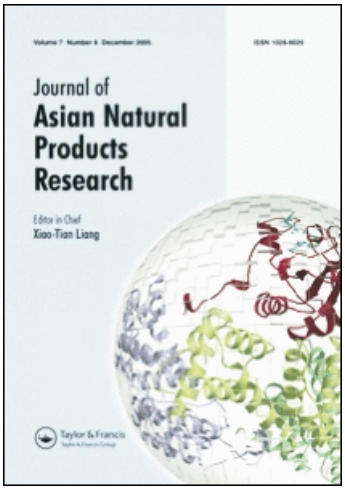

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ORIGINAL ARTICLE

Synthesis and MDR inhibitory activity evaluation of derivatives of schizandrin A

Xiao-Xia Liang[a], Geng-Tao Liu[b], Qiao-Hong Chen[a], Hua Sun[b], Dong-Lin Chen[a] and Feng-Peng Wang[a]*

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Eighteen schizandrin A derivatives, possessing an acyl group at 7-OH and/or halogen(s) at C-4 and C-11, were designed and synthesized for evaluation of their *in vitro* ability to inhibit multidrug resistance (MDR). They exhibit weak ability to restore the intracellular Rhodamine 123 in human hepatocarcinoma MDR cell lines Bel7402 and HCT8 relative to the reference drug verapamil.

Keywords: schizandrin A; lignans; multidrug resistance inhibitory activity

1. Introduction

Multidrug resistance (MDR) constitutes a major limitation in current cancer chemotherapy. Overexpression of P-glycoprotein (P-gp) and MDR-associated protein 1 (MRP1) account for the majority of cases of MDR of cancer cells [1–4]. Both of them are ATP-dependent multidrug transporters [5], displaying cross-resistance to many different anti-cancer drugs [6–8]. However, P-gp transports hydrophobic substrates; while MRP1 targets hydrophilic molecules [9–11].

Numerous compounds have already been discovered to overcome MDR by restoring the intracellular accumulation of antitumor agents in resistant cells. A few of them are currently under clinical evaluation. However, currently available MDR reversal agents still have some limitations including high toxicity, low inhibitory activity, and/or some side effects. The first generation of them (e.g. verapamil and cyclosporine A) modulates MDR at very high concentration with enhanced toxicity to normal cells. The second generation of them (e.g dexniguldipine [12] and dexverapamil [13]) showed more selectivity against P-gp but not more potency. GG918 [14] and PSC833 [15] display a stronger activity compared to cyclosporin A [16], but they can intensify the toxic side effects of anticancer drugs by blocking their metabolizing enzymes or interacting with other drug efflux transporters. Therefore, searching for more potent MDR inhibitors with less toxicity or without side effect is still in need.

It was reported [5,17–19] that schizandrins A (**1**) and B (**2**), and gomisin A (**3**), dibenzocyclooctadiene lignans in *Schisandra chinensis* (Turcz.) Baill, are potent

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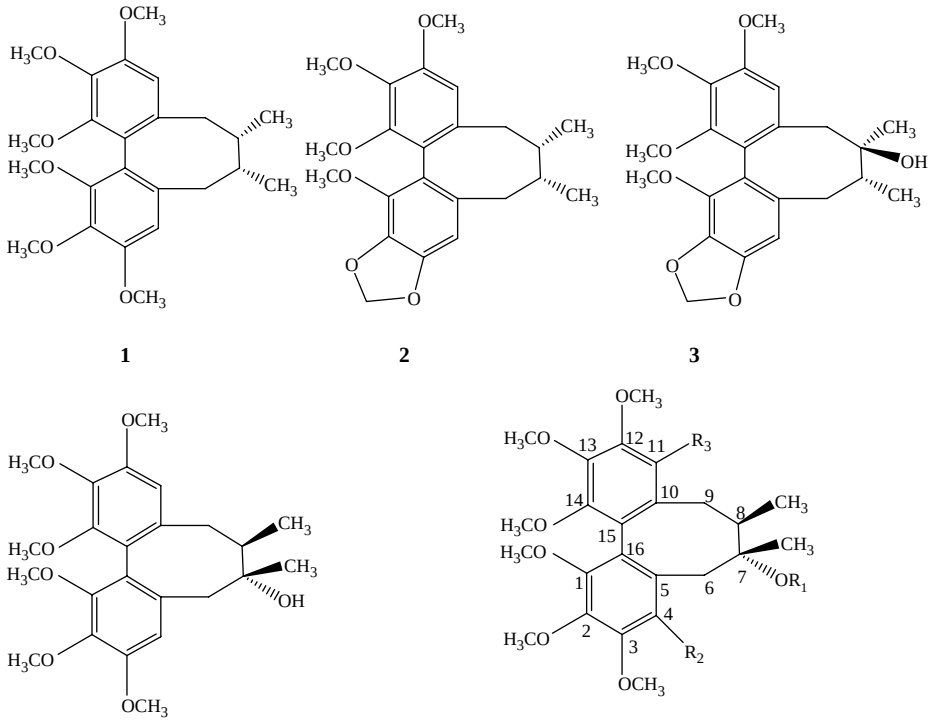

Structure	R_1	R_2	R_3		R_1	R_2	R_3
5	H	Cl	Cl	**14**	H	Br	Br
6	H	Cl	H	**15**	$O{=}CCH_2CH_3$	H	H
7	Ac	H	H	**16**	$O{=}CCH_2CH_3$	Br	Br
8	Ac	Cl	Cl	**17**	$O{=}CCH_2CH_3$	Cl	Cl
9	Ac	Br	H	**18**	$O{=}CCH_2CH_3$	Cl	H
10	H	Br	H	**19**	$O{=}CCH_2CH_3$	H	Cl
11	Ac	H	Br	**20**	$O{=}CCH_2CH_2CH_3$	H	H
12	H	H	Br	**21**	$O{=}CCH_2CH_2CH_3$	Br	Br
13	Ac	Br	Br	**22**	$O{=}CCH_2CH_2CH_3$	H	Cl

Figure 1. The structures of schizandrin A (**4**) and its analogs.

inhibitors of P-gp. In addition, the crude extract of *Fructus schisandrae* [20], with schizandrin A (**4**) as a major constituent, also exhibits good activity against P-gp. Therefore, the MDR inhibitory activity of **4** and its analogs is merited for further investigation. The rational for the design of the derivatives of **4** was based on the expectation that the introduction of an acyl group at 7-OH and/or attachment of halogen(s) at C-4 and C-11 may provide a new class of compounds with MDR inhibitory activity.

2. Results and discussion

Eighteen derivatives (see structures in Figure 1) were prepared from **4**. Esterification of 7-OH of schizandrin A with Ac_2O, propanoic anhydride, or butanoic anhydride provided respective esters **7**, **15**, and **20**. Fifteen halides were generated by reaction of **4**, **7**, **15**, or **20** with

Table 1. Effects of compounds **5**–**22** (10 μM) on the accumulation of Rhodamine 123 in Bel7402 and HCT8 cells.

Compound	Rhodamine 123 accumulation ratio	
	Bel7402	HCT8
5	1.21 ± 0.14	1.29 ± 0.03
6	0.94 ± 0.08	1.00 ± 0.07
7	1.05 ± 0.04	1.03 ± 0.06
8	1.26 ± 0.37	1.41 ± 0.10
9	1.53 ± 0.11	1.44 ± 0.10
10	0.98 ± 0.02	1.25 ± 0.07
11	1.16 ± 0.11	1.05 ± 0.10
12	0.93 ± 0.04	1.06 ± 0.12
13	1.46 ± 0.24	1.35 ± 0.08
14	1.22 ± 0.10	1.08 ± 0.02
15	0.99 ± 0.17	1.06 ± 0.06
16	1.55 ± 0.09	1.27 ± 0.01
17	1.35 ± 0.01	1.26 ± 0.10
18	1.22 ± 0.09	1.38 ± 0.14
19	1.14 ± 0.09	1.05 ± 0.01
20	1.44 ± 0.19	1.52 ± 0.27
21	1.35 ± 0.04	1.32 ± 0.04
22	1.09 ± 0.17	1.17 ± 0.04
Verapamil	2.12 ± 0.01	–

Notes: Data are expressed as the mean ± standard error (SE) of the mean.

N-chlorosuccinimide (NCS) or *N*-bromosuccinimide (NBS) to introduce one or two halogen(s) at C-4 or/and C-11.

The MDR inhibitory activity of these lignans (**5**–**22**) was evaluated by testing their ability to restore the intracellular accumulation of Rhodamine 123, a fluorescent substrate of P-gp. As shown in Table 1, these derivatives exhibit weak ability to restore the intracellular accumulation of Rhodamine 123 in human hepatocarcinoma MDR cell lines Bel7402 and HCT8 relative to the reference drug verapamil. Among them, compound **9**, 7-*O*-acetyl-4-bromo-schizandrin A, is the most potent MDR blocker.

3. Experimental

3.1 General experimental procedures

^{1}H and ^{13}C NMR spectra were acquired on a Bruker AC-E 200 (^{13}C NMR) or a Varian INOVA-400/54 (^{1}H NMR) spectrometer in $CDCl_3$, with TMS as an internal standard; mass spectra were obtained with a Finnigan LCQ and Micromass Auto Spec Ultima-Tof spectrometer; silica GF254 and gel H (10–40 μm, Qingdao Sea Chemical Factory, Qingdao, China) were used for TLC and CC. The starting material schizandrin A was isolated from *S. chinensis* (Turcz.) Baill. Human hepatocarcinoma MDR cell lines Bel7402 and HCT8 were provided by the Graduate Institute of Medicine in Beijing, China.

3.2 Synthesis of the derivatives of schizandrin

3.2.1 Chlorides **5** and **6**

Oxalyl chloride (0.02 ml, 0.21 mmol) in 1 ml dichloromethane and DMSO (0.02 ml, 0.28 mmol) was added to **4** (30 mg, 0.07 mmol) in 1 ml dichloromethane at −40°C. The mixture was stirred for 5 h prior to the addition of Et_3N (0.1 ml, 0.7 mmol) and was extracted with $CHCl_3$. The extract was evaporated and the residue was purified by column chromatography (silica gel, petroleum ether–EtOAc, 7:1) to give **5** (5 mg, 14%) and **6** (16 mg, 49%). **5**: Mp 38–39°C; $[\alpha]_D^{20}$ − 48.4 (c = 1.0, CH_2Cl_2); ^{1}H NMR δ 0.87 (3H, d, J = 7.2 Hz, 8-CH_3), 1.33 (3H, s, 7-CH_3), 1.94 (1H, m, 8-H), 2.58, 2.97 (each 1H, d, J = 14.0 Hz), 2.70 (1H, dd, J = 14.4, 1.2 Hz), 3.10 (1H, dd, J = 14.4, 8.4 Hz), 3.47, 3.60, 3.92, 3.93, 3.96, 3.97 (s, $OCH_3 \times 6$). HR-ESI-MS *m/z*: 523.1268 $[M+Na]^+$ (calcd for $C_{24}H_{30}Cl_2NaO_7$, 523.1261). **6**: Mp 39–41°C; $[\alpha]_D^{20}$ + 22.4 (c = 1.0, CH_2Cl_2); ^{1}H NMR δ 0.81 (3H, d, J = 7.6 Hz, 8-CH_3), 1.33 (3H, s, 7-CH_3), 1.87 (1H, m, 8-H), 2.38 (1H, dd, J = 14.0, 7.6 Hz), 2.53 (1H, d, J = 14.0 Hz), 2.87 (1H, dd, J = 14.0, 1.2 Hz), 2.93 (1H, d, J = 14.0 Hz), 3.51, 3.62, 3.87, 3.89, 3.93, 3.97 (s, $OCH_3 \times 6$), 6.55 (1H, s, 11-H); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 489.1653 $[M+Na]^+$ (calcd for $C_{24}H_{31}ClNaO_7$, 489.1651).

Table 2. ^{13}C NMR spectral data of compounds **6**–**22** (200 MHz).

No.	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
1	152.2	151.7	150.2	152.2	152.0	152.3	152.7	152.3	151.0	151.8	150.9	150.2	152.2	152.0	151.7	150.9	152.1
2	145.3	133.7	145.2	140.2	140.0	144.8	144.7	144.8	145.1	140.2	145.0	145.0	145.1	149.4	140.1	145.0	145.0
3	149.3	151.6	149.5	150.7	151.4	151.2	151.2	150.8	150.5	151.6	150.5	149.8	151.3	150.7	151.6	150.5	149.1
4	127.9	110.1	127.0	110.3	110.5	116.0	116.1	116.2	116.2	110.3	117.3	125.3	110.4	127.0	110.3	117.3	127.3
5	134.2	132.3	132.5	133.5	134.1	134.6	134.6	134.2	134.9	133.7	134.2	132.6	133.6	132.9	133.7	134.2	133.5
6	33.9	34.7	29.7	34.4	33.7	32.5	32.3	32.5	31.3	34.5	32.0	29.4	34.5	29.2	38.0	38.0	36.4
7	73.2	84.9	84.2	84.1	73.0	85.1	72.0	84.3	73.1	84.8	83.6	83.8	83.9	84.4	84.8	83.8	83.9
8	41.9	38.3	36.5	35.5	40.8	39.1	42.3	36.7	41.6	38.5	26.5	36.4	35.6	39.4	38.1	36.3	35.3
9	38.5	38.8	36.5	38.9	38.8	38.7	41.1	39.0	38.7	38.8	38.8	36.4	36.5	38.6	38.9	38.7	38.0
10	132.1	132.3	131.1	132.9	133.5	133.7	131.9	132.9	133.5	132.4	132.9	131.3	131.4	132.3	132.4	132.9	131.4
11	110.7	109.9	126.9	117.0	116.2	110.1	109.8	116.2	116.2	110.3	116.3	124.2	127.4	110.3	110.1	116.2	110.3
12	151.4	140.1	149.8	150.5	150.1	150.5	150.4	151.5	150.7	151.3	150.2	149.5	150.2	151.1	151.1	150.5	150.1
13	140.2	133.7	145.0	144.9	145.0	140.5	140.7	145.1	144.6	140.2	144.8	145.2	149.1	144.8	140.1	144.8	140.7
14	150.8	151.7	149.8	151.1	151.4	151.5	151.7	150.6	151.0	151.6	150.8	149.9	152.2	152.0	151.6	150.8	151.2
15	124.1	122.6	124.1	127.6	128.0	127.3	123.8	127.3	126.9	122.9	127.2	126.9	122.7	123.1	122.9	127.1	122.6
16	122.6	123.5	125.1	122.9	122.7	127.2	127.0	127.5	127.6	123.5	127.5	127.0	125.3	123.8	123.4	127.4	125.3
7-Me	31.3	24.5	25.2	25.8	31.1	22.9	29.2	25.0	30.9	22.8	25.6	25.6	25.7	24.8	24.9	25.8	26.0
8-Me	15.4	15.3	13.4	15.3	15.1	13.3	15.2	13.5	12.8	15.3	13.2	13.1	15.5	12.6	13.6	13.6	13.6
OMe	61.3	60.8	61.1	61.1	61.1	61.3	61.0	61.1	61.1	60.9	61.1	61.1	61.1	61.1	60.8	61.1	61.2
OMe	61.2	60.8	61.1	60.9	60.9	61.2	60.9	61.0	61.1	60.9	61.1	61.1	60.9	61.0	60.8	61.0	60.9
OMe	61.0	60.4	61.0	60.8	60.9	61.0	60.8	60.8	60.9	60.6	60.8	61.0	60.8	60.8	60.8	60.8	60.9
OMe	60.9	60.4	61.0	60.7	60.7	60.7	60.6	60.8	60.9	60.6	60.8	60.9	60.6	60.6	60.5	60.8	60.7
OMe	60.7	55.7	60.7	60.6	60.5	60.7	55.9	60.6	60.8	55.9	60.7	60.7	55.9	55.7	55.8	60.6	60.7
OMe	56.0	55.6	60.7	55.8	55.8	55.9	55.9	60.6	60.6	55.7	60.7	60.7	55.7	55.7	55.6	60.6	55.8
O*CO*	–	170.6	169.9	170.1	–	170.8	–	169.9	–	173.9	173.2	173.1	173.1	173.8	173.1	172.4	172.6
COCH_2	–	–	–	–	–	–	–	–	–	30.0	29.2	29.1	29.1	29.1	34.5	31.7	34.3
CH_2CH_2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	16.3	18.3	18.3
(CO)CH_2CH_3	–	22.7	22.5	22.7	–	22.8	–	22.6	–	9.0	9.0	9.0	9.2	9.0	15.3	13.1	15.2

3.2.2 Compound 7

To a solution of **4** (500 mg, 1.15 mmol) in 5 ml of chloroform, Ac_2O (0.5 ml) and *p*-TsOH (200 mg, 1.16 mmol) were added. The reaction mixture was then stirred at room temperature overnight, neutralized with concentrated NH_4OH solution, and extracted with $CHCl_3$. The crude product was purified by column chromatography (silica gel, petroleum ether–EtOAc, 5:1) to furnish **7** (350 mg, 64%). Mp 121–122°C; $[\alpha]_D^{20}$ + 88.1 (c = 1.0, CH_2Cl_2); ^{1}H NMR δ 0.81 (3H, d, J = 6.8 Hz, 8-CH_3), 1.60 (3H, s, 7-CH_3), 1.88 (3H, s, OCOCH_3), 2.33 (1H, m, 8-H), 2.37 (1H, d, J = 13.6 Hz), 2.55 (1H, d, J = 13.6 Hz), 2.82 (1H, d, J = 12.4 Hz), 3.00 (1H, d, J = 14.0 Hz), 3.56, 3.57, 3.87, 3.87, 3.89, 3.89 (s, OCH_3 × 6), 6.52 (1H, s, 11-H), 6.66 (1H, s, 4-H); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z* 497.2132 $[M+Na]^+$ (calcd for $C_{26}H_{34}NaO_8$, 497.2146).

3.2.3 Compound 8

To compound **7** (50 mg, 0.1 mmol) in 2 ml of glacial HOAc, NCS (25 mg, 0.185 mmol) was added, and the mixture was stirred at room temperature for 40 h, neutralized with concentrated NH_4OH solution, and extracted with $CHCl_3$. The column chromatography (silica gel, petroleum ether–EtOAc, 5:1) of the residue furnished **8** (40 mg, 70%). Mp 46–48°C; $[\alpha]_D^{20}$ − 55.8 (c = 1.0, CH_2Cl_2); ^{1}H NMR δ 0.89 (3H, d, J = 7.2 Hz, 8-CH_3), 1.57 (3H, s, 7-CH_3), 1.85 (3H, s, OCOCH_3), 2.78 (1H, m, 8-H), 2.46 (1H, dd, J = 14.8, 6.0 Hz), 2.52 (1H, d, J = 14.4 Hz), 3.09 (1H, dd, J = 14.4, 8.4 Hz), 3.33 (1H, d, J = 14.4 Hz), 3.54, 3.58, 3.92, 3.94, 3.95, 3.96 (s, OCH_3 × 6); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 565.1356 $[M+Na]^+$ (calcd for $C_{26}H_{32}Cl_2NaO_8$, 565.1366).

3.2.4 Bromides 9, 11, and 13

To a solution of **7** (140 mg, 0.29 mmol) in 5 ml glacial HOAc, NBS (55 mg, 0.31 mmol) was added. After stirring for 15 min, a general work-up as described for the synthesis of **8** and the column chromatography (silica gel, petroleum ether–EtOAc, 15:1) gave **9** (60 mg, 37%), **11** (50 mg, 31%), and **13** (20 mg, 11%). **9**: Mp 145–147°C; $[\alpha]_D^{20}$ − 6.0 (c = 1.0, CH_2Cl_2); ^{1}H NMR δ 0.84 (3H, d, J = 9.2 Hz, 8-CH_3), 1.66 (3H, s, 7-CH_3), 1.86 (3H, s, OCOCH_3), 2.80 (1H, m, 8-H), 2.34 (1H, dd, J = 14.0, 8.0 Hz), 2.61 (1H, d, J = 14.0 Hz), 2.70 (1H, dd, J = 14.0, 2.0 Hz), 3.23 (1H, d, J = 14.0 Hz), 3.56, 3.59, 3.87 3.89, 3.95, 3.95 (s, OCH_3 × 6), 6.52 (1H, s, 11-H); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 575.1233 $[M+Na]^+$ (calcd for $C_{26}H_{33}BrNaO_8$, 575.1251). **11**: Mp 141–143°C; $[\alpha]_D^{20}$ + 10.1 (c = 1.0, CH_2Cl_2); ^{1}H NMR δ 0.84 (3H, d, J = 9.2 Hz, 8-CH_3), 1.57 (3H, s, 7-CH_3), 1.87 (3H, s, OCOCH_3), 2.31 (1H, m, 8-H), 2.60 (1H, d, J = 13.6 Hz), 2.70 (1H, dd, J = 14.8, 7.6 Hz), 3.08 (1H, d, J = 14.0 Hz), 3.13 (1H, dd, J = 14.8, 9.2 Hz), 3.52, 3.55, 3.87, 3.87, 3.92, 3.94 (s, OCH_3 × 6), 6.65 (1H, s, 4-H). ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 575.1235 $[M+Na]^+$(calcd for $C_{26}H_{33}BrNaO_8$, 575.1251). **13**: Mp 147–149°C; $[\alpha]_D^{20}$ − 70.6 (c = 1.0, CH_2Cl_2); ^{1}H NMR δ 0.90 (3H, d, J = 7.2 Hz, 8-CH_3), 1.58 (3H, s, 7-CH_3), 1.87 (3H, s, OCOCH_3), 2.78 (1H, m, 8-H), 2.56 (1H, dd, J = 15.2, 2.0 Hz), 2.61 (1H, d, J = 14.8 Hz), 3.13 (1H, dd, J = 14.8, 8.8 Hz), 3.36 (1H, d, J = 14.4 Hz), 3.53, 3.57, 3.92, 3.93, 3.94, 3.95 (s, OCH_3 × 6); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 653.0335 $[M+Na]^+$ (calcd for $C_{26}H_{32}Br_2NaO_8$, 653.0356).

3.2.5 Compound 10

Hydrolysis of **9** (120 mg, 0.21 mmol) in 10% KOH methanol (60°C, 6 h). The mixture was evaporated and the residue was purified by column chromatography (silica gel, $CHCl_3$–MeOH, 97:3) to give **10** (80 mg, 73%). Mp 138–139°C; $[\alpha]_D^{20}$ + 8.5 (c = 1.0, CH_2Cl_2); ^{1}H NMR δ 0.80 (3H, d, J = 7.2 Hz, 8-CH_3), 1.35 (3H, s, 7-CH_3),

1.88 (1H, m, 8-H), 2.38 (1H, dd, $J = 14.0$, 7.6 Hz), 2.63 (1H, d, $J = 13.6$ Hz,), 2.88 (1H, dd, $J = 14.0$, 1.6 Hz), 2.96 (1H, d, $J = 14.0$ Hz), 3.51, 3.61, 3.87, 3.89, 3.93, 3.96 (s, $OCH_3 \times 6$), 6.55 (1H, s, 11-H); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 533.1136 $[M+Na]^+$ (calcd for $C_{24}H_{31}BrNaO_7$, 533.1145).

3.2.6 Compound **12**

This compound (10 mg, 56%) was prepared from **11**(20 mg, 0.036 mmol) employing a similar procedure as described for the synthesis of **10**. Mp 140–141°C; $[\alpha]_D^{20} - 9.6$ ($c = 1.0$, CH_2Cl_2); ^1H NMR δ 0.89 (3H, d, $J = 7.2$ Hz, 8-CH_3), 1.25 (3H, s, 7-CH_3), 1.92 (1H, m, 8-H), 2.40 (1H, d, $J = 13.6$ Hz), 2.55 (1H, dd, $J = 14.8$, 1.6 Hz), 2.73 (1H, d, $J = 13.6$ Hz), 3.13 (1H, dd, $J = 14.8$, 8.2 Hz), 3.54, 3.58, 3.88, 3.91, 3.92, 3.94 (s, $OCH_3 \times 6$), 6.60 (1H, s, 4-H); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 533.1130 $[M+Na]^+$ (calcd for $C_{24}H_{31}BrNaO_7$, 533.1145).

3.2.7 Compound **14**

This compound (28 mg, 60%) was prepared from **13** (50 mg, 0.079 mmol) by a similar procedure as described for the synthesis of **10**. Mp 139–140°C; $[\alpha]_D^{20} - 54.9$ ($c = 1.0$, CH_2Cl_2); ^1H NMR δ 0.89 (3H, d, $J = 7.2$ Hz, 8-CH_3), 1.34 (3H, s, 7-CH_3), 1.96 (1H, m, 8-H), 2.69 (1H, d, $J = 14.0$ Hz), 2.80 (1H, d, $J = 14.4$ Hz), 3.00 (1H, d, $J = 14.0$ Hz), 3.13 (1H, dd, $J = 14.4$, 8.2 Hz), 3.46, 3.60, 3.92, 3.92, 3.95, 3.96 (s, $OCH_3 \times 6$); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 611.0250 $[M+Na]^+$ (calcd for $C_{24}H_{30}Br_2NaO_7$, 611.0250).

3.2.8 Compound **15**

Using the similar procedure as described for the synthesis of **7**, compound **15** (10 mg, 29%) was synthesized by esterification of **4** (30 mg, 0.069 mmol) with propanoic anhydride (0.05 ml). The residue was purified by column chromatography (silica gel, petroleum ether–EtOAc, 7:1) to give **15**. Mp 122–124°C; $[\alpha]_D^{20} + 60.9$ ($c = 1.0$, CH_2Cl_2); ^1H NMR δ 0.83 (3H, d, $J =$ 6.8 Hz, 8-CH_3), 0.97 (3H, t, $J = 7.2$ Hz, O═C—CH_2CH_3), 1.60 (3H, s, 7-CH_3), 2.13 (2H, m, O═C—CH_2CH_3), 2.38 (1H, m, 8-H), 2.36 (1H, d, $J = 11.2$ Hz), 2.57 (1H, d, $J = 14.0$ Hz), 2.83 (1H, d, $J = 12.4$ Hz), 2.98 (1H, d, $J = 14.0$ Hz), 3.56, 3.56, 3.86, 3.87, 3.87, 3.89 (s, $OCH_3 \times 6$), 6.52 (1H, s, 11-H), 6.65 (1H, s, 4-H); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 511.2288 $[M+Na]^+$ (calcd for $C_{27}H_{36}NaO_8$, 511.2302).

3.2.9 Compound **16**

This compound (20 mg, 61%) was prepared by reaction of **15** (25 mg, 0.05 mmol) in glacial HOAc with NBS (20 mg, 0.11 mmol) as described for the synthesis of **9**. The residue was purified by column chromatography (silica gel, petroleum ether–EtOAc, 9:1) to furnish **16**. Mp 131–133°C; $[\alpha]_D^{20} - 81.0$ ($c = 1.0$, CH_2Cl_2); ^1H NMR δ 0.91 (3H, d, $J = 7.6$ Hz, 8-CH_3), 0.91 (3H, t, $J = 6.8$ Hz, O═C—CH_2CH_3), 1.65 (3H, s, 7-CH_3), 2.15 (2H, m, O═C—CH_2CH_3), 2.86 (1H, m, 8-H), 2.65 (1H, d, $J = 14.0$ Hz), 2.57 (1H, dd, $J = 14.4$, 2.0 Hz), 3.32 (1H, d, $J = 14.4$ Hz), 3.13 (1H, dd, $J = 14.4$, 8.4 Hz), 3.53, 3.57, 3.92, 3.92, 3.94, 3.96 (s, $OCH_3 \times 6$); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 667.0493 $[M+Na]^+$ (calcd for $C_{27}H_{34}Br_2NaO_8$, 667.0513).

3.2.10 Compounds **17** and **18**

17 (12 mg, 52%) and **18** (6 mg, 29%) were prepared by reaction of **15** (20 mg, 0.04 mmol) in glacial HOAc with NCS (20 mg, 0.15 mmol) as described for the synthesis of **8**. The crude product was purified by column chromatography (silica gel, petroleum ether–EtOAc, 10:1) to give **17** and **18**. **17:** Mp 55–57°C; $[\alpha]_D^{20} - 60.2$ ($c = 1.0$, CH_2Cl_2); ^1H NMR δ 0.90 (3H, d, $J = 7.2$ Hz, 8-CH_3), 0.92 (3H, t,

$J = 7.6$ Hz, O═C—CH_2CH_3), 1.61 (3H, s, 7-CH_3), 2.13 (2H, m, O═C—CH_2CH_3), 2.85 (1H, m, 8-H), 2.48 (1H, dd, $J = 14.4$, 2.0 Hz), 2.55 (1H, d, $J = 14.4$ Hz), 3.10 (1H, dd, $J = 14.8$, 8.8 Hz), 3.30 (1H, d, $J = 14.0$ Hz), 3.54, 3.57, 3.93, 3.93, 3.95, 3.97 (s, $OCH_3 \times 6$); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 579.1515 $[M+Na]^+$ (calcd for $C_{27}H_{34}Cl_2NaO_8$, 579.1523). **18**: Mp 50–51°C; $[\alpha]_D^{20} + 18.4$ ($c = 1.0$, CH_2Cl_2); ^{1}H NMR δ 0.84 (3H, d, $J = 7.2$ Hz, 8-CH_3), 0.91 (3H, t, $J = 7.6$ Hz, O═C—CH_2CH_3), 1.65 (3H, s, 7-CH_3), 2.14 (2H, m, O═C—CH_2CH_3), 2.80 (1H, m, 8-H), 2.34 (1H, dd, $J = 14.0$, 8.0 Hz), 2.51 (1H, d, $J = 14.0$ Hz), 2.70 (1H, dd, $J = 14.0$, 2.0 Hz), 3.22 (1H, d, $J = 14.0$ Hz), 3.54, 3.58, 3.92, 3.94, 3.95, 3.96 (s, $OCH_3 \times 6$), 6.52 (1H, s, 11-H); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 545.1892 $[M+Na]^+$ (calcd for $C_{27}H_{35}ClNaO_8$, 545.1913).

3.2.11 Compound **19**

This compound (8 mg, 38%) was prepared by reaction of **15** (20 mg, 0.04 mmol) in glacial HOAc with NCS (8 mg, 0.06 mmol) as described for the synthesis of **8**. The residue was purified by column chromatography (silica gel, petroleum ether–EtOAc, 10:1) to give **19**. Mp 51–53°C; $[\alpha]_D^{20} + 11.1$ ($c = 1.0$, CH_2Cl_2); ^{1}H NMR δ 0.83 (3H, d, $J = 7.2$ Hz, 8-CH_3), 0.96 (3H, t, $J = 7.6$ Hz, O═C—CH_2CH_3), 1.60 (3H, s, 7-CH_3), 2.12 (2H, m, O═C—CH_2CH_3), 2.38 (1H, m, 8-H), 2.63 (1H, d, $J = 13.6$ Hz), 2.64 (1H, dd, $J = 14.8$, 0.8 Hz), 3.03 (1H, d, $J = 14.4$ Hz), 3.10 (1H, dd, $J = 14.8$, 9.2 Hz), 3.53, 3.54, 3.86, 3.87, 3.92, 3.95 (s, $OCH_3 \times 6$), 6.66 (1H, s, 4-H); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 545.1911 $[M+Na]^+$ (calcd for $C_{27}H_{35}ClNaO_8$, 545.1913).

3.2.12 Compound **20**

Using the similar procedure as described for the synthesis of **7**, compound **20** (210 mg, 60%) was synthesized by the esterification of **4** (300 mg, 0.69 mmol) with butanoic anhydride (0.3 ml) as described for the synthesis of **7**. The residue was purified by column chromatography (silica gel, petroleum ether–EtOAc, 7:1) to give **20**. Mp 127–128°C; $[\alpha]_D^{20} + 52.5$ ($c = 1.0$, CH_2Cl_2); ^{1}H NMR δ 0.82 (3H, d, $J = 6.4$ Hz, 8-CH_3), 0.84 (3H, t, $J = 7.2$ Hz, O═C—$CH_2CH_2CH_3$), 1.47 (2H, m, O═C—$CH_2CH_2CH_3$), 2.10 (2H, m, O═C—$CH_2CH_2CH_3$), 1.57 (3H, s, 7-CH_3), 2.37 (hidden, 8-H), 2.37 (1H, d, $J = 12.4$ Hz), 2.57 (1H, d, $J = 14.0$ Hz), 2.82 (1H, d, $J = 12.0$ Hz), 2.97 (1H, d, $J = 14.0$ Hz), 3.56, 3.57, 3.87, 3.87, 3.87, 3.89 (s, $OCH_3 \times 6$), 6.52 (1H, s, 11-H), 6.65 (1H, s, 4-H); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 525.2461 $[M+Na]^+$ (calcd for $C_{28}H_{38}NaO_8$, 525.2464).

3.2.13 Compound **21**

Employing the similar procedure as described for the synthesis of **16**, compound **21** (35 mg, 53%) was prepared from **20** (50 mg, 0.085 mmol). The residue was purified by column chromatography (silica gel, petroleum ether–EtOAc, 40:1) to give **21**. Mp 148–149°C; $[\alpha]_D^{20} - 62.3$ ($c = 1.0$, CH_2Cl_2); ^{1}H NMR δ 0.91 (3H, d, $J = 6.8$ Hz, 8-CH_3), 0.80 (3H, t, $J = 7.2$ Hz, O═C—$CH_2CH_2CH_3$), 1.38 (2H, m, O═C—$CH_2CH_2CH_3$), 2.10 (2H, m, O═C—$CH_2CH_2CH_3$), 1.65 (3H, s, 7-CH_3), 2.90 (1H, m, 8-H), 2.58 (1H, dd, $J = 14.8$, 2.0 Hz), 2.67 (1H, d, $J = 14.0$ Hz), 3.13 (1H, dd, $J = 14.4$, 8.4 Hz), 3.30 (1H, d, $J = 14.0$ Hz), 3.52, 3.57, 3.92, 3.92, 3.94, 3.96 (s, $OCH_3 \times 6$); ^{13}C NMR spectral data see Table 2; HR-ESI-MS *m/z*: 681.0644 $[M+Na]^+$ (calcd for $C_{28}H_{36}Br_2NaO_8$, 681.0669).

3.2.14 Compound **22**

This compound (40 mg, 76%) was prepared by reaction of **20** (50 mg, 0.085 mmol) in HOAc with NCS (12 mg, 0.09 mmol)

as described for the synthesis of **8**. The residue was purified by column chromatography (silica gel, petroleum ether–EtOAc, 20:1) to give **22**. Mp 49–51°C; $[\alpha]$ − 7.5 (c = 1.0, CH_2Cl_2); ^{1}H NMR δ 0.83 (3H, d, J = 7.6 Hz, 8-CH_3), 0.80 (3H, t, J = 7.2 Hz, O═C—$CH_2CH_2CH_3$), 1.38 (2H, m, O═C—$CH_2CH_2CH_3$), 2.09 (2H, m, O═C—$CH_2CH_2CH_3$), 1.64 (3H, s, 7-CH_3), 2.84 (1H, m, 8-H), 2.34 (1H, dd, J = 14.0, 8.0 Hz), 2.51(1H, d, J = 14.0 Hz), 2.70 (1H, d, J = 14.4 Hz), 3.21 (1H, d, J = 14.0 Hz), 3.55, 3.60, 3.87, 3.89, 3.94, 3.96 (s, OCH_3 × 6), 6.52 (1H, s, 11-H); ^{13}C NMR spectral data see Table 2; HR-ESI-MS m/z: 559.2051 $[M+Na]^+$ (calcd for $C_{28}H_{37}ClNaO_8$, 559.2069).

3.3 Measurement of the cellular accumulation of Rhodamine 123

The accumulation of Rhodamine 123 in Bel7402 and HCT8 cells was measured as described previously [17]. Briefly, cells (Bel7402 and HCT8) in 3.5 cm petri dishes were incubated with Rhodamine 123 (final concentration 250 ng/ml) in the absence or the presence of tested compounds (10 μM) for 30 min at 37°C. After incubation, the extracellular Rhodamine 123 was removed through washing with ice-cold PBS twice. The intracellular Rhodamine 123 was determined under laser scanning confocal microscope. A minimum of 50 cells was analyzed for each sample and five random sights were observed. The data were analyzed by computer programs to get the equal fluorescence intensity.

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