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Synthesis and carbonic anhydrase inhibitory effects of new *N*-glycosylsulfonamides incorporating the phenol moiety

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ABSTRACT

A small series of *N*-glycosylsulfonamides incorporating the phenol moiety has been prepared by Ferrier sulfonamidoglycosylation of D-glycals. *N*-Glycosides were tested for the inhibition of four isoforms of carbonic anhydrase. In this study, all compounds showed good inhibitory activity against hCA I and II, with selectivity against the cytosolic hCA II versus the tumor associated isozymes. These results confirm that attaching carbohydrate moieties to CA phenol pharmacophore improves and enhances its inhibitory activity.

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Carbonic anhydrases (CAs, EC 4.2.1.1) are the most studied members of a great family of metalloenzymes. CAs catalyze the reversible hydration of carbon dioxide and they are found in multiple organisms such as vertebrates, bacteria, algae.[1] Five genetically distinct CA families are known to date, the α-, β-, γ-, δ- and ζ-CAs. α-CAs are involved in several physiological processes and have been exploited for the treatment or prevention of various pathologies such as glaucoma, neurological disorders, osteoporosis, obesity and cancer.[2–7] During the last years, the interest in the therapeutic use of carbonic anhydrase inhibitors (CAIs), has improved remarkably due to the validation of several CA isozymes as drug targets.

Phenols inhibit CAs by anchoring to the Zn(II)-bound solvent molecule, that is, a water or hydroxide ion, as initially reported by Christianson's group.[8] Although phenol-based natural and synthetic compounds are largely known to exhibit biological activity (mainly as antioxidants), they have been only recently studied as carbonic anhydrase inhibitors.[9,10]

The use of glycomimetics in the design of CAIs 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.[11,12] 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 bind to enzymes but are not processed to product in the usual way is an active area of research.[13] Usual enzyme-resistant replacement for the glycosidic linkage are the thio, methylene or sulfonamidoglycosides. Recently our group has applied the 'sugar approach' to the preparation of *C*-cinnamoyl phenols, where the carbohydrate moiety is tethered to a phenol CA pharmacophore through a carbon chain.[14] These compounds have been tested as inhibitors of the *Mycobacterium tuberculosis* β-CAs and have shown better inhibitory activity against mtCAs than phenol. Also the antitubercular activity of the *C*-glycosyl phenols was investigated, allowing us to identify the first mtCAs inhibitor with antimycobacterial activity.[15] These glycosides also showed to be very good inhibitors of *Brucella suis* CAs.[16]

In 2007 our group described the Ferrier sulfonamidoglycosylation of peracetylated D-glycals in the presence boron trifluoride etherate.[17] The D-hex-2-enopyranosylsulfonamides were obtained in high yield with very good α-stereoselectivity. Then this methodology was applied to the synthesis of *N*-glycosyl sulfamides, which showed to be potent carbonic anhydrase inhibitors.[18] Recently Winum's group reported on the synthesis and biological activities of carbonic anhydrase glycoinhibitors developed by Ferrier sulfonamidoglycosylation of peracetylated glycals by reaction with hydroxysulfamide[19] or aminoxysulfamide[20] using nitrosyl tetrafluoroborate as catalyst.

Thus, in the search of non-sulfonamide CAIs belonging to different classes of compounds, we report here the synthesis of a series of new *N*-glycosylsulfonamides incorporating phenol moiety

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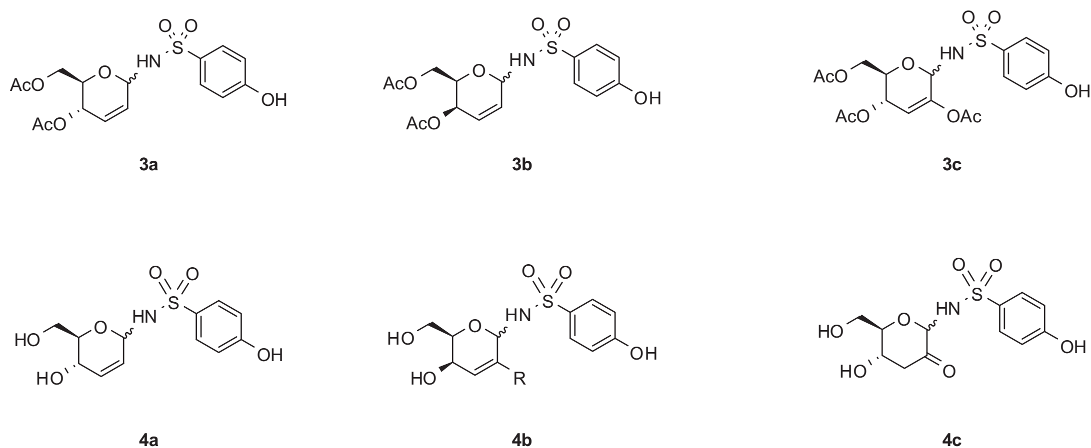
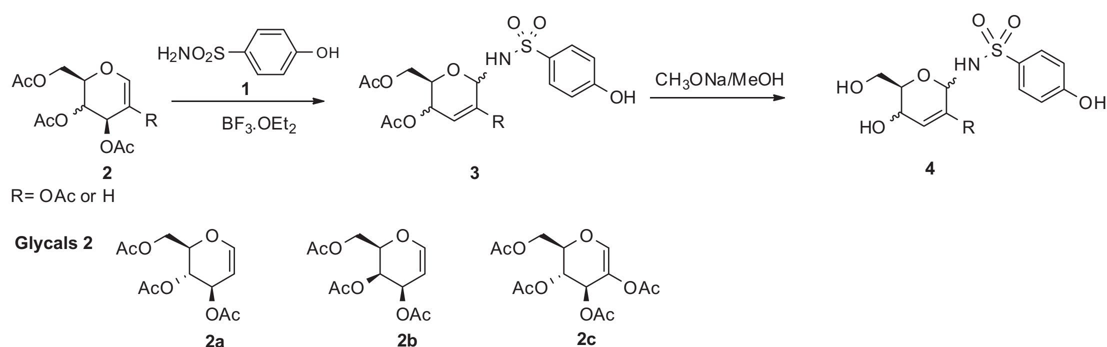


Figure 1. Peracetylated *N*-glycosylsulfonamides (**3a**–**3c**) and fully deprotected derivatives (**4a**–**4c**).



Scheme 1.

(Fig. 1) vía Ferrier sulfonamidoglycosylation of D-glycals and their inhibitory activity against the cytosolic hCA I and II, and tumor-associated hCA IX and XII.

N-Glycosylsulfonamides **3** were synthesized as depicted in Scheme 1. Starting from peracetylated glycals **2**, and using the same methodology previously reported by us on Ferrier sulfonamidoglycosylation, we were able to obtain compounds **3** by reacting **2** with *p*-hydroxybenzenesulfonamide (**1**) in the presence of 1% mol of boron trifluoride etherate in dichloromethane (Scheme 1).[21]

Sulfonamidoglycosides **3** were obtained as a mixture of α and β anomers with a slight selectivity for the α-anomer (see Table 1). Similar selectivities were reported in other sulfonamidoglycosylations. Our group showed that the α-selectivity could be explained in terms of the *endo* and *exo*-anomeric effects, suggesting a thermodynamical control of the reaction.[22]

The α and β anomers of the *threo*-hex-2-enopyranosyl sulfonamide **3b** could be easily separated by flash column chromatography. Although compounds **3a** and **3c** were obtained analytically pure by chromatography, the anomers could not be separated. The anomeric configuration was supported by NOESY experiments (in $CDCl_3$), for example, the configuration of α-anomer of **3a** is consistent with NOEs between NH and H-5, and between H-4 and H-5.

Final deprotection of acetate groups of glycosides **3** with methanolic solution of sodium methoxide led to compounds **4** in very good yields.[23]

Compounds **1**, **3a**–**3c** and **4a**–**4c** as well as clinically used acetazolamide (standard compound) were tested for their inhibitory activity against the two cytosolic CA isoforms hCA I and II and the two membrane tumor-associated isoforms hCA IX and XII using a Stopped-Flow, CO_2 Hydration Assay Method.[24] Results are reported in Table 2.

A number of structure–activity relationships (SARs) were identified in this study and are summarized as follows:

(i) hCA I: *erythro* sulfonamides **3a** and **4a** are nanomolar inhibitors of the hCA I; the other *N*-glycosylsulfonamides are very poor hCA I inhibitors. It should be noted that the β-anomers

Table 1
Synthesis of *N*-glycosylsulfonamides

Glycal	Sulfonamidoglycosylation			Deprotection		
	Reaction time (h)	α:β	Yield (%)	Reaction time (h)	α:β	Yield (%)
2a	1.5	61:39	71	1.5	61:39	80[a]
2b	2	65:45	70	2	–[b]	83α + 85β
2c	5	78:22	72	2	78:22	82[a]

[a] Deprotection of the mixture of anomers.
[b] Anomers of compound **3b** could be separated and thus the pure anomers were deprotected.

Table 2
Inhibition of four CA isoforms: hCA I, II, IX and XII with the *N*-glycosylsulfonamides **3a**–**3c** and **4a**–**4c**, determined by a stopped flow, CO_2 hydration assay method

Compound	K_I[a] (nM)			
	hCA I	hCA II	hCA IX	hCA XII
Phenol[b]	10,200	5500	8800	9200
1	5500	80	235	176
3a	43.1	139	>50,000	>50,000
3bα	6630	695	>50,000	>50,000
3bβ	3840	137	>50,000	>50,000
3c	6690	360	>50,000	>50,000
4a	454	142	>50,000	>50,000
4bα	8850	340	>50,000	>50,000
4bβ	4025	295	>50,000	>50,000
4c	6250	1680	>50,000	>50,000
AAZ	250	12.1	25.3	5.7

[a] Errors in the range of ±5–10% of the reported values, from 3 different assays (data not shown).
[b] From Ref. 25.

of the *threo* sulfonamides **3b** and **4b** are better inhibitors than the α-anomers. It is of great interest to compare the behavior of compounds toward hCA I with the inhibition profile showed by phenol or compound **1**, and it can be concluded that combination of an *erythro*-hex-2-enopyranosyl scaffold and the phenol moiety leads to a steep increase in the inhibitory potency of these compounds against hCA I.

(ii) hCA II: the *N*-glycosides showed to be very good inhibitors in the nanomolar range with the exception of deprotected sulfonamidoglycoside **4c**. It is interesting to compare the inhibitory activity of phenol and the *N*-glycosides. As can be seen in our present Letter, attachment of glycosyl moieties to the phenol scaffold lead to an improvement in the activity.

(iii) Tumor-associated Isozymes hCA IX and XII were not inhibited by *N*-glycosylsufonamides **3a**–**3c** and **4a**–**4c**.

In conclusion, a novel series of sulfonamidoglycosides **3a**–**3c** and **4a**–**4c** containing the phenol scaffold has been synthesized via sulfonamidoglycosylation of D-glycals and investigated as inhibitors against four isozymes of carbonic anhydrases comprising cytosolic, ubiquitous isozymes hCA I and II as well as the transmembrane, tumor-associated isoforms hCA IX and XII. In this study, peracetylated and deprotected *erythro*-hex-2-enopyranosyl sulfonamides **3a** and **4a**, respectively, showed to be nanomolar inhibitors of hCA I. Very good inhibitory activity against hCA II was shown by almost all the *N*-glycosides prepared, showing that attaching carbohydrate moieties to CA phenol pharmacophore improves and enhances its inhibitory activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.07.015.

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21. Experimental procedure for the synthesis of sulfonamidoglycosides **3**: To a solution or suspension of the glycal **2** (1 mmol) and the sulfonamide **1** (1.1 mmol) in 5 mL of dry CH_2Cl_2 was added the catalyst at room temperature. After stirring for the time indicated, the reaction mixture was quenched with aqueous solution of $NaHCO_3$ and the residue dissolved in CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4, filtered and evaporated. The product was purified by column chromatography to give the compounds **3a**–**3c**.
Representative physical and NMR data for **3a**: Colorless syrup. α-Anomer: ^1H NMR (500 MHz, $CDCl_3$) δ 7.79(d, 2H, J = 8.91, ArH), 7.38 (brs, OH), 6.93 (d, 2H, J = 8.83, ArH), 5.97 (d, 1H, J = 8.9 Hz, NH), 5.91 (m, 1H, H-3), 5.83 (dd, 1H, J = 3.0, 2.0 Hz, H-2), 5.59 (ddd, 1H, J = 10.0, 5.6, 4.4 Hz, H-1), 5.29 (dd, 1H, J = 9.2, 1.8 Hz, H-4), 3.89 (dd, 1H, J = 12.1, 3.2 Hz, H-6b), 3.55 (dt, 1H, J = 9.2, 3.0 Hz, H-5), 3.51 (dd, 1H, J = 12.1, 2.8 Hz, H-6a), 2.04 (s, 6H, CH_3COO). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.58 (CH_3COO), 170.26 (CH_3COO), 160.34 (ArC), 132.58 (ArC), 130.40 (ArC), 130.40 (ArC), 129.77 (C-2), 129.51 (C-3), 126.76 (ArC), 126.76 (ArC), 79.61 (C-1), 66.59 (C-5), 64.40 (C-4), 62.22 (C-6), 20.87 (CH_3COO), 20.87 (CH_3COO). β-Anomer: ^1H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, 2H, J = 8.91, ArH), 7.27 (s, 1H, OHβ), 6.87 (d, 2H, J = 8.83, ArH), 5.97 (d, 1H, J = 8.9 Hz, NH), 5.93(m, 1H, H-3), 5.81 (dd, 1H, J = 5.2, 2.5 Hz, H-2), 5.56 (br s, 1H, H-1), 5.19 (m, 1H, H-4), 4.13 (q, 1H, J = 7.2, H-6b), 4.00 (dd, 1H, J = 12.0, 2.6 Hz, H-6a), 3.77 (ddd, 1H, J = 8.4, 5.4, 2.7 Hz, H-5), 2.04 (s, 6H, CH_3COO). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.58 (CH_3COO), 170.26(CH_3COO), 160.25(ArC), 132.69(ArC), 130.07 (C-3), 129.77(ArC), 129.77(ArC), 126.76(C-2), 115.41(ArC), 115.41(ArC) 77.11 (C-1), 73.44(C-5), 66.59(C-5), 64.40(C-4), 62.22 (C-6), 20.87 (CH_3COO), 20.87 (CH_3COO). HRMS m/z: calcd for $C_{16}H_{19}NO_8S$, 385.0831; found, 385.0836. Representative physical and NMR data for compounds **3b**–**3c**: see Supporting information.
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23. Deprotected compounds **4a**–**4c** were prepared by dissolving the corresponding per-*O*-acetylated glycosylsulfonamides **3** in anhydrous methanol with metanolic sodium metoxide (0.05 M final concentration). The mixture was warmed to room temperature and left to stir until full deprotection was evident by TLC. The solution was neutralized with Amberlite IR-120 [H+], filtered and the resin was washed with methanol. The solvent was evaporated under reduced pressure and the product was purified by column chromatography. Representative physical and NMR data for **4a**: Colorless syrup. α-Anomer: ^1H NMR (500 MHz, DMSO-d_6) δ 8.36 (d, 1H, J = 9.2 Hz, NH), 7.68 (d, 2H, J = 8.7 Hz, ArH), 7.11 (s, 1H, OH), 6.89 (d, 2H, J = 4.4 Hz, ArH), 5.31 (dd, 1H, J = 6.4, 2.8 Hz, H-1), 4.63 (d, 1H, J = 6.2 Hz, H-2), 4.49 (t, 1H, J = 4.5 Hz, H-3), 4.22 (t, 1H, J = 4.9 Hz, H-4), 3.98 (m, 1H, H-5), 3.63 (m, 2H, H-6a, H-6b), 3.51 (s, 1H, OH), 3.24 (s, 1H, OH). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.19(ArC), 134.96 (C-3), 132.78(C-2), 129.24(2×ArC), 128.26(ArC), 115.71(ArC), 115.62 (ArC), 86.30 (C-1), 80.95(C-4), 71.93(C-5), 71.46(C-6). β-Anomer: ^1H NMR (500 MHz, DMSO-d_6) δ 8.36 (d, 1H, J = 9.2 Hz, NH), 7.64 (d, 2H, J = 8.6 Hz, ArH), 7.11 (s, 1H, OH), 6.87 (d, 2H, J = 4.5 Hz, ArH), 5.16 (dd, 1H, J = 5.3, 2.2 Hz, H-1), 4.66 (d, 1H, J = 4.5 Hz, H-2), 4.49 (t, 1H, J = 4.5 Hz, H-3), 4.31 (t, 1H, J = 4.8 Hz, H-4), 4.03 (m, 1H, H-5), 3.68 (m, 2H, H-6a, H-6b), 3.51 (s, 1H, OH), 3.24 (s, 1H, OH). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.19(ArC), 134.96 (C-3), 132.78(C-2), 129.24 (2×ArC), 128.26(ArC), 115.71(ArC), 115.62(ArC), 81.93(C-1), 80.95 (C-4), 71.93(C-5), 71.46(C-6). HRMS m/z: calcd for $C_{12}H_{15}NO_6S$, 301.0620; found, 301.0625. Representative physical and NMR data for compounds **4b**–**4c**: see Supporting information.

24. An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity as reported by Khalifah (Khalifah, R.G. *J. Biol. Chem*. **1971**, *246*, 2561). Phenol red (at a concentration of 0.02 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3, and the Cheng–Prussoff equation (Cheng, Y.; Prussoff, W.H. *Biochem. Pharmacol*. **1973**, *22*, 3099) as reported earlier and represent the mean from at least three different determinations.
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