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4-Substituted-2,3,5,6-tetrafluorobenzenesulfonamides as inhibitors of carbonic anhydrases I, II, VII, XII, and XIII

Virginija Dudutienė [a], Asta Zubrienė [a], Alexey Smirnov [a], Joana Gylytė [a], David Timm [a], Elena Manakova [b], Saulius Gražulis [b], Daumantas Matulis [a,*]

[a] Department of Biothermodynamics and Drug Design,Vilnius University Institute of Biotechnology, Graičiūno 8, Vilnius LT-02241, Lithuania
[b] Department of Protein–DNA Interactions, Vilnius University Institute of Biotechnology, Graičiūno 8, Vilnius LT-02241, Lithuania

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ABSTRACT

A series of 4-substituted-2,3,5,6-tetrafluorobenezenesulfonamides were synthesized and their binding potencies as inhibitors of recombinant human carbonic anhydrase isozymes I, II, VII, XII, and XIII were determined by the thermal shift assay, isothermal titration calorimetry, and stop-flow CO_2 hydration assay. All fluorinated benzenesulfonamides exhibited nanomolar binding potency toward tested CAs and fluorinated benzenesulfonamides posessed higher binding potency than non-fluorinated compounds. The crystal structures of 4-[(4,6-dimethylpyrimidin-2-yl)thio]-2,3,5,6-tetrafluorobenzenesulfonamide in complex with CA II and CA XII, and 2,3,5,6-tetrafluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide in complex with CA XIII were determined. The observed dissociation constants for several fluorinated compounds reached subnanomolar range for CA I isozyme. The affinity and the selectivity of the compounds towards tested isozymes are presented.

1. Introduction

The human carbonic anhydrases (CA) are family of zinc metalloenzymes that catalyze the reversible hydration of CO_2.[1,2] Iron and other metals are sometimes found in bacterial and other non-human CAs.[3,4] This reaction regulates a broad range of physiological functions such as respiration, CO_2/bicarbonate transport between lungs and metabolizing tissues, pH and CO_2 homeostasis, electrolyte secretion in many tissues/organs, etc.[5,6] There are 15 carbonic anhydrase isozymes identified in humans—12 catalytically active and 3 inactive, the so called CA-related proteins. The 12 active isoforms have different subcellular localization: 5—cytosolic (I, II, III, VII, and XIII), 3—transmembrane (IX, XII, and XIV), 1—membrane bound (IV), 2—mitochondrial (VA and VB), and 1—secreted (VI). Some of these isozymes (e.g., CA II, IV, VA, VB, VII, IX, XII, XIII and XIV) constitute targets for the development of antiglaucoma, diuretic, antiobesity, anticonvulsant or anticancer drugs.[7–10] Despite the large number of different CA inhibitors (CAIs) that have been synthesized to date, the available pharmaceutical agents have a number of shortcomings. One of them is the non-selective inhibition of many CA isozymes throughout the human body, resulting in various side effects. Therefore, there is a need to improve the inhibition and selectivity profile of the developed CAIs.

The major class of CAIs is aromatic and heterocyclic compounds possessing a primary sulfonamide group.[11] Sulfonamide-class CAIs are widely used as therapeutic agents for the treatment of various diseases. Benzenesulfonamides are the most abundant CAI class possessing a sulfonamide group. Introduction of substituents in various positions of the benzene ring of benzenesulfonamides have been largely investigated from diverse points of view.[9,11,12] General approaches for increasing affinity of benzenesulfonamides for CA are the enhancement of the influence of the arylsulfonamide head group (SO_2NH_2) on their binding and the amplification of interactions between the substituted aryl ring and the hydrophobic pocket of CA.[1]

The contribution towards the affinity of the sulfonamide head group can be explained by the influence of the pK_a of the arylsulfonamide. It is known that the highest-affinity arylsulfonamides should have a pK_a near the pH of buffered medium (~7.4)[1] while nonsubstituted benzenesulfonamide possess pK_a = 10.1.[13] The presence of electronegative substituents decreases the pK_a of sulfonamide and this correlates with an increase in the CA inhibitory properties. Introduction of halogen atoms as substituents on the benzene ring of benzenesulfonamides is one of the possible choices.[11,12]

Fluorine has been used widely in protein ligands. Extensive use of fluorinated compounds in medicinal chemistry is due to the unique properties of fluorine.[14] The physical properties of fluorine that have contributed to its relevance in medicinal chemistry include its slightly larger size compared to hydrogen (fluorine's van

* Corresponding author. Tel.: +370 5 269 1884; fax: +370 5 260 2116.
E-mail addresses: matulis@ibt.lt, daumantas.matulis@bti.vu.lt (D. Matulis).

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der Waals radius is 1.47 Å while hydrogen 1.2 Å, the bond lengths of CH_3–F = 1.382 Å and of CH_3–H = 1.087 Å), its high electronegativity, low polarizability, and the contribution towards increased lipophilicity. These features of fluorine enable the synthesis of compounds possessing unique properties that cannot be attained using any other element.

Numerous previous studies have examined the binding of fluorinated arylsulfonamide ligands to CA. Part of them concentrated on the investigation of a fluorinated 'tail'.[15–24] Another part of them investigated the influence of fluorine incorporated directly into the benezenesulfonamide scaffold. Monofluorination was common approach in the synthesis of CA inhibitors.[21,25–35] However introduction of several fluorine atoms was not as common.[13,36–39]

The study by Olander et al. investigated the binding of pentafluorobenzenesulfonamide (PFBSA) to bovine CA II.[36]

Gerig and co-workers investigated the binding of fluorinated benzenesulfonamides to CA I and CA II by using ^{19}F NMR spectroscopy. Their results proposed a binding stoichiometry of 2:1 for 2-fluoro-, 3-fluoro-, 4-fluoro-, and 2,5-difluorobenzenesulfonamides to CA I and II.[35,37] However, the binding stoichiometry of 1:1 for 3,5-difluorobenzenesulfonamide to human CA I was observed.[38]

Whitesides and co-workers observed 1:1 binding for fluorinated benzenesulfonamides by using biophysical and X-ray crystallographic techniques.[13] Thermodynamic parameters for the association of 2-fluoro-, 3-fluoro-, 4-fluoro-, 2,6-difluoro-, 3,5-difluoro-benzenesulfonamides and PFBSA with bovine CA II were investigated in this study. Their results highlighted the importance of examining the thermodynamic parameters for the association of both the ligand and protein in their active forms. Most other studies have only discussed the experimentally observed thermodynamic parameters without the dissection of intrinsic part and the contribution of linked protonation effects. Furthermore, they concluded that fluorine is the best choice for electron-withdrawing substituents because it decreases the pK_a and also increases hydrophobicity of the ligand.

Supuran and co-workers investigated the binding of halogenated benzenesulfonamides incorporating one or two (equal or different) halogens.[33] Monofluorinated compounds generally were less active than other monohalogenated derivatives. Combination of fluorine with another halogen yielded potent CA IX inhibitors.

A study of binding thermodynamics of benzenesulfonamides bearing chlorine or fluorine atoms was reported by Scott et al.[34] They noted that similar substitutions (e.g., Cl or F in positions 2, 3 or 4) resulted in significantly different changes to the binding thermodynamics, which would be impossible to predict from the structural analysis alone. The presence of fluorine was more favorable than chlorine for the inhibition of CA.

Here we describe the synthesis of benzenesulfonamides possessing as many fluorine atoms as possible in the benzenesulfonamide scaffold. It was expected that fluorine atoms would contribute favorably to CA binding and that highly fluorinated benzenesulfonamides would have a good capacity for nucleophilic aromatic substitution reactions. It has been reported previously that polyfluorobenzenes undergo nucleophilic aromatic substitution reactions[40] and that fluorine is one of the best leaving groups for the substitution. Nucleophilic aromatic substitution reactions of fluorinated benzenes bearing one or several fluorines can be accelerated by electron-withdrawing groups.[41,42] These features allow for the synthesis of a diverse group of compounds from fluorinated benzenes.

Herein, we report the synthesis of 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides and their investigation as inhibitors of carbonic anhydrase isozymes I, II, VII, XII, and XIII. There are many carbonic anhydrase crystal structures solved to atomic resolution since the first CA structure.[43] Here we describe several structures of CA II, XII, and XIII with bound fluorinated ligands.

2. Results and discussion

2.1. Chemistry

A series of 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides were designed and synthesized (Scheme 1). The starting compound for these investigations was pentafluorobenzenesulfonamide which readily underwent nucleophilic substitution reaction and formed the 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides. Such compounds bearing cycloalkylamino or alkylamino groups in the 4-position were described as anticonvulsants[44] but the connection between the anticonvulsant properties and the CA inhibitory properties was not explained.

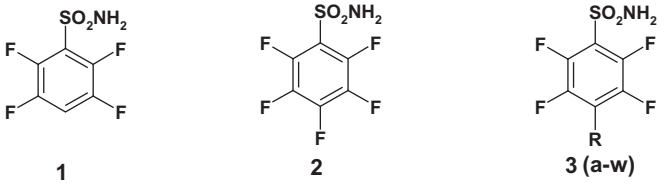
2,3,5,6-Tetrafluorobenzenesulfonamide (compound **1**) was synthesized from 1,2,4,5-tetrafluorobenzene (purchased from Alfa Aesar). First, the 1,2,4,5-tetrafluorobenzene was converted to 2,3,5,6-tetrafluorobenzenesulfonyl chloride.[16] Reaction of obtained sulfonyl chloride with aqueous ammonia yielded the sulfonamide **1**. Similarly, the synthesis of pentafluorobenzenesulfonamide (compound **2**) via amination of pentafluorobenzenesulfonyl chloride (purchased from Alfa Aesar) with aqueous ammonia was obtained. The synthesis of penta- and 2,3,5,6-tetrafluorobenzenesulfonamides from appropriate sulfonyl chloride were reported in the literature,[45] but substantial amounts of disulfonimides were obtained as byproducts. We found that the formation of imide can be avoided by keeping the reaction medium near a neutral pH. The formation of imides is due to low pK_a of penta- and 2,3,5,6-tetrafluorobenzenesulfonamides. Deprotonation of sulfonamide occurs in basic medium and deprotonated sulfonamide reacts readily with the starting material—sulfonyl chloride.

4-Substituted-2,3,5,6-tetrafluorobenzenesulfonamides **3a**, **c**, **e**–**i**, **k**–**o**, and **q**–**w** were obtained from compound **2** by using the appropriate nucleophile in ethanol, methanol or DMSO in the presence of Et_3N or K_2CO_3 (excess of nucleophile was used in several cases instead of the mentioned bases). The compound **3d** was prepared by oxidation of **3c** with $CH_3COOH\backslash H_2O_2$. Oxidation of the compounds **3i**, **3o** with CrO_3 gave **3j**, **3p**. The reaction of **3a** with benzaldehyde led to the formation of **3b**.

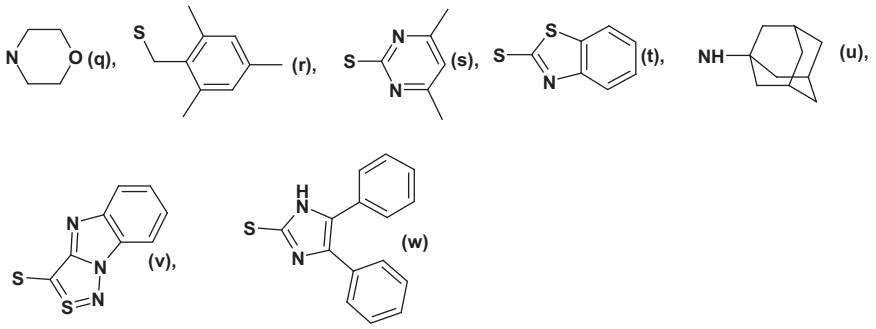
In order to compare with fluorine-free compounds, the 4-substituted benzenesulfonamides **6a**–**c** were synthesized according to the literature[25] (Scheme 2). 4-Bromobenzenesulfonamide (**4**) was converted to compound **5** with *N*,*N*-dimethylformamide dimetyl acetal in acetonitrile. Treatment of protected sulfonamide with thiols led to the replacement of bromine. The deprotection of the sulfonamide group was accomplished by using aqueous sodium hydroxide and thus compounds **6a**–**c** were obtained.

2.2. Binding and inhibition studies

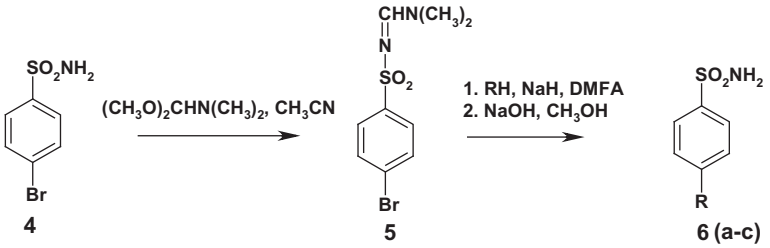
The binding affinities of fluorinated benzenesulfonamides to five carbonic anhydrase isozymes (I, II, VII, catalytic domain of CA XII, and CA XIII) were measured by the thermal shift assay (TSA) and isothermal titration calorimetry (ITC). The dissociation constants for synthesized compounds are listed in Tables 1 and 2. The data for three reference compounds (BSA—benzenesulfonamide, EZA—ethoxzolamide, and AZM—acetazolamide) is provided at the end of the Table 1. Figures 1 and 2 show representative binding data obtained by TSA with isozyme CA I and CA XIII. Figure 3 shows representative binding data obtained by ITC (isozymes CA I and CA XIII). The K_d measured by ITC in most cases was slightly higher than by TSA, especially in the case of very tight binding. This discrepancy could be explained by, as shown in Figure 3a, the



R= $NHNH_2$(a), NHN=CHPh(b), SCH_2CH_2OH (c), $SO_2CH_2CH_2OH$(d), $SCH_2CH_2CH_3$(e), SCH_2COOH(f), SCH_2CH_2COOH(g), $NH(CH_2)_5COOH$ (h), SPh(i), SO_2Ph(j), OPh(k), SCH_2Ph(l), $NHCH_2Ph$(m), $NHCH_2CH_2PhpOH$(n), SCH_2CH_2Ph(o), $SO_2CH_2CH_2Ph$(p),



Scheme 1. Structures of synthesized compounds **1**, **2**, **3a**–**w**.



R= SCH_2CH_2OH (a) $SCH_2CH_2CH_3$(b), SCH_2CH_2Ph(c).

Scheme 2. Synthesis of compounds **6a**–**c**.

integrated curve of the binding of **3d** to CA I, being too steep to be fitted precisely. It was previously discussed[46] that the *c* value ($c = C \times K_b$, where *C* is the molar concentration of the protein, and K_b is the binding constant) for an ITC titration must be between 5 and 500. However, in our ITC experiments the concentration of CA I was 6 μM, and the binding constant of **3d** was shown by TSA to be 3×10^{10} M^{-1}. Thus the *c* value would be 180,000, much higher than ITC limits. The shape of the fitted curve of the ITC isotherm was suitable only for the value of $K_b \leqslant 8.3 \times 10^7$ M^{-1} ($K_d \geqslant 12$ nM). Therefore TSA data was more appropriate to determine tight binding than ITC.

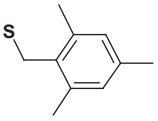
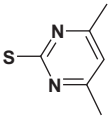
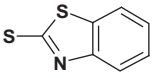
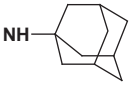
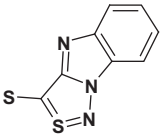
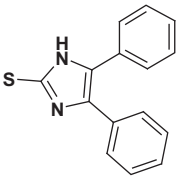
The data in Table 1 shows that fluorine atom substitution and the nature of the substituent on the *para* position of benzenesulfonamide ring significantly influences the binding affinity against CA I, II, VII, XII, and XIII. The 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides (compounds **3a**–**w**) bound CA I, II, VII, and XIII with low nanomolar and subnanomolar affinity, exhibiting especially strong binding to CA I (K_d in the range of 0.05–14 nM). The K_d ranges for CA II were 1.1–91 nM (**3t** and **3a**), for CA VII 0.22–465 nM (**3t** and **3a**), and for CA XIII were 0.22–140 nM (**3o** and **3a**). The weakest binding was observed for CA XII (K_d in the range of 6.67–769 nM as measured by TSA). The most potent CA I inhibitors **3e**, **3j**, **3r** and **3u** bound with K_d in the range of 0.033–0.067 nM. Compound **3a** bearing $NHNH_2$ group in *para* position had the weakest binding affinity for all CAs.

Comparing the fluorinated with nonfluorinated compounds bearing SCH_2CH_2OH (**3c** vs **6a**), $SCH_2CH_2CH_3$ (**3e** vs **6b**), and SCH_2CH_2Ph (**3o** vs **6c**) substituents showed that the fluorination significantly increased the binding affinity to the tested CAs. For example, the K_ds for **3e** and **6b** for CA I varied by more than 150 times (from 0.03 to 4.5 nM), for CA II—6.5 times, for CA VII—17 times, CA XII—10 times, and for CA XIII—29 times.

The influence of the substituent being in *para* position of the benzenesulfonamide ring to binding strength was also evident for compounds of the series **6a**–**c**. Compounds **6a**–**c** had significantly higher binding affinity than benzenesulfonamide (BSA). A significant difference in K_ds was observed between compounds **6a** and **6b** with the first one being much weaker than the latter. Because the changes were only in the hydroxy (**6a**) and methyl group (**6b**) at the end of substituent tail, it is supposed that additional hydrophobic contacts with the enzyme hydrophobic pocket could be formed for compound **6b**.

All compounds used in this study bound to all the tested CAs with 1:1 stoichiometry as demonstrated by ITC and the dosing curves of TSA. This strongly indicated that all inhibitors bound exclusively to the active site of the enzymes. However, direct observation of the inhibition of the CO_2 hydration by the compounds would determine the K_i and confirm that the compounds were inhibitors of the CA. However, the stop-flow hydration assay was significantly more laborious than with TSA. Therefore, the

Table 1
Compound dissociation constants for five human recombinant CA isoforms, determined by TSA (37 °C, pH 7.0)

Compound	R	Dissociation constants K_d (nM), CA isoforms:				
		CA I	CA II	CA VII	CA XII	CA XIII
1	H	2.4	29	11	333	20
2	F	2.2	27	25	500	40
3a	$NHNH_2$	8.3	91	465	769	140
3b	NHN=CHPh	4.0	2.9	43	667	2.2
3c	SCH_2CH_2OH	0.11	6.7	46	220	8.3
3d	$SO_2CH_2CH_2OH$	0.20	17	7.1	250	29
3e	$SCH_2CH_2CH_3$	0.033	2.0	1.1	40	1.7
3f	SCH_2COOH	0.20	11	5.0	50	6.7
3g	SCH_2CH_2COOH	0.40	20	10	91	20
3h	$NH(CH_2)_5COOH$	0.10	67	147	200	14
3i	SPh	0.13	4.0	12	20	1.5
3j	SO_2Ph	0.050	3.3	0.67	17	3.3
3k	OPh	0.10	2.5	1.0	50	1.0
3l	SCH_2Ph	0.25	1.3	1.3	6.7	0.40
3m	$NHCH_2Ph$	0.20	9.1	133	333	5.0
3n	$NHCH_2CH_2PhpOH$	0.17	1.7	0.50	154	0.40
3o	SCH_2CH_2Ph	0.20	1.7	0.83	110	0.22
3p	$SO_2CH_2CH_2Ph$	0.25	1.3	1.3	77	0.40
3q		0.13	18	83	500	25
3r		0.067	3.3	2.5	100	3.3
3s		2.5	10	10	290	2.5
3t		1.1	1.1	0.22	200	0.25
3u		0.050	5.0	1.0	17	0.5
3v		0.40	6.7	13	110	2.5
3w		14	6.7	1.7	670	2.0
6a	SCH_2CH_2OH	200	130	170	2500	1400
6b	$SCH_2CH_2CH_3$	4.5	13	17	400	50
6c	SCH_2CH_2Ph	5.0	25	40	330	290
BSA		7140	1790	6670	12,500	10,000
EZA		14	1.1	0.71	36	13
AZM		1400	38	17	133	50

inhibitory effect K_i on CA II was determined only for the compounds **3c** and **6a**. The compounds were dosed in a stepwise 2× dilution beginning from 1000 nM and ending with a 2 nM concentration of the inhibitor. CA II concentration was 20 nM. These compounds inhibited CA II and the dosing curves exhibited the typical sigmoidal shape with the fitted IC_{50} equal to 10 nM and 60 nM, respectively for **3c** and **6a**. Assuming that the K_M of CA II was 9.3 mM and the half concentration of saturated CO_2 solution at 25 °C was 17 mM, the K_is were equal to 3.5 and 21 nM, respectively. These values are slightly lower than the ones determined by TSA and ITC. The discrepancy could be partially explained by

Table 2
Dissociation constants of selected compounds for five human recombinant CA isoforms, determined by ITC (37 °C, pH 7.0)

Compound	Dissociation constants K_d (nM), CA isoforms:				
	CA I	CA II	CA VII	CA XII	CA XIII
1	18	42	121	588	66
3c	⩽12	⩽12	59	94	18
3d	⩽12	⩽12	37	143	74
3s	⩽12	⩽12	88	150	⩽12
6a	790	330	510	1500	1000

the difference in temperatures—the stop-flow assay was performed at 25 °C and the ITC at 37 °C. However, there was a general agreement between all three methods that both compounds were tight inhibitors of CA II and the fluorinated compound was significantly more potent than the non-fluorinated one.

A detailed binding study of fluorinated benzenesulfonamides (no other substituents were included, general formula $C_6H_nF_{5-n}SO_2NH_2$) with bovine carbonic anhydrase II (BCA) has been previously reported by Whitesides group.[13] They assumed that the thermodynamic parameters of binding could be partitioned into the three different structural interactions between the fluorinated ligand and BCA: the Zn(II) cofactor–sulfonamide bond, the hydrogen bonds between ligand and BCA, and the hydrophobic contacts between the phenyl ring of the ligand and BCA. In this study PFBSA was a 30 times more potent inhibitor than BSA primarily due to their different pK_a (8.2 and 10.1, respectively). The authors assumed that a pK_a of the ligand of about 7.4 should give the lowest observed dissociation constant K_d.

As the pK_as of 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides (compounds **3a**–**w**) should be near that of PFBSA, the additional contacts between the substituent (R) on the benzenesulfonamide ring and CA could result in the increased binding affinity of most compounds of the series **3a**–**w**, as compared to PFBSA.

2.3. Crystallography

Three crystal structures of CAs bound with the fluorinated inhibitors were solved to atomic resolution: CA II and CA XII complexed with compound **3s** and CA XIII with compound **3d**. Data collection and refinement statistics are summarized in Table 3 and the electron densities of the ligands in crystal structures are shown in Figure 4.

The CA XII with compound **3s** structure (PDB ID 4HT2) contained four protein chains in the asymmetric unit. The ligand was observed in all subunits. The superposition of the four subunits showed that the position of the benzene ring of the ligand is nearly identical in the three monomers ('main' orientation), while the conformation in the fourth (subunit A) was rotated by ~80° ('rotated' orientation, Figs. 4C and 5).

The CA XIII with compound **3d** structure (PDB ID 4HU1) contained two protein chains (two monomers) in the asymmetric unit. Orientation of the ligand in both subunits in the asymmetric unit of CA XIII was nearly identical to the 'rotated' orientation of **3s** in CA XII chain A (Figs. 5 and 7B).

The 'main' orientation of the first benzene ring was also observed in the structure of **3s** bound to CA II (PDB ID 4HT0). The structure contained only one protein chain in the asymmetric unit. The first benzene ring fit into the pocket between the side chains of Leu198 and Thr200 in CA II (corresponding residues in CA XII are Leu197 and Thr199). The fluorine atoms made hydrogen bonds with the protein backbone. The opposite side of the fluorinated benzene ring was restricted by Val121 (Val119 in CA XII) and Gln92 in CA II (Gln89 in CA XII). The fluorinated benzene ring also had hydrophobic contacts with His91 in CA XII and Phe131 in CA II. Generally, one side of the benzene ring was sticking to the hydrophobic wall of the protein pocket formed by Val121 (Val119 in CA XII), Leu141 (Leu139 in CA XII), Leu198 (Leu197 in CA XII), and Phe131 in CA II.

The orientation of the fluorinated benzene ring in the ligand binding pocket of CA II was similar to the position of benzenesulfonamide (PDB ID 2WEJ). In 2WEJ, the structure of benzenesulfonamide made van der Waals contacts with Val121, Leu198, His94 and Thr200. There are two crystal structures of the tetrafluorobenzenesulfonamide derivatives bound to CA II available in the PDB (PDB IDs: 3P25 and 3P29) from McKenna group. The

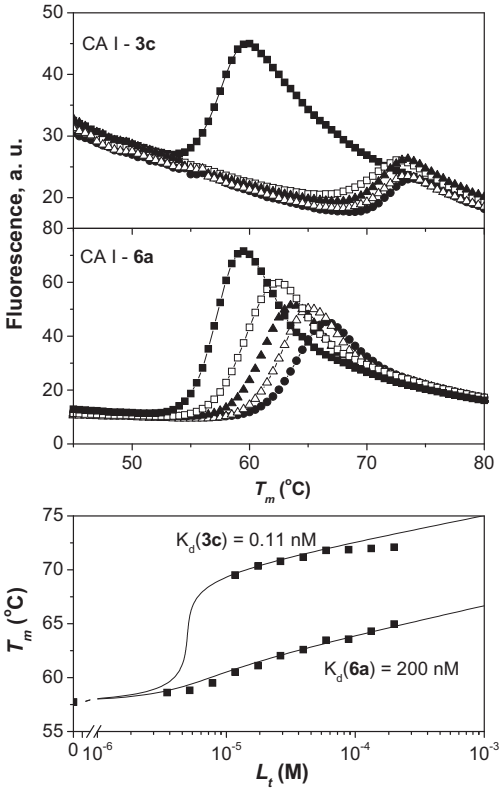


Figure 1. TSA data of representative compound binding to CA I. Panels on the top show thermal shift assay raw melting curves at several added concentrations of **3c** and **6a**. Panel on the bottom shows the dependence of the protein melting temperatures T_m on ligand concentrations. Datapoints are from the experimental values from the upper panel and the curves are simulated according to the model.

tetrafluorobenzene ring of **3s** (PDB ID 4HT0) was bound in the same 'main' orientation as in these two crystal structures.

The second dimethylpyrimidine ring of the ligand **3s** was located in the mainly hydrophobic environment. It contacted hydrophobically Phe131, Val135, Pro202 and Leu204 in CA II (Fig. 6A). Phe131 in CA XII is replaced by Ala129. This variation resulted in different conformations of the bound ligand. So, the bond angle on the sulfur atom connecting the two rings is distorted from the perfect tetrahedral 104° in CA XII, to 146° in CA II, where the second ring should avoid a steric clash with Phe131 (Figs. 6A,B and 7A). In CA XII the second ring also made

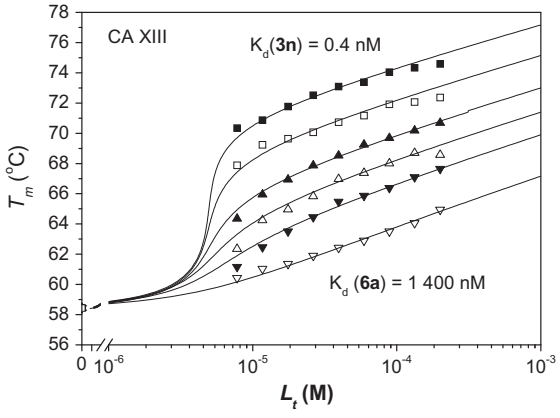


Figure 2. TSA data of **3n**, **3s**, **3c**, **6b**, **3a**, and **6a** binding to CA XIII. The binding affinity of selected compounds ranged from 0.4 nM for **3n** to 1.4 μM for **6a**.

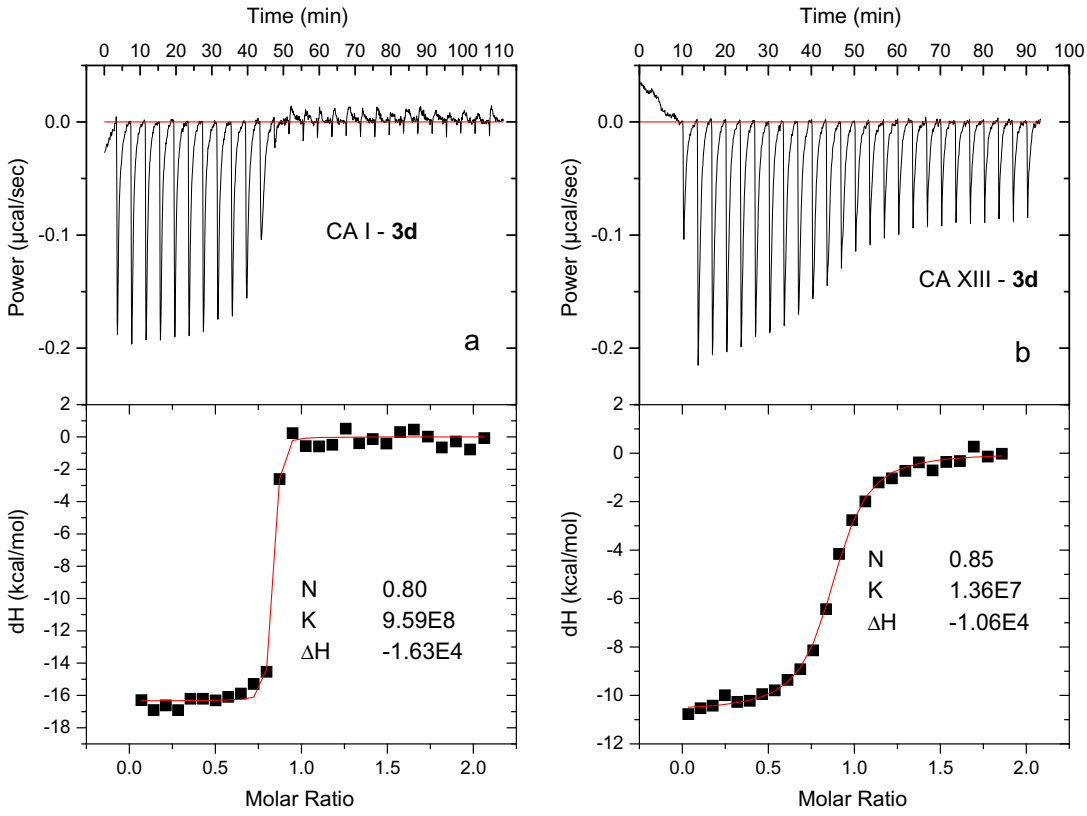


Figure 3. ITC data of **3d** binding to CA I (a) and CA XIII (b) at 37 °C.

Table 3
X-ray crystallographic data collection and refinement statistics. All datasets were collected at 100 K, test set size was 10%

Protein-compound:	CAXII-**3s**	CAXIII-**3d**	CAII-**3s**
PDB ID	4HT2	4HU1	4HT0
Spacegroup	*P*1	*P*212121	*P*21
Unit cell (Å)	a = 46.71, b = 67.26, c = 80.69 α = 81.78°,β = 84.01° γ = 86.48°	a = 56.27, b = 57.39, c = 159.55 $\alpha = \beta = \gamma$ = 90°	a = 42.02, b = 40.90, c = 71.60 $\alpha = \gamma$ = 90°, β = 104.04°
Number of chains	4	2	1
Resolution, (Å) (final shell)	1.45	1.95	1.60
Reflections unique (total)	166,179 (656,979)	38,614 (283,080)	30,222 (227,077)
Completeness (%) overall (final shell)	97.1 (95.6)	100.0 (100.0)	96.5 (97.0)
I/σ_I overall (final shell)	14.1 (4.8)	9.0 (3.6)	21.8 (12.1)
R_{merge} Overall (final shell)	0.039 (0.219)	0.135 (0.486)	0.061 (0.123)
Number of atoms	10,438	4839	2446
Number of solvent molecules	1503	553	270
Number of bounded buffer molecule atoms	15	8	2
R_{cryst} (R_{free})	0.152 (0.186)	0.169 (0.235)	0.168 (0.210)
RMS bonds/angles	0.025 (2.255)	0.024 (2.026)	0.029 (2.562)
Average B-factors (Å^2)	13.1	19.6	15.5
Main chain:	10.0	17.5	12.5
Side chains:	12.1	19.0	15.1
Solvent:	23.2	28.0	26.6
Cofactors:	11.1	19.5	32.8

mostly van der Waals contacts with residues (corresponding CA II residues are shown in parenthesis) Ser130 (Gly132), Ser133 (Val135), and Leu139 (Leu141).

The 'rotated' conformation was found as an alternative conformation of compound **3s** in CA XII (Figs. 6D and 7B) and it was the only orientation of compound **3d** in CA XIII (Figs. 6C and 7B). The fluorinated benzene ring was fixed in the CA XIII binding pocket by mostly hydrophobic interactions with Leu200 (Leu197 in CA XII), Val202 (Thr199 in CA XII), Val145 (Val141 in CA XII), and Phe133 (no contact with Ala129 in CA XII). The other side of the ring was buttressed by the catalytic His96 (His91 in CA XII), Val123 (Val119 in CA XII), and Gln94 (Gln89 in CA XII). Fluorine atoms at one edge were in van der Waals contact with the hydrophobic wall of the pocket. At the opposite edge of the ring there was a net of fixed solvent molecules between the ligand and the protein moiety.

Benzene rings were in the same plane, but in CA XII the ring is rotated ~10° (Fig. 7B). The probable explanation could be the van der Waals interaction of the dimethylpyrimidine ring with residues 129–130 and 133 that form the edge of the ligand binding pocket

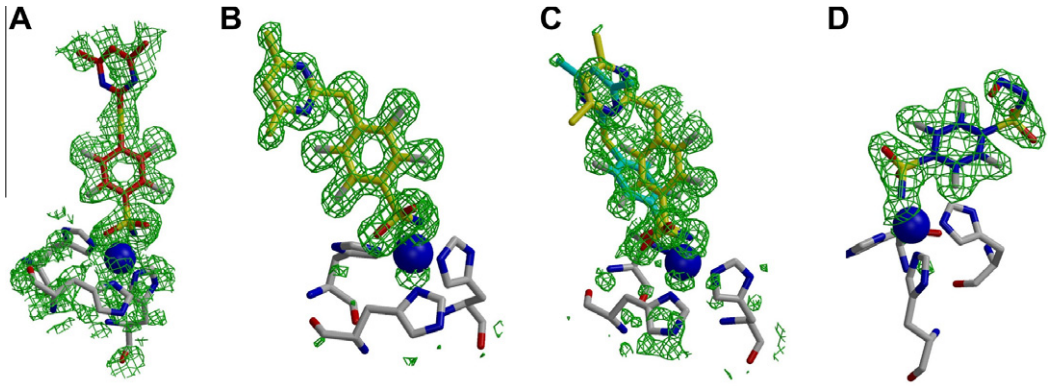


Figure 4. View of the electron density of the compounds **3d** and **3s** located in the active center of CA. The catalytic Zn(II) coordinated by active center histidine residues are shown as blue spheres. The electron density difference maps Fo are contoured at 3σ in B and D, 1σ in A, and 2σ in C. **A**, the compound **3s** bound to CA II. **B**, the 'main' orientation of compound **3s** in protein chain B of CA XII. **C**, two orientations of compound **3s** bound to CA XII, chain A.

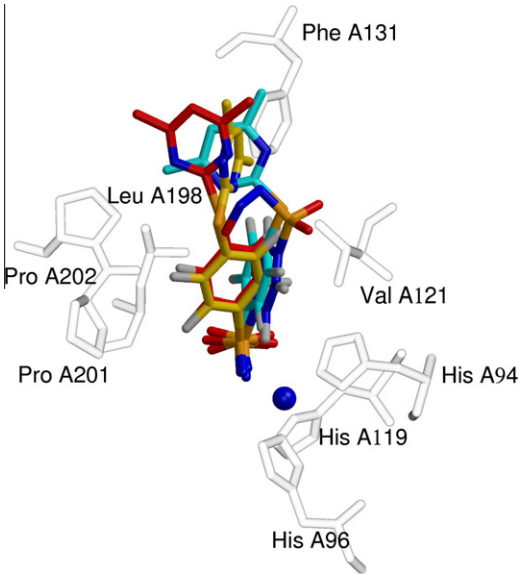


Figure 5. Two orientations of compounds **3d** and **3s** in the active sites of the three crystal structures. All subunits were superimposed by Zn(II) and the three active center histidine residues. Active center residues considered to be most important for binding to CA II are shown transparent. The compound **3s** in chain B of CA XII is colored gold. The compound **3d** in CA XIII and the second 'rotated' orientation of compound **3s** in the chain A of CA XII are shown in blue and cyan, respectively. The ligand **3s** bound by CA II is colored red.

in CA XII. The van der Waals interactions with the side chains of Leu139, Leu197, Asn203, and Pro201 also participated in the binding.

The sulfonyl group of ligand **3d** made water mediated hydrogen bonds with Arg93 and Asn69 and van der Waals bond with Gln94 (Fig. 6C).

In conclusion, the orientation of the benzene ring resulted in a distinct mode of ligand binding in the pocket of the CA active site. Compound **3s** bound in all cases mostly hydrophobically to the inner surface of the ligand binding pockets. This compound in its 'main' orientation found in CA II and CA XII also made hydrogen bonds with the protein backbone as a result of the fluorine atoms of the benzene ring. The second dimethylpyrimidine ring adopted the conformation that made as many hydrophobic contacts as possible. In CA II, the steric clash with Phe131 could be the reason for more elongated conformation of the compound **3s** than in CA XII.

In the 'rotated' orientation of ligand **3s** found in CA XII, the hydrogen bonds with fluorine were absent. Predominantly hydrophobic binding was observed also for compound **3d** in CA XIII, which also exhibited the 'rotated' position of the benzene ring as well. This orientation was fixed in CA XIII by several hydrogen bonds made by the sulfonyl group of the linker.

3. Conclusions

A series of 4-substituted-2,3,5,6-tetrafluorobenezenesulfonamides were synthesized that possess nanomolar affinities toward CA isozymes I, II, VII, catalytic domain XII, and XIII. Most of the compounds showed better affinity for CA I than for CA II, a relatively rare feature among most aromatic sulfonamides. The binding potency of fluorinated benzenesulfonamides was greater than non-fluorinated derivatives. Polyfluorinated benzenesulfonamide scaffold could be used as a tool for CA inhibitor development. X-ray crystallographic cocrystal structures of two fluorinated benzenesulfonamides with CA II, XII, and XIII showed the presence of two possible orientations of these ligands.

4. Experimental part

4.1. Chemistry

All starting materials and reagents were commercial products and were used without further purification. Melting points of the compounds were determined in open capillaries, Thermo Scientific 9100 Series, and are uncorrected. Column chromatography was performed using silica gel 60 (0.04–0.063 mm, Merck). IR spectra were run on a Perkin-Elmer FT-IR spectrophotometer Spectrum BX II in KBr. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova spectrometer (300 and 75 MHz, respectively) with TMS as an internal standard, and proton, carbon chemical shifts were expressed in parts per million (ppm) in the indicated solvent. ^{19}F NMR spectra were recorded on a Varian Unity Inova spectrometer (282 MHz) with $CFCl_3$ as an internal standard, and fluorine chemical shifts were expressed in parts per million (ppm) in the indicated solvent. Multiplicity was defined as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), ddd (double double doublet), m (multiplet), br s (broad singlet), br d (broad doublet), or br t (broad triplet). TLC was performed using silica gel 60 F254 aluminum plates (Merck) and visualized with UV light. High-resolution mass spectra (HRMS) were recorded on a Dual-ESI Q-TOF 6520 mass spectrometer (Agilent Technologies).

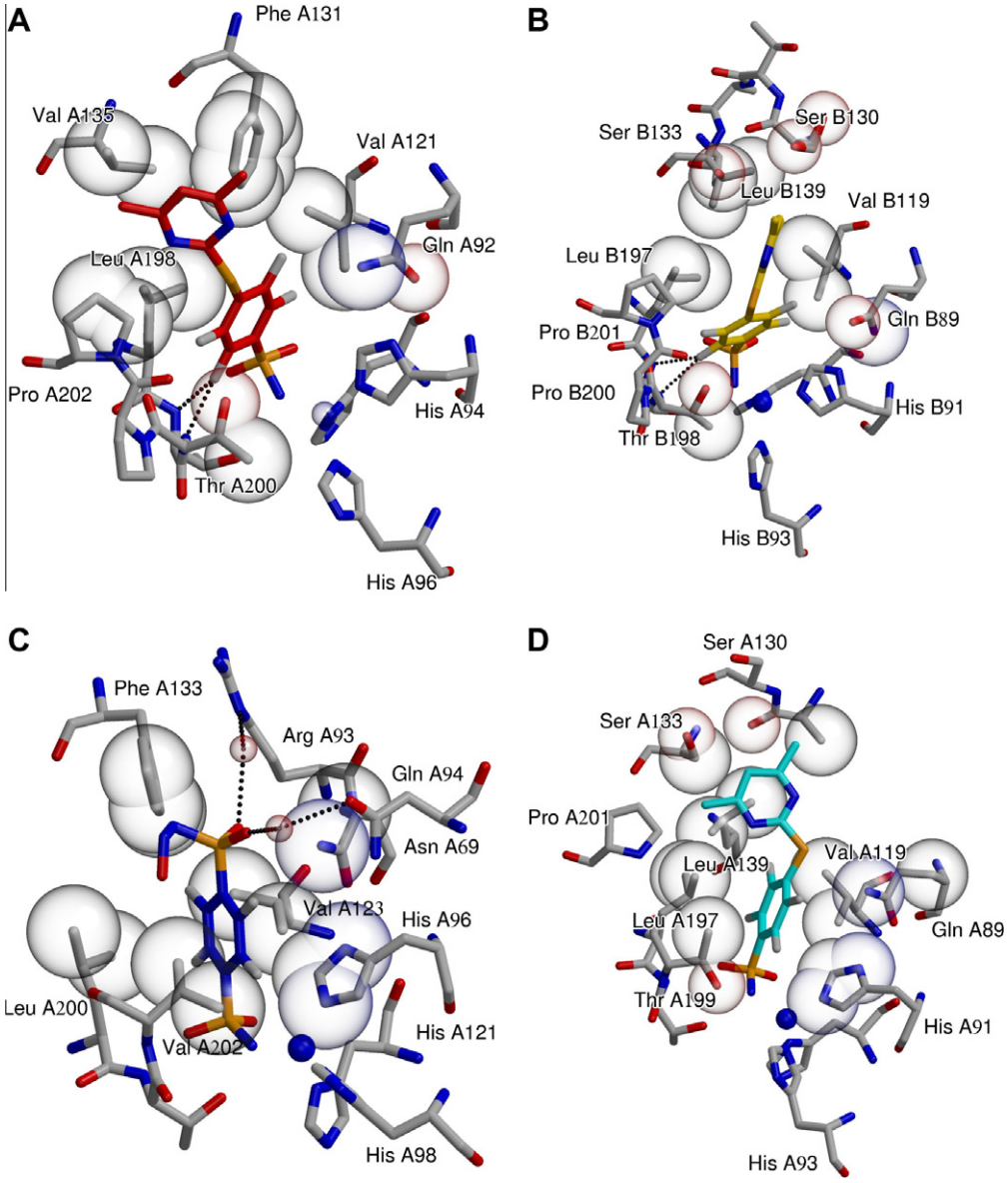


Figure 6. Ligand positions in the active sites of CAs. Protein atoms that are in van der Waals contact with the compounds are shown as CPK. Zn(II) and water molecules are shown as blue and red spheres, respectively. Dashed lines connect the atoms that presumably make hydrogen bonds. (A) the compound **3s** (red) in CA II. (B) the compound **3s** (gold) in CA XII in the 'main' orientation (protein chain B). (C) the compound **3d** (blue) in CA XIII, protein chain A. (D) the compound **3s** (cyan) in CA XII in 'rotated' orientation as modeled in chain A.

4.1.1. General information about ^{13}C NMR spectra

NMR spectra of fluorinated aromatic compounds have been described in the literature.[47] The ^{13}C NMR spectra of investigated compounds were complicated by the fluorine coupling to carbon. Signals of C1 and C4 of 4-substituted-2,3,5,6-tetrafluorobenezenesulfonamides were detected as triplets (the coupling constants were in the range 13–21 Hz) due to *ortho*-coupling of two identical fluorines. Signals of C2, C6 or C3, C5 were detected as ddd, dd or d. The majority of such compounds had signals of d (additional couplings are poorly visible). The first coupling was the coupling of fluorine to the *ipso* position with the coupling constant J (^{19}F–^{13}C) = 228–258 Hz. Other couplings were observed due to the couplings of fluorine to the *ortho* and *meta* positions (the coupling constants were in the range of 12–19 Hz, 4–5 Hz). In the case of 2,3,5,6-tetrafluoro-4-morpholin-4-ylbenzenesulfonamide (**3q**), even the coupling of morpholine N(CH_2)$_2$ group was observed. The signal of the N(CH_2)$_2$ group was detected as a triplet with the coupling constant of 3.6 Hz. An example of a ^{13}C NMR spectrum is given in the Figure 1 of the Supplementary data.

4.1.2. 2,3,5,6-Tetrafluorobenzenesulfonamide (1)

The mixture of 2,3,5,6-tetrafluorobenzenesulfonyl chloride (1 g, 4.02 mmol) and THF (60 mL) was cooled to ~−10 °C and aqueous ammonia (~1.3 mL, 25%) was added dropwise while stirring until the solution was at pH ~7. After stirring for an additional 0.5 h, THF was evaporated in vacuum and the resultant precipitate was washed with cold H_2O. Recrystallization was accomplished from H_2O. Yield: 0.78 g (85%), mp 142–143 °C was close to the value in the literature, mp 145 °C.[45] ^1H NMR (300 MHz, DMSO-d_6): 8.1–8.3 (1H, m, ArH), 8.41 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 110.9 (C4, t, J (^{19}F–^{13}C) = 23 Hz), 124.5 (C1, t, J (^{19}F–^{13}C) = 15 Hz), 143.3 (C3, C5, d, J (^{19}F–^{13}C) = 250 Hz), 146.5 (C2, C6, d, J (^{19}F–^{13}C) = 240 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −137.7: −137.92 (2F, m), −139.4: −139.65 (2F, m).

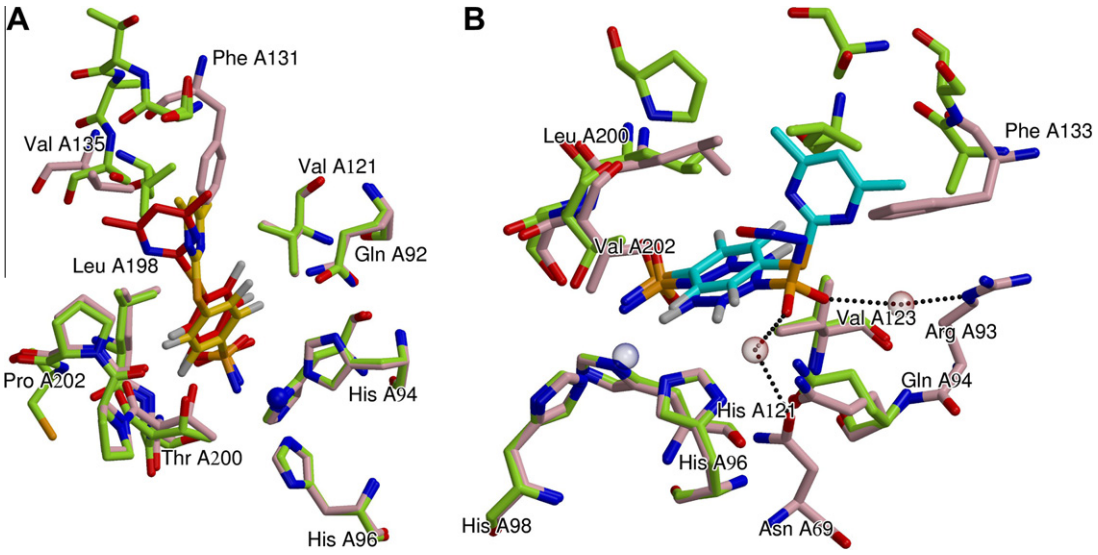


Figure 7. Two orientations of ligands in the active centers of CAs. (A) superposition of active sites of CA II-**3s** complex (pink, the compound **3s** is in red) and CA XII-**3s**, chain B (green, the compound **3s** is in gold). The ligand **3s** is in the 'main' orientation. Amino acids of CA II important for ligand binding are labeled. **B**, superposition of active sites of CA XIII complex with ligand **3d** (pink, the compound **3d** is in blue) and CA XII with alternative orientation of **3s**, chain A (green, **3s** is in cyan). Here ligands are in 'rotated' orientation. CA XIII residues important for ligand binding are labeled.

4.1.3. Pentafluorobenzenesulfonamide (2)

The mixture of pentafluorobenzenesulfonyl chloride (1 g, 3.75 mmol) and THF (60 mL) was cooled to~ −10 °C and aqueous ammonia (~1.2 mL, 25%) was added dropwise while stirring until the solution was at pH ~7. After stirring for an additional 0.5 h, THF was evaporated in vacuum and the resultant precipitate was washed with cold H_2O. Recrystallization was accomplished from H_2O. Yield: 0.84 g (90%), mp 156–157 °C close to the value in the literature, mp 156 °C.[45] ^{1}H NMR (300 MHz, DMSO-d_6): 8.48 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 119.9 (C1, t, J (^{19}F–^{13}C) = 14 Hz), 138.2 (C3, C5, d, J (^{19}F–^{13}C) = 247 Hz), 143.5 (C4, d, J (^{19}F–^{13}C) = 255 Hz), 144 (C2, C6, d, J (^{19}F–^{13}C) = 252 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −139.5 (F2, F6, dd, ^1J = 19 Hz, ^2J = 6 Hz), −149.39 (F4, t, J = 22 Hz), −161.03 (F3, F5, t, J = 20 Hz).

4.1.4. 2,3,5,6-Tetrafluoro-4-hydrazinobenzenesulfonamide (3a)

The mixture of pentafluorobenzenesulfonamide (compound **2**) (0.32 g, 1.295 mmol), $NH_2NH_2{\times}H_2O$ (0.126 mL, 2.59 mmol), and EtOH (10 mL) was stirred at ambient temperature for 24 h. EtOH was evaporated in vacuum and the resultant precipitate was washed with H_2O. Recrystallization was accomplished from H_2O. Yield: 0.2 g, 60%, decomp. at 160–161 °C. IR ν cm^{-1}: 3343, 3260 (NH_2). ^{1}H NMR (300 MHz, DMSO-d_6): 4.68 (2H, s, NH_2), 7.75 (1H, s, NH), 7.98 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 108.6 (C1, t, J (^{19}F–^{13}C) = 14 Hz), 135 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 136 (C3, C5, d, J (^{19}F–^{13}C) = 240 Hz), 144.1 (C2, C6, d, J (^{19}F–^{13}C) = 240 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −143.1 (2F, d, J = 15.8 Hz), −160.2 (2F, d, J = 17.2 Hz). HRMS for $C_6H_5F_4N_3O_2S$ [(M+H)$^+$]: calcd 335.9782, found 335.9780.

4.1.5. 4-(2-Benzylidenehydrazino)-2,3,5,6-tetrafluorobenzenesulfonamide (3b)

The mixture of 2,3,5,6-tetrafluoro-4-hydrazinobenzenesulfonamide (compound **3a**) (0.13 g, 0.5 mmol), benzaldehyde (0.051 mL, 0.5 mmol), and MeOH (10 mL) was stirred at ambient temperature for 4 h. MeOH was evaporated in vacuum. Recrystallization was accomplished from iPrOH. Yield: 0.14 g, 82%, decomp. at 272–273 °C. IR ν cm^{-1}: 3355, 3240 (NH_2). ^{1}H NMR (300 MHz, DMSO-d_6): 7.35–7.55 (3H, m, ArH), 7.6–7.75 (2H, m, ArH), 8.12 (2H, s, SO_2NH_2), 8.25 (1H, s, NH), 10.96 (1H, s, CH). ^{13}C NMR (75 MHz, DMSO-d_6): 111.7 (C1, t, J (^{19}F–^{13}C) = 14 Hz), 127.1 (Ar), 128.5 (C4, t, J (^{19}F–^{13}C) = 13 Hz), 129.5 (Ar), 130.1 (Ar), 135.1 (Ar), 136.3 (C3, C5, d, J (^{19}F–^{13}C) = 244 Hz), 144.5 (C2, C6, d, J (^{19}F–^{13}C) = 251 Hz), 144.7 (CH). ^{19}F NMR (282 MHz, DMSO-d_6): −141.6 (2F, d, J = 16.4 Hz), −156.6 (2F, d, J = 18 Hz). HRMS for $C_{13}H_9F_4N_3O_2S$ [(M+H)$^+$]: calcd 348.0424, found 348.0433.

4.1.6. 2,3,5,6-Tetrafluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (3d)

The mixture of 2,3,5,6-tetrafluoro-4-[(2-hydroxyethyl)thio]-benzenesulfonamide (compound **2c**) (0.1 g, 0.33 mmol), CH_3COOH (2 mL), H_2O (1 mL) was heated at 70 °C for 22 h. H_2O_2 was added by portions (0.2 mL) every 4 h (overall amount 1 mL). The progress of reaction was monitored by TLC. The solvent was then removed in vacuum and crude product was purified by crystallization from H_2O. Yield: 0.067 g, 61%, mp 139–140 °C. IR ν cm^{-1}: 3326, 3481 (NH_2). ^{1}H NMR (300 MHz, DMSO-d_6): 3.75 (2H, t, J = 5.4 Hz, $SO_2CH_2CH_2$), 3.88 (2H, t, J = 5.4 Hz, $SO_2CH_2CH_2$), 5.01 (1H, br s, OH), 8.65 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 55.9 ($SO_2CH_2CH_2$), 60.2 ($SO_2CH_2CH_2$), 123.4 (C1 or C4, t, J (^{19}F–^{13}C) = 15 Hz), 128 (C1 or C4, t, J (^{19}F–^{13}C) = 16 Hz), 143.5 (C3, C5 or C2, C6, dd, ^1J (^{19}F–^{13}C) = 254 Hz, ^2J (^{19}F–^{13}C) = 18 Hz), 145 (C3, C5 or C2, C6, dd, ^1J (^{19}F–^{13}C) = 248 Hz, ^2J (^{19}F–^{13}C) = 15 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −136.7 (2F, dd, ^1J = 26 Hz, ^2J = 12 Hz), −137.6 (2F, dd, ^1J = 26 Hz, ^2J = 12 Hz). HRMS for $C_8H_7F_4NO_5S_2$ [(M−H)$^-$]: calcd 335.9629, found 335.9635.

4.1.7. General procedure for the syntheses of 3c, e–h, l–o, r, v

The mixture of pentafluorobenzenesulfonamide (compound **2**) (0.25 g, 1 mmol), MeOH (10 mL), Et_3N (0.141 mL, 1.01 mmol) and the appropriate nucleophile (1.1 mmol) was refluxed. Compounds **3c**, **g**, **h**, **m**, **o**, **r** were obtained after 8 h, compound **3e** was obtained after 10 h, compounds **3f** and **3n** were obtained after 15 h, compound **3m** was obtained after 4 h, and compound **2v** was obtained after 1 h. MeOH was evaporated in vacuum and the resultant precipitate was washed with H_2O (except **3f**, **g**, **h**).

4.1.7.1. 2,3,5,6-Tetrafluoro-4-[(2-hydroxyethyl)thio]benzenesulfonamide (3c). Recrystallization was accomplished from H_2O. Yield: 0.22 g, 71%, mp 111–112 °C. IR ν cm^{-1}: 3343, 3474

(NH_2). ^1H NMR (300 MHz, DMSO-d_6): 3.16 (2H, t, J = 6 Hz, SCH_2CH_2), 3.6 (2H, k, J = 6 Hz, SCH_2CH_2), 4.97 (1H, t, J = 3.6 Hz, OH), 8.43 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 37.3 (SCH_2CH_2), 61.4 (SCH_2CH_2), 119.95 (C1, t, J (^{19}F–^{13}C) = 14 Hz), 122.9 (C4, t, J (^{19}F–^{13}C) = 15 Hz), 143.2 (C2, C6, ddd, ^1J (^{19}F–^{13}C) = 254 Hz, ^2J (^{19}F–^{13}C) = 17 Hz, ^3J (^{19}F–^{13}C) = 4 Hz), 147 (C3, C5, ddd, ^1J (^{19}F–^{13}C) = 228 Hz, ^2J (^{19}F–^{13}C) = 14 Hz, ^3J (^{19}F–^{13}C) = 4 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −133.5 (2F, dd, ^1J = 26.8 Hz, ^2J = 13.8 Hz), −140 (2F, dd, ^1J = 26.2 Hz, ^2J = 12.7 Hz). HRMS for $C_8H_7F_4NO_3S_2$ [(M−H)$^-$]: calcd 303.9731, found 303.9729.

4.1.7.2. 2,3,5,6-Tetrafluoro-4-(propylthio)benzenesulfonamide (3e). Recrystallization was accomplished from EtOH:H_2O (2:1). Yield: 0.25 g, 81%, mp 120 °C. IR ν cm^{-1}: 3349, 3253 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 0.96 (3H, t, J = 7.2 Hz, CH_3), 1.55 (2H, sext, J = 7.2 Hz, CH_2), 3.04 (2H, t, J = 7.2 Hz, CH_2), 8.41 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 13.3 (CH_3), 23.5 (CH_2), 36.4 (CH_2), 119.2 (C1, t, J (^{19}F–^{13}C) = 20 Hz), 123.1 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 143.2 (C2, C6, dd, ^1J (^{19}F–^{13}C) = 254 Hz, ^2J (^{19}F–^{13}C) = 17 Hz), 147.2 (C3, C5, dd, ^1J (^{19}F–^{13}C) = 244 Hz, ^2J (^{19}F–^{13}C) = 17 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −133.9 (2F, dd, ^1J = 25 Hz, ^2J = 11 Hz), −139.6 (2F, dd, ^1J = 25 Hz, ^2J = 14 Hz). HRMS for $C_9H_9F_4NO_2S_2$ [(M−H)$^-$]: calcd 301.9938, found 301.9940.

4.1.7.3. {[4-(Aminosulfonyl)-2,3,5,6-tetrafluorophenyl]thio}acetic acid (3f). The product was purified by chromatography on a column of silica gel (0.04–0.063 mm) with ethyl acetate. Yield: 0.12 g, 38%, mp 158–159 °C. IR ν cm^{-1}: 3338, 3237 (NH_2), 2925 (OH), 1723 (CO). ^1H NMR (300 MHz, DMSO-d_6): 3.9 (2H, s, CH_2), 8.44 (2H, s, SO_2NH_2), 12.8 (1H, br s, COOH). ^{13}C NMR (75 MHz, DMSO-d_6): 36 (CH_2), 118.8 (C1, t, J (^{19}F–^{13}C) = 20 Hz), 123.3 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 143.1 (C2, C6, dd, ^1J (^{19}F–^{13}C) = 254 Hz, ^2J (^{19}F–^{13}C) = 16 Hz), 147 (C3, C5, d, J (^{19}F–^{13}C) = 249 Hz), 170.3 (COOH). ^{19}F NMR (282 MHz, DMSO-d_6): −133.6 (2F, dd, ^1J = 25 Hz, ^2J = 10 Hz), −139.8 (2F, dd, ^1J = 25 Hz, ^2J = 12 Hz). HRMS for $C_8H_5F_4NO_4S_2$ [(M−H)$^-$]: calcd 317.9523, found 317.9525.

4.1.7.4. 3-{[4-(Aminosulfonyl)-2,3,5,6-tetrafluorophenyl]thio}propanoic acid (3g). The product was purified by chromatography on a column of silica gel (0.04–0.063 mm) with ethyl acetate. Yield: 0.2 g, 59%, mp 168–169 °C. IR ν cm^{-1}: 3372, 3272 (NH_2), 2928 (OH), 1702 (CO). ^1H NMR (300 MHz, DMSO-d_6): 2.59 (2H, t, J = 6.9 Hz, CH_2), 3.21 (2H, t, J = 6.6 Hz, CH_2), 8.42 (2H, s, SO_2NH_2), 12.4 (1H, br s, COOH). ^{13}C NMR (75 MHz, DMSO-d_6): 30.1 (CH_2), 35.4 (CH_2), 118.8 (C1, t, J (^{19}F–^{13}C) = 20 Hz), 123.4 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 143.2 (C2, C6, dd, ^1J (^{19}F–^{13}C) = 254 Hz, ^2J (^{19}F–^{13}C) = 17 Hz), 147.3 (C3, C5, dd, ^1J (^{19}F–^{13}C) = 241 Hz, ^2J (^{19}F–^{13}C) = 19 Hz), 173.1 (COOH). ^{19}F NMR (282 MHz, DMSO-d_6): −133.3 (2F, dd, ^1J = 25 Hz, ^2J = 10 Hz), −139.6 (2F, dd, ^1J = 25 Hz, ^2J = 10 Hz). HRMS for $C_9H_7F_4NO_4S_2$ [(M−H)$^-$]: calcd 331.968, found 331.9683.

4.1.7.5. 6-{[4-(Aminosulfonyl)-2,3,5,6-tetrafluorophenyl]amino}hexanoic acid (3h). The product was purified by chromatography on a column of silica gel (0.04–0.063 mm) with ethyl acetate. Yield: 0.15 g, 42%, mp 142–144 °C. IR ν cm^{-1}: 3422, 3380, 3286 (NH, NH_2), 2945 (OH), 1702 (CO). ^1H NMR (300 MHz, DMSO-d_6): 1.2–1.6 (8H, m, $(CH_2)_4$), 2.22 (2H, t, J = 7.2 Hz, CH_2), 6.7 (1H, s, NH), 7.93 (2H, s, SO_2NH_2), 12.01 (1H, s, COOH). ^{13}C NMR (75 MHz, DMSO-d_6): 24.9 (CH_2), 26.3 (CH_2), 30.7 (CH_2), 34.3 (CH_2), 44.7 (CH_2), 108.2 (C1, t, J (^{19}F–^{13}C) = 15 Hz), 132 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 136.4 (C2, C6, d, J (^{19}F–^{13}C) = 238 Hz), 144.5 (C3, C5, d, J (^{19}F–^{13}C) = 244 Hz), 175.1 (COOH). ^{19}F NMR (282 MHz, DMSO-d_6): −142.7 (2F, d, J = 16.6 Hz), −161.7 (2F, d, J = 18.3 Hz). HRMS for $C_{12}H_{14}F_4N_2O_4S$ [(M−H)$^-$]: calcd 357.0538, found 357.0542.

4.1.7.6. 4-(Benzylthio)-2,3,5,6-tetrafluorobenzenesulfonamide (3l). Recrystallization was accomplished from iPrOH. Yield: 0.23 g, 64%, mp 184 °C. IR ν cm^{-1}: 3395, 3267 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 4.3 (2H, s, CH_2), 7.1–7.5 (5H, m, ArH), 8.44 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 38.6 (CH_2), 118.5 (C1, t, J (^{19}F–^{13}C) = 21 Hz), 123.6 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 128.4 (Ar), 129.3 (Ar), 129.5 (Ar), 137.4 (Ar), 143.1 (C2, C6, dd, ^1J (^{19}F–^{13}C) = 254 Hz, ^2J (^{19}F–^{13}C) = 17 Hz), 147.3 (C3, C5, dd, ^1J (^{19}F–^{13}C) = 248 Hz, ^2J (^{19}F–^{13}C) = 17 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −132.8 (2F, dd, ^1J = 27 Hz, ^2J = 11 Hz), −139.6 (2F, dd, ^1J = 26 Hz, ^2J = 10 Hz). HRMS for $C_{13}H_9F_4NO_2S_2$ [(M−H)$^-$]: calcd 349.9938, found 349.9940.

4.1.7.7. 4-(Benzylamino)-2,3,5,6-tetrafluorobenzenesulfonamide (3m). Recrystallization was accomplished from H_2O:EtOH (1:1). Yield: 0.21 g, 62%, mp 132–133 °C. IR ν cm^{-1}: 3415, 3280 (NH, NH_2). ^1H NMR (300 MHz, DMSO-d_6): 4.56 (2H, d, J = 6.3 Hz, CH_2), 7.2–7.6 (6H, m, NH, ArH), 7.98 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 47.9 (CH_2), 109.1 (C1, t, J (^{19}F–^{13}C) = 17 Hz), 127.2 (Ar), 127.7 (Ar), 129.2 (Ar), 131.7 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 136.5 (C2, C6, d, J (^{19}F–^{13}C) = 244 Hz), 140.5 (Ar), 144.3 (C3, C5, d, J (^{19}F–^{13}C) = 241 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −142.5 (2F, d, J = 18.3 Hz), −160.4 (2F, d, J = 18.3 Hz). HRMS for $C_{13}H_{10}F_4N_2O_2S$ [(M+H)$^+$]: calcd 335.0472, found 335.0472.

4.1.7.8. 2,3,5,6-Tetrafluoro-4-{[2-(4-hydroxyphenyl)ethyl]amino}benzenesulfonamide (3n). Recrystallization was accomplished from EtOH:H_2O (1:2). Yield: 0.25 g, 68%, decomp. at 100 °C. IR ν cm^{-1}: 3397 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 2.74 (2H, t, J = 7.8 Hz, CH_2), 3.45–3.6 (2H, m, CH_2), 6.7 (2H, d, J = 8.4 Hz, ArH), 7.01 (2H, d, J = 8.4 Hz, ArH), 7.96 (2H, s, SO_2NH_2), 9.23 (1H, s, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 36.5 (CH_2), 46.8 (CH_2), 108.4 (C1, t, J (^{19}F–^{13}C) = 16 Hz), 115.9 (Ar), 129.4 (Ar), 130.3 (Ar), 131.9 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 136.5 (C2, C6, d, J (^{19}F–^{13}C) = 240 Hz), 144.4 (C3, C5, d, J (^{19}F–^{13}C) = 240 Hz), 156.5 (Ar). ^{19}F NMR (282 MHz, DMSO-d_6): −142.6 (2F, d, J = 19 Hz), −161.4 (2F, d, J = 18 Hz). HRMS for $C_{14}H_{12}F_4N_2O_3S$ [(M−H)$^-$]: calcd 363.0432, found 363.0435.

4.1.7.9. 2,3,5,6-Tetrafluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (3o). Recrystallization was accomplished from EtOH:H_2O (2:1). Yield: 0.24 g, 71%, mp 121 °C. IR ν cm^{-1}: 3404, 3290 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 2.91 (2H, t, J = 7.2 Hz, SCH_2CH_2), 3.37 (2H, t, J = 7.2 Hz, SCH_2CH_2), 7.1–7.4 (5H, m, ArH), 8.41 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 35.4 (SCH_2CH_2), 36.6 (SCH_2CH_2), 119.1 (C1, t, J (^{19}F–^{13}C) = 20 Hz), 122.9 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 127.2 (Ar), 128.9 (Ar), 129.3 (Ar), 139.7 (Ar), 143.2 (C2, C6, dd, ^1J (^{19}F–^{13}C) = 257 Hz, ^2J (^{19}F–^{13}C) = 17 Hz), 147 (C3, C5, dd, ^1J (^{19}F–^{13}C) = 249 Hz, ^2J (^{19}F–^{13}C) = 17 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −133.5 (2F, dd, ^1J = 26.5 Hz, ^2J = 13.5 Hz), −139.8 (2F, dd, ^1J = 25.6 Hz, ^2J = 10.7 Hz). HRMS for $C_{14}H_{11}F_4NO_2S_2$ [(M−H)$^-$]: calcd 364.0095, found 364.0100.

4.1.7.10. 2,3,5,6-Tetrafluoro-4-[(mesitylmethyl)thio]benzenesulfonamide (3r). Recrystallization was accomplished from EtOH. Yield: 0.29 g, 73%, mp 213–214 °C. IR ν cm^{-1}: 3342, 3264 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 2.23 (3H, s, CH_3), 2.36 (6H, s, $2CH_3$), 4.31 (2H, s, CH_2), 6.89 (2H, s, ArH), 8.47 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 19.6 (CH_3), 21.3 (CH_3), 34.3 (CH_2), 119.2 (C1, t, J (^{19}F–^{13}C) = 21 Hz), 123.6 (C4, t, J (^{19}F–^{13}C) = 18 Hz), 129.4 (Ar), 129.7 (Ar), 137.86 (Ar), 137.93 (Ar), 143.3 (C2, C6, d, J

(^{19}F–^{13}C) = 253 Hz), 147.4 (C3, C5, d, J (^{19}F–^{13}C) = 243 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −133.1 (2F, dd, ^1J = 25 Hz, ^2J = 10 Hz), −139.5 (2F, dd, ^1J = 25 Hz, ^2J = 11 Hz). HRMS for $C_{16}H_{15}F_4NO_2S_2$ [(M−H)$^-$]: calcd 392.0408, found 392.0412.

4.1.7.11. 3-{[4-(Aminosulfonyl)-2,3,5,6-tetrafluorophenyl]thio}-[1,2,3]thiadiazolo[3,4-a]benzimidazole (3v). The obtained compound was washed with MeOH. Yield: 0.13 g, 30%, decomp. at 233–234 °C. IR ν cm^{-1}: 3356 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 7.33 (1H, t, J = 7.8 Hz, ArH), 7.57 (1H, t, J = 8.4 Hz, ArH), 7.81 (1H, d, J = 8.7 Hz, ArH), 8.2 (1H, d, J = 8.1 Hz, ArH), 8.52 (2H, br s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 118.4 (Ar), 120.2 (C1, t, J (^{19}F–^{13}C) = 15 Hz), 125.9 (Ar), 126 (Ar), 127.7 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 130 (Ar), 133.2 (Ar), 133.4 (Ar), 148 (C2, C6, d, J (^{19}F–^{13}C) = 254 Hz), 152 (C3, C5, d, J (^{19}F–^{13}C) = 236 Hz), 158.7 (Ar), 158.8 (Ar). ^{19}F NMR (282 MHz, DMSO-d_6): −127.2 (2F, dd, ^1J = 25.9 Hz, ^2J = 11.3 Hz), −134 (2F, dd, ^1J = 25.9 Hz, ^2J = 10.7 Hz). HRMS for $C_{14}H_6F_4N_4O_2S_3$ [(M+H)$^+$]: calcd 434.9662, found 434.9667.

4.1.8. 2,3,5,6-Tetrafluoro-4-(phenylthio)benzenesulfonamide (3i)

The mixture of pentafluorobenzenesulfonamide (compound **2**) (0.28 g, 1.13 mmol), MeOH (10 mL), Et_3N (0.158 mL, 1.13 mmol) and HSPh (0.116 mL, 1.13 mmol) was stirred at ambient temperature for 2 h. MeOH was evaporated in vacuum and the resultant precipitate was washed with H_2O. Recrystallization was accomplished from EtOH:H_2O (2:1). Yield: 0.28 g, 74%, mp 139 °C. IR ν cm^{-1}: 3400, 3288 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 7.2–7.8 (5H, m, ArH), 8.47 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 117.7 (C1, t, J (^{19}F–^{13}C) = 20 Hz), 124.5 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 128.7 (Ar), 130.3 (Ar), 130.4 (Ar), 132.4 (Ar), 143.5 (C2, C6, ddd, ^1J (^{19}F–^{13}C) = 255 Hz, ^2J (^{19}F–^{13}C) = 17 Hz, ^3J (^{19}F–^{13}C) = 5 Hz), 147.2 (C3, C5, ddd, ^1J (^{19}F–^{13}C) = 240 Hz, ^2J (^{19}F–^{13}C) = 17 Hz, ^3J (^{19}F–^{13}C) = 5 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −132.6 (2F, dd, ^1J = 24.5 Hz, ^2J = 11.6 Hz), −138.8 (2F, dd, ^1J = 24.5 Hz, ^2J = 10.4 Hz). HRMS for $C_{12}H_7F_4NO_2S_2$ [(M−H)$^-$]: calcd 335.9782, found 335.9780.

4.1.9. 2,3,5,6-Tetrafluoro-4-(phenylsulfonyl)-benzenesulfonamide (3j)

The mixture of 2,3,5,6-tetrafluoro-4-(phenylthio)benzenesulfonamide (compound **3i**) (0.2 g, 0.59 mmol), CrO_3 (0.18 g, 1.8 mmol), CH_3COOH (10 mL), H_2O (0.5 mL) was heated at 70 °C for 2 h. The solvent was then removed in vacuum and the resultant precipitate was washed with H_2O. Recrystallization was accomplished from EtOH. Yield: 0.17 g, 77%, mp 266–267 °C. IR ν cm^{-1}: 3389, 3284 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 7.74 (2H, t, J = 7.8 Hz, ArH), 7.87 (1H, t, J = 7.5 Hz, ArH), 8.09 (2H, d, J = 7.8 Hz, ArH), 8.59 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 123 (C1, C4, t, J (^{19}F–^{13}C) = 13 Hz), 128.4 (Ar), 130.7 (Ar), 136.2 (Ar), 140.4 (Ar), 143.8 (C2, C6 or C3, C5, dd, ^1J (^{19}F–^{13}C) = 258 Hz, ^2J (^{19}F–^{13}C) = 18 Hz), 144.4 (C2, C6 or C3, C5, dd, ^1J (^{19}F–^{13}C) = 258 Hz, ^2J (^{19}F–^{13}C) = 18 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −136.6 (4F, s). HRMS for $C_{12}H_7F_4NO_4S_2$ [(M−H)$^-$]: calcd 367.968, found 367.9684.

4.1.10. 2,3,5,6-Tetrafluoro-4-phenoxybenzenesulfonamide (3k)

The mixture of pentafluorobenzenesulfonamide (compound **2**) (0.2 g, 0.81 mmol), sodium phenoxide trihydrate (0.145 g, 0.85 mmol) and DMSO (1 mL) was stirred at ambient temperature for 4 h. The mixture was then diluted with H_2O (20 mL) and the resultant precipitate was filtered, washed with H_2O. Recrystallization was accomplished from EtOH:H_2O (1:2). Yield: 0.07 g, 27%, mp 164–165 °C. IR ν cm^{-1}: 3363, 3277 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 7.0–7.6 (5H, m, ArH), 8.44 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 116.4 (Ar), 120.4 (C1, t, J (^{19}F–^{13}C) = 14 Hz), 125 (Ar), 130.9 (Ar), 136.3 (C4, t, J (^{19}F–^{13}C) = 14 Hz), 141.9 (C3, C5, d, J (^{19}F–^{13}C) = 250 Hz), 144.4 (C2, C6, d, J (^{19}F–^{13}C) = 256 Hz), 157 (Ar). ^{19}F NMR (282 MHz, DMSO-d_6): −139.6 (2F, d, J = 16 Hz), −154 (2F, d, J = 16 Hz). HRMS for $C_{12}H_7F_4NO_3S$ [(M−H)$^-$]: calcd 320.001, found 320.0008.

4.1.11. 2,3,5,6-Tetrafluoro-4-[(2-phenylethyl)sulfonyl]-benzenesulfonamide (3p)

The mixture of 2,3,5,6-tetrafluoro-4-[(2-phenylethyl)thio]benzenesulfonamide (**3o**) (0.1 g, 0.27 mmol), CrO_3 (0.082 g, 0.82 mmol), CH_3COOH (10 mL), H_2O (0.2 mL) was heated at 60 °C for 4 h. The resultant precipitate was filtered, washed with H_2O. Yield: 0.07 g, 64%, mp 248–249 °C. IR ν cm^{-1}: 3356, 3251 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 3.12 (2H, t, J = 7.2 Hz, $SO_2CH_2CH_2$), 3.97 (2H, t, J = 7.8 Hz, $SO_2CH_2CH_2$), 7.1–7.4 (5H, m, ArH), 8.66 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 28.6 ($SO_2CH_2CH_2$), 58 ($SO_2CH_2CH_2$), 121.5 (C1 or C4, t, J (^{19}F–^{13}C) = 15 Hz), 127.5 (Ar), 128.2 (C1 or C4, t, J (^{19}F–^{13}C) = 16 Hz), 128.9 (Ar), 129.2 (Ar), 137.4 (Ar), 143.5 (C2, C6 or C3, C5, dd, ^1J (^{19}F–^{13}C) = 258 Hz, ^2J (^{19}F–^{13}C) = 17 Hz), 144.8 (C2, C6 or C3, C5, dd, ^1J (^{19}F–^{13}C) = 255 Hz, ^2J (^{19}F–^{13}C) = 17 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −136 (2F, dd, ^1J = 25 Hz, ^2J = 12 Hz), −137.1 (2F, dd, ^1J = 25 Hz, ^2J = 12 Hz). HRMS for $C_{14}H_{11}F_4NO_4S_2$ [(M−H)$^-$]: calcd 395.9993, found 395.9996.

4.1.12. 2,3,5,6-Tetrafluoro-4-morpholin-4-ylbenzenesulfonamide (3q)

The mixture of pentafluorobenzenesulfonamide (compound **2**) (0.2 g, 0.809 mmol), MeOH (10 mL), morpholine (0.141 mL, 1.62 mmol) was refluxed for 8 h. MeOH was evaporated in vacuum and the resultant precipitate was washed with H_2O. Recrystallization was accomplished from EtOH:H_2O (1:1). Yield: 0.13 g, 52%, mp 233−234 °C close to the values in the literature, mp 226–227 °C.[44] ^1H NMR (300 MHz, DMSO-d_6): 3.32 (4H, br s, $2CH_2$), 3.7 (4H, br s, $2CH_2$), 8.2 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 51.2 ($N(CH_2)_2$, t, J (^{19}F–^{13}C) = 3.6 Hz), 67.2 ($O(CH_2)_2$), 115.4 (C1, t, J (^{19}F–^{13}C) = 19 Hz), 133.3 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 141.7 (C3, C5, d, J (^{19}F–^{13}C) = 244 Hz), 144.3 (C2, C6, d, J (^{19}F–^{13}C) = 256 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −141.6 (2F, d, J = 15.8 Hz), −151.2 (2F, d, J = 16.6 Hz).

4.1.13. General procedure for the syntheses of 3s, 3t, 3w

The mixture of pentafluorobenzenesulfonamide (compound **2**) (0.1 g, 0.404 mmol), K_2CO_3 (0.056 g, 0.406 mmol), DMSO (2 mL) and appropriate nucleophile (0.404 mmol) was stirred at ambient temperature for 5 h. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (2 × 10 mL). The combined organic phase was dried over Na_2SO_4 and evaporated in vacuum.

4.1.13.1. 4-[(4,6-Dimethylpyrimidin-2-yl)thio]-2,3,5,6-tetrafluorobenzenesulfonamide (3s). Recrystallization was accomplished from EtOH:H_2O (2:1). Yield: 0.12 g, 80%, mp 131–132 °C. IR ν cm^{-1}: 3317 (NH_2). ^1H NMR (300 MHz, DMSO-d_6): 2.34 (6H, s, $2CH_3$), 7.15 (1H, s, ArH), 8.58 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 24 (CH_3), 114.1 (C1, t, J (^{19}F–^{13}C) = 20 Hz), 118.5 (Ar), 125.5 (C4, t, J (^{19}F–^{13}C) = 14 Hz), 143.3 (C2, C6, d, J (^{19}F–^{13}C) = 255 Hz), 147.7 (C3, C5, d, J (^{19}F–^{13}C) = 248 Hz), 166.3 (Ar), 169 (Ar). ^{19}F NMR (282 MHz, DMSO-d_6): −130.7 (2F, dd, ^1J = 25.4 Hz, ^2J = 10.7 Hz), −139.3 (2F, dd, ^1J = 26.2 Hz, ^2J = 11 Hz). HRMS for $C_{12}H_9F_4N_3O_2S_2$ [(M+H)$^+$]: calcd 368.0145, found 368.0142.

4.1.13.2. 4-(1,3-Benzothiazol-2-ylthio)-2,3,5,6-tetrafluorobenzenesulfonamide (3t). Recrystallization was accomplished from EtOH:H_2O (2:1). Yield: 0.11 g, 69%, mp 171 °C. IR ν cm^{-1}: 3426,

3299 (NH_2). ^{1}H NMR (300 MHz, DMSO-d_6): 7.44 (1H, t, J = 8.1 Hz, ArH), 7.51 (1H, t, J = 8.1 Hz, ArH), 7.9 (1H, d, J = 7.5 Hz, ArH), 8.06 (1H, d, J = 8.4 Hz, ArH), 8.65 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 113.8 (C1, t, J (^{19}F–^{13}C) = 20 Hz), 122.7 (Ar), 122.9 (Ar), 126.2 (Ar), 126.5 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 127.5 (Ar), 136 (Ar), 143.5 (C2, C6, dd, ^1J (^{19}F–^{13}C) = 254 Hz, ^2J (^{19}F–^{13}C) = 12 Hz), 147.6 (C3, C5, dd, ^1J (^{19}F–^{13}C) = 250 Hz, ^2J (^{19}F–^{13}C) = 16 Hz), 153.1 (Ar), 162.4 (Ar). ^{19}F NMR (282 MHz, DMSO-d_6): −130.5 (2F, dd, ^1J = 24.8 Hz, ^2J = 11.6 Hz), −137.9 (2F, dd, ^1J = 24.8 Hz, ^2J = 11.6 Hz). HRMS for $C_{13}H_6F_4N_2O_2S_3$ [(M−H)$^-$]: calcd 392.9455, found 392.9457.

4.1.13.3. 4-[(4,5-Diphenyl-1*H*-imidazol-2-yl)thio]-2,3,5,6-tetrafluorobenzenesulfonamide (3w). Recrystallization was accomplished from EtOH:H_2O (2:1). Yield: 0.12 g, 63%, mp 221–222 °C. IR ν cm^{-1}: 3292 (NH_2). ^{1}H NMR (300 MHz, DMSO-d_6): 7.2–7.5 (10H, m, ArH), 8.51 (2H, s, SO_2NH_2), 13.2 (1H, br s, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 116.6 (C1, t, J (^{19}F–^{13}C) = 15 Hz), 124.1 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 128.3 (br s, Ar), 129.3 (br s, Ar), 133.9 (Ar), 143.3 (C2, C6, d, J (^{19}F–^{13}C) = 253 Hz), 146.7 (C3, C5, d, J (^{19}F–^{13}C) = 250 Hz). ^{13}C NMR (75 MHz, DMSO-d_6, CF_3COOH): 115.1 (C1, t, J (^{19}F–^{13}C) = 15 Hz), 125 (C4, t, J (^{19}F–^{13}C) = 16 Hz), 128.7 (Ar), 129.3 (Ar), 129.5 (Ar), 130.2 (Ar), 133.4 (Ar), 134.2 (Ar), 143.5 (C2, C6, d, J (^{19}F–^{13}C) = 255 Hz), 146.9 (C3, C5, d, J (^{19}F–^{13}C) = 255 Hz). ^{19}F NMR (282 MHz, DMSO-d_6): −133.5 (2F, dd, ^1J = 24.8 Hz, ^2J = 10.2 Hz), −139.1 (2F, dd, ^1J = 24.8 Hz, ^2J = 10.2 Hz). HRMS for $C_{21}H_{13}F_4N_3O_2S_2$ [(M+H)$^+$]: calcd 480.0458, found 480.0449.

4.1.14. 4-(1-Adamantylamino)-2,3,5,6-tetrafluorobenzenesulfonamide (3u)

The mixture of pentafluorobenzenesulfonamide (compound **2**) (0.2 g, 0.81 mmol), Et_3N (0.226 mL, 1.62 mmol), adamantanamine hydrochloride (0.15 g, 0.81 mmol) and DMSO (2 mL) was stirred at ambient temperature for 48 h. The mixture was then diluted with H_2O (20 mL) and extracted with EtOAc (2 × 10 mL). The combined organic phase was dried over Na_2SO_4 and evaporated in vacuum. The product was purified by chromatography on a column of silica gel (0.04–0.063 mm) with EtOAc:$CHCl_3$ (1:4), R_f = 0.63. Yield: 0.04 g, 12%, mp 122–123 °C. IR ν cm^{-1}: 3394, 3357, 3255 (NH, NH_2). ^{1}H NMR (300 MHz, $CDCl_3$): 1.6–1.8 (6H, m, adamantane), 1.8–2 (6H, m, adamantane), 2.1–2.25 (3H, m, adamantane), 4.05 (1H, s, NH), 5.59 (2H, s, SO_2NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): 30.1 (adamantane), 36.1 (adamantane), 43.6 (adamantane), 54.8 (adamantane), 110.2 (C1), 130.4 (C4, t, J (^{19}F–^{13}C) = 14 Hz), 138.2 (C2, C6, d, J (^{19}F–^{13}C) = 244 Hz), 144.5 (C3, C5, d, J (^{19}F–^{13}C) = 251 Hz). ^{19}F NMR (282 MHz, $CDCl_3$): −141.5 (2F, d, J = 16 Hz), −152.4 (2F, d, J = 17.5 Hz). HRMS for $C_{16}H_{18}F_4N_2O_2S$ [(M−H)$^-$]: calcd 377.0952, found 377.0952.

4.1.15. 4-Bromo-*N*-[(dimethylamino)methylene]benzenesulfonamide (5)

The compound **5** was synthesized according to the literature.[25] A solution of dimethyl *N*,*N*-dimethylformamide dimetyl acetal (1.65 g, 14 mmol) in CH_3CN (5 mL) was added dropwise to a suspension of **4** (2.83 g, 12 mmol) in CH_3CN (15 mL). The mixture was stirred for 1 h and the solvent was removed in vacuum. The resultant precipitate was washed with H_2O. Yield: 3.44 g, 99%, mp 139–140 °C close to values in the literature mp 141–143 °C.[25]

4.1.16. General procedure for the syntheses of 6a–c

The compounds **6a–c** were synthesized according to the literature.[25] A solution of appropriate thiol (0.68 mmol) was added dropwise to a stirred mixture of NaH (0.033 g, 50% oil dispersion, 0.69 mmol) in anhydrous DMF (1 mL). When gas evolution was complete, **5** (0.1 g, 0.34 mmol) was added and the mixture was heated at 95 °C for 1 h. After removal of the DMF under vacuum, MeOH (0.5 mL) and 10% aqueous NaOH solution (0.5 mL) were added to the residue. This solution was refluxed for 1 h. MeOH was evaporated in vacuum and the resultant precipitate was diluted with H_2O. The obtained solution was washed with petroleum ether and acidified with 10% HCl.

4.1.16.1. 4-[(2-Hydroxyethyl)thio]benzenesulfonamide (6a)

The mixture was extracted with EtOAc (3 × 10 mL). The combined organic phase was dried over Na_2SO_4 and evaporated in vacuum. The product was purified by chromatography on a column of silica gel (0.04–0.063 mm) with EtOAc, R_f = 0.63. Yield: 0.04 g, 50%, mp 109–110 °C close to values in the literature mp 111–112 °C.[25] ^{1}H NMR (300 MHz, DMSO-d_6): 3.15 (2H, t, J = 7 Hz, CH_2), 3.62 (2H, br t, CH_2), 5.04 (1H, br s, OH), 7.34 (2H, s, SO_2NH_2), 7.48 (2H, d, J = 8.7 Hz, ArH), 7.73 (2H, d, J = 8.7 Hz, ArH).

4.1.16.2. 4-Propylthiobenzenesulfonamide (6b)

The resultant precipitate was filtered and washed with H_2O. Recrystallization was accomplished from toluene. Yield: 0.05 g, 63%, mp 124–125 °C. IR ν cm^{-1}: 3349, 3251 (NH_2). ^{1}H NMR (300 MHz, DMSO-d_6): 1.01 (3H, t, J = 7.5 Hz, CH_3), 1.65 (2H, sext, J = 7.2 Hz, CH_2), 3.05 (2H, t, J = 7.2 Hz, CH_2), 7.34 (2H, s, SO_2NH_2), 7.47 (2H, d, J = 8.4 Hz, ArH), 7.74 (2H, d, J = 8.4 Hz, ArH). ^{13}C NMR (75 MHz, DMSO-d_6): 13.9 (CH_3), 22.4 (CH_2), 33.6 (CH_2), 126.9 (Ar), 127.3 (Ar), 141.2 (Ar), 142.8 (Ar). HRMS for $C_9H_{13}NO_2S_2$ [(M−H)$^-$]: calcd 230.0315, found 230.0316.

4.1.16.3. 4-[(2-Phenylethyl)thio]benzenesulfonamide (6c)

The resultant precipitate was filtered and washed with H_2O. Recrystallization was accomplished from toluene. Yield: 0.07 g, 70%, mp 120–121 °C. IR ν cm^{-1}: 3302, 3239 (NH_2). ^{1}H NMR (300 MHz, DMSO-d_6): 2.93 (2H, t, J = 7.2 Hz, CH_2), 3.35 (2H, t, J = 7.2 Hz, CH_2), 7.1-7.4 (7H, m, ArH, SO_2NH_2), 7.51 (2H, d, J = 8.4 Hz, ArH), 7.76 (2H, d, J = 8.4 Hz, ArH). ^{13}C NMR (75 MHz, DMSO-d_6): 33.1 (CH_2), 35 (CH_2), 127 (Ar), 127.1 (Ar), 127.3 (Ar), 129.1 (Ar), 129.3 (Ar), 140.5 (Ar), 141.3 (Ar), 142.5 (Ar). HRMS for $C_{14}H_{15}NO_2S_2$ [(M−H)$^-$]: calcd 292.0471, found 292.0475.

4.2. Protein preparation

Expression and purification of CA I, II, VII, XII and XIII was performed as previously described: CA I in[48] CA II in,[49] CA VII and XIII in,[50] and CA XII in.[51]

4.3. Determination of compound binding and inhibition of CAs

4.3.1. Thermal shift assay

Thermal shift assay experiments were performed in a Corbett Rotor-Gene 6000 (QIAGEN Rotor-Gene Q) RT-PCR instrument using the blue channel (excitation 365 ± 20, detection 460 ± 15 nm). Sample volume was 20 μl containing 5–10 μM protein, 0–200 μM ligand, 50 μM solvatochromic dye ANS (8-anilino-1-naphthalene sulfonate), and 50 mM sodium phosphate buffer containing 100 mM NaCl at pH 7.0, with the final DMSO concentration at 2%. The samples were heated at a constant rate of 1 °C/min. Data analysis was performed as previously described.[48]

4.3.2. Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) experiments were performed using ITC_{200} or VP-ITC instruments (Microcal, Inc., Northampton, USA) with 5–20 μM protein solution in the cell and 50–200 μM of the ligand solution in the syringe. A typical experiment consisted of 18 or 25 injections (2 or 10 μl each) within 2 or 3 min intervals, respectively for the two calorimeters. Experiments were performed at 37 °C in a 50 mM sodium phosphate buffer containing

100 mM NaCl at pH 7.0, with a final DMSO concentration of 2%, equal in the syringe and the cell.

4.3.3. CO_2 hydration assay

The carbon dioxide hydration activity of the recombinant human CA II was measured using Applied Photophysics SX.18MV-R stop-flow spectrometer according to.[9,52] Reaction velocities were measured by recording the absorbance of bromothymol blue dye (40 μM). The reaction buffer contained 10 mM NaCl, 10 mM Hepes, pH 7.4. Saturated CO_2 solution was made by the bubbling of gas in milli-Q water at 25 °C for 1 h. CA II was incubated with inhibitors for 15 min at room temperature. CA II concentration was 20 nM and the final DMSO concentration was less than 0.04%. IC_{50} value was determined by fitting the sigmoidal curve of the data points and then K_i was calculated using the Cheng–Prusoff equation assuming that the K_M = 9.3 mM and half-saturated [CO_2] = 17 mM, according to.[9]

4.4. Crystallography

4.4.1. Crystallization

Human carbonic anhydrases were concentrated to 20–60 mg/ml by ultrafiltration in 20 mM Na-Hepes, pH 7.5, containing 50 mM NaCl. Crystallization by sitting drop method was started by mixing equal volumes of protein solution with reservoir buffer. Reservoir buffer for CA II contained 0.1 M of Na-Bicine, pH 9.0, 0.2 M ammonium sulfate, and 2.0 M Na-malonate, pH 7.5. Reservoir buffer for CA XII contained 0.1 M ammonium citrate, pH 5.0 and 16% PEG4000. Reservoir buffer for CA XIII contained 0.1 M sodium citrate, pH 5.5, 0.1 M sodium acetate pH 4.5 and 26% PEG4000. Crystals were soaked with 1 mM of inhibitor in reservoir buffer for several days. For data collection, the crystals of CA XII and CA XIII were transferred in cryo protecting buffer made of the reservoir mixed with 15–20% ethyleneglycol and flash-cooled after a few minutes.

4.4.2. Data collection and structure determination

Diffraction data for all complexes of CA II, CA XIII, and CA XII with inhibitors were collected at the EMBL beamlines at the DORIS storage ring (DESY, Hamburg). The crystal of CA II complex with compound **3s** and CA XIII with compound **3d** were measured at X11, and the dataset of CA XII complex with compound **3s** was collected at X13 beamline.

Initial phases were obtained by molecular replacement with the protein moiety from PDB entry 3HLJ as an initial model for CA II, protein chain A of the PDB entry 1JD0 was used for phasing of CA XII. PDB entry 2NNO of CA II was used for phasing of CA XIII, and residues that differ between the two proteins were mutated to CA XIII sequence. Datasets were processed using MOSFLM,[53,54] TRUNCATE and SCALA.[55] Structures were refined using REFMAC[56] while COOT[57] was used for model inspection. Atomic coordinates of ligands were generated using molecular editor AVOGADRO.[58] Topology and parameters for structure refinement were generated by LIBREFMAC.[59] Data collection and refinement statistics are presented in Table 3. Coordinates and structure factors were deposited to the RCSB Protein Data Bank and the PDB IDs are listed in Table 3. All molecular representations were made using MOLSCRIPT,[60] RASTER3D[61] and BOBSCRIPT.[62]

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Supplementary data

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References and notes

1. Krishnamurthy, V. M.; Kaufman, G. K.; Urbach, A. R.; Gitlin, I.; Gudiksen, K. L.; Weibel, D. B.; Whitesides, G. M. *Chem. Rev.* **2008**, *108*, 946.
2. Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168.
3. Macauley, S. R.; Zimmerman, S. A.; Apolinario, E. E.; Evilia, C.; Hou, Y.-M.; Ferry, J. G.; Sowers, K. R. *Biochemistry* **2009**, *48*, 817.
4. Xu, Y.; Feng, L.; Jeffrey, P. D.; Shi, Y.; Morel, F. M. *Nature* **2008**, *452*, 56.
5. Hassan, M. I.; Shajee, B.; Waheed, A.; Ahmad, F.; Sly, W. S. *Bioorg. Med. Chem.* **2012**. http://dx.doi.org/10.1016/j.bmc.2012.04.044.
6. Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 603.
7. Supuran, C. T. *Curr. Top. Med. Chem.* **2007**, *7*, 825.
8. Aggarwal, M.; Kondeti, B.; McKenna, R. *Bioorg. Med. Chem.* **2012**. http://dx.doi.org/10.1016/j.bmc.2012.08.019.
9. Alterio, V.; Fiore, A. D.; D'Ambrosio, K.; Supuran, C. T.; Simone, G. D. *Chem. Rev.* **2012**, *112*, 4421.
10. Gieling, R. G.; Williams, K. J. *Biorg. Med. Chem.* **2012**. http://dx.doi.org/10.1016/j.bmc.2012.09.062.
11. Supuran, C. T.; Scozzafava, A.; Conway, J. *Carbonic Anhydrase—Its Inhibitors and Activators*; CRC Press: Boca Raton FL, USA, 2004. pp 1–363.
12. Supuran, C. T.; Winum, J. Y. *Drug Design of Zinc-Enzyme Inhibitors Functional, Structural, and Disease Applications*; Wiley: Hoboken (NJ), 2009.
13. Krishnamurthy, V. M.; Bohall, B. R.; Kim, C.-Y.; Moustakas, D. T.; Christianson, D. W.; Whitesides, G. M. *Chem. Asian J.* **2007**, *2*, 94.
14. Ojima, I. *Fluorine in Medicinal Chemistry and Chemical Biology*; Wiley-Blackwell: West Sussex, UK, 2009.
15. Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 4542.
16. de Leval, X.; Ilies, M.; Casini, A.; Dogne, J. M.; Scozzafava, A.; Masini, E.; Mincione, F.; Starnotti, M.; Supuran, C. T. *J. Med. Chem.* **2004**, *47*, 2796.
17. Vullo, D.; Scozzafava, A.; Pastorekova, S.; Pastorek, J.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2351.
18. Pastorekova, S.; Vullo, D.; Casini, A.; Scozzafava, A.; Pastorek, J.; Nishimori, I.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 211.
19. Fiore, A. D.; Simone, G. D.; Menchise, V.; Pedone, C.; Casini, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1937.
20. Riley, K. E.; Cui, G.; Merz, K. M. *J. Phys. Chem. B* **2007**, *111*, 5700.
21. Vernier, W.; Chong, W.; Rewolinski, D.; Greasley, S.; Pauly, T.; Shaw, M.; Dinh, D.; Ferre, R. A.; Nukui, S.; Ornelas, M.; Reyner, E. *Bioorg. Med. Chem.* **2010**, *18*, 3307.
22. Pacchiano, F.; Aggarwal, M.; Avvaru, B. S.; Robbins, A. H.; Scozzafava, A.; McKenna, R.; Supuran, C. T. *Chem. Commun. (Camb)* **2010**, *46*, 8371.
23. Doyon, J. B.; Jain, A. *Org. Lett.* **1999**, *1*, 183.
24. Doyon, J. B.; Hansen, E. A.; Kim, C. Y.; Chang, J. S.; Christianson, D. W.; Madder, R. D.; Voet, J. G.; Baird, T. A.; Fierke, C. A.; Jain, A. *Org. Lett.* **2000**, *2*, 1189.
25. Shepard, K. L.; Graham, S. L.; Hudcosky, R. J.; Michelson, S. R.; Scholz, T. H.; Schwam, H.; Smith, A. M.; Sondey, J. M.; Strohmaier, K. M.; Smith, R. L., et al *J. Med. Chem.* **1991**, *34*, 3098.
26. Cecchi, A.; Ciani, L.; Winum, J.-Y.; Montero, J.-L.; Scozzafava, A.; Ristori, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3475.
27. Ilies, M. A.; Masereel, B.; Rolin, S.; Scozzafava, A.; Campeanu, G.; Cimpeanu, V.; Supuran, C. T. *Bioorg. Med. Chem.* **2004**, *12*, 2717.
28. Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1551.
29. Winum, J. Y.; Thiry, A.; Cheikh, K. E.; Dogne, J. M.; Montero, J. L.; Vullo, D.; Scozzafava, A.; Masereel, B.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2685.
30. Biswas, S.; Aggarwal, M.; Guzel, O.; Scozzafava, A.; McKenna, R.; Supuran, C. T. *Bioorg. Med. Chem.* **2011**, *19*, 3732.
31. Cecchi, A.; Hulikova, A.; Pastorek, J.; Pastorekova, S.; Scozzafava, A.; Winum, J. Y.; Montero, J. L.; Supuran, C. T. *J. Med. Chem.* **2005**, *48*, 4834.

32. Winum, J.-Y.; Dogne, J.-M.; Casini, A.; de Leval, X.; Montero, J.-L.; Scozzafava, A.; Vullo, D.; Innocenti, A.; Supuran, C. T. *J. Med. Chem.* **2005**, *48*, 2121.
33. Ilies, M. A.; Vullo, D.; Pastorek, J.; Scozzafava, A.; Ilies, M.; Caproiu, M. T.; Pastorekova, S.; Supuran, C. T. *J. Med. Chem.* **2003**, *46*, 2187.
34. Scott, A. D.; Phillips, C.; Alex, A.; Flocco, M.; Bent, A.; Randall, A.; O'Brien, R.; Damian, L.; Jones, L. H. *ChemMedChem* **2009**, *4*, 1985.
35. Dugad, L. B.; Gerig, J. T. *Biochemistry* **1988**, *27*, 4310.
36. Olander, J.; Bosen, S.; Kaiser, E. *J. Am. Chem. Soc.* **1973**, *85*, 1616.
37. Dugad, L. B.; Cooley, C. R.; Gerig, J. T. *Biochemistry* **1989**, *28*, 3955.
38. Veenstra, D.; Gerig, J. *Magn. Reson. Chem.* **1998**, *36*, S169.
39. Mung, Ch. W. K. Patent WO2,008,017,932, 2008.
40. Niembro, S.; Vallribera, A.; Moreno-Manas, M. *New J. Chem.* **2008**, *32*, 94.
41. Tangallapally, R. P.; Yendapally, R.; Lee, R. E.; Lenaerts, A. J. M.; Lee, R. E. *J. Med. Chem.* **2005**, *48*, 8261.
42. Koshio, H.; Hirayama, F.; Ishihara, T.; Kaizawa, H.; Shigenaga, T.; Taniuchi, Y.; Sato, K.; Moritani, Y.; Iwatsuki, Y.; Uemura, T.; Kaku, S.; Kawasaki, T.; Matsumoto, Y.; Sakamoto, S.; Tsukamoto, S. *Bioorg. Med. Chem.* **2004**, *12*, 5415.
43. Liljas, A.; Kannan, K. K.; Bergsten, P. C.; Waara, I.; Fridborg, K.; Strandberg, B.; Carlbom, U.; Jarup, L.; Lovgren, S.; Petef, M. *Nat. New Biol.* **1972**, *235*, 131.
44. Paterson Young, E. H. BE Patent 659,230, 1965.
45. Robson, P.; Smith, T. A.; Stephens, R.; Tatlow, J. C. *J. Chem. Soc.* **1963**, 3692.
46. Zubriene, A.; Matuliene, J.; Baranauskiene, L.; Jachno, J.; Torresan, J.; Michailoviene, V.; Cimmperman, P.; Matulis, D. *Int. J. Mol. Sci.* **2009**, *10*, 2662.
47. Dolbier, W. R. *Guide to Fluorine NMR for Organic Chemists*; Wiley: Hoboken (NJ), 2009.
48. Baranauskiene, L.; Hilvo, M.; Matuliene, J.; Golovenko, D.; Manakova, E.; Dudutiene, V.; Michailoviene, V.; Torresan, J.; Jachno, J.; Parkkila, S.; Maresca, A.; Supuran, C. T.; Grazulis, S.; Matulis, D. *J. Enzyme Inhib. Med. Chem.* **2010**, *25*, 863.
49. Cimmperman, P.; Baranauskiene, L.; Jachimoviciute, S.; Jachno, J.; Torresan, J.; Michailoviene, V.; Matuliene, J.; Sereikaite, J.; Bumelis, V.; Matulis, D. *Biophys. J.* **2008**, *95*, 3222.
50. Sudzius, J.; Baranauskiene, L.; Golovenko, D.; Matuliene, J.; Michailoviene, V.; Torresan, J.; Jachno, J.; Sukackaite, R.; Manakova, E.; Grazulis, S.; Tumkevicius, S.; Matulis, D. *Bioorg. Med. Chem.* **2010**, *18*, 7413.
51. Jogaite, V.; Zubriene, A.; Michailoviene, V.; Gylyte, J.; Morkunaite, V.; Matulis, D. *Bioorg. Med. Chem* **2012**. http://dx.doi.org/10.1016/j.bmc.2012.10.016.
52. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561.
53. Leslie, A. G. W. *Joint CCP4 + ESF-EAMCB Newsletter on Protein Crystallography* **1992**, 26.
54. Leslie, A. G. W. *Acta Crystallogr. D Biol. Crystallogr.* **2006**, *62*, 48.
55. CCP4 (Collaborative Computational Project), *N. Acta Crystallogr. D* **1994**, *50*, 760.
56. Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. *Acta Crystallogr. D Biol. Crystallogr.* **1997**, *53*, 240.
57. Emsley, P.; Cowtan, K. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*, 2126.
58. Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R. *J. Cheminform* **2012**, *4*, 17.
59. Vagin, A. A.; Steiner, R. A.; Lebedev, A. A.; Potterton, L.; McNicholas, S.; Long, F.; Murshudov, G. N. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*, 2184.
60. Kraulis, P. J. *J. Appl. Crystallogr.* **1991**, *24*, 946.
61. Merritt, E. A.; Bacon, D. J. *Methods Enzymol.* **1997**, *277*, 505.
62. Esnouf, R. M. *Acta Crystallogr. D Biol. Crystallogr.* **1999**, *55*, 938.