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OSW-1 analogues: Modification of the carbohydrate moiety

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Abstract—4 OSW-1 analogues featuring modified carbohydrate moieties were prepared. The purpose of these modifications was to assess the importance of certain chemical functions with respect to biological activity. The synthesis and biological activity of the target molecules are shown.

OSW-1 (**1**) (Fig. 1) belongs to a family of saponins endowed with considerable antitumor properties. Considering the complex structure of this molecule, however, discovery of improved, simpler, and more 'drug-like' analogues is a prerequisite to potential clinical uses. To this effect, research efforts aimed at identifying the structural elements required for biological activity have been initiated by several research groups. These studies mainly include modification of the substitution pattern at OH-2 and OH-2′,[1,2] (see Fig. 1 for atom numbering) and replacement of the natural steroidal moiety by other types of aglycones including simple alcohols.[3] In particular, we recently showed that replacement of the natural cholestane aglycone in OSW-1 by an estrane moiety was possible albeit at the cost of some activity loss.[3c]

Regarding the carbohydrate part of the molecule, it has been established that the 2-*O*-acetyl and 2′-*O*-4-methoxybenzoyl (or -cinnamyl) groups (see Fig. 1) are important for biological activity.[4] However, the role (if any) of the remaining OHs on the carbohydrate backbone is not known. To contribute to answering this question we have now synthesized and evaluated a short series of simplified OSW-1 analogues, modified on the disaccharide moiety.[5]

In order to facilitate the comparison of biological activities, the OSW-1 estrane analogue (**2**) that we recently prepared and for which we have accurate biological data was chosen as lead molecule. We then selected the target molecules shown in Figure 2 which were specifically designed to answer specific questions.

In compound **3**, the role of the 4-methoxybenzoyl groups (pMBz) is examined.

In compounds **4** and **5** the role of the substituent on C-4 is examined.

In compound **6**, the key acetate and pMBz groups are present but the xylose moiety is replaced by a simple, semi-rigid spacer.

The preparation of analogue **3** is depicted in Scheme 1: α-benzyl-D-xylopyranoside **7**[6] was first converted to the protected disaccharide **17**. Although the sequence is classical, its efficacy was severely hampered by the facile C2 → C1 silyl migration during the preparation of intermediates **10** and **11**. Compound **11** (containing 40% of isomer **10**) was allowed to react with the partially protected L-arabinoside **12**[6] to afford a mixture of 1 → 3 and 1 → 4 disaccharides (**14** and **13**, respectively).

Keywords: OSW-1; Analogues; Cytotoxicity; Cancer.
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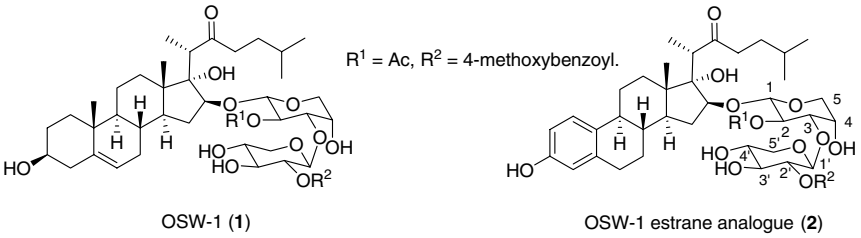


Figure 1. OSW-1 and OSW-1 estrane analogue.

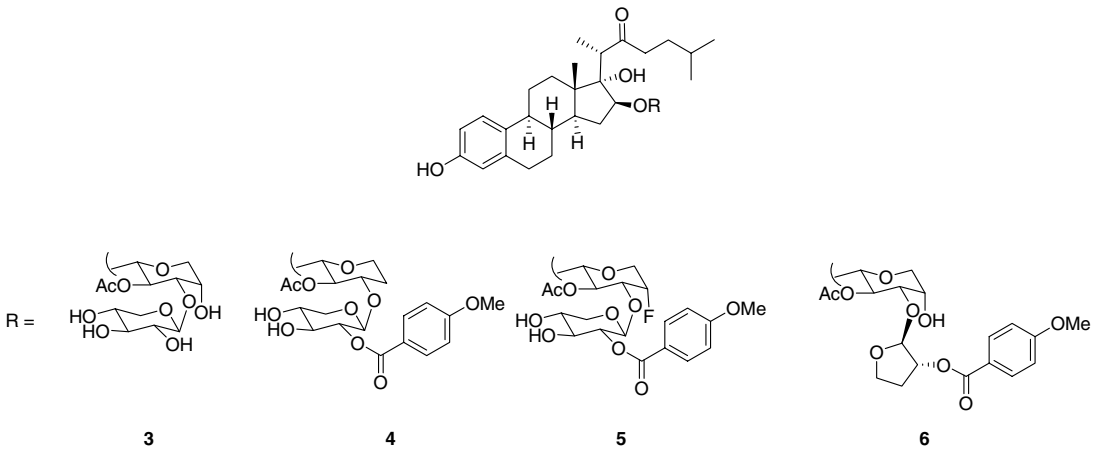
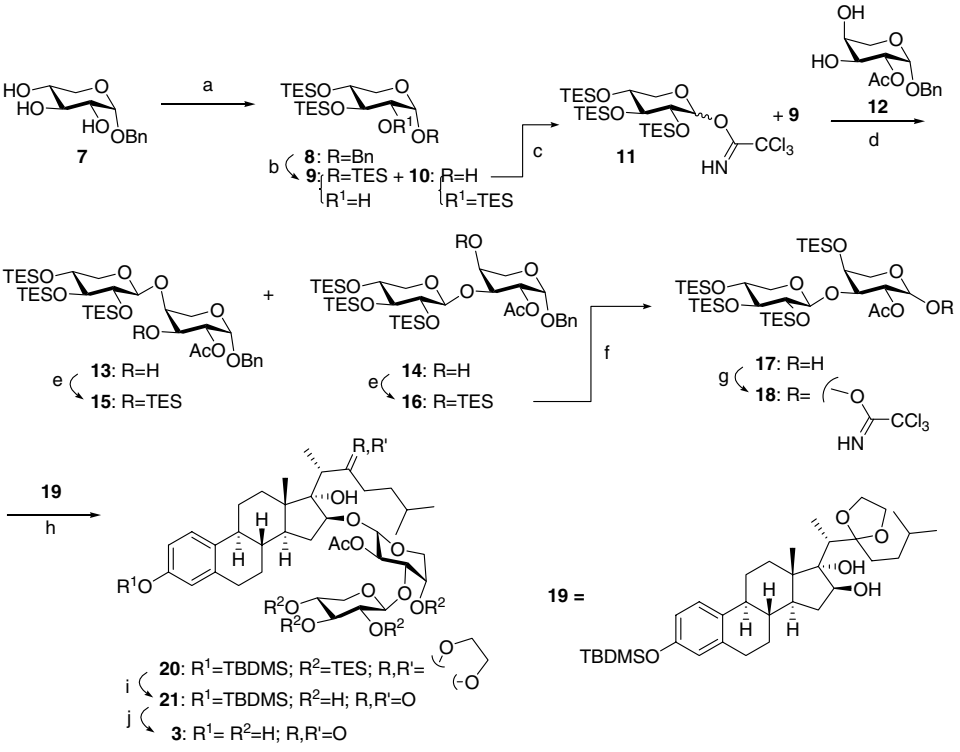


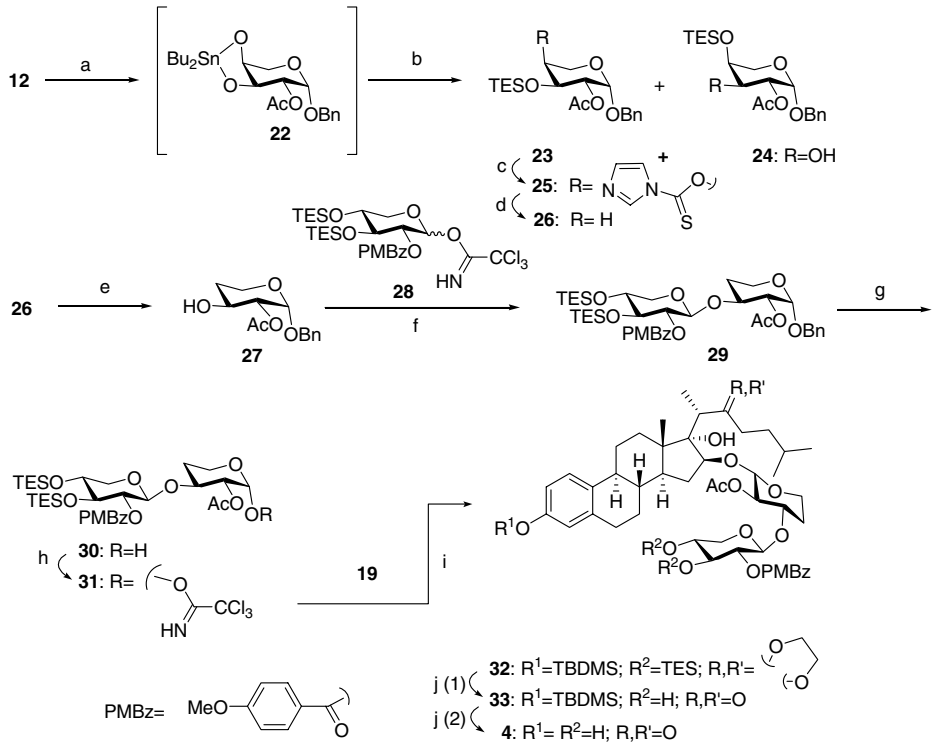
Figure 2. OSW-1 estrane analogues with modified disaccharide moieties.



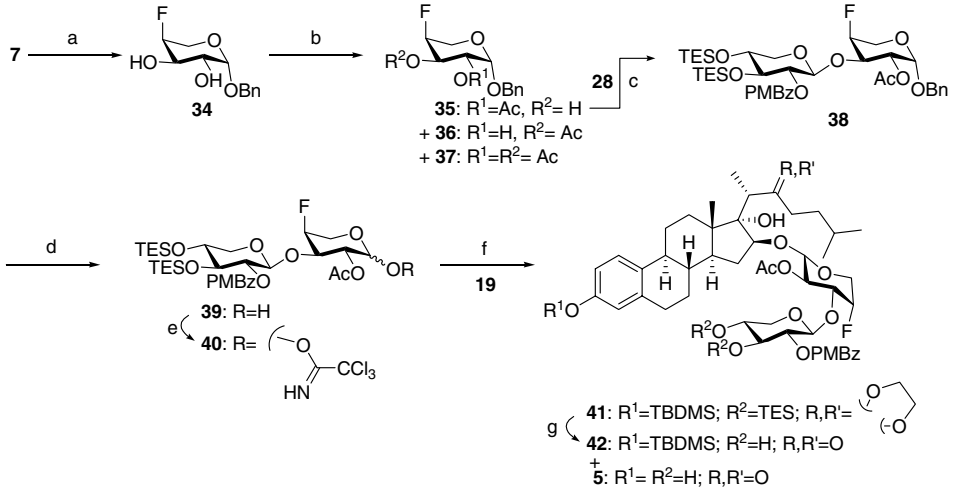
Scheme 1. Reagents and conditions: (a) TESCl, imidazole, DMAP, DMF, 20 °C, 2 h, 70%; (b) $Pd(OH)_2/C$, Na_2CO_3, H_2, AcOEt, 20 °C, 24 h, **8** 35%, **9** 5%, **10** 56%; (c) Cl_3CCN, DBU, CH_2Cl_2, 20 °C, 3 h; (d) BF_3-OEt_2, CH_2Cl_2, 4 mol sieves, −60 °C, 10 min then warm up to 20 °C in the course of 30 min, mixture of **13** + **14** 38% (from **12**); (e) TESOTf, CH_2Cl_2, 2,6-lutidine, −20 °C, 1 h, total yield **15** + **16** 87% ; (f) Na_2CO_3, $Pd(OH)_2/C$, H_2, AcOEt, 20 °C, 22 h, 67%; (g) Cl_3CCN, DBU, CH_2Cl_2, 20 °C, 2 h; (h) TMSOTf, CH_2Cl_2, 4 mol sieves, −20 °C, 1 h; (i) $PdCl_2(CH_3CN)_2$, acetone/H_2O 20/1, air, 20 °C, 15 h; (j) TBAF, THF, 22% (from **19**).

Silylation of the 4-hydroxyl group in **14** furnished **16** and hydrogenolysis of the anomeric benzyl group led to **17** which was converted to trichloroacetimidate **18**. Condensation of the latter with the protected aglycone **19**[3c] afforded the 2′-debenzoylated OSW-1 analogue **20**. Sequential removal of the TES and ethyleneketal protecting groups ($PdCl_2(CH_3CN)_2$) and cleavage of the phenolic TBDMS ether with tetra-*n*-butyl ammonium fluoride (TBAF) followed by chromatography (silica gel, eluent: $MeOH/CH_2Cl_2$ 1:15)[7] provided crude **3**.

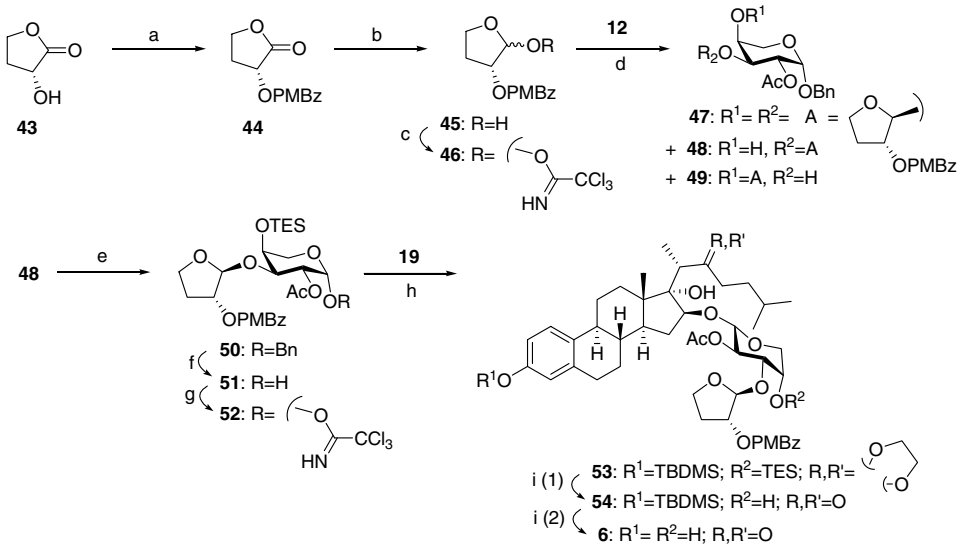
Our next target was the 4-deoxy analogue **4** (Scheme 2). Formation of the 3,4-stannylene **22** followed by exposure to chloro-triethylsilane afforded a ca. 4:1 mixture



Scheme 2. Reagents and conditions: (a) Bu_2SnO, Toluene, reflux; (b) TESCl, NEt_3, −10 → 20 °C, 15 h; (c) $(Imid)_2CS$, DMAP (cat.), CH_2Cl_2, reflux, 4 h, 67% (from **12**); (d) Bu_3SnH, Toluene, reflux, 85%; (e) TBAF, 20 °C, 84%; (f) BF_3/Et_2O, CH_2Cl_2, mol sieves 4 Å, −70 °C, 34%; (g) $Pd(OH)_2/C$, AcOEt, NEt_3, H_2, 15 h, 76%; (h) Cl_3CCN, DBU, CH_2Cl_2, 2 h, 83% (from **29**); (i) TMSOTf (cat.), CH_2Cl_2, mol sieves 4 Å, −20 °C, 1.25 h; (j) 1—$PdCl_2(CH_3CN)_2$, acetone/H_2O 20/1, 20 °C, air, 60 h; 2—TBAF, THF, 25 min, 35% (from **19**).



Scheme 3. Reagents and conditions: (a) DAST, CH_2Cl_2, −45 → 20 °C, 31%; (b) Ac_2O, pyridine, −30 → 20 °C, **37** 14%, **36** 13%, **35** 22%, **34** 49%; (c) BF_3-OEt_2, CH_2Cl_2, 4 Å mol sieves, −60 → 20 °C, 74%; (d) $Pd(OH)_2/C$, $NaHCO_3$, H_2, AcOEt, 15 h, 60%; (e) Cl_3CCN, DBU (cat.), CH_2Cl_2, 2 h, 99%; (f) TMSOTf (cat.), CH_2Cl_2, mol sieves 4 Å, −20 °C, **19** 50%, **41** 45%; (g) $PdCl_2(CH_3CN)_2$, acetone/H_2O 20/1, air, 20 °C, 48 h, **42** 12%, **5** 35% (from **19**).



Scheme 4. Reagents and conditions: (a) PMBzCl, CH_2Cl_2, NEt_3, 75 min, 91%; (b) Dibal-H, THF, −78 °C, 10 min, 81%; (c) Cl_3CCN, DBU (cat.), CH_2Cl_2, 3 h; (d) BF_3-OEt_2, CH_2Cl_2, 4 mol sieves, −60 °C, 10 min then warm up to 20 °C in the course of 30 min, 20 °C, 1 h, **47** 15%, **49** 17%,**48** 44% (from **12**); (e) TESOTf, CH_2Cl_2, 2,6-lutidine, −20 °C → 20 °C, 1 h, 96%. (f) $Pd(OH)_2$/C, NEt_3, H_2, AcOEt, 20 °C, 48 h, 98%; (g) Cl_3CCN, DBU (cat.), CH_2Cl_2, 20 °C, 2 h; (h) TMSOTf (cat.), CH_2Cl_2, mol sieves 4 Å, −20 °C; (i) 1—$PdCl_2(CH_3CN)_2$, acetone/H_2O 20/1, air, 20 °C, 40 h; 2—TBAF, THF, 37% (from **19**).

Table 1. Effects of OSW-1 analogues on tumor cell growth (primary assay) [8]

Compounds	IC_{50} (μM)	
	NCI-H460	MDA-MB-231
Assay 1		
2	0.26	0.68
Cisplatin	0.28	4.44
Assay 2		
3	9.98	6.94
Cisplatin	0.42	5.78
Assay 3		
4	13.7	10.9
5	2.59	1.71
Cisplatin	0.41	4.23
Assay 4		
6	5.75	18.2
Cisplatin	0.58	7.44

of monosilyl ethers **23** and **24** which was treated with 1,1-thiocarbonyldiimidazole. From the resulting mixture of thiocarbonyl derivatives, the desired 3-OTES isomer **25** could be isolated by chromatography and, upon treatment with Bu_3SnH, afforded the corresponding deoxy derivative **26**. Compound **26** was converted to the desired protected disaccharide **30** via a short sequence: removal of the silyl protecting group, coupling with the trichloroacetimidate **28**,[6] and debenzylation of the resulting benzyl glycoside **29** afforded the L-4-deoxy-3-[β-D-xylopyranosyl]-arabinose **30**. The latter was readily converted to the corresponding 1-trichloroacetimidate **31** which was coupled with aglycone **19**. Standard deprotection procedures as for **21** successively gave **32**, **33**, then the 4-deoxyanalogue of OSW-1 **4**.

The fluorinated analogue **5** was prepared as depicted in Scheme 3. The benzyl xyloside **7** was treated with DAST to afford 4-deoxy-4-fluoro-α-L-arabinopyranoside **34**. Acetylation yielded 2-*O*-acetyl-4-deoxy-4-fluoro-α-L-arabinopyranoside **35** as the major product along with 3-*O*-acetyl-4-deoxy-4-fluoro-α-L-arabinopyranoside **36** and 2,3-di-*O*-acetyl-4-deoxy-4-fluoro-α-L-arabinopyranoside **37**. Coupling of **35** with trichloroacetimidate **28** gave the protected, fluorinated disaccharide **38** which

Table 2. Effects of OSW-1 analogues on tumor cell growth (secondary assay)

Compounds	IC_{50} (μM)							
	NCI-H460	T-47D	MDA-MB-231	A498	PC-3	DLD-1	HCT116	UO31
Assay 5								
2	0.44	0.71	1.01	0.43	1.40	1.18	NT	NT
Cisplatin	0.66	11.5	7.85	2.11	3.62	2.21	NT	NT
Adriamycin	0.0071	0.057	0.17	0.048	0.36	0.10	NT	NT
Assay 6								
5	5.34	2.81	0.85	0.30	0.68	1.72	2.90	0.056
Cisplatin	0.48	7.62	3.04	1.99	2.54	1.93	1.71	1.04
Adriamycin	0.0037	0.027	0.10	0.035	0.20	0.13	0.037	0.10

NT, not tested.

led to the OSW-1 analogue **5** via a sequence similar to that used for the preparation of **4**.

To complete this series of structure/activity relationships, we needed to prepare an analogue with a simplified disaccharide moiety in which only the structural elements believed to be required for biological activity are present. The target molecule **6** whose preparation is depicted in Scheme 4 and in which OH-3′ and OH-4′ have been removed fulfils these requirements. Compound **6** features a 3-deoxy-D-erythrofuranose moiety which in our view is the minimal structure allowing the presentation of the key PMBz group while maintaining a high degree of rigidity.

The antitumor activity of the four new OSW-1 analogues was first evaluated using non small-cell lung cancer cells NCI-H460 and breast cancer cells MDA-MB-231 (Table 1). The OSW-1 estrane analogue **2** and cisplatin were used as positive controls. The best compound according to this first series of experiments was then evaluated in additional cellular assays (Table 2). Adryamycin (Doxorubicin) was added as positive control. In the primary assay, the analogue **3** lacking the pMBz group showed only weak activity, in line with results from the literature obtained with related compounds. Surprisingly, an even lower level of activity was observed for **4**, despite the presence of the key Ac and pMBz groups. This strongly suggests that the 4-OH group plays a crucial role for the biological activity (perhaps as a H-bond donor or acceptor). Introduction of the polar fluorine atom at C-4 partially restores activity. Indeed **5** is the only analogue with activity approaching that of the parent molecule **2** in the MDA-MB-231 assay (see Table 1). Finally, compound **6**, which features the key Ac and pMBz groups as well as the arabinose moiety found in OSW-1, showed only weak activity in the NCI-H460 assay and was almost inactive on MDA-MB-231 cells.

The fluorocompound **5** was then submitted to a series of tests using a broad variety of cell lines. Overall, **5** appears to be somewhat less active than the parent molecule **2**. Noteworthy, however, is the good activity of **5** in several assays (columns 5, 6, and 9 in Table 2).

In conclusion, modification of the disaccharide part of an analogue of OSW-1 led to dramatic changes in biological activity. It appears that, besides the two acyl groups (Ac and pMBz), whose role is already documented, additional factors are important for activity. From the present work, OH-4 clearly appears to be crucial and the lack of activity of **6** suggests that the integrity of the acylated xylopyranoside moiety is equally important.

Acknowledgment

We are indebted to Ms. Marie-Christine Matos for help with the synthesis of compound **5**.

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7. Analytical data for analogues **3**–**6**.
Compound **3**. ^{1}H NMR (400 MHz, C_5D_5N) δ = 7.30 (1H, d, J = 8.3 Hz), 7.07 (1H, dd, J = 8.3, 2.3 Hz), 7.00 (1H, d, J = 2.0 Hz), 5.89 (1H, dd, J = 9.0, 7.0 Hz), 4.93 (1H, s), 4.92 (1H, d, J = 7.5 Hz), 4.66 (1H, d, J = 7.0 Hz), 4.46 (1H, bs), 4.34 (1H, dd, J = 11.2, 4.9 Hz), 4.31–4.15 (3H, m), 4.14 (1H, dd, J = 9.4, 4.9 Hz), 4.09 (1H, t, J = 8.6 Hz), 3.85 (1H, t, J = 8.1 Hz), 3.77 (1H, dd, J = 11.8, 1.5 Hz), 3.70 (1H, dd, J = 11.1, 10.0 Hz), 3.35 (1H, q, J = 7.4 Hz), 2.95–2.74 (4H, m), 2.46 (1H, dt, J = 13.1, 7.6 Hz), 2.39 (3H, s), 2.36–2.10 (4H, m), 1.79–1.40 (8H, m), 1.34 (3H, d, J = 7.2 Hz), 1.32–1.22 (2H, m), 0.99 (3H, s), 0.96 (3H, d, J = 5.9 Hz), 0.93 (3H, d, J = 5.9 Hz). ^{13}C NMR (100.62 MHz, C_5D_5N) δ = 218.9, 169.9, 156.7, 138.2, 131.5, 126.9, 116.3, 113.8, 106.8, 101.6, 88.1, 85.7, 80.2, 78.3, 74.2, 72.2, 70.9, 68.9, 67.2, 66.8, 47.3, 47.0, 46.4, 44.0, 39.4, 39.2, 34.8, 33.1, 32.8, 30.1, 28.2, 27.9, 26.8, 22.8, 22.5, 21.5, 13.6, 11.8. MS (FAB) *m/z*:721 (M^++H), 744 (M^++H+Na); HRMS (FAB) Calcd for $C_{38}H_{57}O_{13}$:721.3799 (M^++H), found:721.3829, Calcd for $C_{38}H_{57}NaO_{13}$:744.3697 (M^++H+Na), found:744.3668.
Compound **4**. ^{1}H NMR (400 MHz, C_5D_5N) δ = 8.34 (2H, d, J = 9.0 Hz), 7.30 (1H, d, J = 8.6 Hz), 7.10 (2H, d, J = 8.8 Hz), 7.07 (1H, dd, J = 7.3, 2.5 Hz), 6.99 (1H, d, J = 2.5 Hz), 5.73 (1H, dd, J = 9.0, 7.8 Hz), 5.04 (1H, d, J = 7.8 Hz), 4.98 (1H, dd, J = 6.8, 5.3 Hz), 4.85 (1H, s), 4.51 (1H,d, J = 5.3 Hz), 4.36 (1H, broad d, J = 11.3 Hz), 4.28–4.26 (3H, m), 4.13 (1H, dd, J = 7.6, 5.0 Hz), 4.09–4.01 (2H, m), 3.74 (3H, s), 3.68 (1H, d, J = 7.8 Hz), 3.47 (1H, ddd, J = 11.7, 8.8, 2.9 Hz), 3.22 (1H, q, J = 7.5 Hz), 2.91–2.81 (1H, m), 2.76 (1H, broad dd, J = 16.8, 5.0 Hz), 2.69–2.61 (1H, m), 2.57–2.49 (1H, m), 2.41 (1H, dt, J = 13.4, 7.4 Hz), 2.36–2.10 (6H?, m), 2.02 (3H, s), 2.02–1.93 (1H, m), 1.81–1.70 (2H, m), 1.65–1.44 (4H, m), 1.32 (3H, d, J = 7.6 Hz),1.35–1.25 (1H, m), 1.04 (3H, s), 0.90 (3H, d, J = 6.1 Hz), 0.88 (3H, d,J = 6.1 Hz).^{3}C NMR (100.6 MHz, C_5D_5N) δ = 218.9, 169.1, 165.5, 163.8, 156.6, 138.1, 132.3 (2C atom), 131.5, 126.8, 116.2, 114.1 (2C atom), 113.8, 103.7, 100.8, 88.4, 85.9, 76.8, 76.5, 75.3, 73.1, 71.0, 67.1, 59.6, 55.4, 47.2, 46.9, 46.3, 43.9, 39.2 (2C atom), 34.6, 33.0, 32.6, 31.7, 30.0, 28.2, 27.7, 26.8, 22.5, 22.4, 20.9, 13.7, 11.9. MS (FAB) *m/z*: 839 (M^++H), 862 (M^++H+Na); HRMS (FAB) Calcd for $C_{46}H_{63}O_{14}$: 839.4218 (M^++H), found: 839.4182, Calcd for $C_{46}H_{63}NaO_{14}$: 862.4116 (M^++H+Na), found: 862.4127.

Compound **5**. ^{1}H NMR (400 MHz, C_6D_6) : δ = 8.07 (2H, d, J = 8.6 Hz), 7.10 (1H, d, J = 8.6 Hz), 6.59 (2H, d, J = 8.6 Hz), 6.48 (1H, d, J = 8.3 Hz), 6.34 (1H, broad s,), 5.23 (1H, t, J = 6.5 Hz), 5.16 (1H, t, J = 3.7 Hz), 5.03 (1H, broad s), 4.71 (1H, s), 4.43 (1H, d, J = 49.4 Hz), 4.34 (1H, d, J = 6.0 Hz), 4.27 (near CH_2Cl_2, 1H, d, J = ~11 Hz), 4.16 (1H, t, 6.6 Hz), 3.89 (1H, s), 3.86 (1H, broad s), 3.78 (1H, m), 3.62 (2H, m), 3.46, (1H, dd, J = 12.5, 4.0 Hz), 3.21 (1H, q, J = 7.2 Hz), 3.14 (3H, s), 3.00 (1H, d, J = 4.0 Hz), 2.91 (2H, m), 2.77–2.29 (~7H, m), 2.27–2.14 (3H, m), 1.90 (3H, s), 1.83–1.39 (9H, m), 1.30 (2H, m), 1.25 (3H, d, J = 7.6 Hz), 0.98 (3H, s), 0.92 (3H, d, J = 6.3 Hz), 0.88 (3H, d, J = 6.3 Hz). ^{13}C NMR (100.6 MHz, C_6D_6): δ = 132.2, 115.5, 114.2, 113.0, 100.2, 99.5, 90.1, 71.6, 70.1, 70.7, 69.1, 62.3, 61.7, 54.9, 47.1, 43.7, 39.2, 32.9, 30.0, 27.2, 26.8, 22.8, 22.4, 20.7, 13.9, 11.8. MS (FAB) *m*/*z*: 857 (M^++H), 880 (M^++H+Na); HRMS (FAB) Calcd for $C_{46}H_{62}FO_{14}$: 857.4124 (M^++H), found: 857.4155, Calcd for $C_{46}H_{62}FNaO_{14}$: 880.4021 (M^++H+Na), found: 880.4010.

Compound **6**. ^{1}H NMR (400 MHz, pyridine-d_5)δ = 11.16 (1H, br s), 8.18 (2H, d, J = 8.8 Hz), 7.32 (1H, d, J = 8.6 Hz), 7.10–7.01 (4H, m), 5.74 (1H, s), 5.64–5.53 (2H, m), 4.99 (s very b), 4.92 (1H, s), 4.72 (1H, d, J = 4.8 Hz), 4.38 (1H, m), 4.16–4.29 (5H, m), 3.78 (1H, dd, J = 11.4, 2.3 Hz), 3.72 (3H, s), 3.47 (1H, q, J = 7.3 Hz), 2.93–2.73 (4H, m), 2.52–2.45 (1H, m), 2.29 (3H, m), 2.40–2.14 (5H, m), 2.05–1.98 (1H, m), 1.79 (2H, m), 1.71–1.48 (6H, m), 1.39 (3H, d, J = 7.6 Hz), 1.36–1.24 (2H, m), 1.10 (3H, s), 0.92 (3H, d, J = 6.3 Hz), 0.88 (3H, d, J = 6.3 Hz).
^{13}C NMR (100.6 MHz, pyridine-d_5): δ = 218.9, 171.8, 167.8, 166.2, 158.7, 140.2, 134.1, 133.5, 129.0, 118.3, 116.3, 115.9, 108.5, 103.0, 91.2, 87.9, 80.5, 78.5, 73.1, 69.0, 66.8, 57.5, 49.3, 49.1, 48.6, 46.0, 41.6, 41.4, 36.5, 35.1, 34.8, 32.1, 31.9, 30.2, 29.9, 28.9, 24.7, 24.4, 23.1, 15.8, 14.0. MS (FAB) *m*/*z*: 809 (M^++H), 832 (M^++H+Na); HRMS (FAB) Calcd for $C_{45}H_{61}NaO_{13}$: 832.4010 (M^++H+Na), found: 832.4003.

8. *Materials*. Human cancer cell lines were obtained from followings, *i.e*. breast cancer cell T-47D, MDA-MB-231, renal cancer cell A498, lung cancer cell NCI-H460, colon cancer cell HCT116, and prostate cancer cell PC-3 from the American Type Culture Collection (Manassas, VA 20108, USA), colon cancer cell DLD-1 (JCRB9094) from the Japanese Collection of Research Bioresources (Osaka, Japan), and renal cancer cell UO31 from the National Cancer Institute (Bethesda, MD 20892, USA), respectively. All cells were cultured in RPMI1640 medium (Asahi Techno Glass Corporation, Chiba, Japan) containing 10% fetal bovine serum (Lot No. 49300604, Moregate Bio Tech, Australia). Some supplements were added to the medium as appropriate. Cisplatin (Nippon Kayaku Co., Ltd., Tokyo, Japan) and Adriamycin (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) were used as control in cytotoxic assays.
In vitro cytotoxicity assay. The cytotoxicity against human tumor cell lines was assessed by the methylene blue staining method. Briefly, appropriate numbers of cells were inoculated into 96-well microplates. Following overnight culture at 37 °C under 5% CO_2 condition, serially diluted samples were added into the wells. After a 3-day culture, cells were stained with methylene blue (0.05%) dissolved in Tris buffer (pH 8.5, 10 mM) for 30 min, and then thoroughly washed with distilled water. The stained dye was extracted with HCl (3%), and OD660 was measured with a Benchmark Plus microplate reader (Bio-Rad, USA) to determine cell growth inhibition. All assays were made independently in triplicate. Means of assay data and their standard deviations were calculated (data not shown), and then IC_{50} values were determined. All assays included cisplatin as a positive control, and cispratin IC_{50} values were utilized as a benchmark for the comparison of cytotoxic activity among the independent assays.