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Thiol–ene click chemistry for the synthesis of highly effective glycosyl sulfonamide carbonic anhydrase inhibitors†

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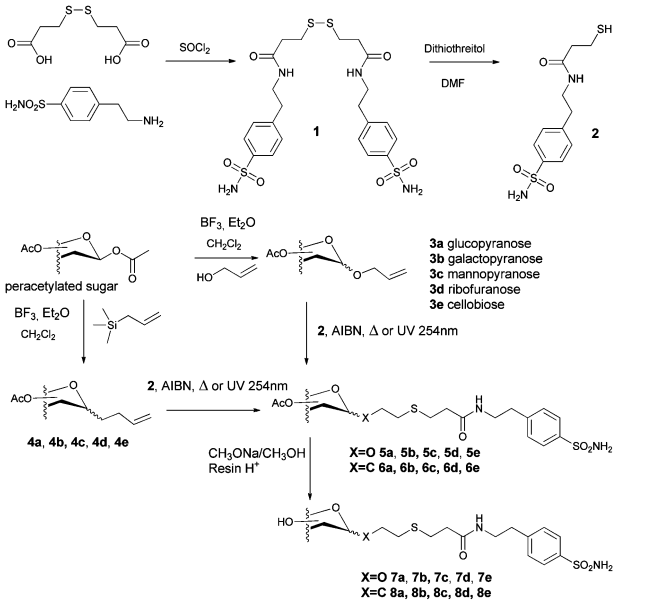
Thiol–ene click chemistry has been applied for obtaining sulfonamide carbonic anhydrase (CA, EC 4.2.1.1) inhibitors incorporating sugar moieties. Most of these new compounds were moderate CA I inhibitors, effective CA II inhibitors, and low nanomolar/subnanomolar inhibitors of the tumor-associated isoforms CA IX and XII.

The classical carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs) are the sulfonamides and their bioisosteres (sulfamates, sulfamides, *etc.*).[1–4] However, most of these compounds indiscriminately inhibit many of the 16 CA isoforms known to date in mammals.[1–3] Thus, efforts have been made to find different classes of sulfonamides with selectivity for isoforms of interest for medicinal chemistry, such as the tumor-associated ones CA IX and XII, which are overexpressed in hypoxic tumors.[4–6] Click chemistry, in its classical form of reacting azides with primary alkynes, has been much used to design novel sulfonamides incorporating 1,2,3-triazole moieties, many of which showed interesting inhibition profiles against isoforms such as CA VA, VB, IX and XII.[7–10] However, variants of this click reaction were not reported so far for the preparation of sulfonamide CA inhibitors (CAIs).

Here we report the use of thiol–ene click chemistry[11] for designing novel sulfonamide CAIs (Scheme 1). The first key synthon was the β-mercaptopropionamide of 4-(2-aminoethyl)-benzenesulfonamide **2**, obtained by routine procedures as shown in Scheme 1, by reaction of 4-(2-aminoethyl)benzenesulfonamide with the disulfide of β-mercaptopropionic acid in the presence of thionyl chloride, leading to **1** which was reduced with dithiothreitol to generate the mercapto-derivative **2**.



Scheme 1 Synthesis of the thiol–sulfonamide intermediate **2** and click chemistry reactions leading to compounds **5–7**.

As the CAIs incorporating sugar moieties were shown to lead to water soluble, isoform-selective compounds (and also to a large chemical diversity, inherent to sugar chemistry)[12,13] we used sugar scaffolds to which double bonds were appended, as the ene component for the click chemistry (Scheme 1). Peracetylated sugars were converted to *O*-allyl ether glycosides **3**, which in the presence of AIBN and UV light (or heating), reacted with thiol **2**, to generate the thioethers **5a–5e.** In order to obtain more chemical diversity, the *C*-glycosides **4a–4e** were obtained by reacting peracetylated sugars with allyltrimethylsilane in the presence of boron trifluoride as catalyst. These alkenes were then converted to thioethers **6a–6e** by reaction with **2** under free-radical conditions (AIBN and UV irradiation). Zemplén deacetylation of glycosidic derivatives **5** and **6** led to the free sugar compounds **7a–7e** and **8a–8e**. The rationale for this drug design was rather straightforward. We used the 4-aminoethyl-benzenesulfonamide

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scaffold due to the fact that many compounds generated from this derivative led to highly effective CAIs against physiologically relevant isoforms.[13,14] The β-mercaptopropionic acid moiety has been attached due to the fact that the SH group is necessary for the ene–thiol click chemistry, and we showed earlier that this moiety is also not detrimental to CA inhibitory properties of compounds incorporating it.[15] In order to generate chemical diversity, five different peracetylated mono- or disaccharides (in pyranosyl or furanosyl form) have been used in the syntheses, *i.e.*, glucopyranose, galactopyranose, mannopyranose, ribofuranose and cellobiose. We have investigated earlier the CA inhibitory properties of sulfanilamides derivatized with such sugar moieties,[12c] which not only showed excellent inhibitory properties against physiologically relevant isoforms, but also possessed very good water solubility and *in vivo* activity as antiglaucoma agents in an animal model of this disease.[12c]

The new compounds reported here were characterized extensively by spectral and physico-chemical methods which confirmed their structures (see ESI† for details).

Sulfonamides of types **5a–8e** reported here were assayed as inhibitors of four physiologically relevant CA isoforms, the cytosolic hCA I and II (h = human isoform), and the transmembrane, tumor-associated hCA IX and XII (Table 1). The clinically employed sulfonamide acetazolamide (**AAZ**, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide) has been used as standard in these measurements, for comparison reasons.

The following should be noted for the CA inhibitory properties of the compounds **5–8** reported here:

(i) Against the abundant, cytosolic isoform hCA I (present mainly in red blood cells and the gastrointestinal tract),[1–3] sulfonamides **5a–8e** showed medium-weak potency as CAIs. Thus, a group of compounds, among which are **5a**, **5b**, **7b**, **7c**, **5e**, **8b**, **6c**, and **8c**, were medium potency hCA I inhibitors with inhibition constants in the range of 79–98 nM. It may be observed that these are glucose, galactose and mannose derivatives. Some of them incorporate peracetylated sugar moieties (**5a**, **5b**, **5e**, **6c**) whereas others contain the free OH moieties of the deprotected sugars. Both *O*-glycosides and *C*-glycosides showed this activity, which makes the structure–activity relationship (SAR) rather complicated.

Table 1 CA inhibition of isoforms hCA I, II, IX and XII with sulfonamides **5–8** reported in this communication, by a CO_2 hydrase stopped-flow assay (ESI)

	K_I[a] (nM)			
Compound	hCA I	hCA II	hCA IX	hCA XII
5a	96	9.5	6.9	5.5
7a	870	66.1	0.82	0.66
5b	94	9.5	7.1	6.7
7b	88	63.2	0.92	0.8
5c	778	53.5	7.9	16.5
7c	98	9.7	0.79	0.64
5d	703	64.6	5.8	7.6
7d	399	38.5	6.8	7.5
5e	97	9.3	7.6	6.4
7e	188	10.2	7.5	5.2
6a	660	61.0	7.4	6.9
8a	418	37.3	8.3	8.5
6b	217	9.8	6.6	7.5
8b	79	7.4	0.69	0.54
6c	87	8.5	8.2	6.9
8c	96	9.4	0.75	0.63
6d	544	45.0	7.7	7.0
8d	170	10.5	8.2	8.3
6e	435	39.5	5.8	6.5
8e	713	60.8	7.5	6.4
AAZ	250	12	25	5.7

[a] Mean from 3 different assays, errors in the range of ±10% of the reported values (data not shown). Acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide, **AAZ**) was used as control.

Indeed, the remaining derivatives were much weaker as hCA I inhibitors with K_Is in the range of 170–870 nM (thus being less efficient CAIs (except for compounds **7e** and **8c**) compared to the clinically used sulfonamide **AAZ**, Table 1). In several cases, the peracetylated compound was a more effective hCA I inhibitor compared to the deacetylated one (*e.g.*, compare the pairs **5a–7a**; **5e–7e**; **6c–8c**) but most of the time, for the remaining pairs, the deprotected, free sugar derivative was the best inhibitor compared to the peracetylated one. There were no substantial differences in activity between the *O*- and *C*-glycosides. Clearly the nature of the sugar incorporated into the scaffold of these sulfonamides was the main determinant of the inhibitory activity, as for similar derivatives reported earlier by this and other groups.[7–10,12,13]

(ii) The physiologically dominant cytosolic isoform hCA II was highly inhibited by several of the new sulfonamides reported here (**5a**, **5b**, **7c**, **5e**, **7e**, **6b**, **8b**, **6c**, **8c**, and **8d**) which had K_Is in the range of 7.4–10.5 nM (comparable to that of **AAZ**, of 12 nM). These derivatives incorporate all the five sugar moieties considered here for obtaining CAIs, as well as both *O*-glycoside and *C*-glycoside derivatives. The remaining derivatives were medium efficient hCA II inhibitors, with K_Is in the range of 37.3–66.1 nM (Table 1). As for the SAR discussed above for hCA I inhibition, there are no regularities regarding the peracetylated/deacetylated compounds in their interaction with the enzyme. In some cases the peracetylated compound was more effective as an hCA II inhibitor compared to the deacetylated one (**5a**, **5b**, **5e**, **6c**, **6e**) whereas in other cases the opposite was true. Both *O*-glycosides and *C*-glycosides were present in the group of highly effective and medium potency hCA II inhibitors. One can conclude, as for hCA I, that the main factor influencing hCA II inhibitory activity is the nature of the sugar moiety present in these sulfonamides.

(iii) The tumor-associated transmembrane isoforms hCA IX and hCA XII were both potently inhibited by all sulfonamides reported here, which showed inhibition constants >10 nM, more precisely, in the range of 0.69–8.2 nM against hCA IX, and of 0.54–16.5 nM against hCA XII, respectively (Table 1). As these are validated antitumor/antimetastatic targets,[5–7] we estimate these results to be highly significant. SAR is again not very simple for the inhibition of these isoforms. For example, the five subnanomolar hCA IX inhibitors, **7a**, **7b**, **7c**, **8b** and **8c**, all incorporate free sugar moieties, and not the peracetylated ones. In fact the corresponding peracetylated compounds are almost one order of magnitude weaker CAIs compared to the deprotected ones. These compounds are glucose, galactose and mannose derivatives, but one may see that the ribose and cellobiose derivatives also show significant hCA IX inhibition

Table 2 Selectivity ratios of sulfonamides **5–8** for the inhibition of hCA II *vs.* hCA IX and hCA XII, respectively

	Selectivity ratio	
Compound	K_I(hCA II)/K_I(hCA IX)	K_I(hCA II)/K_I(hCA XII)
5a	1.37	1.72
7a	80.6	100.15
5b	1.33	1.41
7b	68.69	79
5c	6.77	3.24
7c	12.27	15.15
5d	11.13	8.5
7d	5.66	5.13
5e	1.22	1.44
7e	1.36	1.96
6a	8.24	8.84
8a	4.49	4.38
6b	1.48	1.30
8b	10.72	13.70
6c	1.03	1.23
8c	12.53	14.92
6d	5.84	6.42
8d	1.28	1.26
6e	6.81	6.07
8e	8.10	9.5
AAZ	0.48	2.1

(although, not in the subnanomolar but the low nanomolar range). As for the other isoforms discussed above, *O*-glycosides and *C*-glycosides were present in the group of highly effective and slightly less effective hCA IX inhibitors.

For hCA XII, the subnanomolar inhibitors (**7a**, **7b**, **7c**, **8b** and **8c**) were exactly the same as the hCA IX subnanomolar inhibitors, which is not so much unexpected considering that the two isoforms have a rather high degree of homology (at least in the active site amino acid residues).[16] In fact the SAR for inhibition of the two transmembrane isoforms is rather similar (Table 1).

As hCA II is a very active and ubiquitous CA isoform, with important physiological functions in many tissues/organs, one of the main problems when designing new CAIs is finding compounds which are selective for the target isoform over hCA II (selectivity towards hCA I is a less important issue due to the fact that hCA I is catalytically less efficient compared to hCA II, and also notably inhibited by the chloride and bicarbonate present in plasma).[1,2] Table 2 shows thus the selectivity ratios for inhibiting the transmembrane isoforms hCA IX and XII over the cytosolic one hCA II. It may be seen for example that the clinically used compound **AAZ** is not hCA IX selective (over hCA II) and has only a limited selectivity for inhibiting hCA XII over hCA II (with a factor of 2.1). However, many of the compounds reported in this communication do show indeed excellent selectivity ratios for inhibiting hCA IX over hCA II and hCA XII over hCA II. For example, compounds **7a**, **7b**, **7c**, **5d**, **8b** and **8c** showed selectivity ratios for inhibiting hCA IX over hCA II in the range of 10.72–80.6. As these compounds were also not highly effective as hCA I inhibitors, they can be indeed considered as hCA IX-selective compounds. For the inhibition of hCA XII over hCA IX, selectivity ratios in the range of 13.70–100.15 were registered for compounds **7a**, **7b**, **7c**, **8b** and **8c** (Table 2).

In conclusion, we have reported a series of sulfonamides incorporating sugar moieties, obtained by thiol–ene click chemistry, with excellent hCA IX and XII inhibitory properties, and selectivity for inhibiting the tumor-associated over cytosolic isoforms.

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