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Carbonic Anhydrase Glycoinhibitors belonging to the Aminoxysulfonamide Series

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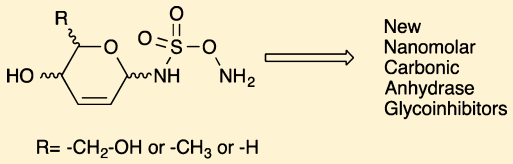
S *Supporting Information*

ABSTRACT: A general approach for the synthesis of carbonic anhydrases glycoinhibitors belonging to an aminoxysulfonamide series is presented using a Ferrier sulfonamidoglycosylation reaction on glycals. All the compounds showed good *in vitro* inhibitory activity against four human carbonic anhydrase isoforms, with selectivity against the cytosolic (hCA II) vs the tumor associated (hCA IX and XII) enzymes.



KEYWORDS: *Glycoinhibitor, carbonic anhydrase, aminoxysulfonamide, zinc binding function, Ferrier rearrangement*

The field of carbonic anhydrase (hCA, EC 4.2.1.1) inhibitors is still currently a very active area of research taking account of the involvement of different isoforms of this enzyme (15 known in human) in major pathological processes found in glaucoma, CNS diseases, obesity, and cancer. Nowadays, the development of new concepts for designing new families of compounds, capable of blocking the process catalyzed by these enzymes (i.e. reversible hydration of CO_2 into bicarbonate and a proton), remains a major concern of the research teams working in this field.[1]

The sugar approach is currently considered as one of the most successful strategies for designing small molecule CAIs targeting all isoforms known to date.[1−5] Throughout the last few years, this approach attracted considerable attention, especially in the field of tumor associated carbonic anhydrase isoforms hCA IX and hCA XII. Extensive searches in the design of selective glycoinhibitors have been accomplished, some of them having shown interesting inhibition profiles and promising antitumoral effects in preclinical experiments.[3−8] Moreover, efforts have been made to find different synthetic methodologies to prepare CAIs incorporating sugar moieties, such as, for example, the use of click reactions and their variants[9,10] or the use of Ferrier sulfonamidoglycosylation of glycals.[11] Recently, we reported an effective synthetic methodology allowing the access to 2,3-unsaturated glycosides in hydroxysulfamide series **A** starting from a peracetylated glycals platform (Figure 1) By using the nonmetallic catalyst nitrosyl tetrafluoroborate, we developed a straightforward Ferrier sulfamidoglycosylation allowing us access to original and effective CAIs in the *N*-glycosyl-*N*-hydroxysulfamides series.[12] In order to develop chemical diversity using the same carbohydrate scaffolds, we focused our research on the aminoxysulfonamide motif as new zinc binding function.[13]

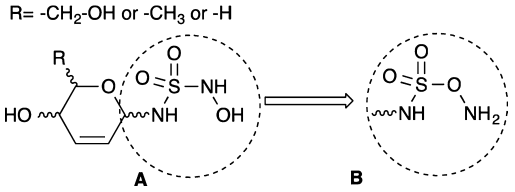
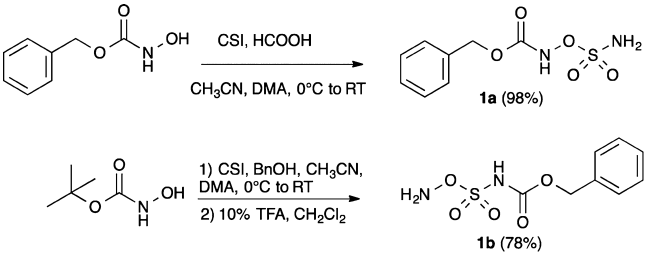


Figure 1. 2,3-Unsaturated glycosides in the hydroxysulfamide series (A) and the aminoxysulfonamide series (B).

Scheme 1. Synthesis of 1a and 1b



In this paper we wish to disclose our latest results toward the synthesis and activity of 2,3-unsaturated glycosides in the aminoxysulfonamide series using a Ferrier sulfonamidoglycosylation reaction with $NOBF_4$ as catalyst.

In order to prepare glycoinhibitor series **B**, aminooxysulfonamide synthons were synthesized according to Scheme 1. Direct sulfamoylation of the commercially available N-Cbz-hydroxylamine with sulfamoyl chloride (prepared *in situ* by

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Scheme 2. Synthesis of glycoinhibitors 4 and 5 in aminoxysulfonamide series

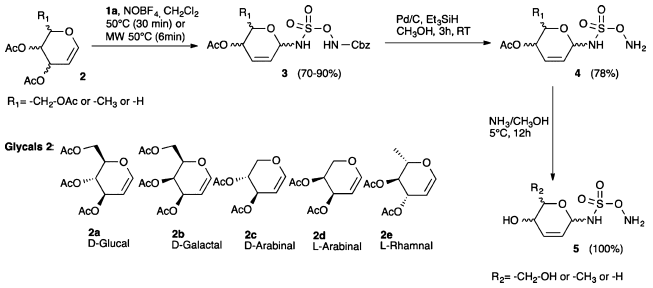

Table 1. Optimized Results for the Ferrier Sulfonamidoglycosylation and Deprotection Steps

Glycals **2**	Step 1[a] yield %/α:β ratio/ reaction time	Steps 2 + 3[b] yield %/ α:β ratio
2a	Δ: 70/35:65/30 min	78/35:65
	MW: 70/35:65/6 min	
2b	Δ: 75/43:57/30 min	78/43:57
	MW: 75/43:57/6 min	
2c	Δ: 80/48:52/30 min	78/48:52
	MW: 86/48:52/6 min	
2d	Δ: 87/47:53/30 min	78/47:53
	MW: 87/47:53/6 min.	
2e	Δ: 90/47:53/30 min	78/47:53
	MW: 93/47: 53/6 min	

[a]Reaction conditions: glycal (1) 1 equiv, **1a** 1.1 equiv, $NOBF_4$ 0.1 equiv, CH_2Cl_2. [b]10% Pd/C, Et_3SiH, MeOH then NH_3/MeOH.

reaction of chlorosulfonyl isocyanate (CSI) with formic acid) led to **1a** with high yield. Compound **1b** was also prepared as reference compound for the inhibitory assay. Reaction of *N*-Cbz-sulfamoyl chloride (prepared *in situ* by reaction of CSI with benzyl alcohol) on *N*-Boc-hydroxylamine followed by acidic deprotection with TFA solution in dichloromethane afforded **1b** in good yields (Scheme 1).

Glycoinhibitors **5** were then synthesized as depicted in Scheme 2. Starting from peracetylated glycals **2**, and using the same methodology previously reported by our group on Ferrier sulfamidoglycosylation,[12] we were able to obtain compounds **3** by reacting **2** with **1a** in the presence of $NOBF_4$ catalyst. By optimizing the amount of $NOBF_4$, the best results were obtained with 0.1 equiv of catalyst, leading to yields ranging from 70 to 90% (Table 1).

Activation of the reaction with microwaves did not allow improvement of the yield of the coupling compared with thermal activation, but an important reduction of reaction time was observed by 5-fold.

Compounds **3** were obtained as a mixture of α- and β-anomers with a slight selectivity for the β-anomer. Interestingly, the selectivity was different for the sulfamidoglycosylation,[12] where the α-anomer was mainly obtained, suggesting a kinetic control of the reaction.[14]

Then removal of the Cbz group was achieved using Pd/C and Et_3SiH in methanol to yield compounds **4**.[15] Final deprotection of acetate groups with a methanolic solution of ammonia led to glycoinhibitors **5** in quantitative yields.

Full characterization of compounds **3**, **4**, and **5** as well as the ratio of α/β-anomers were unambiguously confirmed using ^1H, ^{13}C, 2D COSY pulseprog COSYGPQF (with gradient quadrature mode; time domain size, TD = 2k; relaxation delay, d1 = 1.5 s, and scan number = 1), and HMQC experiments.

Stereochemical assignment of the major diastereoisomer was confirmed by NOESY pulseprog NOESYGPPHPP experiments (time domain size, TD = 2k; the mixing time d8 = 0.7 s; the relaxation delay d1 = 1.5 s, and scan number = 16), by observing a NOE interaction between H-1 and H-5 for the β-anomer (absent for the α-anomer). The NOE effect between H-1 and H-5 allowed discrimination between both anomers, with the chemical shifts in ^1H NMR allowing assignment of which one is predominant (see the Supporting Information).

Compounds **1a**−**1b**, **4a**−**4e**, and **5a**−**5e** 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-ssociated isoforms hCA IX and XII using a Stopped-Flow, CO_2 Hydration Assay Method.[16] Results are reported in Table 2.

Data of Table 2 show that compounds **4** and **5** reported here do show significant CA inhibitory properties. The cytosolic

Table 2. Inhibitory Activity of Compounds 4a−4e and 5a−5e against the Four CA Isoforms (hCA I, II, IX, and XII) Determined by a Stopped-Flow, CO_2 Hydration Assay Method[16,a]

	K_I (nM)[b]				Selectivity ratio	
	hCA I[c]	hCA II[c]	hCA IX[d]	hCA XII[d]	K_I hCA II/K_I hCA IX	K_I hCA II/K_I hCA XII
AAZ	250	12	25	6	0.48	2
1a	31	5	57	49	0.09	0.10
1b	300	1	77	556	0.01	0.001
4a	90	3	90	39	0.03	0.08
4b	69	5	75	43	0.07	0.12
4c	37	6	75	43	0.08	0.14
4d	55	4	86	24	0.05	0.17
4e	88	5	86	24	0.06	0.21
5a	552	8	85	58	0.10	0.14
5b	250	217	61	80	3.55	2.71
5c	50	3	91	41	0.03	0.08
5d	64	6	74	29	0.08	0.21
5e	60	4	61	56	0.06	0.07

[a]Selectivity ratios for the inhibition of the tumor-associated (hCA IX and XII) over the cytosolic (hCA II) isozyme are also reported. [b]Errors in the range of ±5−10% of the reported value from three different determinations. [c]Full length, cytosolic isoform. [d]Catalytic domain, recombinant enzyme.

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isoform hCA I was inhibited with inhibition constants ranging between 37 and 552 nM. There were no regularities in the inhibitory power of the acetylated/deacetylated derivative, and it seems that the nature of the glycal moiety is the main factor influencing the CA inhibitory properties of these compounds. hCA II; the physiologically dominant isoform, was also the most inhibited one by these compounds, which showed K_Is in the range of 3−8 nM (except **5b** which was a much weaker inhibitor, with a K_I of 217 nM).

Thus, most of the scaffolds present in these derivatives led to highly efficient CA II inhibitors (better compared to the standard sulfonamide drug acetazolamide AAZ, which had a K_I of 12 nM against this isoform). The two tumor-associated isoforms, hCA IX and XII; were less well inhibited compared to hCA II; and the K_Is ranged between 61 and 91 nM for hCA IX, and 24−80 nM against hCA XII. Thus, the glycoinhibitors reported here were effective but not highly potent as inhibitors of the transmembrane isoforms hCA IX and XII. They are also nonselective for the inhibition of the tumor-associated versus the cytosolic isoform hCA II; being thus CA II-selective inhibitors (Table 2).

Herein we reported a series a carbonic anhydrase glycoinhibitors in aminoxysulfonamide series. These compounds were prepared according to a Ferrier sulfonamidoglycosylation synthetic methodology using $NOBF_4$ as catalyst.

Inhibition assays against relevant carbonic anhydrase isoforms revealed the nanomolar activity of these inhibitors. Selectivity and strong inhibitory activity with nanomolar activity was observed against hCA II showing that aminoxysulfonamide moiety is a very good zinc binding function.

Future X-ray crystallography study should allow a better understanding of the interaction between this new ZBF and the active site that might be exploited for future investigation of new potent and selective carbonic anhydrase inhibitors.

■ ASSOCIATED CONTENT

Supporting Information

Spectroscopic details of compounds **1**, **3**, **4**, and **5** as well as copies of spectra. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00175.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

hCA, human carbonic anhydrase; CAI, carbonic anhydrase inhibitor; DMA, dimethylacetamide; TFA, trifluoroacetic acid; ZBF, zinc binding function

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