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Letter

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Carbonic Anhydrase Inhibition with Benzenesulfonamides and Tetrafluorobenzenesulfonamides Obtained via Click Chemistry

Nicolino Pala,[†] Laura Micheletto,[†] Mario Sechi,*[,†] Mayank Aggarwal,[‡] Fabrizio Carta,[§] Robert McKenna,[‡] and Claudiu T. Supuran*[,§,∥]

[†]Dipartimento di Chimica e Farmacia, Università di Sassari, Via Vienna 2, 07100 Sassari, Italy

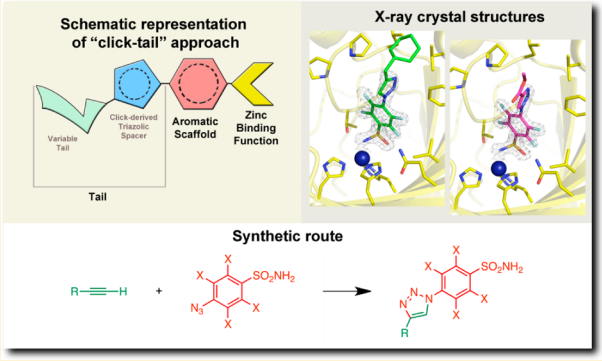
[‡]Department of Biochemistry and Molecular Biology, College of Medicine, University of Florida, Box 100245, Gainesville, Florida 32610, United States

[§]Laboratorio di Chimica Bioinorganica, Università degli Studi di Firenze, Rm 188, Via della Lastruccia 3, 50019 Sesto Fiorentino (Firenze), Italy

[∥]Neurofarba Dept., Università degli Studi di Firenze, Via U. Schiff 6, 50019 Sesto Fiorentino (Firenze), Italy

Supporting Information

ABSTRACT: A series of novel benzene- and 2,3,5,6-tetrafluorobenzenesulfonamide was synthesized by using a click chemistry approach starting from azido-substituted sulfonamides and alkynes, incorporating aryl, alkyl, cycloalkyl, and amino-/hydroxy-/halogenoalkyl moieties. The new compounds were medium potency inhibitors of the cytosolic carbonic anhydrase (CA, EC 4.2.1.1) isoforms I and II and low nanomolar/subnanomolar inhibitors of the tumor-associated hCA IX and XII isoforms. The X-ray crystal structure of two such sulfonamides in adduct with hCA II allowed us to understand the factors governing inhibitory power.



KEYWORDS: *Carbonic anhydrase, click chemistry, human isoform I, human isoform II, human isoform IX, human isoform XII, benzenesulfonamide*

Click chemistry has extensively been used[1−4] to obtain inhibitors of the metallo-enzyme carbonic anhydrase (CA, Ec 4.2.1.1) belonging to the sulfonamide class.[5,6] Recently, thiol-ene click chemistry has been successfully employed to obtain CA inhibitors (CAIs) of the sulfonamide type, which again had excellent inhibitory activity against the tumor-associated isoforms CA IX and XII.[7] Among click techniques, the copper-catalyzed azide−alkyne cycloaddictions (CuAAC) have acquired a prominent role due to their modularity, the short reaction times, and increased yields. By exploiting the high reactivity of aromatic/heterocyclic sulfonamides incorporating azide/alkyne moieties that were reacted with alkynes/azides, a large number of compounds possessing a variety of chemotypes, difficultly available by other procedures, were synthesized (Figure 1). The obtained compounds were assayed as inhibitors of many mammalian CA isoforms of the 16 presently known.[8] For example, sulfonamides incorporating glycosyl moieties (both protected and deprotected at the OH groups of the sugar) of type **C**, **E**, and **F**,[1−4] as well as a heterocyclic or aromatic groups (Chart 1),[9] have been obtained, many of which showed excellent inhibitory activity against physiologically/pathologically relevant isoforms such as CA I, II, IX, and XII.

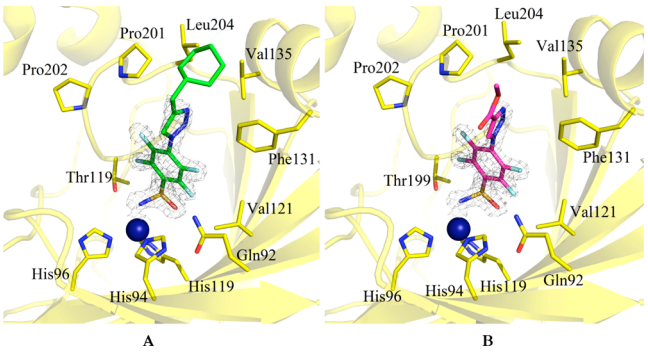


Figure 1. Stick representation showing (A) compound **5c** (green) and (B) compound **5h** (pink) bound in the active site of hCA II. Residues are as labeled. The $|2F_o - F_c|$ electron density is contoured at 1.2σ. Active site Zn is shown as a blue sphere. Figure was made using PyMOL.

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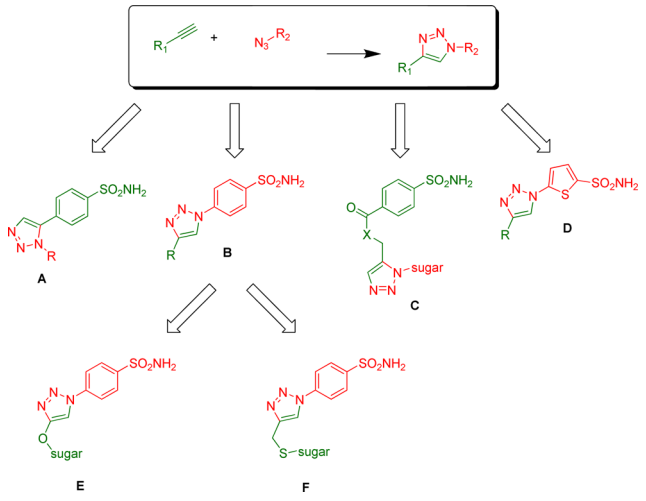
Chart 1. Sulfonamides Obtained via Click Chemistry

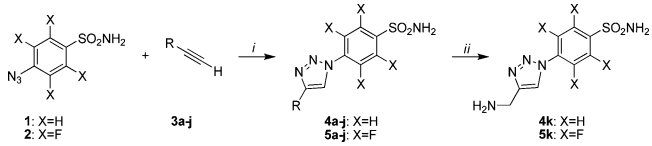


In addition to generating potent CAIs per se, such as compounds **A**–**F** mentioned above, the click chemistry has also been employed for introducing linkers between the sulfonamide fragment of the molecule and toxins such as duocarmycin, as in a recent example of hybrid drug possessing CA inhibitory/toxin fragments for selective tumor targeting.[10] Such CAIs were highly effective in tumor models leading to a synergistic activity compared to the parent sulfonamide or duocarmycin single agents, and produced a sustained and potent inhibition of the tumor growth (in renal cell carcinoma models in mice).

Thus, click chemistry is a powerful tool for generating both chemical diversity as well as novel applications for targeting tumors, based on inhibitors of various CA isoforms with medicinal chemistry applications. However, fluorine has several properties that make it extremely attractive in drug discovery: (a) the small atomic size and the length of the C–F bond make fluorine an unusual substitute of the hydrogen, without affecting significantly on the molecular geometry; (b) the high electronegativity induces substantial changes of the physicochemical properties of the molecules including change in the lipophilicity, decrease of the pK_a, and ability to act as H-bond acceptor. Indeed, fluorine atoms are able to alter, often drastically, the binding mode, the affinity, and the selectivity of the molecule for the respective target. In particular, perfluorination of benzenes dramatically increases the acidity of substituents.

In this letter, we used the click-tailing approach for the synthesis of two homologous series of 4-(*R*-1*H*-1,2,3-triazol-1-yl)-benzenesulfonamides **4** and 2,3,5,6-tetrafluoro-4-(5-*R*-1*H*-1,2,3-triazol-1-il)benzenesulfonamide **5** incorporating a large variety of different moieties. The new compounds were investigated for the inhibition of the physiologically dominant, cytosolic isoforms CA I and II, as well as the transmembrane, tumor-associated ones CA IX and XII. Although both benzenesulfonamides[5,6] and perfluoro benzenesulfonamides[11] were already investigated as CAIs, and their binding to the enzyme has been elucidated using several experimental approaches (for example, many X-ray data for adducts of such compounds with various isoforms are available,[12−17]), this is the first time that two homologous series of compounds acting as CAIs have been compared. The synthesis of the title compounds **4a**–**4j** and **5a**–**5j** was achieved by reacting the azides **1** and **2** [incorporating the sulfamoyl zinc-binding group (ZBG)] with alkynes **3a**–**3j** (Scheme 1) in the presence of nanosized metallic copper as catalyst (Scheme 1). Further

Scheme 1[a]

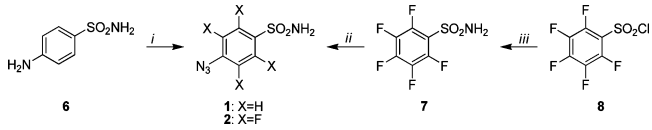


[a]Reagents and conditions: (i) Cu(0) activated nanopowder, TEA, H_2O/*tert*-butanol, r.t., 18 h for **4**, 40 h for **5**; (ii) HCl 4 M in dioxane, room temp, 1.5 h.

derivatives **4k** and **5k** were obtained by *N*-Boc deprotection in acidic conditions from **4j** and **5j**, respectively.

The azidosulfonamide **1** was prepared starting from benzenefulfonamide **6** by routine work. The preparation of the perfluorinated key intermediate **2** started from pentafluorobenzenesulfonyl chloride **8**, which was converted to the corresponding sulfonamide **7** by reaction with concentrated aqueous ammonia. Since the 4-fluoro atom is the most reactive one for nucleophilic substitution reactions, it has been replaced by the azido moiety, as depicted in Scheme 2 (see materials and

Scheme 2[a]



[a]Reagents and conditions: (i) NaN_3, *tert*-butanol, *tert*-butilnitrite, r.t., 56 h; (ii) NaN_3, acetone, H_2O, reflux, 7 h; (iii) 30% aqueous NH_4OH, r.t., 21 h.

methods in Supporting Informations for details). The R moiety present in alkynes **3**, and also in sulfonamides **4** and **5**, was chosen to deeply explore the chemical space. Indeed, the nature of the R moiety present in the new compounds has been planned considering various chemical functionalities that include aromatic, aliphatic, cycloalkyl, and halogeno-, hydroxy- or aminoalkyl moieties (Table 1), in order to achieve a major chemical diversity. It is known from earlier work[11−17] that the nature of the tails present in the sulfonamide CAIs strongly influences their activity since these groups bind in various parts of the active site, making crucial contacts with amino acid residues and water molecules from the cavity, thus influencing the inhibition profile of the compounds in terms of selectivity against various isoforms (see the crystallographic section in Supporting Informations).

Inhibition data with the set of compounds **4a**–**4k** and **5a**–**5k** reported here, against isoforms hCA I, II, IX, and XII, is shown in Table 1. All these isoforms are either drug targets or off-targets for various pharmacologic applications of the CAIs.[5,6,8] The following structure–activity relationship (SAR) can be observed:

(i) Against all four isoforms investigated here, the tetrafluoro-substituted sulfonamides **5** were more effective CAIs compared to the corresponding benzenesulfonamide **4** possessing the same R group at the triazole ring. This is probably due to the fact that the fluorine-substituted sulfonamides are more acidic than the corresponding benzenesulfonamides, which favor

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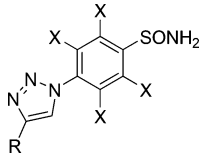
Table 1. Inhibition Data against Isoforms hCA I, II (Cytosolic), IX, and XII (Transmembrane, Tumor-Associated Enzymes) of Sulfonamides 4a−4k and 5a−5k and Acetazolamide AAZ (as Standard) by a Stopped-Flow CO_2 Hydrase Assay



			K_I (nM)[a]			
compd	X	R	hCA I	hCA II	hCA IX	hCA XII
4a	H	*p*-Me−C_6H_4	368	176	22.4	3.6
4b	H	C_6H_5	392	43.9	25.9	6.0
4c	H	cC_6H_{11}	357	50.4	3.0	1.4
4d	H	*p*-C_5H_{11}−C_6H_4	3200	755	5.0	12.4
4e	H	$BrCH_2CH_2$	1250	142	6.9	2.5
4f	H	$Cl(CH_2)_3$	1185	74.8	6.2	2.3
4g	H	$Cl(CH_2)_4$	1350	66.1	5.4	2.0
4h	H	MeOOC	1500	329	127	62.6
4i	H	HO−CH_2	873	235	8.1	3.0
4j	H	BocNH−CH_2	413	32.7	10.5	1.9
4k	H	H_2N−CH_2	765	168	12.1	3.2
5a	F	*p*-Me-C_6H_4	42.1	165	21.3	1.9
5b	F	C_6H_5	41.5	30.1	24.8	5.7
5c	F	cC_6H_{11}	42.5	41.3	1.5	0.8
5d	F	*p*-C_5H_{11}−C_6H_4	1450	648	38.9	10.8
5e	F	$BrCH_2CH_2$	416	98.1	5.3	0.8
5f	F	$Cl(CH_2)_3$	652	56.8	4.7	0.9
5g	F	$Cl(CH_2)_4$	835	46.9	4.5	1.1
5h	F	MeOOC	50.1	450	115	58.9
5i	F	HO−CH_2	258	123	12.8	2.6
5j	F	BocNH−CH_2	44.9	26.9	13.6	3.6
5k	F	H_2N−CH_2	330	142	10.4	2.7
AAZ			250	12	25	5.7

[a]Errors in the range of ±10% of the reported value (from 3 different assays).

deprotonation of the sulfamoyl group (i.e., ZBG) in coordinating the metal ion;

(ii) The cytsolic isoforms hCA I and II were moderately inhibited by most of these sulfonamides, with inhibition constants ranging between 41.5 and 1500 nM against hCA I and ranging between 30.1 and 755 nM against hCA II.

(iii) The tumor associated isoforms were inhibited in the low nanomolar−subnanomolar range by sulfonamides **4** and **5**, with inhibition constants ranging between 1.5 and 38.9 nM against hCA IX and between 0.8 and 12.4 nM against hCA XII. Just two compounds (**4h** and **5h**), possessing the electron withdrawing carboxymethyl group as R moiety, were less active against the two tumor-associated isoforms.

(iv) R groups leading to effective hCA IX/XII inhibitors were all those present in derivatives **4** and **5**, except COOMe, whereas the most effective hCA II inhibitors incorporated cyclohexylmethyl and phenyl moieties. The best hCA I inhibitors contained tosyl, phenyl, cyclohexylmethyl, and COOMe moieties (Table 1).

The crystal structures of human CA II in complex with sulfonamides **5c** and **5h** have been determined (Figure 2) to 1.5 Å resolution.

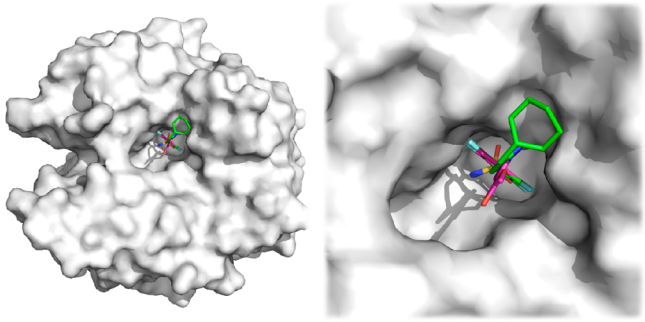


Figure 2. (A) Surface representation of hCA II in complex with **5c** (green) and **5h** (pink) extending out of the active site. Zn is shown as a blue sphere. (B) Zoomed active site details. Figure was made using PyMOL.

The structures were solved using protocols as described by us previously (see Table S1 and experimental details in Supporting Information). The hydrophobic nonplanar compounds were found buried deep into the active site, displacing the catalytic zinc-bound solvent, such that the nitrogen of the sulfonamide group binds directly to the zinc atom of CA II (distance ≈ 2.0 Å). Hence, the overall Zn coordination can be described as a distorted tetrahedron. The O atom of the sulfonamide group lied within hydrogen bonding distance (2.9 Å) from the backbone N atom of Thr199. The tetrafluorophenyl moiety of the inhibitors was stabilized by the surrounding hydrophobic residues (V121, L141, and L198) and also exhibited van der Waals interactions with the side chains of N62, H64, Q92, H94, F131, and P202. These interactions are consistent with those seen with the classical, clinically used sulfonamide CAIs.[11−17] However, as the compounds extend out of the active site (Figure 2A), their tail groups became less ordered, with weaker density seen for the tails in the difference map ($F_o - F_c$) for both compounds. Hence, different orientations were modeled, and the one that best satisfied the observed data was selected as the final refined structure. The nonpolar, puckered cyclohexyl ring in **5c** was found in the hydrophobic pocket lined by residues F131, V135, P202, and L204 (Figure 2A). However, the tail of **5h** was observed to be orientated toward the bulk solvent, not being involved in any hydrophobic or polar interactions with the surface of the protein. Indeed, this compound (**5h**) was 10.9 times less effective as a hCA II inhibitor compared to the cyclohexylmethyl substituted congener **5c** (see Table 1). Compounds **5c** and **5h** bury a total surface area of 394.3 $Å^2$ (75.0% of its total area) and 328.5 $Å^2$ (67.1% of its total area) with the protein interface and have average B-factors of 18.3 and 19.7 $Å^2$, respectively. Both compounds were refined with occupancy of ∼0.70. Some disordered density that could not be assigned was observed around the inhibitor in both the crystal structures. Previous studies have shown a molecule of glycerol bound close to the sulfonamide inhibitors in the active site of CA II.[12,13] In order to avoid the binding of any unwanted ligand in these crystal structures, 20% sucrose instead of the normally used glycerol was used as cryoprotectant prior to freezing the crystals.

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In conclusion, a series of novel benzene- and 2,3,5,6-tetrafluorobenzenesulfonamides, synthesized by using click chemistry approaches, which incorporated aryl, alkyl, cycloalkyl, and amino-/hydroxy-/halogenoalkyl moieties, were synthesized and evaluated as CA inhibitors. The new compounds were medium potency inhibitors of the cytosolic CA isoforms I and II and low nanomolar/subnanomolar inhibitors of the tumor-associated hCA IX and XII isoforms. The X-ray crystal structure of two such sulfonamides in adduct with hCA II allowed us to understand the factors governing inhibitory power.

■ ASSOCIATED CONTENT

Supporting Information

Full characterization of compounds **4a**−**4k** and **5a**−**5k** and crystallographic parameters for the two hCA II adducts (with **5c** and **5h**) are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

PDB accession code for adducts of CA II with **5c** and **5h** are 4DZ7 and 4DZ9, respectively.

■ AUTHOR INFORMATION

Corresponding Authors

*(M.S.) E-mail: mario.sechi@uss.it.

*(C.T.S.) E-mail: claudiu.supuran@unifit.it.

Author Contributions

The manuscript was written through contributions of all authors.

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Notes

The authors declare the following competing financial interest(s): C.T.S. is a coauthor on many patents claiming carbonic anhydrase inhibitors. The compounds from this paper are not patented.

■ ABBREVIATIONS

CA, carbonic anhydrase; AAZ, acetazolamide; NMR, nuclear magnetic resonance; TLC, thin layer chromatography; SAR, structure−activity relationship; ZBG, zinc-binding group

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