Thio- and selenoglycosides as ligands for biomedically relevant lectins: valency-activity correlations for benzene-based dithiogalactoside clusters and first assessment for (di)selenodigalactosides

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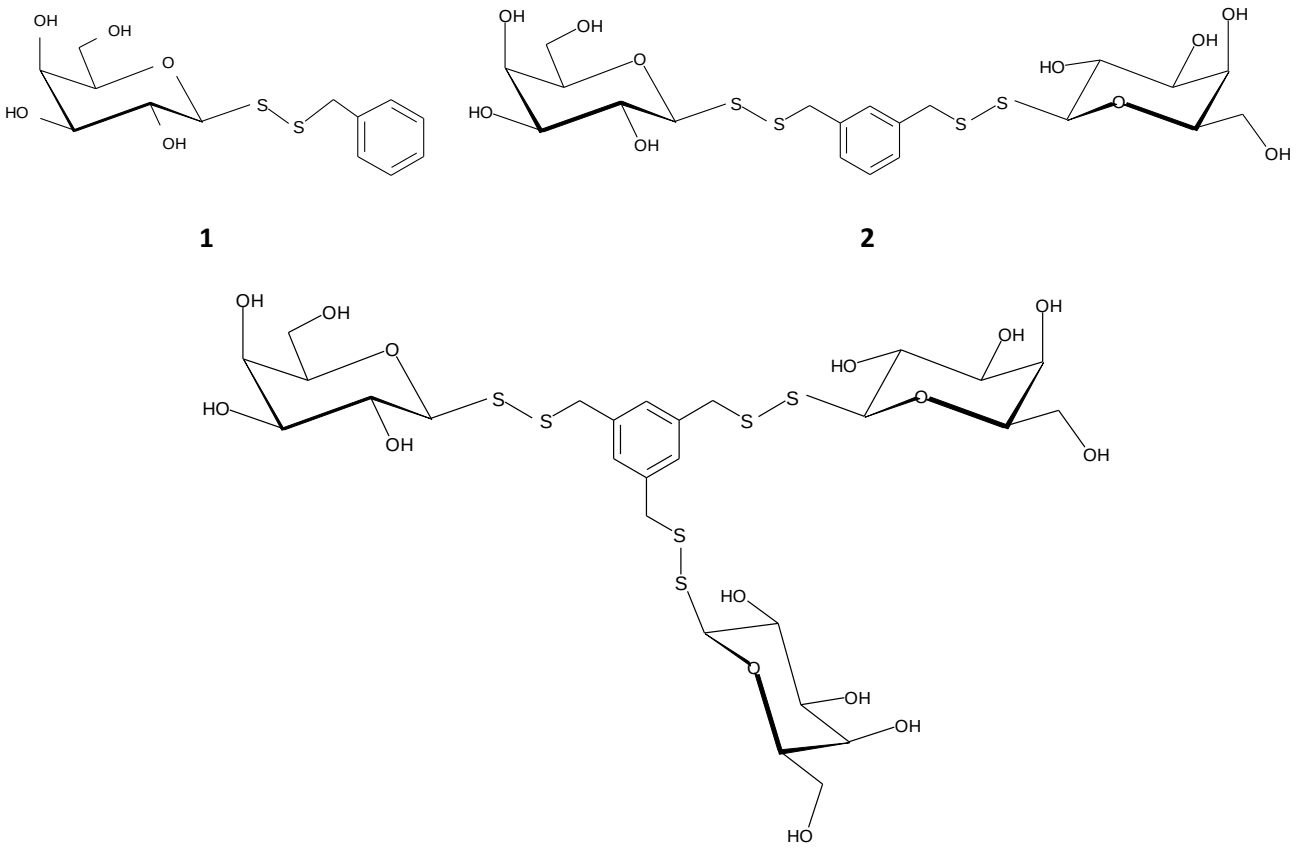
The increasing awareness of biological information coding by glycans, the third alphabet of life,[1] has stimulated efforts to prepare bioactive oligosaccharides. A common route toward this aim starts with thioglycosides.[2] Inspired by their versatility as glycosyl donors, the applicability of a sulfur atom at the anomeric center had been extended to disulfides.[3] They are also readily produced by disulfide exchange in dynamic combinatorial libraries[4] and constitute attractive tools for studying structural properties of a glycosidic linkage in a three-bond system.[5] Along these lines, selenoglycosides and respective selenylsulfides, too, proved their merits for synthetic purposes.[6] Beyond preparative aspects, the resistance to hydrolysis gives reason to examine their capacity for protein-carbohydrate interactions, to define consequences of geometric and electronic changes by substituting oxygen with S/Se.

The symmetric thiodigalactoside (TDG) has gained prominence as potent inhibitor of lectin-dependent haemagglutination in the pioneering studies on detecting and purifying galectins[7], a family of adhesion/growth-regulatory lectins.[8] Interestingly, the corresponding disulfide was much less reactive with galectins but maintained its blocking capacity to a highly toxic plant lectin, i.e. the mistletoe lectin (*Viscum album* agglutinin, VAA) akin to the biohazard ricin.[9] Because computational analysis of flexibility and energy grading of the conformational space of thio- and seleno derivatives of high-affinity lectin ligands, i.e. histo-blood group ABH antigens, inferred an increase of dynamics and access to new secondary conformations,[10] the following questions on S,Se-glycosides needed to be answered:

Will inhibitory potency of disulfides be increased by glycocluster formation?

Will the Se-derivatives of the symmetric digalactoside be lectin ligands, considering the alterations between C-Se/C-S/C-O bond angles (95°/95°/115°) and length (1.9 Å/1.8 Å/1.4 Å)?

To address the first issue a benzene-based core was used as scaffold for presenting one, two and three galactose moieties, synthesized as described (Scheme 1).[11]



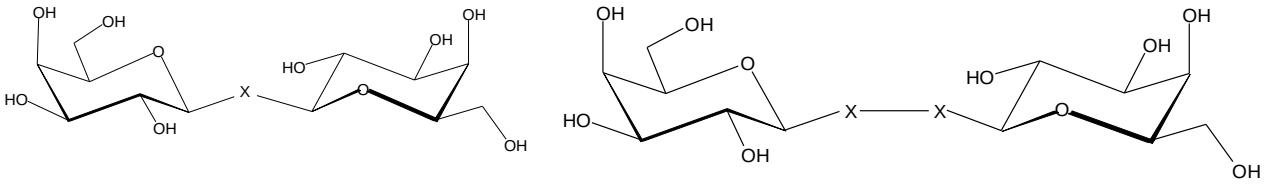
Scheme 1. Structures of the benzene-based mono-, di- and trivalent disulfides presenting D-galactose.

These mono-, di- and trivalent compounds were comparatively tested under identical conditions as inhibitors of lectin binding (the lectins were prepared and controlled for activity as described in detail elsewhere[9a, 12]). In the first setting, a neoglycoprotein (a carrier protein devoid of lectin reactivity, i.e. bovine serum albumin, presenting 28-30 chemically conjugated Lac derivatives[13]) was adsorbed to the surface of microtiter plate wells to establish a lectin-reactive matrix with structurally unambiguously defined sugar part. Binding was saturable and completely dependent on the cognate carbohydrate (not shown). Titrations with increasing additions of the sugar compounds **1, 2** & **3** to the lectin-containing solutions resulted in reduction of the signal recorded by photometry and thus in the determination of the inhibitory concentration (IC) that reduced extent of binding to 50% (IC_{50}). As compiled in Table 1, increase in relative inhibitory potency with valency was pronounced for the

toxin. Inhibitory capacity below the 5 mM threshold was seen for Gal-3, -4 and -9N when exposed to the trivalent compound. Obviously, the individual sugar units of the trivalent glycocluster maintained activity, as had also been reported for the tetrameric concanavalin A based on calorimetric titrations.[14] Increasing the biorelevance of the assay, the three synthetic compounds were next included as inhibitors in cell assays. Here, extent of lectin binding was quantitated by cytofluorometry and the results expressed in percentage of positive cells/mean fluorescence intensity.[9]

The symmetric dithiodigalactoside (DTDG) and the monovalent compound **1** were slightly more active than free Gal, bivalent compound **2** being comparatively strong as in the solid-phase assay (Fig. 1A). This synthetic compound also surpassed the activity of free Lac, but was less potent than trivalent compound **3** (Fig. 1B). Tested at 2 mM, progressive decreases in percentage of positive cells were observed for galectins with increases in valency, e.g. for Gal-3 and colon cancer (SW480) cells graded reductions from 84% (100% value) to 75% (cpd **1**), 68% (cpd **2**) and 61% (cpd **3**) were determined, 52% measured for Lac as reference. Thus, clustered disulfides are effective to block the toxin, with comparatively rather small reactivity to cellular galectins, although a relative increase of inhibitor capacity with valency was noted. Because 1,3,5-triiodobenzene or tris(alkynyloxy)benzene as core for presentation of Lac had yielded cluster effects for Gal-3,[15] the disulfide helps to downregulate the ligand reactivity.

Turning to the second question, we proceeded to synthesize the Se equivalents of TDG/DTDG (Scheme 2).

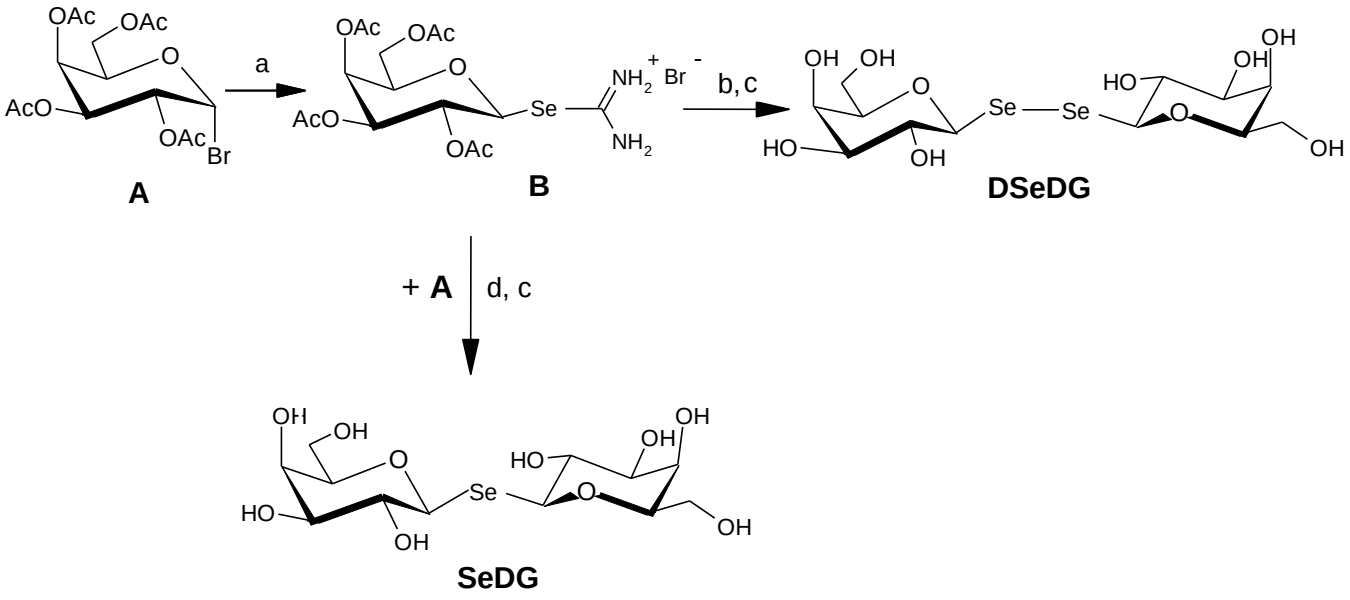


TDG X = S **DTDG** X = S

SeDG X = Se **DSeDG** X = Se

Scheme 2. Structures of the symmetric digalactosides with S/Se-glycosidic linkages.

The synthesis followed the route given in Scheme 3, details given as notes.[16]



a) $(H_2N)_2CSe$, acetone, 60°C, 1 h; b) Et_3N, CH_3CN, reflux, 1 h; c) $NaOCH_3$, methanol, r.t., 10 min, d) acetone, KOH, r.t., 30 min .

Scheme 3. Synthetic pathway to compounds **SeDG/DSeDG** starting from the α-bromo derivative of per-O-acetylated D-galactose.

These two selenides were then introduced to both types of binding assays. The plant toxin was very reactive in the solid-phase assay (Table 1), in cell assays with different lines with similar grading (Fig. 2A, B). The human galectins also bound the selenodigalactoside (**SeDG**), with IC_{50}-values comparable to TDG (Table 1). Cell assays confirmed a slightly better inhibitory potency than Lac for Gal-3 and -9N (Fig. 2C, D) and clearly stronger activity than Lac for Gal-4 (Fig. 3). Thus, bridging of two Gal residues by a Se-glycosidic bond yields a bioactive compound. Following the recent report that a methylseleno-substituted Lac derivative (at the Glc moiety) could be crystallized in complex with Gal-9N so that its structure could be obtained at the resolution of 1.4 Å,[17] and the demonstration that the β-methyl derivative of *N*-acetylglucosamine proved bioactive to form a

complex in crystals with a bacterial adhesin,[18] our data now prove biocompatible Se-incorporation at sites of the ligand, which are in contact with the human bioeffectors. Seleno-carbohydrates, an area of synthesis started in 1921,[19] can thus be designed for testing suitability for biomedical and analytical purposes. This experimental demonstration, solidifying previous indications for several classes of lectin by modeling,[10b] encourages systematic structure-activity exploration for human galectins. Due to availability of full NMR assignments[20] working with ^{15}N-labeled galectins, especially ^{15}N-^{1}H HSQC mapping, is a sensitive tool to detect ligand-specific structural changes, as done for Gal-1 and α-galactosides.[21] In view of our introduction of ^{19}F-bearing carbohydrate ligands into the monitoring of lectin binding by NMR spectroscopy[22] similar (or even combined) exploitation of sensor activity is envisioned for ^{77}Se and human lectins.

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16. (a) Peracetylated **SeDG** was prepared from 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl isoselenuronium bromide (**B**) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**A**) in analogy to the di-(tetraacetyl-1-β-D-glucosyl)-selenide as described [Wagner, G.; Nuhn, P. *Arch. Pharm.* **1964**, *297*, 461.]. This product was directly deacetylated with sodium methoxide in methanol to give **SeDG**.

^{1}H NMR (D_2O, 500 MHz): δ 4.98 (d, 1H, H-1, $J_{1,2}$ 10.0 Hz); 3.94 (dd, 1H, H-4, $J_{3,4}$ 3.3 Hz $J_{4,5}$ ~1 Hz); 3.64 – 3.74 overlapping signals (4H, H-2, H-5, H-6a, H-6b); 3.60 (dd, 1H, H-3, $J_{2,3}$ 9.1 Hz); ^{13}C NMR (D_2O, 125 MHz): δ 80.6 (C-1); 80.4 (C-5); 73.9 (C-3); 70.6 (C-2); 69.1 (C-4); 61.4 (C-6); ^{77}Se NMR (D_2O, 95.4 MHz): δ 394; HRMS: $C_{12}H_{22}O_{10}Se$ $[M+Na]^+$: 429.030, Found: 429.032; (b) peracetylated **DSeDG** was prepared from 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl isoselenuronium bromide (**B**) in analogy to the di-(tetraacetyl-1-β-D-glucosyl)-diselenide as described [Wagner, G.; Nuhn, P. *Arch. Pharm.* **1964**, *297*, 461.]. This product, obtained by a different route as well [Kawai, Y.; Ando, H.; Ozeki, H.; Koketsu,

M.; Ishihara, H. *Org. Lett*., **2005**, *7*, 4653.], gave **DSeDG** on deacetylation with sodium methoxide in methanol.

^{1}H NMR (D_2O, 500 MHz): δ 4.94 (d, 1H, H-1, $J_{1,2}$ 9.8 Hz); 4.04 (br.d, 1H, H-4, $J_{3,4}$ 3.3 Hz); 3.90 (t, 1H, H-2, $J_{2,3}$ 9.8 Hz); 3.75 – 3.84 overlapping signals (3H, H-5, H-6a, H-6b); 3.74 (dd, 1H, H-3); ^{13}C NMR (D_2O, 125 MHz): δ 83.8 (C-1); 80.7 (C-5); 73.8 (C-3); 70.6 (C-2); 69.1 (C-4); 61.2 (C-6); ^{77}Se NMR (D_2O, 95.4 MHz): δ 381; HRMS: $C_{12}H_{22}O_{10}Se_2$ $[M+Na]^+$: 508.943, Found: 508.948.

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Table 1

IC_{50}-values of S/Se-glycosides and free mono- and disaccharides in assays to block binding of labeled lectin to surface-imobilized neoglycoprotein (in mM)

Lectin inhibitor	VAA (3 μg/ml)	Gal-3 (15 μg/ml)	Gal-4 (5 μg/ml)	Gal-8 0.1 (μg/ml)	Gal-9N (15 μg/ml)
1	0.75 (1.2/0.8)	> 5 (n.i./<0.3)	> 5 (n.i./<0.7)	> 5 (n.i./<0.2)	> 5 (n.i./<0.2)
2	0.11 (8.2/5.5)	> 5 (n.i./<0.3)	> 5 (n.i./<0.7)	> 5 (n.i./<0.2)	> 5 (n.i./<0.2)
3	0.06 (15/10)	3.2 (>1.6/0.5)	4.8 (>2.1/0.7)	> 5 (n.i./<0.2)	4 (0.8/0.3)
SeDG	0.25 (3.6/2.4)	1.4 (>3.6/1.1)	1.5 (>6.7/2.3)	n. d.	0.8 (4.0/1.3)
TDG[a]	0.4 (2.3/1.5)	1.1 (>4.5/1.5)	1.8 (>5.6/1.9)	1.4 (7.1/0.9)	0.8 (4.0/1.3)
DSeDG	0.34 (2.6/1.8)	3.8 (>1.3/0.4)	> 5 (n.i./<0.7)	n. d.	2.8 (1.1/0.4)
DTDG[a]	1.1 (0.8/ 0.5)	5.4 (>0.9/0.3)	> 10 (n.i./<0.4)	> 10 (n.i./<0.1)	2.6 (1.2/0.4)
Gal	0.9	> 5	> 10	> 10	3.2
Lac	0.6	1.6	3.5	1.2	1.0

[a] from ref. 9c; titrations were performed in microtiter plate wells using constant concentrations of neoglycoprotein (lactosylated bovine serum albumin; 0.25 μg/well) for coating and of lectin as well as eight concentrations of sugar in triplicates and up to six independent series, reaching an upper limit of 14.6 % for the standard deviation; n. d.: not determined, n.i: not inhibitory. Number in brackets denote the inhibitory capacity relative to free Gal/Lac.

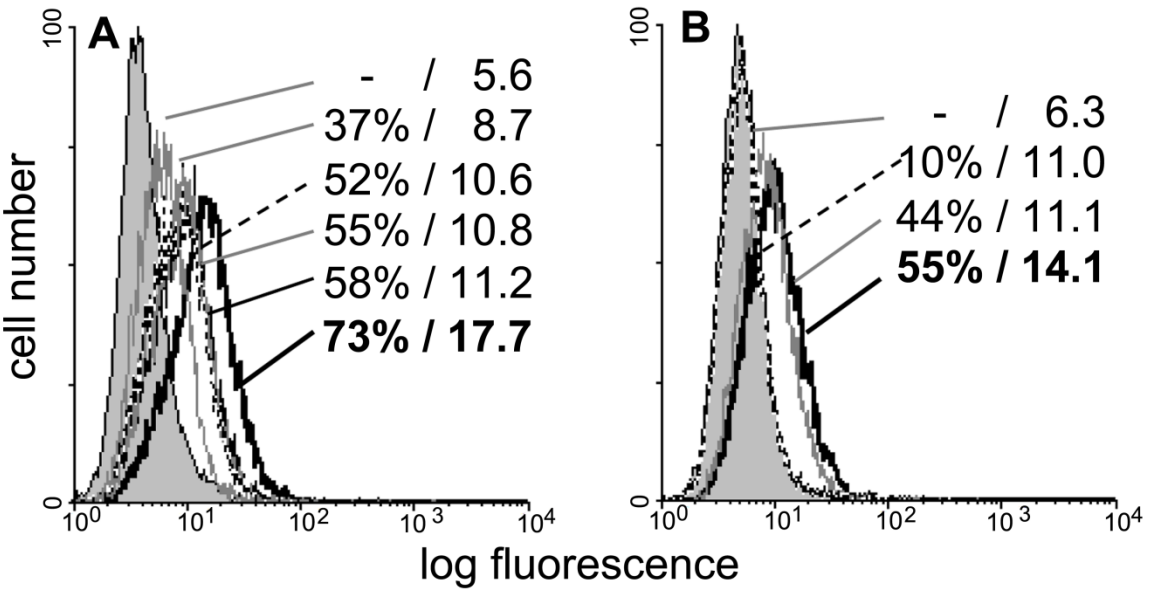

Figure 1. Fluorescent surface staining (percentage of positive cells/mean fluorescence intensity) of human colon adenocarcinoma cells (SW480) by labeled VAA and its decrease by presence of test compounds. Staining profiles obtained with a lectin concentration of 2 µg/ml in the absence of inhibitor (100%-value, bold number) and (listed from bottom to top) in the presence of 2 mM Gal, DTDG, compound **1** and compound **2** (A) or 2 mM Lac and compound **3** (B). The gray area defines lectin-independent staining (0%-value), its fluorescence intensity given at the top of the list.

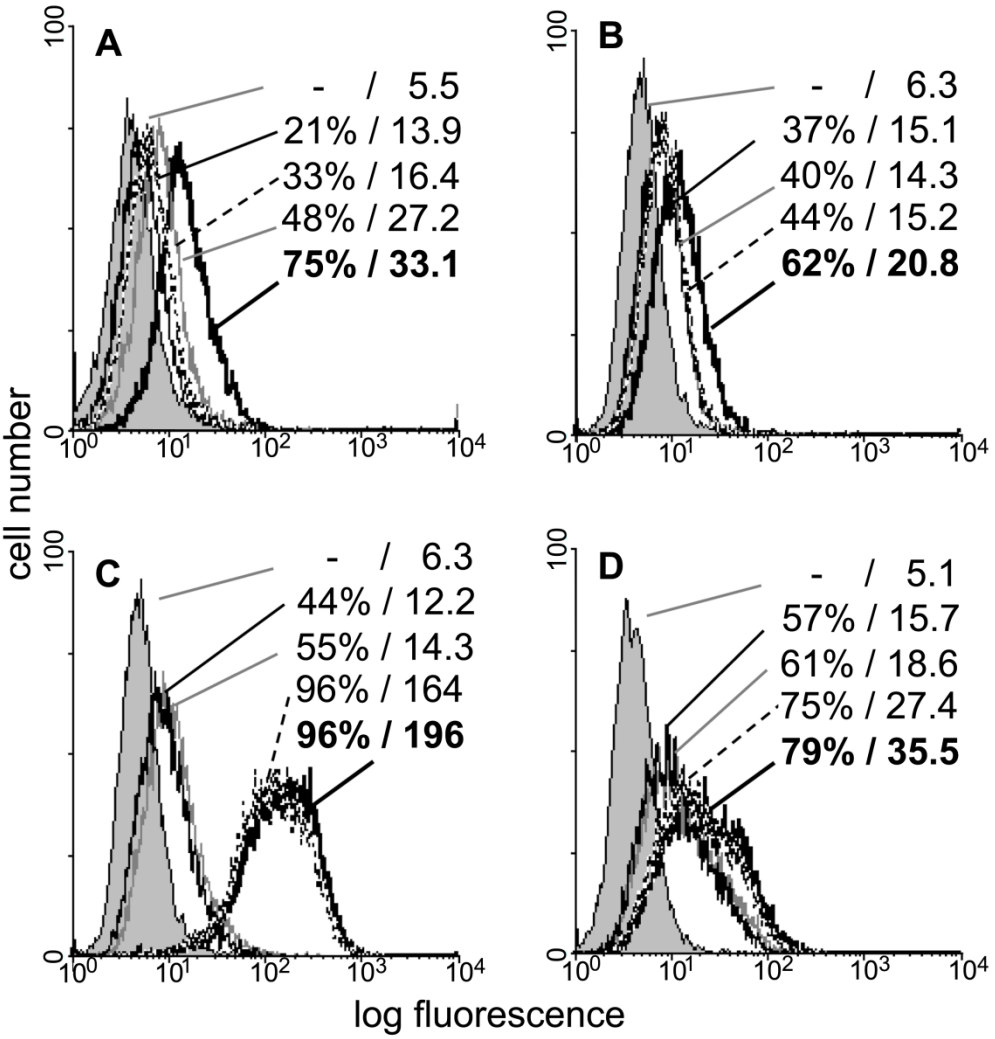

Figure 2. Fluorescent cell surface staining by labeled VAA (A, B), Gal-3 (C) and Gal-9N (D) of human B-lymphoblastoid (Croco II; A) and colon adenocarcinoma cells (SW480, B-D). Staining profiles obtained with a VAA concentration of 1 μg/ml in the absence of inhibitor and in the presence of 2 mM Lac, compound **DSeDG** and compound **SeDG** (A), with a VAA concentration of 2 μg/ml in the absence of inhibitor and 1 mM Lac, compound **DSeDG** and compound **SeDG** (B), with a Gal-3 concentration of 5 μg/ml in the absence of inhibitor and in the presence of 1 mM compound **DSeDG**, Lac and compound **SeDG** (C) and with a Gal-9N concentration of 2 μg/ml in the absence of inhibitor and 5 mM compound **DSeDG**, Lac and compound **SeDG** (D).

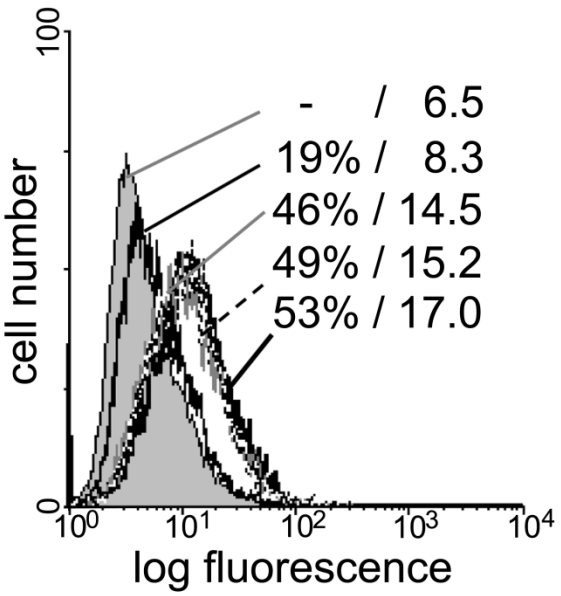

Figure 3. Fluorescent cell surface staining by labeled Gal-4 of human pancreatic adenocarcinoma cells (Capan-1) reconstituted for expression of the tumor suppressor $p16^{INK4a}$ by labeled Gal-4 (20 μg/ml). Test compounds (**DSeDG**, Lac and **SeDG**) were used at the concentration of 5 mM.