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SYNTHESIS AND ANTITUMOR ACTIVITY OF A NOVEL SERIES OF HELICID-PYRROLIDONE DERIVATIVES

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*This paper reports the synthesis of a new series of helicid–pyrrolidone derivatives (**4a–4d**, **5a–5d**, and **6a–6d**), which were tested for antitumor activity against human skov3 cell. In this study, two compounds (**4b** and **6c**) demonstrate high antitumor activity against this cell line having IC_{50} values of 0.22 and 6.5 μM, respectively, which are lower than those of an inhibitor of heat shock protein 90 (17AAG), currently under clinical evaluation for cancer treatment.*

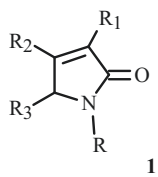
Keywords: antitumor, helicid, pyrrolidone.

Currently, people are increasingly suffering from various diseases, especially cancer. Cancer is a major disease, and according to the World Health Organization, it is considered to be the fourth leading cause of death. Developing effective cancer treatment has been a primary goal of medicine for the last two decades, and chemotherapy is considered to be an effective approach for combating cancer. Although there are a large number of anticancer drugs for clinical use, advancing novel compounds with higher activity that are also active against mutant cells has remained a high priority [1].

The chaperone heat shock protein 90 (Hsp90) is emerging as an important target for antitumor drugs because of its effect on proteins, which plays an important role in maintaining transformation and increasing the survival and growth potential of cancer cells. The most well-known inhibitor of Hsp90, 17-(allylamino)-17-demethoxygeldanamycin (17AAG), is currently in phase II clinical trials for the treatment of cancer in the United States and United Kingdom and has shown early evidence of therapeutic activity when administered alone or in combination with docetaxel [2, 3].

Small and simple heterocyclic structures often have surprisingly complex biological properties. For example, pyrrolidone derivatives represented by the general formula **1** have a neural differentiation induction effect, making them useful as antitumor agents, nootropic agents, etc. [4] for treating nervous diseases. The natural product helicid [4-formylphenyl-β-D-allopyranoside, $C_{13}H_{16}O_7$] (**2**), originally extracted from the fruit of *Helicia nilagirica* Bedd., exhibits various biological activities on the central nervous system, such as hypnotic, anti-inflammatory, and anticonvulsant activity, owing to the rare allopyranoside moiety [5]. Using the principle of combining active groups, we incorporated the pyrrolidone active group into the helicid compounds to form new helicid–pyrrolidone derivatives.

In this paper, we report several new helicid–pyrrolidone derivatives and describe their chemical synthesis. All new compounds, 17AAG, and **2** were tested for antitumor activity against human hepatoma carcinoma cell line 7402, and their IC_{50} values are reported.



1

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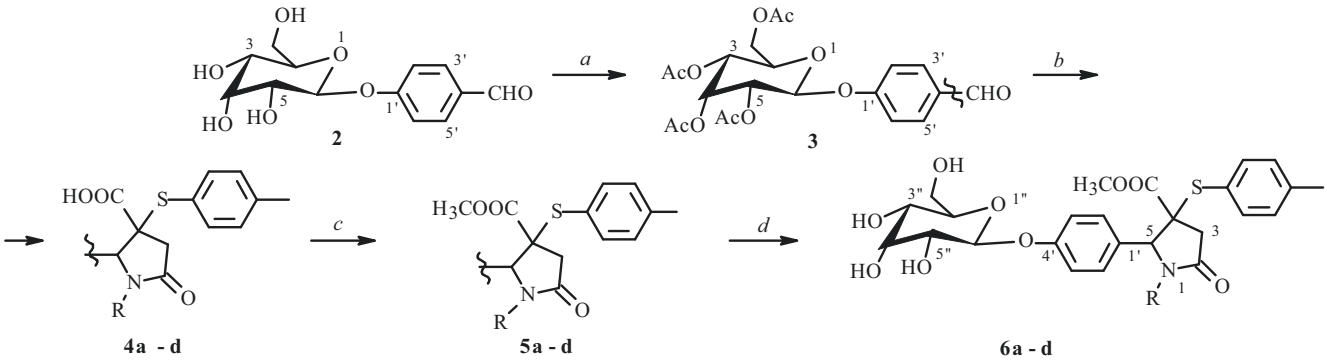
TABLE 1. *In vitro* Activity of Compounds **4a–4d**, **5a–5d**, and **6a–6d** (IC_{50}, μM) against Human Hepatoma Carcinoma Cell Line 7402

Compound	IC_{50}, μM*	Compound	IC_{50}, μM*
17AAG	16.5	**5b**	62.5
2	33.6	**5c**	> 100
4a	62.5	**5d**	> 100
4b	0.22	**6a**	> 100
4c	> 100	**6b**	> 100
4d	> 100	**6c**	6.2
5a	> 100	**6d**	> 100

*IC_{50} values represent the drug concentration required to inhibit cancer cell replication by 50%. The compounds were tested up to 625 μM. IC_{50} values were calculated by probit analysis ($P < 0.05$, χ^2 test).



4a, 5a, 6a: R = n-C_3H_7; **4b, 5b, 6b:** R = *iso*-C_4H_9; **4c, 5c, 6c:** R = cyclohexyl; **4d, 5d, 6d:** R = Bn

a. Ac_2O, TEA, DMF, 96%; *b*. *p*-thiocresol, RNH_2, maleic anhydride, 50°C, 110°C, 45%–56%; *c*. K_2CO_3, CH_3I, r.t., 91%–94%; *d*. NH_3, MeOH, r.t., 87%–90%

Scheme 1.

Helicid–pyrrolidone derivatives **4a–4d**, **5a–5d**, and **6a–6d** and intermediate **3** were synthesized by the method shown in Scheme 1. Compound **3** is an important intermediate and is obtained by the reaction of 4-formylphenyl-*β*-D-allopyranoside (**2**) with acetic anhydride in the presence of triethylamine (TEA) in dimethylformamide (DMF), followed by reduction at room temperature, in good yield (96%, step *a*). Then, helicid–pyrrolidone derivatives **4a–4d** were synthesized through a four-component (primary amine, maleic anhydride, *p*-thiocresol, and intermediate **3**) condensation reaction (step *b*). Helicid–pyrrolidone derivatives **5a–5d** were obtained in good yield by esterification of (**4a–4d**) in which propan-2-one used as a sovlent under alkaline conditions at room temperature (step *c*). Transesterification of helicid–pyrrolidone derivatives **5a–5d** in the presence of NH_3 and CH_3OH at room temperature gave new compounds **6a–6d** (step *d*).

In preliminary screening, compounds **4a–4d**, **5a–5d**, and **6a–6d** were evaluated for their antitumor activity against skov3 cell. The test compounds were dissolved in DMSO and evaluated using five concentrations, the highest being 625 μM (remaining concentrations prepared as fivefold dilutions: 125, 62.5, 25, and 5 μM). Table 1 reports the results obtained expressed as log, considering half maximal inhibitory concentration IC_{50}.

All new compounds were tested to determine their antitumor activity against human skov3 cell with IC_{50} values. Considering the results shown in Table 1, **4b** and **6c** emerged as analogs having higher antitumor activity than 17AAG. Therefore, the development of related helicid–pyrrolidone derivatives merits further attention. The results of pharmacokinetic and toxicological studies on these agents will be published elsewhere.

EXPERIMENTAL

General. Melting points were recorded with a micromelting point tester and are reported uncorrected. ^1H and ^{13}C NMR spectra were obtained in DMSO-d_6 solutions on a Bruker AVII-400 spectrometer operating at 400 and 100 MHz,

respectively. The chemical shift values are given in δ units with reference to internal standard Me_4Si, and signals are designated as s (singlet), br.s (broad singlet), d (doublet), t (triplet), q (quadruplet), qu (quintuplet), h (heptuplet), dt (double triplet), dd (doublet of doublets), ddd (double doublet of doublets), or m (multiplet) with coupling constants given in Hertz (Hz). Infrared spectra (as KBr disks) were recorded using a PerkinElmer 16PC-FT spectrometer. Kieselgel 60F254 (0.25 mm) silica gel and TLC aluminum sheets were used for routine monitoring of reaction samples and for confirming the homogeneity of analytical samples. Silica gel column chromatography was carried out using GF254 (300–400 mesh) silica gel. High-resolution mass spectra were recorded on a Bruker Daltonics ESI-BioTOF Q spectrometer.

Starting Materials. All reagents were commercially available and were used without further purification unless otherwise indicated. Toluene was dried by distillation from sodium and stored over activated molecular sieves (4 Å), and DMF was dried by distillation from magnesium sulfate prior to use.

Synthesis of (2*R*,3*R*,4*R*,5*R*,6*S*)-2-(Acetoxymethyl)-6-(4-formylphenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl Triacetate (3). A solution of 4-formylphenyl-*β*-D-allopyranoside **2** (2.84 g, 10 mmol) was dissolved in anhydrous DMF (8 mL); then acetic anhydride (5 equiv) and triethylamine (4.2 equiv) were added under stirring at 0°C; the resulting mixture was stirred for 2 h at 0°C, then for 12 h at room temperature. The reaction mixture was diluted with water (50 mL). The resulting crystals were collected by filtration, washed with water (10 mL), and dried under vacuum to give 4.37 g (96%) of **3** (white crystals).

Synthesis of Compounds 4a–4d. A solution of succinic anhydride (0.39 g, 4 mmol), RNH_2 (4 mmol), and *p*-thiocresol (0.50 g, 4 mmol) in toluene (10 mL) was stirred at 50°C for 15 min, and **3** (1.84 g, 4 mmol) was added. Then the resulting mixture was stirred for 14 h at 110°C. The reaction mixture was diluted with a large amount of water and extracted with ethyl acetate. The organic layer was purified by column chromatography on silica gel using chloroform–methanol–petroleum ether 5:1:4 as the eluent to give 1.34 g (45–56%) of **4a–4d**.

5-Oxo-1-propyl-3-(*p*-tolylthio)-2-(4-(((2*S*,3*R*,4*R*,5*R*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pyrrolidine-3-carboxylic Acid (4a). Yellow powder. Yield 47%, mp 130–134°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.98–7.20 (8H, m, ArH), 5.63 (1H, dd, $J_{12} = J_{13} = 2.7$, CH), 5.53 (1H, d, J = 8.3, CH), 5.28 (1H, s, CH), 5.03 (1H, dd, $J_{12} = 2.9$, $J_{13} = 8.2$, CH), 4.98 (1H, dd, $J_{12} = 2.9$, $J_{13} = 10.2$, CH), 4.30–4.45 (1H, m, 1/2CH_2), 4.16–4.26 (2H, m, CH + 1/2CH_2), 3.40–3.48 (1H, m, 1/2CH_2), 2.95–3.08 (2H, m, CH_2), 2.37–2.46 (1H, m, 1/2CH_2), 2.25 (3H, s, CH_3), 2.16 (3H, d, J = 1.9, CH_3), 2.02 (3H, s, CH_3), 2.00 (3H, s, CH_3), 1.99 (3H, s, CH_3), 1.23–1.40 (2H, m, CH_2), 0.72 (3H, t, J = 7.3, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 11.07, 19.85, 20.32, 20.44, 20.63, 22.34, 40.27, 40.86, 41.90, 58.76, 61.83, 65.89, 67.35, 68.78, 69.90, 95.68, 115.83, 128.34, 129.20, 130.47, 134.66, 137.87, 156.29, 169.10, 169.93, 169.98, 170.02, 171.75. IR (KBr, ν, cm^{-1}): 3437, 2965, 1754, 1669, 1611, 1511, 1412, 1374, 1227, 1091, 1045, 949, 908, 814, 599, 510. HR-MS (ESI): calcd for $C_{35}H_{41}O_{13}NS$ $[M + H]^+$ 716.2377, found 716.2392; $[M + K]^+$ 754.1936, found 754.1882.

1-Isobutyl-5-oxo-3-(*p*-tolylthio)-2-(4-(((2*S*,3*R*,4*R*,5*R*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pyrrolidine-3-carboxylic Acid (4b). Yellow powder, Yield 51%, mp 134–138°C. IR (KBr, ν, cm^{-1}): 3437, 2961, 1755, 1667, 1610, 1510, 1414, 1372, 1226, 1091, 1045, 949, 907, 814, 598, 509. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.90–7.31 (8H, m, ArH), 5.63 (1H, dd, $J_{12} = J_{13} = 2.7$, CH), 5.53 (1H, d, J = 7.8, CH), 5.30 (1H, s, CH,), 4.95–5.06 (2H, m, 2CH), 4.35–4.45 (1H, m, 1/2CH_2), 4.15–4.26 (2H, m, CH + 1/2CH_2), 3.40–3.57 (1H, m, 1/2CH_2), 3.03–3.27 (2H, m, CH_2), 2.30–2.44 (1H, m, 1/2CH_2), 2.24 (3H, s, CH_3), 2.16 (3H, d, J = 2.9, CH_3), 2.02 (3H, s, CH_3), 2.00 (3H, s, CH_3), 1.99 (3H, s, CH_3), 1.64–1.75 (1H, m, CH), 0.75 (3H, d, J = 6.6, CH_3), 0.72 (3H, d, J = 6.6, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 19.83, 20.15, 20.31, 20.43, 20.63, 25.94, 40.81, 47.71, 58.76, 61.86, 65.93, 67.38, 67.97, 68.79, 69.93, 95.74, 115.87, 128.68, 129.09, 129.72, 130.61, 134.49, 134.55, 137.62, 156.24, 169.07, 169.90, 169.95, 169.98, 172.13. HR-MS (ESI): calcd for $C_{36}H_{43}O_{13}NS$ $[M + H]^+$ 730.2533, found 730.2534; $[M + K]^+$ 768.2092, found 768.2020.

1-Cyclohexyl-5-oxo-3-(*p*-tolylthio)-2-(4-(((2*S*,3*R*,4*R*,5*R*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)- tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pyrrolidine-3-carboxylic Acid (4c). Yellow powder. Yield 45%, mp 158–162°C. IR (KBr, ν, cm^{-1}): 3433, 2934, 1755, 1660, 1609, 1510, 1414, 1373, 1225, 1091, 1044, 948, 906, 813, 596, 509. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.80–7.41 (8H, m, ArH), 5.63 (1H, dd, $J_{12} = J_{13} = 2.8$, CH), 5.53 (1H, d, J = 8.2, CH), 5.38 (1H, s, CH), 4.94–5.06 (2H, m, CH_2), 4.36–4.44 (1H, m, 1/2CH_2), 4.12–4.28 (2H, m, CH + 1/2CH_2), 3.50–3.64 (1H, m, CH), 2.80–3.00 (2H, m, CH_2), 2.25 (3H, s, CH_3), 2.23 (3H, s, CH_3), 2.16 (3H, d, J = 3.9, CH_3), 2.02 (3H, s, CH_3), 1.99 (3H,s, CH_3), 1.98 (3H, s, CH_3), 0.79–1.74 (10H, m, 5CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 20.34, 20.44, 20.63, 24.93, 25.15, 31.22, 40.95, 51.87, 58.76, 59.91, 60.81, 65.90, 66.21, 67.34, 68.83, 69.88, 95.50, 115.53, 128.92, 133.63, 134.28, 134.36, 137.19, 155.80, 155.90, 169.11, 169.95, 170.00, 171.53. HR-MS (ESI): calcd for $C_{38}H_{45}O_{13}NS$ $[M + H]^+$ 756.2690, found 756.2684; $[M + K]^+$ 794.2249, found 794.2169.

1-Benzyl-5-oxo-3-(*p*-tolylthio)-2-(4-(((2*S*,3*R*,4*R*,5*R*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pyrrolidine-3-carboxylic Acid (4d). Yellow powder, Yield 56%, mp 158–162°C. IR (KBr, ν, cm^{-1}): 3427, 2925, 1754, 1663, 1609, 1510, 1412, 1373, 1226, 1091, 1045, 948, 909, 814, 703, 599, 508. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.00–7.25 (13H, m, ArH), 5.64 (1H, dd, $J_{12} = J_{13} = 2.8$, CH), 5.53 (dd, $J_{12} = 4.9$, $J_{13} = 8.1$, CH), 5.08 (1H, s, CH), 4.98–5.06 (2H, m, 2CH), 4.85 (1H, d, J = 15.5, 1/2CH_2), 4.36–4.44 (1H, m, 1/2CH_2), 4.14–4.28 (2H, m, CH + 1/2CH_2), 3.50 (1H, d, J = 15.6, 1/2CH_2), 3.15 (1H, d, J = 15.6, 1/2CH_2), 2.25 (3H, s, CH_3), 2.16 (3H, d, J = 1.8, CH_3), 2.02 (3H, s, CH_3), 2.01 (3H, s, CH_3), 1.99 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 20.34, 20.47, 20.64, 40.82, 42.05, 43.81, 58.80, 61.84, 65.90, 67.36, 68.79, 69.91, 95.69, 115.84, 127.04, 127.22, 128.21, 128.41, 129.17, 127.96, 134.63, 136.22, 137.73, 156.28, 169.11, 169.94, 170.02, 172.33. HR-MS (ESI): calcd for $C_{39}H_{41}O_{13}NS$ $[M + H]^+$ 764.2377, found 764.2365; $[M + K]^+$ 802.1936, found 802.1730.

Synthesis of Compounds 5a–5d. Compound **4** (1 mmol) was dissolved in propan-2-one (5 mL), and K_2CO_3 (1.00 g, 7 mmol) and CH_3I (280 mg, 2 mmol) were added. The mixture was stirred for 24 h at room temperature. The solvent was evaporated, and the residue was purified by flash chromatography (silica gel, propan-2-one–petroleum ether, 2:3, v/v). A yellow powder was obtained: **5a** 91% yield, **5b**, **5c**, and **5d** (92% yield).

(2*R*,3*R*,4*R*,5*R*,6*S*)-2-(Acetoxymethyl)-6-(4-(3-(methoxycarbonyl)-5-oxo-1-propyl-3-(*p*-tolylthio)pyrrolidin-2-yl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl Triacetate (5a). Yellow powder. Yield 94%, mp 68–72°C. IR (KBr, ν, cm^{-1}): 1754, 1698, 1610, 1510, 1412, 1373, 1225, 1091, 1044, 948, 909, 816, 715, 599, 508. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.29 (2H, d, J = 7.6, ArH), 7.12–7.20 (6H, m, ArH), 5.63 (1H, dd, $J_{12} = J_{13} = 2.7$, CH), 5.56 (1H, d, J = 8.2, CH), 5.22 (1H, s, CH), 5.03 (dd, $J_{12} = 2.9$, $J_{13} = 8.1$, CH), 4.99 (1H, dd, $J_{12} = 2.7$, $J_{13} = 10.3$, CH), 4.35–4.42 (1H, m, 1/2CH_2), 4.16–4.26 (2H, m, CH + 1/2CH_2), 3.62 (3H, s, CH_3), 3.40–3.54 (1H, m, 1/2CH_2), 2.94 (1H, d, J = 18.0, 1/2CH_2), 2.85 (1H, d, J = 18.0, 1/2CH_2), 2.40–2.48 (1H, m, 1/2CH_2), 2.29 (3H, s, CH_3), 2.16 (3H, s, CH_3), 2.02 (3H, s, CH_3), 2.01 (3H, s, CH_3), 1.99 (3H, s, CH_3), 1.22–1.38 (2H, m, CH_2), 0.68 (3H, t, J = 7.3, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 10.77, 19.58, 20.32, 20.45, 20.70, 40.35, 41.28, 41.72, 52.95, 58.45, 61.82, 65.88, 67.33, 68.77, 69.93, 95.58, 116.04, 125.91, 128.48, 129.84, 135.75, 139.68, 156.64, 169.10, 169.94, 169.99, 170.03, 170.74, 171.42. HR-MS (ESI): calcd for $C_{36}H_{43}O_{13}NS$ $[M + H]^+$ 730.2533, found 730.2545; $[M + Na]^+$ 752.2353, found 752.2374.

(2*R*,3*R*,4*R*,5*R*,6*S*)-2-(Acetoxymethyl)-6-(4-(1-isobutyl-3-((methylperoxy)methyl)-5-oxo-3-(*p*-tolylthio)pyrrolidin-2-yl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl Triacetate (5b). Yellow powder. Yield 92%, mp 74–78°C. IR (KBr, ν, cm^{-1}): 1754, 1698, 1610, 1374, 1225,1091, 1044, 948, 909, 816, 713, 599, 509. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.13–7.32 (8H, m, ArH), 5.63 (1H, dd, $J_{12} = J_{13} = 2.7$, CH), 5.55 (1H, d, J = 8.2, CH), 5.20 (1H, s, CH), 5.05 (1H, dd, $J_{12} = 2.8$, $J_{13} = 8.1$, CH), 4.99 (dd, $J_{12} = 2.7$, $J_{13} = 10.3$, CH), 4.35–4.45 (1H, m, 1/2CH_2), 4.15–4.26 (2H, m, CH + 1/2CH_2), 3.61 (3H, s, CH_3), 3.40–3.47 (1H, m, 1/2CH_2), 2.96 (1H, d, J = 16.9, 1/2CH_2), 2.87 (1H, d, J = 16.9, 1/2CH_2), 2.22–2.32 (1H, m, 1/2CH_2), 2.29 (3H, s, CH_3), 2.16 (3H, s, CH_3), 2.02 (3H, s, CH_3), 2.00 (3H, s, CH_3), 1.99 (3H, s, CH_3), 1.64–1.80 (1H, m, CH), 0.73 (3H, d, J = 6.6, CH_3), 0.66 (3H, d, J = 6.6, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 19.32, 19.80, 20.32, 20.45, 20.70, 25.71, 41.28, 47.37, 52.97, 58.54, 61.80, 65.86, 66.51, 67.32, 68.77, 69.93, 95.57, 116.14, 125.81, 128.25, 129.85, 135.69, 139.70, 156.67, 169.10, 169.93, 169.99, 170.03, 170.95. HR-MS (ESI): calcd for $C_{37}H_{45}O_{13}NS$ $[M + H]^+$ 744.2690, found 744.2706; $[M + Na]^+$ 766.2509, found 766.2530.

(2*R*,3*R*,4*R*,5R,6*S*)-2-(Acetoxymethyl)-6-(4-(1-cyclohexyl-3-((methylperoxy)methyl)-5-oxo-3-(*p*-tolylthio)pyrrolidin-2-yl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl Triacetate (5c). Yellow powder. Yield 94%, mp 80–84°C. IR (KBr, ν, cm^{-1}): 2857, 1754, 1694, 1610, 1510, 1412, 1373, 1224, 1091, 1044, 949, 909, 815, 715, 598, 509. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.80–7.40 (8H, m, ArH), 5.63 (1H, dd, $J_{12} = J_{13} = 2.8$, CH), 5.57 (1H, d, J = 8.2, CH), 5.24 (1H, s, CH), 4.92–5.05 (2H, m, 2CH), 4.32–4.46 (1H, m, 1/2CH_2), 4.12–4.28 (2H, m, CH + 1/2CH_2), 3.55–3.64 (1H, m, CH), 3.59 (3H, s, CH_3), 2.97 (1H, d, J = 16.4, 1/2CH_2), 2.80 (1H, d, J = 16.4, 1/2CH_2), 2.23 (3H, s, CH_3), 2.16 (3H, d, J = 3.9, CH_3), 2.03 (3H, s, CH_3), 2.01 (3H, s, CH_3), 1.99 (3H, s, CH_3), 0.80–1.74 (10H, m, 5CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 20.14, 20.32, 20.45, 20.70, 25.02, 25.11, 26.41, 29.51, 30.85, 40.60, 41.28, 51.83, 53.05, 59.91, 60.81, 64.69, 65.88, 67.32, 68.83, 69.93, 95.34, 115.88, 125.91, 129.78, 130.13, 130.97, 135.53, 136.08, 139.54, 156.40, 169.09, 169.93, 170.01, 170.14, 171.32. HR-MS (ESI): calcd for $C_{39}H_{47}O_{13}NS$ $[M + H]^+$ 770.2846, found 770.2866; $[M + Na]^+$ 792.2666, found 792.2679.

(2*R*,3*R*,4*R*,5*R*,6*S*)-2-(Acetoxymethyl)-6-(4-(1-benzyl-3-((methylperoxy)methyl)-5-oxo-3-(*p*-tolylthio)pyrrolidin-2-yl)phenoxy)tetrahydro-2*H*-pyran-3,4,5-triyl Triacetate (5d). Yellow powder. Yield 91%, mp 72–76°C. IR (KBr, ν, cm^{-1}): 1754, 1601, 1510, 1412, 1373, 1225, 1091, 1045, 948, 910, 816, 704, 600, 508. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.10–7.35 (11H, m, ArH), 6.95 (2H, d, J = 3.2, ArH), 5.64 (1H, dd, $J_{12} = J_{13} = 2.8$, CH), 5.56 (1H, dd, $J_{12} = 2.9$, $J_{13} = 8.2$, CH), 5.04 (1H, dd, $J_{12} = 2.9$, $J_{13} = 8.1$, CH), 5.02 (1H, dd, $J_{12} = 2.7$, $J_{13} = 10.3$, CH), 4.92 (d, J = 16.1, CH, 1/2CH_2), 4.89 (1H, s,

CH), 4.38–4.45 (1H, m, 1/2CH_2), 4.16–4.24 (2H, m, CH + 1/2CH_2), 3.51 (3H, s, CH_3), 3.50 (1H, d, J = 14.1, 1/2CH_2), 3.01 (1H, d, J = 18.1, 1/2CH_2), 2.94 (1H, d, J = 18.0, 1/2CH_2), 2.29 (3H, s, CH_3), 2.16 (3H, d, J = 2.0, CH_3), 2.03 (3H, s, CH_3), 2.02 (3H, s, CH_3), 2.00 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 20.33, 20.47, 20.70, 40.15, 41.28, 43.62, 52.88, 58.53, 61.82, 65.75, 65.88, 67.35, 68.77, 69.94, 95.58, 116.20, 125.70, 127.30, 127.46, 127.87, 128.53, 129.87, 135.78, 139.75, 156.78, 169.10, 169.93, 170.01, 170.77, 171.22. HR-MS (ESI): calcd for $C_{40}H_{43}O_{13}NS$ $[M + H]^+$ 778.2533, found 778.2509; $[M + K]^+$ 816.2092, found 816.2025.

Synthesis of Compounds 6a–6d. Ammonia water (10 mL, 130 mmol) was heated and the ammonia dissolved in CH_3OH (60 mL); an NH_3-methanol solution was obtained. Compound **5a** (0.5 mmol) was dissolved in the NH_3-methanol solution (5 mL) and stirred at room temperature. The resulting crystals were collected by filtration and purified by flash chromatography (silica gel, chloroform–methanol–petroleum ether 5:1:2, v/v). A yellow powder was obtained **6a**, **6c**, **6d** (87% yield), and **6b** (89% yield).

4-((Methylperoxy)methyl)-1-propyl-4-(*p*-tolylthio)-5-(4-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)- tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pyrrolidin-2-one (6a). Yellow powder. Yield 89%, mp 88–92°C. IR (KBr, ν, cm^{-1}): 3414, 2927, 1731, 1677, 1610, 1510, 1415, 1232, 1177, 1081, 1039, 897, 816, 691, 574, 508. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.22 (2H, d, J = 7.5, ArH), 7.17–7.26 (4H, s, ArH), 7.07 (2H, d, J = 8.2, ArH), 5.17 (1H, s, CH), 5.15 (1H, d, J = 7.8, CH), 5.10 (1H, d, J = 6.6, OH), 4.96 (1H, d, J = 2.9, OH), 4.67 (1H, d, J = 7.4, OH), 4.53 (1H, dd, J_{12} = 5.6, J_{13} = 11.0, OH), 3.93 (1H, m, 1/2CH_2), 3.65–3.75 (2H, m, CH + 1/2CH_2), 3.62 (3H, s, CH_3), 3.40–3.52 (4H, m, 3CH + 1/2CH_2), 2.93 (1H, d, J = 17.0, 1/2CH_2), 2.87 (1H, d, J = 17.0, 1/2CH_2), 2.42 (1H, m, 1/2CH_2), 2.28 (3H, s, CH_3), 1.18–1.40 (2H, m, CH_2), 0.68 (3H, t, J = 7.4, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 10.78, 19.59, 20.71, 40.41, 41.70, 52.95, 58.59, 60.88, 66.09, 66.96, 70.18, 71.45, 74.62, 98.32, 98.49, 115.85, 126.01, 127.29, 129.83, 135.73, 139.62, 158.08, 158.14, 170.72, 171.52. HR-MS (ESI): calcd for $C_{28}H_{35}O_9NS$ $[M + H]^+$ 562.2111, found 562.2108; $[M + Na]^+$ 584.1930, found 584.1931.

1-Isobutyl-4-((methylperoxy)methyl)-4-(*p*-tolylthio)-5-(4-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)- tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pyrrolidin-2-one (6b). Yellow powder. Yield 90%, mp 94–98°C. IR (KBr, ν, cm^{-1}): 3419, 2957, 2925, 1732, 1678, 1610, 1510, 1414, 1240, 1177, 1081, 1040, 898, 816, 689, 574, 508. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.07–7.30 (6H, m, ArH), 7.06 (2H, d, J = 8.1, ArH), 5.18 (1H, s, CH), 5.16 (1H, d, J = 13.2, CH), 5.10 (1H, d, J = 6.6, OH), 4.96 (1H, d, J = 3.2, OH), 4.66 (1H, d, J = 7.4, OH), 4.52 (1H, dd, J_{12} = 5.2, J_{13} = 9.8, OH), 3.93 (1H, m, 1/2CH_2), 3.64–3.76 (2H, m, CH + 1/2CH_2), 3.62 (3H, s, CH_3), 3.38–3.52 (4H, m, 3CH + 1/2CH_2), 2.96 (1H, d, J = 16.9, 1/2CH_2), 2.89 (1H, d, J = 16.9, 1/2CH_2), 2.29 (3H, s, CH_3), 1.66–1.78 (1H, m, CH), 0.73 (3H, d, J = 6.6, CH_3), 0.66 (3H, d, J = 6.6, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 19.31, 19.82, 20.71, 25.71, 39.96, 40.31, 47.35, 52.97, 58.62, 60.89, 66.67, 66.96, 70.18, 71.44, 74.62, 98.31, 98.49, 115.95, 116.44, 125.92, 127.09, 129.85, 135.69, 139.64, 158.12, 158.19, 170.94, 171.57. HR-MS (ESI): calcd for $C_{29}H_{37}O_9NS$ $[M + H]^+$ 576.2267, found 576.2269; $[M + Na]^+$ 598.2087, found 598.2095.

1-Cyclohexyl-4-((methylperoxy)methyl)-4-(*p*-tolylthio)-5-(4-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pyrrolidin-2-one (6c). Yellow powder. Yield 90%, mp 106–110°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.98–7.42 (8H, m, ArH), 5.20 (1H, s, CH), 5.15 (1H, t, J = 7.8, OH), 5.11 (1H, d, J = 6.7, OH), 4.95 (1H, d, J = 3.1, OH), 4.67 (1H, d, J = 7.3, OH), 4.53 (1H, dd, J_{12} = 5.3, J_{13} = 10.7, OH), 3.93 (1H, m, 1/2CH_2), 3.61–3.80 (2H, m, CH + 1/2CH_2), 3.59 (3H, s, CH_3), 3.35–3.54 (3H, m, 3CH), 2.99 (1H, d, J = 16.2, 1/2CH_2), 2.79 (1H, d, J = 16.3, 1/2CH_2), 2.30 (3H, s, CH_3), 0.80–1.70 (10H, m, 5CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 20.70, 24.69, 25.02, 29.51, 30.87, 40.66, 51.79, 53.06, 59.95, 60.86, 64.84, 66.92, 70.20, 71.45, 74.61, 98.31, 98.63, 115.61, 126.00, 129.80, 135.54, 139.51, 157.95, 158.06, 170.17, 171.43. IR (KBr, ν, cm^{-1}): 3427, 2929, 2856, 1732, 1673, 1610, 1510, 1411, 1230, 1177, 1081, 1040, 896, 815, 688, 578, 509. HR-MS (ESI): calcd for $C_{31}H_{39}O_9NS$ $[M + H]^+$ 602.2424, found 602.2421; $[M + Na]^+$ 624.2243, found 624.2239.

1-Benzyl-4-((methylperoxy)methyl)-4-(*p*-tolylthio)-5-(4-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pyrrolidin-2-one (6d). Yellow powder. Yield 87%, mp 92–96°C. IR (KBr, ν, cm^{-1}): 3417, 2927, 1731, 1680, 1609, 1510, 1412, 1243, 1177, 1080, 1038, 888, 816, 704, 608, 507. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.12–7.34 (9H, m, ArH), 7.08 (2H, d, J = 7.6, ArH), 6.95 (2H, d, J = 7.0, ArH), 5.17 (1H, d, J = 7.9, CH), 5.11 (1H, d, J = 6.7, OH), 4.97 (1H, d, J = 3.7, OH), 4.92 (1H, dd, J_{12} = 4.5, J_{13} = 15.3, 1/2CH_2), 4.85 (1H, s, CH), 4.67 (1H, d, J = 7.4, OH), 4.53 (1H, t, J = 5.6, OH), 3.94 (1H, m, 1/2CH_2), 3.61–3.80 (2H, m, CH + 1/2CH_2), 3.51 (3H, s, CH_3), 3.40–3.58 (4H, m, 3CH + 1/2CH_2), 2.98 (2H, s, CH_2), 2.32 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 20.71, 40.19, 43.56, 52.87, 54.87, 58.65, 60.88, 65.85, 66.95, 70.19, 71.46, 74.68, 98.44, 98.50, 116.03, 125.82, 126.66,

127.28, 127.47, 128.55, 129.86, 135.76, 139.69, 158.27, 170.76, 171.32. HR-MS (ESI): calcd for $C_{32}H_{35}O_9NS$ $[M + H]^+$ 610.2111, found 610.2101; $[M + Na]^+$ 632.1930, found 632.1937.

Cells and Culture. Human skov3 cell line was obtained from State Key of Biotherapy, Sichuan University. Logarithmically growing skov3 cells were incubated with 0.05% trypsin at 37°C for about 4 min until cells were nonadherent and formed a single cell suspension. Trypsin activity was neutralized by adding a 20-fold excess of serum-containing medium. Cells were cultured at 37°C in an atmosphere of air and 5% CO_2.

Antitumor Activity. Antitumor activity of the compounds was determined by their positive action on cultured tumor cells. Logarithmically growing cells were plated in 0.1 mL aliquots in 96-well microtiter plates. Cells were plated at an initial density of about 50 000 cells/mL and allowed to acclimatize for 24 h. Cell suspensions were treated with various dilutions of compounds in triplicate, mixed well, and allowed to incubate for 72 h at 37°C in an atmosphere of air and 5% CO_2. To the cell suspension was added 10 μL of CCK-8 reagent, the mixture was incubated for 4 h at 37°C in an atmosphere of air and 5% CO_2, and absorbance at 450 nm was read by the microplate reader. Control plates with serial dilutions of cell types were counted as a control for the assay.

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