MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antitumor activity of novel silibinin and 2,3-dehydrosilybin derivatives with carbamate groups

Qiuchan Wu[1] · Jiang Zeng[2] · Jinfu Dong [iD][3]

Abstract
A novel series of silibinin and 2,3-dehydrosilybin derivatives bearing carbamate groups were designed, synthesized and their in vitro anticancer activities were screened against human cancer cell lines including MCF-7, NCI-H1299, HepG2 and HT29 by CCK-8 assay. The results showed that most of the compounds significantly suppressed the proliferation of tested cancer cells. Among them, compounds **2h**, **3h** and **3f** demonstrated markedly higher antiproliferative activity on MCF-7 cells with IC_{50} values of 2.08, 5.54 and 6.84 μM, respectively. Compounds **3e**, **3g** and **2g** displayed better cytotoxic activity against NCI-H1299 cells with IC_{50} values of 8.07, 8.45 and 9.09 μM, respectively. Compounds **3g**, **3c** and **3h** exhibited a promising inhibitory effect against HepG2 cells with IC_{50} values of 8.88, 9.47 and 9.99 μM, respectively. Compounds **3e**, **2e** and **3c** revealed effective biological potency on HT29 cells with IC_{50} values of 6.27, 9.13 and 9.32 μM, respectively. In addition, the outcomes of the docking studies between compounds **2f**, **2h**, **3e**, **3g** and Hsp90 receptor (PDB ID: 4AWO) suggest the possible mechanism of inhibition against MCF-7 cell lines.

Graphical abstract
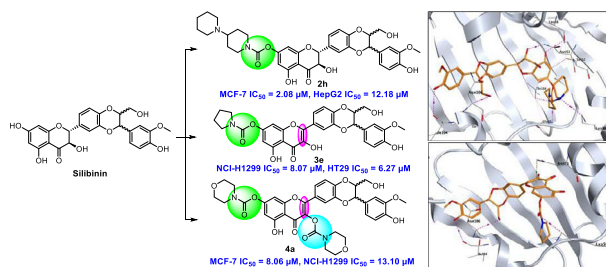
Keywords Silibinin · 2,3-dehydrosilybin · Carbamate · Synthesis · Anticancer · Docking

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Introduction

Cancer is an abhorrent disease with extremely high mortality, caused by abnormal proliferation of human cells [1, 2]. It is estimated that there are approximately19.3 million new cancer cases in 2020 across the world, and
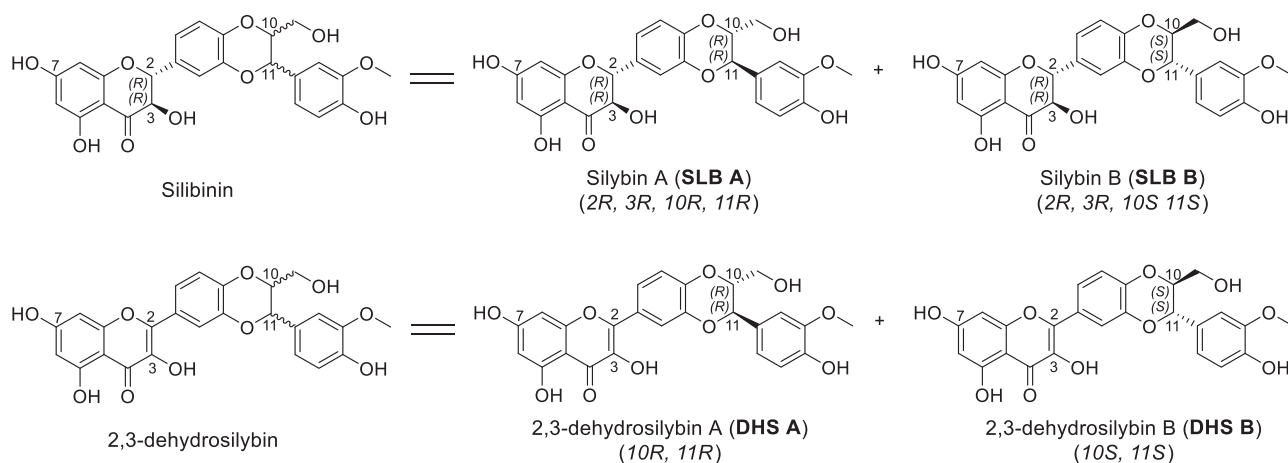
Fig. 1 Chemical structures of silybiin (SLB A, SLB B) and 2,3-dehydrosilybin (DHS A, DHS B)
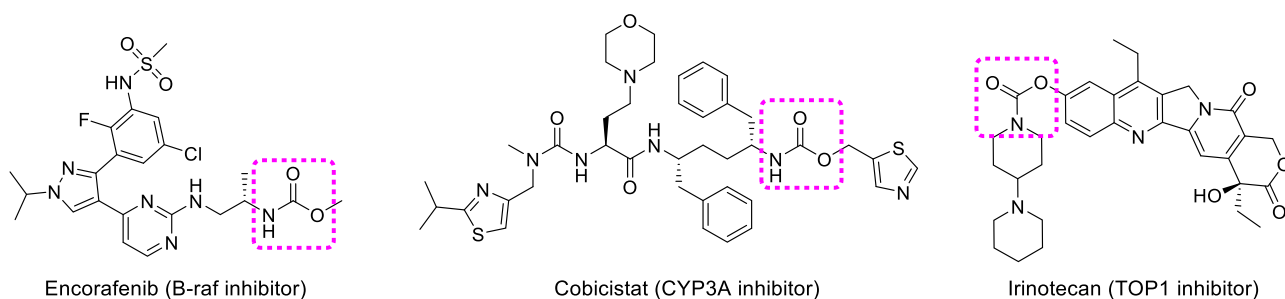
Fig. 2 Chemical structures of encorafenib, cobicistat and irinotecan

this number will surge remarkably to 28.4 million in 2040 [3]. Natural products have been widely investigated and eventually developed into drugs, especially anticancer chemotherapeutics due to their molecular diversity and novelty [4, 5]. Silymarin is an active extract isolated from the seeds of the milk thistle plant, *Silybum marianum*, which contains approximately 65–80% flavonolignans (silybinin, isosilybin, silychristin, silydianin, 2,3-dehydrosilybin) with small amounts of flavonoids and about 20–30% of unidentified polymeric phenolic components [6–8]. Silibinin, the main active ingredient of silymarin, is a roughly 1:1 mixture of two diastereoisomers named as silybin A (SLB A) and silybin B (SLB B), while the 2,3-dehydrosilybin (DHS) consists of a mixture of two enantiomers, namely, DHS A and DHS B [9] (Fig. 1). Silibinin and DHS have been shown to possess anticancerogenic and proapoptotic activity both in vitro and in vivo [10]. Silibinin, as positive control, exhibits anticancer activity with typical IC_{50} value of around 50–200 μM in various cancers [11, 12], while at lower concentrations, DHS induces apoptosis at 30–50 μM when treated alone [13]. Encouragingly, silybin is currently under phase II clinical trials in the US for prostate cancer treatment [14]. Despite these encouraging results, their

use as an antitumor drug is considerably hampered by their small solubility in water which caused by highly hydrophobic characteristic and low acidity [15–19]. To improve the druggability and enhance antiproliferative activity of silibinin and DHS, structural modifications are essential [20, 21].

Organic carbamates are structural motifs of many approved pharmaceutical drugs [22, 23], such as Encorafenib (approved in 2018), Cobicistat (approved in 2013), Irinotecan (approved in 1994), etc (Fig. 2). Structurally, partly owing to the amide-ester hybrid nature, the carbamate functional group displays good chemical and proteolytic stabilities [24]. Furthermore, carbamates have been demonstrated to have the ability to promote inter- and intramolecular interactions with the biological targets. In addition, numerous studies have indicated that structural modifications by introducing carbamate groups greatly improve the water solubility and biological activity of the drugs [25–30]. Based on the above information, a class of silibinin and 2,3-DHS derivatives bearing carbamate groups were designed and synthesized. Their antiproliferative activities were investigated and the structure-activity relationships (SARs) were discussed.
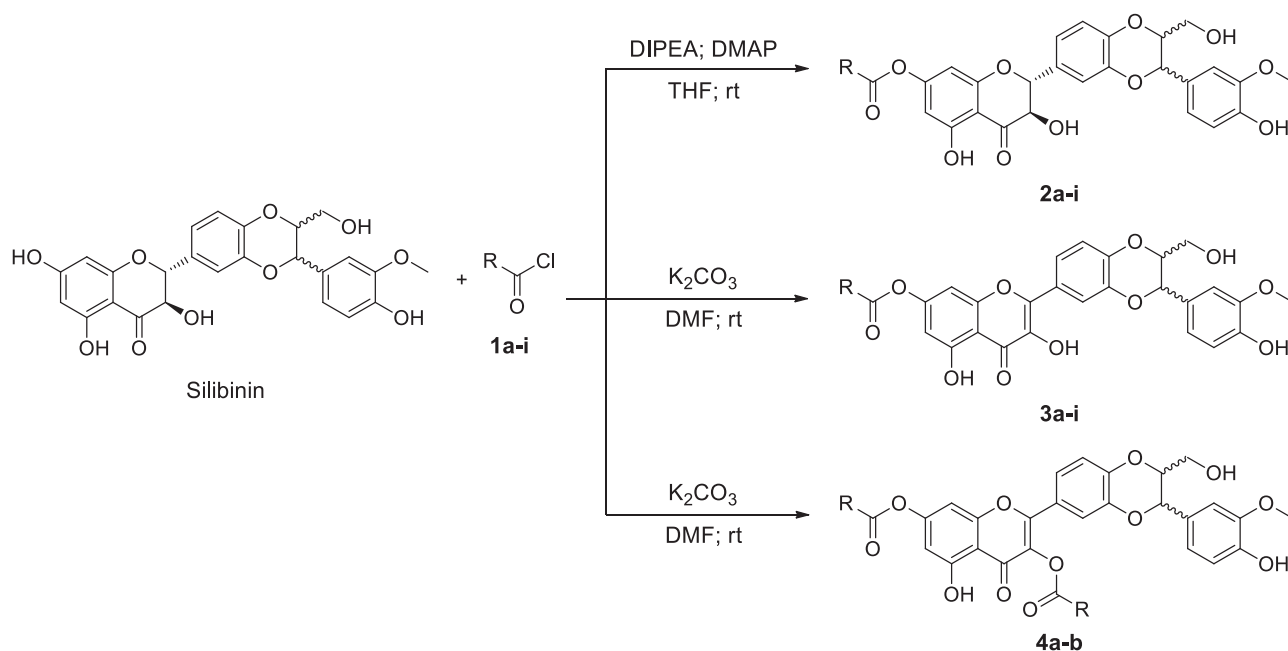
Scheme 1 Synthesis of carbamate-containing silibinin and 2,3-dehydrosilybin derivatives

Results and discussion

Synthesis of silibinin and 2,3-dehydrosilybin derivatives

Scheme 1 presents the protocol for the synthesis of carbamate-containing silibinin and 2,3-DHS derivatives. The reaction of silybin and carbamyl chloride **1a-i** in the presence of N,N-diisopropylethylamine (DIPEA), 4-Dimethylaminopyridine (DMAP) and tetrahydrofuran (THF) as the solvent produced the desired product **2a–i** in yields of 10.8–52.8%. When the reaction base and the solvent were replaced by potassium carbonate (K_2CO_3) and N,N-dimethylformamide (DMF), mono-substituted 2,3-DHS derivatives **3a–i** were produced in yields of 11.2–39.9%. Surprisingly, disubstituted 2,3-DHS derivatives **4a** and **4b** were also synthesized selectively with yields of 11.1 and 27.7%, respectively, by simply increasing the amount of K_2CO_3 and carbamyl chloride **1a-b**. Twenty novel silibinin and 2,3-DHS derivatives bearing carbamate groups were synthesized, and their structures were characterized by ^1H NMR, ^{13}C NMR and mass spectroscopy. As expected, it was found that all target compounds exhibited substantially higher aqueous solubility compared with untreated silibinin and DHS.

In vitro cytotoxicity screening of the synthesized compounds

The in vitro cytotoxicity of the synthesized target compounds (**2a–i**, **3a–i** and **4a–b**) against four human cancer cell lines including MCF-7 (breast carcinoma), NCI-H1299 (lung carcinoma), HepG2 (liver carcinoma) and HT29 (colon carcinoma) was assessed by cell counting kit-8 (CCK-8) assay. The IC_{50} values of the compounds against the tested cell lines are summarized in Table 1. Silibinin and 2,3-DHS were used as control, which exhibited poor anti-proliferative activity against all the four types of cancer cells with almost all IC_{50} values >20 μM. As expected, compared with positive drugs, most of their derivatives bearing carbamate groups exhibited significantly enhanced antitumor activity against the tested tumour cell lines. The majority of the compounds demonstrated antitumor activity with IC_{50} values at low micromolar concentrations. In particular, a series of derivatives exhibited potency in the single-digit micromolar range, such as compounds **2h**, **3h**, **3f**, **3g**, **4b**, **4a** and **2g** showed better cytotoxic activity against MCF-7 cells with IC_{50} values of 2.08, 5.54, 6.84, 7.96, 8.05, 8.06 and 8.24 μM, compounds **3e**, **3g** and **2g** displayed high anti-proliferative activity against NCI-H1299 cells with IC_{50} values of 8.07, 8.45 and 9.09 μM, compounds **3g**, **3c** and **3h** revealed effective biological potency on HepG2 cells with IC_{50} values of 8.88, 9.47 and 9.99 μM, compounds **3e**, **2e**, **3c** and **2f** exhibited a promising inhibitory effect against HT29 cells with IC_{50} values of 6.27, 9.13, 9.32 and 9.86 μM, respectively. Among the twenty screened compounds, seven compounds (**2f**, **2g**, **3b**, **3e**, **3g**, **4a** and **4b**) have exhibited superior potency in anticancer activity on all tested cell lines. Especially compound **2g** (MCF-7 IC_{50} = 8.24 μM, NCI-H1299 IC_{50} = 9.09 μM, HepG2 IC_{50} = 13.96 μM, HT29 IC_{50} = 10.80 μM) and **3g** (MCF-7

Table 1 In vitro antiproliferative activity of silibinin analogues **2a–i**, 2,3-dehydrosilybin derivatives **3a–i** and **4a–b**


Compound	R	IC$_{50}$ (μM)[a]			
		MCF-7	NCI-H1299	HepG2	HT29
Silibinin[b]	—	>20	>20	>20	>20
2,3-dehydrosilybin[b]	—	15.68 ± 0.87	>20	>20	>20
2a		>20	>20	>20	>20
2b		>20	18.45 ± 1.49	>20	>20
2c		>20	>20	>20	14.87 ± 0.70
2d		>20	13.02 ± 0.58	17.47 ± 0.17	10.45 ± 0.56
2e		12.59 ± 0.56	16.83 ± 0.34	>20	9.13 ± 0.54
2f		12.55 ± 0.45	11.21 ± 0.57	13.17 ± 0.24	9.86 ± 0.42
2g		8.24 ± 0.48	9.09 ± 0.24	13.96 ± 0.38	10.80 ± 0.30
2h		2.08 ± 0.26	>20	12.18 ± 0.31	>20
2i		>20	11.84 ± 0.73	>20	>20
3a		>20	>20	>20	>20
3b		14.78 ± 0.32	16.42 ± 1.44	13.30 ± 0.52	12.47 ± 0.58
3c		>20	>20	9.47 ± 0.39	9.32 ± 0.22
3d		>20	11.06 ± 0.45	>20	>20
3e		14.24 ± 0.21	8.07 ± 0.14	11.08 ± 0.06	6.27 ± 0.57
3f		6.84 ± 0.55	11.97 ± 0.18	19.20 ± 0.39	>20
3g		7.96 ± 0.42	8.45 ± 0.62	8.88 ± 0.26	17.23 ± 0.76
3h		5.54 ± 0.37	>20	9.99 ± 0.54	>20
3i		>20	11.98 ± 0.15	15.67 ± 0.49	16.73 ± 0.39
4a		8.06 ± 0.24	13.10 ± 0.18	16.51 ± 0.41	12.44 ± 0.78
4b		8.05 ± 0.56	16.89 ± 1.08	15.69 ± 0.98	10.40 ± 0.95

[a]Values expressed are means ± SEM of three parallel measurements

[b]Reference compound

IC$_{50}$ = 7.96 μM, NCI-H1299 IC$_{50}$ = 8.45 μM, HepG2 IC$_{50}$ = 8.88 μM, HT29 IC$_{50}$ = 17.23 μM) have demonstrated stronger broad-spectrum antitumor activities.

Substituent R plays a crucial role in the biological activity of this group of drugs. According to the structure-activity relationship study, the N,N-dimethylcarbamate
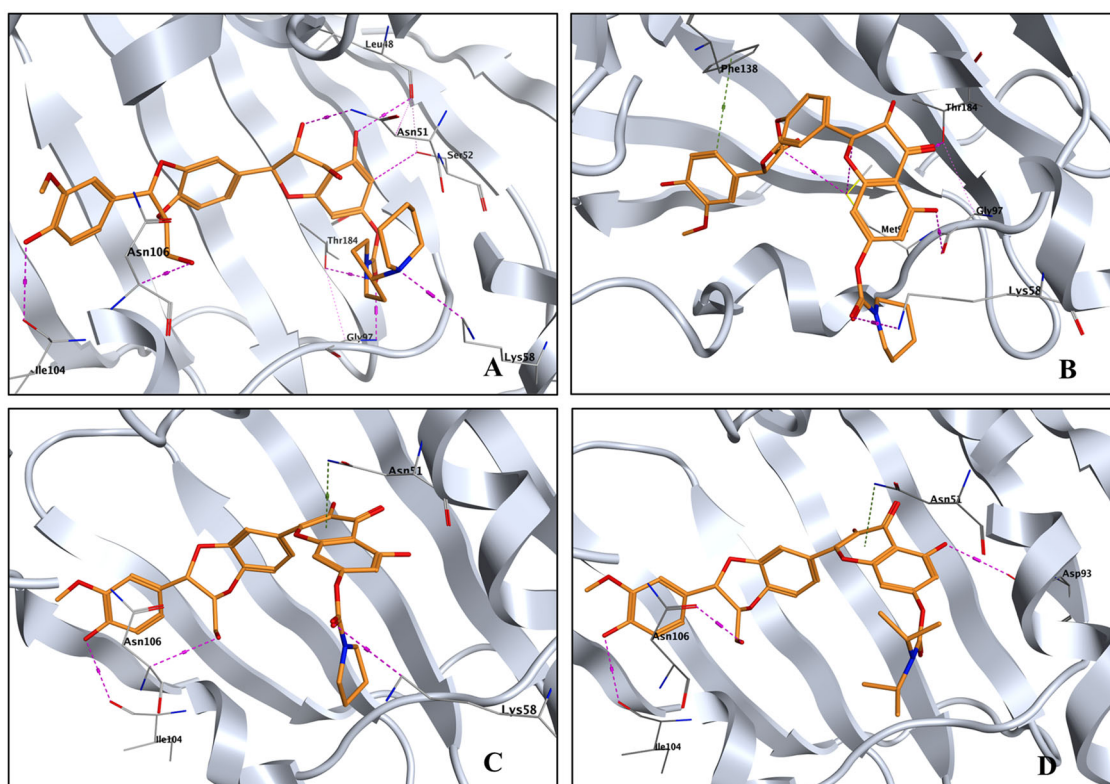
Fig. 3 Docked positions of compound **2h** (A), **2f** (B), **3e** (C) and **3g** (D) inside active site of Hsp90 (PDB ID: 4AWO)

derivative **2b** (18.45 μM), the *N,N*-diethyl carbamate analogue **2d** (13.02 μM), and the *N,N*-diisopropylcarbamate substitute **2g** (9.09 μM) showed a trend toward more potent anticancer activity against NCI-H1299 cells with increasing lipophilicity of the carbamate residues in the molecule. The same tendency in cyclic carbamate derivatives was also observed, with the pyrrolidine-1-carboxylate derivative **3e** (14.24 μM), the piperidine-1-carboxylate analogue **3f** (6.84 μM), and the [1,4′-bipiperidine]-1′-carboxylate substitute **3h** (5.54 μM) showed increasing cytotoxic activity against MCF-7 cells, indicating positive effect of the lipophilic substituents on the activity of these compounds. The derivatives bearing morpholine-4-carboxylate group **2a** and **3a** generally exhibited much higher IC$_{50}$ values (all >20 μM) comparing to compounds bearing the piperidine-1-carboxylate group **2f** and **3f**, which indicated that the additional oxygen atom in the morpholino group interferes greatly with the antiproliferative activities. More interestingly, C3-OH and C7-OH disubstituted derivative of 2,3-DHS **4a** (MCF-7 IC$_{50}$ = 8.06 μM, NCI-H1299 IC$_{50}$ = 13.10 μM, HepG2 IC$_{50}$ = 16.51 μM, HT29 IC$_{50}$ = 12.44 μM) exhibited significantly stronger inhibition potency than C7-OH mono-substituted analogue **3a** (all IC$_{50}$s > 20 μM), which suggested that the double simultaneous substitution strategy is a promising approach in improving anticancer activity. Moreover, it was observed

that silibinin derivatives **2a–2h** had similar activity with the corresponding analogues of 2,3-DHS **3a–3h** against NCI-H1299 cells, which suggested that dehydrogenation of silibinin derivatives at both 2- and 3-positions (CHCH→C=C) had limited the influences on the antiproliferative activities, especially toward target NCI-H1299 cells.

Molecular docking studies

Regarding to the molecular mechanisms of silibinin-induced apoptosis in breast cancer (MCF-7), numerous studies have reported multiple biological targets, such as Hsp90 [31, 32], BCL-2 [33], ERK [34], Akt [34], COX-2 [35], MMP-9 [35, 36], etc. The docking studies of the silibinin and 2,3-DHS analogues (**2f**, **2h**, **3e** and **3g**) were conducted against six protein kinases to investigate the putative mechanism of actions of these novel compounds by MOE. The outcomes of the docking studies showed comparatively higher docking scores against the Hsp90 (4AWO) so that it was chosen for virtual screening study. The results indicated that, **2h** bound strongly to Hsp90 receptor with minimum binding energy −9.88 kcal/mol, while the binding energy of **2f**, **3e** and **3g** are −8.91, −8.39 and −9.11 kcal/mol, respectively.

As illustrated in Fig. 3, docking results revealed that the silibinin analogue **2h** had eight H-bond interactions with the residues Ser52, Leu48, Ile104, Asn51, Asn106, Gly97,

Thr184 and Lys58 at distances of 3.39, 3.05, 3.32, 2.68, 3.24, 2.82, 2.90 and 3.34 Å in the ligand-binding pocket, respectively. Similarly, **2f**-Hsp90 cluster showed six H-bonds with Met98, Gly97, Met98, Thr184, Lys58 and Phe138 amino acids at distances of 4.16 (12CH⋯S bond), 2.87, 3.88 (33CH⋯S bond), 3.21, 3.13 and 4.62 Å, respectively. Four major H-bonds were formed on Ile104, Asn106, Lys58 and Asn51 amino acid residues for compound **3e** with distances of 3.29, 3.25, 3.41 and 3.93 Å, respectively. In addition, **3g** established four H-bonds with Asp93, Asn106, Ile104 and Asn51 amino acids with the corresponding distances being 3.33, 2.82, 3.31 and 3.85 Å, respectively. The docking results are in good agreement with in vitro experimental IC$_{50}$ values against MCF-7 cells shown in Table 1.

Conclusions

Modified on the basis of the silibinin and 2,3-DHS scaffold, 20 novel carbamate derivatives were successfully designed, synthesized, and evaluated against four human cancer cell lines including MCF-7, NCI-H1299, HepG2 and HT29 by employing CCK-8 method. Importantly, compounds **2h**, **3g**, **3c** and **3e** displayed as low as single-digit micromolar activity against different cancer cell lines. Molecular docking between compound **2f**, **2h**, **3e**, **3g** and Hsp90 were in good agreement with in vitro experimental IC$_{50}$ values against MCF-7 cell lines, which disclosed the possible mechanisms of anticancer action. The above findings provided a good starting point for the rational design and development of novel anticancer drugs and Hsp90 inhibitors with silibinin and 2,3-DHS scaffold.

Experimental

All chemicals and reagents were obtained from Chemical Book, Sigma-Aldrich and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing GF-254, and visualization on TLC was achieved by UV light. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 NMR instrument. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on a Qstar mass spectrometer. Melting points were determined with an electro thermal melting point apparatus, and are uncorrected.

General procedure for the synthesis of compounds 2a–i

To a solution of silibinin (241.2 mg, 0.50 mmol, 1.0 eq) in THF (3.6 mL, 15.0 vol) was added DIPEA (129.2 mg, 1.0 mmol, 2.0 eq), 4-DMAP (12.2 mg, 0.10 mmol, 0.2 eq) and carbamyl chloride **1a–i** (0.53 mmol, 1.05 eq). The mixture was stirred at 25 °C under N$_2$ atmosphere for 15 h. After completion of the reaction, the reaction mixture diluted with water (20 mL) and extracted with ethyl acetate (20 mL) twice. The combined organic layer was washed with brine (20 mL), dried over Na$_2$SO$_4$ and filtered. The filtrate was concentrated to give the crude product. The residue was purified by column chromatography (CH$_2$Cl$_2$/MeOH = 15:1) to give the title compound **2a–i**.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl morpholine-4-carboxylate (2a)

White solid (157.2 mg, purity 96.11%, yield 52.8%); mp 148–151 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.69 (s, 1H), 9.16 (s, 1H), 7.12 (d, J = 2.08, 1H), 7.05 (dd, J = 9.96, 1.52, 1H), 7.03 (d, J = 1.52, 1H), 7.00 (d, J = 8.28, 1H), 6.88 (dd, J = 8.23, 1.52, 1H), 6.81 (d, J = 8.08, 1H), 6.40 (d, J = 2.08, 1H), 6.35 (d, J = 2.32, 1H), 5.97 (d, J = 6.28, 1H), 5.24 (d, J = 11.56, 1H), 4.97 (br, 1H), 4.93 (d, J = 7.92, 1H), 4.80–4.74 (m, 1H), 4.20–4.17 (m, 1H), 3.78 (s, 3H), 3.64–3.62 (t, J = 5.04 Hz, 4H), 3.53 (br, 3H), 3.41 (br, 2H), 3.34 (m, 1H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 199.9, 162.3, 162.1, 159.2, 152.0, 148.0, 147.4, 144.2, 143.7, 130.1, 127.8, 121.8, 120.9, 117.1, 116.8, 115.7, 112.0, 104.7, 103.0, 101.8, 83.1, 78.5, 76.3, 72.1, 66.1, 60.6, 56.0, 45.1, 44.3; MS(ESI$^-$) m/z calcd for C$_{30}$H$_{28}$NO$_{12}$ 594.16, found 594.3 (M − H)$^-$.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl dimethylcarbamate (2b)

White solid (126.1 mg, purity 98.58%, yield 45.6%); mp 138–141 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.68 (s, 1H), 9.16 (s, 1H), 7.11 (t, J = 1.96, 1H), 7.04 (d, 1H), 7.02 (s, 1H), 6.99 (d, J = 8.20, 1H), 6.88 (dd, J = 8.08, 1.16, 1H), 6.81 (d, J = 8.08, 1H), 6.36 (d, J = 2.08, 1H), 6.32 (d, J = 2.44, 1H), 5.96 (d, J = 6.40, 1H), 5.23 (d, J = 11.60, 1H), 4.98 (t, J = 5.40, 1H), 4.92 (d, J = 7.92, 1H), 4.79–4.73 (m, 1H), 4.19–4.16 (m, 1H), 3.78 (s, 3H), 3.56–3.51 (m, 1H), 3.37–3.31 (m, 1H), 3.00 (s, 3H), 2.90 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 199.8, 162.3, 162.1, 159.4, 153.0, 148.0, 147.4, 144.2, 143.7, 130.2, 127.8, 121.9, 120.9, 117.1, 116.8, 115.7, 112.0, 104.6, 103.0, 101.8, 83.1, 78.5, 76.3, 72.1, 60.6, 56.0, 36.8, 36.6. MS(ESI$^-$) m/z calcd for C$_{28}$H$_{26}$NO$_{11}$ 552.15, found 552.30 (M − H)$^-$.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl 4-methylpiperazine-1-carboxylate (2c)

White solid (53.0 mg, purity 95.03%, yield 17.4%); mp 137-140 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.68 (s, 1H), 9.16 (s, 1H), 7.11 (d, $J = 2.16$, 1H), 7.04 (d, 1H), 7.02 (s, 1H), 6.99 (d, $J = 8.72$, 1H), 6.88 (dd, $J = 8.23$, 1.52, 1H), 6.81 (d, $J = 8.08$, 1H), 6.37 (d, $J = 2.08$, 1H), 6.33 (d, $J = 2.44$, 1H), 5.96 (d, $J = 6.40$, 1H), 5.23 (d, $J = 11.52$, 1H), 4.98 (t, $J = 5.56$, 1H), 4.92 (d, $J = 7.96$, 1H), 4.79–4.73 (m, 1H), 4.20–4.16 (m, 1H), 3.78 (s, 3H), 3.53 (br, 3H), 3.41 (br, 2H), 3.35 (m, 1H), 2.35 (br, 4H), 2.21 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 199.8, 162.3, 162.1, 159.3, 151.9, 148.0, 147.4, 144.2, 143.7, 130.2, 127.9, 121.7, 120.9, 117.1, 116.8, 115.7, 112.0, 104.7, 102.9, 101.7, 83.1, 78.5, 76.3, 72.5, 60.6, 56.1, 54.5, 54.3, 46.0, 44.6, 44.0. MS(ESI$^+$) m/z calcd for $C_{31}H_{33}N_2O_{11}$ 609.21, found 609.40 (M + H)$^+$.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl diethylcarbamate (2d)

White solid (140.6 mg, purity 97.90%, yield 48.4%); mp 129–132 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.68 (s, 1H), 9.16 (s, 1H), 7.11 (d, $J = 2.24$, 1H), 7.07 (d, 1H), 7.02 (d, $J = 1.36$, 1H), 7.00 (d, $J = 8.88$, 1H), 6.88 (dd, $J = 8.12$, 1.64, 1H), 6.81 (d, $J = 8.08$, 1H), 6.35 (d, $J = 2.08$, 1H), 6.31 (d, $J = 2.32$, 1H), 5.96 (d, $J = 6.32$, 1H), 5.23 (d, $J = 11.60$, 1H), 4.97 (t, $J = 1.00$, 1H), 4.92 (d, $J = 7.92$, 1H), 4.79–4.73 (m, 1H), 4.20–4.16 (m, 1H), 3.78 (s, 3H), 3.52–3.55 (m, 1H), 3.35–3.33 (m, 1H), 3.31–3.26 (q, $J = 6.76$, 4H), 1.17 (t, $J = 6.92$, 3H), 1.12 (t, $J = 6.92$, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 199.8, 162.3, 162.1, 159.5, 152.3, 148.0, 147.4, 144.2, 143.7, 130.2, 127.8, 121.9, 120.9, 117.1, 116.8, 115.7, 112.0, 104.6, 102.8, 101.6, 83.1, 78.5, 76.3, 72.2, 60.6, 56.0, 42.3, 42.1, 14.6, 13.6. MS (ESI$^+$) m/z calcd for $C_{30}H_{31}NO_{11}Na$ 604.18, found 604.40 (M + Na)$^+$.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl pyrrolidine-1-carboxylate (2e)

White solid (75.6 mg, purity 95.75%, yield 26.1%); mp 142–145 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.68 (s, 1H), 9.16 (s, 1H), 7.11 (d, $J = 2.16$ 1H), 7.06 (d, 1H), 7.02 (d, $J = 1.52$, 1H), 6.99 (d, $J = 8.92$, 1H), 6.88 (dd, $J = 8.16$, 1.24, 1H), 6.81 (d, $J = 8.08$, 1H), 6.37 (d, $J = 2.08$, 1H), 6.33 (d, $J = 2.52$, 1H), 5.96 (d, $J = 6.36$, 1H), 5.23 (d, $J =$

11.56, 1H), 4.98 (t, $J = 5.44$, 1H), 4.92 (d, $J = 7.96$, 1H), 4.79–4.73 (m, 1H), 4.19–4.17 (m, 1H), 3.78 (s, 3H), 3.56–3.51 (m, 1H), 3.46–3.43 (t, $J = 6.32$, 2H), 3.37–3.33 (m, 1H), 3.32 (t, $J = 6.12$, 2H), 1.89–1.82 (m, 4H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 199.9, 162.3, 162.1, 159.3, 151.2, 148.0, 147.4, 144.2, 143.7, 130.2, 127.8, 121.9, 120.9, 117.1, 116.8, 115.7, 112.0, 104.6, 102.9, 101.7, 83.1, 78.5, 76.3, 72.1, 60.6, 56.0, 46.7, 46.6, 25.6, 24.8. MS (ESI$^+$) m/z calcd for $C_{30}H_{29}NO_{11}Na$ 602.16, found 602.30 (M + Na)$^+$.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl piperidine-1-carboxylate (2f)

White solid (138.0 mg, purity 97.99%, yield 46.5%); mp 146-147 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.68 (s, 1H), 9.16 (s, 1H), 7.11 (d, $J = 2.00$, 1H), 7.04 (d, 1H), 7.02 (s, 1H), 6.99 (d, $J = 8.72$, 1H), 6.88 (dd, $J = 8.12$, 1.44, 1H), 6.81 (d, $J = 8.04$, 1H), 6.35 (d, $J = 2.08$, 1H), 6.32 (d, $J = 2.44$, 1H), 5.96 (d, $J = 6.36$, 1H), 5.23 (d, $J = 11.56$, 1H), 4.98 (t, $J = 5.44$, 1H), 4.92 (d, $J = 7.96$, 1H), 4.79–4.73 (m, 1H), 4.19–4.16 (m, 1H), 3.78 (s, 3H), 3.56–3.51 (m, 1H), 3.49 (br, 2H), 3.39 (br, 2H), 3.37–3.31 (m, 1H), 1.58–1.53 (m, 6H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 199.9, 162.3, 162.1, 159.5, 151.8, 148.0, 147.4, 144.2, 143.7, 130.2, 127.8, 121.7, 120.9, 117.0, 116.8, 115.7, 111.9, 104.6, 102.9, 101.7, 83.1, 78.5, 76.3, 72.1, 60.6, 56.0, 45.7, 45.1, 25.8, 25.4, 24.0. MS(ESI$^+$) m/z calcd for $C_{31}H_{31}NO_{11}Na$ 616.18, found 616.30 (M + Na)$^+$.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl piperidine-1-carboxylate (2g)

White solid (121.0 mg, purity 98.32%, yield 39.7%); mp 138–141 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.69 (s, 1H), 9.16 (s, 1H), 7.12 (d, $J = 1.00$, 1H), 7.05 (d, 1H), 7.02 (s, 1H), 7.00 (dd, $J = 8.36$, 0.68, 1H), 6.88 (dd, $J = 8.16$, 1.68, 1H), 6.81 (d, $J = 8.08$, 1H), 6.32 (d, $J = 2.08$, 1H), 6.28 (d, $J = 2.32$, 1H), 5.96 (d, $J = 6.36$, 1H), 5.23 (d, $J = 11.60$, 1H), 4.98 (t, $J = 4.92$, 1H), 4.92 (d, $J = 7.92$, 1H), 4.78–4.73 (m, 1H), 4.20–4.16 (m, 1H), 3.91 (br, 2H), 3.78 (s, 3H), 3.55–3.52 (m, 1H), 3.37–3.31 (m, 1H), 1.22 (br, 12H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 199.8, 162.4, 162.2, 159.5, 151.8, 148.0, 147.4, 144.2, 143.7, 130.2, 127.8, 121.8, 120.9, 117.1, 116.8, 115.7, 112.0, 104.4, 102.4, 101.3, 83.1, 78.5, 76.3, 72.2, 60.6, 56.0, 47.0, 46.4, 21.7, 20.4. MS(ESI$^+$) m/z calcd for $C_{32}H_{35}NO_{11}Na$ 632.21, found 632.40 (M + Na)$^+$.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl [1,4′-bipiperidine]-1′-carboxylate (2h)

White solid (37.7 mg, purity 98.22%, yield 10.8%); mp 146–149 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.69 (s, 1H), 9.21 (s, 1H), 7.11 (s, 1H), 7.06–702 (m, 2H), 7.00 (d, $J = 6.96$, 1H), 6.87 (dd, $J = 6.32$, 1H), 6.81 (d, $J = 6.32$, 1H), 6.37 (s,1H), 6.33 (s,1H), 5.99 (d, $J = 4.60$, 1H), 5.23 (d, $J = 9.24$, 1H), 5.00 (br, 1H), 4.92 (d, $J = 6.44$, 1H), 4.79–4.75 (m, 1H), 4.18 (br, 1H), 4.10 (br, 1H), 4.03 (br, 1H), 3.78 (s, 3H), 3.55–3.53 (m, 1H), 3.53–3.35 (m, 3H), 3.00 (t, $J = 10.04$, 1H), 2.87 (t, $J = 9.92$, 1H), 2.50 (m, 3H), 1.79–1.76 (m, 2H), 1.50–1.39 (m, 8H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 199.8, 162.3, 162.1, 159.4, 151.8, 148.0, 147.4, 144.2, 143.7, 130.2, 127.8, 121.9, 120.9, 117.1, 116.8, 115.7, 111.9, 104.6, 103.0, 101.8, 83.1, 78.5, 76.3, 72.1, 61.7, 60.5, 56.0, 50.0, 44.3, 43.8, 27.8, 27.3, 26.1, 24.6. MS(ESI$^+$) m/z calcd for $C_{36}H_{41}N_2O_{11}$ 677.27, found 677.60 (M + H)$^+$.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl methoxy(methyl)carbamate (2i)

White solid (86.0 mg, purity 97.34%, yield 30.2%); mp 178–181 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.69 (s, 1H), 9.16 (s, 1H), 7.12 (d, $J = 1.92$, 1H), 7.07–7.02 (m, 2H), 7.00 (d, $J = 8.64$, 1H), 6.88 (dd, $J = 8.32$, 1.16, 1H), 6.81 (d, $J = 8.08$, 1H), 6.43 (d, $J = 2.08$, 1H), 6.39 (d, $J = 2.32$, 1H), 5.98 (d, $J = 6.36$, 1H), 5.25 (d, $J = 11.64$, 1H), 4.98 (t, $J = 5.44$, 1H), 4.92 (d, $J = 7.96$, 1H), 4.81–4.76 (m, 1H), 4.19–4.17 (m, 1H), 3.78 (s, 3H), 3.71 (s, 3H), 3.56–3.52 (m, 1H), 3.37–3.31 (m, 1H), 3.21 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 199.9, 162.3, 162.2, 158.6, 153.0, 148.0, 147.4, 144.2, 143.7, 130.1, 127.9, 121.7, 120.9, 117.1, 116.8, 115.7, 112.0, 105.0, 103.0, 101.9, 83.1, 78.5, 76.3, 72.1, 61.6, 60.5, 56.1, 35.5. MS(ESI$^-$) m/z calcd for $C_{28}H_{26}NO_{12}$ 568.15, found 568.30 (M − H)$^-$.

General procedure for the synthesis of compounds 3a–i

To a solution of silibinin (241.2 mg, 0.50 mmol, 1.0 eq) in DMF (3.6 mL, 15.0 vol) was added K_2CO_3 (69.2 mg, 0.50 mmol, 1.0 eq) and carbamyl chloride **1a–i** (0.53 mmol, 1.05 eq). The mixture was stirred at 25 °C under N_2 atmosphere for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture diluted with water (4.0 mL) and the resulting precipitate was collected, washed with water. The solid was purified by column chromatography (CH$_2$Cl$_2$/MeOH=15:1) to give the title compound **3a–i**.

3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo [b][1,4]dioxin-6-yl)-4-oxo-4H-chromen-7-yl morpholine-4-carboxylate (3a)

Yellow solid (81.8 mg, purity 98.19%, yield 27.3%); mp 284–286 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.46 (s, 1H), 9.90 (s, 1H), 9.18 (s, 1H), 7.85 (dd, $J = 10.68$, 2.16, 1H), 7.84 (s, 1H), 7.16 (d, $J = 8.40$, 1H), 7.11 (d, $J = 2.00$, 1H), 7.06 (d, $J = 1.76$, 1H), 6.91 (dd, $J = 8.20$, 1.76, 1H), 6.83 (d, $J = 8.04$, 1H), 6.64 (d, $J = 2.04$, 1H), 5.03 (t, $J = 5.44$, 1H), 4.99 (d, $J = 7.92$, 1H), 4.31–4.28 (m, 1H), 3.79 (s, 3H), 3.67 (t, $J = 5.04$, 4H), 3.59–3.56 (m, 3H), 3.44 (br, 2H), 3.40–3.36 (m, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.9, 160.3, 156.6, 155.2, 152.3, 148.0, 147.5, 147.4, 145.8, 143.9, 137.5, 127.6, 123.9, 122.0, 121.0, 117.3, 116.9, 115.7, 112.0, 107.3, 104.3, 101.5, 79.0, 76.3, 66.1, 60.5, 56.1, 45.1, 44.3. MS(ESI$^+$) m/z calcd for $C_{30}H_{28}NO_{12}$ 594.16, found 594.4 (M + H)$^+$.

3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxo-4H-chromen-7-yl dimethylcarbamate (3b)

Yellow solid (109.9 mg, purity 95.75%, yield 39.9%); mp 134–137 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.45(s, 1H), 9.88 (s, 1H), 9.18 (s, 1H), 7.84–7.81 (m, 2H), 7.16 (d, $J = 8.44$, 1H), 7.07 (d, $J = 2.04$, 1H), 7.05 (d, $J = 1.72$, 1H), 6.90 (dd, $J = 8.16$, 1.80, 1H), 6.82 (d, $J = 8.08$, 1H), 6.60 (d, $J = 2.04$, 1H), 5.03 (t, $J = 5.48$, 1H), 4.99 (d, $J = 7.92$, 1H), 4.31–4.27 (m, 1H), 3.79 (s, 3H), 3.60–3.55 (m, 1H), 3.40–3.33 (m, 1H), 3.05 (s, 3H), 2.93 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.9, 160.3, 156.8, 155.2, 153.3, 148.0, 147.4, 147.4, 145.8, 143.9, 137.5, 127.6, 123.9, 122.0, 121.0, 117.3, 116.9, 115.7, 112.0, 107.2, 104.3, 101.4, 79.0, 76.3, 60.5, 56.0, 36.8, 36.6. MS(ESI$^+$) m/z calcd for $C_{28}H_{26}NO_{11}$ 552.15, found 552.40 (M + H)$^+$.

3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxo-4H-chromen-7-yl 4-methylpiperazine-1-carboxylate (3c)

Yellow solid (63.0 mg, purity 97.83%, yield 20.8%); mp 198–201 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.45 (s, 1H), 9.90 (s, 1H), 9.18 (s, 1H), 7.84–7.82 (m, 2H), 7.15 (d, $J = 8.48$, 1H), 7.09 (d, $J = 2.04$, 1H), 7.05 (d, $J = 1.68$, 1H), 6.90 (dd, $J = 8.12$, 1.72, 1H), 6.82 (d, $J = 8.08$, 1H), 6.61 (d, $J = 2.04$, 1H), 5.03 (t, $J = 5.36$, 1H), 4.99 (d, $J = 7.88$, 1H), 4.31–4.27 (m, 1H), 3.79 (s, 3H), 3.58 (br, 3H), 3.44 (br, 2H), 3.40–3.35 (m, 1H), 2.38 (br, 4H), 2.23 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.9, 160.3, 156.7, 155.2, 152.2, 148.1, 147.5, 147.4, 145.8, 143.9, 137.5, 127.6, 123.9, 122.0, 121.0, 117.3, 116.9, 115.7,

112.0, 107.3, 104.2, 101.5, 79.0, 76.3, 60.5, 56.1, 54.6, 54.4, 46.1, 44.7, 44.0. MS(ESI$^+$) m/z calcd for $C_{31}H_{31}N_2O_{11}$ 607.19, found 607.30 $(M + H)^+$.

3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxo-4H-chromen-7-yl diethylcarbamate (3d)

Yellow solid (53.2 mg, purity 97.62%, yield 18.4%); mp 224–227 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.44 (s, 1H), 9.89 (s, 1H), 9.18 (s, 1H), 7.86–7.83 (m, 2H), 7.15 (d, $J = 9.16$, 1H), 7.09 (d, $J = 2.04$, 1H), 7.05 (d, $J = 1.76$, 1H), 6.90 (dd, $J = 8.16, 1.76$, 1H), 6.82 (d, $J = 8.08$, 1H), 6.60 (d, $J = 2.04$, 1H), 5.03 (t, $J = 5.36$, 1H), 4.99 (d, $J = 7.92$, 1H), 4.31–4.27 (m, 1H), 3.79 (s, 3H), 3.60–3.55 (m, 1H), 3.42–3.35 (m, 3H), 3.33–3.28 (m, 2H), 1.21 (t, $J = 7.04$, 3H), 1.15 (t, $J = 7.00$, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.9, 160.3, 156.8, 155.3, 152.6, 148.0, 147.4, 147.4, 145.8, 143.9, 137.5, 127.6, 123.9, 122.0, 121.0, 117.3, 116.9, 115.7, 112.0, 107.2, 104.2, 101.5, 79.0, 76.3, 60.5, 56.0, 42.3, 42.1, 14.6, 13.6. MS(ESI$^+$) m/z calcd for $C_{30}H_{30}NO_{11}$ 580.18, found 580.30 $(M + H)^+$.

3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxo-4H-chromen-7-yl pyrrolidine-1-carboxylate (3e)

Yellow solid (52.6 mg, purity 98.66%, yield 18.2%); mp 133–136 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.44 (s, 1H), 9.88 (s, 1H), 9.18 (s, 1H), 7.84–7.81 (m, 2H), 7.15 (d, $J = 9.16$, 1H), 7.08 (d, $J = 2.00$, 1H), 7.05 (d, $J = 1.72$, 1H), 6.90 (dd, $J = 8.16, 1.76$, 1H), 6.82 (d, $J = 8.04$, 1H), 6.61 (d, $J = 2.04$, 1H), 5.03 (t, $J = 5.44$, 1H), 4.99 (d, $J = 7.92$, 1H), 4.31–4.27 (m, 1H), 3.79 (s, 3H), 3.60–3.55 (m, 1H), 3.52 (t, $J = 6.52$, 2H), 3.39–3.36 (m, 3H), 1.92–1.85 (m, 4H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.9, 160.3, 156.7, 155.3, 151.4, 148.0, 147.5, 147.4, 145.8, 143.9, 137.5, 127.6, 123.9, 122.0, 121.0, 117.3, 116.9, 115.7, 112.0, 107.2, 104.2, 101.4, 79.0, 76.3, 60.5, 56.1, 46.8, 46.6, 25.7, 24.9. MS(ESI$^+$) m/z calcd for $C_{30}H_{28}NO_{11}$ 578.17, found 578.40 $(M + H)^+$.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl piperidine-1-carboxylate (3f)

Yellow solid (67.8 mg, purity 97.07%, yield 22.9%); mp 132–135 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.44 (s, 1H), 9.89 (s, 1H), 9.18 (s, 1H), 7.85–7.82 (m, 2H), 7.15 (d, $J = 8.28$, 1H), 7.08 (d, $J = 1.16$, 1H), 7.05 (d, $J = 1.64$, 1H), 6.90 (dd, $J = 8.16, 1.72$, 1H), 6.82 (d, $J = 8.04$, 1H), 6.60 (d, $J = 1.72$, 1H), 5.03 (t, $J = 5.40$, 1H), 4.99 (d, $J = 7.92$, 1H), 4.31–4.27 (m, 1H), 3.79 (s, 3H), 3.60–3.55 (m, 3H), 3.42–3.35 (m, 3H), 1.56 (br, 6H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.9, 160.3, 156.9, 155.3, 152.1, 148.0, 147.4, 147.4, 145.8, 143.9, 137.5, 127.6, 123.9, 122.0, 121.0, 117.3, 116.9, 115.7, 112.0, 107.2, 104.3, 101.5, 79.0, 76.3, 60.5, 56.0, 45.7, 45.1, 25.9, 25.5, 24.1. MS(ESI$^+$) m/z calcd for $C_{31}H_{30}NO_{11}$ 592.18, found 592.40 $(M + H)^+$.

3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxo-4H-chromen-7-yl diisopropylcarbamate (3g)

Yellow solid (34.1 mg, purity 97.55%, yield 11.2%); mp 125–128 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.44 (s, 1H), 9.89 (s, 1H), 9.18 (s, 1H), 7.88–7.85 (m, 2H), 7.15 (dd, $J = 6.32, 2.84$, 1H), 7.07 (d, $J = 2.04$, 1H), 7.05 (d, $J = 1.76$, 1H), 6.90 (dd, $J = 8.12, 1.72$, 1H), 6.82 (d, $J = 8.08$, 1H), 6.57 (d, $J = 2.04$, 1H), 5.02 (t, $J = 5.48$, 1H), 4.98 (d, $J = 7.92$, 1H), 4.31–4.28 (m, 1H), 3.99 (br, 2H), 3.79 (s, 3H), 3.60–3.55 (m, 1H), 3.40–3.33 (m, 1H), 1.25 (br, 12H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.9, 160.3, 156.8, 155.3, 152.1, 148.1, 147.5, 147.4, 145.8, 143.9, 137.4, 127.6, 123.9, 122.1, 121.0, 117.3, 116.9, 115.7, 112.0, 107.1, 104.0, 101.2, 79.0, 76.3, 60.5, 56.1, 47.0, 46.4, 21.7, 20.4. MS(ESI$^+$) m/z calcd for $C_{32}H_{34}NO_{11}$ 608.21, found 608.30 $(M + H)^+$.

3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxo-4H-chromen-7-yl [1,4′-bipiperidine]-1′-carboxylate (3h)

Yellow solid (102.1 mg, purity 97.30%, yield 30.3%); mp 144–147 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.45 (s, 1H), 9.90 (s, 1H), 9.19 (s, 1H), 7.84–7.82 (m, 2H), 7.15 (d, $J = 8.32$, 1H), 7.08 (d, $J = 1.88$, 1H), 7.05 (d, $J = 1.80$, 1H), 6.90 (dd, $J = 8.20, 1.80$, 1H), 6.82 (d, $J = 8.04$, 1H), 6.61 (d, $J = 1.96$, 1H), 5.03 (t, $J = 5.20$, 1H), 4.99 (d, $J = 7.92$, 1H), 4.31–4.27 (m, 1H), 4.17 (br, 1H), 4.06 (br, 1H), 3.79 (s, 3H), 3.59–3.56 (m, 1H), 3.38–3.36 (m, 3H), 3.05 (t, $J = 13.00$, 1H), 2.90 (t, $J = 12.80$, 1H), 2.49 (br, 3H), 1.79–1.77 (m, 2H), 1.50–1.40 (m, 8H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.9, 160.3, 156.8, 155.2, 152.0, 148.0, 147.5, 147.4, 145.8, 143.9, 137.5, 127.6, 123.9, 122.0, 121.0, 117.3, 116.8, 115.7, 112.0, 107.2, 104.3, 101.5, 79.0, 76.3, 61.7, 60.5, 56.1, 50.0, 44.4, 43.9, 27.9, 27.5, 26.3, 24.7. MS(ESI$^+$) m/z calcd for $C_{36}H_{39}N_2O_{11}$ 675.26, found 675.50 $(M + H)^+$.

(2R,3R)-3,5-dihydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxochroman-7-yl methoxy(methyl)carbamate (3i)

Yellow solid (37.2 mg, purity 97.80%, yield 13.1%); mp 114–117 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.49 (s, 1H),

9.94 (s, 1H), 9.22 (s, 1H), 7.84–7.81 (m, 2H), 7.15 (d, J = 5.12, 1H), 7.14 (d, J = 5.08, 1H), 7.06 (d, J = 1.48, 1H), 6.91 (dd, J = 8.12, 1.52, 1H), 6.83 (d, J = 8.04, 1H), 6.67 (d, J = 1.96, 1H), 5.06 (t, J = 5.16, 1H), 4.98 (d, J = 7.92, 1H), 4.31–4.27 (m, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 3.59–3.56 (m, 1H), 3.38–3.34 (m, 1H), 3.25 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.9, 160.4, 156.0, 155.2, 153.3, 148.0, 147.5, 147.5, 145.8, 143.9, 137.6, 127.6, 123.8, 122.0, 121.0, 117.3, 116.9, 115.7, 112.0, 107.6, 104.3, 101.6, 79.0, 76.3, 61.7, 60.5, 56.1, 35.6. MS(ESI$^+$) m/z calcd for $C_{28}H_{26}NO_{12}$ 568.15, found 568.30 (M + H)$^+$.

General procedure for the synthesis of compounds 4a–b

To a solution of silibinin (241.2 mg, 0.50 mmol, 1.0 eq) in DMF (3.6 mL, 15.0 vol) was added K_2CO_3 (138.2 mg, 1.0 mmol, 2.0 eq) and carbamyl chloride **1a–b** (1.03 mmol, 2.05 eq). The mixture was stirred at 25 °C under N_2 atmosphere for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture diluted with water (20 mL) and extracted with ethyl acetate (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 give the crude product. The residue was purified by column chromatography (CH_2Cl_2/MeOH = 15:1) to give the title compound **4a–b**.

5-hydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b] [1,4]dioxin-6-yl)-4-oxo-4H-chromene-3,7-diyl bis(morpholine-4-carboxylate) (4a)

White solid (39.2 mg, purity 95.71%, yield 11.1%); mp 143–146 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.18 (s, 1H), 9.20 (s, 1H), 7.55 (s, 1H), 7.53 (dd, J = 8.58, 1.96, 1H), 7.21 (d, J = 8.56, 1H), 7.17 (d, J = 2.04, 1H), 7.06 (d, J = 1.76, 1H), 6.90 (dd, J = 8.20, 1.76, 1H), 6.83 (d, J = 8.08, 1H), 6.75 (d, J = 2.04, 1H), 5.05 (t, J = 5.40, 1H), 5.01 (d, J = 7.92, 1H), 4.35–4.31 (m, 1H), 3.79 (s, 3H), 3.68 (t, J = 4.32, 8H), 3.60–3.57 (m, 5H), 3.44–3.36 (m, 5H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.7, 160.6, 157.3, 157.0, 155.8, 152.1, 148.1, 147.5, 147.2, 144.2, 131.4, 127.4, 122.4, 121.8, 121.0, 117.8, 117.3, 115.7, 112.0, 108.0, 105.6, 102.0, 79.0, 76.3, 66.4, 66.1, 60.4, 56.0, 45.4, 45.2, 44.7, 44.3. MS(ESI$^+$) m/z calcd for $C_{35}H_{35}N_2O_{14}$ 707.67, found 707.40 (M + H)$^+$.

5-hydroxy-2-(3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-4-oxo-4H-chromene-3,7-diyl bis(dimethylcarbamate) (4b)

White solid (86.2 mg, purity 92.26%, yield 27.7%); mp 135–138 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.17 (s, 1H), 9.20 (s, 1H), 7.55 (d, J = 8.58, 1H), 7.52 (dd, J = 8.56,

2.12, 1H), 7.19 (d, J = 8.56, 1H), 7.12 (d, J = 2.00, 1H), 7.06 (d, J = 1.72, 1H), 6.90 (dd, J = 8.20, 1.76, 1H), 6.82 (d, J = 8.00, 1H), 6.70 (d, J = 2.04, 1H), 5.04 (t, J = 5.44, 1H), 5.01 (d, J = 7.96, 1H), 4.35–4.31 (m, 1H), 3.78 (s, 3H), 3.60–3.56 (m, 1H), 3.42–3.35 (m, 1H), 3.10 (s, 3H), 3.05 (s, 3H), 2.93 (s, 3H), 2.91 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 176.8, 160.5, 157.5, 156.7, 155.8, 153.1, 152.9, 148.1, 147.5, 147.1, 144.2, 131.8, 127.4, 122.5, 121.9, 121.0, 117.8, 117.3, 115.7, 112.1, 107.8, 105.5, 101.9, 79.0, 76.3, 60.4, 56.1, 37.0, 36.8, 36.8, 36.6. MS(ESI$^+$) m/z calcd for $C_{31}H_{31}N_2O_{12}$ 623.59, found 623.40 (M + H)$^+$.

CCK-8 assay

In vitro cytotoxicity of the synthesized compounds, control drugs silybin and DHS was measured using the CCK-8 assay. In brief, a suspension of cells (around 5000 cells per well for MCF-7, NCI-H1299, HepG2 and HT29) was inoculated into 96-well plates and incubated at 37 °C in an atmosphere with 5% CO_2 for 24 h. Then, solutions (PBS buffer containing 1% DMSO) of the prepared compounds and control drugs at a series of concentrations were added into the 96-well plates. After incubation for another 48 h, 10 μL of CCK-8 solution was added into each well and the plates were again incubated at 37 °C for another 2 h. The optical density (OD) values were measured using a microplate reader at 450 nm and expressed as IC_{50} values which was calculated with SPSS.

Molecular docking

The co-crystal structure of Hsp90 in complex with XL888 [37] (PDB code 4AWO) was obtained from the PDB (http://www.pdb.org) and used for the docking calculation in MOE (version 2020). 3D structures of the ligands were constructed, and their energy minimization were performed. For preparing the protein receptors, the hydrogen atoms were added into the X-ray structure and the Triangle Matcher-force field was employed. The site sphere was determined based on the binding location of XL888 in Hsp90. The ligand XL888 and water were removed from the binding site, and compound **2f**, **2h**, **3e**, **3g** was docked into the prepared site in Hsp90, respectively. After evaluating the types of interactions between the tested compounds and Hsp90, the final binding conformation in Hsp90 was confirmed based on the calculated energy.

Compliance with ethical standards

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