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In vitro antitumor activity of *N*-glycosyl sulfonamides

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ABSTRACT

A series of α-D-hex-2-enopyranosyl sulfonamides was evaluated for their antiproliferative activity against human hepatocellular liver carcinoma (HepG2) and human lung adenocarcinoma (A549) cell lines. The most potent compound (2,4,6-tri-*O*-acetyl-3-deoxy-α-D-*erythro*-hex-2-enopyranosyl ethanesulfonamide) showed antiproliferative properties in the micromolar range. The SARs of these sulfonamidoglycoside which includes the influence of carbohydrate rings and sulfonamide class are described.

Over the past decade, lung and liver cancer have been two of the most common cause of cancer death in the world. Lung and liver cancer cause 1.3 and 0.6 million deaths per year worldwide, respectively.[1] Non-small-cell lung cancer (NSCLC) accounts for over 80% newly diagnosed lung cancer and the majority of patients are diagnosed with advanced and un-resectable disease.[2] The standard treatment for advanced NSCLC is chemotherapy. However, NSCLC is extremely resistant to chemotherapeutic agents[3] and such therapy only modestly increases the survival rate. By other hand, hepatocellular carcinoma (HCC) is one of the most common causes of solid organ malignancy in the world.[4] Definitive treatment of HCC through surgical resection and/or transplantation can provide long-term survival; however, the vast majority of HCC is identified at an advanced stage when successful surgical treatment is no longer feasible. Chemotherapy and other systemic treatments for patients with HCC have been largely ineffective.[5] As a consequence, effective approaches and therapies need to be developed to treat NSCLC and HCC.

Experimental and epidemiological studies have demonstrated the effect of non-steroidal anti-inflammatory drugs in the prevention and treatment of human cancers.[6] Several of these drugs have a sulfonamido functionality in their molecule.[7] *N*-(2-(Cyclohexyloxy)-4-nitrophenyl)-methanesulfonamide (NS398) inhibits the growth of human hepatocellular carcinoma cell line HepG2 by inducing cells cycle arrest and is a potential candidate as an effective chemopreventive tool against human HCC.[8] Celecoxib derivatives have shown to be highly toxic to human non-small-cell lung adenocarcinoma cells line A549 and the results suggest the potential of celecoxib-derived agents to treat NSCLC.[9] Also sulfonamide-containing compounds, such as *N*-pyridinyl- and indole-sulfonamides demonstrated effective inhibition of tubulin polymerization and were found to be potent antimitotic agents.[10]

Since several years ago we have been interested in the synthesis and biological activities of *N*-glycosyl sulfonamides.[11] The incorporation of carbohydrate scaffolds within the design of new compounds, has already contributed to the discovery of lead candidates with anti-infectious, anti-inflammatory, or anticancer activity.[12] In many cases, use of carbohydrates as drugs has an important drawback: they are sensitive to the presence of enzymes and acidic or basic media. Thus, design of mimetics that are not processed to product in the usual way is an active area of research. An unusual enzyme-resistant replacement for the glycosidic linkage is the *sulfonamide* corresponding to the union of a glycosylamine and a sulfonic acid derivative. However, glycosylamines are not stable and are very sensitive to hydrolysis and anomerization.[13] To overcome this problem we have demonstrated that *N*-glycosyl sulfonamides could be prepared by addition of sulfonamides or sulfamide to acetyl-protected glycals via Ferrier rearrangement.[14] Some of these novel compounds have been showed to be carbonic anhydrase (CA) inhibitors.[15] The aim of the present work is to study the in vitro antitumor activity of these α-glycosyl sulfonamides against human hepatocellular liver carcinoma (HepG2) and human lung adenocarcinoma (A549) cell lines.

N-Glycosyl sulfonamides have been prepared by our original procedure, implying the reaction of per-*O*-acetylated glycals **1** and **2** with ethanesulfonamide, *p*-toluenesulfonamide or sulfamide catalyzed by fluoroboronic acid immobilized on silica (Scheme 1).[14b] Unfortunately the reaction of 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-arabinohex-1-enitol (**3**) in these conditions afforded

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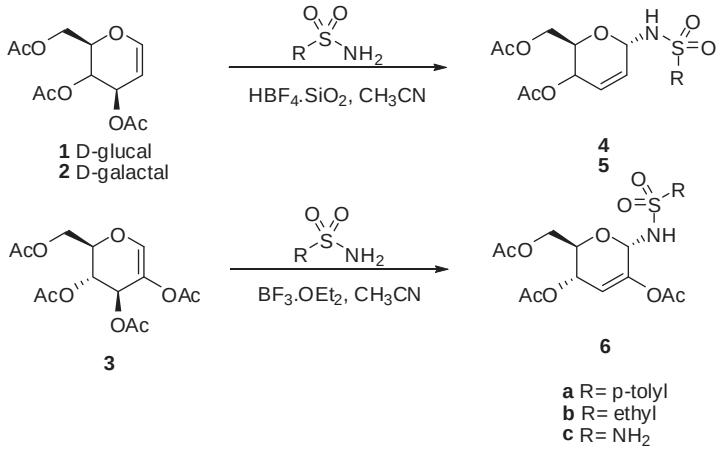

Scheme 1.

Table 1
Cytotoxic activity data for the *N*-glycosyl sulfonamides[a]

Compound	IC_{50} (MM)	
	A549	HepG2
4a	>1000	306.7
4b	>1000	192
4c	557.9	303.6
5a	829.4	156.5
5b	280.1	231.2
5c	703.8	336
6a	34.9	39.2
6b	21.9	14.7

[a] Cytotoxicity as IC_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 560 nm relative to untreated cells using the MTT assay.

several decomposition products. To overcome this problem we have prepared sulfonamidoglycosides **6** in the presence of boron trifluoride etherate as catalyst.[14a,16] The reaction mixtures could be easily purified by flash column chromatography and/or crystallization to afford the pure α anomer. The ^1H and ^{13}C NMR and mass spectral data of the glycosyl sulfonamides were in full accordance with their structure.[14]

These sulfonamidoglycosides were evaluated for their cytotoxicity in vitro towards the human hepatocellular liver carcinoma cell line (HepG2), human lung adenocarcinoma cell line (A549). Cytotoxic activity was determined using the MTT assay, after exposure of cells to the test compounds for 24 h for A549 cells and 48 h for Hep G2 cells.[17] The results are presented in Table 1.

These assays demonstrated that HepG2 cells, in general, are more sensitive to the inhibition by the synthesized compounds. Two of the compounds prepared (**4c** and **5c**) have been showed to be carbonic anhydrase inhibitors.[15] Recent studies revealed that CA isozymes IX and XII are expressed at high levels and with a high prevalence in different tumor tissues, whose normal counterparts do not contain this protein.[19] CA IX significantly correlates with high tumor grade, necrosis, treatment outcome, and poor prognosis in patients with lung carcinoma.[20] Also expression of CA IX has been linked to malignant transformation of hepatobiliary cells.[21] Owing to tumor-associated expression pattern, CA IX and CA XII could be validated as new therapeutic targets for cancer chemotherapy intervention.[22] However, despite the fact that, **4c** and **5c** inhibited CA IX and XII at low nanomolar concentrations,[15] effects on cell proliferation were noted only at low milimolar concentrations for both cell lines. Absence of the effect of CA IX inhibition on cell growth in culture could be explained by the fact that both CA IX and XII grant the survival advantage to hypoxic tumor cells by regulating and maintaining pH. In our cell culture models, cells grew in monolayer and have never become hypoxic. So inhibition of the mechanism that helps survive hypoxic conditions had no effect in cell cultures.[23] Perhaps tumor xenograft model would be more useful to evaluate the effect of **4c** and **5c** on tumor cell survival.

Other interesting feature is the clear activity dependency on the nature of the carbohydrate moiety present in the inhibitor. The *threo* compounds (**5a–b**) are more active than *erythro* ones (**4a–c**) against A549 cells. A similar correlation between activity and configuration of the epimer at C-4 is difficult to find with hepatocellular cell line. It is important to point out that 2-acetyl-D-*erythro*-hex-2-enopyranosyl sulfonamides (**6a–b**) are the most potent antiproliferative agents against both cell lines. These results demonstrate that 2-acetyl group of the glycosyl ring plays a pivotal role in affecting cytotoxicity. Also it is important to note that alkyl sulfonamide derivatives (**b**-type series), in general, are more potent inhibitors of tumor cells than their aryl sulfonamide (compounds of type **a**) and sulfamide analogs (**4c** and **5c**).

To summarize, we have described the antiproliferative activity of eight sulfonamidoglycoside against human hepatocellular liver carcinoma (HepG2) and human lung adenocarcinoma (A549) cell lines. 2-Acetyl-D-*erythro*-hex-2-enopyranosyl sulfonamides **6a** and **6b** displayed the most potent activity. Although compounds **4c** and **5c** are potent inhibitors of the tumor-expressed CA IX, they are poor antiproliferative agents in vitro probably due to cell culture conditions. Further development of glycosyl sulfonamides could be of great interest in finding new effective anticancer agents.

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    The MTT assay was performed according to the method of Mosmann.[18] Since the cell lines employed in the present study had different proliferation rates, the number of cultured cells was adjusted to a density that allowed cells to grow exponentially before initiating the treatment. That is, A549 was inoculated into the 24-well sterile plates at a density of 8000 cell/well for 24 h and Hep G2 was inoculated into 24-well sterile plates at a density of 35 000 cell/well for 48 h. After exposure of the cells to the test drugs for another 24 and 48 h, respectively at 37 °C, 0.5 ml of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Sigma Chemical Co., St. Louis, MO, USA) solution in PBS (0.5 mg/ml) was added, and the cells were incubated for another 2–3 h. Then 0.5 ml of isopropanol + 0.04 N HCl was added, and the absorbance was determined at a wavelength of 560 nm using an ELISA reader. All tests were performed three times in quadruplicate. The IC_{50} values were calculated from curves constructed by plotting cell growth inhibition (%) versus compound concentration.
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