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Improving the carbonic anhydrase inhibition profile of the sulfamoylphenyl pharmacophore by attachment of carbohydrate moieties

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ABSTRACT

One of the most successful approaches for designing carbonic anhydrase (CA, EC 4.2.1.1) inhibitors was denominated 'the sugar approach'. The sugar approach consists in attaching different carbohydrates to CA inhibiting pharmacophores for modulating the physicochemical properties of these pharmacological agents. In line with this approach, in this paper, we present a new class of *C*-glycosides incorporating the sulfamoylphenyl moiety. These compounds have been prepared by sulfamoylation of C-glycosyl phenols, which have been synthetized by aldol reaction of glycosyl ketones with the appropriate aromatic aldehydes. The inhibition profile of the new glycomimetics was determined against four human (h) CA isozymes, comprising hCAs I and II (cytosolic, ubiquitous isozymes), hCA IV and hCA IX (tumor associated isozyme). Peracetylated and deprotected *C*-glycosyl sulfamates showed better inhibition selectivity compared to structurally related phenylsulfamates. In this study, deprotected compound **12** was identified as selective inhibitor of hCA IX. These results confirm that attaching carbohydrate moieties to CA sulfamoylphenyl pharmacophore improves its inhibitory activity.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are among the most studied members of a great family of metalloenzymes [1,2]. CAs catalyze the reversible hydration of carbon dioxide and they are found in multiple organism such as vertebrates and bacteria [1].

Seven genetically distinct CA families are known to date, the α-, β-, γ-, δ-, ζ-, η- and θ-CAs. Mammals posses only α-CAs, while many pathogenic organisms such as bacteria and fungi encoded α-, β- and/or γ-CAs. These enzymes contain a zinc ion (Zn^{2+}) in their active site, which is coordinated by three histidine residues and a water molecule/hydroxide ion (in the α-, γ- and δ-classes) or by two cysteine and one histidine residues (in the β- and ζ-CA classes), by two His and a Gln for the η-CAs, with the fourth ligand being a water molecule/hydroxide ion. Out of the 16 different CA isoforms discovered so far in the α-class, human CA isoforms hCA I and II are cytosolic enzymes that are widespread throughout the human body. Furthermore, the dimeric transmembrane glycoprotein hCA IX is also a human, tumor-associated CA isoform having extracellular active site and is a marker for a broad spectrum of hypoxic tumor types, and a recently validated antitumor target [2,3]. The overexpression of this isoform contributes to the increased acidification of extracellular hypoxic environment (pH = 6.8) in contrast to normal tissues (pH = 7.4) thus promoting tumor cell survival in an acidic condition by decreasing uptake of weakly basic anticancer drugs [4]. Thus specifically targeting the tumor associated isoform hCA IX over the main off target isoforms hCA I and II, which have a physiological relevance, using specific inhibitors is considered to be a promising strategy in the cancer therapy, with a sulfonamide CA IX inhibitor, SLC-0111 in Phase Ib/II clinical trials [5,6].

During the last years, the interest in the therapeutic use of CA inhibitors (CAIs), has improved remarkably due to the validation of several CA isozymes as drug targets [7]. The sulfamate group, a closely related bioisosteric variant of sulfonamide, has demonstrated very attractive possibilities in medicinal chemistry, especially in the carbonic anhydrase field [8]. As proved by X-ray structural data of adducts of such inhibitors with the physiologically most important isozyme (hCA II), sulfamates interact with the zinc ion in deprotonated form. The X-ray crystal structure of

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the adduct of sulfamic acid with hCA II was reported by one of our groups, showing that sulfamic acid is able to bind to the zinc ion as dianion also making a number of favorable contacts in the binding pocket of hCA II [9]. Thus, this compound may be used as a lead molecule for the design of tighter binding CAIs.

Topiramate (2,3:4,5-bis-*O*-(1-methylethylidene)-6-D-fructopyranosyl sulfamate) is a billion dollar drug that is marketed worldwide for the treatment of epilepsy and migraine [10]. Topiramate was shown to behave as a very potent CAI, effectively inhibiting several CA isoforms, especially hCA II, with an inhibitory constant of 5 nM against hCA II [11]. Crystallographic studies revealed that topiramate binds with its sulfamate moiety to the zinc ion, whereas the fructose moiety is entrapped into CA active site, making a large number of favorable interactions (H bonds and hydrophobic ones) with many amino acid ressidues and water molecule from the CA active site [12].

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 [13,14]. Many modifications have been introduced in the structure of carbohydrates to generate glycomimetics with improved drug-like characteristics and stability to enzymatic degradation. Thus, design of mimetics that bind to enzymes but are not processed to products in the usual way is an active area of research [15]. Usual enzyme-resistant replacement for the glycosidic linkage are the thio, methylene or sulfonamidoglycosides. Recently our group has prepared several *C*-cinnamoyl phenols, where the carbohydrate moiety was tethered to a phenol CA pharmacophore through a carbon chain [16]. These compounds have been tested as inhibitors of the *Mycobacterium tuberculosis* β-CAs (mtCAs) and have shown better inhibitory activity against these enzymes compared to the simple phenol. In addition, the antitubercular activity of the *C*-glycosyl phenols was investigated, allowing us to identify the first mtCAs inhibitor with antimycobacterial activity *in vivo* [17]. These glycosides also showed to be effective inhibitors of *Brucella suis* CAs [18]. Then we developed a novel series of *C*-glycosides containing the methoxyaryl scaffold and investigated them as inhibitors against hCA isozymes, allowing us to identify four potent and highly selective inhibitors of hCA IX and XII [19]. We have also investigated the enzyme inhibition profile of these *C*-glycosides against the fungal β-CAs from *Cryptococcus neoformans* [20]. Many compounds showed activities in the micromolar or submicromolar range and excellent selectivity for pathogen CAs over human isozymes.

Thus, in the search of novel glycosidic CAIs belonging to different classes of compounds, we report here the synthesis of a new series of *C*-glycosides incorporating the sulfamoylphenyl moiety (Fig. 1), and their inhibitory activity against hCA I, II, IV and tumor-associated hCA IX enzymes.

2. Results and discussion

2.1. Chemistry

A set of new *C*-cinnamoyl glycosides incorporating the sulfamoylphenyl moiety (Fig. 1) was synthesized as outlined in Scheme 1. β-D-Glycosyl-propan-2-ones were prepared by Knoevenagel condensation with 2,4-pentanedione in the presence of sodium carbonate or sodium bicarbonate using water and THF as solvent [21]. Crude mixtures containing the *C*-glycosyl ketones were acetylated and then purified to afford the peracetylated compounds in very good yields. *C*-glycosides have been prepared by aldol condensation of β-*C*-glucosyl and β-*C*-galactosyl ketones with different aldehydes incorporating the phenol moiety at room temperature in the presence of pyrrolidine as catalyst [22]. The crude mixtures could be easily purified by flash column chromatography and/or crystallization to afford the pure compounds **1**–**4** in good yields.

We attempted to synthesize glycosyl sulfamates by sulfamoylation with sulfamoyl chloride generated *in situ* from formic acid and chlorosulfonylisocyanate, but unfortunately, no reaction was observed to occur. Thus we chose to prepare sulfamoyl chloride from chlorosulfonyl isocyanate and formic acid as described by Appel [23]. In this way, the glycosyl sulfamates **5**–**8** were prepared by the procedure described by Winum [24], employing the reaction of corresponding *C*-glycosyl phenols **1**–**4** with sulfamoyl chloride in *N*,*N*-dimethylacetamide as solvent, to afford the new compounds in good yields (57–87%)

The ^{1}H NMR, ^{13}C NMR, 2D COSY and HSQC were in full agreement with their structures (see Supplementary Information). The *trans* double bond in the glycosides was established by the large coupling constant (J = 16.1 Hz) between the two olefinic protons. The β-configuration of the *C*-glycosyl sulfamates was established by the large coupling constant (J = 9.7–9.9 Hz) observed between H-1′ and H-2′.

Finally, the deprotection of the acetate groups of glycosides **5**–**8** with methanolic solution of sodium methoxide led to fully deprotected *C*-glycosides **9**–**12** in good yields (65–72%).

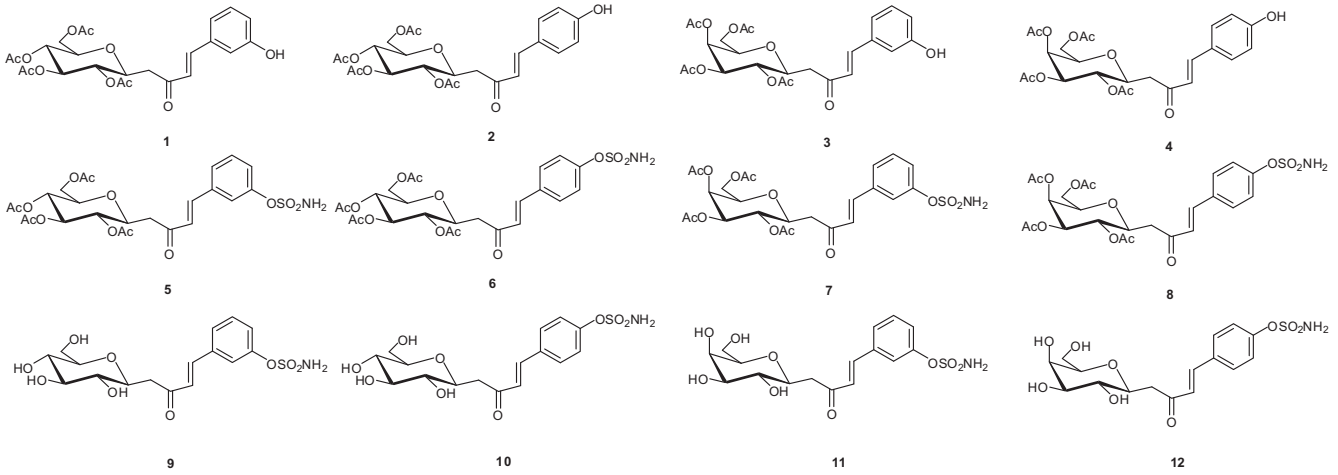
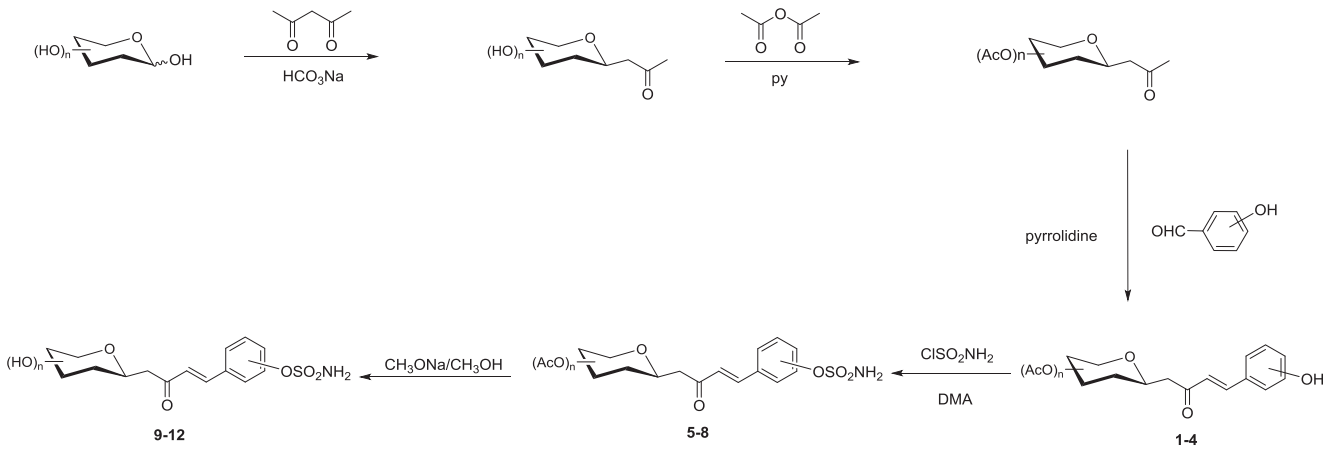


Fig. 1. Per-*O*-acetylated *C*-glycosides (**1**–**8**) and fully deprotected derivatives (**9**–**12**).



Scheme 1. Preparation of *C*-glycosides **1**–**12**.

The inhibitory activities of *C*-glycosides **1**–**12** against cytosolic isoforms hCA I and II as well as the membrane associated isoforms hCA IV and IX was assayed by using stopped flow assay method [25] and the results are presented in Table 1.

A number of structure–activity relationship (SAR) features were identified in this study and are summarized below:

(i) hCA I: Peracetylated *C*-glycosyl phenols **1**–**4** were micromolar inhibitors of the hCA I. The related glycosyl sulfamates **5**–**8** showed to be good hCA I inhibitors in the namolar range. A similar trend was found for the deprotected sugar analogues **9**–**12,** the exception being the *C*-glucosyl compound **10** which was a better hCA I inhibitor. It is of great interest to relate the behaviour of these compounds and phenylsufamate toward hCA I and it can be concluded that the attachment of a glycosyl scaffold to the sulfamate moiety leads to a steep decrease in the inhibitory potency of C-glycosyl sulfamates against hCA I.

(ii) hCA II: The *C*-glycosides showed a very interesting inhibition profile against hCA II. It should be noted that all glycosides incorporating the phenol moiety **1**–**4** were micromolar inhibitors of the hCA II. Peracetylated *C*-glycosyl sulfamates **5**–**8** were good hCA II inhibitors, in the nanomolar range. The isoform hCA II was inhibited in the micromolar range by all the deprotected *C*-glycosyl sulfamates except the *C*-glucosyl derivative **10** which was a nanomolar inhibitor of this isozyme (Table 1).

(iii) hCA IV: Peracetylated *C*-cinnamoyl glycosides incorporating the phenol moiety **1**–**4** were poor inhibitors of hCA IV. A similar trend was found for the deprotected glycosyl sulfamates **9**–**12**. In addition, a very well known sugar sulfamate, topiramate was observed to be a poor inhibitor of this isoform earlier.[11] On the other hand peracetylated *C*-glycosyl sulfamates **9** and **8** incorporating the 4-sulfamoylphenyl moiety are nanomolar inhibitors of hCA IV.

(iv) iii) The tumor associated target isoform hCA IX was inhibited in the micromolar range by all the peracetylated *C*-glycosyl phenols **1**–**4**. The related sulfamates **5**–**8** showed a nanomolar inhibitory activity against hCA IX. The deprotected derivatives incorporating the 4-sufamoylphenyl moiety (**10** and **12**) were micromolar inhibitors of this isozyme.

(v) Selectivity for inhibiting the tumor-associated isoform hCA IX over the widespread cytosolic forms (hCA I and II) is a key issue when designing CAIs. As can be seen in Table 1 only compound **12** showed better activity profile against hCA IX over hCA I and II which is highly desirable when only the tumor-associated isoforms should be targeted. It is interesting to note that all *C*-glycoside sulfamates showed better

Table 1
Inhibition of mammalian α-CA with the *C*-glycosides **1**–**12**.[a]

C-glycoside	K_i (nM)[b]				*Selectivity ratios*	
	hCA I	hCA II	hCA IV	hCA IX	I/IX	II/IX
1[c]	8500	7000	5600	5200	1.63	1.35
2[c]	5700	3900	4900	5900	0.97	0.66
3[c]	5100	7100	7800	3300	1.55	2.15
4[c]	9300	5500	6700	2900	3.24	1.89
5	90.6	5.1	385.8	21.2	4.27	0.24
6	73.3	3.4	65.9	29.6	2.47	0.11
7	57.5	3.4	235.0	15.7	3.66	0.21
8	57.5	4.3	61.1	24.3	2.37	0.18
9	338.6	842.3	>10000	952.9	0.35	0.88
10	76.0	27.6	3242.7	23.1	3.29	1.02
11	585.8	180.4	3285.1	198.9	2.95	0.91
12	340.7	570.9	1902.5	100.9	3.38	5.66
AAZ	250	12	74	25	10	0.48
Topiramate [28]	250	10	4900	3.8	65.79	2.63
Phenylsulfamate	2.1	1.3	nt	63	0.03	0.02

[a] All CAs are recombinant enzymes obtained in the authors' laboratory as reported earlier [5].
[b] Errors in the range of 5–10% of the reported value, from 3 different determinations.
[c] Ref. [14].

selectivity ratios than phenylsulfamate. These results confirm that attaching carbohydrate moieties to CA sulfamoylphenyl pharmacophore improves and enhances its inhibitory activity.

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. The calculated Log P (cLog P) parameter generally provides a good correlation with experimental permeability data, and molecules with cLogP values between 1 and 3 typically have good passive membrane permeability properties while those with cLogP values of <0 are more likely to have a low capacity for penetrating cell membranes [26]. Calculated log P values for the *C*-glycosides (0.653 and -1.285 for compounds **5**–**8** and **9**–**12**, respectively) [27] show that deprotected compounds fall within the range indicative of molecules with poor membrane permeability. Values of acetylated glycosides **5**–**8** are consistent with the incorporated acetyl groups, decreasing the polarity of the resulting carbohydrate moiety. The implications of these values may prove useful for the provision of compounds suited for oral administration. For example, compound **8** is predicted to be orally bioavailable, yet once absorbed, its physicochemical properties altered by esterase activity to give a more polar molecule (compound **12**) that as a consequence selectively targets the extracelular active site of cancer-associated CA IX.

3. Conclusion

A novel series of C-glycosyl sulfamates have been synthesized via sulfamoylation of C-cinnamoyl glycosides incorporating the phenol moiety and investigated as inhibitors of four isozymes of CA, comprising cytosolic, ubiquitous isozymes hCA I and II as well as the membrane-anchored/associated isoforms hCA IV and IX. In this study, the peracetylated and deprotected glycosyl sulfamates showed better inhibition selectivity than phenylsulfamate. Thus, attaching carbohydrate moieties to the sulfamoylphenyl CA pharmacophore improves its inhibitory activity. It is expected that the poor passive membrane permeability of the *C*-glycosides would enhance the preferential inhibition of CAs IX and XII over ubiquitous cytosolic hCA II.

4. Experimental section

4.1. General

All starting materials and reagents, were purchased from commercial suppliers. Reactions were monitored by TLC and TLC plates visualized with short wave UV fluorescence (λ = 254 nm), sulfuric acid stain (5% H_2SO_4 in methanol). Silica gel flash chromatography was performed using silica gel 60 Å (230–400 mesh). All melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 600 (600 and 151 MHz, respectively). Chemical shifts were measured in ppm and coupling constants in Hz. High resolution mass spectra were recorded using electrospray as the ionization technique in positive ion or negative ion modes as stated. All MS analysis samples were prepared as solutions in methanol.

4.1.1. General procedure 1: Synthesis of per-O-acetylated C-glycosides (**5**–**8**)

To a solution of per-*O*-acetylated β-C-cinnamoyl glycosides **1**–**4** (1.0 equiv) in dry DMA was added the sulfamoyl chloride (3.8 equiv) in dry DMA at 0 °C under argon. The reaction was stirred at room temperature until the starting material was consumed. The reaction mixture was diluted in ethyl acetate and washed with water (3×). The aqueous extracts were combined and back extracted with ethyl acetate (1×). The organic extracts were combined, dried over $NaSO_4$, filtered and evaporated. The product was purified by column chromatography (eluant 6:4 hexanes–EtOAc) to give compounds **5**–**8**.

4.1.2. General procedure 2: Deprotection of per-O-acetylated C-glycosides (**5**–**8** → **9**–**12**)

Desprotected compounds **9**–**12** were prepared by dissolving the corresponding per-*O*-acetylated precursor **5**–**8** 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 several times with methanol. The solvent was evaporated under reduced pressure and the product was purified by column chromatography to afforded pure material by ^{1}H NMR and ^{13}C NMR spectroscopy. Yields 65–72%.

*4.1.2.1. (E)-1-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyl)-4-(3-sulfamoylphenyl)but-3-en-2-one (**5**).* The title compound **5** was prepared from compound **1** according to general procedure 1 to give a white solid. Yield: 57% mp = 54–54.5 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (t, 1H, *J* = 1.9 Hz, ArH), 7.52 (d, 1H, *J* = 16.1 Hz, H-4), 7.48 (dd, 1H, *J* = 3.4, 1.8 Hz, ArH), 7.46 (d, 1H, *J* = 7.7 Hz, ArH), 7.39 (m, 1H, ArH), 6.77 (d, 1H, *J* = 16.1 Hz, H-3), 5.61 (br s, 2H, NH_2), 5.23 (t, 1H, *J* = 9.4 Hz, H-3′), 5.08 (t, 1H, *J* = 9.7 Hz, H-4′), 4.98 (t, 1H, *J* = 9.7 Hz, H-2′), 4.27 (dd, 1H, *J* = 12.4, 5.2 Hz, H-6′a), 4.04 (m, 2H, H-1′, H-6′b), 3.71 (ddd, 1H, *J* = 10.1, 5.3, 2.2 Hz, H-5′), 3.01 (dd, 1H, *J* = 15.5, 8.9 Hz, H-1a), 2.66 (dd, 1H, *J* = 15.4, 3.0 Hz, H-1b), 2.06 (s, 3H, CH_3COO), 2.05 (s, 3H, CH_3COO), 2.03 (s, 3H, CH_3COO), 1.94 (s, 3H, CH_3COO). ^{13}C NMR (151 MHz, $CDCl_3$) δ 196.59 (C-2), 171.20 (CH_3COO), 170.27 (CH_3COO), 170.03 (CH_3COO), 169.59 (CH_3COO), 150.67 (ArC), 141.97 (C-4), 136.31 (ArC), 130.51 (ArC), 127.71 (C-3), 127.39 (ArC), 124.45 (ArC), 121.10 (ArC), 75.66 (C-5′), 74.59 (C-1′), 74.05 (C-3′), 71.62 (C-2′), 68.57 (C-4′), 62.15 (C-6′), 42.84 (C-1), 20.78 (CH_3CO), 20.72 (CH_3CO), 20.62 (2 x CH_3CO). HRMS *m*/*z*: calcd for $C_{24}H_{29}NO_{13}S$, 571.1370; found, 571.1382.

*4.1.2.2. (E)-1-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyl)-4-(4-sulfamoylphenyl)but-3-en-2-one (**6**).* The title compound **6** was prepared from compound **2** according to general procedure 1 to give a white solid. Yield: 74% mp = 147–147.5 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (d, 2H, *J* = 8.6 Hz, ArH), 7.52 (d, 1H, *J* = 16.2 Hz, H-4), 7.37 (d, 2H, *J* = 8.6 Hz, ArH), 6.70 (d,1H, *J* = 16.2 Hz, H-3), 5.46 (br s, 1H, NH_2), 5.24 (t, 1H, *J* = 9.4 Hz, H-3′), 5.08 (t, 1H, *J* = 9.7 Hz, H-4′), 4.99 (t, 1H, *J* = 9.7 Hz, H-2′), 4.24 (dd, 1H, *J* = 12.4, 4.9 Hz, H-6′a), 4.11 (ddd, 1H, *J* = 10.0, 8.7, 3.3 Hz, H-1′), 4.03 (dd, 1H, *J* = 12.4, 2.2 Hz, H-6′b), 3.73 (ddd, 1H, *J* = 10.1, 5.0, 2.2 Hz, H-5′), 3.03 (dd, 1H, *J* = 16.1, 8.6 Hz, H-1a), 2.69 (dd, 1H, *J* = 16.2, 3.1 Hz, H-1b), 2.04 (s, 6H, CH_3COO), 2.03 (s, 3H, CH_3COO), 2.01 (s, 3H, CH_3COO). ^{13}C NMR (151 MHz, $CDCl_3$) δ 196.26 (C-2), 170.79 (CH_3COO), 170.28 (CH_3COO), 170.09 (CH_3COO), 169.64 (CH_3COO), 151.58 (ArC), 142.09 (C-4), 133.25 (ArC), 129.87 (2 x ArC), 127.06 (C-3), 122.71 (2 x ArC), 75.71 (C-5′), 74.15 (C-1′), 74.13 (C-3′), 71.69 (C-2′), 68.52 (C-4′), 62.14 (C-6′), 42.65 (C-1), 20.72 (CH_3CO), 20.67 (CH_3CO), 20.63 (CH_3CO), 20.61 (CH_3CO). HRMS *m*/*z*: calcd for $C_{24}H_{29}NO_{13}S$, 571.1370; found, 571.1378.

*4.1.2.3. (E)-1-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-galactopyranosyl)-4-(3-sulfamoylphenyl)but-3-en-2-one (**7**).* The title compound **7** was prepared from compound **3** according to general procedure 1 to give a sticky white solid. Yield: 87% mp = 53–54 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 (t, 1H, *J* = 1.9 Hz, ArH), 7.52 (d, 1H, *J* = 16.2 Hz, H-4), 7.46 (m, 2H, ArH), 7.38 (ddd, 1H, *J* = 7.7, 2.4, 1.4 Hz, ArH), 6.78 (d,

1H, *J* = 16.1 Hz, H-3), 5.81 (brs, 2H, NH_2), 5.45 (dd, 1H, *J* = 3.5, 1.1 Hz, H-4′), 5.19 (t, 1H, *J* = 9.9 Hz, H-2′), 5.08 (dd, 1H, *J* = 10.1, 3.4 Hz, H-3′), 4.19 (dd, 1H, *J* = 10.5, 6.2 Hz, H-6′a), 4.03 (ddd, 1H, *J* = 9.8, 8.9, 3.1 Hz, H-1′), 3.94 (m, 2H, H-6b, H-5′), 3.06 (dd, 1H, *J* = 15.5, 8.9 Hz, H-1a), 2.67 (dd, 1H, *J* = 15.5, 3.0 Hz, H-1b), 2.18 (s, 3H, CH_3COO), 2.06 (s, 3H, CH_3COO), 2.00 (s, 3H, CH_3COO), 1.90 (s, 3H, CH_3COO). ^{13}C NMR (151 MHz, $CDCl_3$) δ 196.77 (C-2), 170.89 ($CH_3\mathbf{C}OO$), 170.29 ($CH_3\mathbf{C}OO$), 170.23 ($CH_3\mathbf{C}OO$), 170.13 ($CH_3\mathbf{C}OO$), 150.72 (ArC), 141.89 (C-4), 136.31 (ArC), 130.46 (ArC), 127.62 (C-3), 127.29 (ArC), 124.39 (ArC), 121.20 (ArC), 75.02 (C-1′), 74.32 (C-5′), 71.92 (C-3′), 69.05 (C-2′), 67.77 (C-4′), 61.65 (C-6′), 43.11 (C-1), 20.81 ($\mathbf{C}H_3CO$), 20.68 ($\mathbf{C}H_3CO$), 20.60 ($\mathbf{C}H_3CO$), 20.55 ($\mathbf{C}H_3$-CO). HRMS *m*/*z*: calcd for $C_{24}H_{29}NO_{13}S$, 571.1370; found, 571.1365.

*4.1.2.4. (E)-1-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-galactopyranosyl)-4-(4-sulfamoylphenyl)but-3-en-2-one (**8**).* The title compound **8** was prepared from compound **4** according to general procedure 1 to give a colorless oil. Yield: 60% ^1H NMR (600 MHz, $CDCl_3$) δ 7.58 (d, 2H, *J* = 8.7 Hz, ArH), 7.52 (d, 1H, *J* = 16.1 Hz, H-4), 7.38 (m, 2H, ArH), 6.71 (d, 1H, *J* = 16.2 Hz, H-3), 6.05 (brs, 2H, NH_2), 5.44 (dd, 1H, *J* = 3.5, 1.2 Hz, H-4′), 5.18 (t, 1H, *J* = 9.9 Hz, H-2′), 5.08 (dd, 1H, *J* = 10.1, 3.5 Hz, H-3′), 4.09 (m, 2H, H-1′, H-6′a), 4.02 (dd, 1H, *J* = 11.3, 6.5 Hz, H-6′b), 3.94 (td, 1H, *J* = 6.6, 1.2 Hz, H-5′), 3.07 (dd,1H, *J* = 16.0, 8.5 Hz, H-1a), 2.70 (dd, 1H, *J* = 16.1, 3.2 Hz, H-1b), 2.16 (s, 3H, CH_3-COO), 2.04 (s, 3H, CH_3COO), 1.99 (s, 3H, CH_3COO), 1.96 (s, 3H, CH_3-COO). ^{13}C NMR (151 MHz, $CDCl_3$) δ 196.39 (C-2), 170.98 (CH_3COO), 170.52 (CH_3COO), 170.26 (CH_3COO), 170.12 (CH_3COO), 151.79 (ArC), 142.05 (C-4), 133.03 (ArC), 129.78 (2 x ArC), 126.91 (C-3), 122.80 (2 x ArC), 74.66 (C-1′), 74.24 (C-5′), 71.95 (C-3′), 69.12 (C-2′), 67.72 (C-4′), 61.44 (C-6′), 42.90 (C-1), 21.47 (CH_3CO), 20.81 (CH_3CO), 20.68 (CH_3CO), 20.60 (CH_3CO). HRMS *m*/*z*: calcd for C_{24}-$H_{29}NO_{13}S$, 571.1370; found, 571.1375.

*4.1.2.5. 1-(β-D-glucopyranosyl)-4-(3-sulfamoylphenyl)but-3-en-2-one (**9**).* The title compound **9** was prepared from compound **5** according to general procedure 2 to give a sticky brown solid. Yield: 65% mp = 128–129 °C ^1H NMR (600 MHz, DMSO) δ 7.50 (d, 1H, *J* = 16.2 Hz, H-4), 7.44 (s, 2H, NH_2), 7.23 (t, 1H, *J* = 7.8 Hz, ArH), 7.15 (d, 1H, *J* = 7.6 Hz, ArH), 7.05 (t, 1H, *J* = 2.0 Hz, ArH), 6.84 (m, 1H, ArH), 6.82 (d,1H, *J* = 16.2 Hz, H-3), 5.03 (d, 1H, *J* = 5.7 Hz, OH), 4.91 (d, 1H, *J* = 4.7 Hz, OH), 4.84 (d, 1H, *J* = 4.7 Hz, OH), 4.32 (t, 1H, *J* = 5.7 Hz, OH), 3.60 (m, 2H, H-1, H-6′a), 3.39 (dt, 1H, *J* = 11.2, 5.4 Hz, H-6′b), 3.17 (td, 1H,*J* = 8.4, 4.6 Hz, H-3′), 3.07 (m, 2H, H-4′, H-5′), 2.95 (m, 2H, H-2′,H-1a), 2.80 (dd, 1H, *J* = 16.1, 8.8 Hz, H-1b). ^{13}C NMR (151 MHz, DMSO) δ 198.49 (C-2), 158.15 (ArC), 142.71 (C-4), 136.25 (ArC), 130.38 (ArC), 127.13 (C-3), 119.88 (ArC), 118.04 (ArC), 115.21 (ArC), 81.16 (C-5′), 78.60 (C-3′), 76.30 (C-1′), 74.04 (C-2′), 70.77 (C-4′), 61.60 (C-6′), 43.83 (C-1). HRMS *m*/*z*: calcd for $C_{16}H_{21}$-NO_9S, 403.0937; found, 403.0945.

*4.1.2.6. 1-(β-D-glucopyranosyl)-4-(4-sulfamoylphenyl)but-3-en-2-one (**10**).* The title compound **10** was prepared from compound **6** according to general procedure 2 to give a white solid. Yield: 67% mp = 212–213 °C ^1H NMR (600 MHz, DMSO) δ 7.56 (d, 2H, *J* = 8.6 Hz, ArH), 7.50 (d, 1H, *J* = 16.1 Hz, H-4) 7.45 (br s, 2H, NH_2), 6.81 (d, 2H, *J* = 8.6 Hz, ArH), 6.74 (d, 1H, *J* = 16.2 Hz, H-3), 5.03 (d, 1H, *J* = 5.8 Hz, OH), 4.90 (d, 1H, *J* = 4.7 Hz, OH), 4.84 (d, 1H, *J* = 4.8 Hz, OH), 4.34 (t, 1H, *J* = 5.7 Hz, OH), 3.60 (m, 2H, H-1′, H-6′a), 3.36 (d, 1H, *J* = 1.8 Hz, H-6′b), 3.17 (t, 1H, *J* = 4.5 Hz, H-3′), 3.06 (m, 2H, H-4′, H-5′), 2.95 (dt, 1H, *J* = 9.1, 4.5 Hz, H-2′), 2.91 (m, 1H, H-1a), 2.75 (dd, 1H, *J* = 16.0, 8.9 Hz, H-1b). ^{13}C NMR (151 MHz, DMSO) δ 198.23 (C-2), 160.29 (ArC), 142.89 (C-4), 130.91 (2 x ArC), 125.93 (ArC), 124.06 (C-3), 116.28 (2 x ArC), 81.13 (C-5′), 78.61 (C-3′), 76.36 (C-1′), 74.04 (C-2′), 70.78 (C-4′), 61.61 (C-6′), 43.66 (C-1). HRMS *m*/*z*: calcd for $C_{16}H_{21}NO_9S$, 403.0937; found, 403.0948.

*4.1.2.7. 1-(β-D-galactopyranosyl)-4-(3-sulfamoylphenyl)but-3-en-2-one (**11**).* The title compound **11** was prepared from compound **7** according to general procedure 2 to give a yellow oil. Yield: 72% ^1H NMR (600 MHz, DMSO) δ 7.48 (d, 1H, *J* = 16.2 Hz, H-4), 7.45 (br s, 2H, NH_2), 7.23 (t, 1H, *J* = 7.8 Hz, ArH), 7.14 (d, 1H, *J* = 7.7 Hz, ArH), 7.05 (t, 1H, *J* = 2.0 Hz, ArH), 6.84 (d, 1H, *J* = 2.5 Hz, ArH), 6.82 (d, 1H, *J* = 16.2 Hz, H-3), 4.87 (d, 1H, *J* = 4.6 Hz, OH), 4.67 (d, 1H, *J* = 4.2 Hz, OH), 4.47 (t, 1H, *J* = 5.6 Hz, OH), 4.31 (d, 1H, *J* = 4.6 Hz, OH), 3.71 (br s, 1H, H-4′), 3.57 (m, 1H, H-1′), 3.46 (m, 1H, H-6′a), 3.36 (br s, 1H, H-6′b), 3.30 (m, 3H, H-2′, H-3′, H-5′), 2.95 (dd, 1H, *J* = 15.9, 2.6 Hz, H-1a), 2.81 (dd, 1H, *J* = 15.2, 8.2 Hz, H-1b). ^{13}C NMR (151 MHz, DMSO) δ 198.74 (C-2), 158.15 (ArC), 142.66 (C-4), 136.25 (ArC), 130.39 (ArC), 127.13 (C-3), 119.88 (ArC), 118.04 (ArC), 115.16 (ArC), 79.22 (C-5′), 76.94 (C-1′), 75.10 (C-3′), 70.95 (C-2′), 69.06 (C-4′), 60.84 (C-6′), 43.94 (C-1). HRMS *m*/*z*: calcd for $C_{16}H_{21}NO_9S$, 403.0937; found, 403.0930.

*4.1.2.8. 1-(β-D-galactopyranosyl)-4-(4-sulfamoylphenyl)but-3-en-2-one (**12**).* The title compound **12** was prepared from compound **8** according to general procedure 2 to give a white solid. Yield: 70% mp = 104–105 °C ^1H NMR (600 MHz, DMSO) δ 7.56 (d, 2H, *J* = 8.6 Hz, ArH), 7.50 (d, 1H, *J* = 16.1 Hz, H-4), 7.45 (br s, 2H, NH_2), 6.81 (d, 2H, *J* = 8.6 Hz, ArH), 6.74 (d, 1H, *J* = 16.1 Hz, H-3), 4.85 (d, 1H, *J* = 4.7 Hz, OH), 4.66 (d, 1H, *J* = 4.6 Hz, OH), 4.46 (t, 1H, *J* = 5.6 Hz, OH), 4.30 (d, 1H, *J* = 4.5 Hz, OH), 3.70 (d, 1H, *J* = 3.2 Hz, H-4′), 3.56 (td, 1H, *J* = 9.1, 2.8 Hz, H-1′), 3.46 (dt, 1H, *J* = 10.6, 6.3 Hz, H-6′a), 3.35 (m, 1H, H-6′b), 3.29 (dt, 3H, *J* = 5.3, 2.6 Hz, H-2′, H-3′, H-5′), 2.90 (dd, 1H, *J* = 15.9, 2.5 Hz, H-1a), 2.76 (dd, 1H, *J* = 15.8, 9.0 Hz, H-1b). ^{13}C NMR (151 MHz, DMSO) δ 198.43 (C-2), 160.28 (ArC), 142.82 (C-4), 130.88 (2 x ArC), 125.94 (ArC), 124.09 (C-3), 116.28 (2 x ArC), 79.22 (C-5′), 77.0 (C-1′), 75.13 (C-3′), 70.95 (C-2′), 69.08 (C-4′), 60.86 (C-6′), 43.75 (C-1). HRMS *m*/*z*: calcd for $C_{16}H_{21}$-NO_9S, 403.0937; found, 403.0931.

4.2. CA Inhibiton studies

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity as reported by Khalifah [18]. 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.; Prusoff, W.H. *Biochem. Pharmacol.* **1973**, *22*, 3099) as reported earlier and represent the mean from at least three different determinations.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bioorg.2017.10.020.

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