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Synthesis and bioactivity of novel xanthone and thioxanthone L-rhamnopyranosides†

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A series of xanthone and thioxanthone rhamnopyranosides were designed and synthesized. Their *in vitro* cytotoxicity and topoisomerase inhibitory activity were evaluated. The bioassay results indicated that the introduction of the 2,3-di-*O*-acetyl-α-L-rhamnopyranosyl moiety to anthracene was helpful to improve the cytotoxicity *in vitro*. The modifications of anthracene had an important effect on the tumor cell growth inhibitory activity. Interestingly, consistency was observed between the cytotoxicity and topo I activity in these anthracene analogs, suggesting that the incorporation of either a polymethyleneamine side chain or a pyrazole ring into the anthraquinone chromophore was able to enhance topo I inhibitory activity as well as cytotoxicity simultaneously. Among them, compound **11** as a new lead compound was discovered, which showed wide *in vitro* cytotoxicity against 12 tumor cell lines and potential antimultidrug resistance capability. It was proved that compound **11** could induce cell apoptosis in KB cells *via* both extrinsic and intrinsic pathways. Flow cytometric analysis exhibited that treatment of KB cells with compound **11** led to cell apoptosis accompanied by cell cycle arrest at the G2/M phase. Furthermore, compound **11** caused a significant and dose-dependent inhibition of topoisomerase I catalytic activity.

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1. Introduction

Natural products are a unique source of chemical complexity and bioactive diversity for drug discovery. More than 70% of anticancer drugs worldwide are from natural products.[1] Recently, considerable attention has been given to natural products due to the increased understanding of their broad spectrum of antitumor activity.[2,3] Xanthones and their derivatives with a planar chromophore are a well known class of secondary metabolites in some microorganisms and higher plants.[4,5] Structural modification of xanthones is attempted to enhance the activity of these compounds.[6–8]

Some improvements were achieved for anthracene compounds by the introduction of a hydrophilic sugar moiety.[9] Notably, a number of xanthone glucosides, such as veratriloside and swertianolin, have shown both potent cytotoxicity and good solubility. It is noteworthy that the addition of sugar chains such as L-rhamnose and 2,3-di-*O*-acetyl-α-L-rhamnopyranosyl residue to the planar chromophore core is an efficient approach to enhance their antitumor activity.[9,10] Thus, we were motivated to design and synthesize a number of xanthone and thioxanthone l-rhamnopyranosides and evaluate their cytotoxic activity.

The natural product anthrone L-rhamnoside "prinoidin" (Fig. 1A) shows cytotoxic activity against KB cells *in vitro* with the IC_{50} value of 0.045 μM, but it exhibited toxic effect on normal cells and led to the death of mice when evaluated *in vivo*.[10] It was proved that the methylene proton at C-10 of anthrone could produce oxygen radicals,[11] which are perhaps the cause of the serious side effects. An efficient approach to the control of oxygen radical formation would be to replace the methylene proton at C-10 with O or S-isosteres of the chromophore. The rationale was that the γ-pyrone moiety should be much more resistant to reduction but should retain some of the planar, spatial, and electronic characteristics of the parent quinonoid system necessary for molecular recognition and intercalative binding.[12] To obtain a preliminary structure–activity relationship, we designed and synthesized a series of xanthone and thioxanthone L-rhamnopyranosides **1–7** (Fig. 1A) structurally related to natural compound prinoidin, for which cytotoxic activity *in vitro* was evaluated against human lung carcinoma cell line (A549), human promyelocytic leukemia cell line (HL-60) and human breast carcinoma cell line (MDA-MB-231). The results showed that the introduction of 2,3-di-*O*-acetyl-α-L-

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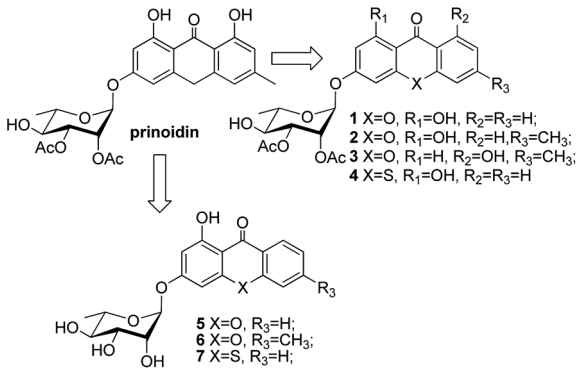
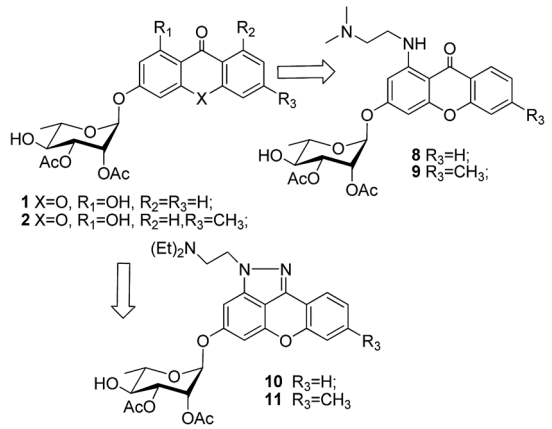


1A



1B

Fig. 1 (A) design strategy for the target compounds **1–7**. (B) design strategy for the target compounds **8–11**.

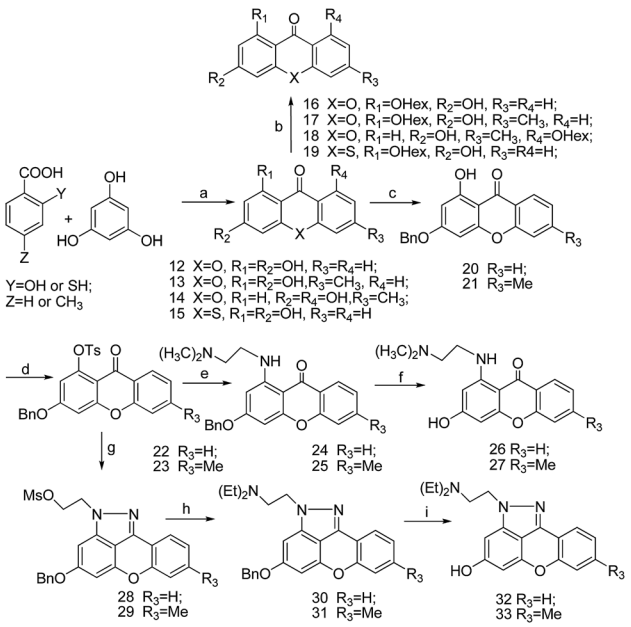
rhamnopyranosyl residue to xanthone could improve *in vitro* cytotoxicity more significantly than the L-rhamnosyl residue.

Based on the above studies, a series of xanthone L-rhamnopyranosides derivatives **8–11** were further synthesized to search for more potentially antitumor lead compounds, and their *in vitro* cytotoxic activity was evaluated. Considering the polymethyleneamine side chain of mitoxantrone plays an important role in antitumor activity,[13] compounds **8** and **9** (Fig. 1B) were designed and synthesized, respectively. Additionally, compounds **10** and **11** (Fig. 1B) were designed and synthesized based on the fact that the incorporation of a pyrazole ring into the anthraquinone chromophore is able to contribute to enhance antitumor activities and reduce side effects.[14] Investigations of the mechanism and *in vitro* cytotoxic activity were performed on the compound **11**. The results obtained have rendered important clues to understanding the cytotoxic profile for these compounds.

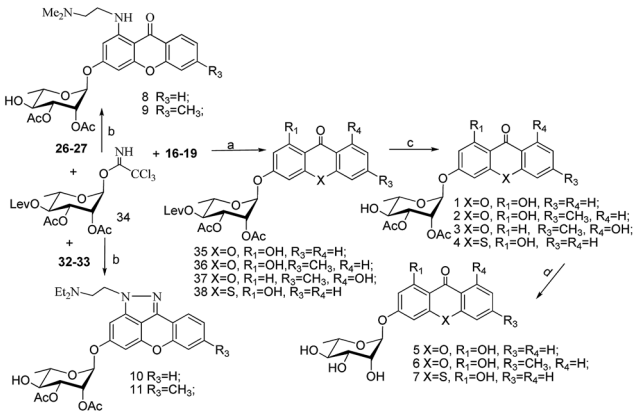
2. Results and discussion

2.1 Chemistry

As shown in Schemes 1 and 2, the synthesis of xanthone and thioxanthone L-rhamnopyranosides **1–11** was achieved. Xanthone **13** or **14** was prepared using a similar procedure as that of **12** (ref. 15) or **15**.[16] For example, the condensation between phloroglucinol and 4-methylsalicylic acid afforded the xanthone **13** in high yield (96%) using Eaton's reagent (phosphorus pentoxide and methanesulfonic acid: P_2O_5/CH_3SO_3H) as the coupling reagent. The anthracene acceptors **16–19** could be obtained easily by employing a procedure similar to that of 2′,3′-di-*O*-acetylfrangulin A.[17] Compounds **12–15** were condensed with *n*-hexanoyl chloride under the promotion of DMAP, followed by deprotection of Hex group at 3-C-OH in the presence of PhSH and a catalytic amount of imidazole to afford the required acceptors **16–19**. The less labile 3-benzyl ether **20** or **21** was prepared upon reaction of **12** or **13** with benzyl chloride in the presence of anhydrous sodium carbonate and sodium iodide. Compound **20** or **21** was changed into the tosylate **22** or **23** with *p*-toluenesulfonyl chloride, then followed by treatment with *N*,*N*-dimethyiethane-1,2-diamine gave the derivative **24** or **25**, respectively. Benzyl group in **24** or **25** was removed by hydrogenolysis using Pd/C to provide the key intermediate **26** or **27**. Treatment of **22** or **23** with 2-hydrohyethylhydrazine, followed by reaction with methanesulfonyl chloride in the presence of triethylamine afforded the compound **28** or **29**. Compound **30** or **31** was obtained upon reaction of **28** or **29** with diethylamine in anhydrous ethanol under reflux. Removal of the benzyl group was effectively accomplished by treatment of **30** or **31** with 1 M BCl_3 in dichloromethane, which furnished the important intermediate **32** or **33**, respectively.



Scheme 1 *Reagents and conditions*: (a) methanesulfonic acid, P_2O_5, 90 °C; (b) (i) *n*-hexanoyl chloride, pyridine, reflux; (ii) PhSH, imidazole, NMP, −10 °C; (c) BnCl, Na_2CO_3, NaI, acetone, reflux; (d) TsCl, Na_2CO_3, acetone, reflux; (e) $(CH_3)_2N(CH_2)_2NH$, DMSO, 150 °C; (f) Pd/C, H_2; (g) (i) 2-hydroxyethylhydrazine, DMSO, 150 °C; (ii) methanesulfonyl chloride, Et_3N, CH_2Cl_2, rt; (h) Et_2NH, EtOH–DMSO, 78 °C; (i) BCl_3, CH_2Cl_2, 0 °C.



Scheme 2 *Reagents and conditions*: (a) $BF_3 \cdot Et_2O$ (1 eq), CH_2Cl_2, 0 °C; (b) (i) $BF_3 \cdot Et_2O$, CH_2Cl_2, 0 °C; (i) AcOH–NH_2NH_2, rt; (c) AcOH–NH_2NH_2, rt; (d) MeONa, MeOH, rt.

Table 1 IC_{50} Cytotoxicity values (μM) of xanthone and thioxanthone rhamnopyranosides derivatives against tumor cells (data derived from the mean of three independent assays)

Compound	A549	HL-60	MDA-MB-231
1	18.20 ± 0.13	16.30 ± 0.26	20.13 ± 0.18
2	17.15 ± 0.26	15.05 ± 0.13	19.11 ± 0.42
3	23.21 ± 0.15	20.25 ± 0.10	25.01 ± 0.88
4	27.13 ± 0.12	26.15 ± 0.18	23.10 ± 0.43
5	28.06 ± 0.12	30.15 ± 0.18	35.24 ± 0.27
6	30.15 ± 0.02	38.06 ± 0.45	35.15 ± 0.38
7	38.01 ± 0.13	>50	>50
8	8.61 ± 0.24	7.52 ± 0.08	8.01 ± 0.03
9	4.10 ± 0.16	6.28 ± 0.26	5.25 ± 0.10
10	5.65 ± 0.08	7.25 ± 0.27	6.02 ± 0.13
11	1.05 ± 0.10	1.20 ± 0.21	2.56 ± 0.15
13, 27, 33	>50	>50	>50
Adriamycin	0.98 ± 0.11	1.02 ± 0.16	10.25 ± 0.21

The preparation of the target compounds **1–11** was outlined in Scheme 2. Glycosylation of **16–19** with the known L-rhamnopyranosyl Schmidt donor **34** (ref. 17) under the promotion of $BF_3 \cdot Et_2O$ (1.0 eq), and then deprotection of the Hex group when the dosage of $BF_3 \cdot Et_2O$ was raised to 3.0 eq provided the intermediates **35–38**, respectively. At last, removal of the Lev group with hydrazine acetate gave the target compounds **1–4**. Treatment of compounds **1**, **2**, and **4** with MeONa afforded efficiently another three target compounds **5–7**, respectively. The synthesis of the target compounds **8–11** followed the similar synthetic route as that of the compounds **1–4**.

2.2 *In vitro* cytotoxic activity

2.2.1 Cytotoxicity against tumor cells *in vitro*. The *in vitro* cytotoxic activity of all glycosylated xanthone derivatives **1–11** was evaluated against the human lung carcinoma cell line A549, the human promyelocytic leukemia cell line HL-60 and the human breast carcinoma cell line MDA-MB-231. For comparative purposes, compounds **13**, **27**, **33** and adriamycin were also included in the assay.

As shown in Table 1, compounds **1–11** exhibited good cytotoxicity against A549, while compounds **13**, **27** and **33** did not show inhibitory activity against the three tumor cells, suggesting the sugar moiety attached on xanthone was a key element for the cytotoxicity. Among compounds **1–7**, compounds **1**, **2** and **4** showed better cytotoxicity than the other xanthone L-rhamnopyranosides **5–7**, indicating subtle modifications of L-rhamnosyl moiety with acetyl group favored the bioactivity. The decreased inhibition of compound **3** against the above three tumor cells comparing with **2** suggested C_1-OH of xanthone was more helpful to improve antitumor activity than C_8-OH. Compound **1** exhibited better cytotoxicity against A549 than compound **4** with the IC_{50} values of 18.2 and 27.1 μM, respectively, suggesting concise chemical modification on the planar chromophore could affect the activity.

The compounds **8–11** showed better cytotoxicity than the xanthone L-rhamnopyranosides **1–4** *in vitro*, indicating either the introduction of the polymethyleneamine side chain or the incorporation of a pyrazole ring into the xanthone was able to contribute to enhance *in vitro* antitumor activity. Compound **9** or **11** showed stronger cytotoxicity compared to its structural analogue **8** or **10**, suggesting C-6 methyl group favored the cytotoxicity when the side chain polymethyleneamine or the pyrazole ring was introduced into the planar chromophore.

Compound **11** displayed the strongest cytotoxicity among all the target compounds. As the potential lead compound, its spectrum of antitumor activity and preliminary anticancer mechanism on KB cell lines were further discussed below.

2.2.2 Cytotoxicity of compound 11 against tumor cells *in vitro*. To examine the broad-spectrum antitumor activity of compound **11**, MTT and SRB assays were employed against a panel of 12 human cell lines, including leukemia, liver cancer, gastric cancer, lung cancer, colon cancer, breast cancer, epithelial carcinoma, prostate cancer, kidney cancer, rhabdomyosarcoma, oral epidermoid carcinoma, and normal cell line HMEC (human microvascular endothelial cell). As shown in Table 2, compound **11** displayed potent and favorable *in vitro* cytotoxicity against the twelve tested tumor cell lines, indicating compound **11** possessed broad-spectrum antitumor activities. Notably, compound **11** showed the stronger cytotoxicity against KB and A549 cells than any other ten cells, suggesting compound **11** had a selective toxicity for different tumor cells.

Table 2 IC_{50} cytotoxicity values (μM) of compound **11** against tumor cells (data derived from the mean of three independent assays)

Tumor cells	IC_{50}	Tumor cells	IC_{50}
HL60	2.20 ± 0.46	A549	1.05 ± 0.10
MDA-MB-231	4.30 ± 0.06	BEL-7402	2.43 ± 1.90
MDA-MB-468	3.47 ± 1.88	MKN45	6.29 ± 0.34
HCT116	2.20 ± 0.21	A431	2.59 ± 1.43
PC3	3.99 ± 0.24	786-O	4.70 ± 0.14
Rh30	2.37 ± 0.52	KB	0.55 ± 0.06

In addition, the IC_{50} value of compound **11** against the normal cell line HEMC was 30.52 ± 0.18 μM, which was almost 6 times higher than that of the tumor cells. The result suggested that the cytotoxicity of the most active compound **11** was found to be selective against cancer cells in comparison to human normal cells.

P-glycoprotein (P-gp) overexpression is reported as a major multidrug resistance (MDR) mechanism, which is the significant impediment to the success of cancer chemotherapy with naturally derived anticancer drugs. To test the antimultidrug resistance capability of compound **11**, two P-gp over-expressed tumor cell lines MCF-7/ADM and KB/VCR and their normal counterparts were employed. High degrees of drug resistance was observed for the two reference compounds adriamycin (RF = 162.93, Table 3) and vincristine (RF = 65.00, Table 3). Compound **11** displayed totally equipotent cytotoxicity in MDR cells and their corresponding parental cells, with RF values of 1.48 and 1.09 (Table 3). The results indicated that compound **11** was not a substrate of the P-gp pump and had potential antimultidrug resistance capability.

2.3 Investigation of apoptosis

2.3.1 Compound 11 derived apoptosis *via* both extrinsic and intrinsic pathway. We evaluated whether compound **11** induced apoptosis in a caspase-dependent manner. The impact of compound **11** on caspase-3 and PARP was preferentially examined. The results showed that compound **11** dose-dependently decreased the expression of caspase-3 and increased the cleaved caspase-3 in KB cells, which resulted in PARP cleavage in a dose-dependent fashion (Fig. 2). After treatment of compound **11** for 24 h, caspase-9, which has been proposed as the initiator caspase in mitochondrial-dependent apoptotic pathway, and caspase-8, which is identified as the apical caspase in apoptosis induced by death receptors, were both activated in KB cells (Fig. 2). These findings favored involvement of both extrinsic and intrinsic apoptosis pathways in the compound **11**-mediated cleavage of caspase-3.

2.3.2 Investigation of cell cycle distribution. G2/M arrest is another factor for inhibition of proliferation. Perturbation of the cell cycle by a 24 h drug treatment was assessed by flow cytometry. As compound **11** concentration increased from 0.1 μM to 1 μM, the proportion of G2/M phase cells increased from 14.28% to 51.71% (Fig. 3), which inferred that compound **11** arrested KB cells in G2/M phase.

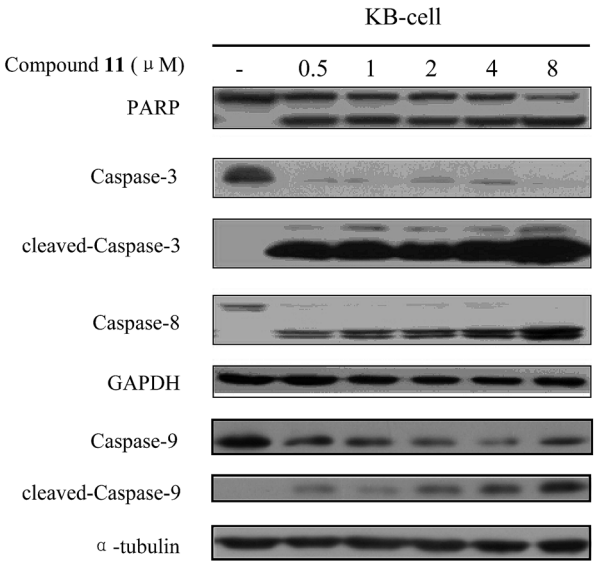


Fig. 2 Concentration-dependent caspases activation was involved in compound **11** induced apoptosis in KB cells. Whole cell lysates were prepared after being incubated with compound **11** (0.5–8 μM) for 24 h. Signals of processed proteins were detected by Western blotting with antibodies specific to caspase-3, -8, and -9, PARP. Data shown are representative of three independent experiments with similar results.

2.4 Inhibition activity of topoisomerase I

We examined the effect of compounds **1**, **5**, **8**, **10** and **11** on the catalytic activity of topoisomerase I by measuring the topoisomerase I-mediated relaxation of supercoiled pBR322 with CPT as a topo I-specific inhibitor.[18] The results indicated that the compounds **8**, **10** and **11** containing the polymethyleneamine side chain or a pyrazole ring exhibited potent inhibitory activity (Fig. 4), whereas the remaining compounds **1** and **5** did not inhibit topo I catalytic activity at 100 μM. The most potent cytotoxic compound **11** caused the most significant and dose-dependent inhibition of topoisomerase I catalytic activity (Fig. 4). These results demonstrated that there was a good correlation between cytotoxicity in cancer cell cultures and topo I inhibitory activity.

2.5 Docking study

Docking study was performed to determine the binding mode of action of the most active compound **11**. The docking study was conducted using Surflex-Dock in Sybyl 7.0 to dock

Table 3 The Inhibition of two pairs of multidrug-resistant tumor cell lines of compound **11** *in vitro* (data derived from the mean of three independent assays)

	IC_{50} (μM ± SD)[a]			IC_{50} (μM ± SD)[a]		
Compound	MCF-7	MCF-7/ADR	RF[b]	KB	KB/VCR	RF[b]
11	4.18 ± 1.51	6.20 ± 0.58	1.48	0.35 ± 0.56	0.38 ± 0.35	1.09
ADR	0.28 ± 0.89	45.62 ± 3.16	162.93	3.02 ± 0.18	96.64 ± 0.58	32.10
VCR	0.04 ± 0.002	4.32 ± 0.16	108.00	0.02 ± 0.01	1.30 ± 0.15	65.00

[a] IC_{50} is the concentration that causes 50% growth inhibition. [b] Relative resistance index (RF) = IC_{50} (drug-resistant)/IC_{50} (drug-sensitive).

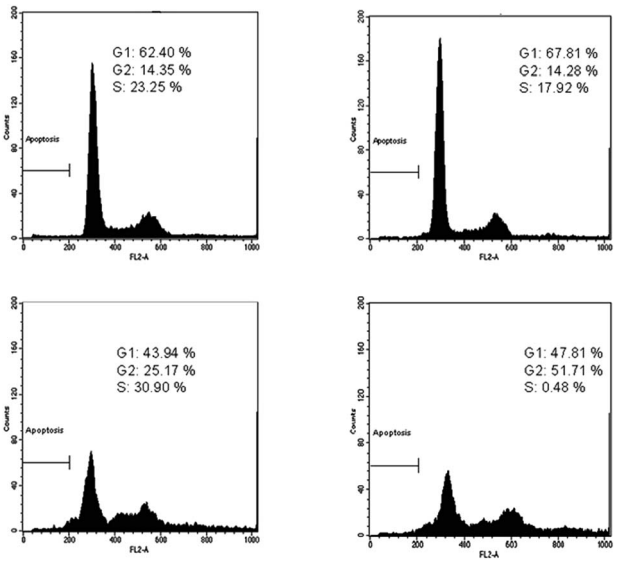


Fig. 3 KB cells treated with or without compound **11** (0, 0.1, 0.5, 1 μM) for 24 h were assayed by flow cytometry after staining with PI.

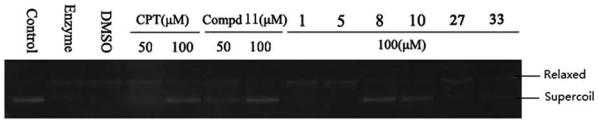


Fig. 4 Topoisomerase I-mediated supercoiled DNA pBR322 relaxation assay. pBR322 DNA was incubated with topoisomerase I (1 unit) in the absence or presence of indicated drugs at 37 °C for 30 min. The position of supercoiled DNA (SC) and relaxed DNA (RLX) are indicated.

compound **11** into the pocket formed after removal of ligand from the 3D crystal structure of topotecan–DNA–topo I (PDB: 1K4T) ternary complexes.[19] The docking result of compound **11** was shown in Fig. 5. From this molecular docking study, the binding mode of action of compound **11** was clarified. As shown in Fig. 5, compound **11** formed a hydrogen bond with LYS-532, which was considered as an important amino acid that interacted with the ligand in the DNA-topo I active site.[20] In addition, the imidazole ring of HIS-632 overlapped the pyrazole ring of the tetracyclic planar scaffold and indicated possible π–π stacking interactions between the aromatic rings.

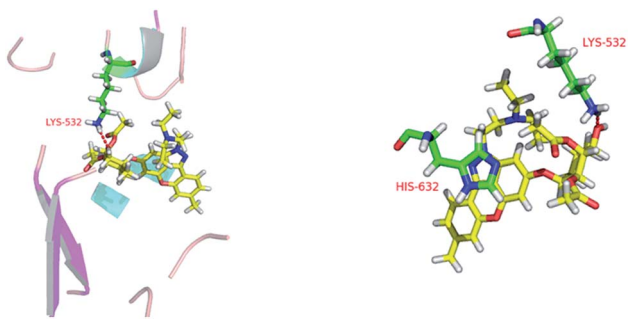


Fig. 5 Docked models of compound **11** in the active site of DNA–topo I complex.

3. Conclusion

In summary, a series of novel xanthone and L-thioxanthone rhamnopyranosides were synthesized in a practical way. Their cytotoxic activity and topo I inhibitory activity were tested *in vitro*. The results showed that the introduction of the 2,3-di-*O*-acetyl-α-L-rhamnopyranosyl residue chain to xanthone and thioxanthone could significantly improve cytotoxicity. As expected, molecules containing the polymethyleneamine side chain or a pyrazole ring into the anthraquinone chromophore exhibited potent topo I inhibitory activity with relatively strong cytotoxicity against three different tumor cell lines. Among them, compound **11** with potent topo I inhibitory activity displayed the strongest cytotoxicity and potential antimultidrug resistance capability. Preliminary mechanistic studies demonstrated that compound **11** could inhibit cell growth by inducing apoptosis, arresting cell cycle progression at the G2/M phase in KB cells. Furthermore, compound **11** could induce apoptosis in KB tumor cell lines *via* both extrinsic and intrinsic pathway, which could cause a significant and dose-dependent inhibition of topoisomerase I catalytic activity. To clarify the binding mode of action of compound **11** with topoisomerase I, molecular docking studies were performed with the Surflex-Dock program to provide the reasonable binding mode of the compound with the binding sites of the DNA–topo I complex.

In further studies of the other constrained structures of pyrazolo-fuzed xanthone analogues, diverse structural modifications structurally related to compound **11** are currently being carried out and these will be reported in due course.

4. Experimental protocols

4.1 General methods

Solvents were purified in a conventional manner. Thin layer chromatography was performed on E-Merck silica gel 60 F254 plates. Flash column chromatography (250 mm × 4 mm i.d.) was performed on silica gel (200–300 mesh, Qingdao, China). Optical rotation data were recorded on a KRUSS P8000 instrument (KRUSS, Karlsruhe, Germany). ^{1}H NMR and ^{13}C NMR spectra were taken on a JEOL JNM-ECP 600 spectrometer (JEOL Ltd., Tokyo, Japan) with tetramethylsilane as an internal standard, and chemical shifts are recorded in ppm values. Mass spectra were measured on a Q-TOF Global mass spectrometer (Bruker, Fallanden, Switzerland).

4.2 Synthesis

General synthetic procedure for compounds 13 and 14. A mixture of phosphorous pentoxide (10 g) and methanesulfonic acid (60 mL) was heated with vigorous stirring at 90 °C until a clear solution was obtained (approximately 1 h). 4-Methylsalicylic acid (3.0 g, 20 mmol) and phloroglucinol (3.2 g, 20 mmol) were then added in one portion and the reaction mixture was heated at 90 °C for 15 min. The mixture was poured into ice, stored at 4 °C for 6 h and the precipitate was filtered, washed with water and dried over $CaCl_2$ to give **13**. Compound **14** was synthesized following the same procedure as **13**.

*1,3-Dihydroxy-6-methyl-9H-xanthen-9-one (**13**).* White solid: yield 95.8%. R_f 0.82 (1 : 1, petroleum ether–EtOAc); m.p. > 280 °C; ^{1}H NMR (DMSO-d_6): δ 12.88 (s, 1H, OH), 11.05 (s, 1H, OH), 7.99 (d, 1H, J = 8.2 Hz, Ar-H), 7.40 (s, 1H, Ar-H), 7.27 (dd, 1H, J = 8.3 Hz, 0.9 Hz, Ar-H), 6.37 (d, 1H, J = 2.3 Hz, Ar-H), 6.20 (d, 1H, J = 2.3 Hz, Ar-H), 2.50 (s, 3H, Ar-CH_3); ^{13}C NMR (DMSO-d_6): δ 180.1, 166.1, 163.3, 157.7, 155.9, 147.3, 126.1, 125.4, 117.9, 117.8, 102.5, 98.5, 94.5, 21.9; HRESIMS calcd for $C_{14}H_9O_4$ 241.0501; found 241.0503.

*1,8-Dihydroxy-3-methyl-9H-xanthen-9-one (**14**).* White solid: yield 88.2%. R_f 0.63 (2 : 1, petroleum ether–EtOAc); m.p. > 280 °C; ^{1}H NMR (DMSO-d_6): δ 12.74 (s, 1H, OH), 11.13 (s, 1H, OH), 8.00 (d, 1H, J = 8.6 Hz, Ar-H), 6.92 (d, 1H, J = 8.5 Hz, Ar-H), 6.83 (s, 2H, Ar-H), 6.01 (s, 1H, Ar-H), 2.50 (s, 3H, Ar-CH_3); ^{13}C NMR (DMSO-d_6): δ 180.6, 165.1, 161.2, 158.0, 156.0, 148.8, 128.0, 114.7, 112.7, 111.2, 107.8, 106.1, 102.6, 22.4; HRESIMS calcd for $C_{14}H_9O_4$ 241.0501; found 241.0502.

General synthetic procedure for compounds 16–19. Preparation of compounds **16–19**: To a solution of **12–15** (1 eq) in dry pyridine was added *n*-hexanoyl chloride (3 eq) and 4-dimethylaminopyridine (DMAP, 0.1 eq) at 0 °C under argon. The reaction mixture was stirred at room temperature for 8 h. The mixture was dissolved in CH_2Cl_2, washed with 1 M HCl, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated to provide the yellow oil. To a solution of the above residue in dry NMP was added imidazole (0.5 eq), followed by PhSH (2 eq) at −5 °C under argon. After stirred for 5 h under this condition, the mixture was diluted with $CHCl_3$ and washed with 1 M HCl, H_2O, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (3 : 1, petroleum ether–EtOAc) to afford **16–19**.

*1-O-n-Hexanoyl-3-hydroxy-9H-xanthen-9-one (**16**).* White solid in 79.1% yield; R_f 0.46 (3 : 1, petroleum ether–EtOAc); m.p. 260–261 °C; ^{1}H NMR ($CDCl_3$): δ 8.20 (dd, 1H, J = 8.2 Hz, 1.4 Hz, Ar-H), 7.60 (td, 1H, J = 8.2, 1.7 Hz, Ar-H), 7.30 (td, 1H, J = 8.2 Hz, 1.1 Hz, Ar-H), 7.24 (d, 1H, J = 8.2 Hz, Ar-H), 6.62 (d, 1H, J = 2.2 Hz, Ar-H), 6.46 (d, 1H, J = 2.2 Hz, Ar-H), 2.82 (t, 2H, J = 7.7 Hz, $COCH_2$), 1.82–1.86 (m, 2H, CH_2), 1.28–1.46 (m, 6H, 3 × CH_2), 0.92 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 175.5, 173.8, 160.3, 158.8, 155.4, 151.6, 134.5, 126.5, 124.1, 122.1, 117.5, 108.9, 108.5, 101.6, 34.5, 31.4, 24.3, 22.5, 14.1; HRESIMS calcd for $C_{19}H_{19}O_5$ 327.1232; found 327.1240.

*1-O-n-Hexanoyl-3-hydroxy-6-methyl-9H-xanthen-9-one (**17**).* White solid in 78.3% yield; R_f 0.67 (2 : 1, petroleum ether–EtOAc); m.p. 254–255 °C; ^{1}H NMR ($CDCl_3$): δ 8.08 (d, 1H, J = 8.2 Hz, Ar-H), 7.11 (d, 1H, J = 8.3 Hz, Ar-H), 7.02 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 6.44 (s, 1H, Ar-H), 2.79–2.80 (m, 2H, CH_2CO), 2.48 (s, 3H, Ar-CH_3), 1.82–1.87 (m, 2H, CH_2), 1.34–1.47 (m, 6H, 3 × CH_2), 0.94 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.5, 169.0. 142.4, 134.3, 131.4, 127.4, 127.2, 123.7, 115.3, 114.7, 101.6, 34.5, 31.4, 24.3, 22.5, 22.0, 14.1; HRESIMS calcd for $C_{20}H_{21}O_5$ 341.1389; found 341.1396.

*1-O-n-Hexanoyl-6-hydroxy-3-methyl-9H-xanthen-9-one (**18**).* White solid in 80.2% yield; R_f 0.65 (2 : 1, petroleum ether–EtOAc); m.p. 256–257 °C; ^{1}H NMR ($CDCl_3$): δ 7.98 (bra, 1H, OH), 7.92 (d, 1H, J = 8.6 Hz, Ar-H), 7.08 (d, 1H, J = 1.8 Hz, Ar-H), 6.79 (1H, J = 1.8 Hz, Ar-H), 6.62 (dd, 1H, J = 8.6 Hz, 2.2 Hz, Ar-H), 6.52 (d, 1H, J = 2.2 Hz, Ar-H), 2.83 (t, 2H, J = 7.6 Hz, CH_2CO), 2.49 (s, 3H, Ar-CH_3), 1.85–1.90 (m, 2H, CH_2), 1.41–1.50 (m, 6H, 3 × CH_2), 0.97 (t, 3H, J = 7.1 Hz, CH_3), 0.94 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 175.3, 173.8, 161.7, 158.8, 155.5, 151.5, 145.9, 126.3, 125.6, 117.3, 108.3, 101.6, 34.3, 31.2, 24.2, 22.5, 22.3, 14.0; HRESIMS calcd for $C_{20}H_{21}O_5$ 341.1389; found 341.1393.

*1-O-n-Hexanoyl-3-hydroxy-9H-thioxanthen-9-one (**19**).* Yellow solid in 83.3% yield; R_f 0.52 (3 : 1, petroleum ether–EtOAc); m.p. 249–250 °C; ^{1}H NMR ($CDCl_3$): δ 8.43 (dd, 1H, J = 7.7 Hz, 1.1 Hz, Ar-H), 7.51 (td, 1H, J = 8.2 Hz, 1.6 Hz, Ar-H), 7.41 (td, 1H, J = 7.7 Hz, 1.1, Ar-H), 7.33 (d, 1H, J = 8.2 Hz, Ar-H), 6.66 (d, 1H, J = 2.2 Hz, Ar-H), 6.47 (d, 1H, J = 2.2 Hz, Ar-H), 2.82 (t, 2H, J = 7.7 Hz, $COCH_2$), 1.83–1.88 (m, 2H, CH_2), 1.40–1.49 (m, 6H, 3 × CH_2), 0.96 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 178.5, 173.6, 159.5, 153.9, 141.4, 135.5, 131.8, 130.3, 129.6, 126.2, 125.1, 115.5, 111.0, 109.3, 34.4, 31.4, 24.2, 22.3, 22.3, 14.0; HRESIMS calcd for $C_{19}H_{19}O_4$ S 343.1004; found 343.1012.

General synthetic procedure for compounds 20 and 21. Preparation of compounds **20** and **21**: To a solution of **12** (2.00 g, 8.76 mmol) in dry acetone (20 mL) were added, under argon, benzyl chloride (1.11 mL, 9.64 mmol), anhydrous sodium carbonate (1.86 g, 17.5 mmol), and anhydrous sodium iodide (1.58 g, 10.5 mmol). The resulting mixture was stirred at reflux temperature for 20 h. The solvent was then vacuum-evaporated, the residue was extracted with CH_2Cl_2–water, and the organic phase was dried (Na_2SO_4) and vacuum-evaporated. The residue was purified by column chromatography (5 : 1, petroleum ether–EtOAc) to give **20**. Compound **21** was synthesized from **13** following the same procedure as **20** from **12**.

*3-Benzyl-1-hydroxy-9H-xanthen-9-one (**20**).* White solid: yield 83.5%; R_f 0.68 (5 : 1, petroleum ether–EtOAc); m.p. 143–144 °C; ^{1}H NMR ($CDCl_3$): δ 8.23 (dd, 1H, J = 8.3 Hz, 1.7 Hz, Ar-H), 7.70 (td, 1H, J = 8.7 Hz, 1.6 Hz, Ar-H), 7.36–7.45 (m, 7H, Ar-H), 6.50 (d, 1H, J = 2.2 Hz, Ar-H), 6.43 (d, 1H, J = 2.2 Hz, Ar-H), 5.15 (s, 2H, Ph-CH_2); ^{13}C NMR ($CDCl_3$): δ 180.9, 165.9, 163.7, 135.8, 135.1, 128.9, 128.5, 127.6, 126.0, 124.1, 117.7, 104.2, 97.9, 93.7, 70.6; HRESIMS calcd for $C_{20}H_{13}O_4$ 317.0814; found 317.0820.

*3-Benzyl-1-hydroxy-6-methyl-9H-xanthen-9-one (**21**).* White solid: yield, 82%; R_f 0.66 (5 : 1, petroleum ether–EtOAc); m.p. 140–141 °C; ^{1}H NMR ($CDCl_3$): δ 12.95 (s, 1H, OH), 8.11 (d, 1H, J = 7.8 Hz, Ar-H), 7.36–7.45 (m, 5H, Ar-H), 7.21 (s, 1H, Ar-H), 7.18 (dd, 1H, J = 8.2 Hz, 0.9 Hz, Ar-H), 6.48 (d, 1H, J = 2.3 Hz, Ar-H), 6.42 (d, 1H, J = 2.3 Hz, Ar-H), 5.15 (s, 2H, Ph-CH_2), 2.50 (s, 3H, Ar-CH_3); ^{13}C NMR ($CDCl_3$): δ 180.8, 165.7, 163.6, 157.8, 156.3, 146.8, 135.9, 128.8, 128.7, 128.5, 127.6, 125.7, 125.6, 118.4, 117.5, 104.1, 97.8, 93.7, 70.6, 22.1; HRESIMS calcd for $C_{21}H_{15}O_4$ 331.0970; found 331.0976.

General synthetic procedure for compounds 22 and 23. Preparation of compounds **22** and **23**: To a solution of **20** (2.13 g, 6.69 mmol) in dry acetone (30 mL) were added, under argon, *p*-toluenesulfonyl chloride (1.53 g, 8.03 mmol) and anhydrous sodium carbonate (1.42 g, 13.38 mmol), and the mixture was refluxed for 40 h. The solvent was then vacuum-evaporated, and the residue was partitioned between EtOAc and NaOH 10%. The

organic phase was washed with water and brine, dried (Na_2SO_4), and concentrated to dryness. The residue was purified by flash chromatography (6 : 1, petroleum ether–EtOAc) to give **22**. Compound **23** was synthesized from **21** following the same procedure as **22** from **20**.

*3-Benzyl-1-[[(4-methylphenyl)sulfonyl]oxy]-9H-thioxanthen-9-one (**22**).* White solid, yield 92.7%; R_f 0.38 (5 : 1, petroleum ether–EtOAc); m.p. 180–181 °C; ^{1}H NMR ($CDCl_3$): δ 8.22 (dd, 1H, J = 7.7 Hz, 2.2 Hz, Ar-H), 7.96 (d, 2H, J = 7.7 Hz, Ar-H), 7.64 (t, 1H, J = 7.7 Hz, Ar-H), 7.43 (d, 3H, J = 7.4 Hz, Ar-H), 7.39 (t, 1H, J = 7.4 Hz, Ar-H), 7.36 (d, 1H, J = 8.8 Hz, Ar-H), 7.32 (d, 3H, J = 7.7 Hz, Ar-H), 6.90 (d, 1H, J = 2.2 Hz, Ar-H), 6.85 (d, 1H, J = 2.2 Hz, Ar-H), 5.14 (s, 2H, Ph-CH_2), 2.41 (s, 3H, Ar-CH_3); ^{13}C NMR ($CDCl_3$): δ 174.0, 162.8, 158.7, 155.2, 149.0, 145.4, 134.4, 129.7, 129.1, 128.9, 128.7, 127.7, 126.9, 124.2, 122.6, 117.3, 108.5, 101.2, 70.9, 21.8; HRESIMS calcd for $C_{27}H_{20}O_6SNa$ 495.0878; found 495.0883.

*3-Benzyl-1-[[(4-methylphenyl)sulfonyl]oxy]-6-methyl-9H-thioxanthen-9-one (**23**).* White solid: yield, 92.1%; R_f 0.56 (4 : 1, petroleum ether–EtOAc); m.p. 180–181 °C; ^{1}H NMR ($CDCl_3$): δ 8.09 (d, 1H, J = 8.1 Hz, Ar-H), 7.94 (d, 2H, J = 8.3 Hz, Ar-H), 7.37–7.43 (m, 4H, Ar-H), 7.31 (d, 2H, J = 7.9 Hz, Ar-H), 7.13–7.15 (m, 3H, Ar-H), 6.89 (d, 1H, J = 2.5 Hz, Ar-H), 6.81 (d, 1H, J = 2.5 Hz, Ar-H), 5.13 (s, 2H, Ph-CH_2), 2.47 (s, 3H, Ar-CH_3), 2.41 (s, 3H, Ar-CH_3); ^{13}C NMR ($CDCl_3$): δ 173.6, 162.4, 158.5, 155.0, 148.8, 145.7, 145.2, 135.2, 132.8, 129.5, 128.8, 128.6, 128.5, 127.6, 126.5, 125.5, 120.2, 116.9, 110.3, 108.1, 101.0, 70.7, 21.8, 21.6; HRESIMS calcd for $C_{28}H_{22}O_6SNa$ 509.1035; found 509.1038.

General synthetic procedure for compounds 24 and 25. Preparation of compounds **24** and **25**: To a solution of **22** (0.60 g, 1.27 mmol) in dry DMSO (5 mL) was added *N*,*N*-dimethyiethane-1,2-diamine (0.70 mL, 6.35 mmol), and the mixture was heated under argon, at 150 °C for 5 h. Upon cooling, the mixture was poured into water and extracted with EtOAc, the organic phase was dried (Na_2SO_4), and the solvent was vacuum-evaporated. The residue was purified by column chromatography (30 : 1, chloroform : methanol) to afford product **24**. Compound **25** was synthesized from **23** following the same procedure as **24** from **22**.

*3-Benzyl-1-[2-(dimethylamino)ethylamino]-9H-xanthen-9-one (**24**).* Yellow solid: yield 66.9%; R_f 0.45 (10 : 1, chloroform : methanol); m.p. 202–203 °C; ^{1}H NMR ($CDCl_3$): δ 9.62 (t, 1H, J = 6.6 Hz, NH), 8.22 (dd, 1H, J = 7.7 Hz, 1.1 Hz, Ar-H), 7.59 (td, 1H, J = 7.7 Hz, 1.6 Hz, Ar-H), 7.45 (d, 2H, J = 7.7 Hz, Ar-H), 7.41 (t, 2H, J = 7.7 Hz, ArH), 7.31–7.36 (m, 2H, Ar-H), 7.28 (t, 1H, J = 7.7 Hz, Ar-H), 6.21 (d, 1H, J = 1.8 Hz, Ar-H), 6.00 (d, 1H, J = 2.2 Hz, Ar-H), 5.15 (s, 2H, Ph-CH_2), 3.31 (q, 2H, J = 6.6 Hz, NCH_2); 2.67 (t, 2H, J = 6.6 Hz, NCH_2); 2.34 (s, 6H, 2 × NCH_3); ^{13}C NMR ($CDCl_3$): δ 178.4, 165.2, 159.9, 155.3, 153.0, 136.4, 123.7, 128.8, 128.3, 127.6, 126.1, 123.5, 122.3, 117.0, 102.7, 90.7, 89.3, 70.2, 57.9, 45.6, 41.1; HRESIMS calcd for $C_{24}H_{25}O_3N_2$ 389.1865; found 389.1872.

*3-Benzyl-1-[2-(dimethylamino)ethylamino]-6-methyl-9H-xanthen-9-one (**25**).* Yellow solid: yield 72.1%; R_f 0.26 (1 : 1, chloroform : acetone); m.p. 197–198 °C; ^{1}H NMR ($CDCl_3$): δ 9.63 (t, 1H, J = 6.6 Hz, N*H*), 8.09 (d, 1H, J = 8.3 Hz, 1.1 Hz, Ar-H), 7.45 (d, 1H, J = 7.3 Hz, Ar-H), 7.41 (t, 2H, J = 7.7 Hz, Ar-H), 7.35 (t, 1H, J = 7.3 Hz, Ar-H), 7.12 (s, 1H, Ar-H), 7.10 (d, 1H, J = 7.8 Hz, Ar-H), 6.19 (d, 1H, J = 2.3 Hz, Ar-H), 5.99 (d, 1H, J = 2.3 Hz, Ar-H), 5.14 (s, 2H, Ph-CH_2), 3.32 (q, 2H, J = 6.4 Hz, NCH_2); 2.68 (t, 2H, J = 6.6 Hz, NCH_2); 2.45 (s, 3H, Ar-CH_3), 2.34 (s, 6H, 2 × NCH_3); ^{13}C NMR ($CDCl_3$): δ 178.5, 165.0, 159.9, 155.4, 152.9, 145.0, 136.4, 128.8, 128.3, 127.6, 125.9, 125.0, 120.0, 116.9, 102.7, 90.7, 89.3, 70.2, 57.9, 45.6, 41.0, 21.9; HRESIMS calcd for $C_{25}H_{27}O_3N_2$ 403.2022; found 403.2025.

General synthetic procedure for compounds 26 and 27. Preparation of compounds **26** and **27**: A suspension of **24** (0.24 g, 0.62 mmol) and Pd/C (50 mg, 10%) in CH_2Cl_2–MeOH (1 : 1, 10 mL) was stirred under H_2 for 5 h and then filtered and concentrated. The residue was purified by silica gel column chromatography (30 : 1, chloroform : methanol) to give **26**. Compound **27** was synthesized from **25** following the same procedure as **26** from **24**.

*1-[2-(Dimethylamino)ethylamino]-9H-xanthen-9-one (**26**).* White solid: yield 92.4%; R_f 0.29 (10 : 1, chloroform : methanol); m.p. 263–264 °C; ^{1}H NMR (DMSO-d_6): δ 9.53 (brs, 1H, OH), 8.06 (d, 1H, J = 7.6 Hz, Ar-H), 7.72 (t, 1H, J = 6.7 Hz, Ar-H), 7.46 (d, 1H, J = 8.0 Hz, Ar-H), 7.37 (t, 1H, J = 7.3 Hz, Ar-H), 6.11 (s, 1H, Ar-H), 5.98 (s, 1H, Ar-H), 4.37 (s, 1H, NH), 2.97–3.46 (m, 4H, 2 × NCH_2), 2.56 (s, 6H, 2 × NCH_3); ^{13}C NMR (DMSO-d_6): δ 176.5, 164.6, 159.0, 154.4, 152.6, 134.0, 125.4, 123.7, 121.5, 116.9, 100.6, 91.0, 90.0, 57.0, 44.9; HRESIMS calcd for $C_{17}H_{17}O_3N_2$ 297.1239; found 297.1232.

*1-[2-(Dimethylamino)ethylamino]-6-methyl-9H-xanthen-9-one (**27**).* White solid; yield, 93.1%; R_f 0.29 (10 : 1, chloroform : methanol); m.p. 259–260 °C ^{1}H NMR ($CDCl_3$): δ 9.58 (t, 1H, J = 4.6 Hz, N*H*), 8.03 (d, 1H, J = 7.8 Hz, Ar-H), 7.05 (d, 1H, J = 8.3 Hz, Ar-H), 7.03 (s, 1H, Ar-H), 6.15 (s, 1H, Ar-H), 6.03 (s, 1H, Ar-H), 3.43 (q, 2H, J = 6.4 Hz, NCH_2), 2.84 (t, 2H, J = 6.4 Hz, NCH_2), 2.49 (s, 6H, 2 × NCH_3), 2.42 (s, 3H, Ph-CH_3); ^{13}C NMR ($CDCl_3$): δ 178.2, 164.5, 159.8, 155.3, 152.8, 144.7, 125.7, 124.7, 119.9, 116.8, 101.8, 91.4, 57.5, 46.0, 45.2, 40.3, 21.8; HRESIMS calcd for $C_{18}H_{19}O_3N_2$ 311.1396; found 311.1391.

General synthetic procedure for compounds 28 and 29. Preparation of compounds **28** and **29**: To a solution of **22** (0.70 g, 1.48 mmol) in dry DMSO (5 mL) was added 2-hydroxyethylhydrazine (0.11 mL, 1.63 mmol), and the mixture was heated under argon at 150 °C for 3 h. Upon cooling, the mixture was poured into water and extracted with CH_2Cl_2, the organic phase was dried (Na_2SO_4), and the solvent was vacuum-evaporated to provide a yellow residue. Methanesulfonyl chloride (0.13 mL, 1.67 mmol) was added to a stirred solution of the above yellow residue and triethylamine (0.29 mL, 2.10 mmol) in dry dichloromethane (20 mL) with cooling to 0 °C. After 30 min, the cooling bath was removed, and the mixture was stirred at room temperature for 4 h. The reaction mixture was partitioned between dichloromethane and 1 N sodium hydroxide. The organic phase was washed with HCl (10%), water, and brine, then dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (50 : 1, chloroform : methanol) to afford **28**. Compound **29** was synthesized from **23** following the same procedure as **28** from **22**.

*2-[4-Benzyloxy-chromeno[4,3,2-c,d]indazol-2-yl]-1-ethanol methanesulfonate (**28**).* white solid: yield 73.8%; R_f 0.56 (1 : 1,

petroleum ether–EtOAc); m.p. 171–172 °C; ^{1}H NMR ($CDCl_3$): δ 7.84 (dd, 1H, J = 7.8 Hz, 1.3 Hz, Ar-H), 7.47 (d, 2H, J = 7.7 Hz, Ar-H), 7.41 (t, 2H, J = 7.3 Hz, Ar-H), 7.35–7.36 (m, 2H, Ar-H), 7.27 (dd, 1H, J = 8.3 Hz, 0.9 Hz, Ar-H), 7.20 (td, 1H, J = 7.3 Hz, 1.4 Hz, Ar-H), 6.43 (d, 1H, J = 1.4 Hz, Ar-H), 6.33 (d, 1H, J = 1.4 Hz, Ar-H), 5.12 (s, 2H, Ph-CH_2), 4.68 (t, 2H, J = 5.0 Hz, NCH_2), 4.56 (t, 2H, J = 5.5 Hz, NCH_2), 2.74 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 163.3, 155.0, 150.1, 141.9, 139.1, 136.6, 130.3, 128.8, 128.2, 127.7, 124.4, 123.2, 118.5, 112.1, 93.6, 86.2, 71.0, 68.3, 48.6, 37.4; HRESIMS calcd for $C_{23}H_{20}O_5N_2SNa$ 459.0991; found 459.0997.

*2-[4-Benzyl-8-methyl-chromeno[4,3,2-c,d]indazol-2-yl]-1-ethanol methane–sulfonate (**29**).* White solid: yield 78.7%; R_f 0.57 (1 : 1, petroleum ether–EtOAc); m.p. 168–169 °C; ^{1}H NMR ($CDCl_3$): δ 7.75 (d, 1H, J = 7.7 Hz, Ar-H), 7.47 (d, 2H, J = 7.1 Hz, Ar-H), 7.41 (t, 2H, J = 7.1 Hz, Ar-H), 7.35 (d, 1H, J = 7.1 Hz, Ar-H), 7.10 (s, 1H, Ar-H), 7.03 (d, 1H, J = 7.1 Hz, Ar-H), 6.42 (d, 1H, J = 1.1 Hz, Ar-H), 6.32 (d, 1H, J = 1.1 Hz, Ar-H), 5.12 (s, 2H, Ph-CH_2), 4.69 (t, 2H, J = 4.9 Hz, NCH_2), 4.56 (t, 2H, J = 5.0 Hz, NCH_2), 2.75 (s, 3H, CH_3), 2.39 (s, 3H, Ar-CH_3); ^{13}C NMR ($CDCl_3$): δ 163.6, 154.9, 150.2, 141.8, 141.3, 139.1, 136.6, 128.8, 128.3, 127.7, 125.4, 123.2, 118.9, 114.8, 111.7, 93.7, 86.1, 71.0, 68.3, 48.5, 37.4, 21.7; HRESIMS calcd for $C_{24}H_{23}O_5N_2S$ 451.1328; found 451.1332.

General synthetic procedure for compounds 30 and 31. Preparation of compounds **30** and **31**: To a solution of **28** (0.58 g, 1.33 mmol) in dry DMSO (5 mL) and absolute ethanol (20 mL) was added diethylamine (1.40 mL, 13.3 mmol), and the mixture was heated under argon at 78 °C for 18 h. The mixture was concentrated *in vacuo*, and the residue was dissolved in chloroform. The organic phase was washed with water and brine, then dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (50 : 1, chloroform : methanol) to afford **30**. Compound **31** was synthesized from **29** following the same procedure as **30** from **28**.

*2-[4-Benzyl-chromeno[4,3,2-c,d]indazol-2-yl]-1-N,N-diethylethanamine (**30**).* Yellow solid: yield 92.7%; R_f 0.34 (1 : 1, chloroform : acetone); m.p. 203–204 °C; ^{1}H NMR ($CDCl_3$): δ 7.87 (dd, 1H, J = 7.7 Hz, 1.6 Hz, Ar-H), 7.46 (d, 2H, J = 7.1 Hz, Ar-H), 7.41 (td, 2H, J = 7.7 Hz, 1.7 Hz, Ar-H), 7.34 (td, 1H, J = 7.1 Hz, 2.2 Hz, Ar-H), 7.31 (dd, 1H, J = 7.1 Hz, 1.6 Hz, Ar-H), 7.24 (dd, 1H, J = 8.2 Hz, 1.1 Hz, Ar-H), 7.17 (td, 1H, J = 7.7 Hz, 1.1 Hz, Ar-H), 6.37 (s-like, 1H, Ar-H), 6.29 (d, 1H, J = 1.7 Hz, Ar-H), 5.12 (s, 2H, Ph-CH_2), 4.34 (t, 2H, J = 7.1 Hz, NCH_2), 2.96 (t, 2H, J = 6.6 Hz, NCH_2), 2.60 (q, 4H, J = 7.1 Hz, 2 × NCH_2–C), 1.03 (t, 6H, J = 7.1 Hz, 2 × N–C–CH_3); ^{13}C NMR ($CDCl_3$): δ 162.8, 154.9, 150.2, 141.1, 136.8, 129.8, 128.7, 128.2, 127.6, 124.2, 123.1, 118.6, 118.3, 112.2, 92.9, 86.4, 71.0, 52.4, 48.2, 47.6, 29.8, 11.9; HRESIMS calcd for $C_{26}H_{28}O_2N_3$ 414.2182; found 414.2186.

*2-[4-Benzyl-8-methyl-chromeno[4,3,2-c,d]indazol-2-yl]-1-N,N-diethylethana-mine (**31**).* Yellow solid: yield, 84.7%; R_f 0.36 (1 : 1, chloroform : acetone); m.p. 201–202 °C; ^{1}H NMR ($CDCl_3$): δ 7.75 (d, 1H, J = 7.7 Hz, Ar-H), 7.47 (d, 2H, J = 7.1 Hz, Ar-H), 7.40 (td, 2H, J = 8.0, 1.7 Hz, Ar-H), 7.34 (t, 1H, J = 7.1 Hz, Ar-H), 7.08 (s, 1H, Ar-H), 7.00 (d, 1H, J = 7.7 Hz, Ar-H), 7.17 (td, 1H, J = 7.7, 1.1 Hz, Ar-H), 6.40 (s-like, 1H, Ar-H), 6.28 (d, 1H, J = 1.1 Hz, Ar-H), 5.12 (s, 2H, Ph-CH_2), 4.38 (t, 2H, J = 7.1 Hz, NCH_2), 3.00 (t, 2H, J = 6.6 Hz, NCH_2), 2.64 (q, 4H, J = 7.1 Hz, 2 × NCH_2–C), 2.38 (s, 3H, Ar-CH_3), 1.04 (t, 6H, J = 7.1 Hz, 2 × N–C–CH_3); ^{13}C NMR ($CDCl_3$): δ 162.8, 154.8, 150.2, 141.1, 140.4, 138.2, 136.8, 128.7, 128.2, 127.6, 125.1, 122.8, 118.8, 115.8, 112.1, 92.9, 86.3, 71.0, 52.3, 47.6, 29.4, 21.6, 11.7; HRESIMS calcd for $C_{27}H_{30}O_2N_3$ 428.2338; found 428.2342.

General synthetic procedure for compounds 32 and 33. Preparation of compounds **32** and **33**: Boron trichloride (1.0 mL, 1 M in dichloromethane) was added dropwise under argon to a stirred solution of **30** (100 mg, 0.24 mmol) in dry dichloromethane (20 mL) with cooling to 0 °C. After 45 min, the cooling bath was removed, and ethanol (1.0 mL) was added dropwise. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography (50 : 1, chloroform : methanol) to afford **32**. Compound **33** was synthesized from **31** following the same procedure as **32** from **30**.

*2-(2-(Diethylamino)ethyl)-2H-chromeno[4,3,2-c,d]indazol-4-ol] (**32**).* Yellow solid: yield 84.5%; R_f 0.15 (1 : 1, chloroform : acetone); m.p. 235–236 °C; ^{1}H NMR ($CDCl_3$): δ 7.83 (dd, 1H, J = 7.7 Hz, 1.6 Hz, Ar-H), 7.27 (td, 1H, J = 7.7 Hz, 1.1 Hz, Ar-H), 7.17 (dd, 1H, J = 7.7 Hz, 1.1 Hz, Ar-H), 7.12 (td, 1H, J = 7.7 Hz, 1.1 Hz, Ar-H), 6.22 (s-like, 1H, Ar-H), 6.12 (s-like, 1H, Ar-H), 4.35 (t, 2H, J = 7.1 Hz, NCH_2), 3.03 (t, 2H, J = 7.1 Hz, NCH_2), 2.66 (q, 4H, J = 7.1 Hz, 2 × NCH_2–C), 1.03 (t, 6H, J = 7.1 Hz, 2 × N–C–CH_3); ^{13}C NMR ($CDCl_3$): δ 161.2, 154.8, 150.3, 141.4, 138.2, 129.9, 124.2, 123.2, 118.3 (two), 111.5, 93.1, 87.4, 51.8, 47.1, 10.9; HRESIMS calcd for $C_{19}H_{20}O_2N_3$ 322.1556; found 322.1563.

*2-(2-(Diethylamino)ethyl)-8-methyl-2H-chromeno[4,3,2-c,d] indazol-4-ol] (**33**).* Yellow solid: yield 84.5%; R_f 0.14 (1 : 1, chloroform : acetone); m.p. 232–233 °C; ^{1}H NMR ($CDCl_3$): δ 7.70 (d, 1H, J = 7.7 Hz, Ar-H), 7.00 (s, 1H, Ar-H), 6.95 (d, 1H, J = 7.7 Hz, Ar-H), 6.25 (s-like, 1H, Ar-H), 6.11 (s-like, 1H, Ar-H), 4.36 (t, 2H, J = 6.6 Hz, NCH_2), 3.05 (t, 2H, J = 7.7 Hz, NCH_2), 2.69 (q, 4H, J = 6.6 Hz, 2 × NCH_2–C), 2.34 (s, 3H, Ar-CH_3), 1.05 (t, 6H, J = 7.7 Hz, 2 × N–C–CH_3); ^{13}C NMR ($CDCl_3$): δ 161.1, 154.8, 150.3, 141.3, 140.4, 138.5, 125.1, 122.9, 118.7, 115.4, 111.3, 93.0, 87.3, 51.7, 47.1, 46.9, 29.8, 29.5, 22.8, 21.6, 10.7; HRESIMS calcd for $C_{20}H_{22}O_2N_3$ 336.1712; found 336.1722.

General procedure for the preparation of 35–38. To a solution of compounds **16–19** (1 eq), the L-rhamnopyranosyl trichloroacetimidate **34** (1.3 eq) and 4 Å molecular sieves in dry CH_2Cl_2 was added $BF_3 \cdot Et_2O$ (1 eq) at 0 °C under argon. The reaction mixture was stirred for 2 h under this condition, while warmed to room temperature for 4 h. To the above solution was added $BF_3 \cdot Et_2O$ (2 eq) at room temperature until TLC indicated that the reaction was complete. The reaction was quenched by Et_3N and concentrated to provide the different yellow residue. Then the residue was purified by silica gel column chromatography (petroleum ether–acetone) to afford compounds **35–38**, respectively.

*1-Hydroxy-3-(2′,3′-di-O-acetyl-4′-levulinyl-α-L-rhamnopyranosyl)-9H-xanthen-9-one (**35**).* White solid in 88.6% yield; R_f 0.29 (2 : 1, petroleum ether–EtOAc); m.p. 172–173 °C; $[\alpha]_D^{25}$ −117.8 (*c* 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.22 (dd, 1H, J = 7.7 Hz, 1.1 Hz, Ar-H), 7.70 (td, 1H, J = 8.2 Hz, 1.1 Hz, Ar-H), 7.42 (d, 1H, J = 8.3 Hz, Ar-H), 7.37 (t, 1H, J = 7.3 Hz, Ar-H), 6.61 (d, 1H, J = 2.2 Hz, Ar-H), 6.51 (d, 1H, J = 2.2 Hz, Ar-H), 5.55 (d, 1H, J = 1.3 Hz, H-1′), 5.50 (dd, 1H, J = 9.9 Hz, 3.3 Hz, H-3′), 5.43 (dd, 1H, J = 3.3 Hz, 1.7 Hz,

H-2′), 5.17 (t, 1H, J = 9.9 Hz, H-4′), 3.93–3.95 (m, 1H, H-5′), 2.70–2.77 (m, 2H, $COCH_2$), 2.49–2.55 (m, 2H, $COCH_2$), 2.19, 2.16, 2.09 (s, each 3H, 3 × CH_3CO), 1.22 (t, 3H, J = 6.0 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 206.1, 181.1, 171.9, 170.3, 170.1, 163.7, 162.5, 157.6, 156.1, 135.3, 126.0, 124.3, 120.6, 117.8, 105.1, 99.1, 95.6, 95.0, 76.9, 69.5, 67.9, 37.8, 29.8, 28.0, 20.9, 20.8, 17.5; HRESIMS calcd for $C_{28}H_{28}O_{12}Na$ 579.1478; found 579.1485.

*1-Hydroxy-3-(2′,3′-di-O-acetyl-4′-levulinyl-α-L-rhamnopyranosyl)-6-methyl-9H-xanthen-9-one (**36**).* White solid in 85.6% yield; R_f 0.28 (2 : 1, petroleum ether–EtOAc); m.p. 169–170 °C; $[\alpha]_D^{25}$ −102.6 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.90 (s, 1H, OH), 8.12 (d, 1H, J = 8.2 Hz, Ar-H), 7.24 (s, 1H, Ar-H), 7.19 (dd, 1H, J = 8.2, 1.1 Hz, Ar-H), 6.62 (d, 1H, J = 2.2 Hz, Ar-H), 6.52 (d, 1H, J = 2.2 Hz, Ar-H), 5.56 (d, 1H, J = 1.6 Hz, H-1′), 5.52 (dd, 1H, J = 10.4 Hz, 3.8 Hz, H-3′), 5.44 (dd, 1H, J = 3.3 Hz, 1.7 Hz, H-2′), 5.18 (t, 1H, J = 9.9 Hz, H-4′), 3.94–3.97 (m, 1H, H-5′), 2.74–2.77 (m, 2H, $COCH_2$), 2.53–2.55 (m, 2H, $COCH_2$), 2.51 (s, 3H, Ar-CH_3), 2.20, 2.19, 2.09 (s, each 3H, each $COCH_3$), 1.24 (d, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 206.1, 181.0, 171.9, 170.3, 170.1, 163.7, 157.6, 156.3, 147.1, 125.8, 118.4, 117.6, 105.1, 99.0, 95.6, 95.0, 70.9, 69.5, 68.5, 67.9, 37.8, 29.8, 28.0, 22.1, 20.9, 20.8, 17.5; HRESIMS calcd for $C_{29}H_{31}O_{12}$ 571.1816; found 571.1818.

*1-Hydroxy-6-(2′,3′-di-O-acetyl-4′-levulinyl-α-L-rhamnopyranosyl)-3-methyl-9H-xanthen-9-one (**37**).* Yellow solid in 84.6% yield; R_f 0.47 (1 : 1, petroleum ether–EtOAc); m.p. 169–170 °C $[\alpha]_D^{25}$ −118.2 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.59 (s, 1H, OH), 8.21 (d, 1H, J = 8.8 Hz, Ar-H), 7.13 (d, 1H, J = 1.7 Hz, Ar-H), 7.10 (dd, 1H, J = 8.8 Hz, 1.9 Hz, Ar-H), 6.73 (d, 1H, J = 2.2 Hz, Ar-H), 6.63 (d, 1H, J = 2.2 Hz, Ar-H), 5.62 (d, 1H, J = 1.6 Hz, H-1′), 5.55 (dd, 1H, J = 10.1 Hz, 3.5 Hz, H-3′), 5.47 (dd, 1H, J = 3.5 Hz, 1.7 Hz, H-2′), 5.21 (t, 1H, J = 10.0 Hz, H-4′), 3.94–3.97 (m, 1H, H-5′), 2.75–2.77 (m, 2H, $COCH_2$), 2.53–2.55 (m, 2H, $COCH_2$), 2.51 (s, 3H, Ar-CH_3), 2.22, 2.19, 2.11 (s, each 3H, each $COCH_3$), 1.25 (d, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 206.0, 180.8, 171.7, 170.3, 170.0, 161.5, 161.0, 157.6, 156.3, 148.5, 127.7, 115.9, 113.9, 111.5, 107.4, 106.8, 103.4, 95.5, 70.7, 69.4, 68.3, 67.9, 37.7, 29.7, 27.9, 22.6, 20.9, 20.8, 17.4; HRESIMS calcd for $C_{29}H_{31}O_{12}$ 571.1816; found 571.1809.

*1-Hydroxy-3-(2′,3′-di-O-acetyl-4′-levulinyl-α-L-rhamnopyranosyl)-9H-thioxanthen-9-one (**38**).* Yellow solid in 85.9% yield; R_f 0.31 (2 : 1, petroleum ether–EtOAc); m.p. 156–157 °C; $[\alpha]_D^{25}$ −148.5 (*c* 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.56 (dd, 1H, J = 7.1 Hz, 1.1 Hz, Ar-H), 7.62 (td, 1H, J = 7.1 Hz, 1.1 Hz, Ar-H), 7.52 (d, 1H, J = 7.1 Hz, Ar-H), 7.48 (t, 1H, J = 7.1 Hz, Ar-H), 6.77 (d, 1H, J = 2.7 Hz, Ar-H), 6.65 (d, 1H, J = 2.7 Hz, Ar-H), 5.57 (d, 1H, J = 1.1 Hz, H-1′), 5.51 (dd, 1H, J = 9.9 Hz, 3.3 Hz, H-3′), 5.42 (dd, 1H, J = 3.3 Hz, 1.6 Hz, H-2′), 5.18 (t, 1H, J = 9.9 Hz, H-4′), 3.93–3.96 (m, 1H, H-5′), 2.74–2.77 (m, 2H, $COCH_2$), 2.52–2.55 (m, 2H, $COCH_2$), 2.20, 2.18, 2.09 (s, each 3H, 3 × CH_3CO), 1.24 (t, 3H, J = 6.0 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 206.1, 184.5, 171.9, 170.3, 170.1, 167.4, 140.6, 137.3, 132.9, 129.4, 128.3, 126.5, 125.5, 110.7, 103.3, 101.9, 95.5, 70.9, 69.5, 68.4, 68.0, 37.8, 29.8, 28.0, 20.9, 20.8, 17.5; HRESIMS calcd for $C_{28}H_{28}O_{11}SNa$ 595.1250; found 595.1262.

General procedure for the preparation of 1–4. To a stirred solution of the different yellow compounds **35–38** (1 eq) in CH_2Cl_2 and CH_3OH (v/v = 1/1) was added NH_2NH_2–HOAc (10 eq). After 3 h, the solution was concentrated. Then the residue was purified by silica gel column chromatography (petroleum ether–acetone) to afford compounds **1–4**, respectively.

*1-Hydroxy-3-(2′,3′-di-O-acetyl-α-L-rhamnopyranosyl)-9H-xanthen-9-one (**1**).* White solid in 85.1% yield; R_f 0.25 (3 : 1, petroleum ether–acetone); m.p. 193–194 °C; $[\alpha]_D^{25}$ −87.8 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.25 (dd, 1H, J = 7.7 Hz, 1.7 Hz, Ar-H), 7.73 (td, 1H, J = 7.7 Hz, 1.6 Hz, Ar-H), 7.44 (d, 1H, J = 8.8 Hz, Ar-H), 7.38 (td, 1H, J = 7.7 Hz, 1.1 Hz, Ar-H), 6.64 (d, 1H, J = 2.2 Hz, Ar-H), 6.54 (d, 1H, J = 2.2 Hz, Ar-H), 5.55 (d, 1H, J = 1.7 Hz, H-1′), 5.43 (dd, 1H, J = 3.3 Hz, 1.7 Hz, H-2′), 5.33 (dd, 1H, J = 9.9 Hz, 3.3 Hz, H-3′), 3.83–3.86 (m, 1H, H-5′), 3.74 (t, 1H, J = 10.2 Hz, H-2′), 2.19, 2.13 (s, each 3H, 2 × CH_3CO), 1.36 (t, 3H, J = 6.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 181.2, 171.2, 170.0, 163.7, 162.7, 157.6, 156.2, 135.3, 126.0, 124.2, 120.7, 117.8, 105.1, 99.1, 95.7, 95.1, 71.8, 71.1, 70.1, 69.6, 31.0, 21.0, 20.9, 17.7; HRESIMS calcd for $C_{23}H_{21}O_{10}$ 457.1135; found 457.1141.

*1-Hydroxy-3-(2′,3′-di-O-acetyl-α-L-rhamnopyranosyl)-6-methyl-9H-xanthen-9-one (**2**).* White solid in 81.6% yield; R_f 0.67 (2 : 1, petroleum ether–acetone); m.p. 191–192 °C; $[\alpha]_D^{25}$ −122.8 (*c* 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.89 (s, 1H, OH), 8.11 (d, 1H, J = 8.2 Hz, Ar-H), 7.23 (brs, 1H, Ar-H), 7.18 (dd, 1H, J = 8.3 Hz, 1.0 Hz, Ar-H), 6.61 (d, 1H, J = 2.3 Hz, Ar-H), 6.52 (d, 1H, J = 2.2 Hz, Ar-H), 5.55 (d, 1H, J = 1.8 Hz, H-1′), 5.43 (dd, 1H, J = 3.7 Hz, 1.9 Hz, H-2′), 5.32 (dd, 1H, J = 10.0 Hz, 3.7 Hz, H-3′), 3.83–3.86 (m, 1H, H-5′), 3.74 (t, 1H, J = 9.6 Hz, H-4′), 2.51 (s, 3H, Ar-CH_3), 2.19, 2.13 (s, each 3H, each $COCH_3$), 1.36 (d, 3H, J = 6.4 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 181.0, 171.2, 170.0, 163.6, 162.5, 157.6, 156.3, 147.0, 125.7, 118.4, 117.6, 105.0, 98.9, 95.7, 95.1, 71.8, 71.1, 70.0, 69.7, 22.1, 21.0, 20.9, 17.7; HRESIMS calcd for $C_{24}H_{23}O_{10}$ 471.1291; found 471.1300.

*1-Hydroxy-6-(2′,3′-di-O-acetyl-α-L-rhamnopyranosyl)-3-methyl-9H-xanthen-9-one (**3**).* White solid in 80.8% yield; R_f 0.35 (3 : 1, petroleum ether–acetone); m.p. 181–182 °C; $[\alpha]_D^{25}$ −112.5 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 12.60 (s, 1H, OH), 8.20 (d, 1H, J = 8.8 Hz, Ar-H), 7.12 (d, 1H, J = 2.2 Hz, Ar-H), 7.09 (dd, 1H, J = 8.8 Hz, 2.2 Hz, Ar-H), 6.73 (s, 1H, Ar-H), 6.62 (s, 1H, Ar-H), 5.60 (d, 1H, J = 1.6 Hz, H-1′), 5.46 (dd, 1H, J = 3.4 Hz, 1.8 Hz, H-2′), 5.36 (dd, 1H, J = 9.8 Hz, 3.5 Hz, H-3′), 3.83–3.88 (m, 1H, H-5′), 3.76 (t, 1H, J = 9.7 Hz, H-4′), 2.43 (s, 3H, Ar-CH_3), 2.21, 2.14 (s, each 3H, each $COCH_3$), 1.38 (d, 3H, J = 6.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 181.0, 171.1, 170.0, 161.6, 161.4, 157.7, 156.3, 148.5, 127.8, 115.8, 114.0, 111.4, 107.4, 106.8, 103.6, 95.6, 71.6, 70.9, 70.0, 69.6, 22.6, 21.0, 20.8, 17.6; HRESIMS calcd for $C_{24}H_{23}O_{10}$ 471.1291; found 471.1300.

*1-Hydroxy-3-(2′,3′-di-O-acetyl-α-L-rhamnopyranosyl)-9H-thioxanthen-9-one (**4**).* Yellow solid in 82.3% yield; R_f 0.28 (3 : 1, petroleum ether–acetone); m.p. 186–187 °C; $[\alpha]_D^{25}$ −138.4 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.55 (dd, 1H, J = 8.2 Hz, 1.1 Hz, Ar-H), 7.61 (td, 1H, J = 8.3 Hz, 1.7 Hz, Ar-H), 7.51 (d, 1H, J = 8.2 Hz, Ar-H), 7.47 (td, 1H, J = 8.2 Hz, 1.1 Hz, Ar-H), 6.76 (d, 1H, J = 2.2 Hz, Ar-H), 6.65 (d, 1H, J = 2.2 Hz, Ar-H), 5.55 (d, 1H, J = 1.7 Hz, H-1′), 5.42 (dd, 1H, J = 3.3 Hz, 1.7 Hz, H-2′), 5.32 (dd, 1H, J = 9.9 Hz, 3.3 Hz, H-3′), 3.82–3.85 (m, 1H, H-5′), 3.73 (t, 1H, J = 10.2 Hz, H-4′), 2.19, 2.13 (s, each 3H, 2 × $COCH_3$), 1.36 (t, 3H, J = 6.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 184.4, 171.2, 170.0, 167.3, 160.7, 140.5, 137.3, 132.8, 129.3, 128.3, 126.5, 125.5, 110.6, 103.4,

101.9, 95.6, 71.8, 71.0, 70.1, 69.6, 21.0, 20.9, 17.7; HRESIMS calcd for $C_{23}H_{21}O_9S$ 473.0906; found 473.0903.

General procedure for the preparation of 5–7. To a stirred solution of compound **1**, **2** or **4** in CH_2Cl_2 and CH_3OH (v/v = 1/1) was added MeONa (cat.). The reaction mixture was stirred at room temperature for 30 min, after which the reaction mixture was neutralized with Dowex 50 × 8 (H^+) resin until pH 7, filtered and concentrated. The residue was recrystallized from absolute ethanol to give compounds **5–7**, respectively.

*1-Hydroxy-3-(α-L-rhamnopyranosyl)-9H-xanthen-9-one (**5**).* White solid in 92.3% yield; R_f 0.39 (10 : 1, chloroform : methanol); m.p. 229–230 °C; $[\alpha]_D^{25}$ −116.0 (*c* 0.10, DMSO); ^{1}H NMR (DMSO-d_6): δ 8.13 (d, 1H, J = 7.7 Hz, Ar-H), 7.85 (t, 1H, J = 7.7 Hz, Ar-H), 7.59 (d, 1H, J = 8.2 Hz, Ar-H), 7.47 (t, 1H, J = 7.7 Hz, Ar-H), 6.63 (s, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 5.56 (s, 1H, H-1′), 5.18 (s, 1H, OH), 4.94 (s, 1H, OH), 4.85 (s, 1H, OH), 3.65 (dd, 1H, J = 8.8 Hz, 2.2 Hz, H-3′), 3.40–3.46 (m, 1H, H-5′), 3.29–3.32 (m, 2H, H-2′, H-4′), 1.12 (d, 3H, J = 6.1 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ 180.5, 163.7, 157.8, 156.0, 136.4, 125.9, 125.0, 120.6, 118.3, 99.6, 99.0, 72.7, 70.8, 70.7, 70.4, 18.5; HRESIMS calcd for $C_{19}H_{17}O_8$ 373.0923; found 373.0932.

*1-Hydroxy-3-(α-L-rhamnopyranosyl)-6-methyl-9H-xanthen-9-one (**6**).* White solid in 91.6% yield; R_f 0.40 (10 : 1, chloroform : methanol); m.p. 227–228 °C; $[\alpha]_D^{25}$ −100.7 (*c* 0.16, DMSO); ^{1}H NMR (DMSO-d_6): δ 8.50 (s, 1H, OH), 8.45 (dd, 1H, J = 8.2, 1.1 Hz, Ar-H), 7.77–7.81 (m, 2H, Ar-H), 7.59 (td, 1H, J = 8.2 Hz, 1.1 Hz, Ar-H), 6.97 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 5.61 (s, 1H, H-1′), 3.85 (dd, 1H, J = 3.3 Hz, 1.6 Hz, H-2′), 3.65 (dd, 1H, J = 9.4 Hz, 3.3 Hz, H-3′), 3.30–3.46 (m, 2H, H-4′, H-5′), 1.12 (d, 3H, J = 6.0 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ 166.8, 161.9, 140.5, 137.2, 134.1, 129.2, 127.4, 126.6, 102.2, 98.9, 72.2, 70.8, 70.7, 70.3, 18.5; HRESIMS calcd for $C_{20}H_{19}O_8$ 387.1080; found 387.1069.

*1-Hydroxy-3-(α-L-rhamnopyranosyl)-9H-thioxanthen-9-one (**7**).* Yellow solid in 92.6% yield; R_f 0.39 (10 : 1, chloroform : methanol); m.p. 214–215 °C; $[\alpha]_D^{25}$ −49.3 (*c* 0.11, DMSO); ^{1}H NMR (DMSO-d_6): δ 8.50 (s, 1H, OH), 8.45 (dd, 1H, J = 8.2 Hz, 1.1 Hz, Ar-H), 7.77–7.81 (m, 2H, Ar-H), 7.59 (td, 1H, J = 8.2 Hz, 1.1 Hz, Ar-H), 6.97 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 5.61 (s, 1H, H-1′), 3.85 (dd, 1H, J = 3.3 Hz, 1.6 Hz, H-2′), 3.65 (dd, 1H, J = 9.4 Hz, 3.3 Hz, H-3′), 3.30-3.46 (m, 2H, H-4′, H-5′), 1.12 (d, 3H, J = 6.0 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ 166.8, 161.9, 140.5, 137.2, 134.1, 129.2, 127.4, 126.6, 102.2, 98.9, 72.2, 70.8, 70.7, 70.3, 18.5; HRESIMS calcd for $C_{19}H_{17}O_7S$ 389.0695; found 389.0699.

General procedure for the preparation of 8–11. To a solution of compounds **26–27** or **32–33** (1 eq), the L-rhamnopyranosyl trichloroacetimidate **34** (1.3 eq) and 4 Å molecular sieves in dry CH_2Cl_2 was added $BF_3{\cdot}Et_2O$ (4 eq) at 0 °C under argon. The reaction mixture was stirred for 2 h under this condition, while warmed to room temperature until TLC indicated that the reaction was complete. The reaction was quenched by Et_3N and concentrated to provide the different yellow residue. To a stirred solution of the different yellow residue in CH_2Cl_2 and CH_3OH (v/v = 1/1) was added NH_2NH_2–HOAc (10 eq). After 3 h, the solution was concentrated. Then the residue was purified by silica gel column chromatography (petroleum ether–acetone) to afford compounds **8–11**, respectively.

*1-[2-(Dimethylamino)ethylamino]-3-(2′,3′-di-O-acetyl-α-L-rhamnopyranosyl)-9H-xanthen-9-one (**8**).* Yellow solid in 82.3% yield; R_f 0.19 (20 : 1, chloroform : methanol); m.p. 223–224 °C; $[\alpha]_D^{25}$ −45.4 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 9.65 (t, 1H, J = 5.5 Hz, N*H*), 8.21 (d, 1H, J = 7.7 Hz, Ar-H), 7.61 (td, 1H, J = 7.7 Hz, 2.2 Hz, Ar-H), 7.34 (d, 1H, J = 8.8 Hz, Ar-H), 7.30 (t, 1H, J = 7.7 Hz, Ar-H), 6.32 (d, 1H, J = 2.2 Hz, Ar-H), 6.11 (d, 1H, J = 2.2 Hz, Ar-H), 5.57 (s-like, 1H, H-1′), 5.43 (dd, 1H, J = 3.3 Hz, 1.7 Hz, H-2′), 5.37 (dd, 1H, J = 9.9 Hz, 3.3 Hz, H-3′), 3.88-3.90 (m, 1H, H-5′), 3.73 (t, 1H, J = 9.9 Hz, H-4′), 3.38 (q, 2H, J = 5.5 Hz, NCH_2), 2.76 (td, 2H, J = 5.5 Hz, 2.2, NCH_2), 2.41 (s, 6H, 2 × NCH_3), 2.19, 2.13 (s, each 3H, each CH_3CO), 1.37 (d, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 178.7, 171.2, 170.1, 162.1, 155.3, 152.9, 133.9, 126.1, 123.6, 122.2, 117.1, 103.4, 95.4, 91.8, 90.7, 71.9, 69.9, 69.8, 57.6, 45.4, 40.8, 29.8, 21.1, 20.9, 17.8; HRESIMS calcd for $C_{27}H_{33}N_2O_9$ 529.2186; found 529.2201.

*1-[2-(Dimethylamino)ethylamino]-3-(2′,3′-di-O-acetyl-α-L-rhamnopyranosyl)-6-methyl-9H-xanthen-9-one (**9**).* Yellow solid in 84.1% yield; R_f 0.18 (20 : 1, chloroform : methanol); m.p. 219–220 °C; $[\alpha]_D^{25}$ −62.9 (*c* 0.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 9.67 (t, 1H, J = 5.5 Hz, N*H*), 8.06 (d, 1H, J = 7.7 Hz, Ar-H), 7.13 (s, 1H, Ar-H), 7.11 (d, 1H, J = 8.8 Hz, Ar-H), 6.31 (d, 1H, J = 2.2 Hz, Ar-H), 6.14 (d, 1H, J = 2.2 Hz, Ar-H), 5.58 (s-like, 1H, H-1′), 5.43 (dd, 1H, J = 3.3 Hz, 2.2 Hz, H-2′), 5.38 (dd, 1H, J = 9.9 Hz, 3.3 Hz, H-3′), 3.91–3.94 (m, 1H, H-5′), 3.72 (t, 1H, J = 9.9 Hz, H-4′), 3.48 (q, 2H, J = 5.5 Hz, NCH_2), 2.88 (t, 2H, J = 5.5 Hz, NCH_2), 2.51 (s, 6H, 2 × NCH_3), 2.46 (s, 3H, Ar-CH_3), 2.19, 2.12 (s, each 3H, each CH_3CO), 1.36 (d, 3H, J = 5.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 178.8, 171.2, 170.2, 161.9, 159.6, 155.4, 152.6, 145.4, 125.9, 125.2, 119.8, 117.1, 103.5, 95.4, 92.1, 91.1, 69.8, 57.0, 44.8, 40.0, 29.8, 21.9, 21.1, 21.0, 17.8; HRESIMS calcd for $C_{28}H_{35}N_2O_9$ 543.2343; found 543.2328.

*2-[4-O-(2′,3′-Di-O-acetyl-α-L-rhamnopyranosyl)-2H-chromeno[4,3,2-c,d]in-dazol-2-yl]-1-N,N-diethylethanamine (**10**).* Yellow solid in 84.7% yield; R_f 0.23 (20 : 1, chloroform : methanol); m.p. 195–196 °C; $[\alpha]_D^{25}$ −83.4 (*c* 0.11, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.87 (d, 1H, J = 7.7 Hz, Ar-H), 7.34 (t, 1H, J = 7.7 Hz, Ar-H), 7.26 (d, 1H, J = 7.7 Hz, Ar-H), 7.19 (t, 1H, J = 7.7 Hz, Ar-H), 6.59 (s-like, 1H, Ar-H), 6.34 (s-like, 1H, Ar-H), 5.48 (s-like, 1H, H-1′), 5.45 (dd, 1H, J = 3.3 Hz, 2.2 Hz, H-2′), 5.37 (dd, 1H, J = 9.9 Hz, 3.3 Hz, H-3′), 4.40 (brs, 2H, NCH_2), 3.93-3.95 (m, 1H, H-5′), 3.73 (t, 1H, J = 9.9 Hz, H-4′), 3.04 (brs, 2H, NCH_2), 2.68 (brs, 4H, 2 × NCH_2–C), 2.19, 2.13 (s, each 3H, each CH_3CO), 1.37 (d, 3H, J = 5.5 Hz, CH_3), 1.07 (t, 6H, J = 6.6 Hz, 2 × N–C–CH_3); ^{13}C NMR ($CDCl_3$): δ 171.3, 170.1, 159.7, 154.8, 150.2, 140.8, 130.0, 124.3, 123.2, 118.4, 113.0, 96.7, 93.5, 89.1, 72.0, 69.7, 52.1, 47.6, 29.8, 21.1, 21.0, 17.7, 11.6; HRESIMS calcd for $C_{29}H_{36}N_3O_8$ 554.2502; found 554.2512.

*2-[4-O-(2′,3′-Di-O-acetyl-α-L-rhamnopyranosyl)-8-methyl-2H-chromeno[4,3,2-c,d]indazol-2-yl]-1-N,N-diethylethanamine (**11**).* Yellow solid in 86.2% yield; m.p. 193–194 °C; $[\alpha]_D^{25}$ −74.3 (*c* 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.75 (d, 1H, J = 7.7 Hz, Ar-H), 7.09 (s, 1H, Ar-H), 7.00 (d, 1H, J = 7.7 Hz, Ar-H), 6.58 (s-like, 1H, Ar-H), 6.33 (s-like, 1H, Ar-H), 5.48 (d, 1H, J = 2.2 Hz, H-1′), 5.45 (dd, 1H, J = 3.3, 2.2 Hz, H-2′), 5.36 (dd, 1H, J = 9.9 Hz, 3.3 Hz, H-3′), 4.39 (brs, 2H, NCH_2), 3.92–3.95 (m, 1H, H-5′), 3.73 (t, 1H, J = 9.9 Hz,

H-4′), 3.04 (t, 2H, $J = 5.5$ Hz, NCH_2), 2.68 (q, 4H, $J = 6.6$ Hz, 2 × NCH_2–C), 2.39 (s, 3H, Ar-CH_3), 2.19, 2.13 (s, each 3H, each CH_3CO), 1.37 (d, 3H, $J = 6.5$ Hz, CH_3), 1.07 (t, 6H, $J = 6.6$ Hz, 2 × N–C–CH_3); ^{13}C NMR ($CDCl_3$): δ 171.3, 170.1, 159.6, 154.8, 150.2, 140.7, 140.6, 138.3, 125.2, 122.9, 118.8, 115.6, 112.9, 96.7, 93.3, 89.0, 71.2, 70.0, 52.1, 47.5, 21.6, 21.1, 21.0, 17.7, 11.6; HRESIMS calcd for $C_{30}H_{38}N_3O_8$ 568.2659; found 568.2658.

4.3 Bioassay

4.3.1 Cytotoxic assay. The cytotoxicity of compounds was examined using a normal cell line, human microvascular endothelial cell line (HMEC) and a panel of human tumor cell lines, including a human lung carcinoma cell line (A549), a human promyelocytic leukemia cell line (HL-60), and a human breast carcinoma cell lines (MDA-MB-231). Cells were seeded into 96-well plates and treated in triplicate with gradient concentrations of compounds at 37 °C for 72 h. Cytotoxicity against leukemia cells was assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay as previously described.[21] Sulforhodamine B (SRB) assay was applied to adherent tumor cells. The cytotoxicity of compounds was expressed as an IC_{50}, determined by the Logit method at least three independent experiments.[22]

4.3.2 Cell cycle assay. The cell cycle was analyzed by flow cytometry. Briefly, KB cells were treated with different concentrations of compound **11** (0.1–1.0 μM) for 24 h. After incubation, a total of 1×10^8 cells were harvested from the treated and normal samples. The cells were washed twice with PBS and fixed in 75% ice-cold ethanol for at least overnight. The sample was concentrated by removing the ethanol and staining the cellular DNA with fluorescent solution (1% (v/v) Triton X-100, 0.01% RNase, 0.05% PI) for 30 min at 4 °C in darkness. The cell cycle distribution was then detected by flow cytometry. All experiments were performed three times.[23]

4.3.3 Western blotting. Tumor cells (2×10^5 mL^{-1}) were treated with compound **11** at the indicated times. The standard Western blotting was performed, and the proteins were recognized with appropriate antibodies such as pro-caspase 3, cleaved caspase 3, PARP, pro-caspase-9, cleaved caspase 9, caspase 8 and visualized by ECL system.[24]

4.3.4 Topoisomerase I-mediated supercoiled pBR322 relaxation. Relaxation assays were carried out in a final volume of 20 μL containing Topoisomerase I reaction buffer (10 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM PMSF and 1 mM mercaptoethanol), 0.25 μg supercoiled pBR322 and 1 unit of Topoisomerase I (1 unit of Topoisomerase I can relax 0.25 μg of supercoiled DNA in 15 min at 37 °C). Reaction were employed at 37 °C for 30 min and terminated by the addition of 2 μL of 10% SDS. Reaction products were separated on a 1% agarose gel for catalytic assays.[25]

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