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5-Substituted-(1,2,3-triazol-4-yl)thiophene-2-sulfonamides strongly inhibit human carbonic anhydrases I, II, IX and XII: Solution and X-ray crystallographic studies

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ABSTRACT

We report here a series of 2-thiophene-sulfonamides incorporating 1-substituted aryl-1,2,3-triazolyl moieties, prepared by click chemistry from 5-ethynylthiophene-2-sulfonamide and substituted aryl azides. The new sulfonamides were investigated as inhibitors of the zinc metalloenzyme CA (EC 4.2.1.1), and more specifically against the human (h) cytosolic isoforms hCA I and II and the transmembrane, tumor-associated ones hCA IX and XII: The new compounds were medium–weak hCA I inhibitors (K_Is in the range of 224–7544 nM), but were compactly, highly effective, low nanomolar hCA II inhibitors (K_Is of 2.2–7.7 nM). The tumor-associated hCA IX was inhibited with K_Is ranging between 5.4 and 811 nM, whereas hCA XII with inhibition constants in the range of 3.4–239 nM. The X-ray crystal structure of the adducts of two such compounds bound to hCA II (one incorporating 1-naphthyl, the other one 3-cyanophenyl moieties) evidenced the reasons of the high affinity for hCA II. Highly favorable, predominantly hydrophobic interactions between the sulfonamide scaffold and the hCA II active site were responsible for the binding, in addition to the coordination of the sulfamoyl moiety to the zinc ion. The tails of the two inhibitors adopted very diverse orientations when bound to the active site, with the naphthyltriazolyl moiety orientated towards the hydrophobic half of the active site, and the 3-cyanophenyl one pointing towards the hydrophilic half. These data may be used for the structure-based drug design of even more effective hCA II inhibitors, with potential use as antiglaucoma agents or as diuretics.

1. Introduction

Carbonic anhydrase (CAs, EC 4.2.1.1) catalyze the interconversion between CO_2 and bicarbonate by using a metal hydroxide nucleophilic mechanism.[1–3] They constitute a superfamily of metalloenzymes with five distinct genetic families known to date, the α-, β-, γ-, δ- and ζ-CAs, in organisms all over the tree of life. These enzymes differ in their preference for metal ions used within the active site for performing the catalysis: Zn(II) ions may be used by all five classes mentioned above, but the γ-CAs are probably Fe(II) enzymes (being active also with bound Zn(II) or Co(II) ions),[4,5] whereas the ζ-class uses Cd(II) or Zn(II) to perform the physiologic reaction catalysis.[6]

The inhibition and activation of CAs are well understood processes, with most classes of inhibitors binding to the metal center,[1–3] whereas activators bind at the entrance of the active site cavity and participate in proton shuttling processes between the metal ion – bound water molecule and the environment.[7] This leads to the enhanced formation of the metal hydroxide, catalytically active species of the enzyme.[7] Inhibitors generally bind to the metal ion from the enzyme active site in deprotonated state (as anions),[1–3] although alternative inhibition mechanisms in which the inhibitor interacts with the zinc-coordinated water molecule/hydroxide ion,[8–10] or does not interact at all with it,[11–13] have been recently described. The zinc-binders are the most investigated classes of CA inhibitors (CAIs), and they include the sulfonamides and their isosteres,[1–3,14,15] the dithiocarbamates,[16] the xanthates,[17] and the hydroxamates/carboxylates[18,19] (although some carboxylates may bind to the enzyme through the alternative inhibition mechanisms, which do not involve directly the metal ion).[8,9,11–13]

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Sulfonamide/sulfamate CAIs are in clinical use for decades as antiglaucoma,[20] diuretic,[21] antiobesity,[22] and antitumor agents,[23] targeting diverse of the various 16 CA isoforms known to date in humans.[1–3] Recently, targeting CAs from parasites (bacteria, fungi and/or protozoa among others) with specific inhibitors was proposed as an alternative for designing antiinfective agents with a new mechanism of action.[24]

Due to the high number of CA isoforms present in most organisms, and because the clinically used sulfonamides such as acetazolamide **AAZ**, methazolamide **MZA**; ethoxzolamide **EZA**, or dichlorophenamide **DCP** do not show selectivity for inhibiting any of the isoforms with pharmacologic applications,[1–3] there is a constant search for new compounds belonging to the CAI class, and the sulfonamides remain one of the most investigated such chemotype, due to their strong affinity for CAs, ease of synthesis, stability and lack of toxicity.[1–3]

Continuing our interest in sulfonamides as CAIs, we report here the synthesis of a series of 5-substituted-(1,2,3-triazol-4-yl)thiophene-2-sulfonamides, prepared by click chemistry from thiophenesulfonamides incorporating an alkyne functionality in the 5th position of the heterocyclic ring and aryl azides.

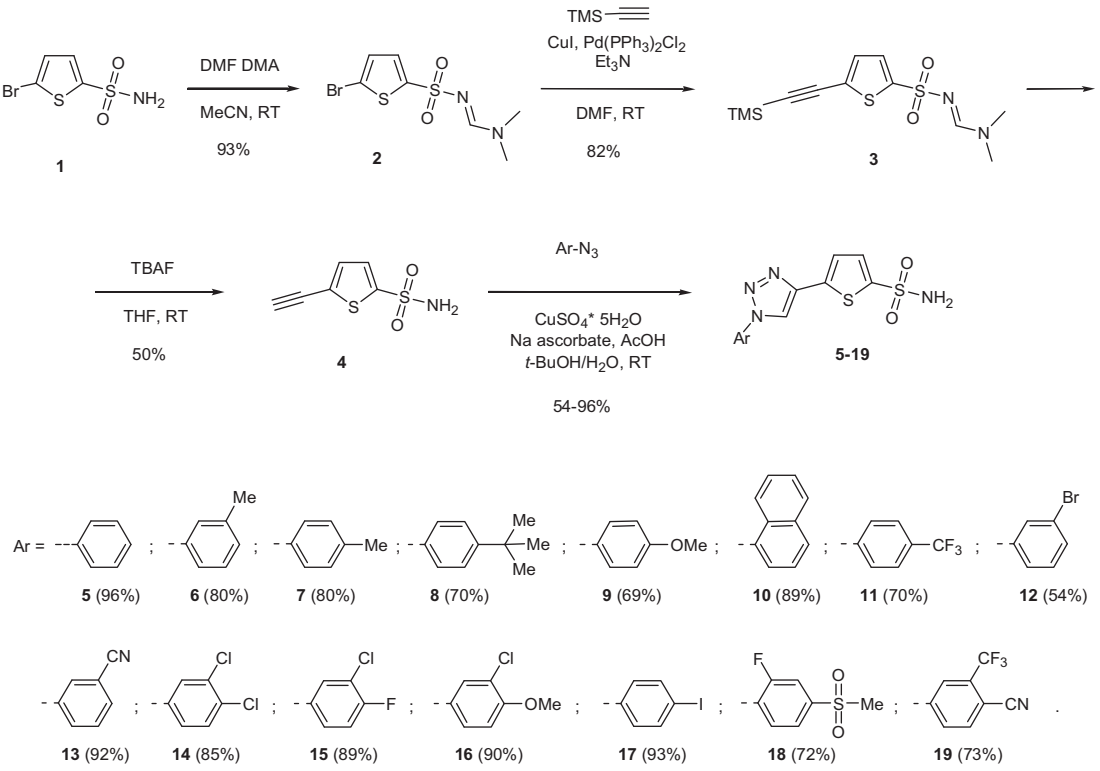
2. Results and discussion

2.1. Chemistry

Thiophene-2-sulfonamides were already investigated in the early 90s by Shepard et al.[25] in the search of topically-acting antiglaucoma CAIs.[26] One of the intermediates reported in this early paper was the dimethylacetamide protected sulfonamide group of 5-bromo-thiophene-2-sulfonamide **1**. This compounds has been used for the preparation of 4-acetylene-thiophene-2-sulfonamide (**4**), the alkyne component used for click chemistry purposes in this work.[27,28] Compound **4** may be prepared in a two step process: the first step involving the Sonogashira reaction of bromide **1** with trimethylsilylacetylene, whereas the second one, the trimethylsilyl (TMS) protecting group cleavage. Unfortunately several attempts to perform Sonogashira reaction with **1**, under various conditions did not provide the cross-coupling product, and only unreacted bromo derivative **1** was detected. The most probable cause of this lack of reactivity might be the unprotected sulfonamide group, therefore protection of sulfonamide NH_2 employing *N*,*N*-dimethylformamide dimethyl acetal was done as reported by Shepard et al.[25] and the protected intermediate **2** was isolated in 93% yield (Scheme 1). Reaction of compound **2** with trimethylsilylacetylene under Sonogashira condition afforded the cross-coupling product **3** in a 82% yield. The desired acetylene intermediate **4** was obtained by treatment of compound **3** with TBAF, when TMS cleavage and the simultaneous deprotection of the sulfonamide group took place, affording the key intermediate **4** in 50% yield.

Cu(I) catalyzed azide–alkyne cycloaddition or 'click chemistry' is a powerful tool for the regioselective synthesis of 1,4-substituted 1,2,3-triazoles.[27] After the first attempts to synthesize compound **5** under click chemistry conditions from alkyne **4** and phenylazide it was observed that the reaction takes place in 99% conversion yield, in 1:1 mixture $^{t}BuOH/H_2O$ and in the presence of sodium ascorbate, at 40 °C in seven days. However, when these reaction conditions were modified by the recently published discovery of using acetic acid as an additive in the Cu(I) catalyzed azide–alkyne cycloaddition,[28] the reaction took place with full conversion in 30 min at room temperature and compound **5** was isolated with a 96% yield. With these optimal conditions in hand, the entire series of triazoles **5**–**19** was prepared in 54–96% yields (Scheme 1).

Apart the phenyl group present in compound **5**, we incorporated various aryl such moieties in the new derivatives reported here, of types **5**–**19**, as it has been observed earlier[29,30] that the nature of the tails present in sulfonamide CAIs strongly influence the inhibitory properties and isoform selectivity of such derivatives. Thus we have chosen variously mono/-di-substituted phenyl



Scheme 1. Preparation of sulfonamides **5**–**19** from 1-bromo-thiophene-5-sulfonamide **1** by using click chemistry.

moieties (in diverse positions of the ring with respect to the azide moiety) as well as the naphthyl moiety (Scheme 1). In this way we introduced chemical diversity in the small library of thiophene-2-sulfonamide derivatives **5**–**19** reported here.

2.2. Carbonic anhydrase inhibition

Sulfonamides **5**–**19** reported here and acetazolamide **AAZ** as standard drug were assayed as inhibitors of four physiologically relevant CA isoforms, the cytosolic hCA I and II, as well as the transmembrane, tumo-associated ones CA IX and XII.[31] It should be mentioned that CA II and XII are targets for developing antiglaucoma drugs,[20] whereas CA IX (and XII) are antitumor drug targets.[23] hCA I is rather widespread in many tissues in humans, and it may be considered as an offtarget isoform.[1–3]

The following structure–activity relationship (SAR) can be evidenced from data of Table 1 regarding the CA inhibitory properties of sulfonamides **5**–**19**:

(i) hCA I was moderately inhibited by sulfonamides **5**–**19**, which showed inhibition constants in the range of 224–7544 nM, being thus similar (or less effective as hCA I inhibitors) to acetazolamide AAZ (K_I of 250 nM). The best inhibitors were **13** and **18** (incorporating 3-cyanophenyl and 4-methylsulfonyl-2-fluorophenyl moieties as Ar group) which showed (K_Is in the range of 224–289 nM, whereas the remaining compounds in the series were less effective, with K_Is in the range of 499–7544 nM (Table 1). It may be thus considered that the relatively weak inhibitory properties against the widespread isoform hCA I constitute a favorable feature of this class of CA is reported here.

(ii) The physiologically dominant isoform hCA II was highly inhibited by the new class of sulfonamides investigated here, with a flat (but excellent) inhibition profile. Indeed, the K_Is only ranged between 2.2 and 7.7 nM, making all these compounds highly effective hCA II inhibitors. Unexpectedly (however, see the X-ray crystallographic part for an explanation of this finding) all the substitution patterns present in compounds **5**–**19** lead to highly effective CAIs. All these compounds were better hCA II inhibitors compared to the clinically used drug **AAZ** (Table 1).

(iii) A more complicate SAR has been evidenced for the inhibition of the tumor-associated isoform hCA IX (Table 1). Several compounds, such as **5**, **9**, **12** and **17**, showed highly effective hCA IX inhibitory properties, with K_Is in the range of 5.4–10.9 nM. They incorporate phenyl, 4-methoxyphenyl-, 3-bromophenyl and 4-iodophenyl moieties. Another group of derivatives, among which **7**, **11**, **13**, **14** and **16**, showed inhibition constants in the range of 29.4–89.1 nM. They incorporate 4-tolyl, 4-trifluoromethylphenyl, 3-cyanophenyl, 3,4-dichlorophenyl and 3-chloro-4-methoxyphenyl moieties. It is obvius from these data that even small modifications of the Ar group from compounds **5**–**19**, lead to important changes in their affinity for CA IX (but not CA II, as mentioned above). For example, introduction of a 3-Cl atom in **9**, leading thus to **16**, had as a consequence a 12.8-times loss of hCA IX inhibitory activity of **16** compared **9** (Table 1). Weaker hCA IX inhibition was observed for compounds **6**, **8**, **10**, **15**, **18** and **19**, which had K_Is in the range of 101–811 nM. Apparently, bulkier Ar moieties (as the 1-naphthyl one present in **10**, the *tert*-butyl-phenyl present in **8**, etc.) lead to a loss of the hCA IX inhibitory activity for this series of sulfonamide CAIs.

(iv) hCA XII was also effectively inhibited by all sulfonamides investigated here, with K_Is in the range of 3.4–239 nM (Table 1). Most of the new sulfonamides were highly effective, low nanomolar hCA XII inhibitors, for example, compounds **5**–**7**, **9**, **10**, **13**, **15**, **17** and **18** (K_Is range of 3.4–9.6 nM). Several other derivatives, such as **8**, **12**, **14**, and **16** showed inhibition constants of 24.7–58.8 nM. Only two derivatives (**11** and **19**, both possessing CF_3 moieties in their molecules) were less effective as hCA XII inhibitors, with K_Is of 107.5–239 nM (Table 1). Thus, as for hCAII; most of the substitution patterns present in these new sulfonamides as Ar moieties (except those from **11** and **19**) lead to quite effective hCA XII inhibitors.

(v) Except for the offtarget isforms hCA I, which is generally poorly inhibited by sulfonamides **5**–**19**, the remaining three isoforms (hCA II, IX and XII) are well inhibited by the compounds reported here, with hCA II inhibition being in the low nanomolar ramnge for all compounds, followed by hCA XII inhibition (most derivatives low nanomolar inhibitors), whereas for hCA IX both highly effective as well as less effective inhibitors have been detected in this small series of derivatives.

Table 1
Inhibition data of isoforms hCA I, II, IX and XII with sulfonamides **5**–**19** and a standard, clinically used sulphonamide (acetazolamide **AAZ**), by a stopped-flow CO_2 hydrase assay[31]

Compound	K_I (nM)[a]			
	hCA I	hCA II	hCA IX	hCA XII
5	499	6.1	7.2	7.3
6	1434	3.8	560	6.2
7	526	3.5	43.6	8.8
8	2540	2.9	713	45.4
9	746	6.3	6.4	8.4
10	750	2.4	101	7.4
11	3055	2.9	89.1	107.5
12	2420	7.7	10.9	30.3
13	289	4.5	29.4	6.0
14	2132	3.2	63.5	24.7
15	2464	3.0	395	6.6
16	4273	2.7	81.9	58.8
17	709	2.9	5.4	9.6
18	224	2.7	140	3.4
19	7544	2.2	811	239
AAZ	250	12	25	5.6

[a] Errors were in the range of 5–10% of the reported values, from three different assays.

2.3. Protein X-ray crystallography

In order to rationalize some of the very interesting inhibition data reported above, we have resolved the high resolution X-ray crystal structures for the adducts of two of these inhibitors (**10** and **13**) bound to hCA II (Tables 2 and 3 and Figs. 1–3).

The crystal structure of hCA II in complex with compound the 1-naphthyl derivative **10** was solved at 1.35 Å resolution (Table 2). The obtained electron density clearly revealed the expected features of inhibitor (Fig. 1). Apart from interaction between the deprotonated sulfonamide moiety and the metal ion, and the hydrogen bond between the NH moiety of the sulfonamide and the OH of Thr199 (present in all other sulfonamide–hCA II complexes)[1a,2a,3a,30,32] **10** was not involved in other polar contacts with protein. This is one of the few cases in which positioning of the ligand within the hCA II active site was achieved solely by hydrophobic and van der Waals interactions (Fig. 1). Interestingly, the binding mode of **10** is quite different from that, observed in another similar compound **13**, (Fig. 2, see latter in the text). The naphthyl moiety of **10** fits perfectly well in a hydrophobic pocket of the protein evidenced earlier for the binding of other sulfonamide CAIs,[1a,2a,3a,30,32] with residues Phe131, Val135, Leu204 and Pro202 being in van der Waals contact with the naphthyl tail of the inhibitor (Fig. 2).

The crystal structure of CA II in complex with compound **13** was solved at 1.82 Å resolution (Table 3). The obtained electron density was good for most of the molecule except for the phenylcyano group, where it was weaker but still interpretable (Fig. 2). Along the interactions with the metal ion and Thr199 (as mentioned

Table 2
Data processing, refinement and validation statistics for the hCA II–**10** complex

Space group	$P2_1$
Cell dimensions	
a (Å)	42.3
b (Å)	41.5
c (Å)	72.2
β (°)	104.3
Resolution (Å)	20–1.34
Highest resolution shell (Å)	1.34–1.41
Number of reflections	50,792
Number of reflections in test set	2615
Completeness (%)	93 (80[a])
R_{merge}	0.05 (0.17)
$\langle I/\sigma I \rangle$	12.7 (4.3)
Average multiplicity	2.0 (1.2)
R-factor	0.13 (0.19)
R_{free}	0.18 (0.24)
Average *B* factor (Å^2)	11.7
Average *B* factor for inhibitor (Å^2)	11.0
<*B*> from Wilson plot (Å^2)	8.5
Number of protein atoms	2094
Number of inhibitor atoms	24
Number of solvent molecules	305
R.m.s. deviations from ideal values	
Bond lengths (Å)	0.022
Bond angles (°)	2.41
Outliers in Ramachandran plot (%)	0.41

[a] Values in parenthesis are for the high resolution bin.

Table 3
Data processing, refinement and validation statistics for the hCA II–**13** complex

Space group	$P2_1$
Cell dimensions	
a (Å)	42.4
b (Å)	41.5
c (Å)	72.2
β (°)	104.2
Resolution (Å)	20–1.82
Highest resolution shell (Å)	1.82–1.86
Number of reflections	20,916
Number of reflections in test set	1122
Completeness (%)	99.3 (86.8[a])
R_{merge}	0.17 (0.36)
$\langle I/\sigma I \rangle$	21.9 (3.4)
Average multiplicity	7.5 (2.8)
R-factor	0.14 (0.21)
R_{free}	0.20 (0.30)
Average *B* factor (Å^2)	12.2
Average *B* factor for inhibitor (Å^2)	15.6
<*B*> from Wilson plot (Å^2)	8.1
Number of protein atoms	2080
Number of inhibitor atoms	22
Number of solvent molecules	296
R.m.s. deviations from ideal values	
Bond lengths (Å)	0.019
Bond angles (°)	2.14
Outliers in Ramachandranplot (%)	0.40

[a] Values in parenthesis are for the high resolution bin.

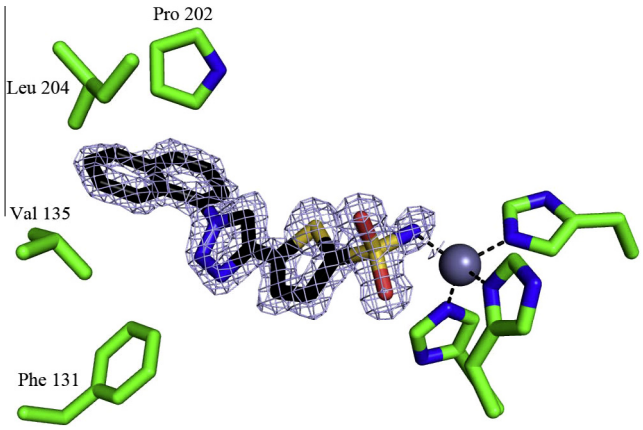


Figure 1. Binding of **10** within the hCA II active site. The zinc ion is shown as a gray sphere and its coordinating histidines (His94, 96 and 119) are shown in green. Residues 131, 135, 204 and 205 participating in hydrophobic and van der Waals contacts with inhibitor are indicated. For the sake of clarity, $2F_o-F_c$ electron density is shown only for ligand, contoured at 1 and calculated in the absence of ligand. The figure prepared by using Pymol (DeLano The PyMOL Molecular Graphics System San Carlos, CA, USA, DeLano Scientific).

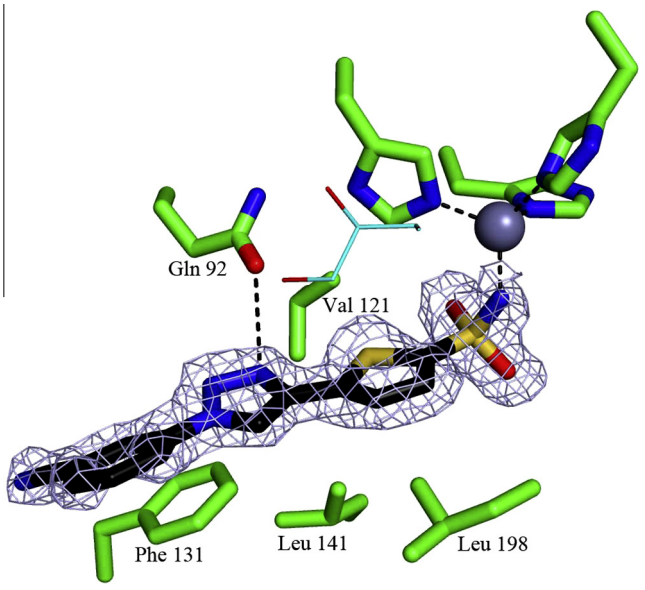


Figure 2. Binding of **13** within the hCA II active site. The zinc ion is shown as a gray sphere and its coordinating histidines (His94, 96 and 119) are shown in green. Residues 92, 121, 131, 141 and 198 participating in hydrogen bonding, hydrophobic and van der Waals contacts with the inhibitor are also indicated. A glycerol molecule bound to the enzyme is shown as a thin stick model with sky blue carbon atoms. For the sake of clarity, $2F_o-F_c$ electron density is shown only for ligand, contoured at 1 and calculated in the absence of ligand. The figure was prepared by using Pymol (DeLano, The PyMOL Molecular Graphics System San Carlos, CA, USA, DeLano Scientific).

above for **10**), compound **13** was involved in another polar contact with the protein, that is, a hydrogen bond with Gln92, an amino acid residues known to be involved in the binding of sulfonamide CAIs.[3a,30,32] A number of hydrophobic and van der Waals interactions are present as well in this adduct, and involved the following amino acid residues: Val121, Leu141, Phe131, and Leu198 (Fig. 2). In the electron density map of active site, we also located a glycerol molecule, in a near identical location as reported earlier in a number of CA II structures by McKenna's group.[33] As shown by these researchers, the glycerol is bound within the enzyme active site during the cryo-protection of the protein crystal, before freezing, and that does not affect the binding of the sulfonamide inhibitor.[33]

It should be also noted the very different orientation of the two inhibitors **10** and **13**, when bound to the hCA II active site (Fig. 3). Only the sulfamoyl moieties of the two compounds are superimposable, whereas the rest of the molecule is not (even at the level of the thiophene ring, rather close to the metal ion). Indeed, the aryl–triazolyl moieties of the two compounds adopt highly diverse orientation within the hCA II active site cavity, being completely non-superimposable. The 3-cyanophenyl group of **13** is oriented more towards the hydrophilic half of the active site and the triazole ring participates in the hydrogen bonding with the oxygen of the $CONH_2$ moiety of Gln92 (Figs. 2 and 3). On the contrary, the naphthyl (and triazole ring) of **10** are orientated towards the

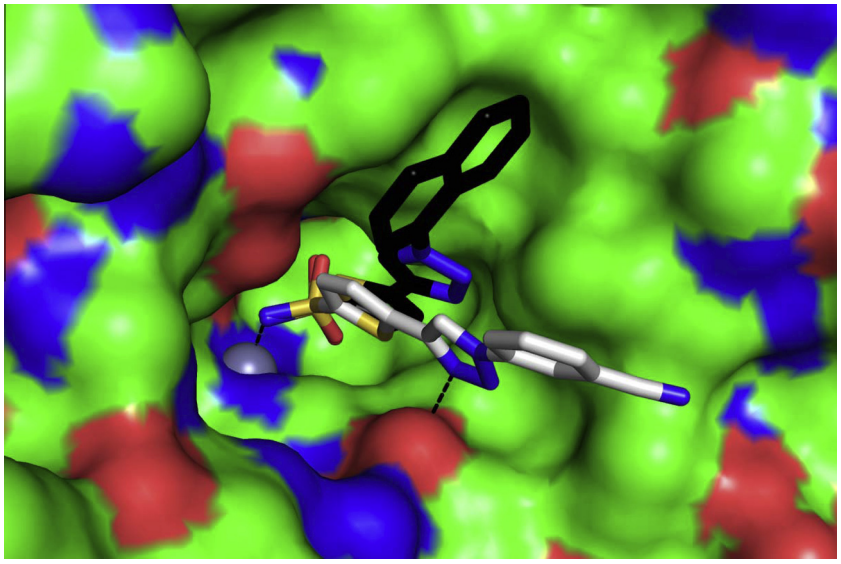


Figure 3. Comparison of the binding modes of **10** and **13** when bound to hCA II. Sulfonamide **10** is shown with black carbons and **13** with gray carbons. The protein is shown as a surface model, coloured according to surface atoms (carbon-green, nitrogen-blue, oxygen-red) and the zinc ion is sown as a gray sphere. Interactions of the sulfonamide group with the metal ion and a hydrogen bond of the triazole ring of **13** to the oxygen of the Gln92 residue are also shown.

hydrophobic half of the hCA II active site, occupying completely this binding pocket. The perfect fit of the aryl–triazolyl moiety of these compounds within the hCA II active site, may explain the excellent inhibition profile of all these compounds against the dominant CA isoforms, as shown above in Section 2.2.

3. Conclusions

We report here a series of 2-thiophene-sulfonamides incorporating 1-substituted aryl-1,2,3-triazolyl moieties, prepared by click chemistry from 5-ethynylthiophene-2-sulfonamide and substituted aryl azides. The new sulfonamides were investigated as inhibitors of the zinc metalloenzyme CA, and more specifically against the cytosolic isoforms hCA I and II and the transmembrane, tumor-associated ones hCA IX and XII: The new compounds were medium–weak hCA I inhibitors (inhibition constants in the range of 224–7544 nM), but were compactly, highly effective, low nanomolar hCA II inhibitors (K_Is in the range of 2.2–7.7 nM). The tumor-associated hCA IX was inhibited with K_Is ranging between 5.4 and 811 nM, whereas hCA XII with inhibition constants in the range of 3.4–239 nM. The X-ray crystal structure of the adducts of two such compounds complexed to hCA II (one incorporating 1-naphthyl, the other one 3-cyanophenyl moieties) evidenced the reasons of the high affinity for this enzyme. Highly favorable, predominantly hydrophobic interactions between the sulfonamide scaffold and the hCA II active site were responsible for the binding, in addition to the coordination of the sulfamoyl moiety to the zinc ion. The tails of the two inhibitors adopted very diverse orientations when bound to the active site, with the naphthyltriazolyl moiety orientated towards the hydrophobic half of the active site, and the 3-cyanophenyl pointing towards the hydrophilic half. These data may be used for the structure-based drug design of even more effective hCA II inhibitors, with potential use as antiglaucoma agents or as diuretics.

4. Experimental protocols

4.1. Chemistry

Reagents and starting materials were obtained from commercial sources and used as received. The solvents were purified and dried by standard procedures prior to use; petroleum ether of boiling range 40–60 °C was used. Flash chromatography was carried out using Merck silica gel (230–400 mesh) or reversed phase PR18 (25–40 μm). Thin-layer chromatography was performed on silica gel, spots were visualized with UV light (254 and 365 nm). Melting points were determined on an OptiMelt automated melting point system. IR spectra were measured on a Shimadzu FTIR IR Prestige-21 spectrometer. NMR spectra were recorded on Varian Mercury (400 MHz) spectrometer with chemical shifts values (δ) in ppm relative to TMS using the residual DMSO-d_6 signal as an internal standard. HRMS data were obtained with a Q-TOF micro high-resolution mass spectrometer with ESI (ESI^+/ESI^-).

*5-Bromo-N-[(E)-(dimethylamino)methylidene]thiophene-2-sulfonamide (**2**)*[25]*:*

To a solution of 5-bromothiophene-2-sulfonamide (**1**) (7.00 g, 28.91 mmol) in acetonitrile (50 mL) *N,N*-dimethylformamide dimethyl acetal (5.50 mL, 41.34 mmol) was added. The mixture was stirred at room temperature for 24 h. Solvent evaporation in vacuum afforded **2** (7.98 g, 93%) as light yellow solid. Mp 104–105 °C. IR (KBr, cm^{-1}) ν_{max}: 1631 (N=C), 1331 (S=O), 1142 (S=O), 1127 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.94 (s, 3H), 3.16 (s, 3H), 7.27 (d, J = 3.9 Hz, 1H), 7.36 (d, J = 3.9 Hz, 1H), 8.21 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 35.3, 41.1, 117.1, 130.6, 131.0, 145.7, 160.0.

*N-[(dimethylamino)methylidene]-5-[2-(trimethylsilyl)ethynyl)]thiophene-2-sulfonamide (**3**):*

To a solution of compound **2** (2.00 g, 6.73 mmol) in dry DMF CuI (0.13 g, 0.68 mmol), $Pd(PPh_3)_2Cl_2$ (0.24 g, 0.34 mmol), trimethylsilylacetylene (1.35 mL, 9.46 mmol) and Et_3N (4.70 mL, 33.78 mmol) were added. The mixture was stirred at room temperature under an argon atmosphere for 48 h, diluted with EtOAc (30 mL), filtered though a celite plug. Saturated aqueous NH_4Cl (50 mL) was added and the product was extracted with EtOAc (3 × 50 mL). The organic extract was washed with H_2O (2 × 50 mL) and brine (50 mL), dried over Na_2SO_4, solvent driven off in vacuum. Purification of the crude product by silica gel chromatography (EtOAc/PE 2:1) afforded **3** (1.75 g, 82%) as light brown solid. R_f = 0.7 (EtOAc/PE 2:1). Mp 120.5–121.5 °C. IR (KBr, cm^{-1}) IR (KBr, cm^{-1}) ν_{max}: 2147 (C≡C), 1635 (N=C), 1345 (S=O), 1148 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.24 (s, 9H), 2.95 (s, 3H), 3.17 (s, 3H), 7.32 (d, J =

3.9 Hz, 1H), 7.43 (d, J = 3.9 Hz, 1H), 8.22 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: −0.45, 35.3, 41.1, 96.0, 101.9, 126.0, 130.0, 133.2, 145.7, 160.0; HRMS (ESI) [M+H]$^+$: m/z Calcd for ($C_{12}H_{19}N_2O_2S_2Si$) 315.0657. Found 315.0652.

*5-Ethynylthiophene-2-sulfonamide (**4**)*[34]*:*

To a solution of compound **3** (1.00 g, 3.18 mmol) in dry THF (30 mL) 1 M TBAF in THF (6.36 mL, 6.36 mmol) was added. The mixture was stirred at room temperature for 3 h, diluted with H_2O (30 mL), extracted with EtOAc (3 × 25 mL). The organic phase was washed with brine (25 mL), dried over Na_2SO_4, solvent was driven off in vacuum. Purification of the crude product by silica gel chromatography (EtOAc/PE 1:1) afforded **4** (0.30 g, 50%) as brown solid. R_f = 0.8 (EtOAc/PE 1:1). Mp 125.5–126.5 °C. IR (KBr, cm^{-1}) ν_{max}: 3330 (NH), 3233 (NH), 2100 (C≡C), 1341 (S=O), 1150 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.81 (s, 1H), 7.38 (d, J = 3.9 Hz, 1H), 7.47 (d, J = 3.9 Hz, 1H), 7.83 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 75.4, 87.6, 125.4, 130.0, 133.3, 146.5.

General procedure for the synthesis of 1,2,3-triazolylderivatives of sulfamoylthiophene

To a solution of 5-ethynylthiophene-2-sulfonamide (**4**) (1 equiv) in 1:1 ^{t}BuOH/H_2O (10 mL per 1.07 mmol of **4**) corresponding arylazide (1 equiv) and solution of $CuSO_4{\cdot}5H_2O$ (1.3 equiv) in H_2O (1.5 mL per 1.36 mmol) were added. The mixture was stirred at room temperature for 5 min before solution of sodium ascorbate (4 equiv) in H_2O (2 mL per 4.27 mmol) was added. The mixture was stirred at room temperature for another 15 min before AcOH (20 equiv) was added and stirring was continued for 30 min. Solvent was driven off in vacuum and the crude product was purified by reversed phase chromatography (C-18, H_2O–MeCN gradient MeCN 10–90%) with followed re-crystallization from MeCN/H2O.

*5-(1-Phenyl-1H-1,2,3-triazol-4-yl)thiophene-2-sulfonamide (**5**)*

Compound **5** was prepared according to the general procedure from **4** (0.20 g, 1.07 mmol), azidobenzene[35,36] (0.13 g, 1.07 mmol), $CuSO_4{\cdot}5H_2O$ (0.34 g, 1.36 mmol), sodium ascorbate (0.85 g, 4.27 mmol) and AcOH (1.22 mL, 21.36 mmol) as a yellowish solid (0.31 g, 96%). Mp 287–288 °C. IR (KBr, cm^{-1}) ν_{max}: 3324 (NH), 3238 (NH), 1335 (S=O), 1151 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.46 (d, J = 3.7 Hz, 1H), 7.50 (d, J = 3.7 Hz, 1H), 7.51–7.56 (m, 1H), 7.61–7.67 (m, 4H), 7.92–7.96 (m, 2H), 9.32 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 120.0, 120.2, 124.1, 129.1, 129.6, 130.0, 135.6, 136.4, 141.7, 147.0; HRMS (ESI) [M+H]$^+$: m/z Calcd for ($C_{12}H_{11}N_4O_2S_2$) 307.0323. Found 307.0325.

*5-[1-(3-Methylphenyl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**6**):*

Compound **6** was prepared according to the general procedure from **4** (0.20 g, 1.07 mmol), 1-azido-3-methylbenzene[37] (0.14 g, 1.07 mmol), $CuSO_4{\cdot}5H_2O$ (0.34 g, 1.36 mmol), sodium ascorbate (0.85 g, 4.27 mmol) and AcOH (1.22 mL, 21.36 mmol) as a yellowish solid (0.27 g, 80%). Mp 222–223 °C. IR (KBr, cm^{-1}) ν_{max}: 3309 (NH), 1340 (S=O), 1151 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.43 (s, 3H), 7.33–7.37 (m, 1H), 7.50 (d, J = 3.9 Hz, 1H), 7.51–7.54 (m, 1H), 7.60 (d, J = 3.9 Hz, 1H), 7.70–7.74 (m, 1H), 7.76–7.79 (m, 3H), 9.33 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.9, 117.3, 120.2, 120.6, 124.2, 129.7, 129.8, 130.8, 136.3, 136.8, 139.8, 141.4, 144.4; HRMS (ESI) [M+H]$^+$: m/z Calcd for ($C_{13}H_{13}N_4O_2S_2$) 321.0480. Found 321.0505.

*5-[1-(4-Methylphenyl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**7**):*

Compound **7** was prepared according to the general procedure from **4** (0.20 g, 1.07 mmol), 1-azido-4-methylbenzene[35] (0.14 g, 1.07 mmol), $CuSO_4{\cdot}5H_2O$ (0.34 g, 1.36 mmol), sodium ascorbate (0.85 g, 4.27 mmol) and AcOH (1.22 mL, 21.36 mmol) as a light brown solid (0.27 g, 80%). Mp 275–276 °C. IR (KBr, cm^{-1}) ν_{max}: 3324 (NH), 3233 (NH), 1340 (S=O), 1153 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.39 (s, 3H), 7.41–7.46 (m, 2H), 7.50 (d, J = 3.9 Hz, 1H), 7.59 (d, J = 3.9 Hz, 1H), 7.78 (s, 2H), 7.79–7.83 (m, 2H), 9.29 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.7, 120.1, 120.2, 124.2, 130.4, 130.9, 134.1, 136.9, 138.9, 141.4, 144.4; HRMS (ESI) [M+H]$^+$: m/z Calcd for ($C_{13}H_{13}N_4O_2S_2$) 321.0480. Found 321.0527.

*5-[1-(4-tert-Butylphenyl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**8**):*

Compound **8** was prepared according to the general procedure from **4** (0.20 g, 1.07 mmol), 1-azido-4-*tert*-butylbenzene[38] (0.19 g, 1.07 mmol), $CuSO_4{\cdot}5H_2O$ (0.34 g, 1.36 mmol), sodium ascorbate (0.85 g, 4.27 mmol) and AcOH (1.22 mL, 21.36 mmol) as a light brown solid (0.27 g, 70%). Mp 213–214 °C. IR (KBr, cm^{-1}) ν_{max}: 3303 (NH), 1341 (S=O), 1152 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.34 (s, 9H), 7.50 (d, J = 3.9 Hz, 1H), 7.59 (d, J = 3.9 Hz, 1H), 7.62–7.67 (m, 2H), 7.78 (s, 2H), 7.81–7.86 (m, 2H), 9.31 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 31.0, 34.6, 120.0, 120.2, 124.2, 126.7, 130.8, 134.0, 136.9, 141.3, 144.3, 151.8; HRMS (ESI) [M+H]$^+$: m/z Calcd for ($C_{16}H_{19}N_4O_2S_2$) 363.0949. Found 363.0952.

*5-[1-(4-Methoxyphenyl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**9**):*

Compound **9** was prepared according to the general procedure from **4** (0.30 g, 1.60 mmol), 1-azido-4-methoxybenzene[36] (0.24 g, 1.60 mmol), $CuSO_4{\cdot}5H_2O$ (0.51 g, 2.04 mmol), sodium ascorbate (1.27 g, 6.41 mmol) and AcOH (1.83 mL, 32.04 mmol) as a light brown solid (0.37 g, 69%). Mp 258-259 °C. IR (KBr, cm^{-1}) ν_{max}: 3337 (NH), 3233 (NH), 1340 (S=O), 1151 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.85 (s, 3H), 7.15–7.20 (m, 2H), 7.49 (d, J = 3.9 Hz, 1H), 7.59 (d, J = 3.9 Hz, 1H), 7.77 (br s, 2H), 7.81–7.86 (m, 2H), 9.25 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.6, 115.0, 120.3, 122.0, 124.1, 129.7, 130.8, 137.0, 141.2, 144.3, 159.6; HRMS (ESI) [M+H]$^+$: m/z Calcd for ($C_{13}H_{13}N_4O_3S_2$) 337.0429. Found 337.0435.

*5-[1-(Naphthalen-1-yl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**10**):*

Compound **10** was prepared according to the general procedure from **4** (0.20 g, 1.07 mmol), 1-azidonaphthalene[39] (0.18 g, 1.07 mmol), $CuSO_4{\cdot}5H_2O$ (0.34 g, 1.36 mmol), sodium ascorbate (0.85 g, 4.27 mmol) and AcOH (1.22 mL, 21.36 mmol) as a brown solid (0.34 g, 89%). Mp 228–229 °C. IR (KBr, cm^{-1}) ν_{max}: 3307 (NH), 1346 (S=O), 1153 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.55 (d, J = 3.9 Hz, 1H), 7.57–7.60 (m, 1H), 7.61 (d, J = 3.9 Hz, 1H), 7.63–7.76 (m, 3H), 7.80 (s, 2H), 7.84 (dd, J = 7.3, 1.2 Hz, 1H), 8.14–8.17 (m, 1H), 8.22–8.26 (m, 1H), 9.22 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 122.0, 124.1, 124.3, 124.7, 125.5, 127.3, 127.8, 128.3, 128.4, 130.7, 130.9, 132.9, 133.7, 137.0, 140.9, 144.4; HRMS (ESI) [M+H]$^+$: m/z Calcd for ($C_{16}H_{13}N_4O_2S_2$) 357.0480. Found 357.0488.

*5-{1-[4-(Trifluoromethyl)phenyl]-1H-1,2,3-triazol-4-yl}thiophene-2-sulfonamide (**11**):*

Compound **11** was prepared according to the general procedure from **4** (0.20 g, 1.07 mmol), 1-azido-4-(trifluoromethyl)benzene[40] (0.20 g, 1.07 mmol), $CuSO_4{\cdot}5H_2O$ (0.34 g, 1.36 mmol), sodium ascorbate (0.85 g, 4.27 mmol) and AcOH (1.22 mL, 21.36 mmol) as a light brown solid (0.28 g, 70%). Mp 252–253 °C. IR (KBr, cm^{-1}) ν_{max}: 3320 (NH), 1340 (S=O), 1152 (S=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.53 (d, J = 3.8 Hz, 1H), 7.61 (d, J = 3.8 Hz, 1H), 7.80 (s, 2H), 8.02–8.07 (m, 2H), 8.17–8.23 (m, 2H), 9.51 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 120.5, 120.7, 123.8 (q, J = 273 Hz), 124.5, 127.4 (q, J = 3.8 Hz), 129.0 (q, J = 33.0 Hz), 130.9, 136.4, 139.1, 141.7, 144.7; HRMS (ESI) [M+H]$^+$: m/z Calcd for ($C_{13}H_{10}F_3N_4O_2S_2$) 375.0197. Found 375.0250.

*5-[1-(3-Bromophenyl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**12**):*

Compound **12** was prepared according to the general procedure from **4** (0.30 g, 1.60 mmol), 1-azido-3-bromobenzene[41] (0.32 g, 1.60 mmol), $CuSO_4{\cdot}5H_2O$ (0.51 g, 2.04 mmol), sodium ascorbate

(1.27 g, 6.41 mmol) and AcOH (1.83 mL, 32.04 mmol) as a yellowish solid (0.33 g, 54%). Mp 246–247 °C. IR (KBr, cm^{-1}) ν_{max}: 3304 (NH), 1342 (S=O), 1152 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ: 7.49 (d, J = 3.9 Hz, 1H), 7.57–7.63 (m, 2H), 7.72–7.76 (m, 1H), 7.79 (br s, 2H), 7.97–8.01 (m, 1H), 8.18–18.21 (m, 1H), 9.43 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 119.2, 120.5, 122.5, 122.8, 124.3, 130.9, 131.8, 131.9, 136.5, 137.4, 141.5, 144.6; HRMS (ESI) $[M+H]^+$: m/z Calcd for ($C_{12}H_{10}BrN_4O_2S_2$) 384.9429. Found 384.9454.

*5-[1-(3-Cyanophenyl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**13**):*

Compound **13** was prepared according to the general procedure from **4** (0.20 g, 1.07 mmol), 3-azidobenzonitrile[37] (0.15 g, 1.07 mmol), $CuSO_4{\cdot}5H_2O$ (0.34 g, 1.36 mmol), sodium ascorbate (0.85 g, 4.27 mmol) and AcOH (1.22 mL, 21.36 mmol) as a yellow solid (0.32 g, 92%). Mp 243–244 °C. IR (KBr, cm^{-1}) ν_{max}: 3331 (NH), 3232 (NH), 2235 (C≡N), 1336 (S=O), 1157 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ: 7.50 (d, J = 3.9 Hz, 1H), 6.61 (d, J = 3.9 Hz, 1H), 7.80 (s, 2H), 7.86 (app t, J = 8.2 Hz, 1H), 8.00–8.04 (m, 1H), 8.32 (ddd, J = 8.2, 2.4, 1.2 Hz, 1H), 8.46–8.48 (m, 1H), 9.45 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 112.9, 117.8, 120.6, 123.7, 124.5, 124.9, 130.9, 131.4, 132.7, 136.4, 136.8, 141.6, 144.7; HRMS (ESI) $[M+H]^+$: m/z Calcd for ($C_{13}H_{10}N_5O_2S_2$) 332.0276. Found 332.0335.

*5-[1-(3,4-Dichlorophenyl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**14**):*

Compound **14** was prepared according to the general procedure from **4** (0.20 g, 1.07 mmol), 4-azido-1,2-dichlorobenzene[37] (0.20 g, 1.07 mmol), $CuSO_4{\cdot}5H_2O$ (0.34 g, 1.36 mmol), sodium ascorbate (0.85 g, 4.27 mmol) and AcOH (1.22 mL, 21.36 mmol) as a white solid (0.34 g, 85%). Mp 260–261 °C. IR (KBr, cm^{-1}) ν_{max}: 3325 (NH), 3218 (NH), 1343 (S=O), 1155 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ: 7.49 (d, J = 3.9 Hz, 1H), 7.60 (d, J = 3.9 Hz, 1H), 7.79 (s, 2H), 7.93 (d, J = 8.7 Hz, 1H), 7.99 (dd, J = 8.7, 2.4 Hz, 1H), 8.29 (d, J = 2.4 Hz, 1H), 9.44 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 120.3, 120.6, 122.0, 124.4, 130.9, 131.4, 131.9, 132.4, 135.8, 136.4, 141.6, 144.7; HRMS (ESI) $[M+H]^+$: m/z Calcd for ($C_{12}H_9Cl_2N_4O_2S_2$) 374.9544. Found 374.9597.

*5-[1-(3-Chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**15**):*

Compound **15** was prepared according to the general procedure from **4** (0.20 g, 1.07 mmol), 4-azido-2-chloro-1-fluorobenzene[42] (0.18 g, 1.07 mmol), $CuSO_4{\cdot}5H_2O$ (0.34 g, 1.36 mmol), sodium ascorbate (0.85 g, 4.27 mmol) and AcOH (1.22 mL, 21.36 mmol) as a light yellow solid (0.34 g, 89%). Mp 240–241 °C. IR (KBr, cm^{-1}) ν_{max}: 3314 (NH), 1340 (S=O), 1152 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ: 7.48 (d, J = 3.9 Hz, 1H), 7.60 (d, J = 3.9 Hz, 1H), 7.72 (app t, J = 9.0 Hz, 1H), 7.79 (s, 2H), 7.99 (ddd, J = 9.0, 4.3, 2.7 Hz, 1H), 8.24 (dd, J = 6.3, 2.7 Hz, 1H), 9.38 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 118.3 (d, J = 22.8 Hz), 120.7, 120.9 (d, J = 19.3 Hz), 121.2 (d, J = 7.9 Hz), 122.7, 124.4, 130.9, 133.3 (d, J = 3.1 Hz), 136.5, 141.5, 144.6, 157.1 (d, J = 249 Hz); HRMS (ESI) $[M+H]^+$: m/z Calcd for ($C_{12}H_9ClFN_4O_2S_2$) 358.9839. Found 358.9866.

*5-[1-(3-Chloro-4-methoxyphenyl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**16**):*

Compound **16** was prepared according to the general procedure from **4** (0.10 g, 0.53 mmol), 4-azido-2-chloro-1-methoxybenzene[43] (0.10 g, 0.53 mmol), $CuSO_4{\cdot}5H_2O$ (0.17 g, 0.68 mmol), sodium ascorbate (0.42 g, 2.14 mmol) and AcOH (0.61 mL, 10.68 mmol) as a light brown solid (0.20 g, 90%). Mp 262–263 °C. IR (KBr, cm^{-1}) ν_{max}: 3332 (NH), 1336 (S=O), 1156 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ: 3.95 (s, 3H), 7.39 (d, J = 9.0 Hz, 1H), 7.47 (d, J = 3.9 Hz, 1H), 7.59 (d, J = 3.9 Hz, 1H), 7.78 (s, 2H), 7.90 (dd, J = 9.0, 2.7 Hz, 1H), 8.05 (d, J = 2.7 Hz, 1H), 9.31 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 56.7, 113.6, 120.4, 120.5, 121.9, 122.0, 124.2, 129.8, 130.9, 136.8, 141.3, 144.4, 154.9; HRMS (ESI) $[M+H]^+$: m/z Calcd for ($C_{13}H_{12}ClN_4O_3S_2$) 371.0039. Found 371.0047.

*5-[1-(4-Iodophenyl)-1H-1,2,3-triazol-4-yl]thiophene-2-sulfonamide (**17**):*

Compound **17** was prepared according to the general procedure from **4** (0.10 g, 0.53 mmol), 1-azido-4-iodobenzene[35,36] (0.13 g, 0.53 mmol), $CuSO_4{\cdot}5H_2O$ (0.17 g, 0.68 mmol), sodium ascorbate (0.42 g, 2.14 mmol) and AcOH (0.61 mL, 10.68 mmol) as a light brown solid (0.20 g, 90%). Mp 274–275 °C. IR (KBr, cm^{-1}) ν_{max}: 3349 (NH), 3246 (NH), 1342 (S=O), 1150 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ: 7.50 (d, J = 3.9 Hz, 1H), 7.59 (d, J = 3.9 Hz, 1H), 7.74–7.77 (m, 2H), 7.78 (s, 2H), 7.99–8.03 (m, 2H), 9.37 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 94.9, 120.2, 122.1, 124.4, 130.9, 136.0, 136.6, 138.8, 141.6, 144.5; HRMS (ESI) $[M+H]^+$: m/z Calcd for ($C_{12}H_{10}IN_4O_2S_2$) 432.9290. Found 432.9306.

*5-{1-[2-Fluoro-4-(methylsulfonyl)phenyl]-1H-1,2,3-triazol-4-yl}thiophene-2-sulfonamide (**18**):*

Compound **18** was prepared according to the general procedure from **4** (0.10 g, 0.53 mmol), 1-azido-2-fluoro-4-(methylsulfonyl)benzene (0.12 g, 0.53 mmol) prepared: To a solution of 2-fluoro-4-(methylsulfonyl)aniline (1.00 g, 5.28 mmol) in mixture of H_2O (6.7 mL) and conc. HCl (3.3 mL) aqueous 4 M $NaNO_2$ (1.72 mL, 6.87 mmol) dropwise was added at 0 °C. The mixture was stirred at 0 °C for 10 min before aqueous 4 M NaN_3 (1.59 mL, 6.36 mmol) dropwise was added at 0 °C and stirring was continued at the same temperature for 1 h. H_2O (15 mL) was added and the product was extracted with Et_2O (2 × 25 mL). The organic phase was washed with H_2O (25 mL) and dried over Na_2SO_4. Solvent evaporation afforded 1-azido-2-fluoro-4-(methylsulfonyl)benzene (0.89 g, 78%) as orange solid.

IR (KBr, cm^{-1}) ν_{max}: 2142 (N_3), 2101 (N_3), 1318 (S=O), 1148 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ: 3.26 (s, 3H), 7.58 (app t, J = 8.3 Hz, 1H), 7.77 (ddd, J = 8.3, 2.0, 0.8 Hz, 1H), 7.88 (dd, J = 10.6, 2.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 43.3, 115.7 (d, J = 21.9 Hz), 122.3 (d, J = 1.6 Hz), 124.3 (d, J = 3.6 Hz), 133.1 (d, J = 0.9 Hz), 137.7 (d, J = 3.9 Hz), 153.0 (d, J = 252 Hz).), $CuSO_4{\cdot}5H_2O$ (0.17 g, 0.68 mmol), sodium ascorbate (0.42 g, 2.14 mmol) and AcOH (0.61 mL, 10.68 mmol) as a light brown solid (0.15 g, 72%). Mp 279–280 °C. IR (KBr, cm^{-1}) ν_{max}: 3343 (NH), 3261 (NH), 1349 (S=O), 1298 (S=O), 1164 (S=O), 1133 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ: 7.59–7.62 (m, 2H), 7.80 (s, 2H), 8.01–8.05 (m, 1H), 8.20–8.28 (m, 2H), 9.27 (d, J = 2.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 43.1, 116.7 (d, J = 22.9 Hz), 123.3 (d, J = 5.5 Hz), 124.4 (d, J = 3.5 Hz), 125.0, 126.6, 128.3 (d, J = 10.8 Hz), 130.9, 136.1, 141.5, 142.9 (d, J = 6.2 Hz), 144.8, 153.1 (d, J = 257 Hz); HRMS (ESI) $[M+H]^+$: m/z Calcd for ($C_{13}H_{12}FN_4O_4S_3$) 403.0005. Found 402.9996.

*5-{1-[4-Cyano-3-(trifluoromethyl)phenyl]-1H-1,2,3-triazol-4-yl}thiophene-2-sulfonamide (**19**):*

Compound **19** was prepared according to the general procedure from **4** (0.20 g, 1.07 mmol), 4-azido-2-(trifluoromethyl)benzonitrile (0.23 g, 1.07 mmol) prepared: To a solution of 4-amino-2-(trifluormetyl)benzonitrile (1.00 g, 5.37 mmol) in mixture of H_2O (6.7 mL) and conc. HCl (3.3 mL) aq 4 M $NaNO_2$ (1.75 mL, 6.98 mmol) dropwise was added at 0 °C. The mixture was stirred at 0 °C for 10 min before aq 4 M NaN_3 (1.61 mL, 6.44 mmol) dropwise was added at 0 °C and stirring was continued at the same temperature for 1 h. H_2O (15 mL) was added and the product was extracted with Et_2O (2 × 25 mL). The organic phase was washed with H_2O (25 mL), dried over Na_2SO_4. Solvent evaporation afforded 4-azido-2-(trifluoromethyl)benzonitrile (0.71 g, 62%) as light brown solid. IR (KBr, cm^{-1}) ν_{max}: 2229 (C≡N), 2133 (N_3); ^1H NMR (400 MHz, DMSO-d_6) δ: 7.61–7.66 (m, 2H), 8.17 (d, J = 8.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 103.7 (q, J = 2.2 Hz), 115.4, 118.3 (q, J = 4.8 Hz), 122.1 (q, J = 274 Hz), 123.7, 132.5 (q, Jv32.7 Hz), 137.0, 145.8.), $CuSO_4{\cdot}5H_2O$ (0.34 g, 1.36 mmol), sodium ascorbate

(0.85 g, 4.27 mmol) and AcOH (1.22 mL, 21.36 mmol) as a yellow solid (0.31 g, 73%). Mp 254–255 °C. IR (KBr, cm^{-1}) ν_{max}: 3368 (NH), 3276 (NH), 2230 (CN), 1340 (S=O), 1138 (S=O); ^1H NMR (400 MHz, DMSO-d_6) δ: 7.52 (d, J = 3.9 Hz, 1H), 7.62 (d, J = 3.9 Hz, 1H), 7.81 (s, 2H), 8.45–8.56 (m, 3H), 9.65 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 108.2, 115.0, 118.5 (q, J = 4.9 Hz), 120.9, 122.0 (q, J = 274 Hz), 124.1, 124.7, 131.0, 132.7 (q, J = 32.7 Hz), 136.0, 137.6, 139.4, 142.0, 145.0; HRMS (ESI) $[M+H]^+$: m/z Calcd for ($C_{14}H_9F_3N_5O_2S_2$) 400.0150. Found 400.0161.

4.2. Protein production and purification

hCA I, hCA II, hCA IX and hCA XII were produced and purified as described earlier by our groups.[8,9,11]

4.3. CA inhibition assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity.[31] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with 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, as reported earlier,[8] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.[8,9,11]

4.4. Crystallization and data collection

Protein was concentrated to 10.8 mg/ml in 20 mM tris–HCl pH 8.0 using 10 kDa cutoff Amicon concentrator. Crystallization was done by sitting drop technique in 96-well MRC plates (Molecular Dimensions). 1 µl of protein was mixed with 1 µl of bottom solution, (1.5 M Na citrate, 80 mM tris–HCl pH 9.0) and 0.2 µl of 100 mM inhibitor in 100% DMSO. The obtained crystals were flash-frozen in liquid nitrogen. Data were collected at beamline I911-3, MAX-lab synchrotron, Lund, Sweden.

4.5. Structure determination

Images were processed by MOSFLM[44] and scaled by SCALA.[45] The structure was refined by REFMAC[46] using unliganded CA II mutant (PDB code 3DC3)[9] as an initial model. The parameter files for sulfonamides 10 and 13 were generated by LIBCHECK.[47] The ligand was fitted in electron density in COOT (Emsley and Cowtan 2004),[48] followed by further REFMAC runs. Data scaling, refinement, and validation statistics are listed in Table 1. Atomic coordinates and structure factors were deposited in PDB with accession code 4bf1 and 4bf6.

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