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SYNTHESIS AND *IN VITRO* ANTICANCER EFFICACY OF NOVEL SILIBININ DERIVATIVES

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Abstract – A series of eleven novel silibinin derivatives bearing alkynyl or sulfonyl groups were successfully designed and synthesized, and most of them suppressed the proliferation of MCF-7, NCI-H1299, HepG2 and HT29 by CCK-8 assay. Especially **3a** and **5b** were determined to be the most effective derivatives in the tested series with IC_{50} values of 7.41 and 3.37 μM against MCF-7 and HepG2 cells, respectively. In addition, a structure-activity relationship (SAR) study revealed that monosubstituted derivatives exhibited more potent anticancer activity than multiple substituted derivatives. The proliferation inhibitory activity can be improved by oxidative dehydrogenation of silibinin derivatives. The present findings suggest C7-OH monosubstituted silybin or 2,3-dehydrosilibinin derivatives with alkynyl or sulfonyl groups would be promising for further development as an anticancer agent.

Silymarin is a mixture extracted from the small fruits and seeds of *Silybum marianum*, a medicinal plant in the chrysanthemum family of the genus *Silybum*.[1] Silibinin (**1**, **Figure 1**) exists in these extracts, accounts for approximately 50%-70% of the total composition, and is the most important active ingredient.[2] Structurally, it is an approximately 1:1 mixture of two diastereoisomers named as silybin A (2*R*, 3*R*, 10*R*, 11*R*) and silybin B (2*R*, 3*R*, 10*S*, 11*S*). It has a variety of biological activities, such as

antioxidant, antiviral and anti-inflammatory effects,[3] which is used as a typical liver protectant and dietary supplement in Europe and Asia.[4] The antitumor application of silibinin is particularly useful.[5] *In vitro* and *in vivo* studies have shown that silibinin can significantly inhibit the proliferation of human liver cancer cells and prostate cancer cells, where the antitumor effect may be related to a series of molecular biological processes, including preventing the cancer cell cycle and reducing metastatic invasion.[6] Interestingly, the oxidized form of silibinin, 2,3-dehydrosilibinin (DHS, **2**, **Figure 1**), exhibits more pronounced anticancer activity than silibinin.[7] Despite these encouraging results, moderate efficacy and poor bioavailability have posed challenges to the development of silibinin and DHS as anticancer drugs.[8] At present, there are few developments on the structural modification of silibinin and DHS.

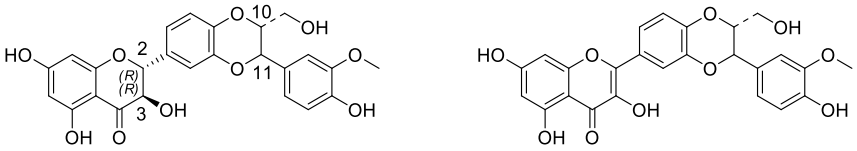


Figure 1. Structures of silibinin (**1**) and 2,3-dehydrosilibinin (**2**)

Alkynyl groups are commonly found in antitumor drug structures, such as Icotinib Hydrochloride (EGFR antagonist) and Acalabrutinib (BTK inhibitors) (**Figure 2**). This structural feature plays multiple roles in drug activity, which include significant potency enhancement (by a complementary interaction with a receptor binding pocket), reactive warheads (for irreversibly inhibiting target proteins), isosteric to a wide range of functions (i.e., carboxamide, vinyl, cyano, ethyl, chloro, iodo, carbonyl, and cyclopropyl groups), and improvement of drug metabolism pharmacokinetic (DMPK) properties.[9] Sulfonyl-containing compounds occupy an important position in the therapeutic drugs, such as Belzutifan (EPAS1 inhibitor) and Ceritinib (INSR/ROS inhibitor) (**Figure 2**). Sulfonyl groups possess unique physical and chemical properties, which can adjust the solubility and acidity of pharmacologically active molecules when they are introduced into structures.[10] The rational introduction of the sulfonyl group that may provide two hydrogen bond acceptors is able to improve the biological activity of the drug by strengthening the hydrogen bond interaction between the molecule and the target.[11] Thus, we attempted to introduce propargyl or sulfonyl groups into silibinin or DHS to enhance their antitumor activities. Eleven novel silibinin derivatives, including three alkynyl-substituted and eight sulfonyl-substituted compounds, were successfully designed and synthesized. All the target compounds were assessed for antiproliferative effects and the corresponding structure-activity relationship (SAR) were discussed.

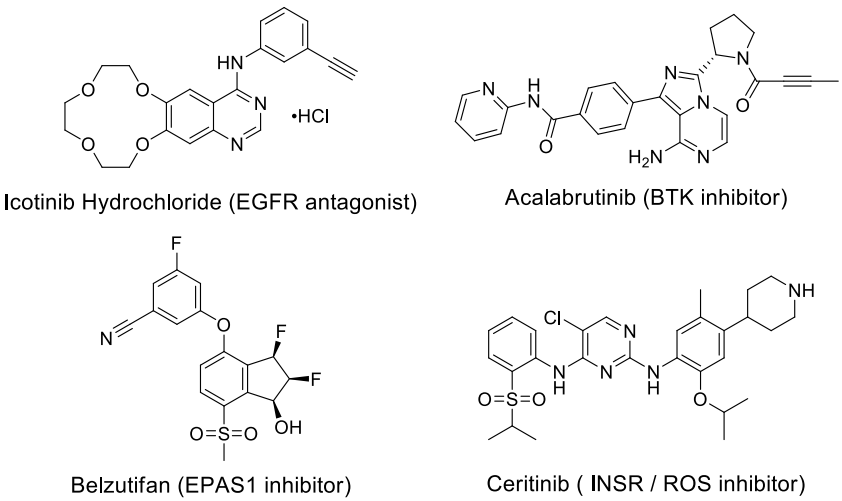
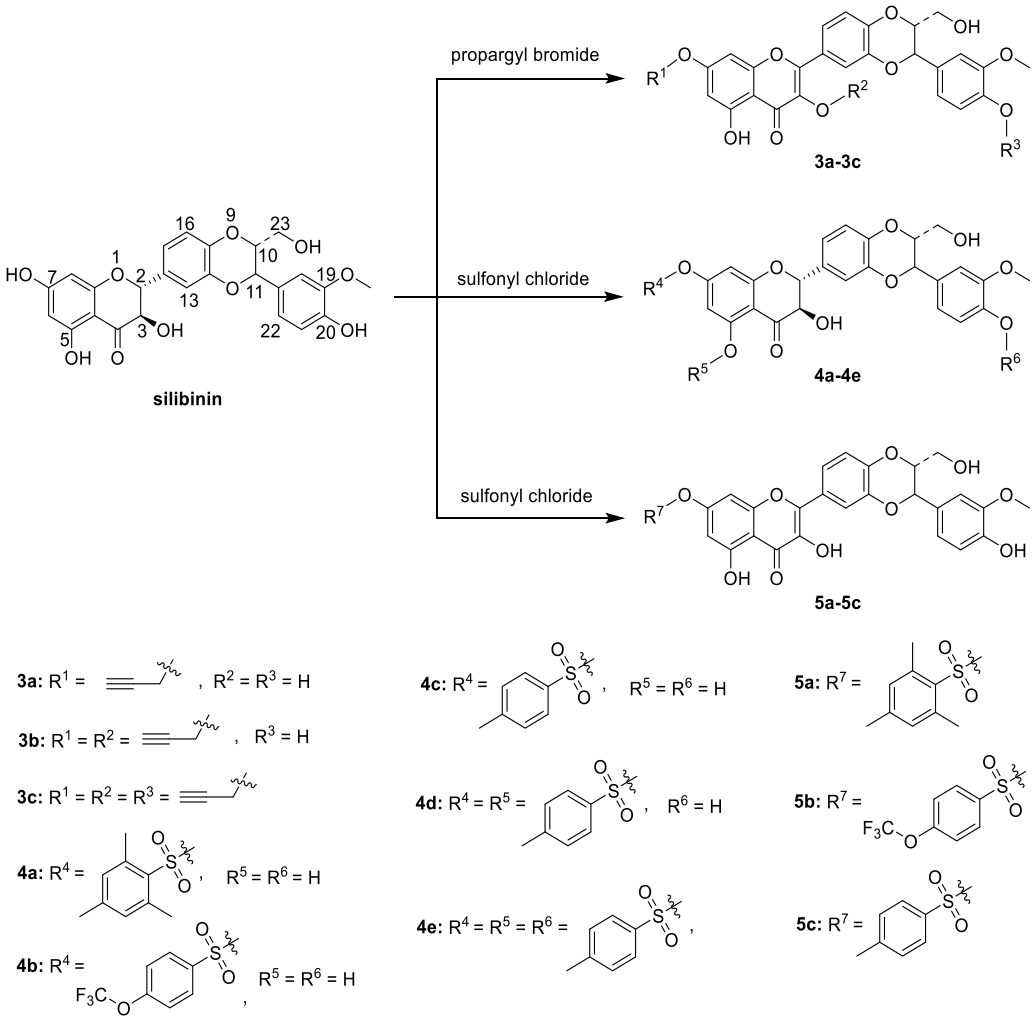


Figure 2. Chemical structures of Icotinib Hydrochloride, Acalabrutinib, Belzutifan and Ceritinib

Scheme 1 presents the protocol for the synthesis of alkynyl or sulfonyl-containing silibinin and 2,3-dehydrosilybin derivatives. Treatment of silibinin with 3-bromoprop-1-yne in DMF at 45 °C under N_2 atmosphere for 6 h gave the desired product **3a** in 17.4% yield. When the reaction base was replaced by sodium hydride, 2,3-dehydro derivatives **3b**-**3c** were produced at 25 °C under N_2 atmosphere for 6 h in yields of 11.4% and 11.8%. It should be noted that the amount of sodium hydride should be strictly controlled. Interestingly, The reaction of silibinin with sulfonyl chloride and sodium hydride at 0-5 °C under N_2 atmosphere for 2 h provided the corresponding silibinin derivatives (**4a**, **4c**) with yields of 22.4% and 23.8%. Similarly, 2,3-dehydrosilybin derivatives **4d** and **4e** were synthesized in yields of 48.9% and 15.6%, respectively, by increasing the amount of sodium hydride and 4-methylbenzenesulfonyl chloride. In order to improve the yield, the reaction conditions were optimized during the synthesis of **4b**. It was gratifying that the yield was significantly improved when *N*,*N*-diisopropylethylamine and 4-dimethylaminopyridine were used as bases, which may be due to the fact that weak bases can reduce the occurrence of side reactions such as polysubstitution. The reaction of silibinin and sulfonyl chloride in the presence of potassium carbonate and DMF as the solvent at 25 °C under N_2 atmosphere for 12 h provided the corresponding 2,3-dehydro derivatives (**5a**-**5c**) in yields of 22.6-26.0%. Silibinin is susceptible to auto-oxidation, which had been reported by many authors in the literatures.[12] Actually, it is found that auto-oxidation of silibinin or its derivatives was observed in all reactions to synthesize the target products. The degree of auto-oxidation is closely related to the reaction temperature, the basic strength of the reaction system and the length of the reaction time. When the reaction temperature or alkalinity decreased, the auto-oxidation reaction proceed slowly, so that the silibinin derivative can be isolated as the main product, such as **4a**-**4e**. Conversely, when the reaction temperature or alkalinity increased, the auto-oxidation reaction can be promoted, so that the 2,3-dehydro derivatives can be easily obtained, such as **5a**-**5c**. Therefore, it is necessary to strictly control the

temperature, basic strength and time of the reaction when synthesizing silibinin derivatives that are not oxidatively dehydrogenated.

The structures of the target compounds were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. In the supplemental material the spectral data of the target compounds were given.



Scheme 1. Synthesis of silibinin derivatives

The antiproliferative activities of the synthesized silibinin derivatives were evaluated by CCK-8 assay on four human cancer cell lines, namely, MCF-7 human breast cancer cells, NCI-H1299 human non-small cell lung cancer cells, Hep G2 human hepatoma cells and HT29 human colon cancer cells. The results are summarized in Table 1. As expected, most silibinin derivatives bearing side chains enhanced growth inhibitory effects compared with silibinin. In particular, compounds **3a** and **5b** performed better cytotoxic activity against tested cancer cell lines with IC_{50} value of 7.41, 11.89, 12.40, 9.23 μM and 17.02, 2.17, 3.37, 18.95 μM against MCF-7, NCI-H1299, HepG2 and HT29, respectively. According to the structure-activity relationship (SAR) study, 7-monosubstituted alkynyl derivative **3a** exhibited significantly stronger inhibition potency than 3,7-disubstituted and 3,7,20-trisubstituted alkynyl

derivatives **3b**, **3c** (all IC_{50} values >20 μM). The same tendency in sulfonyl analogues was also observed, 7-monosubstituted sulfonyl derivative **4c** showed more potent anticancer activity than 3,5-disubstituted and 3,5,20-trisubstituted sulfonyl derivatives **4d**, **4e** (almost all IC_{50} values >20 μM), indicating negative effect of the multiple substituents on the activity of these compounds. 2,4,6-Trimethylbenzenesulfonyl derivative **4a**, 4-methylbenzenesulfonyl derivative **4c** and 4-(trifluoromethoxy)benzenesulfonyl derivative **4b** showed a trend toward increasing cytotoxic activity against NCI-H1299, HepG2 and HT29 cell lines, which suggest that compounds containing electron-withdrawing groups on the benzene ring can effectively improve antitumor cell activity compared with compounds containing electron-donating groups. 2,3-Dehydrosilybin derivatives bearing mono substituted sulfonyl groups **5a**, **5b** and **5c** generally exhibited lower IC_{50} values comparing to the corresponding silibinin derivatives bearing the same sulfonyl groups **4a**, **4b** and **4c**, which indicate that oxidative dehydrogenation of silibinin analogues has positive influences on the proliferation inhibitory activity.

Table 1. Antiproliferative activity of silibinin, DHS and target compounds on four human cancer cell lines

Compd.	MCF-7		NCI-H1299		HepG2		HT29	
	IC_{50}	$GI_{20\ \mu M}$	IC_{50}	$GI_{20\ \mu M}$	IC_{50}	$GI_{20\ \mu M}$	IC_{50}	$GI_{20\ \mu M}$
1	>20	7.29	>20	19.63	>20	17.26	>20	23.88
2	14.88±0.87	58.03	>20	49.23	>20	42.89	>20	26.67
3a	7.41±0.58	93.06	11.89±0.50	71.52	12.40±0.24	84.70	9.23±0.12	86.24
3b	>20	25.89	>20	13.46	>20	7.10	>20	5.13
3c	>20	4.66	>20	10.49	>20	8.62	>20	7.07
4a	>20	42.79	10.53±0.22	73.50	16.67±0.44	71.08	>20	36.99
4b	>20	49.94	5.24±0.33	71.09	6.60±0.35	88.93	8.95±0.30	98.85
4c	15.98±0.78	53.12	7.05±0.35	77.66	14.13±0.44	80.02	14.42±0.26	96.67
4d	>20	32.77	3.28±0.43	72.86	9.82±0.87	73.20	>20	47.09
4e	>20	3.21	>20	8.37	>20	13.08	>20	7.57
5a	>20	46.90	2.97±0.17	75.77	4.47±0.20	83.91	6.00±0.04	97.57
5b	17.02±1.35	57.18	2.17±0.55	75.52	3.37±0.24	93.95	18.95±1.60	62.65
5c	>20	47.95	2.06±0.18	71.03	3.82±0.25	87.20	3.26±0.33	97.51

IC_{50} values expressed in μM as the mean values of triplicate wells from three experiments and reported as the mean ± standard error.

$GI_{20\ \mu M}$ (%) = growth inhibition rate at a 20 μM concentration.

In conclusion, eleven novel silibinin derivatives bearing alkynyl or sulfonyl groups were successfully designed and synthesized, and most of them enhanced antiproliferative activity in the tested cancer cell lines compared with silibinin or DHS. Interestingly, monosubstituted derivatives exhibited more potent anticancer activity than multiple substituted derivatives. The proliferation inhibitory activity can be improved by oxidative dehydrogenation of silibinin derivatives. We investigated and summarized SAR of silibinin or DHS derivatives containing novel substituents, which may be helpful in finding more efficient and selective anticancer drugs.

EXPERIMENTAL

In this article the reagents and solvents used are all commercially available chemical or analytical grades and used without further purification. Reactions were monitored by TLC using UV light. ^{1}H NMR spectra were recorded on a Bruker DRX 400 NMR instrument. ^{13}C NMR spectra were recorded on a Bruker DRX 126 NMR instrument. Chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane or the solvent signal. Mass spectra were recorded using a Agilent mass spectrometer in the positive electrospray ionization (ESI) mode. Melting points were determined with an electrothermal melting point apparatus made in Shanghai Shenguang Instrument Co., Ltd.

7-Propargyloxy-2,3-dehydrosilibinin (3a). Potassium carbonate (17.3 mg, 0.125 mmol) and 3-bromoprop-1-yne (30.0 mg, 0.25 mmol) were added to a solution of silibinin (120.6 mg, 0.25 mmol) in DMF (1.8 mL). The mixture was stirred at 45 °C under N_2 atmosphere for 6 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (10 mL) twice. The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the crude product (167.6 mg). The residue was purified by prep-TLC (CH_2Cl_2:MeOH = 15/1) to afford the title compound **3a** as a yellow solid, yield 17.4%, mp 224-227 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.42 (s, 1H), 9.76 (s, 1H), 9.18 (s, 1H), 7.82 (s, 1H), 7.82-7.80 (dd, 1H), 7.16-7.14 (d, J = 8.6 Hz, 1H), 7.06-7.05 (d, J = 1.8 Hz, 1H), 6.91-6.88 (dd, J = 8.2, 1.8 Hz, 1H), 6.85-6.86 (d, J = 1.8 Hz, 1H), 6.83-6.81 (d, J = 8.0 Hz, 1H), 6.42-6.41 (d, J = 2.1 Hz, 1H), 5.01 (br, 1H), 4.99-4.97 (d, J = 7.9 Hz, 1H), 4.93-4.92 (d, J = 2.1 Hz, 2H), 4.31-4.27 (m, 1H), 3.80 (s, 3H), 3.69-3.67 (t, J = 2.3 Hz, 1H), 3.60-3.56 (m, 1H), 3.39-3.38 (m, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz) : δ 176.68, 163.28, 160.84, 156.35, 148.11, 147.54, 146.77, 145.68, 143.93, 137.22, 127.67, 124.09, 121.87, 121.04, 117.37, 116.72, 115.78, 112.13, 104.93, 98.44, 93.59, 79.49, 79.02, 78.91, 76.37, 60.55, 56.67, 56.16. HRMS (ESI) calcd for $C_{28}H_{23}O_{10}$ $[M+H]^+$ 519.1286; found 519.1293.

3,7-Bis(propargyloxy)-2,3-dehydrosilibinin (3b). Sodium hydride (12.0 mg, 0.30 mmol, 60% dispersion in mineral oil) and 3-bromoprop-1-yne (30.0 mg, 0.25 mmol) were added to a solution of silibinin (120.6 mg, 0.25 mmol) in DMF (1.8 mL) at 0-5 °C. The mixture was stirred at 25 °C under N_2

atmosphere for 6 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (10 mL) twice. The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the crude product (170.8 mg). The residue was purified by prep-TLC (CH_2Cl_2:MeOH = 18/1) to afford the title compound **3b** as a light yellow solid, yield 11.4%, mp 132-134 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.46 (s, 1H), 9.21 (s, 1H), 7.78-7.75 (dd, J = 8.4 Hz, 1.36, 1H), 7.75 (s, 1H), 7.17-7.15 (d, J = 8.4 Hz, 1H), 7.07-7.06 (d, J = 1.0 Hz, 1H), 6.92-6.89 (m, 2H), 6.84-6.82 (d, J = 7.9 Hz, 1H), 6.47-6.46 (d, J = 2.2 Hz, 1H), 5.03 (br, 1H), 5.00-4.98 (d, J = 7.9 Hz, 1H), 4.97-4.96 (d, J = 2.4 Hz, 2H), 4.95-4.94 (d, J = 2.3 Hz, 2H), 4.36-4.33 (m, 1H), 3.79 (s, 3H), 3.70-3.68 (t, J = 2.2 Hz, 1H), 3.61-3.57 (m, 1H), 3.56-3.55 (t, J = 2.4 Hz, 1H), 3.40-3.39 (m, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 178.39, 163.58, 161.27, 156.54, 156.40, 148.12, 147.60, 146.74, 143.96, 136.16, 127.55, 122.96, 122.92, 121.12, 117.61, 117.39, 115.82, 112.25, 105.81, 99.02, 94.00, 80.08, 79.54, 79.07, 79.00, 78.80, 76.37, 60.51, 59.42, 56.77, 56.18. HRMS (ESI) calcd for $C_{31}H_{25}O_{10}$ $[M+H]^+$ 557.1442; found 557.1447.

3,7,20-Tri(propargyloxy)-2,3-dehydrosilibinin (3c). Sodium hydride (22.0 mg, 0.55 mmol, 60% dispersion in mineral oil) and 3-bromoprop-1-yne (60.0 mg, 0.50 mmol) were added to a solution of silibinin (120.6 mg, 0.25 mmol) in DMF (1.8 mL) at 0-5 °C. The mixture was stirred at 25 °C under N_2 atmosphere for 6 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (10 mL) twice. The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the crude product (151.4 mg). The residue was purified by prep-TLC (CH_2Cl_2:MeOH = 20/1) to afford the title compound **3c** as a light-yellow solid, yield 11.8%, mp 124-126 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.46 (s, 1H), 7.79-7.77 (dd, J = 7.7, 2.2 Hz, 1H), 7.77 (s, 1H), 7.19-7.16 (d, J = 9.2 Hz, 1H), 7.15-7.14 (d, J = 1.7 Hz, 1H), 7.10-7.08 (d, J = 8.4 Hz, 1H), 7.06-7.03 (dd, J = 8.2, 1.6 Hz, 1H), 6.91-6.90 (d, J = 2.2 Hz, 1H), 6.47-6.46 (d, J = 1.0 Hz, 1H), 5.07-5.05 (d, J = 7.8 Hz, 2H), 4.97-4.96 (d, J = 2.4 Hz, 2H), 4.95-4.94 (d, J = 2.3 Hz, 2H), 4.82-4.83 (d, J = 2.3 Hz, 2H), 4.41-4.37 (m, 1H), 3.80 (s, 3H), 3.70-3.68 (t, J = 2.3 Hz, 1H), 3.62-3.61 (m, 1H), 3.61-3.59 (t, J = 2.4 Hz, 1H), 3.56-3.55 (t, J = 2.4 Hz, 1H), 3.43-3.39 (m, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 178.39, 163.58, 161.28, 156.54, 156.35, 149.67, 147.33, 146.68, 143.87, 143.84, 136.18, 130.22, 123.02, 122.98, 120.58, 117.62, 117.43, 114.30, 112.08, 105.81, 99.02, 94.00, 80.08, 79.71, 79.53, 79.00, 78.95, 78.92, 78.84, 76.16, 60.46, 59.43, 56.49, 56.12. HRMS (ESI) calcd for $C_{34}H_{27}O_{10}$ $[M+H]^+$ 595.1599; found 595.1605.

7-(2,4,6-Trimethylbenzenesulfonic acid)-silibinin ester (4a). Sodium hydride (22.0 mg, 0.55 mmol, 60% dispersion in mineral oil) and 2,4,6-trimethylbenzenesulfonyl chloride (109.4 mg, 0.5 mmol) were added to a solution of silibinin (120.6 mg, 0.25 mmol) in DMF (1.8 mL) at 0-5 °C. The mixture was stirred at 0-5 °C under N_2 atmosphere for 2 h. After completion of the reaction (as monitored by TLC),

the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (10 mL) twice. The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the crude product (200.1 mg). The residue was purified by prep-TLC (CH_2Cl_2:MeOH = 20/1) to afford the title compound **4a** as a white solid, yield 22.4%, mp 179-182 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.62 (s, 1H), 9.17 (s, 1H), 7.19 (s, 2H), 7.08 (s, 1H), 7.02 (s, 1H), 6.99-6.96 (m, 2H), 6.88-6.86 (d, J = 9.6 Hz, 1H), 6.82-6.80 (d, J = 8.0 Hz, 1H), 6.22-6.20 (d, J = 2.2 Hz, 1H), 6.18-6.17 (d, J = 2.1 Hz, 1H), 5.99-5.98 (br, 1H), 5.25-5.22 (d, J = 11.6 Hz, 1H), 4.97 (br, 1H), 4.92-4.90 (d, J = 7.9 Hz, 1H), 4.79-4.76 (m, 1H), 4.20-4.16 (m, 1H), 3.78 (s, 3H), 3.55-3.53 (m, 1H), 3.36-3.32 (m, 1H), 2.54 (s, 6H), 2.31 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 199.66, 162.43, 162.30, 155.94, 148.07, 147.46, 145.18, 144.30, 143.77, 140.10, 132.52, 130.18, 129.85, 129.80, 127.88, 121.95, 121.78, 120.98, 117.12, 116.88, 115.73, 112.06, 106.05, 102.27, 101.32, 83.23, 78.58, 76.32, 72.09, 60.60, 56.10, 22.56, 21.08. HRMS (ESI) calcd for $C_{34}H_{33}O_{12}S$ $[M+H]^+$ 665.1687; found 665.1691.

7-(4-Trifluoromethoxybenzenesulfonic acid)-silibinin ester(4b). *N*,*N*-Diisopropylethylamine (129.2 mg, 1.00 mmol), 4-dimethylaminopyridine (12.2 mg, 0.10 mmol) and 4-(trifluoromethoxy)benzenesulfonyl chloride (136.8 mg, 0.525 mmol) were added to a solution of silibinin (241.2 mg, 0.50 mmol) in THF (3.6 mL). The mixture was stirred at 0-5 °C under N_2 atmosphere for 2 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (20 mL) twice. The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the crude product (398.8 mg). The residue was purified by prep-TLC (CH_2Cl_2:MeOH = 22/1) to afford the title compound **4b** as a white solid, yield 55.1%, mp 141-144 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.65 (s, 1H), 9.19 (s, 1H). 8.13-8.11 (d, J = 8.7 Hz, 2H), 7.70-7.68 (d, J = 8.8 Hz, 2H), 7.09 (s, 1H), 7.02 (s, 1H), 6.99-6.97 (m, 2H), 6.88-6.86 (dd, J = 8.2, 1.4 Hz, 1H), 6.82-6.80 (d, J = 8.1 Hz, 1H), 6.32-6.31 (d, J = 2.2 Hz, 1H), 6.26-6.25 (d, J = 0.7 Hz, 1H), 6.01 (br, 1H), 5.25-5.22 (d, J = 11.8 Hz, 1H), 4.99 (br, 1H), 4.93-4.91 (d, J = 7.9 Hz, 1H), 4.81-4.77 (d, J = 4.8 Hz, 1H), 4.21-4.16 (m, 1H), 3.78 (s, 3H), 3.57-3.54 (m, 1H), 3.36-3.33 (m, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 199.79, 162.47, 162.30, 155.55, 153.18, 148.06, 147.44, 144.31, 144.29, 143.76, 133.25, 131.66, 129.87, 127.88, 122.33, 121.91, 120.97, 117.14, 116.87, 115.72, 112.04, 106.36, 103.03, 102.03, 83.23, 78.56, 76.31, 72.14, 60.60, 56.08. HRMS (ESI) calcd for $C_{32}H_{26}F_3O_{13}S$ $[M+H]^+$ 707.1041; found 707.1046.

7-(4-Methylbenzenesulfonic acid)-silibinin ester (4c). Sodium hydride (12.0 mg, 0.30 mmol, 60% dispersion in mineral oil) and 4-methylbenzenesulfonyl chloride (47.7 mg, 0.25 mmol) were added to a solution of silibinin (120.6 mg, 0.25 mmol) in DMF (1.8 mL) at 0-5 °C. The mixture was stirred at 0-5 °C under N_2 atmosphere for 2 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (10 mL) twice. The combined organic

layer was washed with brine (10 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the crude product (173.5 mg). The residue was purified by prep-TLC (CH_2Cl_2:MeOH = 12/1) to afford the title compound **4c** as a white solid, yield 23.8%, mp 144-146 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.63 (s, 1H), 9.17 (s, 1H), 7.85-7.83 (d, J = 8.1 Hz, 2H), 7.52-7.50 (d, J = 8.0 Hz, 2H), 7.09 (s, 1H), 7.02 (s, 1H), 7.00-6.97 (m, 2H), 6.89-6.86 (dd, J = 8.2, 1.4 Hz, 1H), 6.82-6.80 (d, J = 8.0 Hz, 1H), 6.27-6.25 (d, J = 2.2 Hz, 1H), 6.23-6.22 (d, J = 1.7 Hz, 1H), 5.99-5.97 (d, J = 6.4 Hz, 1H), 5.25-5.22 (d, J = 11.7 Hz, 1H), 4.99 (br, 1H), 4.93-4.91 (d, J = 7.9 Hz, 1H), 4.79-4.77 (m, 1H), 4.20-4.17 (m, 1H), 3.79 (s, 3H), 3.56-3.53 (m, 1H), 3.34-3.33 (m, 1H), 2.43 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 179.55, 162.40, 162.27, 155.95, 148.09, 147.47, 146.72, 144.31, 143.78, 131.79, 130.90, 129.88, 128.67, 127.90, 121.94, 120.98, 117.15, 116.88, 115.76, 112.06, 106.17, 102.89, 101.79, 83.25, 78.58, 76.32, 72.12, 60.62, 56.13, 21.67. HRMS (ESI) calcd for $C_{32}H_{29}O_{12}S$ $[M+H]^+$ 637.1374; found 637.1380.

5,7-Bis(4-methylbenzenesulfonic acid)-silibinin ester (4d) and 5,7,20-tris(4-methylbenzenesulfonic acid)-silibinin ester (4e). Sodium hydride (22.0 mg, 0.55 mmol, 60% dispersion in mineral oil) and 4-methylbenzenesulfonyl chloride (95.4 mg, 0.50 mmol) were added to a solution of silibinin (120.6 mg, 0.25 mmol) in DMF (1.8 mL) at 0-5 °C. The mixture was stirred at 0-5 °C under N_2 atmosphere for 2 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (10 mL) twice. The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the crude product (272.4 mg). The residue was purified by prep-TLC (CH_2Cl_2:MeOH = 15/1) to afford the title compound **4d** and compound **4e**, both as white solids. Compound **4d**: yield 48.9%, mp 133-135 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.17 (s, 1H), 7.80-7.78 (d, J = 8.0 Hz, 2H), 7.73-7.71 (d, J = 7.8 Hz, 2H), 7.53-7.51 (d, J = 8.7 Hz, 2H), 7.51-7.48 (d, J = 9.3 Hz, 2H), 7.06 (s, 1H), 7.02 (s, 1H), 6.97 (br, 2H), 6.88-6.86 (dd, J = 8.2, 1.6 Hz, 1H), 6.84-6.83 (dd, J = 4.0, 2.3 Hz, 1H), 6.82-6.80 (d, J = 8.1 Hz, 1H), 6.41-6.40 (d, J = 2.2 Hz, 1H), 5.82-5.80 (d, J = 5.2 Hz, 1H), 5.19-5.16 (d, J = 11.6 Hz, 1H), 4.97 (br, 1H), 4.92-4.90 (d, J = 7.88 Hz, 1H), 4.53-4.47 (m, 1H), 4.20-4.16 (m, 1H), 3.78 (s, 3H), 3.56-3.53 (m, 1H), 3.32-3.36 (m, 1H), 2.45 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 191.08, 162.63, 153.19, 148.14, 148.09, 147.48, 147.04, 146.64, 144.33, 143.77, 131.88, 131.23, 130.98, 130.61, 129.56, 128.75, 128.69, 127.89, 121.83, 120.99, 117.14, 116.88, 115.75, 112.94, 112.07, 110.79, 109.95, 83.32, 78.58, 76.31, 72.83, 60.61, 56.13, 21.69. HRMS (ESI) calcd for $C_{39}H_{38}NO_{14}S_2$ $[M+NH_4]^+$ 808.1728; found 808.1741. Compound **4e**: yield 15.6%, mp 148-150 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.80-7.78 (d, J = 7.8 Hz, 2H), 7.73-7.71 (d, J = 7.8 Hz, 4H), 7.53-7.51 (d, J = 8.6 Hz, 3H), 7.48-7.46 (m, 3H), 7.17 (s, 1H), 7.17-7.14 (d, J = 8.6 Hz, 1H), 7.09 (s, 1H), 7.07-7.05 (d, J = 8.4 Hz, 1H), 6.98 (br, 2H), 6.84-6.83 (dd, J = 3.9, 2.2 Hz, 1H), 6.41-6.40 (d, J = 2.2 Hz, 1H), 5.82-5.81 (d, J = 4.0 Hz, 1H), 5.20-5.17 (d, J = 11.6 Hz, 1H), 5.05 (br, 1H), 5.05-5.03 (d, J = 7.6 Hz, 1H), 4.53-4.48 (m, 1H), 4.21-4.17 (m, 1H), 3.57-3.54 (m, 1H), 3.53 (s, 3H),

3.34-3.29 (m, 1H), 2.46 (s, 3H), 2.43 (s, 6H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 191.05, 162.61, 153.21, 151.84, 148.16, 147.03, 146.63, 146.05, 144.20, 143.36, 138.14, 137.52, 132.64, 131.91, 131.26, 130.97, 130.60, 130.28, 129.80, 129.76, 128.74, 128.68, 123.91, 122.07, 121.89, 120.43, 117.15, 116.96, 113.13, 112.94, 110.77, 109.99, 83.27, 78.27, 75.81, 72.87, 60.36, 56.17, 21.68, 21.63. HRMS (ESI) calcd for $C_{46}H_{41}O_{16}S_3$ $[M+H]^+$ 945.1551; found 945.1552.

7-(2,4,6-Trimethylbenzenesulfonic acid)-2,3-dehydrosilibinin ester (5a). Potassium carbonate (69.1 mg, 0.5 mmol) and 2,4,6-trimethylbenzenesulfonyl chloride (109.4 mg, 0.5 mmol) were added to a solution of silibinin (241.2 mg, 0.50 mmol) in DMF (3.6 mL). The mixture was stirred at 25 °C under N_2 atmosphere for 12 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (20 mL) twice. The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the crude product (142.0 mg). The residue was purified by prep-TLC (CH_2Cl_2:MeOH = 20/1) to afford the title compound **5a** as a yellow solid, yield 22.8%, mp 123-126 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.52 (s, 1H), 10.01 (s, 1H), 9.19 (s, 1H), 7.84 (s, 1H) , 7.84-7.82 (dd, 1H), 7.19 (s, 2H), 7.15-7.13 (d, J = 9.5 Hz, 1H), 7.05 (s, 2H), 6.90-6.88 (dd, J = 8.2, 1.7 Hz, 1H), 6.83-6.81 (d, J = 8.1 Hz, 1H), 6.36 (s, 1H), 5.03-5.00 (t, J = 5.4 Hz, 1H), 4.98-4.96 (d, J = 8.0 Hz, 1H), 4.32-4.28 (m, 1H), 3.79 (s, 3H), 3.61-3.56 (m, 1H), 3.39-3.36 (m, 1H), 2.55 (s, 6H), 2.31 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 176.79, 160.65, 155.14, 153.36, 148.10, 147.87, 147.52, 146.00, 145.18, 143.92, 140.31, 137.85, 132.48, 129.83, 127.58, 123.66, 122.22, 121.05, 117.36, 116.98, 115.75, 112.05, 108.62, 103.51, 101.92, 79.04, 76.35, 60.51, 56.11, 22.64, 21.07. HRMS (ESI) calcd for $C_{34}H_{31}O_{12}S$ $[M+H]^+$ 663.1531; found 663.1537.

7-(4-Trifluoromethoxybenzenesulfonic acid)-2,3-dehydrosilibinin ester (5b). Potassium carbonate (69.1 mg, 0.5 mmol) and 4-(trifluoromethoxy)benzenesulfonyl chloride (136.8 mg, 0.525 mmol) were added to a solution of silibinin (241.2 mg, 0.50 mmol) in DMF (3.6 mL). The mixture was stirred at 25 °C under N_2 atmosphere for 12 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (20 mL) twice. The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the crude product (150.0 mg). The residue was purified by prep-TLC (CH_2Cl_2:MeOH = 20/1) to afford the title compound **5b** as a yellow solid, yield 26.0%, mp 119-122 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.56 (s, 1H), 10.05 (s, 1H), 9.21 (s, 1H), 8.13-8.05 (d, J = 8.9 Hz, 2H), 7.83-7.81 (m, 2H), 7.69-7.68 (d, J = 7.8 Hz, 2H), 7.15-7.12 (d, J = 9.0 Hz, 1H), 7.09-7.08 (d, J = 2.2 Hz, 1H), 7.05-7.04 (d, J = 1.9 Hz, 1H), 6.91-6.88 (dd, J = 8.2, 1.9 Hz, 1H), 6.83-6.81 (d, J = 8.1 Hz, 1H), 6.47-6.46 (d, J = 2.1 Hz, 1H), 5.05-5.02 (t, J = 5.3 Hz, 1H), 4.98-4.96 (d, J = 7.96 Hz, 1H), 4.32-4.28 (m, 1H), 3.80 (s, 3H), 3.60-3.56 (m, 1H), 3.40-3.39 (m, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 176.84, 160.80, 155.15, 153.18, 148.09, 147.96, 147.52, 146.04, 143.94, 137.90, 133.01, 131.78, 127.58, 123.64, 122.34, 122.21, 121.49,

121.04, 118.90, 117.37, 116.98, 115.74, 112.05, 108.90, 104.12, 102.25, 79.04, 76.34, 60.51, 56.10. HRMS (ESI) calcd for $C_{32}H_{24}F_3O_{13}S$ $[M+H]^+$ 705.0884; found 705.0888.

7-(4-Methylbenzenesulfonic acid)-2,3-dehydrosilibinin ester (5c). Potassium carbonate (69.1 mg, 0.5 mmol) and 2,4,6-trimethylbenzenesulfonyl chloride (95.3 mg, 0.50 mmol) were added to a solution of silibinin (241.2 mg, 0.50 mmol) in DMF (3.6 mL). The mixture was stirred at 25 °C under N_2 atmosphere for 12 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (20 mL) twice. The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford the crude product (166.0 mg). The residue was purified by prep-TLC (CH_2Cl_2:MeOH = 20/1) to give the title compound **5c** as a yellow solid, yield 22.6%, mp 234-237 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.53 (s, 1H), 10.01 (s, 1H), 9.19 (s, 1H), 7.85-7.82 (m, 4H), 7.51-7.49 (d, J = 8.0 Hz, 2H), 7.15-7.13 (d, J = 8.4 Hz, 1H), 7.09-7.08 (d, J = 1.9 Hz, 1H), 7.05-7.04 (d, J = 1.7 Hz, 1H), 6.91-6.88 (dd, J = 8.2, 1.8 Hz, 1H), 6.83-6.81 (d, J = 8.0 Hz, 1H), 6.41-6.40 (d, J = 2.1 Hz, 1H), 5.03-5.01 (t, J = 5.4 Hz, 1H), 4.98-4.96 (d, J = 7.9 Hz, 1H), 4.32-4.28 (m, 1H), 3.79 (s, 3H), 3.60-3.55 (m, 1H), 3.40-3.35 (m, 1H), 2.42 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ 176.84, 160.67, 155.16, 153.52, 148.10, 147.94, 147.52, 146.74, 146.02, 143.93, 137.85, 131.49, 130.88, 128.80, 127.59, 123.67, 122.24, 121.05, 117.39, 117.02, 115.75, 112.07, 108.72, 103.97, 102.11, 79.04, 76.35, 60.51, 56.12, 21.67. HRMS (ESI) calcd for $C_{32}H_{27}O_{12}S$ $[M+H]^+$ 635.1218; found 635.1220.

Antiproliferative assay.

Inhibition of cell growth inhibition was determined using a CCK-8 assay. Briefly, cells were planted in 96-well plates (5000 cells/well) and cultured at 37 °C for 24 h. The cells were treated with silibinin derivatives at different concentrations and incubated for 48 h. A volume of 10 μl of CCK-8 solution was added to each well. The cells were then incubated at 37 °C for 2 h. The absorbance was measured at a wavelength of 450 nm using a microplate reader.

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