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Synthesis of the A,B-ring-truncated OSW saponin analogs and their antitumor activities

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Abstract—The A,B-ring-truncated OSW saponin analogs (**1**, **18a**, and **18b**) were synthesized. These greatly simplified *trans*-hydrindane disaccharides retained considerable inhibitory activity against the growth of HeLa and Jurkat T cells (IC_{50} = 0.8–21.1 μM).

Stimulated by the exceptionally potent antitumor activities of the OSW-1 saponin (Fig. 1),[1] extensive efforts have been devoted to the access to its natural congeners[2] and synthetic analogs to understand the structure–activity relationships (SAR) of this class of cholestane glycosides.[3–6] The following SAR have been deciphered: (1) The disaccharide part is crucial to the antitumor activities of the molecule. Absence of the 2′-*O*-acetyl or 2″-*O*-methoxybenzoyl (or -cinnamoyl) groups,[1b,2,5b] presence of an additional glucopyranosyl residue on the 4″-OH,[2b] or truncation on the xylopyranosyl residue greatly diminishes the activities.[3e,6b] (2) The steroidal C17-side chain can tolerate certain modifications without significant loss in the antitumor potency.[3c,4] In particular, the easily accessible 22-ester analogs (e.g., the 23-oxa-OSW-1, Fig. 1) exhibit inhibitory activities against certain tumor cell lines with potency comparable to that of OSW-1.[4] (3) The steroidal A,B-ring can also be modified without a significant loss in the antitumor potency. For example, the 3-*O*-glucopyranosyl-[2] and 5,6-dihydro-OSW-1[3d] derivatives are as active as the parent compound. (4) However, replacement of the aglycone with disparate steroids leads to inactive compounds.[3a,b] (5) An analog with an aromatic A-ring is as potent as cisplatin, although it is much lower than that of the parent OSW-1.[6] Despite the above SAR information, it was unclear whether removal of the steroidal A,B-ring of the 23-oxa-OSW-1 will cause a complete loss of the antitumor activities. Herein we report the synthesis of such compounds (e.g., **1**, **18a**, and **18b**) and the determination of their inhibitory activities against the growth of HeLa and Jurkat cells.

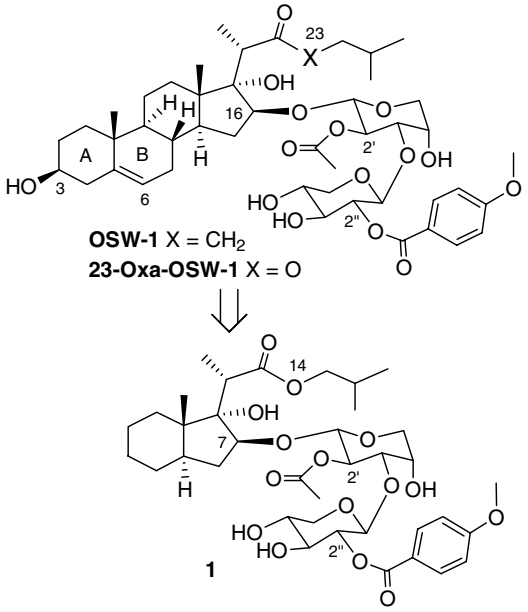

Figure 1. The OSW-1 saponin and its synthetic 23-*O*- and A,B-ring-truncated analogs.

Keywords: OSW saponins; Glycosides; *trans*-Hydrindane; Synthesis; Antitumor activity.
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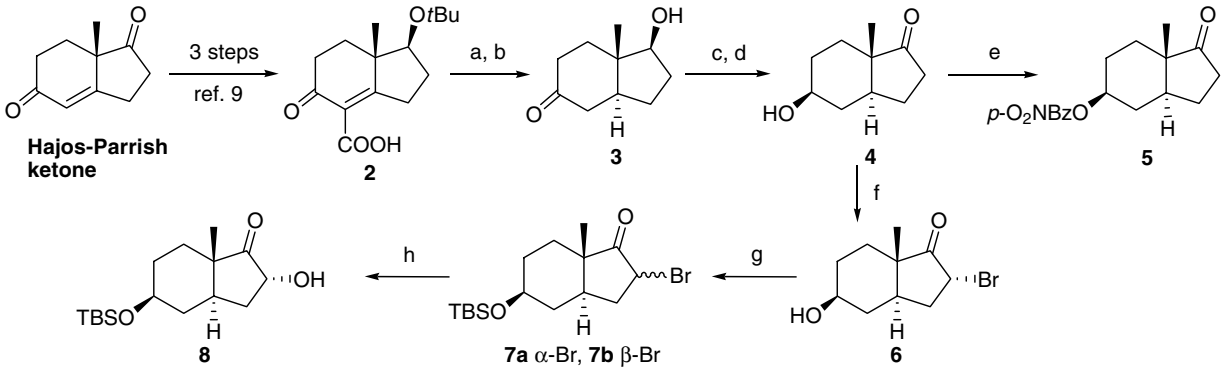


Scheme 1. Reagents and conditions: (a) H_2 (3 atm), 5% Pd–$BaSO_4$, MeOH, 0 °C; then high vacuo, 90 °C, 73%; (b) 2 N HCl, MeOH, reflux, 100%; (c) PCC, CH_2Cl_2, rt, 100%; (d) $NaBH_4$, isopropanol, 0 °C, 72%; (e) *p*-NO_2PhCOOH, EDCI, DMAP, CH_2Cl_2, 100%; (f) $CuBr_2$, MeOH, reflux, 89%; (g) TBSCl, imidazole, DMF, rt, 100%; (h) aq NaOH–DMF–CH_2Cl_2, 0 °C, rt, 96%.

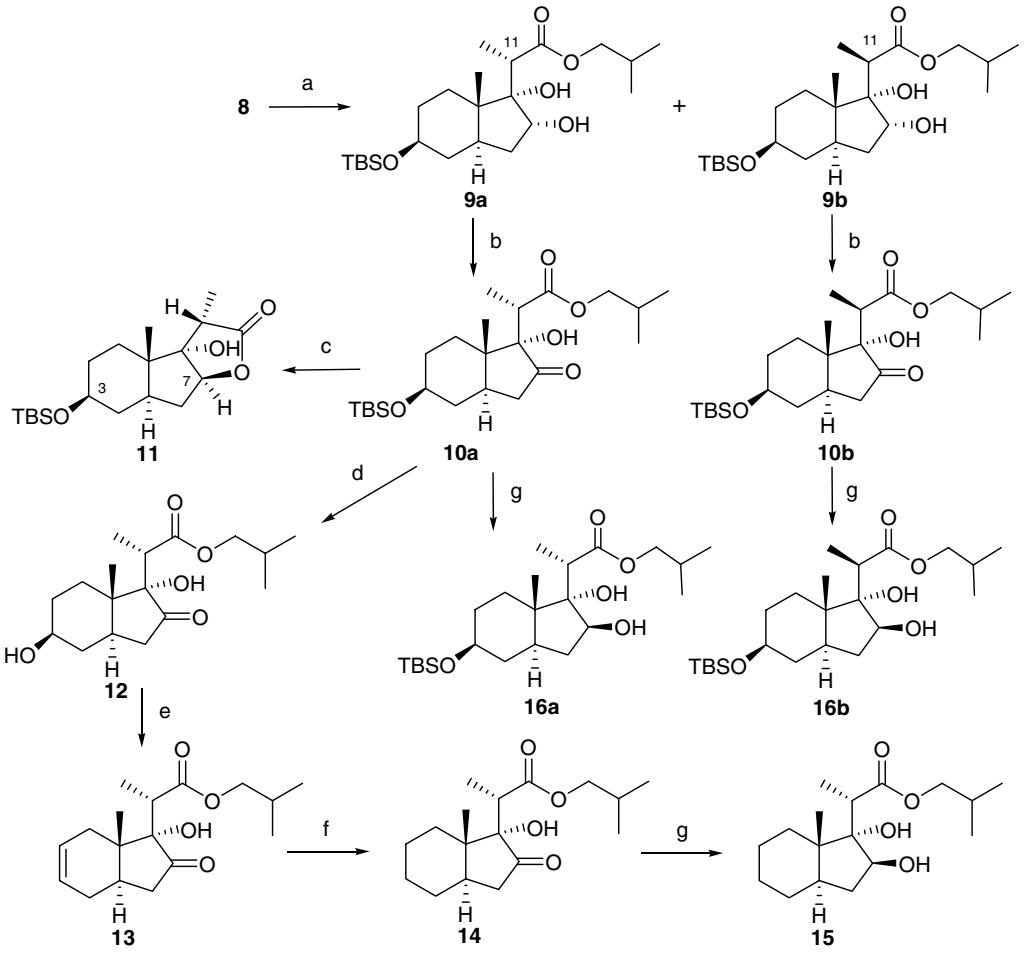
We started the synthesis with the ready available optically pure Hajos–Parrish ketone[7] (Scheme 1). However, elaboration of the hydrindene derivative into the desired *trans* ring junction is not a trivial task.[8] Thus, modification of the most reliable literature protocol involving a five-step temporary substitution of the 4-position with a carboxyl group (leading to compound **2**) was adopted.[9] Hydrogenation of **2** over Pd–$BaSO_4$ formed the *trans*-hydrindane derivative as the major product. The 4-carboxyl group was then removed under high vacuo at 90 °C. Subsequent cleavage of the 8-*O*-*tert*-butyl group with HCl provided 3-ketone-8-ol **3**.[10] PCC oxidation of 8-ol **3** gave a dione, which was reduced selectively with $NaBH_4$ in isopropanol to provide the 3-ol-8-ketone **4**.[11] A single crystal was grown from the *p*-nitrobenzoyl ester **5**,[12] thus confirming the *trans* ring junction in **4**.

8-One **4**, in analog to the steroid dehydroisoandrosterone, was then subjected to the transformations we have developed for the 23-oxa-OSW-1 synthesis.[4] Thus, treatment of **4** with $CuBr_2$ in MeOH afforded the 7-α-bromide **6** in high yield (89%). Protection of the 3-OH in **6** with TBSCl in the presence of imidazole in DMF led to the 3-*O*-TBS derivative as a 1:1 mixture of the 7-α/β-bromide epimers (**7a/7b**). Hydrolysis of the bromide epimers **7a/7b** with NaOH led to the 7-α-ol **8** exclusively in 96% yield.[4,13] These results imply that the epimerization of the 7-bromide in the *trans*-hydrindane-8-one derivatives takes place readily in basic conditions and only the 7-β-bromide is vulnerable to the S_N2 substitution (here by a hydroxide ion).

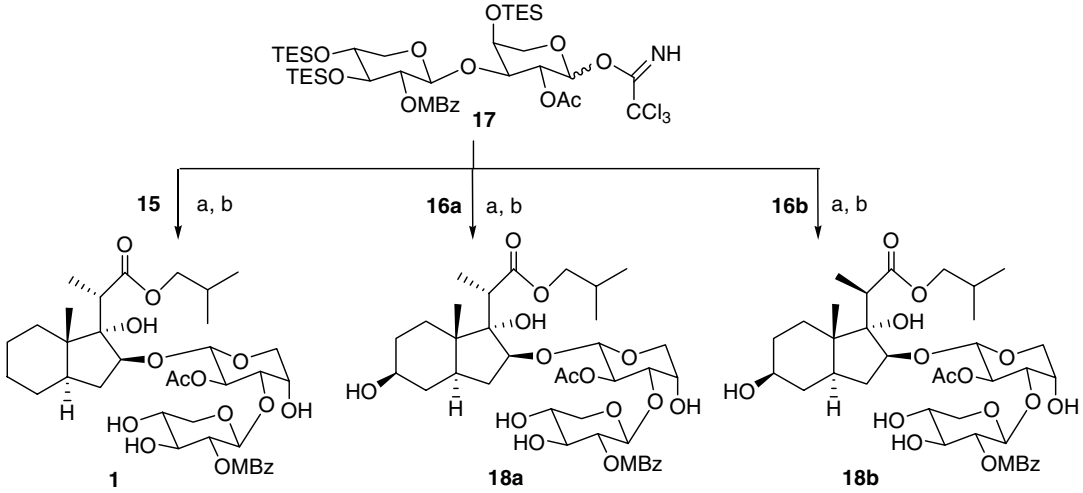
Aldol condensation of the *trans*-hydrindane-7-α-ol-8-one derivative **8** with the in situ prepared isobutyl propionate *E*/*Z*-enolates under conditions similar to those employed in the relevant steroid substrates formed analogously a pair of the 11-*R*/*S* adducts in favor of the desired 11-*S*-isomer (**9a/9b** = 5:1) in good yield (Scheme 2).[4] Separation of the isomers was achieved by careful chromatography on silica gel. Subjection of **9a** and **9b**, respectively, to the oxidation with TPAP/NMO gave 7-one **10a** or **10b** in nearly quantitative yield. The structure of **10b** was confirmed by a single crystal diffraction analysis.[12] Reduction of **10a** or **10b** with $NaBH_4/CeCl_3$ in THF at −10 °C, upon quenching at low temperature (−78 °C) with strong acid (1 N HCl), afforded the 7-β-ol product (**16a** or **16b**) in good yield. However, if the reduction was quenched at a higher temperature (0 °C) with a weak acid (MeOH), a lactonization product (e.g., **11** from **10a**) was obtained as the major product.[4] NOE analysis of lactone **11** also confirmed the nascent stereochemistries in the aldol adduct **9a**. Removal of the 3-*O*-TBS group on **10a** with HF·pyridine gave 3-ol derivative **12**, which was treated with $PhNTf_2$ in the presence of DMAP in pyridine,[14] leading to the 2-ene product **13** in a high 80% yield. Saturation of the nascent 2,3-double bond with H_2 over Pd/C, followed by reduction of the 7-ketone under the conditions for **10** → **16**, furnished the desired aglyone (**15**) of the target molecule **1**.

Glycosylation of the *trans*-hydrindane-7β,8α-diol derivatives **15**, **16a**, and **16b** with the disaccharide trichloroacetimidate **17**, which has been extensively used in the synthesis of OSW saponins,[3–6] in the presence of a catalytic amount of TMSOTf afforded the expected 7-*O*-α-glycosides in satisfactory yields (~50%) (Scheme 3). Subsequent removal of the silyl groups with HF·pyridine furnished the desired compounds **1**, **18a**, and **18b** cleanly.[15]

The in vitro activities of the synthetic A,B-ring-truncated OSW saponin analogs (**1**, **18a**, and **18b**) against the growth of two human cancer cell lines, Jurkat (human T cell leukemia) and HeLa (human cervical cancer), with 23-oxa-OSW-1 as a positive control, were determined by following the incorporation of [3H]thymidine.[16] As shown in Table 1, the simplified hydrindane glycosides (**1**, **18a**, and **18b**) showed much weaker activity against the Jurkat cells when compared to the steroid counterpart (IC_{50} = 1.5 nM). However, they still possess considerable activity, with IC_{50} at the μM level. For HeLa cells, the hydrindane glycosides (**1**, **18a**, and **18b**) showed activity that is only slightly weaker than that of the parent saponin derivative (IC_{50} = 0.24 μM). These three hydrindane glycosides differ from one another only at the presence or absence of the 3-OH (**1** vs **18a**) or the configuration of the 11-methyl group (**18a** vs **18b**). The 3-hydroxy derivatives (**18a/18b**) are stronger toward the HeLa



Scheme 2. Reagents and conditions: (a) *i*-Pr_2NH, *n*-BuLi, THF, −78 °C; isobutyl propionate, −78 °C; then **8**; 82% (**9a/9b** = 5:1); (b) TPAP, NMO, 4 Å MS, rt, 98%; (c) $CeCl_3{\cdot}7H_2O$, $NaBH_4$, THF, −10 °C; then MeOH at 0 °C, 56%; (d) HF·pyridine, CH_2Cl_2, rt, 100%; (e) $PhNTf_2$, DMAP, pyridine, 80%; (f) H_2 (1 atm), 10% Pd/C, EtOH–EtOAc–Et_3N, 100%; (g) $CeCl_3{\cdot}7H_2O$, $NaBH_4$, THF, −10 °C; then 1 N HCl, −78 °C, 65–75%.



Scheme 3. Reagents and conditions: (a) TMSOTf, 4 Å MS, CH_2Cl_2, −20 °C; (b) HF·pyridine, CH_2Cl_2, rt, 48–56% (for two-steps).

cells, while the 3-deoxy compound (**1**) is a slightly better agent against the growth of the Jurkat cells. The change of the configuration at C-11 does not change the level of potency, but slightly reverses the selectivity toward the two cell lines. The cell line-dependent variation of the SAR among these truncated analogs is similar to that observed with other structural classes of OSW-1 analogs.[4]

Table 1. The inhibitory activity of the A,B-ring-truncated OSW-1 analogs (**1**, **18a**, and **18b**) against the growth of tumor cells

	IC_{50} (μM)			
	1	**18a**	**18b**	23-Oxa-OSW-1
Jurkat	8.9	14.5	21.1	0.0013
HeLa	14.9	2.6	0.8	0.24

In summary, based on the previous SAR data on the highly potent antitumor OSW saponins, we designed and synthesized the A,B-ring-truncated OSW saponin analogs (**1**, **18a**, and **18b**). The previous chemistry for the synthesis of 23-oxa-OSW saponin derivatives was successfully applied to the synthesis of the present *trans*-hydrindane derivatives. The greatly simplified and easily accessible compounds (**1**, **18a**, and **18b**) retained considerable inhibitory activity against the growth of HeLa and Jurkat cells (IC_{50} = 0.8–21.1 μM). These results suggest that an intact steroid ring is not absolutely required for the biological activity of the OSW-1 family of saponin analogs.

Acknowledgments

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12. Full crystallographic details of compounds **5** and **10b** have been deposited with the Cambridge Crystallographic Data Centre (CCDC 627944 and 627945, respectively). Copies of these data may be obtained free of charge from the director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).
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15. Analytical data for the final compounds **1**, **18a**, and **18b**. Compound **1**: $[\alpha]_D^{26}$ −41(*c* 0.1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.99 (d, *J* = 9.0 Hz, 2H), 6.90 (d, *J* = 9.3 Hz, 2H), 4.99 (s, 1H), 4.84 (s, 2H), 4.04–3.88 (m, 2H), 3.85 (s, 3H), 3.87–3.72 (m, 5H), 3.70–3.63 (m, 1H), 3.50–3.33 (m, 4H), 2.70 (q, *J* = 7.5 Hz, 1H), 2.20–2.05 (m, 1H), 1.90 (s, 3H), 1.92–1.56 (m, 6H), 1.53–1.37 (m, 4H), 1.33–1.14 (m, 5H), 1.08 (d, *J* = 6.9 Hz, 3H), 0.92–0.80 (m, 1H), 0.80–0.72 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.9, 169.5, 165.6, 163.9, 132.1, 121.4, 113.8, 100.0, 99.7, 89.2, 84.7, 76.3, 72.9, 72.6, 70.3, 69.7, 69.2, 65.1, 63.4, 61.7, 55.4, 45.8, 41.8, 40.9, 37.0, 32.8, 29.7, 27.5, 26.0, 25.5, 21.3, 20.6, 19.0, 18.9, 12.9, 12.3; HRESI-MS: *m/z* Calcd $[M+K^+]$ for $C_{37}H_{54}O_{15}K$: 777.3094. Found 777.3091. Compound **18a**: $[\alpha]_D^{26}$ −24 (*c* 0.4, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 8.00 (d, *J* = 7.5 Hz, 2H), 6.92 (d, *J* = 7.5 Hz, 2H), 4.98 (s, 1H), 4.84 (d, *J* = 4.8 Hz, 2H), 4.21 (s, 1H), 4.15 (d, *J* = 12.0 Hz, 1H), 4.00 (t, *J* = 9.3 Hz, 1H), 3.92 (s, 1H), 3.90–3.68 (m, 8H), 3.85 (s, 3H), 3.66–3.52 (m, 1H), 3.51–3.33 (m, 3H), 2.69 (q, *J* = 7.2 Hz, 1H), 2.17–1.94 (m, 2H), 1.91 (s, 3H), 1.85–1.65 (m, 4H), 1.55–1.20 (m, 5H), 1.08 (d, *J* = 6.9 Hz, 3H), 0.82 (s, 3H), 0.77 (t, *J* = 4.9 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.7, 169.5, 165.5, 163.9, 132.1, 121.6, 113.8, 100.1, 99.8, 89.5, 84.0, 77.2, 72.8, 72.6, 71.1, 70.4, 69.7, 69.1, 65.1, 63.6, 61.8, 55.4, 45.3, 40.8, 40.2, 36.2, 34.6, 30.9, 30.1, 29.7, 27.5, 22.7, 20.6, 19.0, 18.9, 14.1, 12.8, 12.5; HRESI-MS: *m/z* Calcd $[M+Na^+]$ for $C_{37}H_{54}O_{16}Na$: 777.3304. Found 777.3326. Compound **18b**: $[\alpha]_D^{26}$ −85 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 5.11 (t, *J* = 7.5 Hz, 1H), 4.99 (t, *J* = 7.5 Hz, 1H), 4.66 (d, *J* = 7.2 Hz, 1H), 4.24 (d, *J* = 7.5 Hz, 1H), 4.14–3.96 (m, 4H), 3.85 (s, 3H), 3.88–3.77 (m, 4H), 3.77–3.70 (m, 2H), 3.70–3.60 (m, 2H), 3.58 (s, 1H), 3.60–3.50 (m, 1H), 3.50–3.42 (m, 1H), 3.36 (t, *J* = 7.7 Hz, 1H), 2.70 (q, *J* = 6.9 Hz, 1H), 2.18–1.70 (m, 8H),1.59 (s, 3H), 1.10 (d, *J* = 6.9 Hz, 3H), 0.92 (dd, *J* = 2.1, 6.3 Hz, 6H), 0.83 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ 177.7, 169.4, 165.8, 163.8, 132.1, 130.9, 121.6, 113.7, 103.0, 101.9, 87.6, 84.6, 80.4, 74.4, 73.6, 71.1, 71.0, 70.5, 69.6, 67.9, 65.2, 64.9, 55.5, 46.0, 40.2, 38.9, 36.3, 34.4, 30.7, 29.7, 29.6, 27.4, 20.2, 19.1, 19.0, 14.1, 12.7; HRESI-MS: *m/z* Calcd $[M+Na^+]$ for $C_{37}H_{54}O_{16}Na$: 777.3304. Found 777.3309.
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