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Carbonic anhydrase inhibitors. Inhibition of cytosolic isoforms I and II, and extracellular isoforms IV, IX, and XII with sulfamides incorporating sugar moieties

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Abstract—A series of glycosylated sulfamides possessing a diverse substitution pattern, with benzylated, peracetylated, and unsaturated six- and five-membered ring sugar moieties attached to the $NHSO_2NH_2$ zinc binding group is reported. These derivatives were tested for the inhibition of five human carbonic anhydrase (hCA, EC 4.2.1.1) isoforms, hCA I, II, IV, IX, and XII. Against hCA I the sulfamides behaved as weak inhibitors, whereas they showed low nanomolar activity against hCA II, IX, and XII, being slightly less effective as hCA IV inhibitors. One compound showed selectivity for inhibiting the tumor-associated isoforms hCA IX and XII over the ubiquitous cytosolic hCA II. The sulfamide zinc binding group may thus indeed lead to very effective glycosylated inhibitors targeting several physiologically relevant isozymes.

The attachment of sugar moieties to the scaffold of aromatic sulfonamides has recently been proven[1–3] to constitute a new approach for designing potent inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1).[4–7] Indeed, derivatization of 4-carboxy-benzenesulfonamide or sulfanilamide by means of tails incorporating various simple or more complex sugar moieties, such as in derivatives **A**–**J**,[1–3] led to sulfonamides with excellent inhibitory activity against the cytosolic isoforms CA I, and II, as well as the transmembrane, tumor-associated enzymes CA IX and XII, and the other extracellular isoform, CA XIV. Furthermore, the X-ray crystal structure of the adduct of compound **J** with the human isozyme hCA II recently reported by Di Fiore et al.[8] showed a lot of previously unknown favorable interactions between the sugar scaffold of the inhibitor and the enzyme active site, which can be exploited to obtain tighter binding and eventually isozyme-specific CA inhibitors (CAIs).[8]

Clinically used CAIs such as acetazolamide **AZA** or topiramate **TPM** show applications as antiglaucoma,[9] antiepileptic,[10] or antiobesity agents.[11] Other types of compounds, targeting the tumor-associated isoforms CA IX and XII, are being developed as diagnostic tools or for the treatment of hypoxic tumors.[7,12–15] Thus, there is great interest in this type of pharmacological agents, also due to the fact that the presently available drugs or many such investigational compounds generally indiscriminately inhibit most of the known mammalian isoforms (16 CAs are characterized in mammals until now).[4–7]

It has also been demonstrated mainly by this group[16] that in addition to the sulfonamide zinc binding group (ZBG), potent CAIs can be obtained by using sulfamate or sulfamide moieties as ZBGs. Only one sugar sulfamide derivative was investigated up to now for its interaction with various CAs, the sulfamide analogue of topiramate **TPM**.[1b] As that compound showed very interesting enzyme inhibitory properties,[1b] affording

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Keywords: Carbonic anhydrase; Isoforms; Tumor-associated isozyme; Sulfamide; Sugar.

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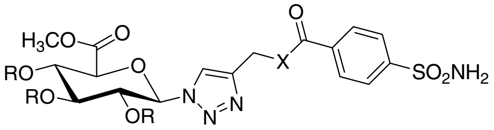
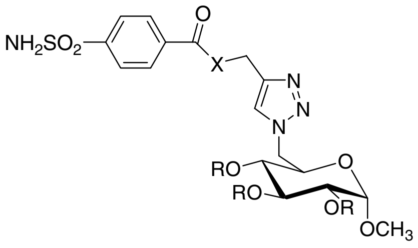
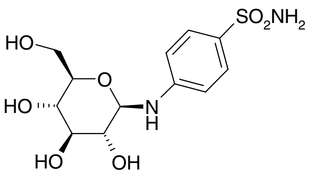


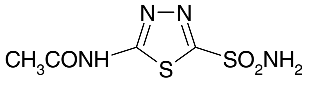
A X = NH, R = Ac, **C** X = NH, R = H
B X = O, R = Ac, **D** X = O, R = H



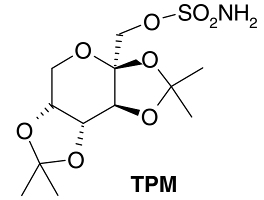
E X = NH, R = Ac, **G** X = NH, R = H
F X = O, R = Ac, **H** X = O, R = H



J



AZA



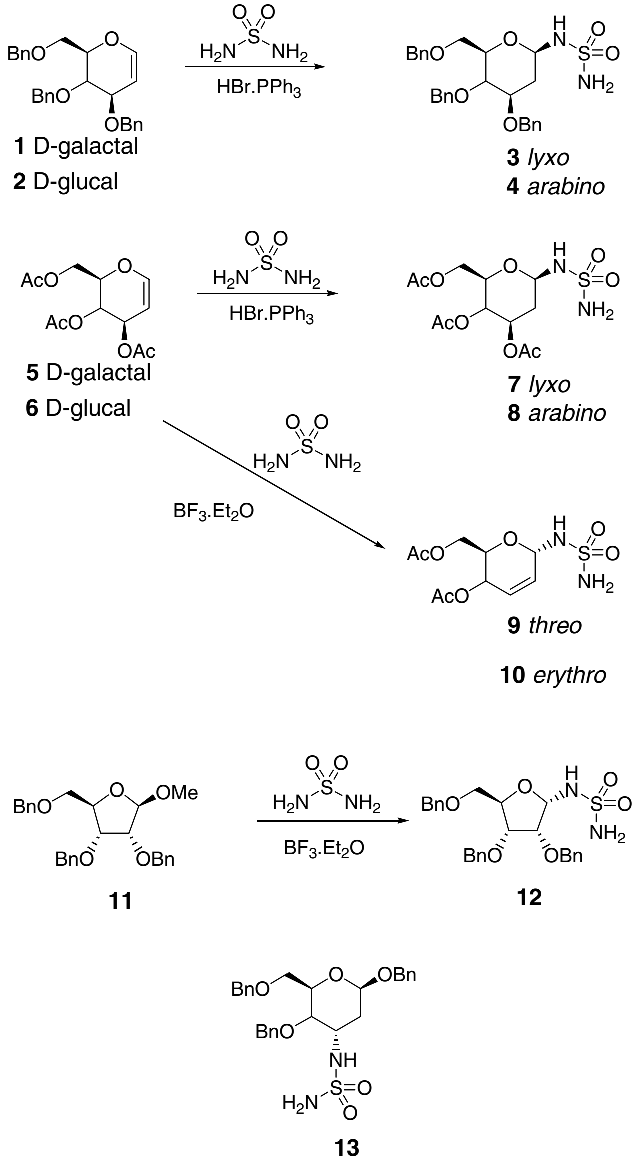
TPM

the possibility to obtain inhibitors with lower affinity for the ubiquitous isozyme CA II (but excellent affinity for other isoforms such as CA VA, VB, VII, XIII, and XIV), we decided to investigate in more detail the design of sugar sulfamides as CAIs. Here we report the preparation of a series of such sugar sulfamide derivatives as well as their interaction with five physiologically relevant CA isoforms, the cytosolic hCA I and II as well as the extracellular hCA IV, hCA IX, and hCA XII (the last two are predominantly tumor-associated enzymes).[4–7]

Benzylated *N*-glycosylsulfamides **3**–**4** have been prepared by an original procedure recently described by Colinas and Bravo,[17] implying the reaction of glycals **1**, **2** with sulfonamides catalyzed by triphenylphosphine hydrobromide (Scheme 1). Although the sulfonamidoglycosylation with sulfamide was not reported in the original procedure, this methodology afforded the *N*-glycosylsulfamides **3** and **4** with excellent yields and stereoselectivity. We extended the reaction conditions to the *O*-acetyl protected compounds **5** and **6**, and found that sulfamide reacted smoothly with tri-*O*-acetyl glycals **5**, **6** to give the *N*-glycosylsulfonamides **7**, **8** in very good yields, with the β-anomer as the preponderant product. The *N*-glycosyl sulfamides **9**–**10** were prepared by Ferrier sulfonamidoglycosylation of glycals in the presence of boron trifluoride etherate as catalyst.[19] The obtained mixture of anomeric sulfamides could be easily purified by crystallization from ethyl acetate/hexane to afford the pure α anomers. The related *O*-benzyl 2,3-unsaturated glycosyl sulfamides could not be prepared due to the preponderant formation of compound **13** by reaction of the sulfonamide with the carbenium ion at C (3) followed by the addition of the benzyl alcohol to the double bond.[17] The ribofuranosyl sulfamide **12** was prepared by sulfonamidoglycosylation of methyl benzyl ribofuranoside **11**.[18] The anomeric mixture was purified by MPLC to afford the pure α anomer. This compound was previously reported in the literature[18] but has not been tested as a CAI. All new compounds reported here were fully characterized by NMR spectroscopy and mass spectrometry (data not shown).

The new sulfamides **3**–**12** reported here and the two standard drugs acetazolamide **AZA** and topiramate **TPM** were tested for the in vitro inhibition of purified, recombinant isoforms hCA I, II (cytosolic), IV (membrane-bound), IX and XII (transmembrane, tumor-associated enzymes) (Table 1).[20,21]

The following SAR should be noted from the data of Table 1: (i) sulfamides **3**–**12**, showed weak hCA I inhibitory activity, with inhibition constants in the range of 420–830 nM, being thus less effective as compared to acetazolamide and topiramate (which possess a K_I of 250 nM against this isoform); (ii) the new sugar–sulfamides behaved on the other hand as quite effective inhibitors of the physiologically relevant and dominant isoform hCA II,[4–7] with K_Is in the low nanomolar range (of 6–23 nM). Only the *erythro* derivative **10** was a slightly weaker inhibitor (K_I of 58 nM), but anyhow appreciably inhibited this isoform too. It may be observed that both the benzyl-substituted epimers **3**



Scheme 1. Preparation of sulfamides **3**–**12**.

and **4**, as well as the corresponding peracetylated ones **7** and **8** showed comparable inhibitory power, with the less bulky peracetylated derivatives being slightly more effective than the benzylated ones. Furthermore, the configuration at C-4 of the two pairs did not influence substantially the inhibitory power, with both pairs having practically the same activity (around 20 nM for the benzylated ones **3** and **4**, and around 12 nM for the peracetylated ones **7** and **8**, respectively). However, this was not the case with the next pair *erythro*/*threo* derivatives, the hex-2-enopyranosyl sulfamides **9** and **10**: whereas **9** was a very effective hCA II inhibitor (two times more effective than **AZA** or **TPM**, K_I of 6 nM), its epimer **10** was a almost 10 times less effective enzyme inhibitor, with a K_I of 58 nM. Obviously the 4-acetyl moiety from **10** probably participates in a clash with an amino acid residue within the hCA II active site, which leads to this decreased affinity, whereas in its *threo* epimer **9**, this negative interaction is not present, and the inhibitor shows a very good binding to the enzyme. The same phenomenon has been observed for the strong CA inhibitor topiramate, over the 210 times weaker hCA II inhibitor topiramate–sulfamide (the compound analogous to **TPM** but having a $NHSO_2NH_2$ ZBG instead of the OSO_2NH_2 of **TPM**).[1b] In that case, by means of X-ray crystallography, we detected a strong clash between a methyl group of the inhibitor and the methyl of Ala65, an amino acid unique to the hCA II active site, which explained the diminished affinity of the sulfamide as compared to the cognate sulfamate **TPM**.[1b] Returning to the investigated compounds from this series of sulfamides, it may be observed that the five-membered ring derivative **12** showed comparable activity against hCA II with the corresponding benzylated six-membered ring derivatives **3** and **4** (Table 1); (iii) the new sulfamides **3**–**12** showed efficient hCA IV inhibitory activity, with K_Is in the range of 43–85 nM, in the same range as the clinically used compound **AZA** (K_I of 74 nM), but being much more effective inhibitors as compared to **TPM** (K_I of 4900 nM). Again the less bulky peracetylated derivatives **7** and **8** were better inhibitors as compared to the corresponding perbenzylated isomers **3** and **4**, whereas the unsaturated compounds **9** and **10** showed this time a very similar behavior, with no significant differences between the two epimers. The ribose derivative **12** was also an effective inhibitor, with a potency quite similar to that of **AZA**; (iv) an excellent inhibitory activity was shown by sulfamides **3**–**12** against the two tumor-associated isoforms hCA IX and XII. Thus, the inhibition constants of the new derivatives were in the range of 9–45 nM against the first isoform, and in the range of 4.8–32 nM against the second one. In this case, the least effective inhibitor was against both isozymes the furanose derivative **12**, whereas the other substitution patterns (perbenzylated, peracetylated,

Table 1. Inhibition of cytosolic isozymes hCA I, and II, membrane-bound isoform hCA IV, and transmembrane isozymes hCA IX and hCA XII with acetazolamide **AZA**, topiramate **TPM** and the new sugar sulfamides **3**–**12**

Isozymes	K_I [a] (nM)								
	AZA	**TPM**	**3**	**4**	**7**	**8**	**9**	**10**	**12**
hCA I[b]	250	250	800	720	830	830	590	780	420
hCA II[b]	12	10	20	21	12	11	6	58	23
hCA IV[c]	74	4900	85	54	43	57	63	58	76
hCA IX [d]	25	58	10	17	13	16	11	9	45
hCA XII[d]	5.7	3.8	5.4	7.2	4.8	7.8	9.8	11	32

[a] Errors in the range of 5–10% of the shown data, from three different assays, by a CO_2 hydration stopped-flow assay.[20]

[b] Human, recombinant isozymes.

[c] Human truncated (−20 aminoterminal residues) recombinant isozyme.

[d] Catalytic domain of human, cloned isoform.[13,21]

unsaturated) and the configuration at C-4 did not contribute much to activity, the compounds showing a rather compact behavior of very good inhibitor. Indeed, except for **12**, many of the new compounds investigated here are more effective inhibitors than acetazolamide and much better ones than **TPM** (which is less effective as a CA IX inhibitor). Against CA XII, the activities of these sulfamides, that of the sulfonamide AZA, and the sulfamate TPM were comparable, all these compounds being very effective inhibitors; (v) no selectivity for the inhibition of the tumor-associated isozymes over that of the ubiquitous, cytosolic one (CA II) has been observed for these compounds, except for **10**, which is an effective hCA IX and hCA XII inhibitor (K_Is of 9–11 nM) but a medium potency hCA II inhibitor (K_I of 58 nM). Thus, its selectivity ratio for the inhibition of hCA IX over hCA II is 6.44, and that for the inhibition of hCA XII over hCA II 5.27. This is a rather important result, since these ratios for acetazolamide are 0.48 and 2.10, respectively. Thus, compound **10** is practically a 6.5 times better hCA IX than hCA II and a 5.3 times better hCA XII than hCA II inhibitor. The inhibition of the house-keeping enzyme hCA II is generally considered deleterious when the aim is to inhibit selectively only the tumor-associated isoforms hCA IX and/or hCA XII.[22,23]

In conclusion, we report here a small series of glycosylated sulfamides possessing a diverse substitution pattern, with benzylated, peracetylated, and unsaturated six- and five-membered ring sugars attached to the $NHSO_2NH_2$ zinc binding group. These derivatives were tested for the inhibition of five CA isoforms: hCA I, II (cytosolic), IV (membrane-bound), IX, and XII (transmembrane, tumor-associated enzymes). Except for hCA I against which the sulfamides behaved as weak inhibitors, these new compounds showed low nanomolar activity against hCA II, IX, and XII, and were slightly less effective as hCA IV inhibitors. One compound with selectivity for inhibiting the tumor-associated isoforms hCA IX and XII over the ubiquitous hCA II has also been evidenced. Thus, the sulfamide ZBG may indeed lead to very effective inhibitors targeting several physiologically relevant CA isozymes.

Acknowledgments

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20. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561, An applied photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining constant the ionic strength), following 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 0.1 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, as reported earlier,[13] and represent means from at least three different determinations. Enzyme concentrations in the assay system were: 9.2 nM for hCA I, 7.6 nM for hCA II, 12.5 nM for hCA IV, 14 nM for hCA IX, and 13 nM for hCA XII.

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