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New highly active taxoids from 9β-dihydrobaccatin-9,10-acetals. Part 5

Yasuyuki Takeda,[a] Kouichi Uoto,[a] Michio Iwahana,[b] Takeshi Jimbo,[b] Motoko Nagata,[b] Ryo Atsumi,[c] Chiho Ono,[c] Noriko Tanaka,[d] Hirofumi Terasawa[b] and Tsunehiko Soga[a,*]

[a]*Medicinal Chemistry Research Laboratory, Daiichi Pharmaceutical Co., Ltd, Tokyo R&D Center, 16-13 Kita-Kasai 1-Chome, Edogawa-ku, Tokyo 134-8630, Japan*
[b]*New Product Research Laboratories III, Daiichi Pharmaceutical Co., Ltd, Tokyo R&D Center, 16-13 Kita-Kasai 1-Chome, Edogawa-ku, Tokyo 134-8630, Japan*
[c]*Drug Metabolism & Physicochemical Property Research Laboratory, Daiichi Pharmaceutical Co., Ltd, Tokyo R&D Center, 16-13 Kita-Kasai 1-Chome, Edogawa-ku, Tokyo 134-8630, Japan*
[d]*Research Planning & Administration Department, Daiichi Pharmaceutical Co., Ltd, Tokyo R&D Center, 16-13 Kita-Kasai 1-Chome, Edogawa-ku, Tokyo 134-8630, Japan*

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Abstract—To improve the metabolic stability of **3**, which exhibited both in vitro antitumor activity and in vivo efficacy by both iv and po administration, we designed and synthesized new taxane analogues. Most of the synthetic compounds maintained excellent antitumor activity and were scarcely metabolized by human liver microsomes. And some compounds exhibited potent antitumor effects against B16 melanoma BL6 in vivo by both iv and po administration similarly to **3**.

1. Introduction

Paclitaxel (**1**, Taxol®)[1] and docetaxel (**2**, Taxotere®)[2] inhibit cell growth by interacting with microtubules and are particularly effective against ovarian and breast cancer and Kaposi's sarcoma. However, their low water solubility requires the co-injection of a detergent, such as Cremophor® EL or Tween® 80. These detergents frequently cause untoward hypersensitivity reaction, and patients receiving these drugs require premedication. In addition, neither is suitable for oral administration because each has low oral bioavailability. Under these circumstances, we had attempted to discover orally active taxane analogues and found that a new taxane analogue, 9-β-dihydro-9,10-*O*-acetal taxane **3**, exhibited marked in vivo efficacy by both iv and po administration.[3] However, during our study of the biological properties of **3**, we found that the incubation of **3** with monkey or human liver microsomes rapidly provided the metabolite M-1 (**4**). In our previous paper, to improve the metabolic stability of **3**, we synthesized taxane analogues having substituents introduced onto the metabolic position, exemplified by **5** and **6**, which maintained excellent antitumor activity and were scarcely metabolized by human liver microsomes.[4]

We investigated yet another approach to obtain the compound, which possessed potent antitumor efficacy and high metabolic stability. We report here the synthesis and antitumor activities of new taxane analogues designed to improve the metabolic stability of **3**.

2. Chemistry

Firstly, we tried to replace the morpholine moiety of **3** with the other amine groups. Oxidation of **7**,[3] the precursor of **3**, with OsO_4 followed by cleavage with $NaIO_4$ gave the aldehyde, to which the amine moiety was introduced by reductive amination to afford the targeted compounds (**8a**–**d**)[5] (Scheme 1).

* Corresponding author. Tel.: +81-3-3680-0151; fax: +81-3-5696-8344; e-mail: sogatf7t@daiichipharm.co.jp

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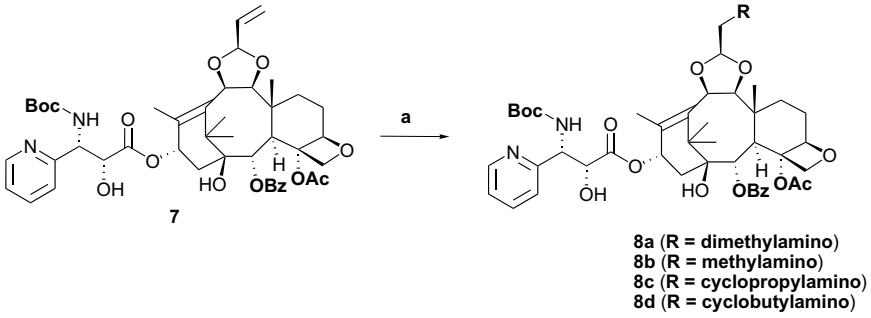
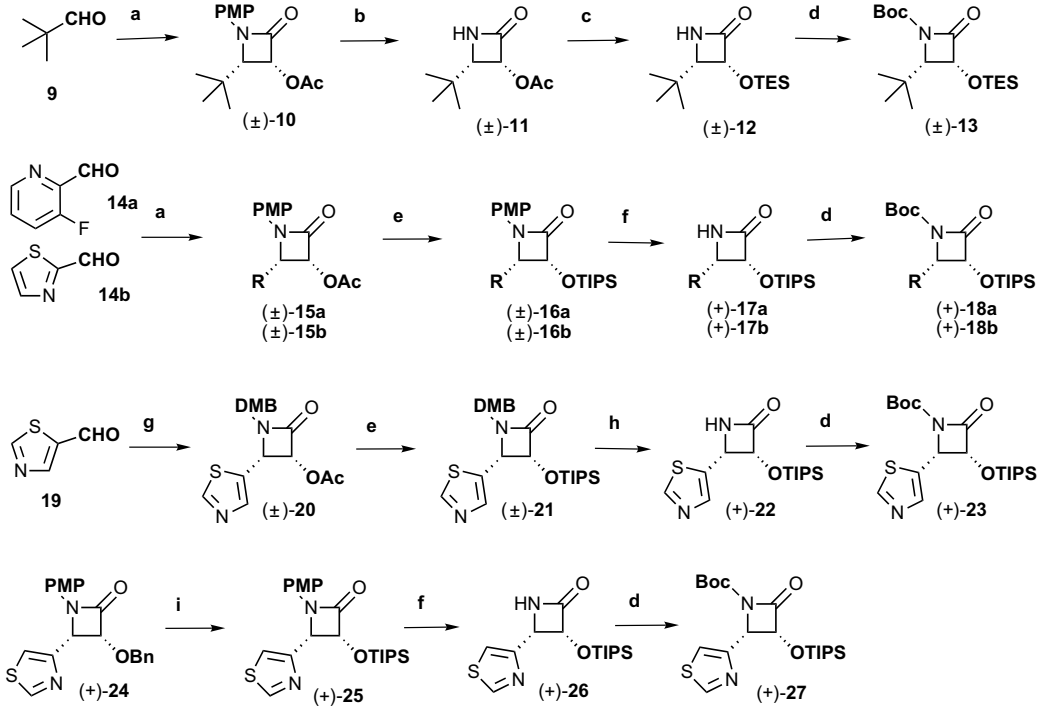
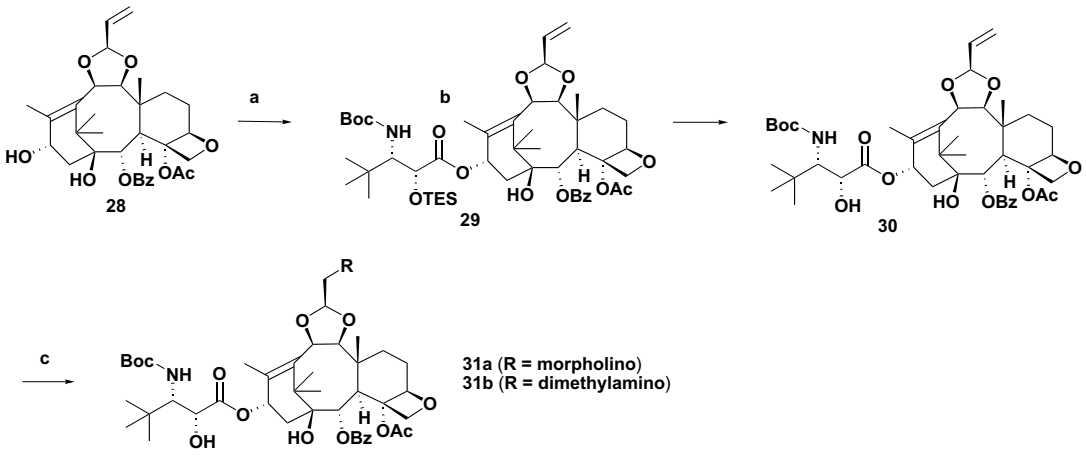


Scheme 1. Reagents and conditions: (a) (i) OsO_4, NMO, THF, acetone, H_2O; (ii) $NaIO_4$, THF, MeOH, H_2O; (iii) amine, AcOH, $NaBH_3CN$, EtOH (82% for **8a**, 12% for **8b**, 60% for **8c**, 56% for **8d**).

Next, to avoid the formation of the hydroxypyridine ring through metabolism by human liver microsomes, we tried to replace the pyridine ring with *tert*-butyl, 3-fluoropyridyl, and thiazolyl groups. The synthesis of the key β-lactam intermediates (**13**, **18a**,**b**, **23**, **27**) is shown in Scheme 2. The β-lactams (**10**, **15a**,**b**) were synthesized by the Staudinger reaction between the imines, which were derived from the corresponding aldehydes (**9**, **14a**,**b**) and *p*-anisidine, and the ketene derived in situ from 2-acetoxyacetyl chloride. The *p*-methoxyphenyl moiety of **10** was removed with ceric ammonium nitrate (CAN) and sequential deacetylation and silylation gave the β-lactam **12**. Finally, acylation of **12** was accomplished with di-*tert*-butyl dicarbonate ($(Boc)_2O$) and 4-dimethylaminopyridine (DMAP) to afford the targeted β-lactam **13** as a racemic mixture. Compounds **15a** and **15b** were converted to **16a** and **16b**, respectively, by the sequential deacetylation and silylation. After removal of the *p*-methoxyphenyl moieties, the racemic mixtures were resolved by using a chiral HPLC column to afford the optically pure β-lactams ((+)-**17a**, (+)-**17b**). Acylations of chiral β-lactams (**17a**,**b**) with $(Boc)_2O$ afforded the desired β-lactams (**18a**,**b**) in good yield. The chiral β-lactam **23** was obtained from **19** by following a procedure similar to that described for the preparation of **18b** from **14b** by replacing *p*-anisidine with 2,4-dimethoxybenzylamine. The chiral β-lactam **27**, possessing a 4-thiazolyl group, was derived from the reported chiral β-lactam[6] **24** in three steps.



Scheme 2. Reagents and conditions: (a) (1) 4-anisidine, Na_2SO_4, benzene, (2) $2\text{-AcOCH}_2\text{COCl}$, Et_3N, CH_2Cl_2 (54% for **10**, 61% for **15a**, 41% for **15b**); (b) CAN, CH_3CN, H_2O (72%); (c) (1) K_2CO_3, THF, MeOH, (2) TESCl, imidazole, DMF (71%); (d) $(Boc)_2O$, DMAP, THF (97% for **13**, quant. for **18a**, 96% for **18b**, 98% for **23**, 98% for **27**); (e) (1) K_2CO_3, THF, MeOH, (2) TIPSCl, imidazole, DMF (74% for **16a**, 70% for **16b**, 38% for **21**); (f) (1) CAN, CH_3CN, H_2O, (2) Chiralcel OD (24% for **17a**, 31% for **17b**, 56% for **26** from **24**); (g) (1) 2,4-dimethoxylbenzylamine, Na_2SO_4, benzene, (2) $2\text{-AcOCH}_2\text{COCl}$, Et_3N, CH_2Cl_2 (48%); (h) (1) $K_2S_2O_8$, Na_2HPO_4, CH_3CN, H_2O, (2) Chiralcel OD (20%); (i) (1) $BC1_3$, CH_2Cl_2, (2) TIPSCl, imidazole, DMF.



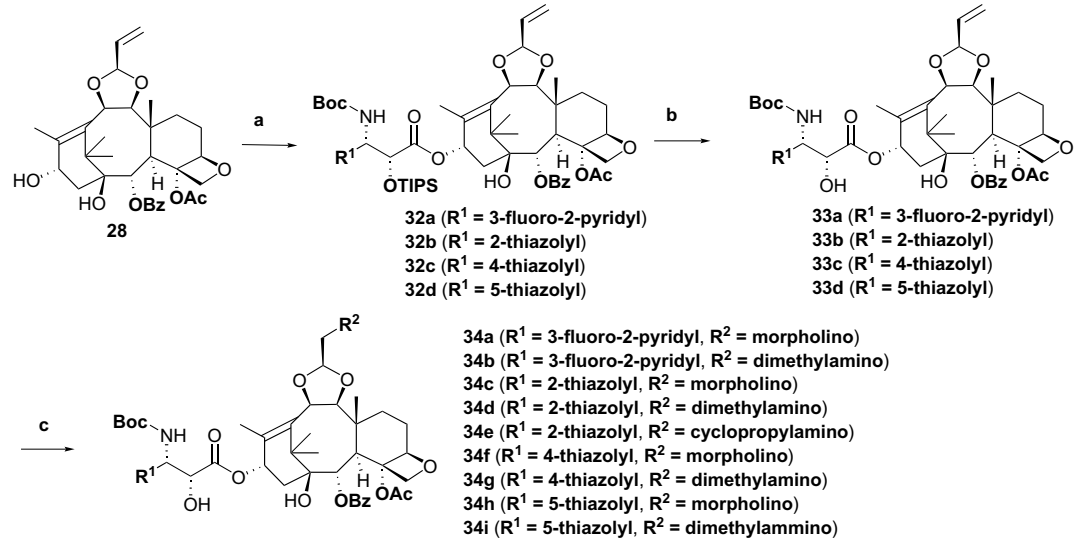
Scheme 3. Reagents and conditions: (a) **13**, LiHMDS, THF (86%); (b) TBAF, THF (96%); (c) (1) OsO_4, NMO, THF, acetone, H_2O, (2) $NaIO_4$, THF, MeOH, H_2O, (3) amine, AcOH, $NaBH_3CN$, EtOH (73% for **31a**, 96% for **31b**).

The synthesis of the taxane analogues possessing a *tert*-butyl group at the C-13 side chain is depicted in Scheme 3. The coupling of **28**[3] with the 3 equiv of the racemic β-lactam **13** was carried out following a procedure similar to that reported by Ojima et al.[7] to afford the single isomer **29** in good yield. Subsequent removal of the protecting group at C-2′ gave the compound **30**. Oxidation of **30** with OsO_4 followed by cleavage with $NaIO_4$ gave the aldehyde, which was converted to the desired compounds **31a** and **31b** by reductive amination.[8]

The targeted compounds (**34a**–**i**) were synthesized according to a procedure similar to that described for the preparation of **31a** utilizing **28** and the 1.2 equiv of corresponding chiral β-lactams (**18a**,**b**, **23**, **27**) (Scheme 4).[9]

3. Results and discussion

Activities of the synthetic compounds were evaluated in cytotoxicity assays against five cell lines (P388, PC-6, PC-12, PC-6/VCR29-9, and PC-6/VP1-1), and activities were compared with those of paclitaxel, docetaxel, and **3** (Table 1). Among the compounds having another amine group than the morpholine of **3**, the methylamino derivative **8b** exhibited significantly decreased cytotoxicity against cancer cell lines expressing P-glycoprotein (PC-12, PC-6/VCR29-9, and PC-6/VP1-1). It should be noted that compounds in which the pyridine ring of **3** was replaced by a *tert*-butyl or other hetero ring groups exhibited nearly the same cytotoxicity as **3** and good metabolic stability. The cytotoxicity of the other three compounds (**8a**,**c**,**d**) was slightly weaker than that of **3**, but stronger than those of paclitaxel and docetaxel.



Scheme 4. Reagents and conditions: (a) **18a**, **18b**, **23**, or **27**, LiHMDS, THF (97% for **32a**, 77% for **32b**, 57% for **32c**, 84% for **32d**); (b) TBAF, THF (94% for **33a**, 98% for **33b**, 84% for **33c**, 95% for **33d**); (c) (1) OsO_4, NMO, THF, acetone, H_2O, (2) $NaIO_4$, THF, MeOH, H_2O, (3) amine, AcOH, $NaBH_3CN$, EtOH (78% for **34a**, 78% for **34b**, 70% for **34c**, 64% for **34d**, 69% for **34e**, 57% for **34f**, 88% for **34g**, 54% for **34h**, 51% for **34i**).

Table 1. Cytotoxicity and metabolic stability of 7-deoxy-9,10-*O*-acetal taxane analogues

Compd	Cytotoxicity GI_{50} (ng/mL)[a]					Remaining rate (%)[b]
	P388	PC-6	PC-12	PC-6/VCR29-9	PC-6/VP1-1	
1	2.93	1.27	539	455	1000	NT
2	0.78	0.26	19.1	62.1	442	88.7
3	0.18	0.26	0.13	2.43	19.3	60.2
8a	0.41	0.38	0.74	4.04	54.2	84.8
8b	0.60	0.54	5.82	43.2	265	NT
8c	0.17	0.54	0.30	2.22	49.4	94.4
8d	0.56	0.83	1.45	4.88	81.7	92.0
31a	0.34	0.70	0.13	0.85	5.56	92.1
31b	0.73	0.67	0.50	1.42	15.7	93.1
34a	0.20	0.29	0.14	0.96	13.7	85.2
34b	0.67	0.38	0.62	2.58	21.3	91.4
34c	0.06	0.12	0.13	1.37	19.7	94.8
34d	0.25	0.27	0.35	2.81	39.1	91.4
34e	0.06	0.42	0.12	1.16	39.4	84.9
34f	0.03	0.65	0.08	1.02	9.13	91.2
34g	0.06	0.35	0.19	1.10	17.3	91.2
34h	0.04	0.07	0.12	0.66	20.5	86.4
34i	0.07	0.30	0.17	1.63	23.8	87.4

NT: not tested.

[a] Concentration that inhibited the growth of cells by 50% at 72 h continuous exposure for the five cell lines [mouse leukemia (P388), human lung cancer cell lines (PC-6 and PC-12), and resistant cancer cell lines (PC-6/VCR29-9 and PC-6/VP1-1)].[11] Resistance factors (GI_{50} of the drug for the selection for the resistance cell line/that for the parent cell line) of PC-6/VCR29-9 and PC-6/VP1-1 were 842 and 20, respectively.

[b] Remaining rate of the substrate after 5 min of incubation with human liver microsomes.

Comparing the cytotoxicity of **8c** and **8d**, **8d** exhibited weaker cytotoxicity against all cell lines than **8c**. This decrease in cytotoxicity might be due to a steric hindrance. As compared with the cytotoxicity of compounds possessing the dimethylamino moiety on their acetal moiety, the compounds possessing the morpholine moiety on their acetal moiety showed slightly stronger cytotoxicity.

The metabolic stability of all the synthetic compounds except **8b** was examined, and the results were shown as the rate remaining after 5 min of incubation with human liver microsomes. All compounds in which the pyridine ring was replaced by a *tert*-butyl or other hetero ring groups also exhibited good metabolic stability as we had expected. On the other hand, the metabolic stability of **35** (Fig. 1),[3] whose C-13 side chain is the same as that of docetaxel, got worse.[10] These results clarify that the substitution of the pyridine ring, which is considered main metabolic position, with a suitable moiety such as a *tert*-butyl or other hetero ring groups may contribute to the improvement of metabolic stability. Interestingly, the compounds in which the morpholine moiety of **3**, which was not the metabolic position, was replaced by another amine group exhibited good metabolic stability. Hence, it was elucidated that the modification of the pyridine ring was not the only method to improve the metabolic stability of **3**.

To evaluate the antitumor effects in vivo, we used B16 melanoma BL6 cells subcutaneously implanted into mice, and the activities of all the synthetic compounds

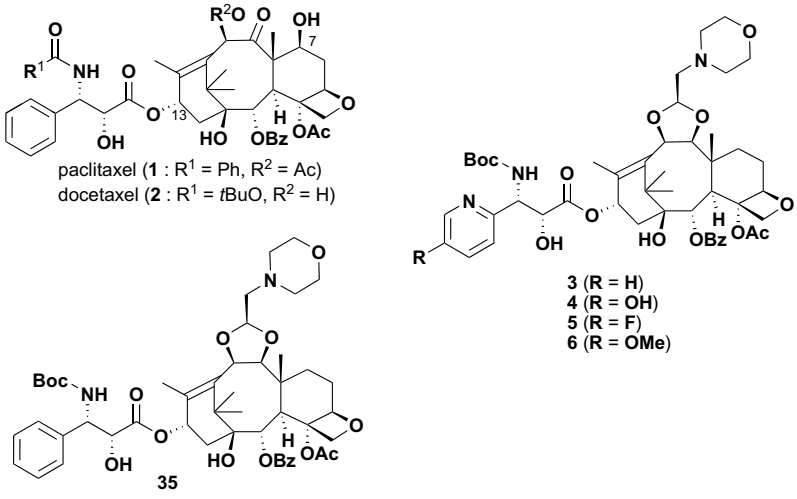


Figure 1. Structures of taxane analogues.

except **8b**,**c** and **34e**,**g**,**i** were compared with those of docetaxel and **3** when administered intravenously (iv) and orally (po) (Table 2). Our synthetic compounds exhibited potent antitumor effects by both iv and po administration; however, the effective dose ranges of some compounds, including **5** and **6**, were narrow. They showed potent antitumor activity at only one dosage by oral administration; whereas the higher dosage resulted in death and the lower dosage did not exhibit the high antitumor effect. On the other hand, compounds **8a**, **31a**, and **34a**,**b**,**c** exhibited potent antitumor effects over a wide dosage range by both iv and po administration as **3**. The detailed data of these six compounds (**3**, **8a**, **31a**, and **34a**,**b**,**c**) are shown in Table 3. Compounds **8a** and **34a**,**b** could be assumed to have good oral bioavailabilities, because these compounds administered orally gave nearly the same antitumor effects and body weight losses as compared with those when they were administered intravenously. In contrast, docetaxel when administered orally at a dose of 600 mg/kg exhibited no antitumor effect and no body weight loss as a result of its poor oral bioavailability.

In summary, we designed and synthesized new taxane analogues to improve the metabolic stability of **3**, and it was suggested that some compounds had nearly the same biological properties as **3** and were scarcely metabolized by human liver microsomes. We expect to find an optimal compound to be selected for clinical development among these compounds.

Table 2. Antitumor activity against B16 melanoma BL6[a]

Compd	Effective dose range (mg/kg)[b]	
	po	iv
3	12.0–27.0	5.3–18.0
5	7.9	3.5
6	13.3	5.9
8a	7.9–17.8	7.9–17.8
8c	NT[c]	NT[c]
8d	11.9–27.6	7.9
31a	11.9–17.8	7.8–11.9
31b	11.9	11.9
34a	11.9–17.8	11.9–17.8
34b	7.9–11.0	5.3–11.9
34c	11.9–26.7	7.9–17.8
34d	11.9	7.8–17.8
34e	NT[c]	NT[c]
34f	7.9	3.5–5.3
34g	NT[c]	NT[c]
34h	None	7.9
34i	NT[c]	NT[c]

[a] Cultured B16 melanoma BL6 was kindly provided by Dr. Tsuruo (Institute of Molecular and Cellular Biosciences, University of Tokyo) by courtesy of Dr. Fidler (The University of Texas M. D. Andersen Cancer Center).[12] B16 melanoma BL6 cells were subcutaneously inoculated into C57BL/6 mice (six mice per group) on day 0. Compounds were administered intravenously or orally on day 4 (single administration). Tumor masses were weighed on day 15.

[b] Effective dose range means that the doses resulted in inhibition rate of more than 58% without both body weight loss of less than 20% and no death due to toxicity.

[c] NT: not tested. Compounds **8c** and **34e** could not be administered due to their low solubility. Maximum tolerated dose (MTD) of **34g** and **34i** were significantly decreased in a preliminary test.

Table 3. Antitumor activity of selected five compounds against B16 melanoma BL6

Compd	Route	Dose (mg/kg)	IR (%)[a]	BWLmax (%)[b]	Mortality	Route	Dose (mg/kg)	IR (%)[a]	BWLmax (%)[b]	Mortality
2	po	600.0	6.2	<0	0/6	iv	100.0	95.1	<0	0/6
3	po	27.0	97.3	4.5	0/6	iv	18.0	95.6	3.7	0/6
		18.0	93.3	0.3	0/6		12.0	94.4	<0	0/6
		12.0	84.9	<0	0/6		8.0	87.2	<0	0/6
		8.0	27.2	<0	0/6		5.3	62.4	<0	0/6
8a	po	26.7	97.5	13.1	1/6	iv	26.7	—	—	6/6
		17.8	97.0	8.8	0/6		17.8	97.7	8.6	0/6
		11.9	88.8	<0	0/6		11.9	95.0	1.0	0/6
		7.9	72.8	<0	0/6		7.9	82.3	<0	0/6
31a	po	26.7	—	—	6/6	iv	17.8	—	—	6/6
		17.8	90.3	11.7	0/6		11.9	93.8	10.5	0/6
		11.9	63.4	0.4	0/6		7.8	71.9	1.2	0/6
		7.9	25.5	<0	0/6		5.3	28.6	<0	0/6
34a	po	26.7	97.4	16.6	1/6	iv	26.7	—	—	6/6
		17.8	88.5	2.2	0/6		17.8	94.7	11.9	0/6
		11.9	65.9	<0	0/6		11.9	85.7	<0	0/6
		7.9	−16.3	0.8	0/6		7.9	28.6	0.3	0/6
34b	po	17.8	94.8	13.9	5/6	iv	17.8	—	—	6/6
		11.9	97.4	12.9	0/6		11.9	95.6	12.0	0/6
		7.9	91.5	3.8	0/6		7.9	91.5	2.9	0/6
		5.3	42.4	<0	0/6		5.3	67.6	2.0	0/6
34c	po	40.0	—	—	6/6	iv	26.7	98.3	15.0	2/6
		26.7	97.8	10.0	0/6		17.8	96.9	7.1	0/6
		17.8	97.0	8.8	0/6		11.9	85.7	<0	0/6
		11.9	77.9	<0	0/6		7.9	79.1	1.5	0/6

[a] IR(%) = (1 − TWt/TWc)×100. TWt: the mean tumor weight of the treated group. TWc: the mean tumor weight of the control group.

[b] BWLmax (%): Maximum rate of body weight loss (<0 indicates no body weight loss).

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5. Spectral data of **8a–d** are as follows. Compound **8a**: mp 148–149 °C. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, s), 1.44 (9H, s), 1.48 (3H, s), 1.61 (3H, s), 1.72 (3H, s), 1.88–1.94 (3H, m), 2.05–2.13 (2H, m), 2.32–2.39 (2H, m), 2.38 (6H, s), 2.38 (3H, s), 2.63–2.76 (2H, m), 2.93 (1H, d, $J = 4.4$ Hz), 4.12 (1H, d, $J = 7.3$ Hz), 4.22 (1H, d, $J = 8.3$ Hz), 4.33 (1H, d, $J = 7.2$ Hz), 4.90–4.92 (2H, m), 5.01 (1H, t, $J = 5.4$ Hz), 5.24 (1H, d, $J = 6.4$ Hz), 5.36 (1H, d, $J = 9.3$ Hz), 5.94–5.99 (2H, m), 6.09 (1H, t, $J = 8.7$ Hz), 7.23 (1H, dd, $J = 4.9$, 7.3 Hz), 7.41 (1H, d, $J = 7.8$ Hz), 7.47 (2H, t, $J = 7.8$ Hz), 7.60 (1H, t, $J = 7.3$ Hz), 7.72 (1H, t, $J = 7.3$ Hz), 8.12 (2H, d, $J = 7.8$ Hz), 8.54 (1H, d, $J = 4.9$ Hz), FAB-MS (m/z): 864 $(M+H)^+$. Anal. Calcd for $C_{46}H_{61}N_3O_{13}\cdot 0.5H_2O$: C, 63.29; H, 7.16; N, 4.81. Found: C, 63.12; H, 7.06; N, 4.70; IR: 3450, 2946, 1722, 1712, 1592, 1506, 1469, 1452, 1438 cm^{-1}; $[\alpha]_D^{25}$ −4.6 (c 0.5, $CHCl_3$). Compound **8b**: mp 139–142 °C. ^{1}H NMR ($CDCl_3$) δ: 1.27 (3H, s), 1.44 (9H, s), 1.49 (3H, s), 1.61 (3H, s), 1.72 (3H, s), 1.84–2.13 (5H, m), 2.26–2.39 (2H, m), 2.35 (3H, s), 2.55 (3H, s), 2.94–2.95 (3H, m), 4.16 (1H, d, $J = 7.3$ Hz), 4.22 (1H, d, $J = 8.3$ Hz), 4.33 (1H, d, $J = 8.3$ Hz), 4.90–4.92 (2H, m), 5.00 (1H, br s), 5.26 (1H, d, $J = 6.4$ Hz), 5.36 (1H, d, $J = 8.3$ Hz), 5.94–5.99 (2H, m), 6.09 (1H, t, $J = 8.3$ Hz), 7.22–7.25 (1H, m), 7.42 (1H, d, $J = 7.8$ Hz), 7.47 (2H, t, $J = 7.8$ Hz), 7.60 (1H, t, $J = 7.8$ Hz), 7.72 (1H, t, $J = 7.8$ Hz), 8.12 (2H, d, $J = 7.8$ Hz), 8.54 (1H, d, $J = 3.9$ Hz); FAB-MS (m/z): 850 $(M+H)^+$; HR-MS Calcd for $C_{45}H_{60}N_3O_{13}$: 850.4126. Found, 850.4165; IR: 3423, 2931, 1712, 1592, 1492, 1434, 1367 cm^{-1}; $[\alpha]_D^{24}$ −4.8 (c 0.1, $CHCl_3$). Compound **8c**: mp 210–212 °C. ^{1}H NMR ($CDCl_3$) δ: 0.37–0.41 (2H, m), 0.44–0.48 (2H, m), 1.26 (3H, s), 1.43 (9H, s), 1.49 (3H, s), 1.61 (3H, s), 1.72 (3H, s), 1.78–2.11 (6H, m), 2.25–2.34 (2H, m), 2.35 (3H, s), 2.93 (1H, d, $J = 4.9$ Hz), 3.04 (2H, d, $J = 4.9$ Hz), 4.15 (1H, d, $J = 6.8$ Hz), 4.22 (1H, d, $J = 8.3$ Hz), 4.32 (1H, d, $J = 8.8$ Hz), 4.90–4.92 (2H, m), 4.97 (1H, t, $J = 4.9$ Hz), 5.25 (1H, d, $J = 6.8$ Hz), 5.35 (1H, d, $J = 9.3$ Hz), 5.94–5.99 (2H, m), 6.09 (1H, t, $J = 8.3$ Hz), 7.22–7.25 (1H, m), 7.42 (1H, d, $J = 7.8$ Hz), 7.47 (2H, t, $J = 7.8$ Hz), 7.60 (1H, t, $J = 7.8$ Hz), 7.72 (1H, t, $J = 7.8$ Hz), 8.12 (2H, d, $J = 7.8$ Hz), 8.53 (1H, d, $J = 4.4$ Hz); FAB-MS (m/z): 876 $(M+H)^+$. Anal. Calcd for $C_{47}H_{61}N_3O_{13}\cdot 0.5H_2O$: C, 63.79; H, 7.06; N, 4.75. Found: C, 63.73; H, 7.01; N, 4.58; IR: 3542, 2973, 1724, 1596, 1529, 1442 cm^{-1}; $[\alpha]_D^{22}$ −8.2 (c 0.34, $CHCl_3$). Compound **8d**: mp 211–212 °C. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, s), 1.44 (9H, s), 1.47 (3H, s), 1.61 (3H, s), 1.72 (3H, s), 1.65–2.31 (13H, m), 2.35 (3H, s), 2.87 (2H, d, $J = 5.4$ Hz), 2.94 (1H, d, $J = 5.4$ Hz), 3.33–3.36 (1H, m), 4.15 (1H, d, $J = 7.3$ Hz), 4.22 (1H, d, $J = 8.3$ Hz), 4.33 (1H, d, $J = 8.3$ Hz), 4.90–4.92 (2H, m), 4.95 (1H, t, $J = 4.9$ Hz), 5.24 (1H, d, $J = 6.8$ Hz), 5.35 (1H, d, $J = 7.9$ Hz), 5.94–6.00 (2H, m), 6.09 (1H, t, $J = 8.3$ Hz), 7.22–7.25 (1H, m), 7.42 (1H, d, $J = 7.8$ Hz), 7.47 (2H, t, $J = 7.8$ Hz), 7.60 (1H, t, $J = 7.8$ Hz), 7.72 (1H, t, $J = 7.8$ Hz), 8.13 (2H, d, $J = 7.8$ Hz), 8.54 (1H, d, $J = 3.9$ Hz); FAB-MS (m/z): 890 $(M+H)^+$. Anal. Calcd for $C_{48}H_{63}N_3O_{13}\cdot 0.25H_2O$: C, 64.45; H, 7.15; N, 4.70. Found: C, 64.29; H, 7.15; N, 4.89; IR: 3264, 2983, 1735, 1698, 1596, 1529, 1479, 1446 cm^{-1}; $[\alpha]_D^{22}$ −6.0 (c 0.27, $CHCl_3$).
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8. Spectral data of **31a** and **31b** are as follows. Compound **31a**: mp 159–160 °C. ^{1}H NMR ($CDCl_3$) δ: 1.05 (9H, s), 1.25 (3H, s), 1.39 (9H, s), 1.49 (3H, s), 1.60 (3H, s), 1.79 (3H, s), 1.64–2.03 (5H, m), 2.31–2.38 (2H, m), 2.34 (3H, s), 2.59–2.68 (4H, m), 2.70–2.82 (2H, m), 2.92 (1H, d, $J = 4.8$ Hz), 3.74 (4H, t, $J = 4.9$ Hz), 3.93 (1H, d, $J = 10.3$ Hz), 4.07 (1H, s), 4.13 (1H, d, $J = 6.8$ Hz), 4.24 (1H, d, $J = 8.3$ Hz), 4.33 (1H, d, $J = 8.3$ Hz), 4.60 (1H, s), 4.93 (1H, s), 5.06 (1H, t, $J = 4.9$ Hz), 5.10 (1H, d, $J = 10.3$ Hz), 5.24 (1H, d, $J = 6.3$ Hz), 6.00 (1H, d, $J = 4.8$ Hz), 6.05 (1H, t, $J = 8.3$ Hz), 7.48 (2H, t, $J = 7.8$ Hz), 7.61 (1H, t, $J = 7.8$ Hz), 8.13 (2H, d, $J = 7.8$ Hz); FAB-MS (m/z): 885 $(M+H)^+$. Anal. Calcd for $C_{47}H_{68}N_2O_{14}\cdot H_2O$: C, 62.51; H, 7.81; N, 3.10. Found: C, 62.70; H, 7.73; N, 2.99; IR: 3450, 2956, 1712, 1602, 1492, 1452, 1367 cm^{-1}; $[\alpha]_D^{25}$ −10.6 (c 0.39, $CHCl_3$). Compound **31b**: mp 142–144 °C. ^{1}H NMR ($CDCl_3$) δ: 1.05 (9H, s), 1.25 (3H, s), 1.39 (9H, s), 1.49 (3H, s), 1.62 (3H, s), 1.80 (3H, s), 1.86–2.06 (5H, m), 2.30–2.38 (2H, m), 2.34 (3H, s), 2.38 (6H, s), 2.66 (1H, dd, $J = 5.4$, 13.2 Hz), 2.75 (1H, dd, $J = 3.9$, 13.2 Hz), 2.93 (1H, d, $J = 4.9$ Hz), 3.93 (1H, d, $J = 10.8$ Hz), 4.14 (1H, d, $J = 7.4$ Hz), 4.25 (1H, d, $J = 8.3$ Hz), 4.33 (1H, d, $J = 8.3$ Hz), 4.60 (1H, s), 4.94 (1H, s), 5.04 (1H, t, $J = 4.9$ Hz), 5.09 (1H, d, $J = 10.8$ Hz), 5.26 (1H, d, $J = 6.9$ Hz), 6.00 (1H, d, $J = 4.9$ Hz), 6.05 (1H, t, $J = 7.8$ Hz), 7.48 (2H, t, $J = 7.8$ Hz), 7.61 (1H, t, $J = 7.8$ Hz), 8.13 (2H, d, $J = 7.8$ Hz); FAB-MS (m/z): 843 $(M+H)^+$. Anal. Calcd for $C_{45}H_{66}N_2O_{13}\cdot H_2O$: C, 62.77; H, 7.96; N, 3.25. Found: C, 62.97; H, 7.94; N, 2.98; IR: 3450, 2954, 1712, 1602, 1492, 1452, 1367 cm^{-1}; $[\alpha]_D^{24}$ −21.2 (c 0.2, $CHCl_3$).
9. Spectral data of **34a–i** are as follows. Compound **34a**: mp 166–167 °C. ^{1}H NMR ($CDCl_3$) δ: 1.29 (3H, s), 1.40 (9H, s), 1.49 (3H, s), 1.61 (3H, s), 1.79 (3H, s), 1.70–2.03 (5H, m), 2.30–2.44 (2H, m), 2.35 (3H, s), 2.61–2.65 (4H, m), 2.70–2.82 (2H, m), 2.94 (1H, d, $J = 4.8$ Hz), 3.75 (4H, t, $J = 4.9$ Hz), 4.14 (1H, d, $J = 7.3$ Hz), 4.23 (1H, d, $J = 8.3$ Hz), 4.33 (1H, d, $J = 7.8$ Hz), 4.67 (1H, s), 4.92 (1H, s), 5.05 (1H, t, $J = 4.9$ Hz), 5.25 (1H, d, $J = 7.3$ Hz), 5.65 (1H, d, $J = 7.8$ Hz), 5.99 (1H, d, $J = 5.4$ Hz), 6.09 (1H, t, $J = 7.8$ Hz), 6.20 (1H, d, $J = 8.3$ Hz), 7.29–7.33 (1H, m), 7.43–7.49 (3H, m), 7.60 (1H, t, $J = 7.3$ Hz), 8.13 (2H, d, $J = 7.3$ Hz), 8.40 (1H, d, $J = 4.9$ Hz); FAB-MS (m/z): 924 $(M+H)^+$. Anal. Calcd for $C_{48}H_{62}FN_3O_{14}\cdot 0.25H_2O$: C, 62.09; H, 6.78; N, 4.53; F, 2.05. Found: C, 62.09; H, 6.77; N, 4.61; F, 2.01; IR: 3565, 2950, 1722, 1693, 1600, 1500, 1448 cm^{-1}; $[\alpha]_D^{24}$ −17.9 (c 0.45, $CHCl_3$). Compound **34b**: mp 147–149 °C. ^{1}H NMR

($CDCl_3$) δ: 1.29 (3H, s), 1.41 (9H, s), 1.49 (3H, s), 1.63 (3H, s), 1.79 (3H, s), 1.86–2.08 (5H, m), 2.32–2.38 (2H, m), 2.34 (3H, s), 2.38 (6H, s), 2.66 (1H, dd, $J = 5.4$, 13.6 Hz), 2.75 (1H, dd, $J = 3.9$, 13.6 Hz), 2.94 (1H, d, $J = 4.9$ Hz), 4.14 (1H, d, $J = 6.9$ Hz), 4.23 (1H, d, $J = 8.3$ Hz), 4.33 (1H, d, $J = 8.3$ Hz), 4.68 (1H, d, $J = 2.9$ Hz), 4.92 (1H, s), 5.02 (1H, t, $J = 4.9$ Hz), 5.25 (1H, d, $J = 6.8$ Hz), 5.65 (1H, d, $J = 8.3$ Hz), 6.00 (1H, d, $J = 4.9$ Hz), 6.09 (1H, t, $J = 7.8$ Hz), 6.21 (1H, d, $J = 8.3$ Hz), 7.28–7.33 (1H, m), 7.43–7.49 (3H, m), 7.60 (1H, t, $J = 7.3$ Hz), 8.14 (2H, d, $J = 7.3$ Hz), 8.40 (1H, d, $J = 4.4$ Hz); FAB-MS (m/z): 882 $(M+H)^+$. Anal. Calcd for $C_{46}H_{60}FN_3O_{13}\cdot 1.5H_2O$: C, 60.78; H, 6.99; N, 4.62; F, 2.09. Found: C, 60.86; H, 6.94; N, 4.54; F, 2.17; IR: 3575, 2946, 1722, 1702, 1602, 1492, 1446 cm^{-1}; $[\alpha]_D^{22}$ −18.9 (c 1.0, $CHCl_3$). Compound **34c**: mp 152–156 °C. ^1H NMR ($CDCl_3$) δ: 1.25 (3H, s), 1.21–1.73 (2H, m), 1.45 (9H, s), 1.48 (3H, s), 1.60 (3H, s), 1.73 (3H, s), 1.78–2.11 (3H, m), 2.34 (3H, s), 2.31–2.37 (2H, m), 2.57–2.68 (4H, m), 2.70–2.82 (2H, m), 2.93 (1H, d, $J = 4.9$ Hz), 3.74 (4H, t, $J = 4.9$ Hz), 4.12 (1H, d, $J = 6.8$ Hz), 4.22 (1H, d, $J = 8.3$ Hz), 4.32 (1H, d, $J = 7.8$ Hz), 4.92 (1H, br s), 5.00 (1H, d, $J = 2.0$ Hz), 5.04 (1H, t, $J = 4.4$ Hz), 5.23 (1H, d, $J = 6.8$ Hz), 5.60 (1H, d, $J = 9.3$ Hz), 5.92 (1H, d, $J = 9.3$ Hz), 5.99 (1H, d, $J = 4.9$ Hz), 6.12 (1H, t, $J = 7.8$ Hz), 7.31 (1H, d, $J = 3.4$ Hz), 7.47 (2H, t, $J = 7.8$ Hz), 7.60 (1H, t, $J = 7.8$ Hz), 7.76 (1H, d, $J = 3.4$ Hz), 8.12 (2H, d, $J = 7.8$ Hz); FAB-MS (m/z): 912 $(M+H)^+$. Anal. Cacld for $C_{46}H_{61}N_3O_{13}\cdot H_2O$: C, 59.40; H, 6.83; N, 4.52; S, 3.45. Found: C, 59.53; H, 6.82; N, 4.23; S, 3.51; IR: 3438, 2956, 1718, 1602 cm^{-1}; $[\alpha]_D^{25}$ −9.0 (c 0.25, $CHCl_3$). Compound **34d**: mp 151–154 °C. ^1H NMR ($CDCl_3$) δ: 1.25 (3H, s), 1.21–1.75 (2H, m), 1.45 (9H, s), 1.48 (3H, s), 1.61 (3H, s), 1.74 (3H, s), 1.75–2.08 (3H, m), 2.32–2.39 (2H, m), 2.34 (3H, s), 2.38 (6H, s), 2.66 (1H, dd, $J = 5.4$, 13.6 Hz), 2.75 (1H, dd, $J = 3.9$, 13.6 Hz), 2.93 (1H, d, $J = 4.9$ Hz), 4.12 (1H, d, $J = 6.8$ Hz), 4.22 (1H, d, $J = 8.3$ Hz), 4.32 (1H, d, $J = 8.3$ Hz), 4.92 (1H, s), 4.99–5.03 (2H, m), 5.24 (1H, d, $J = 6.8$ Hz), 5.60 (1H, d, $J = 9.3$ Hz), 5.92 (1H, d, $J = 9.3$ Hz), 5.99 (1H, d, $J = 4.9$ Hz), 6.13 (1H, t, $J = 7.8$ Hz), 7.31 (1H, d, $J = 2.9$ Hz), 7.47 (2H, t, $J = 7.8$ Hz), 7.60 (1H, t, $J = 7.8$ Hz), 7.76 (1H, d, $J = 2.9$ Hz), 8.12 (2H, d, $J = 7.8$ Hz); FAB-MS (m/z): 870 $(M+H)^+$. Anal. Calcd for $C_{44}H_{59}N_3O_{13}S\cdot H_2O$: C, 59.51; H, 6.92; N, 4.73; S, 3.61. Found: C, 59.56; H, 6.71; N, 4.76; S, 3.60; IR: 3409, 2948, 1712, 1602, 1490, 1452, 1367 cm^{-1}; $[\alpha]_D^{24}$ −13.8 (c 0.2, $CHCl_3$). Compound **34e**: mp 209–210 °C. ^1H NMR ($CDCl_3$) δ: 0.37–0.51 (4H, m), 1.25 (3H, s), 1.45 (9H, s), 1.49 (3H, s), 1.61 (3H, s), 1.74 (3H, s), 1.82–2.12 (6H, m), 2.25–2.29 (1H, m), 2.33–2.37 (1H, m), 2.35 (3H, s), 2.94 (1H, d, $J = 5.4$ Hz), 3.04 (2H, d, $J = 4.9$ Hz), 4.15 (1H, d, $J = 7.3$ Hz), 4.23 (1H, d, $J = 8.3$ Hz), 4.33 (1H, d, $J = 8.3$ Hz), 4.92 (1H, br s), 4.97–5.00 (2H, m), 5.26 (1H, d, $J = 7.3$ Hz), 5.59 (1H, d, $J = 9.8$ Hz), 5.93 (1H, d, $J = 9.8$ Hz), 6.00 (1H, d, $J = 4.9$ Hz), 6.13 (1H, t, $J = 8.3$ Hz), 7.31 (1H, d, $J = 2.9$ Hz), 7.47 (2H, t, $J = 7.8$ Hz), 7.60 (1H, t, $J = 7.8$ Hz), 7.76 (1H, d, $J = 2.9$ Hz), 8.12 (2H, d, $J = 7.8$ Hz); FAB-MS (m/z): 882 $(M+H)^+$. Anal. Calcd for $C_{45}H_{59}N_3O_{13}S\cdot 0.5H_2O$: C, 60.66; H, 6.79; N, 4.72; S, 3.60. Found: C, 60.61; H, 6.59; N, 4.74; S, 3.59; IR: 3490, 2967, 1741, 1706, 1602, 1531, 1454, 1369 cm^{-1}; $[\alpha]_D^{24}$ −12.4 (c 0.21, $CHCl_3$). Compound **34f**: mp 159–160 °C. ^1H NMR ($CDCl_3$) δ: 1.26 (3H, s), 1.43 (9H, s), 1.48 (3H, s), 1.60 (3H, s), 1.71 (3H, s), 1.82–2.12 (6H, m), 2.31–2.39 (1H, m), 2.35 (3H, s), 2.59–2.67 (4H, m), 2.72 (1H, dd, $J = 4.9$, 13.2 Hz), 2.80 (1H, dd, $J = 3.9$, 13.2 Hz), 2.92 (1H, d, $J = 4.9$ Hz), 3.74 (4H, t, $J = 4.9$ Hz), 4.12 (1H, d, $J = 7.3$ Hz), 4.22 (1H, d, $J = 8.8$ Hz), 4.32 (1H, d, $J = 8.3$ Hz), 4.56 (1H, br s), 4.92 (1H, s), 4.93 (1H, d, $J = 2.4$ Hz), 5.04 (1H, t, $J = 4.9$ Hz), 5.23 (1H, d, $J = 6.8$ Hz), 5.49 (1H, d, $J = 9.2$ Hz), 5.75 (1H, d, $J = 9.8$ Hz), 5.99 (1H, d, $J = 4.9$ Hz), 6.10 (1H, t, $J = 7.8$ Hz), 7.32 (1H, s), 7.47 (2H, t, $J = 7.8$ Hz), 7.60 (1H, t, $J = 7.8$ Hz), 8.12 (2H, d, $J = 7.8$ Hz), 8.80 (1H, d, $J = 1.9$ Hz); FAB-MS (m/z): 912 $(M+H)^+$. Anal. Calcd for $C_{46}H_{61}N_3O_{14}S\cdot 0.5H_2O$: C, 59.98; H, 6.78; N, 4.56; S, 3.48. Found: C, 60.04; H, 6.58; N, 4.56; S, 3.60; IR: 3559, 2948, 1720, 1697, 1687, 1600, 1500, 1452 cm^{-1}; $[\alpha]_D^{24}$ −5.2 (c 0.42, $CHCl_3$). Compound **34g**: mp 141–144 °C. ^1H NMR ($CDCl_3$) δ: 1.26 (3H, s), 1.43 (9H, s), 1.48 (3H, s), 1.62 (3H, s), 1.72 (3H, s), 1.82–2.12 (6H, m), 2.34–2.38 (1H, m), 2.34 (3H, s), 2.38 (6H, s), 2.66 (1H, dd, $J = 4.9$, 13.2 Hz), 2.75 (1H, dd, $J = 3.9$, 13.2 Hz), 2.93 (1H, d, $J = 4.9$ Hz), 4.12 (1H, d, $J = 6.9$ Hz), 4.22 (1H, d, $J = 8.3$ Hz), 4.32 (1H, d, $J = 8.3$ Hz), 4.92 (1H, s), 4.93 (1H, d, $J = 1.9$ Hz), 5.02 (1H, t, $J = 4.9$ Hz), 5.24 (1H, d, $J = 6.8$ Hz), 5.49 (1H, d, $J = 9.2$ Hz), 5.75 (1H, d, $J = 9.8$ Hz), 5.99 (1H, d, $J = 4.9$ Hz), 6.10 (1H, t, $J = 8.3$ Hz), 7.31 (1H, d, $J = 1.4$ Hz), 7.47 (2H, t, $J = 7.8$ Hz), 7.60 (1H, t, $J = 7.8$ Hz), 8.13 (2H, d, $J = 7.8$ Hz), 8.80 (1H, d, $J = 1.4$ Hz); FAB-MS (m/z): 870 $(M+H)^+$. Anal. Calcd for $C_{44}H_{59}N_3O_{13}S\cdot 0.75H_2O$: C, 59.81; H, 6.90; N, 4.76; S, 3.63. Found: C, 59.84; H, 6.75; N, 4.48; S, 3.56; IR: 3423, 2948, 1712, 1602, 1492, 1452, 1367 cm^{-1}; $[\alpha]_D^{24}$ −16.3 (c 0.15, $CHCl_3$). Compound **34h**: mp 152–155 °C. ^1H NMR ($CDCl_3$) δ: 1.23 (3H, s), 1.19–1.72 (2H, m), 1.42 (9H, s), 1.47 (3H, s), 1.60 (3H, s), 1.63 (3H, s), 1.75–2.06 (3H, m), 2.31 (3H, s), 2.32–2.38 (2H, m), 2.57–2.68 (4H, m), 2.70–2.82 (2H, m), 2.91 (1H, d, $J = 4.9$ Hz), 3.74 (4H, t, $J = 4.9$ Hz), 4.09 (1H, d, $J = 6.8$ Hz), 4.24 (1H, d, $J = 8.4$ Hz), 4.33 (1H, d, $J = 8.4$ Hz), 4.60 (1H, br s), 4.94 (1H, br s), 5.04 (1H, t, $J = 4.9$ Hz), 5.22 (1H, d, $J = 6.9$ Hz), 5.62–5.68 (2H, m), 6.00 (1H, d, $J = 4.9$ Hz), 6.10 (1H, t, $J = 7.8$ Hz), 7.48 (2H, t, $J = 7.8$ Hz), 7.59 (1H, t, $J = 7.8$ Hz), 7.92 (1H, s), 8.12 (2H, d, $J = 7.8$ Hz), 8.75 (1H, s); FAB-MS (m/z): 912 $(M+H)^+$. Anal. Calcd for $C_{46}H_{61}N_3O_{14}S\cdot 1.25H_2O$: C, 59.12; H, 6.85; N, 4.50; S, 3.43. Found: C, 59.16; H, 6.58; N, 4.51; S, 3.47; IR: 3423, 2956, 1712, 1600, 1490, 1452, 1367 cm^{-1}; $[\alpha]_D^{24}$ −3.4 (c 0.1, $CHCl_3$). Compound **34i**: mp 153–157 °C. ^1H NMR ($CDCl_3$) δ: 1.23 (3H, s), 1.20–1.69 (2H, m), 1.42 (9H, s), 1.47 (3H, s), 1.61 (3H, s), 1.65 (3H, s), 1.75–2.05 (3H, m), 2.30–2.39 (2H, m), 2.31 (3H, s), 2.38 (6H, s), 2.66 (1H, dd, $J = 4.9$, 13.5 Hz), 2.75 (1H, dd, $J = 3.9$, 13.5 Hz), 2.91 (1H, d, $J = 4.9$ Hz), 4.10 (1H, d, $J = 6.8$ Hz), 4.24 (1H, d, $J = 8.3$ Hz), 4.33 (1H, d, $J = 8.3$ Hz), 4.60 (1H, br s), 4.94 (1H, br s), 5.02 (1H, t, $J = 4.4$ Hz), 5.23 (1H, d, $J = 6.8$ Hz), 5.62–5.71 (2H, m), 6.01 (1H, d, $J = 4.9$ Hz), 6.10 (1H, t, $J = 7.8$ Hz), 7.48 (2H, t, $J = 7.8$ Hz), 7.61 (1H, t, $J = 7.8$ Hz), 7.92 (1H, s), 8.12 (2H, d, $J = 7.8$ Hz), 8.75 (1H, s); FAB-MS (m/z): 870 $(M+H)^+$. Anal. Calcd for $C_{44}H_{59}N_3O_{13}S\cdot H_2O$: C, 59.51; H, 6.92; N, 4.73; S, 3.61. Found: C, 59.37; H, 6.67; N, 4.77; S, 3.57; IR: 3430, 2948, 1710, 1600, 1490, 1452, 1367 cm^{-1}; $[\alpha]_D^{24}$ −18.7 (c 0.2, $CHCl_3$).

10. The condition of the metabolic stability test of **35** differed from those of the other compounds. Under this condition, the remaining rates of **35** and **3** after 5 min of incubation with human liver microsomes were about 66% and 84%, respectively.
11. PC-6/VCR29-9: PC-6 cell line, which is resistant to Vincristine®. PC-6/VP1-1: PC-6 cell line, which is resistant to VP-16 (Etoposide®).
12. Poste, G.; Doll, J.; Hart, I. R.; Fidler, I. J. *Cancer Res.* **1980**, *40*, 1636.