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Synthesis of Azasugar–Sulfonamide conjugates and their Evaluation as Inhibitors of Carbonic Anhydrases: the Azasugar Approach to Selectivity

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Dedicated to Professor Franco Cozzi on the occasion of his 70th birthday.

Carbonic Anhydrases (CAs; EC 4.2.1.1) are zinc metalloenzymes which play a pivotal role both in physiological and pathological processes in humans (h). Therefore, modulation of the activity of hCAs represents an appealing target for drug development, which is highly challenging due to the large number of isozymes expressed and the requirement in discovery of selective inhibitors. By following the "sugar approach" and in light of our recent disclosure of two selective hCAs inhibitors based on nitrogen containing glycomimetic–sulfonamide conjugates, twelve new azasugar–benzenesulfonamides have been synthesized. These compounds were prepared by connecting several benzenesulfonamides to a triazole armed azasugar, varying in the chain length and type of linking moiety (ureido, amido or thioureido) to probe their influence on the inhibition profile. The *in vitro* biological assays highlighted that such structural changes have remarkable effects on the hCAs inhibition profile. Several new compounds behave as selective inhibitors, and four of them are particularly effective on the therapeutically relevant hCAs II and VII isoforms.

Introduction

The carbonic anhydrases (CA, EC 4.2.1.1) are widespread zinc(II) metalloenzymes that catalyze the reversible hydration of carbon dioxide (CO_2) to give bicarbonate (HCO_3^-) and a proton (H^+).[1] This fundamental physiological reaction underpins a multitude of essential cellular processes such as respiration and acid-base regulation, electrolyte secretion, bone resorption, calcification and biosynthetic reactions.[2] Fifteen different isozymes, designated as hCAs, have been identified in humans. Five are cytosolic (hCA I, II, III, VII and XIII), four are membrane-bound or transmembrane proteins (hCA IV, IX, XII and XIV), two are mitochondrial (hCA VA and VB), and one is secreted (hCA VI). Besides being involved in physiological processes, different hCA isoforms have also been recognized to play important roles in several pathologies, such as renal disorders, elevated intraocular pressure, arteriosclerosis, memory loss, depression, obesity and oncogenesis. For example, hCA IX and XII have a fundamental role in hypoxic tumor sustainment, progression, and metastasis in several carcinomas, such as those of the cervix, colon, lungs, esophagus and breast.[3]

Therefore, hCAs have become important targets for the design of inhibitors or activators with biomedical applications. The primary sulfonamide group ($R\text{-}SO_2NH_2$) is the most important and widely used functionality for the design of CA inhibitors (CAIs), since it acts as an efficient zinc-binding moiety upon displacement of the metal-coordinated water molecule within the active site. Due to the large number of different hCA isoforms possessing highly preserved active site, there is a constant need to improve the inhibition and the selectivity profile of the new compounds, in order to avoid side effects derived from undesired altered activity of isoforms not involved in a given pathology. This is not an easy task, since the high degree of preservation in active site of isoenzymes suggests focusing on diversified secondary allosteric interactions. Therefore, the search for sulfonamide inhibitors with pendant units

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able to induce such differentiation is of paramount importance. One promising strategy that has emerged in recent years to differentiate transmembrane hCA IX from the physiologically dominant cytosolic isozymes hCA I and II has been the design of inhibitors with polar or charged tails, which hamper their ability to diffuse through lipid membranes.

An original approach to this issue, the so-called "sugar approach" suggested by Winum and co-workers,[4] opened a promising direction. The "sugar approach" is revolutionary since carbohydrates offer a vast steric and structural diversity that can be exploited to discriminate the subtle differences in the topology of the active sites surroundings of the different hCA isoforms.[5] Given their good hydrosolubility, the presence of carbohydrate moieties in the tails modifies the physicochemical properties of the inhibitors, improving their membrane permeation and thus affecting the pharmacokinetics and the mechanism of action.[6] On the other hand, use of carbohydrates in the development of new drugs has been largely hampered by the susceptibility of the glycosidic bond to hydrolysis, besides their often weak binding energies to enzyme or receptor aminoacidic residues.

Following our long-lasting experience on the synthesis of nitrogenated glycomimetics[7] and searching for more stable carbohydrates analogues that could selectively inhibit the different hCAs, we envisaged that iminosugars or azasugars[8] might offer a good alternative and by-pass the shortcomings of carbohydrates. Exploiting this assumption, we have recently synthesized the azasugar sulfonamides **1** and **2** (Figure 1), which showed potent and selective hCAs inhibition.[9] In particular, the azasugar **1** was a very strong and selective inhibitor of the central nervous system (CNS) abundantly expressed hCA VII ($K_i = 7.4$ nM) and showed a remarkable selectivity profile towards this isoform. Isoform hCA VII and the CNS physiologically dominant isoform hCA II have been recently identified as promising targets to alleviate neuropathic pain.[10] Adding a hydrolytically stable carbohydrate derived moiety, such as a levoglucosenone derivative, to give compound **2** resulted in gaining activity towards some isoforms, such as the tumor associated hCA IX ($K_i = 35.9$ nM) and mitochondrial hCA VA ($K_i = 34.1$ nM), but in an overall less satisfying selectivity profile.[9]

In this work we present our progresses in this field extending our survey on the paradigm of azasugar-benzenesulfonamide CAs inhibitors. To this objective, several new sulfonamide-azasugar conjugates structurally related to the triazole linked sulfonamide-azasugar **1** have been synthesized and their inhibitory activities have been evaluated. A structural study of the complex of one of the new inhibitors with hCA II is also reported.

Results and Discussion

Compounds **1** and **2** have been synthesized as reported in our preliminary communication (Scheme 1).[9] With the aim of understanding the subtle factors responsible for their activity, we undertook a preliminary X-ray crystallographic study in order to analyse the mode of binding of compounds **1** and **2** in complex with hCA II, one of the physiologically most relevant isoforms (data not shown). Albeit the data refinement has not been fully optimized, the electron densities clearly showed the presence of the inhibitors inside the active site of the enzyme

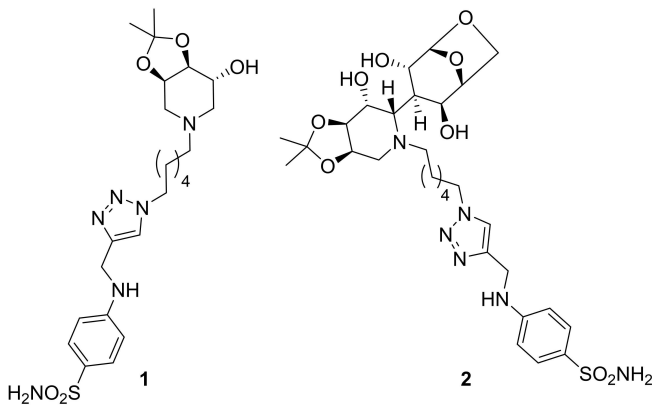
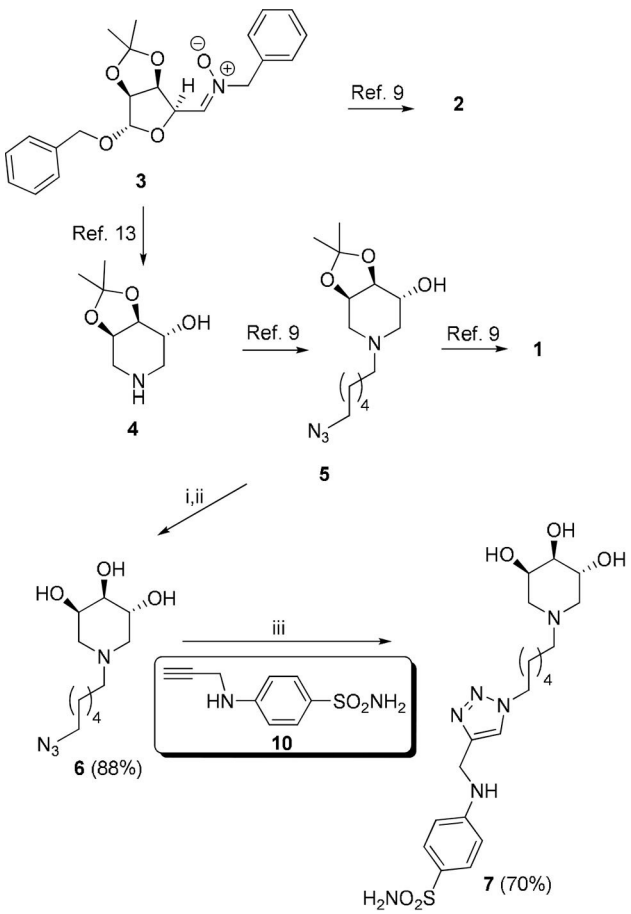


Figure 1. Azasugar-sulfonamide chimeric compounds previously synthesized as CAs inhibitors.



Scheme 1. Reagents and conditions: i) 12 M HCl, MeOH, r.t., 18 h; ii) Ambersep 900-OH, MeOH, r.t., 40 min; iii) sulfonamide **10**; $CuSO_4$, sodium ascorbate, THF:H_2O 2:1, r.t., 16 h, then Quadrasil MP® resin, MeOH, r.t., 1 h.

and fixed up to their triazole rings. Conversely, scarce electron density at the level of the inhibitors long tails testifies for a high disorder of this portion of the molecules. Very similar results have been obtained for one of the newly synthesized inhibitors (see below for details of the crystal structure of its complex with hCA II).

Based on the better selectivity profile of compound **1** towards the CAs tested and its potent inhibition of the relevant isoform hCA VII, we decided to further investigate the synthesis of other azasugar-sulfonamide conjugates shown in Figure 2. The target compounds were designed to maintain the trihydroxypiperidine skeleton (either free or partially protected as acetonide) with its configuration and linkage to triazole and to explore variations concerning the connection between the benzenesulfonamide and the triazole moieties. This part of the molecule should interact more directly with the near enzyme catalytic site and is therefore expected to have a major impact on the compound bioactivity.

We aimed in particular at replacing the amine with amide and urea or thiourea based linkers (Figure 2), accordingly to previous results obtained by some of us.

For instance, some potent inhibitors of hCAs II and IV were developed by inserting peptide residues via formation of an amide bond in *para* to the benzenesulfonamide.[6] Moreover, it was reported that the presence of an ureido linker[6] at that position favors inhibitors in establishing H-bonding interactions within the enzyme cavity.[11] Numerous examples of inhibitors deriving from 4-aminobenzenesulfonamide functionalized with thiourea linkers obtained from isothiocyanates have also been reported, some of which have proved to be effective drugs for the treatment of glaucoma.[12]

While targeting these variations, we aimed simultaneously at exploring the effects of different chain lengths connecting the triazole ring to the sulfonamide unit (Figure 2).

The key intermediate **5** used for the synthesis of the new compounds **7–9** was obtained through *N*-alkylation of piperidine **4** with 1-azido-6-bromohexane as previously reported (Scheme 1).[9] In turn, the partially protected piperidine **4** was

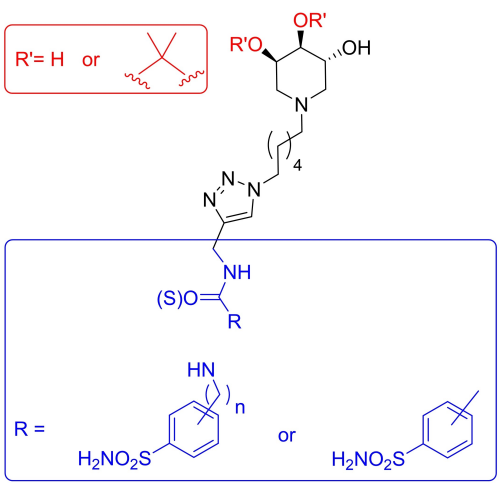


Figure 2. New azasugar-sulfonamide synthetic targets.

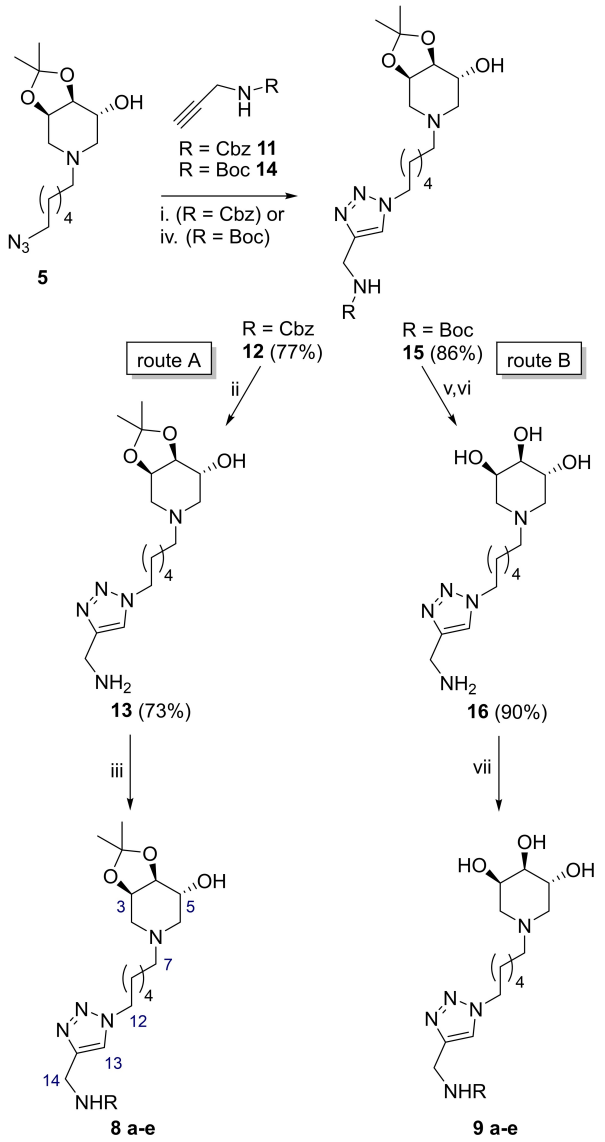
obtained by a reductive amination (RA) of nitrone **3**,[13] which occurred quantitatively and performed more satisfactorily than the direct double reductive amination (DRA) starting from its aldehyde precursor.[14]

First, we aimed at synthesizing the sulfonamide-azasugar **7**, the deprotected analog of **1**, in order to assess the effect of the acetonide moiety on inhibition. Sulfonamides are often difficult to manipulate, so that deprotection of **5** to **6** (88% yield) with HCl was firstly carried out (Scheme 1), followed by copper catalyzed azide-alkyne cycloaddition (CuAAC)[15] with sulfonamide **10** (70% yield).

Two different synthetic routes were then followed in order to obtain the two series of partially protected **8** (route A) and fully deprotected **9** (route B) azasugar-benzenesulfonamide inhibitors containing the different amido, ureido, and thioureido linkers considered (Scheme 2). Both strategies started with a CuAAC reaction of piperidine **5** with an *N*-protected propargylamine, since several attempts using unprotected propargylamine met with little success, leading to low yields of product. The two strategies differed in the protecting group, with route A utilizing an orthogonal carbobenzyloxy (Cbz) protection with respect to the acetonide while route B involved a *tert*-butoxycarbonyl (Boc) group removable under the same acidic conditions.

The triazole-azasugar **12** was obtained in 77% yield through the CuAAC reaction of piperidine **5** with the *N*-Cbz protected propargylamine **11**. Hydrogenolytic removal of the Cbz protecting group over $Pd(OH)_2/C$ gave the amine **13** (73% yield), which was coupled with several benzenesulfonamide derivatives bearing a carbamate (**17**), an activated ester (**18**) or an isothiocyanate group (**19-21**), affording five different azasugar-benzenesulfonamides **8a–e** with ureido, amido or thioureido groups connecting the triazole moiety to the benzenesulfonamide (Scheme 2 and Table 1). In particular, the urea **8a** was obtained from **13** by means of an amine-carbamate coupling reaction with sulfonamide **17** performed at 78 °C in 64% yield. The amide **8b** was obtained in 75% yield through a coupling reaction with sulfonamide **18** in the presence of triethylamine. The thioureas **8c**, **8d** and **8e** were obtained by amine-isothiocyanate coupling reactions with sulfonamides **19**, **20** and **21** in the presence of triethylamine in 50%, 96% and 92% yield, respectively. Only the synthesis of **8e** required heating the reaction mixture at 78 °C, the other coupling reactions occurring to completion at room temperature.

For the synthesis of the corresponding azasugar-benzenesulfonamide inhibitors **9a–e** with the free hydroxy groups at the piperidine ring, an initial CuAAC reaction of piperidine **5** was carried out as before with the *N*-Boc protected propargylamine **14** to afford the triazole **15** in 86% yield. Removal of both Boc and acetonide by acid treatment afforded the azasugar **16** in 90% yield with free amino group (Scheme 2). Coupling of the amine **16** with the same benzenesulfonamide derivatives **17–21** as above afforded five different azasugar derivatives **9a–e** displaying an ureido, amido or thioureido linked benzenesulfonamide to the triazole moiety (Scheme 2 and Table 1). In particular, the urea **9a** was obtained in 81% yield by means of an amine-carbamate coupling reaction at



Scheme 2. Reagents and conditions: i) $CuSO_4$, sodium ascorbate, THF:H_2O 2:1, r.t., 16 h, then Quadrasil MP® resin, MeOH, r.t., 1 h; ii) $Pd(OH)_2/C$, MeOH, r.t., H_2, 19 h; iii) CH_3CN:EtOH 1:1; see Table 1; iv) $CuSO_4$, sodium ascorbate, THF:H_2O 2:1, r.t., 23 h, then Quadrasil MP® resin, MeOH, r.t., 1 h; v) 12 M HCl, MeOH, r.t., 18 h; vi) Ambersep 900-OH, MeOH, r.t., 40 min; vii) CH_3CN:EtOH 1:1; see Table 1.

78 °C with sulfonamide **17**. The amide **9b** was obtained in 87% yield through the amine-carboxylic coupling reaction at room temperature with sulfonamide **18** in the presence of triethylamine. The thioureas **9c**, **9d** and **9e** were obtained by addition to the isothiocyanate sulfonamides **19**, **20** and **21** in 75%, 88% and 73% yields, respectively, in the presence of triethylamine and heating at 78 °C.

Biological evaluation

The newly synthesized compounds **7**, **8a–e** and **9a–e** were tested towards eleven isoforms of hCA in order to evaluate the effect of the different functional groups and linkers on the enzymatic inhibitory activity. The results are reported in Table 2 and compared to the previously reported compound **1**. In particular, concerning the effect of deprotection at the trihydroxy piperidine ring, it is interesting to note an inversion of the selectivity inhibitory profiles for compounds **1** and **7** bearing the amine linker. Deprotected **7** was a less potent inhibitor of hCA VII (K_i = 23.4 nM) compared to **1** (K_i = 7.4 nM), but showed much more powerful towards hCA XII (K_i = 7.1 nM *vs* K_i = 541.0 nM). The same trend was observed for the thiourea **8d** *vs* its deprotected analog **9d** towards hCA VII (K_i = 9.6 nM *vs* K_i = 53.3 nM) and hCA XII (K_i = 69.5 nM *vs* K_i = 8.5 nM). This difference was not observed for the protected urea **8a** *vs* its deprotected analog **9a**, both good inhibitors of hCAs VII (overexpressed in the CNS) and XII (tumor associated), nor for the compounds **8b** and **9b** bearing the amide moiety. Notably, the deprotected thiourea **9a** was a 7-fold more potent inhibitor (K_i = 9.3 nM) than the reference acetazolamide AAZ (K_i = 63.0 nM) towards hCA VA.

Regarding the deprotected thiourea linked benzenesulfonamides **9c-e**, structural changes and substitution of the benzenesulfonamide derivatives remarkably affected the inhibitory profile. Indeed, the *para* substituted **9c** showed good inhibitory activity for the hCA VA (K_i = 25.1 nM, 2.5 times smaller than that of the reference AAZ), while the *meta* substituted **9d** showed a potent and selective inhibitory activity for hCA XII (K_i = 8.5 nM). Interestingly, the *para* homologue with a two-carbon atom linker between the thiourea and the benzene ring **9e** showed good inhibitory activity towards hCA I (K_i = 27.0 nM, 9 times smaller than that of the reference AAZ), VA (K_i = 24.8 nM, almost 3 times smaller than that of the reference AAZ) and XII (K_i = 9.7 nM), therefore being less selective. Remarkably, the protected **8e** was a potent inhibitor of hCA II (K_i = 5.0 nM), doubling the potency of reference AAZ (K_i = 12.1 nM).

As mentioned above, inhibitors of isoforms CA II and VII recently showed neuropathic pain attenuating effects.[10] Considering the selectivity profile observed on hCAs VII and II for the amino-linked prototype compound **1**, together with its thioureido analogs **8d** and **8e** and its amido analog **8b**, we deem that these compounds may have promising therapeutic applications in CNS-related pathologies.

Crystallographic study

An X-ray crystallographic study was undertaken in order to analyze the binding mode of this class of compounds in complex with hCA II.

The results for the complex **8b**·hCA II are depicted in Figure 3. A well-defined electron density inside the active site of the protein was present in the Fo–Fc map, compatible with the presence of the inhibitor (Figure 3A). On the other hand, the long tail of inhibitor presents high disorder, which suggests the absence of stable interactions with the rim out of the active site of hCA II. However, compound **8b** appears well fixed up to the triazole ring.

Table 1. Reagents, reaction conditions and yields for the preparation of compounds **8a-e** and **9a-e** in Scheme 2.

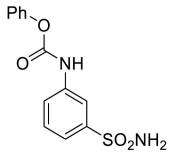
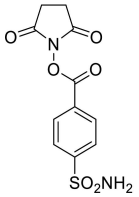
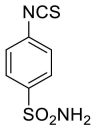
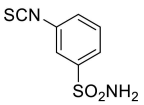
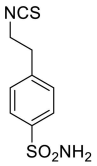
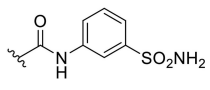
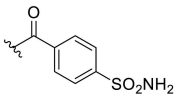
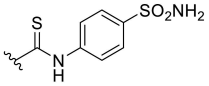
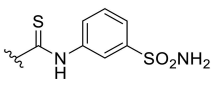
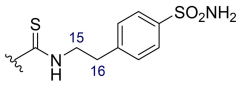
Reagent					
	17	**18**	**19**	**20**	**21**
R =					
Product **a**	**8a** (64%)	**8b** (75%)	**8c** (50%)	**8d** (96%)	**8e** (92%)
Conditions for step iii	r.t.-> 78 °C, 4 d	NEt_3, r.t., 16 h	NEt_3, r.t., 23 h	NEt_3, r.t., 18 h	NEt_3, r.t.-> 78 °C, 47 h
Product **b**	**9a** (81%)	**9b** (87%)	**9c** (75%)	**9d** (88%)	**9e** (73%)
Conditions for step vii	78 °C, 3 d	NEt_3, r.t., 18 h	NEt_3, 78 °C, 3 d	NEt_3, 78 °C, 3 d	NEt_3, 78 °C, 3 d

Table 2. Inhibition data of inhibitors **1**, **7**, **8a–e** and **9a–e** on hCAs I, II, III, IV, VA. VB, VI, VII, IX, XII and XIII by a Stopped Flow CO_2 Hydrase Assay. Acetazolamide (AAZ) was used as reference inhibitor.

	K_i (nM)[a]										
Cpd	hCA I	hCA II	hCA III	hCA IV	hCA VA	hCA VB	hCA VI	hCA VII	hCA IX	hCA XII	hCA XIII
1	2880.0	80.3	n. d.[b]	80.9	70.3	812.7	68.7	**7.4**	2980.0	541.0	65.3
7	3792.0	769.0	> 10000	7693.0	84.5	484.7	83.8	23.4	316.1	**7.1**	450.8
8a	576.9	523.6	> 10000	8209.0	83.5	294.1	92.5	32.3	84.6	**19.1**	87.1
9a	2676	783.5	> 10000	7452.0	**9.3**	371.6	77.7	**18.8**	428.7	22.6	280.0
8b	70.5	41.3	> 10000	4301.0	90.5	346.5	91.2	27.9	365.7	**9.6**	92.7
9b	73.4	64.7	> 10000	5463.0	88.3	380.4	188.2	22.3	363.3	**19.4**	261.2
8c	265.4	52.1	> 10000	440.5	348.0	425.3	478.9	76.1	434.8	797.0	82.5
9c	69.1	80.0	> 10000	5824.0	**25.1**	596.6	213.2	44.9	234.4	34.9	829.3
8d	2899.0	629.7	> 10000	767.1	127.0	61.3	157.2	**9.6**	395.5	69.5	52.2
9d	675.7	956.2	> 10000	7042.0	66.0	223.3	94.8	53.3	158.9	**8.5**	89.9
8e	45.3	**5.0**	> 10000	681.5	151.0	70.0	39.3	87.0	133.1	19.7	53.8
9e	**27.0**	80.7	> 10000	5298.0	**24.8**	412.3	86.4	51.0	483.6	**9.7**	720.0
AAZ	250.0	12.1	> 10000	74.0	63.0	54.0	11.0	2.5	25.8	5.7	17.0

[a] Mean from three different assays, by a stopped flow technique (errors were in the range of ±5–10% of the reported values). [b] n. d. = not determined.

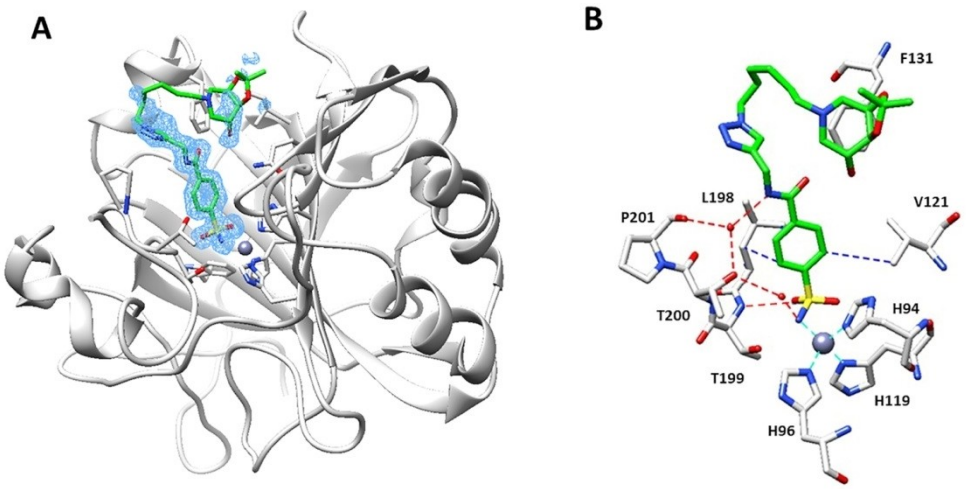


Figure 3. X-ray crystal structure of hCA II bound with compound **8b** (Part **A**, PDB: 7NH8). Part **B** shows its interactions in the active site (hydrogen bonds and hydrophobic interactions are labelled in red and blue, respectively). Residues involved in the binding of inhibitors are also shown and the gray sphere represents the zinc atom in the active site of the proteins.

The sulfonamide moiety showed the canonical tetrahedral coordination with the zinc ion reported for all CA-sulfonamide adducts characterized by this technique to date.[16]

The zinc bound sulfonamide NH makes also a strong H-bond with the OH of Thr199, an interaction observed in all CA-sulfonamide adducts investigated so far.

In addition, several hydrophobic interactions of the aromatic ring bound to the sulfonamide group and the side chains of Leu198 and Val121 stabilize the complex. Furthermore, a network of water bridge bonds is present which stabilizes the amide moiety (Figure 3B).

On the other hand, the long tail of the inhibitor, protruding outside of the cavity, likely presents high disorder due to the absence of stable interactions with the active site rim, as inferred from the scarce electron density present in the maps for this portion of the molecule. Only in proximity of the azasugar moiety a somewhat better defined electron density was revealed, which may denote a subtle role played by the trihydroxypiperidine ring in modulating the inhibitors activity.

Conclusion

The large number of hCA isoforms involved in different physiological contexts triggers a continuous and essential need for improving the profile of new inhibitors to address selectively the isoform(s) connected to a given pathology. To this aim, we have advanced the concept of the "sugar approach" to an "azasugar approach", extending our previous study on an azasugar-benzenesulfonamide prototype to a series of structural analogs. Switching from a carbohydrate to an azasugar moiety is significant: not only the latter is hydrolytically stable, but also the presence of a basic nitrogen may help inhibitors in establishing stronger electrostatic interactions with enzyme residues at physiological pH values, experiencing protonation and conformational changes.[17] Besides the prototypical inhibitor **1** and its fully deprotected parent compound **7**, other ten derivatives **8a**–**e** and **9a**–**e** have been obtained by connecting five different benzenesulfonamide moieties to a partially protected or fully deprotected triazole-armed azasugar, respectively. The triazole ring has been conserved, since preliminary structural studies indicated its firm setting inside the hCA II enzyme cavity. Conversely, modifications in the linking functional group (ureido, amido or thioureido) to the benzenesulfonamide and its orientation and chain length have been considered in order to highlight subtle structural factors for improving the products selectivity profile. The introduced structural changes in the new compounds pointed up a remarkable effect on their selectivity profile and allowed to identify several new selective inhibitors of therapeutically relevant hCA isoforms. In particular, the prior azasugar-benzenesulfonamide **1** and its thioureido analogs **8d** and **8e** behave as selective inhibitors of hCAs II and VII, which are involved in neuropathic pain. An X-ray crystallographic study of the complex **8b**·hCA II showed the binding of the azasugar-benzenesulfonamide into the enzyme catalytic site and its rigidity up to the triazole ring. While its tail is associated with a high disorder, more defined electron density is suggestive for a role of the azasugar moiety in achieving secondary interactions with the enzyme residues.

Experimental Section

General methods: Commercial reagents were used as received. All reactions were carried out under magnetic stirring and monitored by TLC on 0.25 mm silica gel plates (Merck F254). Column chromatographies were carried out on Silica Gel 60 (32–63 μm) or on silica gel (230–400 mesh, Merck). Yields refer to spectroscopically and analytically pure compounds unless otherwise stated. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 200 MHz, a Varian Mercury 400 MHz or on a Varian INOVA 400 MHz instruments at 25 °C. Chemical shifts are reported relative to $CDCl_3$ (^{13}C: δ = 77.0 ppm). Integrals are in accordance with assignments, coupling constants are given in Hz. For detailed peak assignments 2D spectra were measured (COSY, HSQC, NOESY, and NOE as necessary). Small scale microwave assisted syntheses were carried out in a microwave apparatus for synthesis (CEM Discover) with an open reaction vessel and external surface sensor. IR spectra were recorded with a BX FTIR Perkin-Elmer system spectrophotometer. ESI-MS spectra were recorded with a Thermo Scientific™ LCQ fleet ion trap mass spectrometer. Elemental analyses were performed with a Thermo Finnigan FLASH EA 1112 CHN/S analyzer. Optical rotation measurements were performed on a JASCO DIP-370 polarimeter. Names of compounds follow the IUPAC nomenclature rules; however, the assignment of H and C atoms in NMR characterizations reflects the numbering of chemical structures in Scheme 2 and in the Supporting Information for practical reasons.

(3a*R*,7*R*,7a*S*)-2,2-Dimethylhexahydro[1,3]dioxolo[4,5-*c*]pyridin-7-ol (4):[13] To a solution of nitrone **3** (915 mg, 2.39 mmol) in dry MeOH (150 mL) acid acetic (2 equivalents) and Pd/C (458 mg) were added under nitrogen atmosphere. The mixture was stirred at room temperature under hydrogen atmosphere (balloon) for 2 days, until a control by ^{1}H NMR spectroscopy attested the presence of acetate salt of **4**. The mixture was filtered through Celite® and the solvent was removed under reduced pressure. The corresponding free amine was obtained by dissolving the residue in MeOH, then the strongly basic resin Ambersep 900-OH was added, and the mixture was stirred for 40 minutes. The resin was removed by filtration to afford compound **4** (414 mg, 2.39 mmol, 100% yield) as a white solid with spectral data in agreement with those reported previously.[14] ^{1}H NMR (200 MHz, CD_3OD): δ = 4.26-4.16 (m, 1H, H-3), 3.91 (pt, J = 6.1 Hz, 1H, H-4), 3.75-3.62 (m, 1H, H-5), 3.13 (dd, J = 14.5, 2.5 Hz, 1H, Ha-2), 3.01–2.94 (m, 1H, Ha-6), 2.94–2.86 (m, 1H, Hb-2), 2.38 (dd, J = 13.1, 9.2 Hz, 1H, Hb-6), 1.50 (s, 3H, *Me*), 1.35 (s, 3H, *Me*) ppm.

(3a*R*,7*R*,7a*S*)-5-(6-Azidohexyl)-2,2-dimethylhexahydro[1,3]dioxolo[4,5-*c*]pyridin-7-ol (5):[9] To a solution of compound **4** (48 mg, 0.28 mmol) in acetonitrile (2 mL) and milliQ water (0.6 mL), 1-azido-6-bromohexane (86 mg, 0.41 mmol) and potassium carbonate (57 mg, 0.41 mmol) were added. The mixture was stirred under microwave irradiation at 120 °C for 2.5 hours, until a TLC control attested the disappearance of the starting material (CH_2Cl_2: MeOH 10:1). The mixture was filtered through Celite® and the solvent was removed under reduced pressure. The crude reaction was purified by silica gel flash column chromatography (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 50:1:0.1, R_f = 0.2) obtaining compound **5** (45 mg, 0.15 mmol, 55% yield) as a yellow oil with spectral data in agreement with those reported previously.[9] ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.27 (pq, J = 5.7 Hz, 1H, H-3), 4.01 (pt, J = 4.3 Hz, 1H, H-4), 3.93–3.91 (m, 1H, H-5), 3.24 (t, J = 6.7 Hz, 2H, H-12), 2.70 (dd, J = 11.8, 5.8 Hz, 1H, Ha-2), 2.56 (dd, J = 11.8, 3.0 Hz, 1H, Ha-6), 2.42–2.34

(m, 4H, Hb-2, Hb-6, H-7), 1.61–1.54 (m, 2H, H-11), 1.50–1.29 (m, 12H, H-8, H-9, H-10, *Me*, *Me*) ppm.

(3*R*,5*R*)-1-(6-Azidohexyl)piperidine-3,4,5-triol (6): A solution of compound **5** in MeOH (0.015 M) was left stirring with 12 M HCl (120 μL) at room temperature for 18 h. The crude mixture was concentrated to yield the hydrochloride salt of **6**. The corresponding free amine was obtained by dissolving the residue in MeOH, then the strongly basic resin Ambersep 900-OH was added, and the mixture was stirred for 40 minutes. The resin was removed by filtration and the crude product was purified by silica gel flash column chromatography (CH_2Cl_2:MeOH:NH_4OH (6%) from 15:1:0.1, R_f = 0.20) obtaining compound **6** (83.4 mg, 0.323 mmol, 88% yield) as a colorless oil. $[\alpha]_D^{22}$: −25.3 (MeOH, *c* = 0.8). ^{1}H NMR (400 MHz, CD_3OD): δ = 3.94–3.91 (m, 1H, H-3), 3.84–3.80 (m, 1H, H-5), 3.43–3.35 (m, 1H, H-4), 3.29 (t, *J* = 6.9 Hz, 2H, H-12), 2.86–2.83 (m, 2H, Ha-2, Ha-6), 2.47–2.14 (m, 4H, Hb-2, Hb-6, H-7), 1.64–1.29 (m, 8H, H-8, H-9, H-10, H-11) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 75.0 (d, 1 C, C-4), 69.3 (d, 1 C, C-5), 68.2 (d, 1 C, C-3), 58.9 (t, 1 C, C-7), 57.9 (t, 1 C, C-6), 57.3 (t, 1 C, C-2), 52.3 (t, 1 C, C-12), 29.7 (t, 1 C, C-11), 28.0 (t, 1 C), 27.6 (t, 1 C), 27.2 (t, 1 C) ppm. $C_{11}H_{22}N_4O_3$: MS-ESI (m/z, %): 259.24 (100, $[M+H]^+$). Calcd.: C, 51.15; H, 8.58; N, 21.69; found: C, 51.42; H, 8.74; N, 21.41.

4-{[(1-{6-[(3*R*,5*R*)-3,4,5-Trihydroxypiperidin-1-yl]hexyl}-1*H*-1,2,3-triazol-4-yl)methyl]amino}benzenesulfonamide (7): Compound **10** (43.6 mg, 0.207 mmol), $CuSO_4$ (9.05 mg, 0.056 mmol) and sodium ascorbate (22 mg, 0.113 mmol) was added to a solution of compound **6** (48.7 mg, 0.188 mmol) in THF (2 mL) and milliQ water (1 mL). The reaction mixture was stirred at room temperature for 16 hours, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2: MeOH: NH_4OH (6%) 10:1:0.1). The mixture was filtered through Celite®, the solvent was removed under reduced pressure and subsequently the crude mixture was treated with 'Quadrasil MP®' resin keeping the mixture under stirring at room temperature in the minimum amount of MeOH for 1 hour (1 g of resin for each mmol of copper). The crude was purified by silica gel flash column chromatography in a gradient eluent (eluent CH_2Cl_2:MeOH:NH_4OH (6%) from 15:1:0.1 to 3:1:0.1) obtaining compound **7** (62.7 mg, 0.134 mmol, R_f = 0.08 eluent CH_2Cl_2:MeOH:NH_4OH (6%) 15:1:0.1, 70% yield) as a yellow oil. $[\alpha]_D^{22}$: −8.5 (MeOH, *c* = 0.7). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.86 (s, 1H, H-13), 7.61 (d, *J* = 8.4 Hz, 2H, *Ar*), 6.69 (d, *J* = 8.4 Hz, 2H, *Ar*), 4.45 (s, 2H, H-14), 4.35 (t, *J* = 7.0 Hz, 2H, H-12), 3.93–3.92 (m, 1H, H-3), 3.85–3.80 (m, 1H, H-5), 3.48–3.40 (m, 1H, H-4), 2.85–2.78 (m, 2H, Ha-2, Ha-6), 2.45–2.14 (m, 4H, Hb-2, Hb-6, H-7), 1.89–1.82 (m, 2H, H-11), 1.47–1.27 (m, 6H, H-8, H-9, H-10) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 152.7 (s, 1 C, *Ar*), 146.9 (s, 1 C, *Ar*), 131.3 (s, 1 C, *triazole*), 128.8 (d, 2 C, *Ar*), 124.1 (d, 1 C, C-13), 112.7 (d, 2 C, *Ar*), 74.8 (d, 1 C, C-4), 69.3 (d, 1 C, C-5), 68.7 (d, 1 C, C-3), 58.8 (t, 1 C, C-7), 57.8 (t, 1 C, C-6), 57.0 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 39.4 (t, 1 C, C-14), 31.1 (t, 1 C, C-11), 27.7 (t, 1 C), 27.2 (t, 1 C), 27.0 (t, 1 C) ppm. $C_{20}H_{32}N_6O_5S$: MS-ESI (m/z, %): 469.25 (100, $[M+H]^+$), 491.33 (78, $[M+Na]^+$), 958.17 (40, $[2\,M+Na]^+$). Calcd: C, 51.27; H, 6.88; N, 17.94; found: C, 51.50; H, 7.01; N, 17.72.

Benzyl [(1-{6-[(3a*R*,7*R*,7a*S*)-7-hydroxy-2,2-dimethyltetrahydro[1,3] dioxolo[4,5-*c*]pyridin-5(4*H*)-yl]hexyl}-1*H*-1,2,3-triazol-4-yl) methyl]carbamate (12): Compound **11** (97 mg, 0.51 mmol), $CuSO_4$ (22 mg, 0.14 mmol) and sodium ascorbate (56 mg, 0.28 mmol) was added to a solution of compound **5** (139 mg, 0.47 mmol) in THF (2 mL) and milliQ water (1 mL). The reaction mixture was stirred at room temperature for 16 hours, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1). The mixture was filtered through Celite®, the solvent was removed under reduced pressure and subsequently the crude mixture was treated with 'Quadrasil MP®' resin keeping the mixture under stirring at room temperature in the minimum amount of MeOH for 1 hour (1 g of resin for each mmol of copper). The crude was purified by silica gel flash column chromatography (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 25:1:0.1, R_f = 0.40) obtaining compound **12** (175 mg, 0.36 mmol, 77% yield) as a yellow oil. $[\alpha]_D^{24}$: −18.1 (MeOH, *c* = 0.8). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.82 (s, 1H, H-13), 7.34–7.28 (m, 5H, *Ar*), 5.09 (s, 2H, O-C$\underline{H}_2$-Ph), 4.37–4.33 (m, 4H, H-14, H-12), 4.27 (*pq*, *J* = 3.8 Hz, 1H, H-3), 3.84–3.77 (m, 2H, H-4, H-5), 3.00 (d, *J* = 12.5 Hz, 1H, Ha-2), 2.72 (dd, *J* = 12.2, 3.8 Hz, 1H, Ha-6), 2.42–2.32 (m, 3H, Hb-2, H-7), 1.99 (dd, *J* = 11.2, 8.9 Hz, 1H, Hb-6), 1.90–1.85 (m, 2H, H-11), 1.48–1.33 (m, 12H, H-8, H-9, H-10, *Me, Me*) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 158.7 (s, 1 C, CO), 146.8 (s, 1 C, *Ar*), 138.2 (s, 1 C, *triazole*), 129.5 (d, 2 C, *Ar*), 129.0 (d, 1 C, *Ar*), 128.8 (d, 2 C, *Ar*), 123.9 (d, 1 C, C-13), 110.2 (s, 1 C, *acetonide*), 80.2 (d, 1 C, C-4), 74.5 (d, 1 C, C-3), 70.5 (d, 1 C, C-5), 67.6 (t, 1 C, O-C$\underline{H}_2$-Ph), 58.9 (t, 1 C, C-7), 57.7 (t, 1 C, C-6), 54.9 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 37.1 (t, 1 C, C-14), 31.1 (t, 1 C, C-11), 28.5 (q, 1 C, *Me*), 27.8 (t, 1 C), 27.2 (t, 2 C), 26.6 (q, 1 C, *Me*) ppm. IR (MeOH): 3610, 3300, 3068, 3012, 2966, 2929, 2806, 2758, 1705, 1526, 1042 cm^{-1}. $C_{25}H_{37}N_5O_5$: MS-ESI (m/z, %): 488.29 (100, $[M+H]^+$), 510.32 (73, $[M+Na]^+$). Calcd.: C, 61.58; H, 7.65; N, 14.36; found: C, 61.33; H, 7.81; N, 14.57.

(3a*R*,7*R*,7a*S*)-5-{6-[4-(Aminomethyl)-1*H*-1,2,3-triazol-1-yl]hexyl}-2,2-dimethylhexahydro[1,3]dioxolo[4,5-*c*]pyridin-7-ol (13): To a solution of compound **12** (163 mg, 0.33 mmol) in dry MeOH (9 mL) $Pd(OH)_2$/C (82 mg) was added under nitrogen atmosphere. The mixture was stirred at room temperature under hydrogen atmosphere (balloon) for 19 hours, until a control by ^{1}H NMR spectroscopy attested the disappearance of the starting material. The mixture was filtered through Celite® and the solvent was removed under reduced pressure. The crude was purified by silica gel flash column chromatography (eluent CH_2Cl_2:MeOH:NH_4OH (6%) from 10:1:0.1 to 7:1:0.1) obtaining compound **13** (85.3 mg, 0.24 mmol, R_f = 0.45 eluent CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1, 73% yield) as a colorless oil. $[\alpha]_D^{24}$: +8.6 ($CHCl_3$, *c* = 0.6). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.89 (s, 1H, H-13), 4.40 (t, *J* = 7.0 Hz, 2H, H-12), 4.28 (*pq*, *J* = 3.7 Hz, 1H, H-3), 3.91 (s, 2H, H-14), 3.85-3.77 (m, 2H, H-4, H-5), 3.01 (d, *J* = 13.1 Hz, 1H, Ha-2), 2.72 (dd, *J* = 11.8, 3.9 Hz, 1H, Ha-6), 2.44–2.33 (m, 3H, Hb-2, H-7), 1.99 (dd, *J* = 11.4, 9.2 Hz, 1H, Hb-6), 1.95–1.88 (m, 2H, H-11), 1.48–1.34 (m, 12H, H-8, H-9, H-10, *Me, Me*) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 149.1 (s, 1 C, *triazole*), 123.4 (d, 1 C, C-13), 110.1 (s, 1 C, *acetonide*), 80.3 (d, 1 C, C-4), 74.5 (d, 1 C, C-3), 70.5 (d, 1 C, C-5), 58.9 (t, 1 C, C-7), 57.7 (t, 1 C, C-6), 55.0 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 37.4 (t, 1 C, C-14), 31.2 (t, 1 C, C-11), 28.5 (q, 1 C, *Me*), 27.7 (t, 1 C), 27.3 (t, 2 C), 26.6 (q, 1 C, *Me*) ppm. IR ($CHCl_3$): 3848, 3670, 3597, 3379, 3018, 2991, 2939, 2825, 1284 cm^{-1}. $C_{17}H_{31}N_5O_3$: MS-ESI (m/z, %): 354.30 (61, $[M+H]^+$), 376.34 (65, $[M+Na]^+$), 728.99 (100, $[2\,M+Na]^+$). Calcd.: C, 57.77; H, 8.84; N, 19.81; found: C, 57.90; H, 8.62; N, 19.70.

3-[({[(1-{6-[(3a*R*,7*R*,7a*S*)-7-Hydroxy-2,2-dimethyltetrahydro[1,3] dioxolo[4,5-*c*]pyridin-5(4*H*)-yl]hexyl}-1*H*-1,2,3-triazol-4-yl)methyl] amino}carbonyl)amino]benzenesulfonamide (8a): Compound **17** (14.5 mg, 0.049 mmol) was added to a solution of compound **13** (17.4 mg, 0.049 mmol) in EtOH (1.5 mL) and CH_3CN (1.5 mL). The reaction mixture was stirred for 17 hours at room temperature, until a TLC control attested the appearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 8:1:0.1). The reaction mixture was heated at 78 °C for 3 days, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 8:1:0.1). The mixture was concentrated under reduced pressure, the crude was purified by silica gel flash column chromatography in an eluent gradient (eluent CH_2Cl_2:MeOH:NH_4OH at 6% from 15:1:0.1, R_f = 0.31) obtaining the compound **8a** (17.4 mg, 0.032 mmol, 64% yield) as a colorless oil. $[\alpha]_D^{24}$: −17.4 (MeOH, *c* = 1.2). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.04-8.02 (m, 1H, *Ar*), 7.89 (s, 1H, H-13), 7.54-7.48 (m, 2H, *Ar*), 7.40 (td, *J* =

7.9, 1.3, 1H, *Ar*), 4.46 (s, 2H, H-14), 4.38 (t, $J=6.8$ Hz, 2H, H-12), 4.28–4.25 (m, 1H, H-3), 3.83–3.76 (m, 2H, H-4, H-5), 3.00 (d, $J=13.2$ Hz, 1H, Ha-2), 2.72 (dd, $J=11.9$, 4.1 Hz, 1H, Ha-6), 2.42–2.32 (m, 3H, Hb-2, H-7), 1.99 (*p*t, $J=10.2$ Hz, 1H, Hb-6), 1.93–1.86 (m, 2H, H-11), 1.47–1.33 (m, 12H, H-8, H-9, H-10, *Me, Me*) ppm. ^{13}C NMR (50 MHz, CD_3OD): $\delta=157.6$ (s, 1 C, CO), 147.1 (s, 1 C, *Ar*), 145.5 (s, 1 C, *Ar*), 141.7 (s, 1 C, *triazole*), 130.5 (d, 1 C, Ar), 124.0 (d, 1 C, C-13), 123.1 (d, 1 C, *Ar*), 120.6 (d, 1 C, *Ar*), 117.2 (d, 1 C, *Ar*), 110.2 (s, 1 C, *acetonide*), 80.2 (d, 1 C, C-4), 74.5 (d, 1 C, C-3), 70.5 (d, 1 C, C-5), 58.9 (t, 1 C, C-7), 57.6 (t, 1 C, C-6), 54.9 (t, 1 C, C-2), 51.3 (t, 1 C, C-12), 36.1 (t, 1 C, C-14), 31.1 (t, 1 C, C-11), 28.5 (q, 1 C, *Me*), 27.8 (t, 1 C), 27.2 (t, 2 C), 26.5 (q, 1 C, *Me*) ppm. IR (MeOH): 3622, 3387, 3142, 2981, 2946, 2769, 1668, 1554, 1462, 1400, 1373, 1159 cm^{-1}. $C_{24}H_{37}N_7O_6S$: MS-ESI (m/z, %): 552.27 (100, $[M+H]^+$), 574.26 (56, $[M+Na]^+$). Calcd.: C, 52.25; H, 6.76; N, 17.77; found: C, 52.50; H, 6.98; N, 17.59.

4-(Aminosulfonyl)-*N*-[(1-{6-[(3a*R*,7*R*,7a*S*)-7-hydroxy-2,2-dimethyl tetrahydro[1,3]dioxolo[4,5-*c*]pyridin-5(4*H*)-yl]hexyl}-1*H*-1,2,3-triazol-4-yl)methyl]benzamide (8 b): Compound **18** (23.7 mg, 0.08 mmol) and triethylamine (15 μL, 0.11 mmol) were added to a solution of compound **13** (18.7 mg, 0.053 mmol) in anhydrous EtOH (1.5 mL) and anhydrous CH_3CN (1.5 mL). The reaction mixture was stirred for 16 hours in an inert atmosphere at room temperature, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 8:1:0.1). The mixture was concentrated under reduced pressure, the crude was purified by silica gel flash column chromatography in an eluent gradient (eluent CH_2Cl_2:MeOH:NH_4OH (6%) from 15:1:0.1 to 10:1:0.1) obtaining the compound **8 b** (21.4 mg, 0.040 mmol, $R_f=0.31$ eluent CH_2Cl_2:MeOH:NH_4OH (6%) 15:1:0.1, 75% yield) as a colorless oil. $[\alpha]_D^{24}$: −17.6 (MeOH, $c=0.6$). ^{1}H NMR (400 MHz, CD_3OD): $\delta=8.00$-7.96 (m, 4H, *Ar*), 7.94 (s, 1H, H-13), 4.65 (s, 2H, H-14), 4.39 (t, $J=$ 7.0 Hz, 2H, H-12), 4.27 (*p*q, $J=3.7$ Hz, 1H, H-3), 3.83–3.76 (m, 2H, H-4, H-5), 3.00 (d, $J=13.0$ Hz, 1H, Ha-2), 2.71 (dd, $J=11.8$, 4.1 Hz, 1H, Ha-6), 2.41–2.33 (m, 3H, Hb-2, H-7), 1.97 (dd, $J=11.4$, 9.2 Hz, 1H, Hb-6), 1.92–1.87 (m, 2H, H-11), 1.48–1.33 (m, 12H, H-8, H-9, H-10, *Me, Me*) ppm. ^{13}C NMR (50 MHz, CD_3OD): $\delta=168.7$ (s, 1 C, CO), 147.9 (s, 1 C, *Ar*), 146.1 (s, 1 C, *Ar*), 138.7 (s, 1 C, *triazole*), 129.1 (d, 2 C, *Ar*), 127.3 (d, 2 C, *Ar*), 124.4 (d, 1 C, C-13), 110.2 (s, 1 C, *acetonide*), 80.2 (d, 1 C, C-4), 74.5 (d, 1 C, C-3), 70.5 (d, 1 C, C-5), 58.9 (t, 1 C, C-7), 57.7 (t, 1 C, C-6), 55.0 (t, 1 C, C-2), 51.3 (t, 1 C, C-12), 36.3 (t, 1 C, C-14), 31.2 (t, 1 C, C-11), 28.5 (q, 1 C, *Me*), 27.8 (t, 1 C), 27.3 (t, 2 C), 26.6 (q, 1 C, *Me*) ppm. IR (MeOH): 3593, 3266, 3224, 3019, 2955, 2932, 2830, 1653, 1535, 1340, 1016 cm^{-1}. $C_{24}H_{36}N_6O_6S$: MS-ESI (m/z, %): 537.24 (100, $[M+H]^+$), 559.25 (24, $[M+Na]^+$). Calcd.: C, 53.71; H, 6.76; N, 15.66; found: C, 53.93; H, 6.62; N, 15.40.

4-[({[(1-{6-[(3a*R*,7*R*,7a*S*)-7-Hydroxy-2,2-dimethyltetrahydro [1,3] dioxolo[4,5-*c*]pyridin-5(4*H*)-yl]hexyl}-1*H*-1,2,3-triazol-4-yl) methyl] amino}carbonothioyl)amino]benzenesulfonamide (8 c): Compound **19** (8.51 mg, 0.040 mmol) and triethylamine (2 μL, 0.012 mmol) were added to a solution of compound **13** (14 mg, 0.040 mmol) in EtOH (1.5 mL) and CH_3CN (1.5 mL). The reaction mixture was stirred for 23 hours at room temperature, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 3:1:0.1). The mixture was concentrated under reduced pressure, the crude was purified by silica gel flash column chromatography (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 15:1:0.1, $R_f=0.20$) obtaining the compound **8 c** (11.3 mg, 0.019 mmol, 50% yield) as a colorless oil. $[\alpha]_D^{20}$: −21.8 (MeOH, $c=$ 1.0). ^{1}H NMR (400 MHz, CD_3OD): $\delta=8.00$ (s, 1H, H-13), 7.83 (d, $J=$ 8.6 Hz, 2H, *Ar*), 7.17 (d, $J=8.6$ Hz, 2H, *Ar*), 4.87 (s, 2H, H-14), 4.39 (t, $J=6.9$ Hz, 2H, H-12), 4.29–4.26 (m, 1H, H-3), 3.83–3.76 (m, 2H, H-4, H-5), 3.01 (d, $J=12.4$ Hz, 1H, Ha-2), 2.74–2.70 (m, 1H, Ha-6), 2.42–2.34 (m, 3H, Hb-2, H-7), 2.01–1.96 (m, 1H, Hb-6), 1.94–1.87 (m, 2H, H-11), 1.50–1.28 (m, 12H, H-8, H-9, H-10, *Me, Me*) ppm. ^{13}C NMR (50 MHz, CD_3OD): $\delta=182.7$ (s, 1 C, CS), 145.9 (s, 1 C, *Ar*), 144.2 (s, 1 C, *Ar*), 140.2 (s, 1 C, *triazole*), 128.0 (d, 2 C, *Ar*), 124.6 (d, 2 C, *Ar*), 123.6 (d, 1 C, C-13), 110.2 (s, 1 C, *acetonide*), 80.2 (d, 1 C, C-4), 74.5 (d, 1 C, C-3), 70.5 (d, 1 C, C-5), 58.9 (t, 1 C, C-7), 57.6 (t, 1 C, C-6), 55.0 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 40.4 (t, 1 C, C-14), 31.1 (t, 1 C, C-11), 28.5 (q, 1 C, *Me*), 27.8 (t, 1 C), 27.2 (t, 2 C), 26.6 (q, 1 C, *Me*) ppm. IR (MeOH): 3406, 3371, 3153, 2953, 2918, 2792, 1535, 1346, 1161 cm^{-1}. $C_{24}H_{37}N_7O_5S_2$: MS-ESI (m/z, %): 568.30 (100, $[M+H]^+$). Calcd.: C, 50.77; H, 6.57; N, 17.27; found: C, 50.49; H, 6.80; N, 17.56.

3-[({[(1-{6-[(3a*R*,7*R*,7a*S*)-7-Hydroxy-2,2-dimethyltetrahydro [1,3] dioxolo[4,5-*c*]pyridin-5(4*H*)-yl]hexyl}-1*H*-1,2,3-triazol-4-yl) methyl] amino}carbonothioyl)amino]benzenesulfonamide (8 d): Compound **20** (26.15 mg, 0.122 mmol) and triethylamine (5 μL, 0.036 mmol) were added to a solution of compound **13** (43 mg, 0.122 mmol) in EtOH (1.5 mL) and CH_3CN (1.5 mL). The reaction mixture was stirred for 18 hours at room temperature, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 6:1:0.1). The mixture was concentrated under reduced pressure, the crude was purified by silica gel flash column chromatography (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 15:1:0.1, $R_f=0.20$) obtaining the compound **8 d** (66.3 mg, 0.117 mmol, 96% yield) as a colorless oil. $[\alpha]_D^{21}$: −19.8 (MeOH, $c=$ 0.6). ^{1}c (400 MHz, CD_3OD): $\delta=8.05$–8.03 (m, 1H, *Ar*), 7.98 (s, 1H, H-13), 7.67–7.63 (m, 2H, *Ar*), 7.49 (td, $J=8.0$, 1.0, 1H, *Ar*), 4.88 (s, 2H, H-14), 4.38 (t, $J=6.7$ Hz, 2H, H-12), 4.28–4.25 (m, 1H, H-3), 3.84–3.77 (m, 2H, H-4, H-5), 3.00 (d, $J=13.2$ Hz, 1H, Ha-2), 2.74–2.70 (m, 1H, Ha-6), 2.42–2.32 (m, 3H, Hb-2, H-7), 1.99 (*p*t, $J=10.0$ Hz, 1H, Hb-6), 1.90–1.87 (m, 2H, H-11), 1.47–1.32 (m, 12H, H-8, H-9, H-10, *Me, Me*) ppm. ^{13}C NMR (50 MHz, CD_3OD): $\delta=182.9$ (s, 1 C, CS), 145.8 (s, 1 C, *Ar*), 145.3 (s, 1 C, *Ar*), 140.9 (s, 1 C, *triazole*), 130.5 (d, 1 C, Ar), 128.3 (d, 1 C, C-13), 124.5 (d, 1 C, *Ar*), 123.4 (d, 1 C, *Ar*), 122.2 (d, 1 C, *Ar*), 110.2 (s, 1 C, *acetonide*), 80.1 (d, 1 C, C-4), 74.5 (d, 1 C, C-3), 70.4 (d, 1 C, C-5), 58.9 (t, 1 C, C-7), 57.6 (t, 1 C, C-6), 54.8 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 40.6 (t, 1 C, C-14), 31.1 (t, 1 C, C-11), 28.5 (q, 1 C, *Me*), 27.8 (t, 1 C), 27.2 (t, 2 C), 26.6 (q, 1 C, *Me*) ppm. IR (MeOH): 3606, 3504, 2953, 2918, 2779, 1535, 1346, 1163 cm^{-1}. $C_{24}H_{37}N_7O_5S_2$: MS-ESI (m/z, %): 568.147 (100, $[M+H]^+$). Calcd.: C, 50.77; H, 6.57; N, 17.27; found: C, 50.46; H, 6.83; N, 17.59.

4-{2-[({[(1-{6-[(3a*R*,7*R*,7a*S*)-7-Hydroxy-2,2-dimethyltetrahydro [1,3] dioxolo[4,5-*c*]pyridin-5(4*H*)-yl]hexyl}-1*H*-1,2,3-triazol-4-yl) methyl] amino}carbonothioyl)amino]ethyl}benzenesulfonamide (8 e): Compound **21** (25 mg, 0.102 mmol) and triethylamine (4.3 μL, 0.031 mmol) were added to a solution of compound **13** (36.1 g, 0.102 mmol) in EtOH (1.5 mL) and CH_3CN (1.5 mL). The reaction mixture was stirred for 18 hours at room temperature, until a TLC control attested the appearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 6:1:0.1). The reaction mixture was heated at 78 °C for 29 hours, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 6:1:0.1). The mixture was concentrated under reduced pressure, the crude was purified by silica gel flash column chromatography (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 15:1:0.1, $R_f=0.20$) obtaining the compound **8 d** (55 mg, 0.092 mmol, 92% yield) as a colorless oil. $[\alpha]_D^{21}$: −17.4 (MeOH, $c=$ 0.7). ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.89$ (s, 1H, H-13), 7.81 (d, $J=$ 8.0 Hz, 2H, *Ar*), 7.40 (d, $J=8.2$ Hz, 2H, *Ar*), 4.74 (s, 2H, H-14), 4.37 (t, $J=6.9$ Hz, 2H, H-12), 4.28–4.25 (m, 1H, H-3), 3.84–3.75 (m, 4H, H-4, H-5, H-15)„ 2.99–2.96 (m, 3H, Ha-2, H-16), 2.73–2.69 (dd, $J=3.5$, 11.8 Hz, 1H, Ha-6), 2.42–2.32 (m, 3H, Hb-2, H-7), 2.01–1.96 (m, 1H, Hb-6), 1.90–1.87 (m, 2H, H-11), 1.47–1.33 (m, 12H, H-8, H-9, H-10, *Me, Me*) ppm. ^{13}C NMR (50 MHz, CD_3OD): $\delta=184.0$ (s, 1 C, CS), 146.2 (s, 1 C, *Ar*), 145.3 (s, 1 C, *Ar*), 143.0 (s, 1 C, *triazole*), 130.5 (d, 2 C, *Ar*), 127.3 (d, 2 C, *Ar*), 124.3 (d, 1 C, C-13), 110.2 (s, 1 C, *acetonide*), 80.2 (d, 1 C, C-4), 74.5 (d, 1 C, C-3), 70.5 (d, 1 C, C-5), 58.9 (t, 1 C, C-7), 57.6 (t, 1 C, C-6), 54.9 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 46.2 (t, 1 C, C-15), 40.2 (t, 1 C, C-14), 36.0 (t, 1 C, C-16), 31.1 (t, 1 C, C-11), 28.5 (q,

1 C, *Me*), 27.8 (t, 1 C), 27.2 (t, 2 C), 26.6 (q, 1 C, *Me*) ppm. IR (MeOH): 3604, 3377, 3221, 2953, 2918, 2792, 1543, 1342, 1163 cm^{-1}. $C_{26}H_{41}N_7O_5S_2$: MS-ESI (m/z, %): 596.17 (100, $[M+H]^+$), 618.17 (58, $[M+Na]^+$). Calcd.: C, 52.42; H, 6.94; N, 16.46; found: C, 52.70; H, 7.20; N, 16.69.

***tert*-Butyl [(1-{6-[(3a*R*,7*R*,7a*S*)-7-hydroxy-2,2-dimethyltetrahydro [1,3] dioxolo[4,5-*c*]pyridin-5(4*H*)-yl]hexyl}-1*H*-1,2,3-triazol-4-yl) methyl] carbamate (15)**: Compound **14** (30 mg, 0.190 mmol), $CuSO_4$ (8.28 mg, 0.052 mmol) and sodium ascorbate (21 mg, 0.104 mmol) was added to a solution of compound **5** (51.6 mg, 0.173 mmol) in THF (2 mL) and milliQ water (1 mL). The reaction mixture was stirred at room temperature for 23 hours, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 10:1:0.1). The mixture was filtered through Celite®, the solvent was removed under reduced pressure and subsequently the crude mixture was treated with 'Quadrasil MP®' resin keeping the mixture under stirring at room temperature in the minimum amount of MeOH for 1 hour (1 g of resin for each mmol of copper). The crude was purified by silica gel flash column chromatography (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 20:1:0.1, R_f = 0.20) obtaining compound **15** (67.2 mg, 0.148 mmol, 86% yield) as a colorless oil. $[\alpha]_D^{22}$: −2.0 ($CHCl_3$, c = 0.6). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50 (s, 1H, H-13), 5.19 (bs, 1H, OH), 4.38–4.25 (m, 5H, H-3, H-14, H-12), 4.04 (*p*t, J = 4.4 Hz, 1H, H-4), 4.00–3.89 (m, 1H, H-5), 2.88 (bs, 1H, –NH), 2.54 (dd, J = 11.8, 5.8 Hz, 1H, Ha-2), 2.72 (dd, J = 11.6, 2.4 Hz, 1H, Ha-6), 2.46–2.30 (m, 4H, Hb-2, Hb-6, H-7), 1.91–1.84 (m, 2H, H-11), 1.49–1.29 (m, 21H, H-8, H-9, H-10, 5*Me*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.0 (s, 1 C, CO),145.5 (s, 1 C, *triazole*), 121.9 (d, 1 C, C-13), 109.4 (s, 1 C, *acetonide*), 108.6 (interference), 79.8 (s, 1 C, *Cq-Boc*), 76.9 (d, 1 C, C-4), 77.2 (d, 1 C, C-3), 67.7 (d, 1 C, C-5), 57.4 (t, 1 C, C-7), 56.0 (t, 1 C, C-2), 55.6 (t, 1 C, C-6), 50.3 (t, 1 C, C-12), 36.2 (t, 1 C, C-14), 30.3 (t, 1 C, C-11), 28.5 (q, 3 C, *Me*), 28.4 (q, 1 C, *Me*), 26.7 (t, 1 C), 27.5 (t, 2 C), 26.4 (q, 1 C, *Me*) ppm. IR (MeOH): 3452, 3012, 2939, 1707, 1504, 1055 cm^{-1}. $C_{22}H_{39}N_5O_5$: MS-ESI (m/z, %): 454.13 (70, $[M+H]^+$), 476.11 (100, $[M+Na]^+$), 928.68 (30, $[2M+Na]^+$). Calcd.: C, 58.26; H, 8.67; N, 15.44; found: C, 58.52; H, 8.96; N, 15.73.

(3*R*,5*R*)-1-{6-[4-(Aminomethyl)-1*H*-1,2,3-triazol-1-yl]hexyl}piperidine-3,4,5-triol (16): A solution of compound **15** in MeOH (0.02 M) was left stirring with 12 M HCl (50 μL) at room temperature for 18 h. The crude mixture was concentrated to yield the hydrochloride salt of **16**. The corresponding free amine was obtained by dissolving the residue in MeOH, then the strongly basic resin Ambersep 900-OH was added, and the mixture was stirred for 40 minutes. The resin was removed by filtration and the crude product was purified by silica gel flash column chromatography (eluent CH_2Cl_2:MeOH:NH_4OH (6%) from 4:1:0.1 to 2:1:0.1) obtaining compound **16** (43 mg, 0.137 mmol, R_f = 0.09 eluent CH_2Cl_2:MeOH:NH_4OH (6%) 4:1:0.1, 90% yield) as a colorless oil. $[\alpha]_D^{22}$: −19.5 (MeOH, c = 0.4). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.90 (s, 1H, H-13), 4.40 (t, J = 7.0 Hz, 2H, H-12), 3.95–3.88 (m, 3H, H-3, H-14), 3.82–3.77 (m, 1H, H-5), 3.45–3.35 (m, 1H, H-4), 2.86–2.69 (m, 2H, Ha-2, Ha-6), 2.42–2.08 (m, 4H, Hb-2, Hb-6, H-7), 1.94–1.87 (m, 2H, H-11), 1.52–1.29 (m, 6H, H-8, H-9, H-10) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 148.7 (s, 1 C, *triazole*),123.5 (d, 1 C, C-13), 75.2 (d, 1 C, C-4), 69.5 (d, 1 C, C-5), 69.1 (d, 1 C, C-3), 59.0 (t, 1 C, C-7), 58.2 (t, 1 C, C-6), 57.5 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 37.3 (t, 1 C, C-14), 31.2 (t, 1 C, C-11), 27.9 (t, 1 C), 27.3 (t, 2 C) ppm. $C_{14}H_{27}N_5O_3$: MS-ESI (m/z, %): 314.17 (70, $[M+H]^+$), 336.17 (98, $[M+Na]^+$), 648.83 (100, $[2M+Na]^+$). Calcd.: C, 53.65; H, 8.68; N, 22.35; found: C, 53.93; H, 8.99; N, 22.02.

3-[({[(1-{6-[(3*R*,5*R*)-3,4,5-Trihydroxypiperidin-1-yl]hexyl}-1*H*-1,2,3-triazol-4-yl)methyl]amino}carbonyl)amino]benzenesulfonamide (9a): Compound **17** (20 mg, 0.068 mmol) was added to a solution of compound **16** (21.2 mg, 0.068 mmol) in EtOH (1.5 mL) and CH_3CN (1.5 mL). The reaction mixture was heated at 78 °C for 3 days, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 3:1:0.1). The mixture was concentrated under reduced pressure, the crude was purified by silica gel flash column chromatography in an eluent gradient (eluent CH_2Cl_2:MeOH:NH_4OH (6%) from 6:1:0.1 to 4:1:0.1) obtaining the compound **9a** (28.3 mg, 0.055 mmol, R_f = 0.06 eluent CH_2Cl_2:MeOH:NH_4OH (6%) 6:1:0.1, 81% yield) as a colorless oil. $[\alpha]_D^{22}$: −5.4 (MeOH, c = 0.6). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.05 (s, 1H, Ar), 7.98 (s, 1H, H-13), 7.54–7.38 (m, 3H, *Ar*), 4.46 (s, 2H, H-14), 4.38 (t, J = 7.0 Hz, 2H, H-12), 3.93–3.92 (m, 1H, H-3), 3.85–3.80 (m, 1H, H-5), 3.51–3.40 (m, 1H, H-4), 2.87–2.80 (m, 2H, Ha-2, Ha-6), 2.46–2.22 (m, 4H, Hb-2, Hb-6, H-7), 1.91–1.87 (m, 2H, H-11), 1.51–1.29 (m, 6H, H-8, H-9, H-10) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 157.6 (s, 1 C, CO), 147.1 (s, 1 C, *Ar*), 145.4 (s, 1 C, *Ar*), 141.7 (s, 1 C, *triazole*), 130.4 (d, 1 C, *Ar*), 124.0 (d, 1 C, *Ar*), 123.1 (d, 1 C, C-13), 120.5 (d, 1 C, *Ar*), 117.2 (d, 1 C, *Ar*), 74.6 (d, 1 C, C-4), 69.2 (d, 1 C, C-5), 68.5 (d, 1 C, C-3), 58.7 (t, 1 C, C-7), 57.6 (t, 1 C, C-6), 57.0 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 36.1 (t, 1 C, C-14), 31.1 (t, 1 C, C-11), 27.7 (t, 1 C), 27.2 (t, 1 C), 26.8 (t, 1 C) ppm. $C_{21}H_{33}N_7O_6S$: MS-ESI (m/z, %): 512.20 (34, $[M+H]^+$), 534.19 (100, $[M+Na]^+$), 1044.75 (70, $[2M+Na]^+$). Calcd.: C, 49.30; H, 6.50; N, 19.16; found: C, 49.63; H, 6.20; N, 19.42.

4-(Aminosulfonyl)-*N*-[(1-{6-[(3*R*,5*R*)-3,4,5-trihydroxypiperidin-1-yl] hexyl}-1*H*-1,2,3-triazol-4-yl)methyl]benzamide (9b): Compound **18** (46.1 mg, 0.154 mmol) and triethylamine (29 μL, 0.205 mmol) were added to a solution of compound **16** (32.2 mg, 0.103 mmol) in anhydrous EtOH (1.5 mL) and anhydrous CH_3CN (1.5 mL). The reaction mixture was stirred for 18 hours in an inert atmosphere at room temperature, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 3:1:0.1). The mixture was concentrated under reduced pressure, the crude was purified by silica gel flash column chromatography in an eluent gradient (eluent CH_2Cl_2:MeOH:NH_4OH (6%) from 5:1:0.1 to 3:1:0.1) obtaining the compound **9b** (44.6 mg, 0.089 mmol, R_f = 0.25 eluent CH_2Cl_2:MeOH:NH_4OH (6%) 5:1:0.1, 87% yield) as a colorless oil. $[\alpha]_D^{22}$: −12.1 (MeOH, c = 0.7). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.00–7.96 (m, 5H, Ar, H-13), 4.65 (s, 2H, H-14), 4.39 (t, J = 7.0 Hz, 2H, H-12), 3.97-3.95 (m, 1H, H-3), 3.87–3.83 (m, 1H, H-5), 3.52–3.41 (m, 1H, H-4), 2.93–2.85 (m, 2H, Ha-2, Ha-6), 2.55–2.35 (m, 4H, Hb-2, Hb-6, H-7), 1.91–1.88 (m, 2H, H-11), 1.55–1.29 (m, 6H, H-8, H-9, H-10) ppm. ^{13}C NMR (50 MHz, CD_3OD): δ = 168.6 (s, 1 C, CO), 147.7 (s, 1 C, *Ar*), 146.1 (s, 1 C, *Ar*), 138.6 (s, 1 C, *triazole*), 129.1 (d, 2 C, *Ar*), 127.3 (d, 2 C, *Ar*), 124.4 (d, 1 C, C-13), 74.1 (d, 1 C, C-4), 69.0 (d, 1 C, C-5), 68.1 (d, 1 C, C-3), 58.7 (t, 1 C, C-7), 57.1 (t, 1 C, C-6), 56.6 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 36.3 (t, 1 C, C-14), 31.0 (t, 1 C, C-11), 27.5 (t, 1 C), 27.1 (t, 1 C), 26.6 (t, 1 C) ppm. $C_{21}H_{32}N_6O_6S$: MS-ESI (m/z, %): 519.17 (100, $[M+Na]^+$). Calcd.: C, 50.79; H, 6.50; N, 16.92; found: C, 50.48; H, 6.79; N, 16.65.

4-[({[(1-{6-[(3*R*,5*R*)-3,4,5-Trihydroxypiperidin-1-yl]hexyl}-1*H*-1,2,3-triazol-4-yl)methyl]amino}carbonothioyl)amino] benzenesulfonamide (9c): Compound **19** (23 mg, 0.105 mmol) and triethylamine (5 μL, 0.031 mmol) were added to a solution of compound **16** (33 mg, 0.105 mmol) in EtOH (1.5 mL) and CH_3CN (1.5 mL). The reaction mixture was heated at 78 °C for 3 days, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 3:1:0.1). The mixture was concentrated under reduced pressure, the crude was purified by silica gel flash column chromatography in an eluent gradient (eluent CH_2Cl_2:MeOH:NH_4OH (6%) from 6:1:0.1 to 2:1:0.1) obtaining the compound **9c** (41.4 mg, 0.078 mmol, R_f = 0.50 eluent CH_2Cl_2:MeOH:NH_4OH (6%) 2:1:0.1, 75% yield) as a colorless oil. $[\alpha]_D^{22}$: −10.2 (MeOH, c = 0.8). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.99 (s, 1H, H-13), 7.83 (d, J = 8.4 Hz, 2H, *Ar*), 7.70 (d, J = 8.4 Hz, 2H, *Ar*), 4.88 (s, 2H, H-14), 4.39 (t, J = 6.9 Hz, 2H, H-12), 3.98–3.95 (m, 1H, H-3), 3.88–3.84 (m, 1H, H-5), 3.55–3.47 (m, 1H, H-4), 2.98–2.87 (m, 2H, Ha-

2, Ha-6), 2.61–2.41 (m, 4H, Hb-2, Hb-6, H-7), 1.92–1.88 (m, 2H, H-11), 1.58–1.30 (m, 6H, H-8, H-9, H-10) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 182.7 (s, 1 C, CS), 145.8 (s, 1 C, *Ar*), 144.2 (s, 1 C, *Ar*), 140.1 (s, 1 C, *triazole*), 127.9 (d, 2 C, *Ar*), 124.6 (d, 2 C, *Ar*), 123.6 (d, 1 C, C-13), 73.8 (d, 1 C, C-4), 68.8 (d, 1 C, C-5), 67.9 (d, 1 C, C-3), 58.6 (t, 1 C, C-7), 56.8 (t, 1 C, C-6), 56.4 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 40.4 (t, 1 C, C-14), 31.0 (t, 1 C, C-11), 27.5 (t, 1 C), 27.1 (t, 1 C), 26.4 (t, 1 C) ppm. $C_{21}H_{33}N_7O_5S_2$: MS-ESI (m/z, %): 528.12 (100, $[M+H]^+$), 550.15 (32, $[M+Na]^+$), 1076.64 (40, $[2\,M+Na]^+$). Calcd: C, 47.80; H, 6.30; N, 18.58; found: C, 47.49; H, 6.62; N, 18.89.

3-[({[(1-{6-[(3*R*,5*R*)-3,4,5-Trihydroxypiperidin-1-yl]hexyl}-1*H*-1,2,3-triazol-4-yl)methyl]amino}carbonothioyl)amino] benzenesulfonamide (9 d): Compound **20** (29 mg, 0.134 mmol) and triethylamine (6 μL, 0.040 mmol) were added to a solution of compound **16** (42 mg, 0.134 mmol) in EtOH (1.5 mL) and CH_3CN (1.5 mL). The reaction mixture was heated at 78 °C for 3 days, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 3:1:0.1). The mixture was concentrated under reduced pressure, the crude was purified by silica gel flash column chromatography in an eluent gradient (eluent CH_2Cl_2:MeOH:NH_4OH (6%) from 6:1:0.1 to 2:1:0.1) obtaining the compound **9 d** (61.4 mg, 0.116 mmol, R_f = 0.50 eluent CH_2Cl_2:MeOH:NH_4OH (6%) 2:1:0.1, 88% yield) as a colorless oil. $[\alpha]_D^{22}$: −11.0 (MeOH, *c* = 0.7). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.04–8.02 (m, 1H, *Ar*), 8.00 (s, 1H, H-13), 7.69–7.48 (m, 3H, *Ar*), 4.39 (t, *J* = 6.8 Hz, 2H, H-12), 3.95–3.94 (m, 1H, H-3), 3.86–3.81 (m, 1H, H-5), 3.52–3.41 (m, 1H, H-4), 2.91–2.84 (m, 2H, Ha-2, Ha-6), 2.54–2.31 (m, 4H, Hb-2, Hb-6, H-7), 1.93–1.89 (m, 2H, H-11), 1.56-1.29 (m, 6H, H-8, H-9, H-10) ppm. ^{13}C NMR (50 MHz, CD_3OD): δ = 183.1 (s, 1 C, CS), 146.0 (s, 1 C, *Ar*), 145.4 (s, 1 C, *Ar*), 141.1 (s, 1 C, *triazole*), 130.6 (d, 1 C, *Ar*), 128.4 (d, 1 C, *Ar*), 124.6 (d, 1 C, C-13), 123.4 (d, 1 C, *Ar*), 122.4 (d, 1 C, *Ar*), 74.3 (d, 1 C, C-4), 69.1 (d, 1 C, C-5), 68.3 (d, 1 C, C-3), 58.7 (t, 1 C, C-7), 57.3 (t, 1 C, C-6), 56.8 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 40.6 (t, 1 C, C-14), 31.1 (t, 1 C, C-11), 27.6 (t, 1 C), 27.1 (t, 1 C), 26.7 (t, 1 C) ppm. $C_{21}H_{33}N_7O_5S_2$: MS-ESI (m/z, %): 528.16 (44, $[M+H]^+$), 550.14 (100, $[M+Na]^+$), 1076.67 (80, $[2\,M+Na]^+$). Calcd: C, 47.80; H, 6.30; N, 18.58; found: C, 47.53; H, 6.68; N, 18.84.

4-{2-[({[(1-{6-[(3*R*,5*R*)-3,4,5-Trihydroxypiperidin-1-yl]hexyl}-1*H*-1,2,3-triazol-4-yl)methyl]amino}carbonothioyl)amino]ethyl} benzene sulfonamide (9 e): Compound **21** (25 mg, 0.103 mmol) and triethylamine (4 μL, 0.031 mmol) were added to a solution of compound **16** (32.3 mg, 0.103 mmol) in EtOH (1.5 mL) and CH_3CN (1.5 mL). The reaction mixture was heated at 78 °C for 3 days, until a TLC control attested the disappearance of the starting material (eluent CH_2Cl_2:MeOH:NH_4OH (6%) 3:1:0.1). The mixture was concentrated under reduced pressure, the crude was purified by silica gel flash column chromatography in an eluent gradient (eluent CH_2Cl_2:MeOH:NH_4OH (6%) from 6:1:0.1 to 3:1:0.1) obtaining the compound **9 e** (37.4 mg, 0.067 mmol, R_f = 0.30 eluent CH_2Cl_2:MeOH:NH_4OH (6%) 3:1:0.1, 73% yield) as a colorless oil. $[\alpha]_D^{22}$: −12.2 (MeOH, *c* = 0.8). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.90 (s, 1H, H-13),), 7.81 (d, *J* = 7.9 Hz, 2H, *Ar*), 7.40 (d, *J* = 7.9 Hz, 2H, *Ar*), 4.75 (s, 2H, H-14), 4.37 (t, *J* = 6.9 Hz, 2H, H-12), 3.92–3.88 (m, 1H, H-3), 3.82–3.75 (m, 3H, H-5, H-15), 3.44–3.38 (m, 1H, H-4), 2.97 (t, *J* = 6.8 Hz, 2H, H-16) 2.83–2.66 (m, 2H, Ha-2, Ha-6), 2.42–2.13 (m, 4H, Hb-2, Hb-6, H-7), 1.89–1.86 (m, 2H, H-11), 1.49–1.32 (m, 6H, H-8, H-9, H-10) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 184.0 (s, 1 C, CS), 146.3 (s, 1 C, *Ar*), 145.4 (s, 1 C, *Ar*), 142.9 (s, 1 C, *triazole*), 130.5 (d, 2 C, *Ar*), 127.3 (d, 2 C, *Ar*), 124.4 (d, 1 C, C-13), 75.0 (d, 1 C, C-4), 69.3 (d, 1 C, C-5), 68.8 (d, 1 C, C-3), 58.9 (t, 1 C, C-7), 58.0 (t, 1 C, C-6), 57.3 (t, 1 C, C-2), 51.2 (t, 1 C, C-12), 46.1 (t, 1 C, C-15), 40.2 (t, 1 C, C-14), 36.0 (t, 1 C, C-16), 31.1 (t, 1 C, C-11), 27.8 (t, 1 C), 27.2 (t, 1 C), 26.1 (t, 1 C) ppm. $C_{23}H_{37}N_7O_5S_2$: MS-ESI (m/z, %): 556.18 (42, $[M+H]^+$), 578.19 (100, $[M+Na]^+$), 1132.59 (54, $[2\,M+Na]^+$). Calcd.: C, 49.71; H, 6.71; N, 17.64; found: C, 49.42; H, 6.39; N, 17.36.

Carbonic anhydrases inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[18] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mMHepes (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 the assay buffer. 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-Prusoff equation, as reported earlier[19] and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier.[19]

Crystallization and X-ray data collection

Crystals of hCA II were obtained by the hanging drop vapor diffusion method using 24 well Linbro plate. 2 μl of 10 mg/ml solution of hCA II in Tris-HCl 20 mM pH 8.0 were mixed with 2 μl of a solution of 1.5 M sodium citrate, 0.1 M Tris pH 8.0 and were equilibrated against the same solution at 296 K. The complexes were prepared soaking the hCA II native crystals in the mother liquor solution containing the inhibitors at concentration of 10 mM for two days. The crystals were flash-frozen at 100 K using a solution obtained by adding 15% (v/v) glycerol to the mother liquor solution as cryoprotectant. Data on crystals of the complexes were collected using synchrotron radiation at the XRD2 beamline at Elettra Synchrotron (Trieste, Italy) with a wavelength of 1.000 Å and a DECTRIS Pilatus 6 M detector. Data were integrated and scaled using the program XDS.[20] Data processing statistics are showed in supporting information.

Structural determination

The crystal structure of hCA II (PDB accession code: 4FIK) without solvent molecules and other heteroatoms was used to obtain initial phases of the structure using Refmac5.[21] 5% of the unique reflections were selected randomly and excluded from the refinement data set for the purpose of Rfree calculations. The initial |Fo-Fc| difference electron density maps unambiguously showed the inhibitor molecules. The inhibitor was introduced in the model with 1.0 occupancy. Refinements proceeded using normal protocols of positional, isotropic atomic displacement parameters alternating with manual building of the models using COOT.[22] The quality of the final models were assessed with COOT and RAMPAGE.[23] Crystal parameters and refinement data are summarized in Electronic Supplementary Information (ESI). Atomic coordinates for the complex hCA II·**8 b** were deposited in the Protein Data Bank (PDB accession code: 7NH8). Graphical representations were generated with Chimera.[24]

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Azasugars · Benzenesulfonamides · Carbonic anhydrases · Glycomimetics · Enzyme inhibitors

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