Bioorganic & Medicinal Chemistry 21 (2013) 1489–1494

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of *C*-cinnamoyl glycosides and their inhibitory activity against mammalian carbonic anhydrases

Leonardo E. Riafrecha [a], Oscar M. Rodríguez [a], Daniela Vullo [b], Claudiu T. Supuran [b,*], Pedro A. Colinas [a,*]

[a] *LADECOR, Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115, 1900 La Plata, Argentina*
[b] *Universitá degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3,I-50019 Sesto Fiorentino (Firenze), Italy*

ARTICLE INFO

Article history:
Available online 11 September 2012

Keywords:
C-Glycosides
Carbonic anhydrase
Phenol
Enzyme inhibition

ABSTRACT

A small series of *C*-cinnamoyl glycosides incorporating the phenol moiety has been prepared by reaction of glycosyl ketones with the appropriate benzaldehydes. Glycosides were tested for the inhibition of twelve mammalian isoforms of carbonic anhydrase. This is the first study in which α-CAs have been investigated for their interaction with *C*-glycosides, a novel carbohydrate scaffold in the design of carbonic anhydrase inhibitors. The *C*-cinnamoyl glycosides were generally effective CA inhibitors, with inhibition constants in the low micromolar range against CA I, II, IV, VA, VB, VI, VII, IX, XII, XIII, XIV and ineffective inhibitors of CA III. These results confirm that attaching carbohydrate moieties to CA phenol pharmacophore improves its inhibitory activity.

Carbonic anhydrases (CAs, EC 4.2.1.1) are a superfamily of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide to give bicarbonate and a proton.[1] CAs constitute an excellent example of convergent evolution, and in addition to α-CAs from mammals there are four distinct, unrelated families (β-, γ-, δ- and ξ-CA families that encode these metalloenzymes in organisms across the phylogenetic tree.[2]

Inhibition of carbonic anhydrases has pharmacologic applications in the field of antiglaucoma, anticonvulsant, and anticancer agents but is emerging also for designing antifungal and antibacterial agents with a novel mechanism of action. CA inhibitors (CAIs) belong to four main classes: (i) sulfonamides and their isosteres (sulfamates/sulfamides), which bind in deprotonated form, as anions, to the Zn(II) ion from the enzyme active site in tetrahedral geometry,[3] (ii) coumarins which exhibited a completely unprecedent binding mode (in hydrolized form), with no interactions between the inhibitor molecule and the active site Zn(II) ion observed,[4] (iii) polyamines, which bind by anchoring to the water molecule/hydroxide ion coordinated to Zn(II)[5] and (iv) phenols which bind rather similar but not identical to polyamines, by interacting with a zinc-bound water molecule through hydrogen bonding and with no direct interaction between the inhibitor and the zinc.[6] This binding mode was reported by Christianson and colleagues in a very elegant study of the X-ray structure for the adduct of hCA II with phenol.[7] Also they showed that the inhibitor bound in the active site mainly because of van der Waals contacts with side chain residues of the hydrophobic side of the active site. Supuran's group has recently investigated the interaction of phenol and some derivatives with α- and β-carbonic anhydrases enzymes displaying low micromolar/submicromolar inhibition.[6,8] The different mechanism of inhibition of phenols as compared to other inhibitors and their inhibition profile, make this class of derivatives of great interest to design novel CA inhibitors with selectivity and/or specificity for some of the medicinal targets belonging to this enzyme family.

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] Attaching a carbohydrate moiety should induce the desired physicochemical properties such as water solubility, low permeability, etc. Also the stereochemical diversity across the carbohydrate tails provides the opportunity for interrogation of subtle differences in active site topology of CA isozymes. A particular problem for the application of carbohydrates as drugs is the lability of the glycosidic bond. To circumvent metabolic problems in the organism, stable mimetics (glycomimetics) have to be prepared.[10] In recent years a variety of replacement for the glycosidic linkage have raised considerable interest as stable carbohydrate mimetics due to their enormous stability to enzymatic degradation and the resulting possibilities as enzyme inhibitors. The most common modifications with respect to the linkage are *N*-glycosides, *C*-glycosides and *S*-glycosides.[11] On the last years one of our groups has developed several synthesis of *N*-glycosyl sulfonamides by sulfonamidoglycosylation of carbohydrate derivatives.[12] Recently we reported the synthesis of a series

* Corresponding authors. Tel.: +39 055 4573005; fax: +39 055 4573385 (C.T.S.); tel.: +54 221 4243104; fax: +54 221 4226947 (P.A.C.).
E-mail addresses: claudiu.supuran@unifi.it (C.T. Supuran), pcolinas@quimica.unlp.edu.ar (P.A. Colinas).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.09.002

of α-D-hex-2-enopyranosyl sulfonamides, which was evaluated against human hepatocellular liver carcinoma (HepG2) and human lung adenocarcinoma (A549) cell lines and showed antiproliferative activity in the micromolar range.[13] Very recently we have prepared novel *N*-β-glycosyl sulfamides and shown that they selectively target cancer-associated CAs (IX and XII) with K_is in the low nanomolar range.[14]

Now our approach is based on the synthesis of *C*-cinnamoyl glycosides, where the carbohydrate moiety is tethered to a phenol CA pharmacophore through a carbon chain. The replacement of the naturally occurring *O*-glycoside bond by *C*-glycoside bond is an approach practiced in the synthesis of carbohydrate-containing compounds for the downstream biological applications.[12] This isosteric replacement seeks to enhance stability of the small molecule glycoside toward enzymatic hydrolysis of the glycosidic bond while retaining vital molecular recognition interactions with the biological target.[15] *C*-cinnamoyl glycosides derivated from β-*C*-glycosyl ketones were shown to possess α-glucosidase inhibition activity[16] and to be effective as antimycobacterial agents.[17]

Thus, in the search of non-sulfonamide CAIs belonging to different classes of compounds, we report here the synthesis of a series of new *C*-cinnamoyl glycosides incorporating the phenol moiety, and their inhibitory activity against the 12 catalytically active mammalian CA isozymes.

1. Chemistry

A set of new *C*-cinnamoyl glycosides (Fig. 1) was synthesized as outlined in Scheme 1. 1-(β-D-Glucopyranosyl)-propan-2-one was prepared by Knoevenagel condensation with 2,4-pentanedione in the presence of sodium carbonate using water as solvent.[18] We found that the reaction of D-galactose in these conditions resulted in a mixture of several compounds that were difficult to separate due to their similar polarity. Thus β-*C*-galactosyl ketone was synthesized by the same reaction but using sodium bicarbonate following an earlier protocol.[19] Crude mixtures containing the *C*-glycosyl ketones were acetylated and then purified to afford the peracetylated compounds in good yields. 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannosyl)-propan-2-one was found as by-product in the Knoevenagel condensation of D-glucose. The formation of the *C*-mannosyl ketone could be rationalized considering that D-mannose could be obtained by alkaline epimerization of D-glucose.

C-cinnamoyl glycosides have been prepared by aldol condensation of β-*C*-glucosyl and β-*C*-galactosyl ketones with different aromatic aldehydes incorporating the phenol moiety at room temperature in the presence of pyrrolidine as catalyst.[16,17] We found that reaction times and the yields are highly dependent on the position of the hydroxyl group in the aromatic ring. Thus the reaction gave the *C*-cinnamoyl glycosides in good yields with 3-, and 4-hydroxybenzaldehydes. When salicylaldehyde was used as starting material, very complex mixtures were found and no product could be isolated. This may be due to the presence of hydroxyl group at the *o*-position to the aldehyde group, which induces the steric hindrance. On the other hand, the reaction of 3,4-dihydroxybenzaldehyde with *C*-glucosyl or *C*-galactosyl ketones afforded a dark red tar and no reactives could be recovered from the reaction mixtures. No reaction was found when triethylamine was used as catalyst.

The reaction mixtures were easily purified by flash chromatography and/or crystallization to afford the pure *C*-cinnamoyl glycosides **1**–**4**. The ^{1}H NMR, ^{13}C NMR, 2D COSY and HSQC were in full agreement with their structures. The *trans* double bond in the glycosides was established by the large coupling constant (J = 16 Hz) between the two olefinic protons.

The *O*-acetate protecting groups of the carbohydrate moiety were next removed using triethylamine in methanol/water to afford the fully deprotected *C*-glycosides **5**–**8** in very good yields.

The inhibitory activity of *C*-glycosides **1**–**8** and phenol against all the catalytically active mammalian α-CA isozymes are presented in Table 2.

In contrast to standard CA inhibitors, the inhibition profile with α-CAs for the peracetylated *C*-glycosyl compounds **1**–**4** is flat and there is small variations in the *K*i values observed across all CA isozymes investigated, leading to *K*i selectivity ratios of ~1.0 across isozymes, that is, they are nonselective CA inhibitors. On the other hand deprotected glycosides **5**–**8** showed a more complicated inhibition profile.

The following should be noted regarding the inhibition of the mammalian α-CA isozime with *C*-glycosides **1**–**8**:

(i) All investigated *C*-cinnamoyl glycosides were efficient, micromolar hCA I and hCA II inhibitors with inhibition constants in the range of 3.6–9.3 μM (CA I) and of 3.1–8.8 μM (CA II).

(ii) Isozyme hCA III was not inhibited by *C*-cinnamoyl glycosides with only compound **8** behaving as an effective inhibitor (*K*i of 8.4 μM.) Glycosides, similarly to other CA inhibitors, may have steric clashes with Phe198, a bulky amino acid residue place in the middle of the CA II active site, explaining their inefficient inhibitory activity.[20]

(iii) Peracetylated *C*-glycosides **1**–**4** act as good inhibitors of the mitochondrial isoforms hCA VA and VB. A quite similar inhibition was observed with the deprotected compounds **5**–**8**, which inhibited hCA VA with K_is of 4.4–8.0 μM. On the other hand *C*-glycosides **5**–**8** behave as ineffective inhibitors of hCA VB. One should note the important differences in the pattern of inhibition of the two mitochondrial

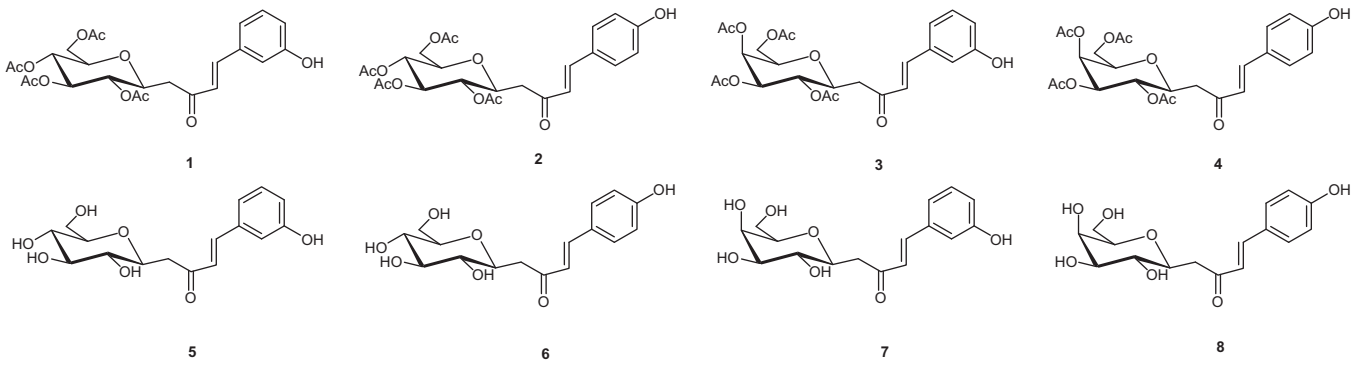
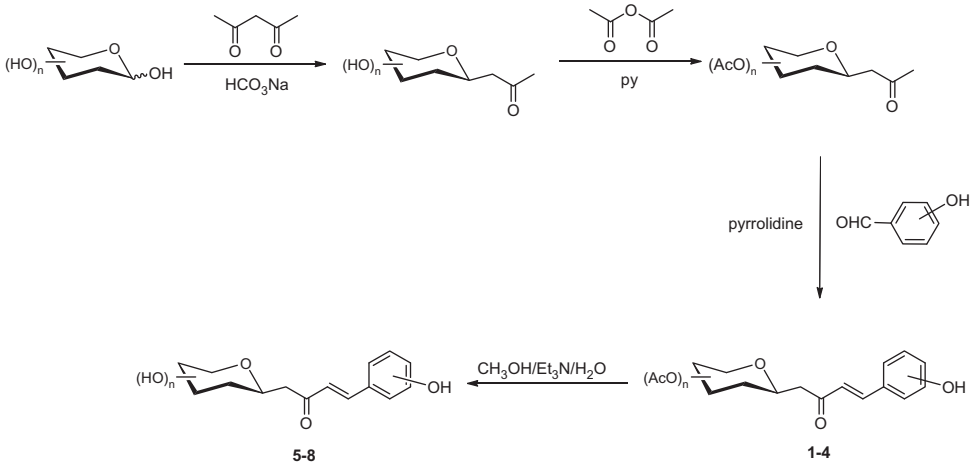


Figure 1. Per-*O*-acetyl *C*-glycosides (1–4) and fully deprotected derivatives.



Scheme 1. Preparation of *C*-glycosides.

Table 1
Synthesis of *C*-cinnamoyl glycosides

Ketone	Benzaldehyde	Reaction time (hs.)	Product	Yield (%)
C-Glucosyl	2-Hydroxy	28	—	—
	3-Hydroxy	1.5	1	68
		24[a]		n.r.
	4-Hydroxy	48	2	44
	3,4-Dihydroxy	24	—	—
C-Galactosyl	2-Hydroxy	28	—	—
	3-Hydroxy	4	3	62
		24[a]		n.r.
	4-Hydroxy	48	4	40
	3,4-Dihydroxy	24	—	—

[a] Reaction performed using triethylamine as catalyst.

enzymes (hCA VA and hCA VB) by the deprotected *C*-glycosides, in contrast with sulfonamides which are strong inhibitors for both of them.

(iv) *C*-cinnamoyl glycosides **1**–**4** are good inhibitors of the secreted isozyme hCA VI (K_is of 6.2–8.8 μM). On the other hand only deprotected glycoside **7** showed a high affinity for this isozyme.

(v) Isoform hCA VII was also inhibitied by compounds **1**–**8** with K_is in the range 4.9–9.5 μM, showing a flat SAR with less variation of the inhibitory power.

(vi) The two tumor-associated isoforms hCA IX and XII showed a similar inhibition profile with compounds **1**–**8**. Thus, these derivatives inhibited hCA IX with inhibition constants in the range of 2.9–9.2 μM, and hCA XII with K_is of 3.9–8.7 μM. In the development of anti-cancer compounds that target selectively the membrane bound isoform hCA IX (and hCA) versus the ubiquitous isoform hCA II, the design of membrane non-permeant inhibitors is crucial. In recent years, several parameters have been introduced for membrane permeability prediction.[21] 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.[22] Molecules with a TPSA greater than 140 Å^2 are likely to have a low capacity for penetrating cell membranes. We had calculated the lipophilicity and topological polar surface area for the *S*-glycosyl sulfonamides showing that all compounds fall within the range indicative of molecules with poor membrane permeability.[23] Though *C*-glycosides showed no selectivity for the cancer associated CAs, their pysicochemical properties would lead to preferential inhibition of the transmembrane CA IX over cytosolic CA II.

(vii) *C*-glycosides **1**–**7** were good inhibitors of mCA XIII, another cytosolic isoform (K_is in the range 4.9–8.8 μM) whereas the remaining derivative behaved as an ineffective inhibitor.

(viii) The last human isoform hCA XIV (transmembrane one, similarly to hCA IX and XII, but not tumor associated)

Table 2
Inhibition of mammalian α-CA with the *C*-cinnamoyl glycosydes **1**–**8** and phenol

Isozyme[a]	K_i (μM)[b]								
	1	2	3	4	5	6	7	8	Phenol
hCA I	8.5	5.7	5.1	9.3	6.8	3.7	3.6	5.5	10.2
hCA II	7.0	3.9	7.1	5.5	7.8	8.8	3.1	6.8	5.5
hCA III	>100	>100	>100	>100	>100	>100	>100	8.4	2.7
hCA IV	5.6	4.9	7.8	6.7	8.3	7.1	9.2	8.4	9.5
hCA VA	9.8	8.4	9.5	9.3	7.4	4.4	3.4	8.0	218
hCA VB	6.0	4.0	6.9	5.2	>100	>100	>100	>100	543
hCA VI	8.8	6.2	7.7	7.9	>100	>100	8.1	>100	208
hCA VII	9.5	6.3	7.1	5.8	4.9	8.5	9.0	9.3	710
hCA IX	5.2	5.9	3.3	2.9	4.5	8.2	9.2	8.2	8.8
hCA XII	6.7	6.2	3.9	4.2	7.4	8.7	8.4	6.8	9.2
hCA XIII	5.1	4.9	7.2	6.7	8.8	8.6	8.6	>100	697
hCA XIV	5.9	5.6	4.6	2.3	4.3	7.1	>100	>100	11.5

[a] All CAs are recombinant enzymes obtained in the authors' laboratory as reported earlier.[4]
[b] Errors in the range of 5–10% of the reported value, from 3 different determinations.

was generally well inhibited by the *C*-cinnamoyl glycosides **1**–**6**, which showed K_is in the range of 2.3–7.1 μM. Deprotected galactosyl derivatives **7**–**8** were not all inhibitory.

Neither the stereochemistry presented by the differing carbohydrate moiety nor the nature of the carbohydrate hydroxyl groups, either as the *C*-glycosides (**5**–**8**) or less polar and bulkier acetylated sugar (**1**–**4**) impacted to alter enzyme inhibition characteristics (with the exception of isozymes hCA VB and hCA VI). Also there is no influence of the position of hydroxyl in the aromatic ring on the inhibition profile. However per-*O*-acetyl and deprotected *C*-glycosides showed better inhibitory activity against almost all α-CA than phenol. Thus, this confirms that attaching carbohydrate moieties to CA phenol pharmacophore could improve its inhibitory activity.

2. Conclusions

We have investigated the enzyme inhibition characteristics of a small series of *C*-cinnamoyl glycosides incorporating the phenol moiety, against mammalian CAs. This is the first study in which α-CAs have been investigated for their interaction with *C*-glycosides, a novel carbohydrate scaffold in the design of carbonic anhydrase inhibitors. The *C*-cinnamoyl glycosides were generally effective CA inhibitors, with inhibition constants in the low micromolar range against CA I, II, IV, VA, VB, VI, VII, IX, XII, XIII, XIV and ineffective inhibitors of CA III. These results confirm that attaching carbohydrate moieties to CA phenol pharmacophore improves its inhibitory activity.

The physicochemical properties of the glycosides tested would enhance the preferential inhibition of transmembrane isozymes in vivo. Free *C*-cinnamoyl glycosydes could be useful for chemotherapy if they are delivered through a route of intravenous administration. For oral delivery peracetylated glycosides may be used as ester prodrugs. Once in the body, the ester groups could be readily hydrolyzed by ubiquitous esterases.[24]

3. Experimental section

3.1. 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 Varian Mercury 200 (200.055 and 50.309 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.

3.2. General procedure 1: Synthesis of per-*O*-acetylated *C*-cinnamoyl glycosides (1–4)

To a solution of per-*O*-acetylated β-*C*-glucopyranosyl or *C*-galactopyranosyl ketone (1.0 equiv) in dry dichloromethane was added the desired benzaldehyde (1.0 equiv) and pyrrolidine (0.2 equiv). The reaction was stirred at room temperature until the starting material was consumed as evidenced by TLC (see times in Table 1). The reaction mixture was concentrated and the residue 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 4:6 to 1:1 hexanes–EtOAc) to give compounds **1**–**4**.

3.3. General procedure 2: Deprotection of per-*O*-acetylated *C*-glycosides (1–4 → 5–8)

Deprotected compounds **5**–**8** were prepared by dissolving the corresponding per-*O*-acetylated precursor **1**–**4** in methanol/triethylamine/water (8:2:1). The reaction was kept at room temperature overnight and then concentrated. The product was purified by column chromatography (eluant 5:1 EtOAc–MeOH) to afforded pure material by ^{1}H NMR and ^{13}C NMR spectroscopy. Yields 75–95%.

3.4. (*E*)-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-(3-hydroxyphenyl)but-3-en-2-one (1)

The title compound **1** was prepared from 1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-propan-2-one and 3-hydroxybenzaldehyde according to general procedure 1 to give a white solid. Mp = 152–153 °C ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.50 (d, 1H, *J* = 16.2 Hz, H-4), 7.27 (dd, 1H, *J* = 9.2, 6.5 Hz, ArH), 7.07 (t, *J* = 4.4 Hz, 2H, ArH), 6.91 (m, 1H, ArH), 6.71 (d,1H, *J* = 16.2 Hz, H-3), 6.69 (s, 1H, OH), 5.22 (d, 1H, *J* = 9.2 Hz, H-3′), 5.11 (d, 1H, *J* = 9.8 Hz, H-4′), 4.96 (d, 1H, *J* = 9.3 Hz, H-2′), 4.27 (dd, 1H, *J* = 12.4, 4.7 Hz, H-6′a), 4.11 (ddd, 1H, *J* = 9.7, 8.6, 2.8 Hz, H-1′), 4.05 (dd, 1H, *J* = 12.4, 2.2 Hz, H-6′b), 3.73 (ddd, 1H, *J* = 9.7, 4.7, 2.2 Hz, H-5′), 3.02 (dd, 1H, *J* = 16.3, 8.6 Hz, H-1a), 2.68 (dd, 1H, *J* = 16.3, 3.1 Hz, H-1b), 2.04 (s, 3H, CH_3COO), 2.03 (s, 3H, CH_3COO), 2.02 (s, 3H, CH_3COO), 2.01 (s, 3H, CH_3COO). ^{13}C NMR (50 MHz, $CDCl_3$) δ 196.99 (C-2'), 171.27 (CH_3COO), 170.63 (CH_3COO), 170.37 (CH_3COO), 169.95 (CH_3COO), 156.83 (ArC), 144.22 (C-4), 135.79 (ArC), 130.44 (ArC), 126.46 (C-3), 121.29 (ArC), 118.46 (ArC), 114.86 (ArC), 75.84 (C-5′), 74.40 (C-3′), 74.30 (C-4′), 71.84 (C-1′), 68.67 (C-2′), 62.29 (C-6′), 42.74 (C-1), 20.96 (CH_3COO), 20.93 (CH_3COO), 20.878 (CH_3COO), 20.878 (CH_3COO). HRMS *m*/*z*: calcd for $C_{24}H_{28}O_{11}$, 492.1632; found, 492.1630

3.5. (*E*)-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-(4-hydroxyphenyl)but-3-en-2-one (2)

The title compound **2** was prepared from 1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-propan-2-one and 4-hydroxybenzaldehyde according to general procedure 1 to give a white solid. Mp = 176–177 °C ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.50 (d, 1H, *J* = 16.1 Hz,H-4), 7.43 (d, 2H, *J* = 8.6 Hz, ArH), 6.97 (s, 1H, OH), 6.87 (d, 2H, *J* = 8.6 Hz, ArH), 6.59 (d,1H, *J* = 16.1 Hz, H-3), 5.24 (t, 1H, *J* = 9.2 Hz, H-3′), 5.10 (d, 1H, *J* = 9.8 Hz, H-4′), 5.00 (dd, 1H, *J* = 13.6, 5.5 Hz, H-2′), 4.27 (dd, 1H, *J* = 12.4, 4.8 Hz, H-6′a), 4.13 (ddd, 1H, *J* = 11.1, 9.1, 3.9 Hz, H-1′), 4.03 (dd, 1H, *J* = 12.4, 2.1 Hz, H-6′b), 3.72 (ddd, 1H, *J* = 9.8, 4.8, 2.1 Hz, H-5′), 3.01 (dd, 1H, *J* = 16.3, 8.3 Hz, H-1a), 2.67 (dd, 1H, *J* = 16.3, 3.2 Hz, H-1b), 2.03 (s, 3H, CH_3COO), 2.02 (s, 3H, CH_3COO), 2.01 (s, 3H, CH_3COO), 2.01 (s, 3H, CH_3COO). ^{13}C NMR (50 MHz, $CDCl_3$) δ 196.84 (C-2), 171.16 (CH3COO), 170.59 (CH3COO), 170.41 (CH3COO), 169.94 (CH3COO), 159.18 (ArC), 144.42 (C-4), 130.75 (ArC), 126.67 (C-3), 123.84 (ArC), 116.34 (ArC), 75.89 (C-5′), 74.45 (C-3′),74.37 (C-4′), 71.98 (C-1′), 68.74 (C-2′), 62.31 (C-6′), 42.57 (C-1), 20.95 (CH_3COO), 20.95 (CH_3COO), 20.85 (CH_3COO), 20.85 (CH_3COO). HRMS *m*/*z*: calcd for $C_{24}H_{28}O_{11}$, 492.1632; found, 492.1651

3.6. (*E*)-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-(3-hydroxyphenyl)but-3-en-2-one (3)

The title compound **3** was prepared from 1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-propan-2-one and 3-hydroxybenzal-

dehyde according to general procedure 1 to give a sticky white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.51 (d, 1H, J = 16.1 Hz, H-4), 7.26 (t, 1H, J = 7.9 Hz, ArH), 7.08 (d, 2H, J = 6.8 Hz, ArH), 6.92 (d, 1H, J = 8.9 Hz, ArH), 6.72 (d, 1H, J = 16.1 Hz, H-3), 5.45 (dd, 1H, J = 3.2, 0.9 Hz, H-4′), 5.20 (m, 1H, H-2), 5.08 (dd, 1H, J = 10.0, 3.3 Hz, H-3′), 4.04 (m, 4H, H-1′, H-5′, 2 × H-6′), 3.06 (dd, J = 16.1, 8.5 Hz, 1H, H-1a), 2.69 (dd, J = 16.1, 3.2 Hz, 1H, H-1b), 2.16 (s, 3H, CH_3COO), 2.04 (s, 3H, CH_3COO) 1.99 (s, 3H, CH_3COO), 1.96 (s, 3H, CH_3COO). ^{13}C NMR (50 MHz, $CDCl_3$) δ 197.19 (C-2), 170.99 (CH_3COO), 170.61 (CH_3COO), 170.57 (CH_3COO), 170.44 (CH_3COO), 156.88 (ArC), 144.12 (C-4), 135.85 (ArC), 130.39 (ArC), 126.49 (C-3), 121.23 (ArC), 118.42 (ArC), 114.86 (ArC), 74.87 (C-5′), 74.43 (C-1′), 72.24 (C-3′), 69.33 (C-2′), 67.99 (C-4′), 61.71 (C-6′), 42.98 (C-1), 21.03 (CH_3COO), 20.89 (CH_3COO), 20.82 (CH_3COO), 20.82 (CH_3COO). HRMS m/z: calcd for $C_{24}H_{28}O_{11}$, 492.1632; found, 492.1630.

3.7. (*E*)-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-(4-hydroxyphenyl)but-3-en-2-one (4)

The title compound **4** was prepared from 1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-propan-2-one and 4-hydroxybenzaldehyde according to general procedure 1 to give a pale yellow solid. mp = 119-120 °C ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.51 (d, 1H, J = 16.2 Hz, H-4), 7.43 (d, 2H, J = 8.6 Hz, ArH), 6.88 (d, 2H, J = 8.6 Hz, ArH), 6.61 (d, 1H, J = 16.1 Hz, H-3), 5.45 (d, 1H, J = 2.5 Hz, H-4′), 5.20 (m, 1H, H-2′), 5.07 (dd, 1H, J = 10.0, 3.2 Hz, H-3′), 4.06 (m, 4H, H-1′, H-5′, 2 × H-6′), 3.06 (dd, 1H, J = 16.1, 8.4 Hz, H-1a), 2.68 (dd, 1H, J = 16.1, 3.3 Hz, H-1b), 2.16 (s, 3H, CH_3COO), 2.04 (s, 3H, CH_3COO), 1.99 (s, 3H, CH3COO), 1.97 (s, 3H, CH_3COO). ^{13}C NMR (50 MHz, $CDCl_3$) δ 197.17 (C-2), 170.96 (CH_3COO), 170.67 (CH_3COO), 170.49 (CH_3COO), 159.40 (ArC), 144.48 (C-4), 130.75 (ArC), 123.79 (C-3), 116.35 (ArC), 74.91 (C-5′), 74.40 (C-1′), 72.26 (C-3′), 69.40 (C-2′), 67.99 (C-4′), 61.69 (C-6′), 42.75 (C-1), 21.06 (CH_3COO), 20.92 (2 × CH_3COO), 20.84 (CH_3COO). HRMS m/z: calcd for $C_{24}H_{28}O_{11}$, 492.1632; found, 492.1626.

3.8. (*E*)-1-β-D-glucopyranosyl-4-(3-hydroxyphenyl)but-3-en-2-one (5)

The title compound **5** was prepared from compound **1** according to general procedure 2 to give a white solid. Mp = 172–173 °C ^{1}H NMR (200 MHz, D_2O) δ 7.51 (d, 1H, J = 16.2 Hz,H-4), 7.28 (t, 1H, J = 7.8 Hz,ArH), 7.12 (d, 1H, J = 7.8 Hz,ArH), 7.03 (d, 1H, J = 2.0 Hz,ArH), 6.92 (dd, 1H, J = 8.1, 1.6 Hz,ArH), 6.73 (d, 1H, J = 16.2 Hz,H-3), 3.72 (m, 3H, H-5′, 2 × H-6′), 3.35 (m, 4H, H-1′,H-2′,H-3′,H-4′), 3.07 (dd, 1H, J = 16.2, 5.4 Hz,H-1a), 2.89 (dd, 1H, J = 16.2, 8.8 Hz,H-1b). ^{13}C NMR (50 MHz, D_2O) δ 202.65 (C-2), 156.23 (ArC), 145.30 (C-4), 135.93 (ArC),130.70 (ArC),126.31 (C-3), 121.51 (ArC), 118.54 (ArC), 115.06 (ArC), 79.85 (C-5′), 77.67 (C-3′), 76.0 2 (C-4′), 73.58 (C-1′), 70.09 (C-2′), 61.16 (C-6′), 42.74 (C-1). HRMS (m/z): [M+Na]$^+$ calculated for $C_{16}H_{20}O_7Na$ 347.1107, found 347.1126.

3.9. (*E*)-1-β-D-glucopyranosyl)-4-(4-hydroxyphenyl)but-3-en-2-one (6)

The title compound **6** was prepared from compound **2** according to general procedure 2 to give a white solid. Mp = 123–124 °C. ^{1}H NMR (200 MHz, D_2O) δ 7.64 (d, 1H, J = 16.2 Hz, H-4), 7.56 (d, 2 H, J = 8.5 Hz, ArH), 6.90 (d, 2 H, J = 8.5 Hz, ArH), 6.73 (d, 1H, J = 16.2 Hz, H-3), 3.68 (m, 3H, H-5′, 2 × H-6′), 3.27 (m, 4H, H-1′, H-2′, H-3′, H-4′), 3.06 (dd, 1H, J = 16.2, 5.3 Hz, H-1a), 2.87 (dd, 1H, J = 16.2, 8.7 Hz, H-1b). ^{13}C NMR (50 MHz, D_2O) δ 202.80 (C-2), 156.23 (ArC), 146.28 (C-4), 131.13 (ArC), 126.44 (C-3), 116.13 (ArC), 79.68 (C-5′), 77.40 (C-1′), 76.05 (C-3′), 73.15 (C-2′), 69.81 (C-4′), 60.83 (C-6′), 42.36 (C-1). HRMS (m/z): [M+Na]$^+$ calculated for $C_{16}H_{20}O_7Na$ 347.1107, found 347.1112.

3.10. (*E*)-1-β-D-galactopyranosyl-4-(3-hydroxyphenyl)but-3-en-2-one (7)

The title compound **7** was prepared from compound **3** according to general procedure 2 to give a pale yellow solid. Mp = 126.5–127 °C ^{1}H NMR (200 MHz, D_2O) δ 7.53 (d, 1H, J = 16.3 Hz, H-4), 7.29 (t, 1H, J = 7.8 Hz, ArH), 7.14 (d, 1H, J = 7.8 Hz, ArH), 7.04 (d, 1H, J = 1.6 Hz, ArH), 6.93 (dd, 1H, J = 7.6, 2.1 Hz, ArH), 6.75 (d, 1H, J = 16.3 Hz, H-3), 3.95 (d, 1H, J = 3.2 Hz, H-2′), 3.66 (m, 6H, H-1′, H-3′, H-4′, H-5′, 2 × H-6′), 3.18 (dd, 1H, J = 16.2, 3.1 Hz, H-1a), 2.94 (dd, 1H, J = 16.3, 8.7 Hz, H-1b). ^{13}C NMR (50 MHz, D_2O) δ 202.80 (C-2), 156.22 (ArC), 145.28 (C-4), 135.97 (ArC), 130.70 (ArC), 126.32 (C-3), 121.51 (ArC), 118.51 (ArC), 115.05 (ArC), 78.84 (C-5′), 76.46 (C-1′), 74.27 (C-4′), 70.98 (C-3′), 69.48 (C-2′), 61.49 (C-6′), 42.89(C-1). HRMS (m/z): [M+Na]$^+$ calculated for $C_{16}H_{20}O_7Na$ 347.1107, found 347.1128.

3.11. (*E*)-1-β-D-galactopyranosyl)-4-(4-hydroxyphenyl)but-3-en-2-one (8)

The title compound **8** was prepared from compound **4** according to general procedure 2 to give a pale yellow solid. Mp = 162–163 °C

^{1}H NMR (200 MHz, D_2O) δ 7.51 (dd, 3H, J = 14.5, 12.5 Hz, H-4, ArH), 6.86 (d, 2H, J = 8.6 Hz, ArH), 6.64 (d,1H, J = 16.1 Hz, H-3), 3.95 (d, 1H, J = 3.2 Hz, H-2′), 3.64 (m, 6H, H-1′, H-3′, H-4′, H-5′, 2 × H-6′), 3.14 (dd, 1H, J = 13.3, 6.6 Hz, H-1a), 2.90 (dd, 1H, J = 16.1, 8.8 Hz, H-1b). ^{13}C NMR (50 MHz, D_2O) δ 202.84 (C-2), 159.01 (ArC), 146.02 (C-4), 131.27 (ArC), 126.63 (C-3), 116.31 (ArC), 78.84 (C-5′), 76.65 (C-1′), 74.28 (C-3′), 71.02 (C-2′), 69.49 (C-4′), 61.50 (C-6′), 42.70 (C-1). HRMS (m/z): [M+Na]$^+$ calculated for $C_{16}H_{20}O_7Na$ 347.1107, found 347.1115.

4. CA Inhibiton studies

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity as reported by Khalifah.[25] 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[26] as reported earlier and represent the mean from at least three different determinations.

Acknowledgments

This work was financed in part by an EU grant (Metoxia) to C.T.S., and by UNLP and CONICET (Argentina). P.A.C. is member of the Scientific Research Career of CONICET.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.09.002.

References and notes

1. Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168–181.
2. Supuran, C. T. *Future Med. Chem.* **2011**, *3*, 1165.
3. Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3467–3474.
4. (a) Maresca, A.; Temperini, C.; Vu, H.; Pham, N. B.; Poulsen, S. A.; Scozzafava, A.; Quinn, R. J.; Supuran, C. T. *J. Am. Chem. Soc.* **2009**, *131*, 3057; (b) Maresca, A.; Temperini, C.; Pochet, L.; Masereel, B.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2010**, *53*, 335; (c) Temperini, C.; Innocenti, A.; Scozzafava, A.; Parkkila, S.; Supuran, C. T. *J. Med. Chem.* **2010**, *53*, 850; (d) Maresca, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4511; (e) Maresca, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7255.
5. Carta, F.; Temperini, C.; Innocenti, A.; Scozzafava, A.; Kaila, K.; Supuran, C. T. *J. Med. Chem.* **2010**, *53*, 5511.
6. (a) Innocenti, A.; Vullo, D.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1583; (b) Innocenti, A.; Hilvo, M.; Scozzafava, A.; Parkkila, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3593; (c) Innocenti, A.; Vullo, D.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem.* **2008**, *16*, 7424; (d) Bayram, E.; Senturk, M.; Kufrevioglu, O. I.; Supuran, C. T. *Bioorg. Med. Chem.* **2008**, *16*, 9101; (e) Sarikaya, S. B. O.; Topal, F.; Şentürk, M.; Gülcin, I.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4259.
7. Nair, S. K.; Ludwig, P. A.; Christianson, D. W. *J. Am. Chem. Soc.* **1994**, *116*, 3659.
8. Davis, R. A.; Hofmann, A.; Osman, A.; Hall, R. A.; Mühlschlegel, F. A.; Vullo, D.; Innocenti, A.; Supuran, C. T.; Poulsen, S.-A. *J. Med. Chem.* **2011**, *54*, 1682.
9. Winum, J.-Y.; Poulsen, S. A.; Supuran, C. T. *Med. Res. Rev.* **2009**, *29*, 419.
10. Ernst, B.; Magnani, J. L. *Nat. Rev. Drug Disc.* **2009**, *8*, 661.
11. Koester, D. C.; Holkenbrink, A.; Werz, D. B. *Synthesis* **2010**, 3217.
12. Colinas, P. A. *Curr. Org. Chem.* **2012**, *16*, 1670.
13. Crespo, R.; de Bravo, M. G.; Colinas, P. A.; Bravo, R. D. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6469.
14. Rodríguez, O. M.; Maresca, A.; Témpera, C. A.; Bravo, R. D.; Colinas, P. A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4447.
15. Asensio, J. L.; Cañada, F. J.; Cheng, X.; Khan, N.; Mootoo, D. R.; Jiménez-Barbero, J. *Chem. Eur. J.* **2000**, *6*, 1035.
16. Bisht, S. S.; Pandey, J.; Sharma, A.; Tripathi, R. P. *Carbohydr. Res.* **2008**, *343*, 1399.
17. Mugunthan, G.; Ramakrishna, K.; Sriram, D.; Yogeeswari, P.; Kartha, K. P. R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3947.
18. Wang, J.; Li, Q.; Ge, Z.; Li, R. *Tetrahedron* **2012**, *68*, 1315.
19. Bragnier, N.; Scherrmann, M.-C. *Synthesis* **2005**, 814.
20. Biswas, S.; Aggarwal, M.; Güzel, O.; Scozzafava, A.; McKenna, R.; Supuran, C. T. *Bioorg. Med. Chem.* **2011**, *19*, 3732.
21. Vistoli, G.; Pedretti, A.; Testa, B. *Drug Discov. Today* **2008**, *13*, 285.
22. Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
23. Calculated physicochemical properties of *C*-glycosides **1**–**8**. TPSA (Å^2) compounds **1**–**4**: −0.15; compounds **5**–**8**: −1.1. *c*Log*P* compounds **1**–**4**: 152; compounds **5**–**8**: 127. Values calculated using ChemBioDraw Ultra 12.0.
24. Rautio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Järvinen, T.; Savolainen, J. *Nat. Rev. Drug Disc.* **2008**, *7*, 255.
25. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561.
26. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.