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CARBONOYLOXY ANALOGS OF THE ANTI-METASTATIC DRUG SWAINSONINE

ACTIVATION IN TUMOR CELLS BY ESTERASES

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Abstract—Swainsonine (SW), a plant alkaloid and inhibitor of α-mannosidases, has been shown to inhibit N-linked oligosaccharide processing and to block tumor cell metastasis in mice. In this study, a series of SW analogs were chemically synthesized and compared for inhibition of complex-type N-linked oligosaccharide processing in cultured MDAY-D2 tumor cells, for inhibition of α-mannosidases *in vitro*, and for stimulation of bone marrow proliferation *in vivo*. Carbonoyloxy substitutions at the 2 and 8 carbons of SW reduced inhibitor activity by 2–3 orders of magnitude for Jack Bean and MDAY-D2 tumor cell lysosomal α-mannosidases *in vitro*. However, 2-*p*-nitrobenzoyloxy-, 2-octanoyloxy- and 2-butanoyloxy-derivatives of SW retained full activity as inhibitors of Golgi oligosaccharide processing in viable MDAY-D2 tumor cells. Inhibition of oligosaccharide processing was reduced by the esterase inhibitor diethyl *p*-nitrophenyl phosphate, suggesting that although 2-*p*-nitrobenzoyloxy-SW, 2-octanoyloxy-SW and 2-butanoyloxy-SW are relatively poor inhibitors of α-mannosidases *in vitro*, the compounds enter cells at a rate comparable to that of SW, and are converted to SW by cellular esterases. The more lipophilic esters, 2-benzoyloxy-SW, 2-toluoyloxy-SW, 8-palmitoyloxy-SW and 8-myristinoyloxy-SW, showed IC_{50} values at least 10 times higher for inhibition of Golgi oligosaccharide processing, probably due to less efficient entry of the compounds into tumor cells. The anti-metastatic activities of SW and two analogs were tested and shown to correlate with the IC_{50} values for inhibition of Golgi oligosaccharide processing in cultured tumor cells. *In vivo*, SW and the analogs were administered intraperitoneally to mice and found to have comparable activities as stimulators of bone marrow cell proliferation. Carbonoyloxy substitutions at the 2- or 8-position of SW with other chemical groups may lead to new drugs with improved pharmacokinetics and anti-cancer activity.

Swainsonine (SW)¶ is an indolizidine alkaloid found in Australian *Swainsona canescens* [1], North American plants of the genera *Astragalus* and *Oxytropis* [2], and also the fungus *Rhizoctonia leguminicola* [3]. The alkaloid is a potent inhibitor of the Golgi enzyme α-mannosidase II, an enzyme required for maturation of N-linked oligosaccharides on newly synthesized glycoproteins. SW also blocks lysosomal α-mannosidases causing the accumulation of oligomannose chains in cells exposed to the drug [4]. The SW-block in Golgi processing prevents expression of the β1-6 D-*N*-acetylglucosamine (β1-6GlcNAc) branched "complex-type" N-linked oligosaccharides that have been observed to increase following malignant transformation in human and rodent cells [5, 6]. The branched oligosaccharides appear to play a role in cancer metastasis, as loss or truncation of the structures due to somatic mutations in metastatic tumor cell lines results in greatly reduced metastasis and slower solid tumor growth [7, 8]. Furthermore, SW-treated murine tumor cells are less metastatic in both organ-colonization and spontaneous metastasis assays in mice [9, 10]. SW has been shown to block tumor cell invasion through extracellular matrix *in vitro* [11, 12]. The anti-metastatic effect of SW is enhanced by administering the drug to mice in their drinking water [9]. SW administered either orally or by mini-osmotic pumps to athymic nude mice inhibits the growth rate of human MeWo melanoma and HT29m colon carcinoma tumor xenografts in the mice [13, 14]. The alkaloid has positive effects on cellular immunity in mice [reviewed in Refs. 15 and 16]. In particular, SW has been shown to alleviate both chemically induced and tumor-associated immune suppression [17], increase NK cell [18] and LAK cell activities [19], and increase splenic and bone marrow (BM) cell proliferation [20–22]. The drug has also been shown to be hemorestorative in mice following treatment with both cycle-specific and nonspecific chemotherapeutic agents [23]. SW is currently being

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¶ Abbreviations: SW, swainsonine; Gal, D-galactose; GlcNAc, D-*N*-acetylglucosamine; Man, D-mannose; BM, bone marrow; CFU, colony forming units; FBS, fetal bovine serum; PBS, phosphate-buffered saline; and IL, interleukin.

tested in phase I clinical trials of cancer patients, as a 5-day intravenous infusion.* The preliminary results of this study suggest that the drug is well tolerated with this regimen.

The aim of the present study was to identify analogs of SW with improved specificity for Golgi α-mannosidase II and/or improved pharmacokinetics that may enhance anti-tumor and immune stimulatory activity, while reducing the potential long-term side-effects of the drug, the latter presumably being associated with inhibition of lysosomal α-mannosidases. The results suggest that 2- and 8-carbonoyloxy esters of SW are approximately 2–3 orders of magnitude less active than SW as α-mannosidase inhibitors, but esters with solubility properties that allow entry into cells in tissue culture were readily converted to SW by esterases, and therefore may be useful as prodrugs. Three compounds with these properties, 2-*p*-nitrobenzoyloxy-SW, 2-octanoyloxy-SW and 2-butanoyloxy-SW, showed potencies similar to that of SW as inhibitors of oligosaccharide processing *in vitro*. SW and the ester analogs also showed similar activities as stimulators of BM cell proliferation, *in vivo*.

MATERIALS AND METHODS

Analogs of SW were synthesized by Toronto Research Chemicals and included 2-benzoyloxy-SW, 2-toluoyloxy-SW, 2-.*p*-nitrobenzoyloxy-SW, 2-octanoyloxy-SW, 2-butanoyloxy-SW, 8-palmitoyloxy-SW, 8-myristinoyloxy-SW, and 6,7-dihydroxy-SW (Fig. 1). The structures of all new compounds utilized in this paper were confirmed by proton nuclear magnetic resonance (NMR) spectroscopic analysis, using a combination of 2D COSY experiments that allowed unambiguous assignment of the 1D spectras and by Rotating Frame NOE spectroscopy (ROESY) experiments using methods described previously [24]. Chemical shifts were also obtained for intermediates in the chemical synthesis of the analog. The analogs were purified by recrystallization to constant melting point, and purity was confirmed by NMR to be greater than 98%. Chemically synthesized SW and the natural product from *R. leguminicola* have been shown previously to be equally effective immunomodulators [18, 20]. SW isolated from *R. leguminicola* by Drs. Warren Croom and Winston Hagler (North Carolina State University) was used in the BM proliferation assays and chemically synthesized SW was used in all other experiments. L-PHA (i.e. leukoagglutinin) was purchased from Pharmacia and [^{3}H]thymidine from Amersham. Jack Bean α-mannosidase and diethyl *p*-nitrophenyl phosphate were obtained from Sigma.

MDAY-D2 is a highly metastatic DBA/2 strain lymphoreticular tumor cell line [25], and B16F10 is a metastatic subline of the C57B1/6J strain melanoma [26]. Tumor cells were grown in Modified Eagle's Medium (MEM) plus 10% fetal bovine serum (FBS).

Inhibition of Jack Bean and lysosomal α-mannosidases by SW and analogs. SW and analogs were serially diluted into a volume of 75 μL in 96-well Elisa plates followed by the addition of 37.5 μL of 100 mM sodium acetate, pH 5.0, and 37.5 μL of 1.0 mM *p*-nitrophenyl α-D-mannospyranoside. Jack Bean α-mannosidase (Sigma, 38 U/mL) was diluted 1/800, 10 μL was added to each well, and the plates were incubated for 20 min at 37°. The reaction was stopped by the addition of 150 μL of 0.5 M sodium carbonate, and formation of *p*-nitrophenol was measured with a plate set at 405 nm.

The effects of SW and analogs on cellular acid α-mannosidases (i.e. lysosomal enzyme) were measured using the same assay where Jack Bean α-mannosidase was replaced by 10 μL MDAY-D2 tumor cell lysate (10 μg/μL of protein) prepared in 0.9% NaCl, 50 mM Tris, pH 7.0, 0.5% Triton X-100 and 1.0 mM diethyl *p*-nitrophenyl phosphate.

Inhibition of L-PHA toxicity by SW and analogs. MDAY-D2 tumor cells were inoculated into 96-well micro-test plates at 2×10^4 cells/well, and contained serial dilutions of SW or SW analogs in MEM plus 10% FBS. The cells were cultured for 18–20 hr, followed by the addition of L-PHA at 25 μg/mL for an additional 24 hr. Cell proliferation was measured by adding 0.5 μCi of [^{3}H]thymidine/well for 3–4 hr, and harvesting onto glass fiber disks using a Titertek

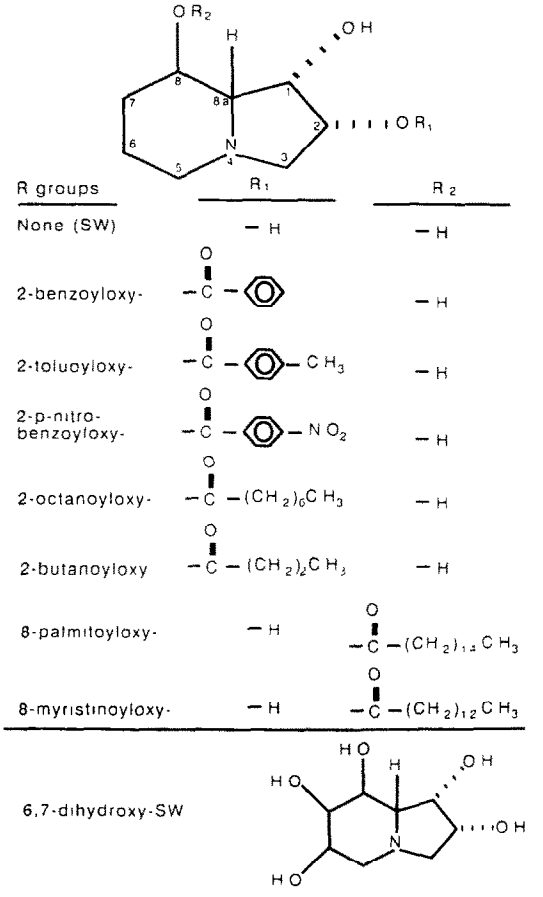


Fig. 1. Structures of SW and analogs.

* Goss P, Baker M and Dennis J, Submitted for publication.

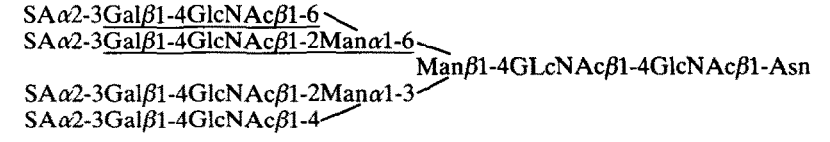
harvester; the disks were counted in a liquid scintillation counter. The apparent IC_{50} values for SW and the analogs are the drug concentrations showing 50% protection from L-PHA toxicity; that is, 50% [^{3}H]thymidine incorporated compared with cells grown in the absence of L-HPA. In experiments using the esterase inhibitor diethyl *p*-nitrophenyl phosphate, the compound was added to MDAY-D2 cells 4 hr prior to the α-mannosidase inhibitors and was present throughout the experiment.

Lung colonization assay. B16F10 melanoma cells were maintained in log-phase growth in MEM plus 10% FBS (Gibco) either untreated or treated for 48 hr with SW or analogs of SW. Cells were harvested with trypsin/EDTA (Gibco), washed, and 10^5 melanoma cells in 100 μL of phosphate-buffered saline (PBS) were injected into the lateral tail vein of male C57B1/6J mice. The lungs were removed from the mice and tumor nodules were counted at death or on day 28 when the experiment was terminated.

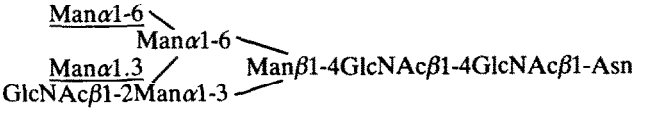
Bone marrow cell proliferation assay. Pathogen-free C57B1/6J mice, 8- to 14-weeks-old, were maintained according to NIH ethical guidelines. Groups of 2–3 mice received intraperitoneal injections of the PBS, SW or designated analog twice daily for 4 days. As we have reported for swainsonine [22], 10 μg was found to be the optimal dose for all of the responsive analogs. Eighteen hours after the last SW injection, mice were killed and BM cells were harvested. Marrow cell suspensions were prepared under sterile conditions using femurs and tibias of both legs from each donor. BM cells were flushed from the marrow cavities with ice-cold RPMI 1640, washed and resuspended at desired concentrations in RPMI-1640 supplemented with 5% FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL). Cell viability was monitored by staining with trypan blue. The ability of the analogs to stimulate BM proliferation was measured by two methods. First, cellularity was determined by directly counting BM cells after they were flushed from the tibias and femurs. Second, enhancement in proliferation was assessed by the increase in the proportion of BM progenitor cells present in the BM preparations as measured by colony formation in soft agar (CFU). BM cells were processed according to the procedures of the GIBCO-BRL Mouse Bone Marrow Stem Cell Proliferation Kit, which employs suspension of mononuclear cells in a semi-solid methylcellulose medium containing FBS, 2-mercaptoethanol and the growth factors, interleukin-3 (IL-3) (75 U/mL) and erythropoietin (20 U/mL). The plates were incubated for 10–14 days at 37° in a humidified atmosphere of 5% CO_2 and 95% air, and colonies consisting of at least 40 cells were enumerated using an inverted microscope. The potential colonies that form in the semi-solid medium are the granulocyte, erythrocyte, monocyte, megakaryocyte (i.e. CFU-GEMM); the granulocyte, macrophage (i.e. CFU-GM), and the burst forming unit-erythroid (i.e. BFUe).

RESULTS

The toxic plant-lectin L-PHA binds to complex-type N-linked oligosaccharides and requires the underlined portion of the structure shown below [27].



Golgi α-mannosidase II removes the underlined mannose residues in the structure shown below, allowing Golgi processing to proceed to complex-type oligosaccharides of the type shown above [28]. In SW-treated cells, this enzyme is blocked, and therefore complex-type structures are replaced by hybrid-type oligosaccharides [4] (see below) which lack the L-PHA reactive sequence. SAα-Galβ1-4 is added to the lower arm to complete the hybrid structure observed in SW-treated cells.



Consequently, cells cultured in the presence of SW show greatly reduced L-PHA binding, and increased resistance to the toxic effects of the L-PHA. The ID_{50} for L-PHA toxicity on MDAY-D2 cells is 5 μg/mL, whereas cells cultured in the presence of SW are resistant to >100 μg/mL of L-HPA [9]. To compare SW and the carboxoyloxy analogs of SW for inhibition of oligosaccharide processing (i.e. Golgi α-mannosidase II) in viable cells, the drugs were titrated into cultures to determine the concentration that is required to protect MDAY-D2 cells from 25 μg/mL of L-PHA (Fig. 2). The apparent IC_{50} for SW was 0.20 μM, and the value for the SW analogs ranged from 0.23 to >5.0 μM (Table 1).

Measurement of IC_{50} in the L-PHA toxicity assay is a compilation of drug entry into the cells, potency

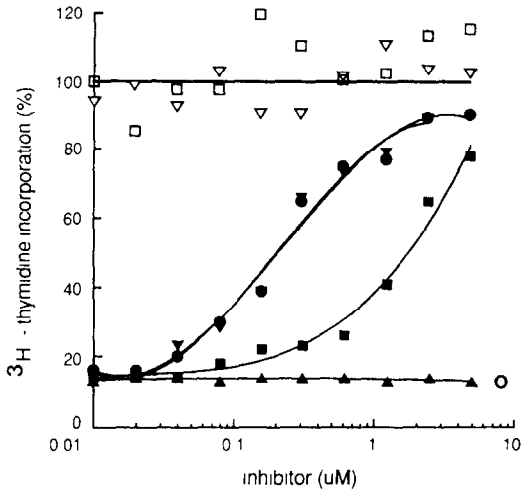


Fig. 2. L-PHA (25 μg/mL) sensitivity of MDAY-D2 tumor cells cultured in the presence of SW (●), 2-octanoyloxy-SW (▼), 2-benzoyloxy-SW (■), and 8-myristinoyloxy-SW (▲). SW and analogs in the absence of L-PHA had no effect on cell growth; open symbols (□, ▽) are examples showing SW and 2-octanoyloxy-SW in the absence of L-PHA. L-PHA sensitivity in the absence of drug is shown by an open circle (○).

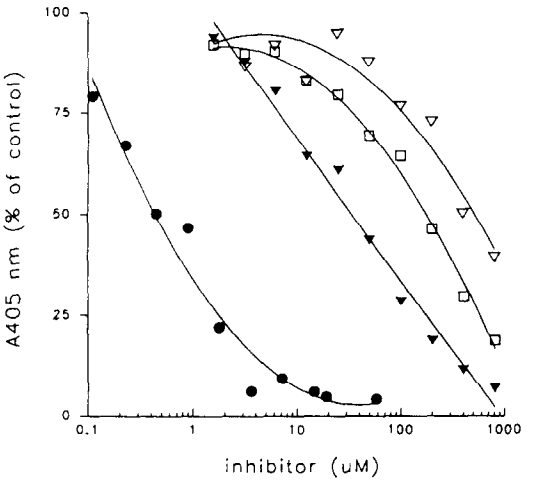


Fig. 3. Inhibition of Jack Bean α-mannosidase by SW (●), 2-toluoyloxy-SW (▽), 2-butanoyloxy-SW (□), and 6,7-dihydroxy-SW (▼). The absorbance value for the control was 0.63.

as an α-mannosidase inhibitor, and drug catabolism and efflux from the cells. To determine whether the IC_{50} values of the analogs in the L-PHA assay correlated with inhibitor potencies, the compounds were compared as inhibitors of Jack Bean α-mannosidase *in vitro* (Fig. 3). The carbonoyloxy substitutions of SW at carbons 2 or 8 greatly reduced inhibitor activity (i.e. 300- to >1000-fold compared to SW) (Table 1). Although 6,7-hydroxy-SW, was the most active of the analogs in the Jack Bean α-mannosidase assay, it was 100 times less active than SW. Inhibition by the analogs of lysosomal α-mannosidases in lysates of MDAY-D2 tumor cells (i.e. activity measured at pH 5.0) showed a similar rank order of activity as that observed for the Jack Bean enzyme (Table 1).

Although 2-*p*-nitrobenzoyloxy-SW, 2-octanoyloxy-SW and 2-butanoyloxy-SW showed a 300-fold greater IC_{50} for α-mannosidase *in vitro*, the compounds had activities similar to that of SW for protection of MDAY-D2 tumor cells against L-PHA toxicity (Table 1). It is possible that either the analogs were much more efficient inhibitors of Golgi α-mannosidase than the Jack Bean and lysosomal enzymes or, more likely, that cellular esterases converted the analogs to SW inside the cells. To examine the latter possibility, the L-PHA toxicity assay was preformed in the presence of the esterase inhibitor diethyl *p*-nitrophenyl phosphate. The IC_{50} for inhibition of Golgi oligosaccharide processing by 2-butanoyloxy-SW was increased 10-fold by 0.5 mM diethyl *p*-nitrophenyl phosphate (Fig. 4A). Similar results were obtained for 2-octanoyloxy-SW, and the

Table 1. Inhibition of oligosaccharide processing and α-mannosidases by SW-analogs

SW derivative	IC_{50} for inhibition of L-PHA toxicity (μM)	IC_{50} for inhibition of J.B. α-mannosidase (μM)	IC_{50} for inhibition of MDAY-D2 α-mannosidase (μM)
SW	0.20	0.4	0.5
2-benzoyloxy-SW	1.80	350.0	720.0
2-toluoyloxy-SW	3.40	350.0	69.0
2-*p*-nitrobenzoyloxy-SW	0.23	123.0	35.0
2-octanoyloxy-SW	0.23	123.0	35.0
2-butanoyloxy-SW	0.23	123.0	35.0
8-palmitoyloxy-SW	>2.50	240.0	500.0
8-myristinoyloxy-SW	>2.50	>500.0	>500.0
6,7-dihydroxy-SW	>5.00	50.0	10.0

The IC_{50} values for inhibition of L-PHA toxicity were determined using MDAY-D2 tumor cells as described in Materials and Methods (also see Fig. 2). The IC_{50} values for inhibition of Jack Bean (J.B.) and MDAY-D2 lysosomal α-mannosidases were determined as described in Materials and Methods (also see Fig. 3).

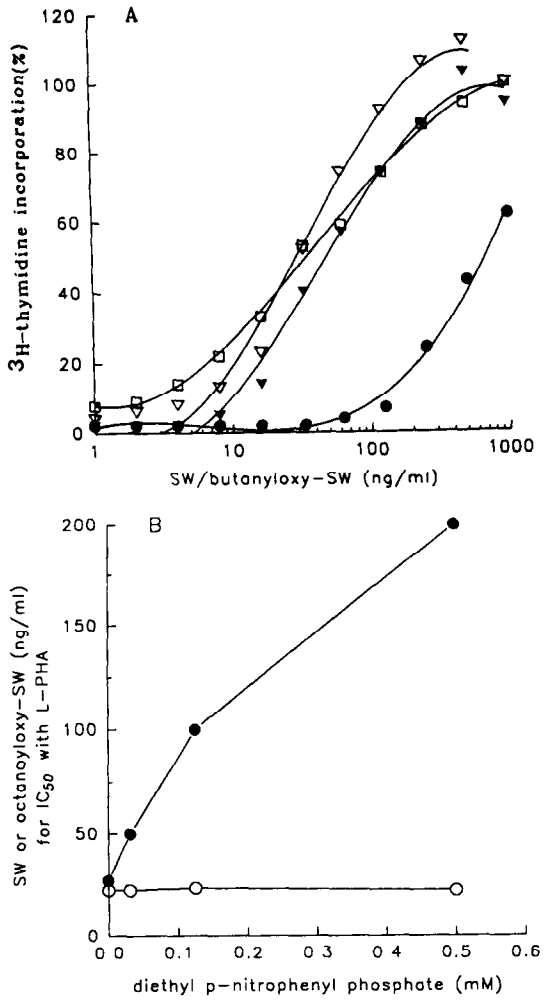


Fig. 4. Blockade of *in vivo* activity of SW-esters by diethyl *p*-nitrophenyl phosphate, an esterase inhibitor. (A) L-PHA sensitivity of MDAY-D2 tumor cells was compared in the presence of SW (▽, □) and 2-butanoyloxy-SW (▼, ●) either without (open symbols) or in the presence of 0.5 mM diethyl *p*-nitrophenyl phosphate (closed symbols). (B) SW (○) and 2-octanoyloxy-SW (●) concentrations required for 50% inhibition of L-PHA toxicity as a function of diethyl *p*-nitrophenyl phosphate concentration.

results in Fig. 4B show a titration for the effect of diethyl *p*-nitrophenyl phosphate on IC_{50} on the L-PHA toxicity assay.

SW ($IC_{50} = 0.2\ \mu M$) was compared with 2-toluoyloxy-SW ($IC_{50} = 3.4\ \mu M$) and 2-octanoyloxy-SW ($IC_{50} = 0.23\ \mu M$) for inhibition of lung colonization by B16F10 melanoma cells. Tumor cells were cultured in the presence of 20, 100 or 1000 ng/mL of drug for 48 hr, then washed in PBS, and injected intravenously into syngeneic mice. SW at 1000 ng/mL has been shown previously to inhibit organ colonization by B16F10 melanoma cells by >90%, yet the drug does not reduce viability or clonigenicity of the tumor cells *in vitro* [9, 13]. The survival of mice 28 days after being injected with SW-treated or 2-oxtanoyloxy-SW treated tumor cells was significantly higher than mice injected with the same number of 2-toluoyloxy-SW-treated or untreated B16F10 cells (Table 2). The anti-metastatic activity of the compounds correlated with their IC_{50} for inhibition of L-PHA toxicity, that is, SW ≈ 2-octanoyloxy-SW > 2-toluoyloxy-SW.

To compare the myeloproliferative activity of the SW analogs *in vivo*, mice were injected intraperitoneally with the compound twice daily for 4 days, a regimen previously shown to be optimal for SW-induced BM proliferation [22]. BM cells were flushed from the femurs of the mice, and the total number of cells recovered was determined to be 2- to 3-fold greater for mice injected with SW or the analogs (Table 3). To determine whether the analogs of SW affected the number of pluripotent hematopoietic stem cells present in unfractionated BM, *in vitro* progenitor assays were performed. The CFU increased 2- to 3-fold in BM from SW- and analog-treated mice compared with vehicle-treated mice. The stimulatory effects of the compounds were dose and time dependent. Dose curves were performed for each compound (i.e. 5, 10, 20, and 40 μg/injection, data not shown), and the optimum for both BM cellularity and CFU was observed at 10 μg/injection, similar to that reported previously for SW [22]. Maximum stimulation was observed after 5–6 days of treatment. The response was not increased by prolonged administration of any of the analogs (data not shown).

Table 2. Lung colonization by B16F10 melanoma cells cultured in the presence of SW, 2-toluoyloxy-SW, and 2-octanoyloxy-SW

Treatment	Dose (μg/mL)	Survivors at day 28	Lung colonies [mean (range)]
None		0/8	>100
SW	1.0 (5.8 μM)	4/5	3 (0–4)
	0.1	4/5	5 (2–10)
	0.02	2/5	23 (15–31)
2-octanoyloxy-SW	1.0 (3.3 μM)	2/5	1 (0–2)
	0.1	3/5	13 (8–20)
	0.02	3/5	9 (5–17)
2-toluoyloxy-SW	1.0 (3.4 μM)	0/5	>100
	0.1	0/5	>100

B16F10 melanoma cells were injected into the lateral tail vein of C57B1/6J mice at 105 cells/mouse. Prior to the injection, tumor cells had been cultured for 48 hr either untreated or treated with the indicated concentration of SW, or 2-octanoyloxy-SW, or 2-toluoyloxy-SW. The experiment was terminated on day 28, and lung colonies in the mice were counted.

Table 3. Stimulation of bone marrow cell proliferation *in vivo* by SW and SW-analogs

Treatment	BM cellularity (cells × 10^7)	CFU (colonies/10^7 cells)
PBS	1.46 ± 0.08	145 ± 4
SW	4.50 ± 0.42	540 ± 34
2-benzoyloxy-SW	5.77 ± 0.00	421 ± 44
2-toluoyloxy-SW	3.40 ± 0.25	384 ± 25
2-*p*-nitrobenzoyloxy-SW	3.60 ± 0.42	380 ± 7
2-octanoyloxy-SW	3.88 ± 0.43	482 ± 22
2-butanoyloxy-SW	3.80 ± 0.91	545 ± 59
8-palmitoyloxy-SW	5.10 ± 0.57	553 ± 32
8-myristinoyloxy-SW	4.80 ± 0.24	226 ± 6
6,7-dihydroxy-SW	5.20 ± 0.03	538 ± 5

Mice were given twice daily intraperitoneal injections of PBS, SW or the analogs at 10 μg/injection. After 4 days of injections, BM cells were flushed from the femurs and tibias and counted. CFUs growing in soft agar consisting of at least 40 cells were enumerated. The results are the means ± SEM of 4 experiments.

DISCUSSION

We have tested a series of carbonoyloxy esters of SW for activity as inhibitors of α-mannosidases *in vitro*, for inhibition of oligosaccharide processing in cultured tumor cells, and for stimulation of BM cell proliferation in mice. The 2- and 8-carbonoyloxy esters of SW were 2–3 orders of magnitude less active than SW as α-mannosidase inhibitors *in vitro*. The potency of SW as a α-mannosidase inhibitor has been shown previously to be dependent upon the presence and stereochemistry of the three hydroxyls at positions 1, 2 and 8 [29]; and the present results show that carbonoyloxy substitutions at 2 and 8 while maintaining the stereochemistry also greatly reduce activity.

2-Ester analogs with favorable solubility properties for entry into cells appear to be converted to SW by intracellular esterases. In particular, 2-*p*-nitrobenzoyloxy-SW, 2-octanoyloxy-SW and 2-butanoyloxy-SW showed activities similar to that of SW when measured *in vivo* as inhibitors of oligosaccharide processing. In contrast, the more hydrophobic esters 2-benzoyloxy-SW and 2-toluoyloxy-SW were at least 20 times less active. This interpretation was supported by the observation that diethyl *p*-nitrophenyl phosphate, an esterase inhibitor, reduced the activity of 2-*p*-nitrobenzoyloxy-SW and 2-octanoyloxy-SW as inhibitors of oligosaccharide processing in tissue culture cells. The results suggest that carbonoyloxy esters of SW may be described as prodrugs that require activation by esterases. SW and the SW-esters showed similar dose responses in the BM cell proliferation assay (data not shown), suggesting that the analogs may be activated by esterases in the peritoneal cavity of mice.

6,7-Dihydroxyl-SW was 20- to 100-fold less active at inhibiting α-mannosidase *in vitro* and Golgi oligosaccharide processing *in vivo*, but the compound showed activity similar to that of SW in the CFU assay. As 6,7-dihydroxyl-SW is not an ester, it is unclear whether the compound is active or must be converted to SW for stimulation of BM proliferation. Although the action of SW and the analogs on tumor cells as inhibitors of organ colonization appears to be directly related to the block in Golgi processing, the mechanism of the action of SW on BM stimulation *in vivo* is much less clear. SW is an analog of a disaccharide Manα1-Man and, therefore, may interact with a carbohydrate-binding protein which mediates a change in cellular phenotype. Alternatively, the SW-induced block in Golgi processing may sensitize cells to cytokines with carbohydrate binding activities. IL-1, IL-2 and tumor necrosis factor have been shown to have carbohydrate-binding activity with specificity for oligomannose, sequences that are exposed in the hybrid-type structures of SW-treated cells [30, 31]. This may explain the observations that macrophages cultured in the presence of SW show increased killer cell activity and increased surface Ia expression [32], as well as increased sensitivity to tumor necrosis factor [30]. Similarly, human lymphocytes cultured in the presence of swainsonine showed increased lymphokine-activated killer (LAK) cell activity, and induction of this activity can be suppressed by anti-IL-2 antibodies [19]. It is well known that immunomodulatory substances, such as IL-1, IL-3, IL-6, muramyl dipeptides, bacterial lipopolysaccharides and granulocyte/macrophage-colony stimulating factor (GM-CSF), confer chemoradioprotection because of their ability to enhance hematopoietic and immune functions [33–37]. In combination with chemical agents commonly used in the treatment of human malignancies, SW also confers protection against the toxic effects of high-dose chemotherapy in mice [23]. Swainsonine-induced recovery from myelosuppression caused by lethal doses of chemotherapeutic agents such as methotrexate and doxirubicin is dependent on timing and sequence of administration as has been demonstrated for IL-1 and recombinant human GM-CSF [38, 39].

SW partitions well between lipid membrane and aqueous phases, and has been shown previously to

rapidly enter cells, and effects inhibition of α-mannosidase without requiring activation [40]. The carbonoyloxy esters with more lipophilic R groups such as 2-toluoyloxy, 2-benzoyloxy, 8-palmitoyloxy and 8-myristinoyloxy were 3–10 times less active as inhibitors of Jack Bean α-mannosidase, and 10–20 times less active in protecting cells against L-PHA toxicity compared with 2-*p*-nitrobenzoyloxy-SW, 2-octanoyloxy-SW and 2-butanoyloxy-SW. These observations suggest that lipophilic groups attached to the 2 or 8 position of SW reduce binding to α-mannosidase, and also decrease entry of the compounds into the cells as compared with 2-*p*-nitrobenzoyloxy-SW, 2-octanoyloxy-SW and -butanoyloxy-SW. It is also possible that lipophilic R groups reduce hydrolysis of the analogs by intracellular esterases and contribute in this manner to a loss of activity *in vivo*.

Castanospermine, an α-glucosidase inhibitor that also blocks N-linked oligosaccharide processing, has been shown to have anti-viral activity (i.e. HIV-1 and -2, MOLV, and FLV). The anti-viral activity of castanospermine has been improved 20- to 30-fold by substitution of the 6 position with a butanoyloxy group [41]. The IC_{50} for inhibition of α-glucosidase *in vitro* was similar for both compounds, and therefore the improved anti-viral activity measured in cell culture assays appeared to be due to improved membrane solubility of butanyloxyl-castanospermine. In this study, similar substitutions to SW did not result in improved activity as reflected in IC_{50} for inhibition of oligosaccharide N-linked processing, or *in vivo* in the BM cell proliferation assay. However, 2-*p*-nitrobenzoyloxy-SW, 2-octanoyloxy-SW and 2-butanoyloxy-SW or similar analogs may be useful as prodrugs with 300-fold less activity until they are cleaved by esterases. Ideally, 2- or 8-substitutions to SW that are preferentially hydrolyzed in tumor cell and/or lymphoid cells would be expected to have improved pharmacologic properties and reduced side-effects. Furthermore, carbonoyloxy-linked groups at the 2- or 8-position of SW, which have anti-tumour or immune-stimulatory activity on their own, may be designed to create even more potent prodrugs.

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