44.2, 44.2, 55.5, 56.2, 64.6, 71.9, 86.7, 127.2, 128.1, 129.1, 145.0.

2.1.5. 3,7-Dioxo-24-triphenylmethoxy-5β-cholan ***5***

Chromium trioxide (5 eq., 4.74 g) was added to a dry dichloromethane (100 mL) solution containing pyridine (10 eq., 7.64 mL). Under the condition of stirring, a solution of **4** (5.87 g, 9.47 mmol) in dry dichloromethane (10 mL) was dropped in one portion. The mixture was stirred for overnight at room temperature, and the reaction progress was monitored by TLC. The mixture was then poured into a silica gel column and chromatographied using dichloromethane to afford **5** in 90% yield as colorless oil (5.25 g). $[\alpha]^{25}_D$ −25.0 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3445, 3280, 2946, 2880, 1435, 1310, 1112, 702, 535 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ 0.67 (3H, s, 18-CH_3), 0.92 (3H, d, J = 6.5 Hz, 21-CH_3), 1.25 (3H, s, 19-CH_3), 0.95–2.87 (m, 26H), 3.02 (2H, m, 24-H), 7.19–7.45 (m, 15H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.5, 19.2, 22.6, 22.9, 25.2, 27.0, 28.7, 32.8, 35.8, 35.8, 35.8, 37.2, 39.3, 43.0, 43.3, 43.4, 45.4, 48.2, 49.3, 50.0, 55.4, 64.6, 86.7, 127.2, 128.1, 129.1, 145.0, 210.6, 211.6. HRMS (EI): *m/z* calcd for $C_{43}H_{52}O_3$ [M^+], 616.3916; found, 616.3920.

2.1.6. 3,7-Dioxo-5β-cholan-24-ol ***6***

To a solution of 5 (5.25 g, 8.52 mol) in acetone (100 mL), 4-toluenesulfonic acid (1.05 eq., 1.49 g) were added and stirred under room temperature for 0.5 h (monitored by TLC). The solvent was removed under reduced pressure. The residue obtained was purified by column chromatography using 25% ethyl acetate in *n*-hexane to afford **6** in quantitative yield as colorless oil (3.19 g). $[\alpha]^{25}_D$ −15.5 °C (c 0.35, $CHCl_3$); IR (KBr) ν_{max} 3447, 3278, 2946, 2882, 1425, 1350, 1098, 699, 550 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ 0.70 (3H, s, 18-CH_3), 0.94 (3H, d, J = 6.5 Hz, 21-CH_3), 1.31 (3H, s, 19-CH_3), 0.97–2.90 (m, 26H), 3.60 (2H, m, 24-H). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.5, 19.1, 22.5, 22.8, 25.2, 28.8, 29.8, 32.2, 35.8, 35.8, 35.8, 37.1, 39.3, 43.0, 43.2, 43.3, 45.4, 48.1, 49.2, 50.0, 55.4, 63.7, 210.8, 211.8. HRMS (TOF ES$^+$): *m/z* calcd for $C_{24}H_{38}NaO_3^+$ [(M+Na)$^+$], 397.2713; found, 397.2706.

2.1.7. 3,7-Dioxo-24-cholan-methansulfonate ***7***

To a 8.52 mmol solution of **6** in 100 mL of CH_2Cl_2 at 0 °C was added 17 mmol (2 eq., 2.37 mL) of Et_3N followed by slow addition of 10.22 mmol (1.2 eq., 816 μL) of methanesulfonyl chloride. The mixture was stirred for 1 h at 0 °C and diluted with cold water. The solution was extracted twice with 100 ml of CH_2Cl_2. The organic layer was washed successively with H_2O to neutral pH and with brine. The solution was dried over anhydrous $MgSO_4$, filtered, and concentrated to afford crude product which was chromatographed on silica gel using 30% ethyl acetate in *n*-hexane to afford **7** in 93% yield as amorphous solid (3.58 g). $[\alpha]^{25}_D$ −17.5 °C (c 0.25, $CHCl_3$); IR (KBr) ν_{max} 3445, 3272, 2947, 2872, 1422, 1321, 1085, 732, 521 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ 0.63 (3H, s, 18-CH_3), 0.88 (3H, d, J = 6.5 Hz, 21-CH_3), 1.24 (3H, s, 19-CH_3), 0.91–2.84 (m, 26H), 2.94 (3H, s, -OSO_2CH_3), 4.12 (2H, m, 24-H). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.5, 19.0, 22.5, 22.8, 25.1, 26.2, 28.7, 31.9, 35.5, 35.8, 35.8, 37.1, 37.7, 39.2 43.0, 43.2, 43.3, 45.4, 48.1, 49.2, 49.9, 55.2, 71.0, 210.6, 211.6. HRMS (TOF ES$^+$): *m/z* calcd for $C_{25}H_{40}NaO_5S^+$ [(M+Na)$^+$], 475.2489; found, 475.2482.

2.1.8. 3,7-Dioxo-24-azido-5β-cholan ***8***

NaN_3 (2 eq., 1.03 g) was added to a solution of mesylate **7** (3.58 g, 7.92 mmol) in DMF (30 mL) and stirred overnight at room temperature. Water (50 mL) was added and the aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with saturated aqueous NaCl solution (2 × 100 mL) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure. Silica gel column chromatography using 10% ethyl acetate in *n*-hexane to afford **8** in 82% yield as colorless oil (2.59 g). $[\alpha]^{25}_D$ −9.5 °C (c 0.3, $CHCl_3$); IR (KBr) ν_{max} 3455, 3280, 2945, 2870, 1325, 1095, 625, 533 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.73 (3H, s, 18-CH_3), 0.98 (3H, d, J = 6.5 Hz, 21-CH_3), 1.28 (3H, s, 19-CH_3), 1.01–3.00 (m, 26H), 3.48 (2H, m, 24-H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 12.4, 19.1, 22.5, 22.8, 25.1, 25.9, 28.7, 33.3, 35.7, 35.8, 35.8, 37.1, 39.2, 43.0, 43.2, 43.3, 45.4, 48.1, 49.2, 49.9, 52.2, 55.2, 210.6, 211.5. HRMS (TOF ES$^+$): *m/z* calcd for $C_{24}H_{37}N_3NaO_2^+$ [(M+Na)$^+$], 422.2778; found, 422.2776.

2.1.9. 3,7-Dioxo-5β-cholan-24-amide ***9***

A mixture of **8** (2.59 g, 6.49 mmol), 5% Pd-C (137 mg, 5% mol) in 100 mL methanol was hydrogenated under stirring overnight by using hydrogen balloon. After hydrogenation, the reaction mixture was filtered. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography over silica gel using 2% MeOH in CH_2Cl_2 to get the amine **9** in 80% yield as amorphous solid (1.94 g). $[\alpha]^{25}_D$ −11.0 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3450, 3281, 2949, 2868, 1410, 1320, 1100, 661, 512 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ 0.73 (3H, s, 18-CH_3), 0.90 (3H, d, J = 6.5 Hz, 21-CH_3), 1.26 (3H, s, 19-CH_3), 0.94–2.85 (m, 26H), 3.19 (2H, m, 24-H). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.3, 18.9, 22.0, 22.2, 24.0, 24.7, 28.3, 32.7, 35.1, 35.2, 35.3, 36.7, 38.8, 42.0, 42.6, 42.9, 44.9, 47.2, 48.9, 49.1, 54.9, 63.2, 210.2, 211.5. HRMS (EI): *m/z* calcd for $C_{24}H_{39}NO_2$ [M^+], 373.2981; found, 373.2975.

2.1.10. General procedure for the synthesis of N-sulfonyl-3,7-dioxo-5β-cholan-24-amides ***10a–o***

A mixture of compounds **9** (100 mg, 0.268 mol) and a series of sulfonyl chloride compounds in dry pyridine (2.0 mL) was stirred overnight at room temperature (monitored by TLC). The solvent was removed under reduced pressure. The residue obtained was purified by column chromatography over silica gel using 2% acetone in *n*-hexane to get the sulfonamides **10a–o** in 78–94% yield as colorless solid.

*2.1.10.1. N-methanesulfonyl-3,7-dioxo-5β-cholan-24-amide (**10a**).* 111 mg (92% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −15.6 °C (c 0.25, $CHCl_3$); IR (KBr) ν_{max} 3456, 3283, 2946, 2872, 1707, 1616, 1442, 1325, 1140, 1096, 1050 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.70 (3H, s, 18-CH_3), 0.94 (3H, d, *J* = 6.5 Hz, 21-CH_3), 1.31 (3H, s, 19-CH_3), 0.98–2.91 (m, 26H), 2.95 (3H, s, –$OSO_2C\underline{H}_3$), 3.09 (2H, m, 24-H), 4.73 (1H, m, –N$\underline{H}SO_2$–). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.5, 19.1, 22.5, 22.8, 25.1, 27.2, 28.8, 33.2, 35.7, 35.8, 35.8, 37.1, 39.2, 40.6, 43.0, 43.2, 43.3, 44.2, 45.4, 48.1, 49.2, 49.9, 55.3, 210.8, 211.8. HRMS (TOF ES^-): *m/z* calcd for $C_{25}H_{40}NO_4S^-$ $[(M-H)^-]$, 450.2684; found, 450.2687.

*2.1.10.2. N-ethanesulfonyl-3,7-dioxo-5β-cholan-24-amide (**10b**).* 112 mg (90% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −7.5 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3456, 3287, 2944, 2868, 1706, 1633, 1450, 1319, 1143, 1097, 1052, 725 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.69 (3H, s, 18-CH_3), 0.94 (3H, d, *J* = 6.5 Hz, 21-CH_3), 1.31 (3H, s, 19-CH_3), 1.36 (3H, t, *J* = 7.2 Hz, –$OSO_2CH_2C\underline{H}_3$), 0.94–2.91 (m, 26H), 3.03 (2H, q, *J* = 7.2 Hz, –$OSO_2C\underline{H}_2CH_3$), 3.07 (2H, m, 24-H), 4.63 (1H, t, *J* = 5.6 Hz, –N$\underline{H}SO_2$–). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 8.7, 12.5, 19.1, 22.5, 22.8, 25.1, 27.5, 28.8, 33.2, 35.7, 35.8, 35.8, 37.1, 39.3, 43.0, 43.3, 43.3, 44.1, 45.4, 47.2, 48.1, 49.2, 49.9, 55.3, 210.7, 211.6. HRMS (TOF ES^-): *m/z* calcd for $C_{26}H_{42}NO_4S^-$ $[(M-H)^-]$, 464.2840; found, 464.2845.

*2.1.10.3. N-n-butanesulfonyl-3,7-dioxo-5β-cholan-24-amide (**10c**).* 112 mg (85% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −8.5 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3457, 3285, 2946, 2872, 1706, 1625, 1452, 1319, 1143, 1097, 1053, 929, 894, 732 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.69 (3H, s, 18-CH_3), 0.93 (3H, d, *J* = 6.5 Hz, 21-CH_3), 0.96 (3H, t, *J* = 7.4 Hz, –$OSO_2CH_2CH_2CH_2C\underline{H}_3$), 1.31 (3H, s, 19-$CH_3$), 1.00–3.02 (m, 30H), 3.00 (2H, t, *J* = 7.8 Hz, –$OSO_2C\underline{H}_2$-$CH_2CH_2CH_3$), 3.07 (2H, m, 24-H), 4.63 (1H, t, *J* = 5.6 Hz, –N$\underline{H}SO_2$–). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.4, 14.0, 19.1, 21.9, 22.5, 22.8, 25.1, 26.1, 27.5, 28.8, 33.2, 35.7, 35.8, 35.8, 37.1, 39.3, 43.0, 43.3, 43.3, 44.1, 45.4, 48.1, 49.3, 49.9, 52.7, 55.4, 210.6, 211.6. HRMS (TOF ES^-): *m/z* calcd for $C_{28}H_{46}NO_4S^-$ $[(M-H)^-]$, 492.3153; found, 492.3155.

*2.1.10.4. N-benzenesulfonyl-3,7-dioxo-5β-cholan-24-amide (**10d**).* 126 mg (92% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −13.5 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3458, 3281, 2945, 2871, 1708, 1445, 1328, 1160, 1090, 735, 585 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.65 (3H, s, 18-CH_3), 0.84 (3H, d, *J* = 6.4 Hz, 21-CH_3), 1.30 (3H, s, 19-CH_3), 0.92–2.90 (m, 26H), 2.93 (2H, m, 24-H), 5.06 (1H, t, *J* = 6.0 Hz, –N$\underline{H}SO_2$–), 7.52 (2H, m), 7.58 (1H, m), 7.87 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.4, 19.0, 22.5, 22.8, 25.1, 26.7, 28.7, 33.1, 35.6, 35.8, 35.8, 37.1, 39.2, 43.0, 43.3, 43.3, 44.1, 45.4, 48.1, 49.2, 49.9, 55.3, 127.4, 127.4, 129.4, 129.4, 132.9, 140.6, 210.7, 211.6. HRMS (TOF ES^+): *m/z* calcd for $C_{30}H_{43}NNaO_4S^+$ $[(M+Na)^+]$, 536.2805; found, 512.2800.

*2.1.10.5. N-(1′-phenylmethanesulfonyl)-3,7-dioxo-5β-cholan-24-amide (**10e**).* 123 mg (87% yield) as a colorless amorphous solid. IR (KBr) ν_{max} 3460, 3287, 2943, 2871, 1708, 1450, 1323, 1146, 1078, 699, 543 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.70 (3H, s, 18-CH_3), 0.92 (3H, d, *J* = 6.4 Hz, 21-CH_3), 1.32 (3H, s, 19-CH_3), 1.00–2.91 (m, 26H), 2.94 (2H, m, 24-H), 4.26 (2H, s, –$NHSO_2C\underline{H}_2C_6H_5$), 4.41 (1H, br.s., –N$\underline{H}SO_2$–), 7.41 (5H, m). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.5, 19.1, 22.5, 22.8, 25.2, 27.5, 28.8, 33.1, 35.7, 35.8, 35.8, 37.1, 39.3, 43.0, 43.3, 43.3, 44.6, 45.4, 48.1, 49.3, 50.0, 55.3, 59.1, 129.1, 129.2, 129.2, 130.0, 131.0, 131.0, 210.6, 211.6. HRMS (TOF ES^+): *m/z* calcd for $C_{31}H_{45}NNaO_4S^+$ $[(M+Na)^+]$, 550.2962; found, 550.2958.

*2.1.10.6. N-(4′-methylbenzenesulfonyl)-3,7-dioxo-5β-cholan-24-amide (**10f**).* 120 mg (85% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −9.0 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3478, 3281, 2944, 2872, 1708, 1646, 1325, 1157, 549 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.60 (3H, s, 18-CH_3), 0.79 (3H, d, *J* = 6.5 Hz, 21-CH_3), 1.24 (3H, s, 19-CH_3), 0.89–2.45 (m, 26H), 2.37 (3H, s, 4′-CH_3), 2.83 (2H, m, 24-H), 5.17 (1H, t, *J* = 5.9 Hz, –N$\underline{H}SO_2$–), 7.25 (2H, d, *J* = 8.2 Hz, 3′-ArH and 5′-ArH), 7.70 (2H, d, *J* = 8.2 Hz, 2′-ArH and 6′-ArH). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.2, 19.0, 21.8, 22.4, 22.7, 25.1, 26.5, 28.6, 33.1, 35.6, 35.7, 35.7, 37.1, 39.2, 43.0, 43.2, 43.2, 44.0, 45.4, 48.0, 49.2, 49.9, 55.2, 127.4, 127.4, 130.0, 130.0, 137.6, 143.5, 210.8, 211.7. HRMS (TOF ES^+): *m/z* calcd for $C_{31}H_{45}NNaO_4S^+$ $[(M+Na)^+]$, 550.2962; found, 550.2958.

2.1.10.7. N-(4′-bromobenzenesulfonyl)-3,7-dioxo-5β-cholan-24-amide (10g). 140 mg (88% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −11.0 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3450, 3281, 2949, 2868, 1707, 1606, 1432, 1322, 1156, 825, 529 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.63 (3H, s, 18-CH_3), 0.82 (3H, d, *J* = 6.5 Hz, 21-CH_3), 1.27 (3H, s, 19-CH_3), 0.90–2.48 (m, 26H), 2.87 (2H, m, 24-H), 5.20 (1H, t, *J* = 5.9 Hz, –N$\underline{H}SO_2$–), 7.63 (2H, d, *J* = 8.4 Hz, 3′-ArH and 5′-ArH), 7.71 (2H, d, *J* = 8.2 Hz, 2′-ArH and 6′-ArH). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.4, 19.0, 22.5, 22.8, 25.1, 26.6, 28.7, 33.1, 35.6, 35.8, 35.8, 37.1, 39.2, 43.0, 43.3, 43.3, 44.1, 45.4, 48.1, 49.2, 49.9, 55.2, 127.8, 129.0, 129.0, 132.7, 132.7, 139.8, 210.8, 211.7. HRMS (TOF ES^+): *m/z* calcd for $C_{30}H_{42}BrNNaO_4S^+$ $[(M+Na)^+]$, 614.1910; found, 614.1914.

*2.1.10.8. N-(4′-chlorobenzenesulfonyl)-3,7-dioxo-5β-cholan-24-amide (**10h**).* 115 mg (78% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −18.0 °C (c 0.15, $CHCl_3$); IR (KBr) ν_{max} 3476, 3281, 2945, 2872, 1708, 1331, 1161, 1088, 752, 621 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.66 (3H, s, 18-CH_3), 0.85 (3H, d, *J* = 6.5 Hz, 21-CH_3), 1.30 (3H, s, 19-CH_3), 0.93–2.87 (m, 26H), 2.90 (2H, m, 24-H), 5.16 (1H, t, *J* = 5.8 Hz, –N$\underline{H}SO_2$–), 7.49 (2H, d, *J* = 8.4 Hz, 3′-ArH and 5′-ArH), 7.81 (2H, d, *J* = 8.4 Hz, 2′-ArH and 6′-ArH). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.4, 19.0, 22.5, 22.8, 25.1, 26.6, 28.7, 33.1, 35.6, 35.8, 35.8, 37.1, 39.2, 43.0, 43.3, 43.3, 44.1, 45.4, 48.1, 49.2, 49.9, 55.2, 128.9, 128.9, 129.7, 129.7, 139.2, 139.3, 210.8, 211.7. HRMS (TOF ES^+): *m/z* calcd for $C_{30}H_{42}ClNNaO_4S^+$ $[(M+Na)^+]$, 570.2415; found, 570.2416.

*2.1.10.9. N-(4′-fluorobenzenesulfonyl)-3,7-dioxo-5β-cholan-24-amide (**10i**).* 1271 mg (89% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −14.0 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3456, 3281, 2946, 2872, 1708, 1592, 1331, 1158, 1091, 837, 550 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.59 (3H, s, 18-CH_3), 0.78 (3H, d, *J* = 6.5 Hz, 21-CH_3), 1.24 (3H, s, 19-CH_3), 0.86–2.45 (m, 26H), 2.83 (2H, m, 24-H), 5.35 (1H, t, *J* = 5.8 Hz, –N$\underline{H}SO_2$–), 7.13 (2H, t, *J* = 8.6 Hz, 3′-ArH and 5′-ArH), 7.83 (2H, dd, *J* = 8.6, 5.0 Hz, 2′-ArH and 6′-ArH). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.4, 18.9, 22.4, 22.7, 25.1, 26.5, 28.6, 33.1, 35.5, 35.7, 35.7, 37.1, 39.2, 42.9, 43.2, 43.3, 44.0, 45.4, 48.1, 49.2, 49.9, 55.2, 116.5, 116.7, 130.1, 130.1, 136.7, 164.2, 166.3, 210.9, 211.8. HRMS (TOF ES^+): *m/z* calcd for $C_{30}H_{42}FNNaO_4S^+$ $[(M+Na)^+]$, 554.2711; found, 554.2710.

*2.1.10.10. N-(3′-fluorobenzenesulfonyl)-3,7-dioxo-5β-cholan-24-amide (**10j**).* 128 mg (90% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$

−16.5 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3452, 3283, 2946, 2872, 1708, 1593, 1432, 1333, 1223, 1155, 1081, 794, 690, 587 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.66 (3H, s, 18-CH_3), 0.86 (3H, d, *J* = 6.4 Hz, 21-CH_3), 1.30 (3H, s, 19-CH_3), 0.93–2.92 (m, 26H), 2.95 (2H, m, 24-H), 5.31 (1H, t, *J* = 5.7 Hz, –N<u>H</u>SO_2–), 7.29 (1H, m), 7.52 (1H, m), 7.57 (1H, d, *J* = 8.1 Hz), 7.67 (1H, d, *J* = 7.8 Hz). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.4, 19.0, 22.5, 22.8, 25.1, 26.6, 28.7, 33.1, 35.6 35.8, 35.8, 37.1, 39.2, 43.0, 43.2, 43.3, 44.2, 45.4, 48.1, 49.2, 49.9, 55.2, 114.7, 114.9, 120.0, 120.2, 123.2, 131.3, 142.7, 161.8, 163.8, 211.0, 211.9. HRMS (TOF ES^+): *m/z* calcd for $C_{30}H_{42}FNNaO_4S^+$ [$(M+Na)^+$], 554.2711; found, 554.2705.

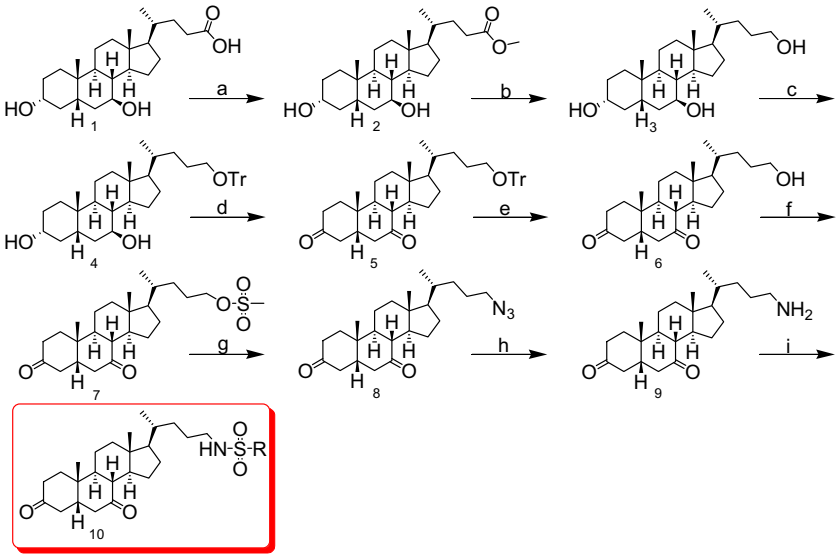
*2.1.10.11. N-(2′-fluorobenzenesulfonyl)-3,7-dioxo-5β-cholan-24-amide (**10k**).* 124 mg (87% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −15.0 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3290, 2946, 2872, 1708, 1595, 1466, 1336, 1162, 1079, 764, 591 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.65 (3H, s, 18-CH_3), 0.84 (3H, d, *J* = 6.4 Hz, 21-CH_3), 1.29 (3H, s, 19-CH_3), 0.92–2.89 (m, 26H), 2.96 (2H, m, 24-H), 5.15 (1H, br.s., –N<u>H</u>SO_2–), 7.20 (1H, t, *J* = 9.4 Hz), 7.28 (1H, m), 7.57 (1H, m), 7.88 (1H, t, *J* = 7.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.4, 19.0, 22.5, 22.8, 25.1, 26.6, 28.6, 33.1, 35.6 35.8, 35.8, 37.1, 39.2, 43.0, 43.3, 43.3, 44.2, 45.4, 48.1, 49.2, 49.9, 55.2, 117.2, 117.4, 124.8, 128.7, 130.7, 135.2, 158.2, 160.2, 210.8, 211.7. HRMS (TOF ES^+): *m/z* calcd for $C_{30}H_{42}FNNaO_4S^+$ [$(M+Na)^+$], 554.2711; found, 554.2704.

*2.1.10.12. N-(4′-nitrobenzenesulfonyl)-3,7-dioxo-5β-cholan-24-amide (**10l**).* 126 mg (84% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −15.5 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3456, 3285, 2945, 2868, 1706, 1623, 1334, 1011, 842, 551 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.63 (3H, s, 18-CH_3), 0.83 (3H, d, *J* = 6.5 Hz, 21-CH_3), 1.25 (3H, s, 19-CH_3), 0.88–2.89 (m, 26H), 2.96 (2H, m, 24-H), 5.37 (1H, t, *J* = 5.8 Hz, –N<u>H</u>SO_2–), 8.05 (2H, t, *J* = 8.7 Hz, 2′-ArH and 6′-ArH), 8.35 (2H, d, *J* = 8.7 Hz, 3′-ArH and 5′-ArH). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.4, 19.0, 22.5, 22.8, 25.1, 26.7, 28.7, 33.0, 35.5, 35.8, 35.8, 37.1, 39.2, 43.0, 43.3, 43.3, 44.3, 45.4, 48.1, 49.3, 49.9, 55.2, 124.8, 124.8, 128.7, 128.7, 146.7, 150.4, 210.9, 211.8. HRMS (TOF ES^-): *m/z* calcd for $C_{30}H_{41}N_2O_6S^-$ [$(M-H)^-$], 557.2691; found, 557.2686.

*2.1.10.13. N-(4′-(acetamide)benzenesulfonyl)-3,7-dioxo-5β-cholan-24-amide (**10m**).* 119 mg (78% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −30.0 °C (c 0.35, $CHCl_3$); IR (KBr) ν_{max} 3472, 3281, 2946, 2872, 1708, 1610, 1451, 1321, 1110, 862, 712 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.63 (3H, s, 18-CH_3), 0.84 (3H, d, *J* = 6.2 Hz, 21-CH_3), 1.24 (3H, s, 19-CH_3), 2.10 (3H, s, –NHCOC<u>H</u>$_3$), 1.00–2.96 (m, 26H), 3.40 (2H, m, 24-H), 7.41 (1H, t, *J* = 5.5 Hz, -N<u>H</u>SO_2-), 7.71 (2H, d, *J* = 8.6 Hz, 2′-ArH and 6′-ArH), 7.77 (2H, d, *J* = 8.6 Hz, 3′-ArH and 5′-ArH), 10.32 (1H, s, –N<u>H</u>$COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.2, 18.9, 22.0, 22.2, 24.4, 24.7, 26.0, 28.2, 32.8, 35.0, 35.2, 35.3, 36.7, 38.7, 42.0, 42.6, 42.9, 43.4, 44.9, 47.2, 48.9, 49.0, 54.9, 119.0, 119.0, 128.0, 128.0, 134.8, 143.0, 169.3, 210.2, 211.5. HRMS (TOF ES^-): *m/z* calcd for $C_{32}H_{45}N_2O_5S^-$ [$(M-H)^-$], 569.3055; found, 569.3060.

*2.1.10.14. N-(4′-(trifluoromethoxy)benzenesulfonyl)-3,7-dioxo-5β-cholan-24-amide (**10n**).* 131 mg (83% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −11.2 °C (c 0.25, $CHCl_3$); IR (KBr) ν_{max} 3476, 3283, 2947, 2868, 1709, 1434, 1258, 1215, 1163, 1092, 819, 695, 603 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.66 (3H, s, 18-CH_3), 0.84 (3H, d, *J* = 6.4 Hz, 21-CH_3), 1.31 (3H, s, 19-CH_3), 1.00–2.96 (m, 26H), 2.92 (2H, m, 24-H), 5.37 (1H, t, *J* = 5.8 Hz, -N<u>H</u>SO_2-), 7.35 (2H, d, *J* = 8.4 Hz, 3′-ArH and 5′-ArH), 7.94 (2H, d, *J* = 8.4 Hz, 2′-ArH and 6′-ArH). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.4, 18.9, 22.5, 22.7, 25.1, 26.6, 28.7, 33.1, 35.6 35.8, 35.8, 37.1, 39.2, 43.0, 43.3, 43.3, 44.1, 45.4, 48.1, 49.2, 49.9, 55.2, 121.4, 121.4, 129.6, 129.6, 139.1, 139.1, 152.3, 211.0, 211.8. HRMS (TOF ES^-): *m/z* calcd for $C_{31}H_{41}F_3NO_5S^-$ [$(M-H)^-$], 596.2663; found, 596.2660.

*2.1.10.15. N-(thiophene-2-sulfonyl)-3,7-dioxo-5β-cholan-24-amide (**10o**).* 111 mg (80% yield) as a colorless amorphous solid. $[\alpha]^{25}_D$ −18.0 °C (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3278, 2945, 2871, 1707, 1430, 1333, 1156, 1081, 1014, 727, 589 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$-*d*) δ 0.67 (3H, s, 18-CH_3), 0.87 (3H, d, *J* = 6.4 Hz, 21-CH_3), 1.30 (3H, s, 19-CH_3), 0.94–2.90 (m, 26H), 3.01 (2H, m, 24-H), 5.09 (1H, br.s., –N<u>H</u>SO_2–), 7.10 (1H, m), 7.60 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$-*d*) δ 12.4, 19.0, 22.5, 22.8, 25.1, 26.5, 28.7, 33.2, 35.6, 35.8, 35.8, 37.1, 39.2, 43.0, 43.3, 43.3, 44.4, 45.4, 48.1, 49.2, 50.0, 55.3, 127.8, 132.0, 132.3, 141.7, 210.8, 211.7. HRMS (TOF ES^-): *m/z* calcd for $C_{28}H_{40}NO_4S_2^-$ [$(M-H)^-$], 518.2404; found, 518.2408.



Scheme 1. Synthesis of *N*-sulfonyl-3,7-dioxo-5β-cholan-24-amides. Reagents and conditions: Reagents and conditions: (a) $SOCl_2$, CH_3OH, reflux, 3 h, 95%; (b) $LiAlH_4$, THF, rt, 1 h, 92%; (c) triphenylmethyl chloride (TrCl), pyridine, 90 °C, 24 h, 85%; (d) CrO_3, pyridine, CH_2Cl_2, rt, 0.5 h, 95%; (e) TsOH, acetone, rt, 10 min, quantitative; (f) MsCl, CH_2Cl_2, 0 °C, 1 h, 92%; (g) NaN_3, DMF, 70 °C, 3 h, 80%; (h) H_2, Pd/C, CH_3OH, rt, 5 h, 82%; (i) RSO_2Cl, pyridine, rt, 12 h, 78–94%.

2.2. Biology

The tumor cell lines (HCT-116, MCF-7, K562, and SGC-7901) were obtained from Shanghai Institute of Pharmaceutical Industry. The cytotoxic activity *in vitro* was measured using the MTT assay. The MTT solution (10.0 μL/well) was added in RPMI-1640 media (Sigma, St. Louis, MO) after cells were treated with the drug for 72 h, and cells were incubated for further 4 h at 37 °C. The purple formazan crystals were dissolved in 100.0 μL DMSO. After 10 min, the plates were read on an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT) at 570 and 630 nm. Assays were performed in triplicate on three independent experiments. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software "Dose–Effect Analysis with Microcomputers". The tumor cell lines panel consisted of HCT-116, MCF-7, K562, and SGC-7901. In all of these experiments, three replicate wells were used to determine each point.

3. Results and discussion

3.1. Chemistry

The synthetic route of target compounds is shown in Scheme 1. Synthesis of amine **9** started from commercially available ursodeoxycholic acid. A solution of ursodeoxycholic acid and thionyl chloride was refluxed for 2 h. After cooling to room temperature, the solution was evaporated to dryness to form ester **2** [20]. Subsequently, the ester group of compound **2** was reduced by lithium aluminum hydride in dry tetrahydrofuran to generate corresponding compound **3**. Then, **3** was selectively protected in the position of primary alcohol by treatment with triphenylmethyl chloride (TrCl) in the presence of catalytic amount of 4-dimethylaminopyridine in pyridine to provide the key precursor **4** [21]. Next, Collins oxidation furnished the ketone **5**, followed by deprotection of the Tr ether to afford **6** in quantitative yield. The mesylate **7** was prepared from **6** by treatment with methanesulfonyl chloride, followed by conversion of **7** in dimethylformamide with sodium azide to give the azide **8**. Thereafter, hydrogenation of **8** with Pd/C in methanol provided amine **9**. Finally, amine **9** was treated with various sulfuryl chloride and refluxed in pyridine for 2–5 h to afford various, *N*-sulfonyl-3,7-dioxo-5β-cholan-24-amides. All the new compounds synthesized were characterized by ^1H NMR, ^{13}C NMR, HRMS, and IR spectroscopy.

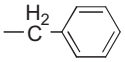
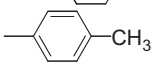
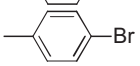
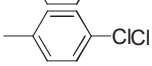
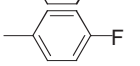
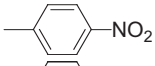
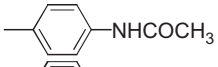
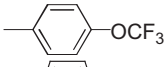
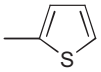
3.2. Biological activity

All the synthesized *N*-sulfonyl-3,7-dioxo-5β-cholan-24-amides s were evaluated *in vitro* against a panel of four cancer cell lines including HCT-116 (human colon cancer cell line), MCF-7 (human breast adenocarcinoma cell line), K562 (human leukemia cell line), and SGC-7901 (human gastric cancer cell line) by the standard microculture tetrazolium (MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay using 5-FU(5-Fluorouracil) as a positive control. Antitumor potency of the compounds was indicated by IC_{50} values that were calculated by linear regression analysis of the concentration–response curves obtained for each compound. The results of the cytotoxicity studies are summarized in Table 1 (IC_{50} value, defined as the concentration corresponding to 50% growth inhibition).

As shown in Table 1, all of *N*-sulfonyl-3,7-dioxo-5β-cholan-24-amides exhibited potent inhibitory effects on HCT-116, MCF-7, and K562 cell lines. Sulfonamides **10a–o**, compared to amide **9**, displayed a higher cytotoxicity. However, these compounds displayed low cytotoxicity against SGC-7901 cell line in the investigated concentration range (>100 μM), with only **10b** having IC_{50} below 50 μM. For the four cell lines, all sulfonamide compounds displayed the higher cytotoxicity to K562 cell, which was superior to 5-FU (IC_{50} = 45.4 μM). Among **10a–o**, **10g** was found to have the best activity on K562 cell line (IC_{50} = 2.39 μM). Interestingly, the result that all compounds showed potent activity for human colon cancer cell line (HCT-16) was consistent to our design. In addition, the comparisons of **7** and **10a** revealed that the cytotoxicities of *N*-sulfonyl-3,7-dioxo-5β-cholan-24-amides were more potent than that of 3,7-dioxo-5β-cholan-24-sulfonate.

The results presented in Table 1 implied that the nature of the substituent groups at the sulfonamide core play a critical role in antitumor potency. Compounds **10a**, **10b**, and **10c** with the aliphatic chain of methyl, ethyl, and *n*-butyl, respectively, exhibited increasing cytotoxicity against MCF-7 and K562 cancer cells, suggesting that the length of the aliphatic chain of the sulfonamide groups is critical for the compound's cytotoxic activity. Nevertheless, for HCT-116, ethyl substitution was superior to the others. In addition, replacement of the aliphatic groups of the sulfonamide

Table 1
Human cancer cell line growth inhibition values *in vitro*[a] (IC_{50}, μM/mL[b]) for the synthesized compounds.

Compounds	R	IC_{50}/(μM)			
		HCT-116	MCF-7	K562	SGC-7901
6	–	70.23	76.38	39.44	73.26
7	–	31.61	42.4	>100	>100
8	–	88.5	65.2	24.34	>100
9	–	24.11	38.5	61.29	63.32
10a	$-CH_3$	13.74	24.47	16.58	53.04
10b	$-CH_2CH_3$	3.39	15.89	12.75	26.79
10c	$-CH_2CH_2CH_2CH_3$	9.07	8.99	6.54	59.2
10d		15.46	15.29	6.76	72.75
10e		12.81	35.04	10.73	>100
10f		3.71	8.62	4.94	>100
10g		8.86	9.34	2.39	>100
10h		9.05	10.36	4.51	>100
10i		8.49	16.27	4.68	>100
10j		16.4	18.28	6.98	>100
10k		12.06	9.3	5.19	>100
10l		11.97	16.4	7.37	72.36
10m		6.16	13.84	11.37	78.49
10n		50	14.77	5.89	>100
10o		50	31.31	9.48	68.97
5-fu		1.93	14.18	45.4	2.19

[a] Cytotoxicity as IC_{50} for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.
[b] Data represent the mean values of three independent determinations.

moiety with the aromatic groups resulted in derivative **10d–o**, which overall had similar activity potency against four cells as **10a–c**.

Apparently, introduction of an additional group at the phenyl ring of the sulfonamide moiety had a profound influence on cytotoxic activity. The *para*-methyl derivative **10f** was 1- to 3-fold more potent than no substituent **10d**. The effect on potency of different halogen substitutents at *para*-position of phenyl ring is in approximate order of –Br (**10g**) > –Cl (**10h**) > –F (**10i**) against MCF-7 and K562 cancer cells. Additionally, the cytotoxicities of **10n** and **10o** against HCT-116, MCF-7, and K562 cell lines were ever-increasing, reflecting the excellent selectivity for the three cancer cells.

4. Conclusions

In summary, a series of *N*-sulfonyl-3,7-dioxo-5β-cholan-24-amides, ursodeoxycholic acid analogs, have been prepared and evaluated against HCT-116, MCF-7, K562, and SGC-7901 cell lines. The results showed that more of the prepared sulfonamides displayed the selective cytotoxicity toward HCT-116, MCF-7 and K562 cell lines. From the structure–activity relationships we may conclude that the introduction sulfonamide groups at 24-position in 3,7-dioxo-5β-cholan is associated with enhanced cytotoxic activity. This study may provide valuable information for further designing and developing more potent anticancer agents.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2012.09.009.

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