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Synthesis and Pharmacological Evaluation of Vinyl Sulfone Based Anticancer Agents

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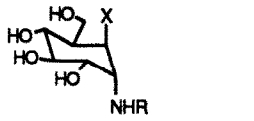
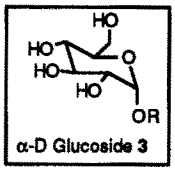
Abstract: Several vinyl sulfone based substrates exhibited excellent *in vitro* activity against the HT-29 colon cancer cell line and also demonstrated *in vitro* differential cytotoxicity against ras transformed rat kidney epithelial cell lines. Evaluation of three agents in animal trials revealed no anticancer activity.

Abnormal patterns of glycolipids and glycoproteins invariably accompany the transformation of normal cells to their cancerous state.[1] Variations in glycoprotein structure are also strongly implicated in the metastatic process.[2] A series of recent papers have demonstrated that attenuation of the process of glycoprotein maturation can result in substantially decreased metastasis. These papers have shown that glycosidase inhibitors, such as castanospermine, 1-deoxynojirimycin, swainsonine, and mannostatin A, interfere with the development of pulmonary metastasis in mice.[3,4,5,6] Except for mannostatin A, these naturally occurring glycosidase inhibitors are polyhydroxylated nitrogen alkaloids resembling either the corresponding glycosides or the glycosyl cations.



1 Dihydroacarbose (X=H; R=α-4Fuc1->βGlc1->βGlcOH)

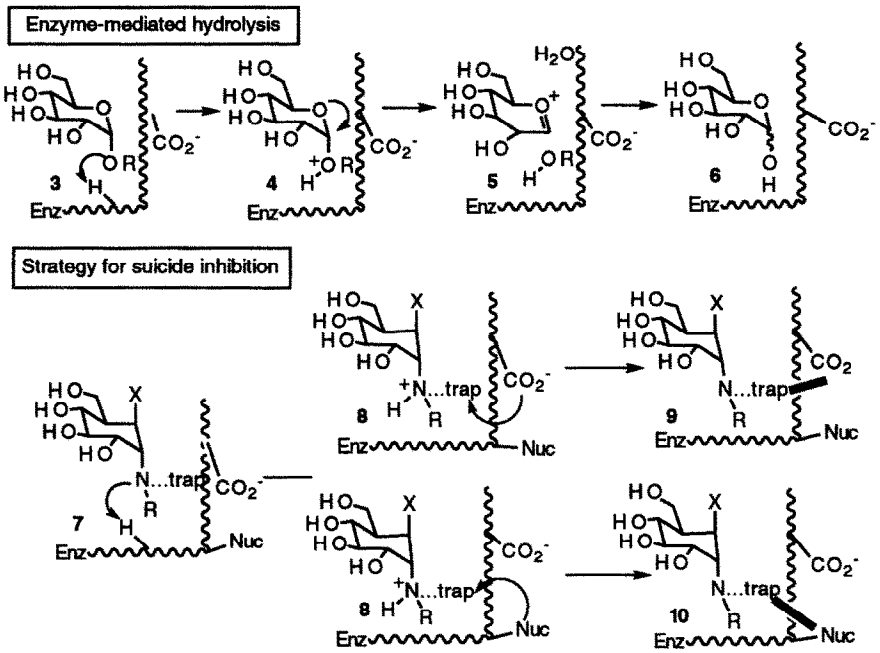
2 Oligiostatin (X=OH; R=α-4Fuc1->βGlc1->βGlcOH)



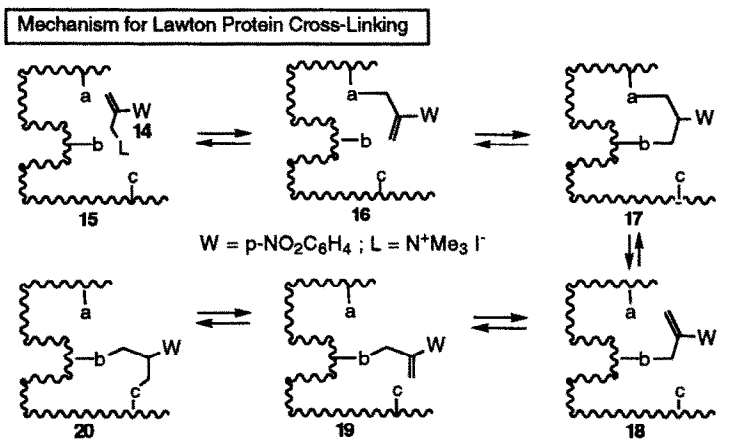
α-D Glucoside 3

Recently, we initiated a new program to exploit the use of inhibitors of glycoprotein processing enzymes as anticancer agents. Specifically, an effort was directed towards the synthesis of tailored substrates which would be delivered to the enzymatic site by virtue of their topographical similarity to the glycosides, thereby being positioned to inactivate the enzyme by irreversible covalent bond formation (i.e., suicide inhibition).[7] A typical substrate consists of a recognition unit and a nitrogen-tethered trapping moiety (*vide infra*). Due to their close resemblance to the generic α-D-glucoside **3**, the pseudoglycoside portions of α-1,2-glucosidase I inhibitors **1** and **2** were considered to be attractive recognition units.

The mechanism of enzyme-mediated hydrolysis of the generic glucoside **3** presumably involves an enzyme-directed protonation (**3** to **4**) followed by lone-pair participation (**4** to **5**) and addition of water to afford lactol **6**. A carboxylate is shown in proximity to the site of action since the enzyme-protonated nitrogenous inhibitors such as **1** and **2** may derive their high binding constants by virtue of formation of a tight ion pair with the nearby carboxylate. The basic tenant employed in the design of the targeted suicide inhibitors of the above enzyme is that protonation of the axial amino moiety will activate the nitrogen-tethered trapping group toward covalent bond formation by either the carboxylate (**8** to **9**) or a fortuitously disposed nucleophilic site on the enzyme surface (**8** to **10**).



Before undertaking the syntheses of the nitrogen-containing carbocyclic units of **1** and **2**, it was deemed necessary to employ a more easily available pseudoglycoside surrogate during the development of the specific suicide traps. Racemic axial amino alcohols **11**[8] and **12** (see page 4 of this letter) were chosen for this purpose, which were prepared from 4-t-butylcyclohexanol in 26% and 25% overall yields, respectively.[9]

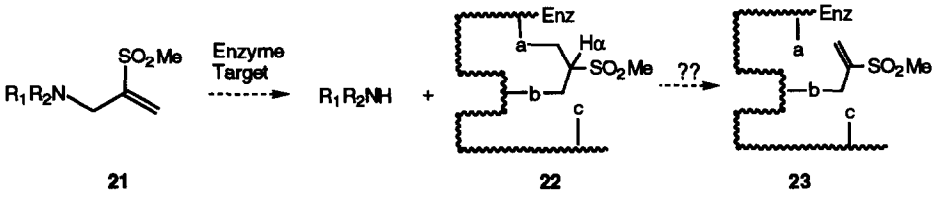


The S_N2' chemistry of allyl groups which bear an additional electron-withdrawing substituent on the olefin has been pioneered by Lawton and co-workers.[10] An important extension of this concept was provided by Lawton in 1979, where he demonstrated that such reagents are capable of providing cross-linked proteins.[11] The cross-linking occurs via an equilibrium transfer mechanism where an accessible nucleophilic

site at the enzyme surface (typically an ε-amino group of a lysine or the mercaptan moiety of a cysteine) undergoes bond formation to provide initial adduct **16**. Subsequent steps occur under equilibrium control and the site of thermodynamic cross-linking **20** is fixed by a final chemical step involving reduction of the aryl nitro "W" group.[12]

It was envisaged that substrates of structural type **21** might foster the Lawton reaction in the enzyme cavity.[13] The methanesulfonyl activating group was selected so as to render the α-sulfonyl proton (H_α of **22**) as non-acidic as possible, which should slow the formation of intermediate **23**. While this could circumvent the full thermodynamic equilibrium as shown in the above scheme, it should also retard loss of the cross-linking moiety via the action of exogenous nucleophiles, thereby favoring the desired suicide inactivation of the target enzyme.

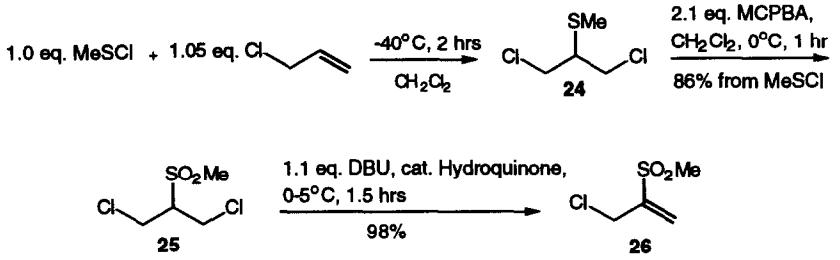
Plan for Enzyme Inactivation via Formation of Cross-Linked Sulfones

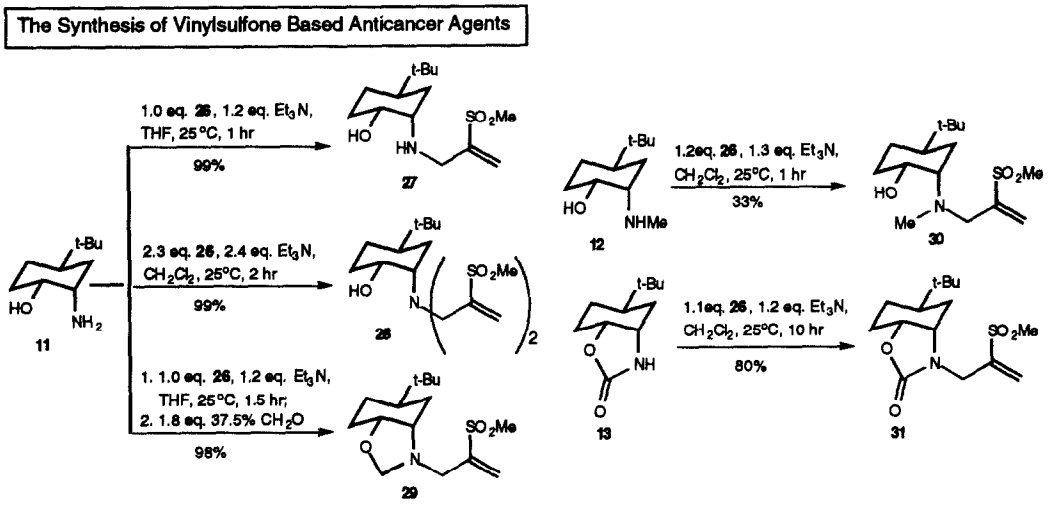


21 **22** **23**

In practice, the trapping agent **26** was readily prepared from methansulfenyl chloride and allyl chloride in 84% overall yield.[13a,14] After a brief experimentation, the selective synthesis of **27** or **28** was achieved by judicious choices of solvent and reagent addition sequence.[9] The undesired oxygen alkylation and the intramolecular cyclization products were not observed.

Preparation of the Vinyl Sulfone Based Trapping Agent 26





Encouraged by the preliminary anticancer testing results of **27** and **28**, three additional substrates (**29-31**) were synthesized and tested. The cytotoxicity of **27-31**, **11** and **26** was tested using the MTT assay. MTT assay is a quick *in vitro* calorimetric assay employed to screen possible antitumorigenic agents against a variety of well established human tumor cell lines.[15] In the event, five different human tumor cell lines were included in this test, the results are summarized in Table 1.[16] These numbers are single determinations and should be regarded as having a standard deviation of ca. ±1/2 log.

Table 1. The *in vitro* Human Tumor Cytotoxicity Assay Results (ED_{50})[a]

Substrates	A-549[b]	MCF-7[c]	HT-29[d]	SK-MEL-5[e]	Malme-3M[f]
11	1.6×10^{-4}	2.6×10^{-4}	>100	4.5×10^{-4}	>100
26	1.9×10^{-5}	6.5×10^{-6}	2.2×10^{-6}	3.2×10^{-6}	6.5×10^{-6}
27	6.7×10^{-7}	4.7×10^{-7}		1.9×10^{-7}	6.0×10^{-7}
28	1.3×10^{-6}	3.3×10^{-7}		4.6×10^{-7}	4.2×10^{-6}
29	1.2×10^{-6}	2.4×10^{-7}		9.6×10^{-7}	1.0×10^{-6}
30	1.4×10^{-5}	1.7×10^{-7}		5.6×10^{-7}	4.8×10^{-7}
31	2.9×10^{-5}	2.7×10^{-6}		1.3×10^{-6}	1.1×10^{-5}

[a]The ED_{50} values are expressed in moles/l. DMSO was used as the solvent. Substrate **30** turned into a gel in DMSO while running the MTT assay. [b]Lung carcinoma. [c]Breast carcinoma. [d]Colon adenocarcinoma. [e]Melanoma, metastasis of the auxiliary node. [f]Melanoma, metastasis to lung.

Table 1 showed excellent *in vitro* activity against HT-29 for **27-31**. It is particularly interesting to note that **11** - the delivery agent for **27-28** is essentially non-toxic to the tumor cells. Compounds **27-31** were further tested for *in vitro* differential cytotoxicity at Eli Lilly. In recent years, progress has been made in detection of genetic changes involved in cancers. The ras gene family is important in the etiology of a large variety of human cancers, particularly pancreas, lung and colon cancers.[17] In these tumors, the proteins encoded by the ras genes are mutated and are required for the maintenance of the transformed

phenotype.[17b,18] Due to the importance of mutated ras in cancers, the *in vitro* cytotoxicity of **27-31** was evaluated against normal and ras transformed epithelial cells.

For these studies, normal rat kidney epithelial (NRKE) cells[19] and the ras transformed cell lines K/1-NRK, H/1.2-NRK and N/4.2-NRK were employed. The K/1-NRK line, the H/1.2-NRK line, and the N/4.2-NRK line were produced by transformation of NRKE cells with the v-K-ras gene (gly^{12} to val^{12}), the mutated c-H-ras gene (gly^{12} to val^{12}), and the mutated c-N-ras gene (gly^{12} to val^{12}), respectively. NRKE cells are not tumorigenic in several strains of immunologically deficient mice, whereas the H/1.2-NRK, K/1-NRK and N/4.2-NRK cells produce carcinomas in mice. A clonogenicity assay was used to determine the cytotoxicity of **27-31** against the parental NRKE and the transformed H-1.2-NRK, K/1-NRK and N/4.2-NRK cells.

These *in vitro* differential cytotoxicity tests were run in triplicate of media-control, DMSO solvent-control, and substrate (drug) treatment groups. Each drug was added 24 hours after seeding of the cell lines and the resulting culture was assayed five days later. Cell kill was calculated from the quotient for colony number of drug-treated cultures divided by colony number of solvent-control cultures. The LC_{50} values in Table 2 were obtained from linear interpolation of semilogarithmic plots of % cell kill (arithmetic axis) against dosages of drugs administrated (logarithmic axis). At 3.3 and 33.00 μM, **27** and **30** had no differential cytotoxicity. In contrast, **28-29** and **31** were more cytotoxic against K/1-NRK cells than H/1.2-NRK or NRKE cells (see Table 2). In addition, **28-29**, and **31** exhibited differential cytotoxicity against Lewis lung carcinoma cells (3LL). The ratios of LC_{50} (NRKE) to LC_{50} (cancer cells) give the differential indices (DI). In our experience, a DI of 3 or higher generally warrants further *in vivo* investigation.[20]

Table 2. *In vitro* Cytotoxicity of **28-29** and **31** Against NRKE, H/1.2-NRK, K/1-NRK and 3LL Cells (LC_{50})*

Substrates	NRKE	H/1.2-NRK	DI	K/1-NRK	DI	N/4.2-NRK	DI	3LL	DI
28	2.4	2.5	1.0	0.6	[illegible]	1.4	0.6	0.8	[illegible]
29	1.4	2.1	0.6	0.5	[illegible]	1.8	0.8	0.5	[illegible]
31	4.4	4.7	0.9	2.6	1.7	5.0	0.9	3.1	1.4

*The LC_{50} values are expressed in μM. The maximum standard deviation is ±20%.

Due to the excellent *in vitro* activity against HT-29 colon cancer, **28-29** and **31** were also evaluated at their maximally tolerated doses (MTD) and 1/3 MTD against HT-29 colon tumor cells *in vivo*. The tumor was grown as a xenograft in 16 to 18 g, female, C57xBALB/C immunologically deficient mice (8 animals per group). The tumor cells were implanted approximately 0.5 cm below the left, anterior, mammary gland. Seven days after tumor implantation, the three substrates were given ip daily up to the their MTD respectively for ten days. On the eighteenth day, length (L) and width (W) measurements of tumors were made. Unfortunately, no apparent inhibition on the tumor growth by these substrates was observed. Only one toxic death was reported when **31** was administrated with the MTD of 100 mg/kg/day (Table 3). The inability of these substrates to penetrate tumor cell membrane is likely the culprit of the poor *in vivo* cytotoxicity.[21] Substituting the β-amino alcohol unit with a glycoside mimic, such as the pseudoglycoside portion of **1** or **2**, may improve the cell-permeability, thereby enhancing the *in vivo* anticancer activity of these tailored substrates.

Table 3. The *in vivo* Anticancer Testing Results for **28-29** and **31**

Substrate	Dose (mg/kg)	% Inhibition	Toxic Death/Total animals per group
28	11	0	0/8
28	33	0	0/8
29	3	0	0/8
29	10	0	0/8
31	33	0	0/8
31	100	0	1/8

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