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N-β-Glycosyl sulfamides are selective inhibitors of the cancer associated carbonic anhydrase isoforms IX and XII

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ABSTRACT

The transmembrane isoforms of carbonic anhydrase (CA IX and XII) have been shown to be linked to carcinogenesis and their inhibition to arrest primary tumor and metastases growth. In this Letter, we present a series of peracetylated and deprotected *N*-β-glycosyl sulfamides that were tested for the inhibition of **4** carbonic anhydrase isoforms: the cytosolic hCA I and hCA II and transmembrane tumor-associated IX and XII. Compounds **1–4** and **6–8** selectively target cancer-associated CAs (IX and XII) with K_Is in the low nanomolar range.

Treatment of cancer has changed over the last decade with the advent of targeted therapies. Whereas traditional chemotherapy was directed toward all rapidly dividing cells (cancerous or not), several new anti-cancer drugs are mainly tailored to specific genetic pathways of cancer cells. Ideally, the goal of these new therapies is to improve the management of cancer with a specific targeting of the malignant cell and fewer side effects than traditional chemotherapy.[1]

Recently the zinc metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1), which catalyzes the reversible hydration of cell-generated carbon dioxide into protons and bicarbonate ions, has emerged as a potential target in cancer therapy.[2] Mammalian cells express different carbonic anhydrase isozymes, which differ in their tissue distribution and cellular localization.[3] Membrane-bound 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.[4]

Hypoxia often develops in solid tumors due to insufficient supply of oxygen by aberrant vasculature.[4b] CA IX belongs to the mostly strongly induced proteins in response to hypoxia and was therefore suggested to serve as a marker of tumor hypoxia with possible diagnostic, prognostic and therapeutic value.[4]

Maintenance of pH balance in solid tumors is challenged by their high metabolism and inadequate vasculature. The acid that is removed from cells leads to increased extracellular acidity (pH_e). Low pH_e may favor tumor aggressiveness and metastasis. From a therapeutic point of view, low pH_e decrease in the absorption of basic anti-cancerous drugs so modulating the answer of the tumor cells to chemo- and radio-therapy.[5]

The high catalytic activity of CA IX isozyme leading to formation of protons by the hydration of CO_2, was demonstrated to participate to the tumor microenvironment acidification by maintaining low pH_e.[6] It is expected that CA XII, another hypoxia-induced extracellular isoform of CA, should show similar physiology to CA IX. Overexpression of CA IX (or IX and XII) due to hypoxia has a strong impact on cancer progression, because maintenance of neutral intracellular pH is vital for cell proliferation and survival, whereas low pH_e contributes to aggressive tumor phenotype by promoting invasion and metastasis.[6] Neri's and our groups showed that targeting of CA IX (and XII) with sulfonamide or coumarin potent and specific inhibitors, leads to effective inhibition of both primary tumor and metastases growth, and that this may provide a novel anti-cancer therapy.[6–8]

The use of carbohydrate scaffolds in the design of CA inhibitors has proven to be a successful approach and now constitutes one of the most attractive ways to develop new generations of effective and selective inhibitors.[9] The role of the sugar is to facilitate selective or preferential inhibition of transmembrane CAs over cytosolic CAs. The stereochemical diversity across the carbohydrate tails also provides the opportunity for interrogation of subtle differences in

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active site topology of CA isozymes. However, the use of carbohydrates as drugs has an important drawback: they are sensitive to the presence of glycoside hydrolases and acidic or basic media.[10] Thus, design of mimetics that circumvent metabolic in vivo problems, is an active area of research.[11] An unusual enzyme-resistant replacement for the glycosidic linkage is the *sulfonamide* corresponding to the union of a glycosylamine carrying a sulfonyl group at nitrogen. However, glycosylamines are not stable and are very sensitive to hydrolysis and anomerization.[12] To overcome this problem one of our groups have developed several sulfonamidoglycosylations of sugar derivatives.[13]

Very recently, we reported the synthesis of *N*-β-glycosyl sulfamides, a new class of glycosides which possess the unnatural sulfamide functionality at the pyranose anomeric center.[14] Therein we described the synthesis of sulfamideglycosides from per-*O*-acetylated pyranoses in only one step. Previous studies demonstrated that the sulfamide moiety is a suitable zinc binding function (ZBF) for CA inhibitors and that such derivatives could be useful in the design of potent/selective compounds of this type.[15] As our compounds possess a stereochemically rich carbohydrate scaffold attached to the ZBF, we were interested in investigating these novel derivatives for CA inhibition. Herein we present the inhibition profile of a series of pyranosyl sulfamide against the cancer associated (hCA IX and XII) and physiologically dominant (hCA I and II) carbonic anhydrase isozymes (offtargets).

A set of *N*-β-glycosyl sulfamides (Fig. 1) was prepared as outlined in Scheme 1 and described by us previously.[14] Per-*O*-acetylated pyranose derived from the monosaccharides D-glucose, D-galactose, D-mannose and D-rhamnose, were reacted with boron trifluoride diethyl etherate and sulfamide to provide the corresponding sulfamideglycosides **1–4** in good yields and with complete β-stereoselectivity.[14] The *O*-acetate protecting groups of the carbohydrate moiety were next removed using Zemplen's condition to afford the fully deprotected sulfamide glycosides **5–6** in nearly quantitative yield.

Enzyme inhibition data was determined for physiologically dominant hCA I and II and cancer-associated hCA IX and XII by assaying the CA catalyzed hydration of CO_2. Inhibition and isozyme selectivity ratio data for the *N*-β-glycosyl sulfamides **1–8** as well as for carbohydrate sulfamate drug topiramate and their sulfamide analogue (**STA**) are presented in Table 1.[16] The structures for topiramate and **STA** are in Figure 2.

Topiramate (**TPM**), an anti-epileptic fructopyranose sulfamate, has been shown to be a good inhibitor of CAs.[17] The sulfamide analogue of topiramate, **11**, is a 210 times less potent inhibitor of isozyme II compared to **TPM**. Its weak binding to CA II is due to a clash between one methyl group of the inhibitor and Ala65, an amino acid unique to the hCA II active site.[18] These compounds present ZBFs tethered to the anomeric pyranose center and therefore could be useful to include them for comparison with the novel *N*-glycosyl sulfamide reported in this study.

Per-*O*-acetylated *N*-β-glycosyl sulfamides **1–4** were micromolar inhibitors of hCA I with K_is in the same range of **STA** but being less effective inhibitors compared to topiramate. Deprotected carbohydrate derivatives **5–8** showed a diminished affinity for isozyme I. The new acetylated glycosyl sulfamides behaved as quite effective inhibitors of the physiologically relevant and dominant isoform hCA II with K_is in the low nanomolar range (of 76–85 nM).Our compounds showed better activity against hCA II as compared with the sulfamide glycoside **STA**. Deprotected compounds were very weak inhibitors of hCA II, with inhibition constants in the micromolar range (Table 1).

The acetylated *N*-β-glycosyl sulfamides were very good hCA IX and exhibited a narrow range of K_is, from 5.0 to 7.7 nM. This inhibition is 10 times stronger than for hCA II. On the other hand the deacetylated glycosides were weaker inhibitors of hCA IX, but anyhow inhibited selectively this isoform over the ubiquitous hCA II (except for compound **5**). The protected glycosyl sulfamides were very good inhibitors of hCA XII in the low nanomolar range (5.4–6.5 nM), while the deprotected ones were weaker inhibitors (K_is of 19.70–99 μM).

Recently we have reported on the synthesis and CA inhibition of peracetylated 2-deoxy-D-glucose and D-galactose sulfamides (**9** and **10**).[13g] These inhibitors differ by the nature of the substituent at C-2 and are useful for comparison with the glycosyl sulfamides of the present study. The glycosyl sulfamides exhibit similar inhibition profiles against the various isoforms. They are typically weak hCA I inhibitors (micromolar K_is) and good hCA II, hCA IX and hCA XII inhibitors (low nanomolar K_is). We do however observe a variation in the selectivity profile, as the 2-deoxy compounds do not show selectivity for inhibiting the tumor-associated isoforms over cytosolic hCA II.

Some important features of the above SAR should be remarked:

(i) Acetylated glycosyl sulfamides **1–4** showed micromolar affinity for the inhibition of CA I, but nanomolar binding to CAs II, IX and XII. They are selective inhibitors of CA IX and CA XII in the range of 10- to 17-fold.

(ii) Protected 2-deoxy glycosyl sulfamides **9–10** were potent but non-selective inhibitors of all CA isozymes investigated here.

(iii) Deprotected glycosides **5–8** were weaker inhibitors of the CA isoforms investigated in this study, in the micromolar range, and showed selectivity for CA IX and CA XII.

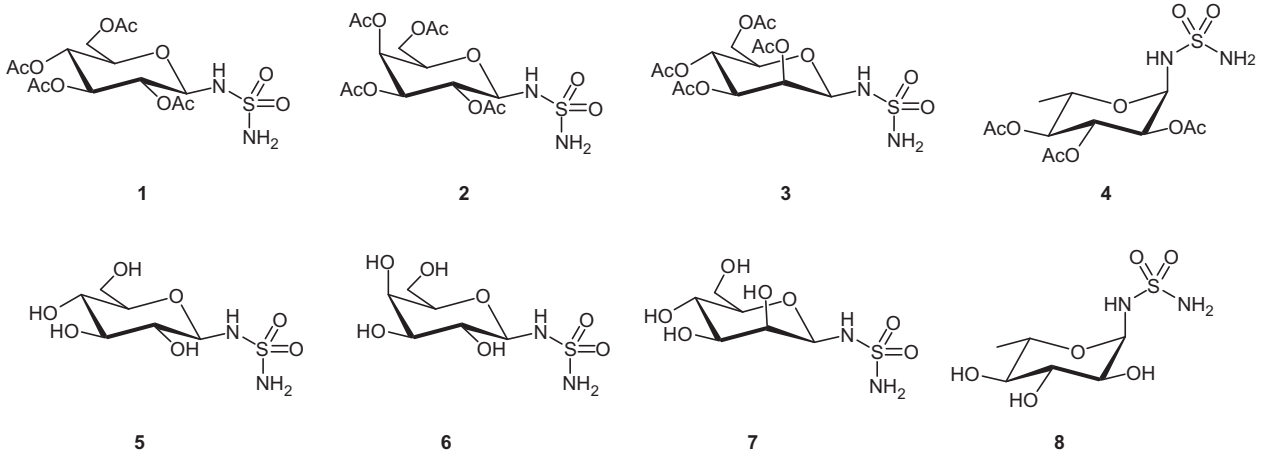
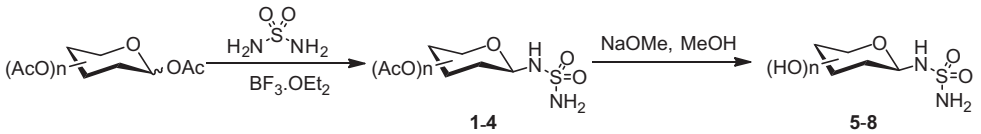


Figure 1. Per-*O*-acetyl glycosyl sulfamides (**1–4**) and fully protected derivatives (**5–8**).



Scheme 1. Preparation of glycosyl sulfamides **1**–**8**.

Table 1
Inhibition of cytosolic isozymes hCA I and II, and transmembrane isozymes hCA IX and hCA XII with topiramate, sulfamide **STA** and the *N*-β-glycosyl sulfamides **1**–**8**

Compound	K_i (nM)*				Selectivity ratios	
	hCA I	hCA II	hCA IX	hCA XII	hCA I/hCA IX	hCA II/hCA IX
Topiramate	250	5	58	3.8	4.3	0.09
STA	3,450	2,135	4,580	1,875	0.75	0.5
1	3,714	81	7.7	5.4	482.3	10.5
2	8,637	76	7.2	6.5	1198.6	10.5
3	9,703	85	5.0	5.6	1940.6	17
4	9,530	82	5.3	5.8	1798.1	15
5	75,400	4680	6,470	1,970	11.6	0.7
6	65,800	48,500	940	8,230	70	51.6
7	91,900	21,200	1,790	5,440	51.3	11.8
8	$>0.5 \times 10^5$	22,300	3,140	9,900	>15.9	7.1
9	830	12	13	4.8	63.8	0.9
10	830	11	16	7.8	51.9	0.7

* Errors in the range of 5–10% of the reported value, from three different determinations.

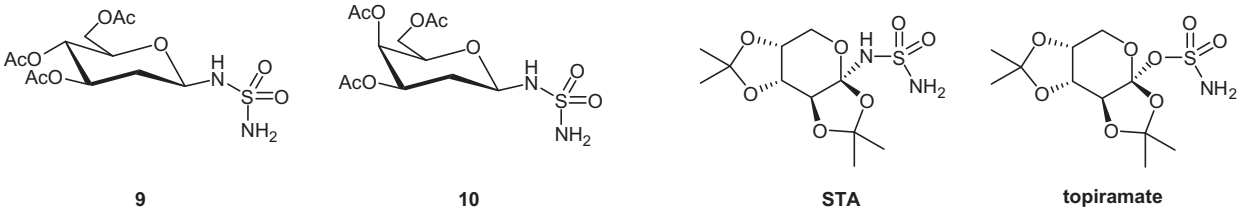


Figure 2. Compounds **9**, **10** topiramate (**TPM**) and its sulfamide analogue (**STA**).

In the development of anti-cancer compounds that target selectively the membrane bound isoform CA IX and CA XII versus the ubiquitous isoform CA II, the design of membrane non-permeant inhibitors is crucial.

In recent years, several parameters have been introduced for membrane permeability prediction.[19] Topological polar surface area (TPSA) is now been recognized as a good indicator of drug absorbance in the intestines, Caco-2 monolayers penetration, and blood–brain barrier crossing.[20] Molecules with a TPSA greater than 140 A^2 are likely to have a low capacity for penetrating cell membranes. As can be seen in Table 2 all our compounds, except **8** are above the threshold for good passive membrane diffusion. Log *P* represents intrinsic lipophilicity, and compounds with log *P* <0 have good solubility but poor lipid bilayer permeability. Calculated log *P* values for the *N*-β-glycosides sulfamides show that all compounds fall within the range indicative of molecules with poor membrane permeability. Values of acetylated glycosides **1–4** are consistent with the incorporated acetyl groups, decreasing the polarity of the resulting carbohydrate moiety. It is expected that glycosides **1–7** would have poor passive membrane permeability and thus lead to preferential inhibition of CAs IX and XII over ubiquitous cytosolic hCA II.

Table 2
Calculated physicochemical properties of glycosides **1**–**8**

Compound	Molecular weight	TPSA[a] (A^2)	*c* Log *P*[a]
1	426.4	186	−0.2
2	426.4	186	−0.2
3	426.4	186	−0.5
4	368.4	160	−0.7
5	258.2	162	−2.7
6	258.2	162	−2.7
7	258.2	162	−2.7
8	242.2	142	−2.1

[a] Calculated using ChemBioDraw Ultra 12.0.

In conclusion, we report here a series of peracetylated and deprotected glycosyl sulfamides. These derivatives were tested for the inhibition of four CA isoforms: cytosolic hCA I and hCA II and transmembrane tumor-associated IX and XII. Compounds **1–4** and **6–8** selectively target cancer-associated CAs (IX and XII). Also the physicochemical properties of the glycosides tested would enhance the preferential inhibition in vivo. Free glycosyl sulfamides could be useful for chemotherapy if they are delivered through a route of intravenous administration. For oral delivery peracetylated *N*-β-glycosyl sulfamides may be used as ester prodrugs. Once in the body, the ester groups could be readily hydrolyzed by ubiquitous esterases.[21]

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