Sulfonamide/sulfamate switch with a series of piperazinylureido derivatives: Synthesis, kinetic and in silico evaluation as carbonic anhydrase isoforms I, II, IV, and IX inhibitors

Alessio Nocentini, Davide Moi, Alessandro Deplano, Sameh M. Osman, Zeid A. AlOthman, Gianfranco Balboni, Claudiu T. Supuran, Valentina Onnis

PII: S0223-5234(19)31048-7

DOI: https://doi.org/10.1016/j.ejmech.2019.111896

Reference: EJMECH 111896

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 16 October 2019

Revised Date: 15 November 2019

Accepted Date: 15 November 2019

Please cite this article as: A. Nocentini, D. Moi, A. Deplano, S.M. Osman, Z.A. AlOthman, G. Balboni, C.T. Supuran, V. Onnis, Sulfonamide/sulfamate switch with a series of piperazinylureido derivatives: Synthesis, kinetic and in silico evaluation as carbonic anhydrase isoforms I, II, IV, and IX inhibitors, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.111896.

Sulfonamide/sulfamate switch with a series of piperazinylureido derivatives: synthesis, kinetic and *in silico* evaluation as carbonic anhydrase isoforms I, II, IV, and IX inhibitors

Alessio Nocentini [a,§], Davide Moi [b,§], Alessandro Deplano [c], Sameh M. Osman[d], Zeid A. AlOthman[d] Gianfranco Balboni [b], Claudiu T. Supuran [a,*], Valentina Onnis [b,*]

[a] *Department NEUROFARBA – Pharmaceutical and Nutraceutical Section, University of Firenze, via Ugo Schiff 6, I-50019 Sesto Fiorentino, Firenze, Italy*

[b] *Department of Life and Environmental Sciences, Unit of Pharmaceutical, Pharmacological and Nutraceutical Sciences, University of Cagliari, via Ospedale 72, I-09124 Cagliari, Italy*

[c] *Pharmacelera, Placa Pau Vila, 1, Sector 1, Edificio Palau de Mar, Barcelona 08039, Spain*

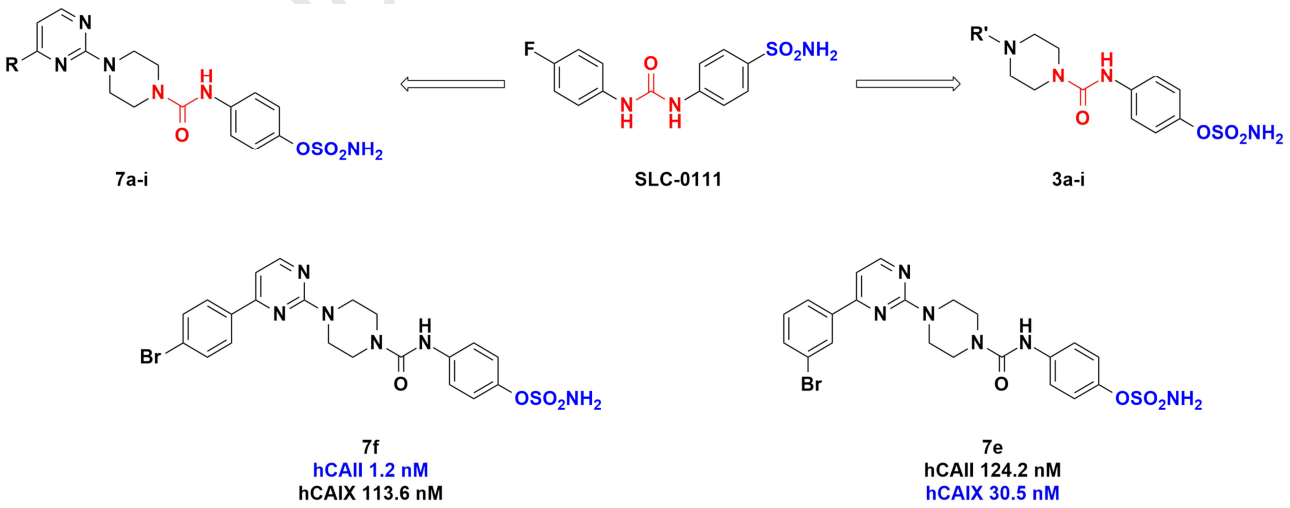
[d] *Chemistry Department, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia.*

Highlights

* Piperazinylureido aryl sulfamates were designed and synthesized.
* Sulfamates were assayed *in vitro* as human Carbonic Anhydrase (CA) isoforms inhibitors.
* Synthesized compounds show weak activity against off-target CA I and CA IV isoforms
* Some sulfamates are CA II/IX selective inhibitors representing leads for the development of new anticancer agents.
* Docking revealed that CA-selective inhibition is related to distinguished interactions.

Keywords: metalloenzyme; inhibitor; bioisoster; antitumor; selectivity.

Graphical abstract



Abstract

We report here a thorough structure-activity relationship (SAR) with piperazinylureido sulfamates as inhibitors of human (h) carbonic anhydrase (CA, EC 4.2.1.1). A SAR investigation over the structure of reported anti-cancer zinc-binder CAIs such as **SLC-0111** and **S4** was carried out by including the urea outer nitrogen atom into a substituted piperazine ring reducing the linker flexibility. The derivatives were assessed for the inhibition of CA I, II and IV (off-target isoforms) and the tumor-associated CA IX (anticancer drug target). CA I and IV were not effectively inhibited, whereas many low nanomolar inhibitors were evidenced against CA II (K_Is in the range of 1.0-705.5 nM), and IX (K_Is in the range of 0.91-155.9 nM). Interestingly, a subset of CA II/IX selective inhibitors was detected which might represent interesting lead for the development of new anticancer strategies.

1. Introduction

Carbonic anhydrases (CAs; EC 4.2.1.1) are a superfamily of metalloenzymes that catalyze the CO_2 hydration/dehydration reaction, and are classified into eight genetically distinct families, named α-, β-, γ-, δ-, ζ-, η-, ɵ- and ι-CAs [1,2]. Among α-class enzymes are all human (h) CAs, that are fifteen different human isoforms identified to date [3]. Human CAs are also sorted into four different subsets depending on their subcellular localization: CA I, II, III, VII, VIII, X, XI, XIIII are cytosolic proteins, CA VA and VB are present in the mitochondrial matrix, CA VI is a secreted enzyme, CA IV is a glycosylphosphatidylinositol (GPI)-anchored protein and CA IX, XII and XIV are trans-membrane isoforms [1-3]. As these enzymes are disseminated in the human body and are implicated in a multitude of crucial physiological processes, serious pathological conditions might occur due to their dysregulated expression and/or abnormal activity [2]. The isoform CA IX is at the forefront of the research over CAs since its validation as marker of disease progression in many aggressive hypoxic tumors. Its targeted inhibition is associated with a significant reduction of the growth of both primary tumors and metastases [4].

CA inhibitors (CAIs) are grouped in different categories according to their inhibition mechanism. The zinc-binders, among which sulfonamides and their bioisosteres sulfamates, are the most effective and utilized for drug-design purposes, but lack of isoform specificity [5]. By applying drug-design methods such as the tail approach, it has been possible to endow derivatives of this category with a significant degree of isoform selectivity to apply for the treatment of the target disease reducing side effect related to promiscuous CAs inhibition. In this context, an ureidobenzenesulfonamide successfully completed Phase I clinical trials for the treatment of advanced, metastatic hypoxic tumors over-expressing hCA IX, and is currently in Phase Ib/II

clinical trials in a multi-center, open-label study of oral **SLC-0111** (Figure 1) in combination with gemcitabine (administered i.v.) in subjects affected by metastatic pancreatic ductal adenocarcinoma [6-8]. By switching the zinc-binding group in the series of ureidobenzenesulfonamide to sulfamate, Gieling et al. described a set of ureidophenyl sulfamates able to inhibit migration and spreading of tumor cells under oxygen-decreased conditions. In addition, the low dose maintenance therapy with one such sulfamates (**S4**, Figure 1) strongly inhibited the development of MDA-MB-231 metastases in lung with no signs of toxicity [9].

We recently reported a series of piperazinylureidosulfonamides (**A**, Figure 1) as analogs of **SLC-0111**, detecting several low nanomolar inhibitors of CA IX depending on the substitution pattern at the piperazine ring [2]. The considerable flexibility of the urea linker was constrained by including the outer nitrogen atom into a substituted piperazine ring to observe the effect on the CA inhibitory potency.

Therefore, to investigate the impact on CA inhibition of a sulfonamide/sulfamate switch, such as that producing **S4** from **SLC-0111**, a new series ureidopiperazinylsulfamates (B, Figure 1) was designed [11]. Based on promising preliminary CA inhibition outcomes with these derivatives (**B**) [11], we extend here the series to provide thorough SAR for this series of piperazinylureidosulfamates (**3a-l** and **7a-i**).

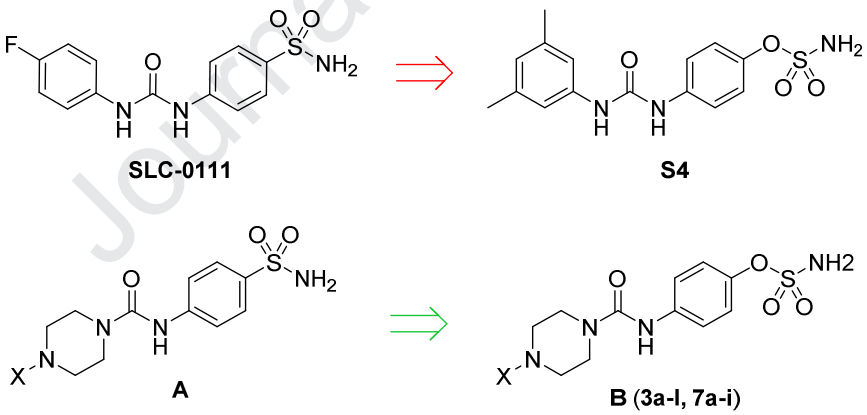
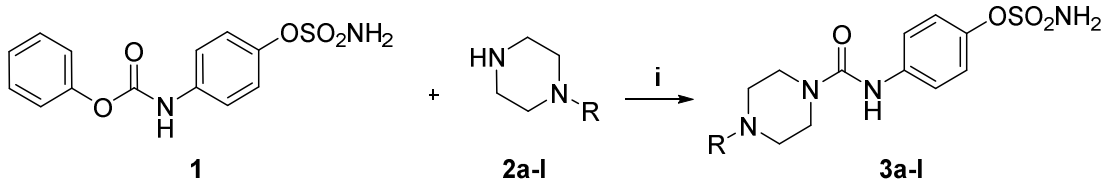


Figure 1. Drug design of ureidobenzenesulfamates **3a-l**, **7a-i** from sulfonamide of series **A** based on **S4** development from **SLC-0111**.

2. Results and discussion

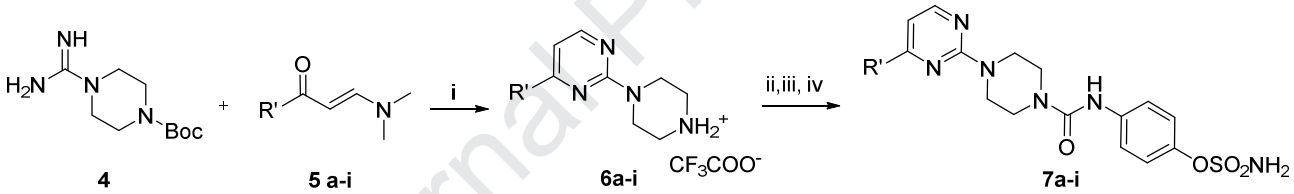
2.1. Chemistry

The synthesis of the new compounds is reported in Schemes 1 and 2. Coupling of 1-substituted piperazines **2a–l** with 4-(phenoxycarbonyl)aminophenylsulfamate **1** in anhydrous DMSO gave the piperazinylurea derivatives **3a–l** in 38-98% yields (Scheme 1).



Scheme 1. General synthetic procedure for sulfamates subsets **3**. Reagents and conditions: (i) anhydrous DMSO, r.t., 24 h.

Enaminones are versatile synthons to yield a variety of heterocycles, and their preparation has been recognized for some time. The convenient synthesis of β-dimethylaminovinyl ketones from the reaction of aryl ketones and formamide acetal has already been described [12]. Accordingly, enaminones **5a-i** were prepared from the appropriate acetophenone and *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA). The pyrimidinyl-piperazines **6a–i** were obtained by heterocyclization of 4-Boc-piperazine-1-carboxamidine (**4**) with enaminones **5a-i** in boiling ethanol in the presence of sodium methoxide, followed by a trifluoroacetic acid (TFA)-mediated deprotection in dichloromethane (DCM) solution (Scheme 2). Then piperazinyl urea derivatives **7a-i** we prepared using the same reaction method described for sulfamates **3**.



Scheme 2. General synthetic procedure for sulfamates of subset **7**. Reagents and conditions: (i) DMF-DMA, toluene, 1h, reflux then r.t., 24 h; (ii) 30% MeONa in MeOH, EtOH, 8 h, reflux; (iii) CF_3COOH, CH_2Cl_2, r.t. 12 h; iv) compound **1**, anhydrous DMSO, r.t., 24 h.

2.2. Carbonic anhydrase inhibition

Derivatives **3a-l** and **7a-i** were assayed for their inhibition of the target CA IX and off-target isoforms such as CA I, II and IV by a stopped flow CO_2 hydrase assay [13] (details are showed in supporting information). Acetazolamide (**AAZ**) was as standard inhibitor. The inhibition profiles were assembled to those previously reported and are shown in Table 1. The following structure–activity relationships (SAR) were worked out: the 4-fluorophenyl substituted sulfamate **3e** was a potent CA I inhibitor, with an inhibition constant of 9.4 nM, whereas the replacement of the fluorine with a chlorine atom led to a reduction in activity (**3f** and **3g**). Likewise, the unsubstituted compound **3a** and methyl substituted compounds **3b** and **3c** showed weak efficacy against CA I with inhibition constants (K_Is) ranging between 692 and 897 nM.

Table 1. Inhibition data of human CA isoforms I, II, IV and IX with sulfamates **3a-l** and **7a-i** and the standard sulfonamide inhibitor acetazolamide (**AAZ**) by a stopped flow CO_2 hydrase assay.

		K_I (nM)[a]			
Compound	**R/R'**	CA I	CA II	CA IV	CA IX
3a	C_6H_5	896.8[b]	71.9[b]	-	11.1[b]
3b	2-CH_3-C_6H_4	692.0	56.9	759.3	29.7
3c	3-CH_3-C_6H_4	851.5[b]	15.9[b]	-	0.91[b]
3d	2,3-$(CH_3)_2$-C_6H_3	63.5[b]	11.1[b]	1087.1	32.3[b]
3e	4-F-C_6H_4	9.4[b]	18.2[b]	890.3	61.5[b]
3f	4-Cl-C_6H_4	581.3[b]	16.9[b]	2592.2	10.4[b]
3g	3,4-Cl_2-C_6H_3	918.8	45.4	1641.6	90.3
3h	3-OCH_3-C_6H_4	88.1[b]	11.2[b]	994.3	34.1[b]
3i	4-OCH_3-C_6H_4	282.4[b]	9.1[b]	1625.6	114.1[b]
3j	2-methylbenzofuryl	760.3	36.2	422.3	32.7
3k	*n*-octyl	752.3	15.9	4797.5	31.4
3l	*n*-decyl	680.1	21.0	4189.5	32.2
7a	C_6H_5	2370.9	705.5	4097.6	155.9
7b	3-CH_3-C_6H_4	4069.9	206.9	1317.3	93.3
7c	3-CF_3-C_6H_4	3472.9	58.7	457.7	27.1
7d	4-CF_3-C_6H_4	290.9[b]	1.1[b]	-	24.3[b]
7e	3-Br-C_6H_4	7769.1	124.2	831.3	30.5
7f	4-Br-C_6H_4	277.2[b]	1.2[b]	-	113.6[b]
7g	4-OCH_3-C_6H_4	3930.4	29.1	384.1	31.8
7h	3,4,5-$(OCH_3)_3$-C_6H_2	5284.0	6.8	279.8	24.2
7i	4-benzofuran-2-yl	70.2[b]	1.0[b]	-	6.7[b]
AAZ	-	250	12	74	25

[a]Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10 % of the reported values). [b]values taken from [11]

On the contrary, the presence of a 2,3-dimethylphenyl group (compound **3d**) or 3-methoxyphenyl group (compound **3h**) restored the activity. The displacement of the methoxy group in 4-position produced about a third reduction in activity (**3i**, K_I of 282 nM). The replacement of the aryl ring with an heteroaryl as in compound **3j** or with alkyl chains as in compound **3k** and **3l** also afforded weak inhibitors of this slow cytosolic CA isoform. In sulfamates of subset **7**, the presence of the pyrimidine ring generally afforded poor CA I inhibitors except for compound **7i** and in part for the 4-trifluoromethyl and 4-bromine substituted **7d** and **7f**, displaying about the same potency of **AAZ**. Interestingly, the shift of these groups in 3-position to give the analogs **7c** and **7e** led drop in activity against CA I isoform maintaining at the same time high potency on tumor expressed CA IX isoform. As CAI is basically an off- target isoform, its weak inhibition with sulfamates **7c** and **7e**

reported here might be considered of interest for the development of anticancers based on CA inhibition.

CA II is the physiologically dominant isoform, being ubiquitous and involved in a variety of physiologic functions [2]. CA II is considered target for antiglaucoma drugs, but off-target when the tumor-associated isoform CA IX should be inhibited [1]. The sulfamates of series **3** and **7** behaved as weak to potent CA II inhibitors depending by the nature and position of substituents on the piperazine N-4 atom. In the sulfamate subseries **3**, the unsubstituted compound **3a** showed both the worse K_I against CA II and an approximately 7-fold II/IX selectivity. The 3-methylphenyl compound **3c** showed a very potency to **AAZ** against CA II and a 16-fold II/IX selectivity. The shift of the methyl in the 2-position to give the analog **3b** produced about a 4-fold reduction of activity against CA II as compared to compound **3c**, while the selectivity toward CAIX was worsened. The alkyl substituted compound **3l** and 4-chlorine derivative **3f** showed about the same K_Is values against CA II and CA IX (21.2 and 32.2 nM; 16.9 and 10.4 nM, respectively). The introduction of a second chlorine atom as in compound **3g** reduced the activity against both isoforms (45.4 and 90.3 nM, respectively). In the sulfamate subset **7**, the 4-trifluoromethyl and 4-bromine substituted compounds **7d** and **7f** showed activity against CA II about 10-fold greater than **AAZ** and an approximately 20-fold and 100-fold selectivity for CA IX over CA II. The shift of the bromine from 4- to 3-position to afford compound **7e** led to about a 100-fold reduction of CA II inhibition and a 4-fold increase of action against CAIX. Likewise, the 3-trifluoromethylated compound **7c** showed about a 60-fold reduced CAII inhibition as compared with its analog **7d**, while the two compounds showed very similar K_Is against CA IX. The presence on the aryl ring of three methoxy groups (sulfamate **7h**, K_I of 6.8 nM) led to about a 4-fold increase in activity against CA II as compared with the 4-methoxy analog **7g**. On the contrary, both compounds **7g** and **7h** showed about the same K_Is against the CA IX (31.8 and 24.2 nM).

All assayed sulfamates of series **3** and **7** showed low inhibitory activity against the membrane-bound CA IV in comparison to CA II and IX. This low CA IV inhibitory activity might be related to the position of the enzyme α-helix including residues 126-136. In many CAs, this is located in the direction of the binding site stabilizing the binding orientations of substituents at N-4 of piperazine ring. Conversely, in CA IV the helix is replaced by a loop that extends away from the active site preventing the binding stabilization (Figure 1S).

2.3. Molecular Docking

Selected compounds were docked in the isoforms CA II and CA IX to explore the interactions behind the CA isoform selectivity showed by some compounds. In this regard, four compounds of

series **7** were selected to evaluate the effect on activity of substituents on 3- and 4-position of the phenyl ring. Compounds that clearly show a modification on selectivity related to the shift of the substituent from 3- to 4-position are the trifluoromethylphenyl- (**7c**, **7d**) and bromophenyl substituted (**7e**, **7f**). These four compounds were docked in CA II (pdb 4g0c) and CA IX (pdb 3iai), and the obtained poses were analyzed to understand the reason behind this behavior. All compounds are characterized by the same phenylsulfamate moiety that establishes comparable sets of interactions in both isoforms. The sulfamate moiety fits deeply into the active site where the negative charged nitrogen coordinates the zinc. The hydrogen atom bonded to the sulfamate nitrogen forms a H-bond with the hydroxyl group of Thr199, whereas the amidic hydrogen atom of the residue was H-bonded to the S=O of the ligand. An important difference highlighted by the docking was the orientation of the urea oxygen atom (Figure 2).

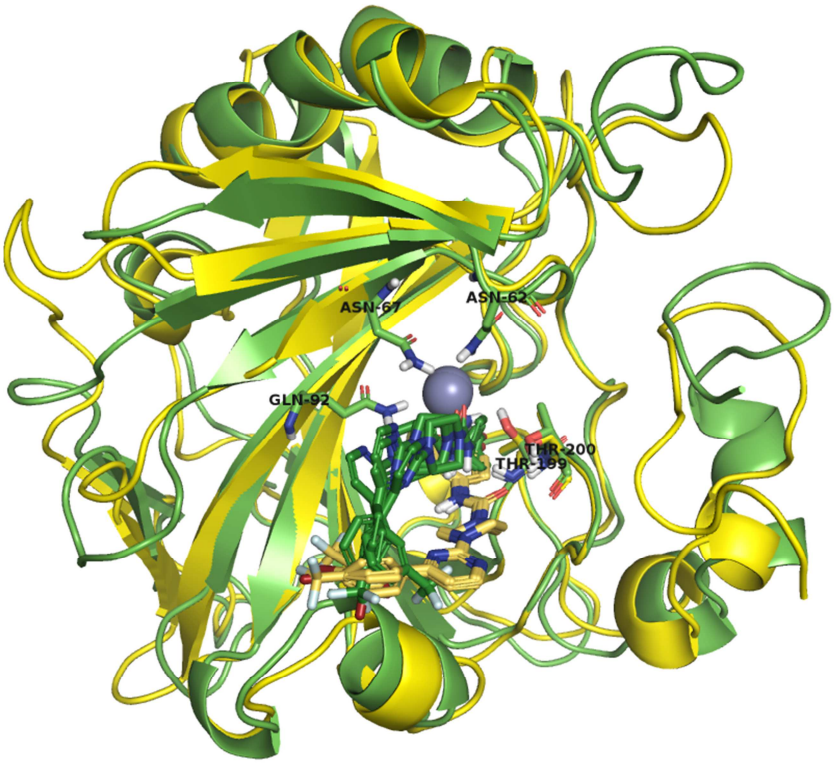


Figure 2. Focus on the different orientation of the best poses of compounds **7c**, **7d**, **7e** and **7f** on hCAII (green, PDBID: 4g0c) and hCAIX (yellow, PDBID: 3iai).

In CA II, the oxygen atom points towards Asn62, Asn67 and Gln92 establishing at least one H-bond with this orientation allowing at the same time the generation of another H-bond between the Thr200 and the oxygen Ph-O-S. Conversely, in CA IX the carbonyl is 90° degrees rotated with the oxygen pointing in the direction of Thr200 establishing a long-range H-bond (3.6 - 3.8 Å).

For what concerns selectivity and influence for the activity of the position of the substituent on the phenyl ring, the main differences are on the 4-arylpyrimidine portion of the molecules. The docking experiment with CA II highlights differences on the stability of poses; in detail the 4-substituted phenyl compounds **7d** and **7f** showed lower RMSD between the best scored pose and all the others, 0.472±0.33 Å and 0.825±0.57 Å respectively, compared to the 3-substitutend analogs **7c** and **7e** (1.595±0.75 Å and 1.52±1.11 Å, respectively). Analyzing the docking poses, two main aspects can explain the stability of the 4-substitued compounds. First, the presence in 4-position of the phenyl ring of halogens allow the establishing of H-bonds and/or van de Waals interaction that stabilize the tail of the inhibitors (Figure 3A). These kinds of interaction do not appear optimal for the 3-phenyl substituted analogs. The docking poses of these compounds showed two possible tail positions, one that generates steric clash and the second that lets the tail of the compounds more exposed to the solvent than the 4-substituted analogs (Figure 3B).

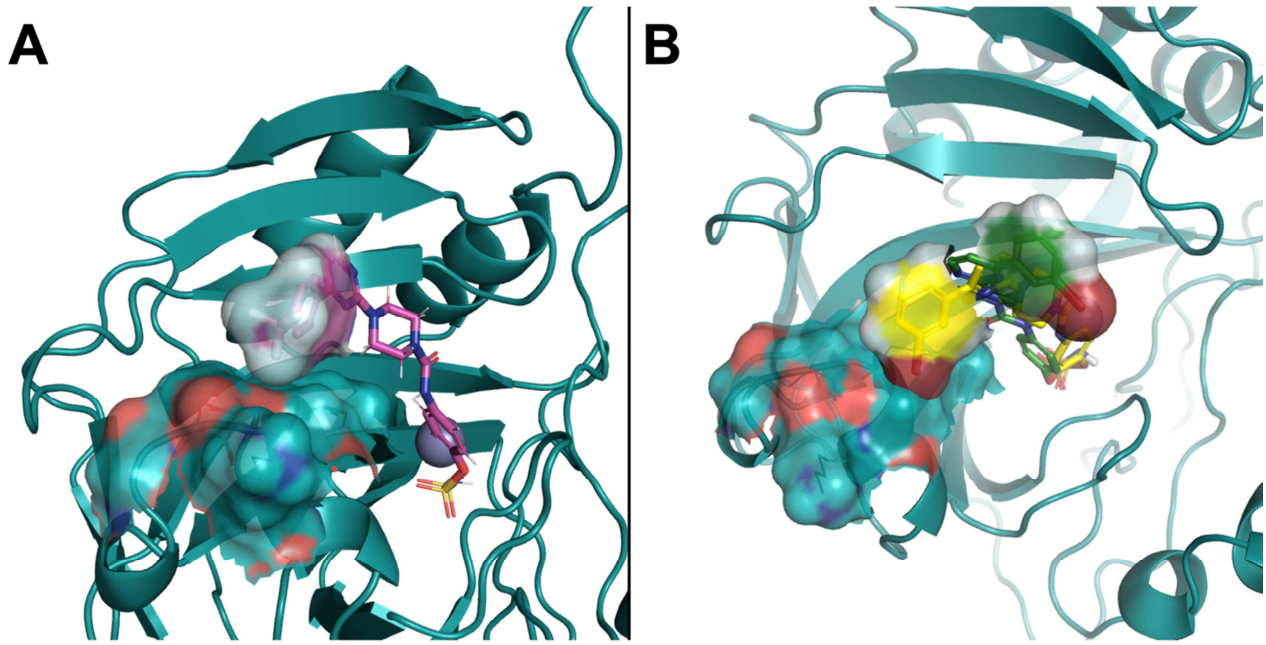


Figure 3. A) Best scored pose for compound **7d** on hCAII (PDBID: 4g0c); **B)** Two proposed poses of compound **7e** on hCAII (PDBID: 4g0c); in yellow one generating steric clash, in green one with high solvent exposure.

This could explain the possible reason why compounds **7d** and **7f** are better CA II inhibitor respect to their 3-substituted analogs **7c** and **7e**. About the docking results with CA IX the variability of the poses RMSD obtained with the CA II disappeared, all compound showed similar RMSD values (0.67±1.2 Å, 0.69±0.62 Å, 0.41±0.25 Å, 0.52±0.31 Å respectively for **7c**, **7d**, **7e** and **7f**) confirming the flat CA IX inhibitory activity showed by all compounds except **7f**. The poses of all four

compounds present similar binding mode. H-Bond between the urea oxygen atom and Thr200 pushes the molecules on the lower portion of the binding site and the presence of smaller residue on position 131 (a Val instead of a Phe) allows 3-phenylsubstituted compounds **7c** and **7e** to assume a similar position to 4-phenylsubstituted analogs **7d** and **7f**. The minor activity of compound **7f** could be assumed due to bromine atom steric effects.

Conclusions

We report here an extension of a series of piperazinylureido sulfamates as inhibitors of hCA I, II, IV and IX providing a thorough SAR investigation over the structure of **SLC-0111** and **S4**. The urea outer nitrogen atom was included into a substituted piperazine ring to explore the effect of a reduction of the linker flexibility on the CAs inhibition. CA I and IV were not effectively inhibited, whereas many low nanomolar inhibitors were evidenced against CA II (K_Is in the range of 1.0-705.5 nM), and IX (K_Is in the range of 0.91-155.9 nM). A subset of CA II/IX selective inhibitors were detected which might represent interesting lead for the development of new anticancer strategies.

4. Experimental section

4.1. Chemistry

All commercially available solvents and reagents were used without further purification. NMR spectra were recorded on an Inova 500 spectrometer (Varian, Palo Alto, CA, USA). The chemical shifts (δ) are reported in part per million downfield from tetramethylsilane (TMS), which was used as internal standard, and the

spectra were recorded in hexadeuteriodimethylsulphoxide (DMSO-d_6). Infrared spectra were recorded on a Vector 22 spectrometer (Bruker, Bremen, Germany) in Nujol mulls. The main bands are given in $cm^{-}1$. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing MAT 95 instrument (Finnigan, Waltham, MA, USA) with BE geometry. Melting points (mp) were determined with a SMP1 Melting Point apparatus (Stuart Scientific, Stone, UK) and are uncorrected. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. The purity of the tested compounds was determined by combustion elemental analyses conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara with a MT-5 CHN recorder elemental analyzer (Yanagimoto, Kyoto, Japan) and the values found were within 0.4% of theoretical values. 4-(Phenoxycarbonyl)aminophenylsulfamate (**1**) [11] and 4-Boc-piperazine-1-carboxamidine (**4**) [14], sulfamates **3a**, **3c-f**, **3h**, **3i**, **7d**, **7f**, and **7i** [11], **3k** and **3l** [15] were synthesized as previously described.

*4.1.1. General procedure for the synthesis of 4-(piperazinocarbonyl)aminosulfamates (**3a-l**)*

A mixture of 4-(phenoxycarbonyl)aminophenylsulfamate **1** (0,31 g, 1 mmol) and substituted 1-substituted piperazine **2a-l** (1 mmol), in anhydrous DMSO (3 mL) was stirred at room temperature for 24 h. Then, water (10 mL) was added and the mixture was stirred at room temperature until a solid is formed. The formed solid was filtered off, washed with water, air dried and recrystallized from EtOH to give ureas **3a-l**.

*4.1.1.1. 4-(4-(2-Methylphenyl)piperazinocarbonyl)aminophenylsulfamate (**3b**).* Following the general procedure, the title compound was obtained from **2b** in 72% yield, mp 170-171°C. ESIMS (m/z): 391 $(M+H)^+$. ^{1}H NMR: δ 2.29 (s, 3H, CH_3), 2.85 (s, 4H, CH_2), 3.60 (s, 4H, CH_2), 6.98 (d, *J* = 7.0 Hz, 1H, Ar), 7.04 (d, *J* = 8.5 Hz, 1H, Ar), 7.15 (d, *J* = 8.0 Hz, 2H, Ar), 7.18 (d, *J* = 7.0 Hz, 2H, Ar), 7.52 (d, *J* = 8.0 Hz, 2H, Ar), 7.86 (s, 2H, NH_2), 8.70 (s, 1H, NH); IR (Nujol) 3408 (NH), 3329 (NH), 3096 (NH), 1647 (C=O), 1607 (C=C) cm^{-1}. Anal. Calcd for $C_{18}H_{22}N_4O_4S$: C, 55.37; H, 5.68; N,14.35. Found: C, 55.31; H, 5.66; N, 14.29.

*4.1.1.2. 4-(4-(3,4-Dichlorophenyl)piperazinocarbonyl)aminophenylsulfamate (**3g**).* Following the general procedure, the title compound was obtained from **2g** in 98% yield, mp 152-154°C. ESIMS (m/z): 447, 445 $(M+H)^+$. ^{1}H NMR: δ 2.63 (m, 2H, CH_2), 2.74 (m, 2H, CH_2), 3.23 (m, 2H, CH_2), 3.58 (m, 2H, CH_2), 6.97 (s, 1H, Ar), 7.14 (d, *J* = 8.5 Hz, 2H, Ar), 7.20 (m, 2H, Ar), 7.52 (d, *J* = 8.5 Hz, 2H, Ar), 7.87 (s, 2H, NH_2), 8.72 (s, 1H, NH); IR (Nujol) 3390 (NH), 3341 (NH), 3126 (NH), 3032 (NH), 1718 (C=O), 1649 (C=O) cm^{-1}. Anal. Calcd for $C_{17}H_{18}Cl_2N_4O_4S$: C, 45.85; H, 4.07; N,12.58. Found: C, 45.91; H, 4.06; N, 12.63.

*4.1.1.3. 4-(4-(Benzofuran-2-ylmethyl)piperazine-1-carboxamido)phenyl sulfamate (**3j**).* Following the general procedure, the title compound was obtained from **2j** in 40% yield, mp 152-154°C. ESIMS (m/z): 431 $(M+H)^+$. ^{1}H NMR: δ 2.54 (s, 2H, CH_2), 3.30 (m, 4H, CH_2), 3.51 (m, 4H, CH_2), 6.81 (s, 1H, Ar), 7.13 (d, *J* = 9.0 Hz, 1H, Ar), 7.16 (d, *J* = 9.0 Hz, 1H, Ar), 7.26 (d, *J* = 9.5 Hz, 1H, Ar), 7.43 (d, *J* = 7.0 Hz, 1H, Ar), 7.46 (d, *J* = 7.0 Hz, 1H, Ar), 7.49 (d, *J* = 9.5 Hz, 1H, Ar), 7.67 (m, 2H, Ar), 7.88 (s, 2H, NH_2), 8.61 (s, 1H, NH); IR (Nujol) 3386 (NH), 1724 (C=O), 1646 (C=O), 1607 (C=C) cm^{-1}. Anal. Calcd for $C_{20}H_{22}N_4O_5S$: C, 55.80; H, 5.15; N,13.02. Found: C, 55.74; H, 5.17; N, 13.07.

*4.1.2. General procedure for the synthesis of arylpropenones (**5a-i**)*

A mixture of 1-arylethanone (5 mmol) and DMF-DMA (1.79 g, 15 mmol) in anhydrous toluene (10 mL) was refluxed for 1 h, then was allowed to reach the room temperature and stirred for additional 24 h. The mixture was carefully concentrated in vacuum to give the title compounds.

*4.1.2.1. (E)-3-(Dimethylamino)-1-(phenyl)prop-2-en-1-one (**5a**)*

Following the general procedure, the title compound was obtained from 1-phenylethanone in 87 % yield. mp 90-91 °C (n-hexane), lit. [16] 89-90 °C. ESIMS (m/z): 176 $(M+H)^+$. ^{1}H NMR: δ 2.88 (s, 3H, CH_3), 2.92 (s, 3H, CH_3), 5.77 (d, *J* = 12.5 Hz, 1H, CH), 7.37-7.74 (m, 5H, Ar), 7.80 (d, *J* = 12.5 Hz, 1H, CH); IR (Nujol) 3350, 1643 cm^{-1}. Anal. Calcd for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N,7.99. Found: C, 75.34; H, 7.51; N, 7.95.

*4.1.2.2. (E)-3-(Dimethylamino)-1-(m-tolyl)prop-2-en-1-one (**5b**)*

Following the general procedure, the title compound was obtained from 1-(m-tolyl)ethanone in 92 % yield. Oil. ESIMS (m/z): 135 $(M+H)^+$. ^{1}H NMR: δ 2.36 (s, 3H, CH_3), 2.90 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 5.79 (d, *J* = 12.5 Hz, 1H, CH), 7.29 (m, 2H, Ar), 7.67 (m, 3H, Ar); IR (Nujol) 3348, 1642 cm^{-1}. Anal. Calcd for $C_{12}H_{15}NO$: C, 76.16; H, 7.99; N,7.40. Found: C, 76.09; H, 8.03; N, 7.44.

*4.1.2.3. (E)-3-(Dimethylamino)-1-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (**5c**)*

Following the general procedure, the title compound was obtained from 1-(3-(trifluoromethyl)phenyl)propan-1-one in 80 % yield. mp 44-46 °C Lit. [16] ESIMS (m/z): 244 $(M+H)^+$. ^{1}H NMR: δ 2.92 (s, 3H, CH_3), 3.14 (s, 3H CH_3), 5.88 (d, J = 12.5 Hz, 1H, CH), 7.43 (d, J = 7.0 Hz, 1H, Ar), 7.70 (d, *J* = 8.0 Hz, 1H, Ar), 7.80 (d, *J* = 12.5 Hz, 1H, CH), 7.88 (d, *J* = 7.0 Hz, 1H, Ar), 8.04 (s, 1H, Ar); IR (Nujol) 1643, 1594 cm^{-1}. Anal. Calcd for $C_{12}H_{12}F_3NO$: C, 59.26; H, 4.97; N, 5.76. Found: C, 59.32; H, 4.79; N, 5.74.

*4.1.2.4. (E)-1-(3-Bromophenyl)-3-(dimethylamino)prop-2-en-1-one (**5e**)*

Following the general procedure, the title compound was obtained from 1-(3-bromophenyl)ethanone in 90% yield. mp 53-54°C. ESIMS (m/z): 255 $(M+H)^+$. ^{1}H NMR: δ 2.94 (s, 3H, CH_3), 3.16 (s, 3H, CH_3), 5.82 (d, *J* = 12.0 Hz, 1H, CH), 7.40 (d, *J* = 7.5 Hz, 1H, Ar), 7.67 (d, *J* = 8.0 Hz, 1H, Ar), 7.72 (d, *J* = 12.0 Hz, 1H, CH), 7.90 (d, *J* = 7.5 Hz, 1H, Ar), 8.02 (s, 1H, Ar); IR (Nujol) 1641, 1573 cm^{-1}. Anal. Calcd for $C_{11}H_{12}BrNO$: C, 51.99; H, 4.76; N,5.51. Found: C, 52.05; H, 4.77; N, 5.48.

*4.1.2.5. (E)-3-(Dimethylamino)-1-(4-methoxyphenyl)-prop-2-en-1-one (**5g**)*

Following the general procedure, the title compound was obtained from 1-(4-methoxyphenyl)ethanone in 87% yield. mp 113-115°C. Lit. 89-91 °C [17] ESIMS (m/z): 206 $(M+H)^+$. ^{1}H NMR: δ 2.93 (s, 3H, CH_3), 3.15 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 5.83 (d, *J* = 12.0 Hz, 1H, CH), 7.20 (d, *J* = 8.0 Hz, 2H, Ar), 7.68 (d, *J* = 12.0 Hz, 1H, CH), 8.12 (d, *J* = 8.12 Hz, 2H, Ar); IR (Nujol) 3330, 1642, 1601, 1581 cm^{-1}. Anal. Calcd for $C_{11}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.15; H, 7.40; N, 6.80.

*4.1.2.6. (E)-3-(Dimethylamino)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**5h**)*

Following the general procedure, the title compound was obtained from 1-(3,4,5 trimethoxyphenyl)ethanone in 76 % yield. mp 123-125 °C, Lit. 125-127 °C [18] ESIMS (m/z): 206 $(M+H)^+$. ^1H NMR: δ 2.94 (s, 3H, CH_3), 3.15 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 3.83 (s, 6H, CH_3), 5.81 (d, *J* = 12.0 Hz, 1H, CH), 7.18 (s, 2H, Ar), 7.70 (d, *J* = 12.0 Hz, 1H, CH); IR (Nujol) 1638, 1548 cm^{-1}. Anal. Calcd for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.43; H, 7.20; N, 5.31.

*4.1.3. General procedure for the synthesis of 1-(4-arylpyrimidin-2-yl)piperazines (**6a-i**)*

A solution of 1-(aryl)-3-(dimethylamino)prop-2-en-1-one (**5a-i**) (2 mmol), 4-(tert-butoxycarbonyl)piperazine-1-carboxamidine (**4**) (1.02 g, 2.2 mmol) and sodium methylate 30% MeOH solution (0.8 ml, 4 mmol) in anhydrous EtOH (5 mL) was refluxed 8 h. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was treated with ethyl acetate (20 mL) and washed with water (3 x 10 mL) and brine (10 mL). After drying over sodium sulphate the solvent was removed under reduced pressure. Then the residue was solubilised in anhydrous dichloromethane (10 mL) and trifluoroacetic acid (5 mL) was added. The mixture was stirred at room temperature overnight and after evaporation of the solvent, the residue was treated with isopropyl ether to obtain a solid, that was filtered off and dried. The formed solid was used in the next step without further purification.

*4.1.3.1. 4-Phenyl-2-(piperazin-1-yl)pyrimidine trifluoroacetate (**6a**)*

Following the general procedure, the title compound was obtained from **5a** in 42% yield, mp 119-120 °C. ESIMS (m/z): 241 $(M+H)^+$. ^1H NMR: δ 3.22 (s, 4H, CH_2), 4.04 (s, 4H, CH_2), 7.31 (d, *J* = 5.0 Hz, 2H, Ar), 7.53 (m, 2H, Ar), 8.15 (d, *J* = 5.0 Hz, 2H, Ar), 8.49 (d, *J* = 5.0 Hz, 1H, Ar), 8.93 (s, 1H, NH); IR (Nujol) 3367, 1687 cm^{-1}. Anal. Calcd for $C_{16}H_{17}F_3N_4O_2$: C, 54.24; H, 4.84; N, 15.81. Found: C, 54.18; H, 4.85; N, 15.84.

*4.1.3.2. 2-(Piperazin-1-yl)-4-(m-tolyl)pyrimidine trifluoroacetate (**6b**)*

Following the general procedure, the title compound was obtained from **5b** in 58% yield, mp 113-114 °C. ESIMS (m/z): 255 $(M+H)^+$. ^1H NMR: δ 2.09 (s, 1H, NH), 2.41 (s, 3H, CH_3), 3.23 (s, 4H, CH_2), 4.05 (s, 4H, CH_2), 7.31 (d, *J* = 5.0 Hz, 1H, Ar), 7.35 (d, *J* = 7.0 Hz, 1H, Ar), 7.40 (d, *J* = 7.5 Hz, 1H, Ar), 7.95 (d, *J* = 7.5 Hz, 2H, Ar), 8.49 (d, *J* = 5.0 Hz, 1H, Ar), 9.16 (s, 1H, OH); IR (Nujol) 2502,1673 cm^{-1}. Anal. Calcd for $C_{17}H_{19}F_3N_4O_2$: C, 55.43; H, 5.20; N, 15.21. Found: C, 55.49; H, 5.22; N, 15.17.

*4.1.3.3. 2-(Piperazin-1-yl)-4-(3-(trifluoromethyl)phenyl)pyrimidine trifluoroacetate (**6c**)*

Following the general procedure, the title compound was obtained from **5c** in 60% yield, mp 105-106 °C. ESIMS (m/z): 309 $(M+H)^+$. ^1H NMR: δ 2.08 (s, 1H, NH), 3.24 (s, 4H, CH_2), 4.06 (s, 4H,

CH_2), 7.46 (d, *J* = 5.0 Hz, 1H, Ar), 7.77 (d, *J* = 7.5 Hz, 1H, Ar), 7.91 (d, *J* = 7.5 Hz, 1H, Ar), 8.86 (d, *J* = 8.0 Hz, 2H, Ar), 8.57 (d, *J* = 5.0 Hz, 1H, Ar), 9.03 (s, 1H, OH); IR (Nujol) 3371, 2514, 1668, 1589 cm^{-1}. Anal. Calcd for $C_{17}H_{16}F_6N_4O_2$: C, 48.35; H, 3.82; N, 13.27. Found: C, 48.39; H, 3.80; N, 13.24.

*4.1.3.4. 4-(3-Bromophenyl)-2-(piperazin-1-yl)pyrimidine trifluoroacetate (**6e**)*

Following the general procedure, the title compound was obtained from **5e** in 40% yield, mp 108-109 °C. ESIMS (m/z): 321, 319 $(M+H)^+$. ^1H NMR: δ 2.08 (s, 1H, NH), 3.23 (s, 4H, CH_2), 4.05 (s, 4H, CH_2), 7.38 (d, *J* = 5.0 Hz, 1H, Ar), 7.49 (d, *J* = 7.0 Hz, 1H, Ar), 7.74 (d, *J* = 7.0 Hz, 1H, Ar), 8.16 (d, *J* = 6.5 Hz, 1H, Ar), 8.33 (m, 1H, Ar), 8.54 (d, *J* = 5.0 Hz, 1H, Ar), 9.15 (s, 1H, OH); IR (Nujol) 3368, 2510, 1671 cm^{-1}. Anal. Calcd for $C_{16}H_{16}BrF_3N_4O_2$: C, 44.36; H, 3.72; N, 12.93. Found: C, 44.30; H, 3.74; N, 12.97.

*4.1.3.5. 2-(Piperazin-1-yl)-4-(4-methoxyphenyl)pyrimidine trifluoroacetate (**6g**)*

Following the general procedure, the title compound was obtained from **5g** in 38% yield, mp > 240 °C. ESIMS (m/z): 385 $(M+H)^+$. ^1H NMR: δ 3.68 (s, 4H, CH_2), 3.83 (s, 4H, CH_2), 4.02 (s, 3H, OCH_3), 7.06 (d, *J* = 7.0 Hz, 2H, Ar), 7.38 (d, *J* = 5.0 Hz, 1H, Ar), 7.65 (d, J = 7.0 Hz, 2H, Ar), 8.43 (d, J = 5.0 Hz, 1H, Ar); IR (Nujol) 3320, 1662 cm^{-1}. Anal. Calcd for $C_{17}H_{19}F_3N_4O_3$: C, 53.12; H, 4.98; N, 14.58. Found: C, 53.18; H, 4.97; N, 14.55.

*4.1.3.6. 2-(Piperazin-1-yl)-4-(3,4,5-trimethoxyphenyl)pyrimidine trifluoroacetate (**6h**)*

Following the general procedure, the title compound was obtained from **5h** in 36% yield, mp > 240 °C. ESIMS (m/z): 331 $(M+H)^+$. ^1H NMR: δ 3.73 (s, 4H, CH_2), 3.79 (s, 4H, CH_2), 3.88 (s, 9H, OCH_3), 7.41 (d, *J* = 6.5 Hz, 1H, Ar), 7.44 (s, 2H, Ar), 8.35 (d, *J* = 6.5 Hz, 1H, Ar); IR (Nujol) 2485, 1662 cm^{-1}. Anal. Calcd for $C_{19}H_{23}F_3N_4O_5$: C, 51.35; H, 5.22; N, 12.61. Found: C, 51.28; H, 5.25; N, 12.65.

*4.1.4. General procedure for the synthesis of 4-(4-(4-aryl)pyrimidin-2-yl)piperazinocarbonyl)aminophenyl sulfamate (**7a-i**)*

A mixture of 4-(phenoxycarbonyl)aminophenylsulfamate **1** (0,31 g, 1 mmol) and pyrimidines **6a-i** (1 mmol), in anhydrous DMSO (3 mL) was stirred at room temperature for 24 h. Then, water (10 mL) was added and the mixture was stirred at room temperature until a solid is formed. The formed solid was filtered off, washed with water, air dried and recrystallized from EtOH to give ureas **7a-i**.

*4.1.4.1. 4-(4-(4-Phenylpyrimidin-2-yl)piperazine-1-carboxamido)phenyl sulfamate (**7a**)*

Following the general procedure, the title compound was obtained from **6a** in 25% yield, mp 134-135 °C. ESIMS (m/z): 455 $(M+H)^+$. ^1H NMR: δ 3.55 (m, 4H, CH_2), 3.87 (m, 4H, CH_2), 6.65 (s, 1H, CH), 7.22 (m, 4H Ar), 7.25 (d, *J* = 5.0 Hz, 2H, Ar), 7.39-7.52 (m, 4H, Ar), 8.15 (s, 2H, NH_2), 8.47

(s, 1H, NH); IR (Nujol) 3336 (NH), 1701 (C=O), 1634 (C=N), 1567 (C=C) cm^{-1}. Anal. Calcd for $C_{21}H_{22}N_6O_4S$: C, 55.49; H, 4.88; N, 18.49. Found: C, 55.55; H, 4.87; N, 18.52.

*4.1.4.2. 4-(4-(4-(m-Tolyl)pyrimidin-2-yl)piperazine-1-carboxamido)phenyl sulfamate (**7b**)*

Following the general procedure, the title compound was obtained from **6b** in 31% yield, mp 164-165 °C. ESIMS (m/z): 469 $(M+H)^+$. ^1H NMR: δ 2.42 (s, 3H, CH_3), 3.60 (m, 4H, CH_2), 3.89 (m, 4H, CH_2), 7.16 (d, *J* = 6.0 Hz, 1H, Ar), 7.27 (d, *J* = 6.0 Hz, 1H, Ar), 7.37 (m, 4H, Ar), 7.51 (d, *J* = 8.0 Hz, 2H Ar), 7.53 (d, *J* = 8.0 Hz, 2H Ar), 7.88 (s, 2H, NH_2), 8.71 (s, 1H, NH); IR (Nujol) 3295 (NH), 1644 (C=O) cm^{-1}. Anal. Calcd for $C_{22}H_{24}N_6O_4S$: C, 56.40; H, 5.16; N, 17.94. Found: C, 56.34; H, 5.18; N, 17.97.

*4.1.4.3.4-(4-(4-(3-(Trifluoromethyl)phenyl)pyrimidin-2-yl)piperazine-1-carboxamido)phenyl sulfamate (**7c**)*

Following the general procedure, the title compound was obtained from **6c** in 41% yield, mp 174-175 °C. ESIMS (m/z): 523 $(M+H)^+$. ^1H NMR: δ 3.51-3.60 (m, 4H, CH_2), 3.90 (m, 4H, CH_2), 7.16 (d, *J* = 7.0 Hz, 2H, Ar), 7.40 (d, *J* = 5.0 Hz, 1H, Ar), 7.53 (m, 3H, Ar), 7.78 (d, *J* = 7.2 Hz, 2H, Ar), 7.90 (s, 2H, NH_2), 8.44 (s, 1H, Ar), 8.55 (d, *J* = 5.0 Hz, 1H, Ar), 8.72 (s, 1H, NH); IR (Nujol) 3307 (NH), 1642 (C=O), 1598 (C=N), 1571 (C=C) cm^{-1}. Anal. Calcd for $C_{22}H_{21}F_3N_6O_4S$: C, 50.57; H, 4.05; N, 16.08. Found: C, 50.63; H, 4.02; N, 16.12.

*4.1.4.4. 4-(4-(4-(3-Bromophenyl)pyrimidin-2-yl)piperazine-1-carboxamido)phenyl sulfamate (**7e**)*

Following the general procedure, the title compound was obtained from **6e** in 26% yield, mp 163-164 °C. ESIMS (m/z): 533, 535 $(M+H)^+$. ^1H NMR: δ 3.59 (m, 4H, CH_2), 3.89 (m, 4H, CH_2), 7.17 (m, 2H, Ar), 7.31 (d, *J* = 5.0 Hz, 1H, Ar), 7.53 (m, 3H, Ar), 7.73 (d, *J* = 7.5 Hz, 1H, Ar), 7.87 (s, 2H, NH_2), 8.16 (d, *J* = 7.5 Hz, 1H Ar), 8.31 (s, 1H, Ar), 8.52 (d, *J* = 5.0 Hz, 1H, Ar), 8.72 (s, 1H, NH); IR (Nujol) 3323 (NH), 1646 (C=O), 1605 (C=N), 1571 (C=C) cm^{-1}. Anal. Calcd for $C_{22}H_{21}BrN_6O_4S$: C, 47.29; H, 3.97; N, 15.76. Found: C, 47.25; H, 3.98; N, 15.80.

*4.1.4.5. 4-(4-(4-(4-Methoxyphenyl)pyrimidin-2-yl)piperazine-1-carboxamido)phenyl sulfamate (**7g**)*

Following the general procedure, the title compound was obtained from **6g** in 40% yield, mp 93-95 °C. ESIMS (m/z): 485 $(M+H)^+$. ^1H NMR: δ 3.59 (s, 4H, CH_2), 3.64 (s, 4H, CH_2), 3.85 (s, 3H, CH_3), 7.07 (d, *J* = 8.0 Hz, 2H, Ar), 7.19 (m, 4H, Ar), 7.53 (d, *J* = 8.5 Hz, 2H, Ar), 8.13 (d, *J* = 8.5 Hz, 2H, Ar), 7.88 (s, 2H, NH_2), 8.73 (s, 1H, NH); IR (Nujol) 3404 (NH), 3317 (NH), 3182 (NH), 1650 (C=O), 1569 (C=N) cm^{-1}. Anal. Calcd for $C_{22}H_{24}N_6O_5S$: C, 54.53; H, 4.99; N, 17.34. Found: C, 54.47; H, 5.01; N, 17.38.

*4.1.4.6. 4-(4-(4-(3,4,5-Trimethoxyphenyl)pyrimidin-2-yl)piperazine-1-carboxamido)phenyl sulfamate (**7h**)*

Following the general procedure, the title compound was obtained from **6h** in 31% yield, mp 119-120 °C. ESIMS (m/z): 545 $(M+H)^+$. ^{1}H NMR: δ 3.59 (s, 4H, CH_2), 3.74 (s, 4H, CH_2), 3.90 (s, 9H, CH_3), 7.16 (d, *J* = 7.5 Hz, 2H, Ar), 7.29 (m, 1H, Ar), 7.45 (m, 2H, Ar), 7.53 (d, *J* = 7.5 Hz, 2H, Ar), 7.88 (s, 2H, NH_2), 8.45 (s, 1H, Ar), 8.72 (s, 1H, NH); IR (Nujol) 3330 (NH), 3175 (NH), 1632 (C=O), 1552 (C=N) cm^{-1}. Anal. Calcd for $C_{24}H_{28}N_6O_7S$: C, 52.93; H, 5.18; N, 15.43. Found: C, 52.97; H, 5.20; N, 15.40.

4.2. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity [13]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mMHepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in 10% DMSO aqueous solution and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier [19-21] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier [22-24].

4.3. Molecular Docking

Molecular Docking simulations were carried out as previously described [25] using rDock [26]. The crystal structures of CAII (pdb 4G0C) and CAIX (pdb 3IAI) were retrieved from RCSB Protein Data Bank web server (http://www.rcsb.org/). Proteins preparation was executed using HTMD (HighThroughput MD) tool [27] to add hydrogens, ionize side chain of amino acids at physiological pH using propKa, deleting water molecules and co-crystallized small molecules (AAZ included). 3D ligands were prepared using an in-house python script developed using RDKit toolkit [28] and minimized using MMFF94 forcefield. Considering the high conservation of the binding mode of the sulfamide moiety of co-crystallized compounds a tethered docking was executed [29] to enforce the partial binding modes of the sulfamates group. The ‘dock_solv’ rDock protocol was used, this protocol allows a full docking search but using the desolvation scoring function. Docking grid with

radius of 10.0 Å was centered on the co-crystalized ligand. Number of poses was set to 10. Docking protocol validation was described in our previous paper [26]. It contemplates the re-docking of the co-crystallized ligand AAZ and the evaluation of the RMSD with the crystallographic pose.

Acknowledgements

This study was funded by FIR grants from the University of Cagliari, the Italian Ministero dell'Istruzione, Università e della Ricerca (PRIN 2017, Prot. No. 2010E84AA4_002) (to V.O. and G.B.) and by the Researchers Supporting Project No. (RSP-2019/1) King Saud University, Riyadh, Saudi Arabia.

Conflict of interest

The authors declare no conflict of interest.

References

[1] A. Nocentini, C.T. Supuran. Advances in the structural annotation of human carbonic anhydrases and impact on future drug discovery. Expert Opin. Drug Discov. 14 (2019) 1175-1197.

[2] C.T. Supuran. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat. Rev. Drug. Discov. 7 (2008) 168–181.

[3] V. Alterio, A. Di Fiore, K. D'Ambrosio, C.T. Supuran, G. De Simone G. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem. Rev. 112 (2012) 4421–4468.

[4] A. Nocentini, C.T. Supuran. Carbonic anhydrase inhibitors as antitumor/antimetastatic agents: a patent review (2008-2018). Expert Opin. Ther. Pat. 28 (2018) 729-740.

[5] C.T. Supuran. How many carbonic anhydrase inhibition mechanisms exist? J. Enzyme Inhib. Med. Chem. 31 (2016) 345-360.

[6] https://clinicaltrials.gov/ct2/show/NCT02215850 (last updated on May 17, 2016); last accessed on October 10, 2019.

[7] https://clinicaltrials.gov/ct2/show/NCT03450018 (last updated on March 1, 2018); last accessed on October 10, 2019.

[8] F. Pacchiano, F. Carta, P.C. McDonald, Y. Lou, D. Vullo, A. Scozzafava, S. Dedhar, C.T. Supuran. Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis. J. Med. Chem. 54 (2011) 1896-1902.

[9] R.G. Gieling, M. Babur, L. Mamnani, N. Burrows, B.A. Telfer, F. Carta, J.Y. Winum, A. Scozzafava, C.T. Supuran, K.J. Williams. Antimetastatic effect of sulfamate carbonic anhydrase IX inhibitors in breast carcinoma xenografts. J. Med. Chem. 14 (2012) 5591-5600.

[10] C. Congiu, V. Onnis, A. Deplano, G. Balboni, N. Dedeoglu, C. T. Supuran. Synthesis of sulfonamides incorporating piperazinyl-ureido moieties and their carbonic anhydrase I, II, IX and XII inhibitory activity. Bioorg. Med. Chem. Lett. 25 (2015) 3850-3853.

[11] C. Congiu, V. Onnis, A. Deplano, G. Balboni, M. Ceruso, C.T. Supuran. Synthesis and carbonic anhydrase I, II, IX and XII inhibitory activity of sulfamates incorporating piperazinyl-ureido moieties. Bioorg. Med. Chem. 23 (2015) 5619-5625.

[12] (a) S-S. Tseng, J. W. Epstein, H. J. Brabander, G. Francisco. A simple regioselective synthesis of pyrimido[1,2□a]benzimidazoles. J. Heterocycl. Chem. 24 (1987) 837-843; (b) S. Chimichi, M. Boccalini, M. M. M. Hassan, G. Viola, F. Dall'Acqua, M. Curini. Synthesis, structural determination and photo-antiproliferative activity of new 3-pyrazolyl or -isoxazolyl substituted 4-hydroxy-2(1*H*)-quinolinones. Tetrahedron 62 (2006) 90-96; (c) C. Reidlinger, R. Dworczak, H. Junek. Cyanoacetophenone as a Synthon for 1,4,5-Substituted Pyrazoles. Monatsh. Chem. 129 (1998) 1207-1211; (d) F. Al-Omram, A.-Z. A. Elassar, A. A. El-Khair. Synthesis of condensed heteroaromatics: novel synthesis of aminoquinolizinone derivatives as anti-HIV agents. Tetrahedron 57 (2001) 10163-10170.

[13] R.G. Khalifah. The carbon dioxide hydration activity of carbonic anhydrase. J. Biol. Chem. 246 (1971) 2561–2573.

[14] D. L. Coffen, M. P. Dillon, A. P. D. W. Ford, Z. Li, T. J. Williams. US Patent 2002, 6355641 B1.

[15] D. Moi, P. A. Foster, L. G. Rimmer, A. Jaffri, A. Deplano, G. Balboni, V. Onnis, B. V.L. Potter. Synthesis and in vitro evaluation of piperazinyl-ureido sulfamates as steroid sulfatase inhibitors. Eur. J. Med. Chem. 182 (2019) 111614.

[16] F. A. Rosa, P. Machado, H. G. Bonacorso, N. Zanatta, M. A. P. Martins. Reaction of β-Dimethylaminovinyl Ketones with Hydroxylamine: a Simple and Useful Method for Synthesis of 3- and 5-Substituted Isoxazoles. J. Heterocyclic Chem. 45 (2008) 879-885.

[18] A. Balbi, M. Anzaldi, C. Maccio, C. Aiello, M. Mazzei, R. Gangemi, P. Castagnola, M. Miele, C. Rosano, M. Viale. Synthesis and biological evaluation of novel pyrazole derivatives with anticancer activity. Eur. J. Med. Chem. 46 (2011) 5293-5309.

[19] L. E. Kiss, H. S. Ferreira, L. Torrao, M. J. Bonifacio, P. N. Palma, P. Soares-da-Silva, D.A. Learmonth. Discovery of a Long-Acting, Peripherally Selective Inhibitor of Catechol-O-methyltransferase. J. Med. Chem. 53 (2010) 3396-3411.

[20] D. Vullo, S. Del Prete, A. Nocentini, S.M. Osman, Z. AlOthman, C. Capasso, M. Bozdag, F. Carta, P. Gratteri, C.T. Supuran, Dithiocarbamates effectively inhibit the b-carbonic anhydrase from the dandruff-producing fungus Malassezia globose. Bioorg. Med. Chem. 25 (2017) 1260–1265.

[21] Y. Entezari Heravi, S. Bua, A. Nocentini, S. Del Prete, A. A. Saboury, H. Sereshti, C. Capasso, P. Gratteri, C.T. Supuran. Inhibition of Malassezia globosa carbonic anhydrase with phenols. Bioorg. Med. Chem. 25 (2017) 2577-2582.

[22] A. Nocentini, A. Bonardi, P. Gratteri, B. Cerra, A. Gioiello, C.T. Supuran. Steroids interfere with human carbonic anhydrase activity by using alternative binding mechanisms. J. Enzyme Inhib. Med. Chem. 33 (2018)1453-1459.

[23] A. Nocentini, D. Moi, G. Balboni, V. Onnis, C.T. Supuran. Discovery of thiazolin-4-one-based aromatic sulfamates as a new class of carbonic anhydrase isoforms I, II, IV, and IX inhibitors. Bioorg. Chem. 77 (2018) 293-299.

[24] A. Bonardi, A.B. Vermelho, V. da Silva Cardoso, M.C. de Souza Pereira, L.L. da Silva, S. Selleri, P. Gratteri, C.T. Supuran, A. Nocentini. N-Nitrosulfonamides as Carbonic Anhydrase Inhibitors: A Promising Chemotype for Targeting Chagas Disease and Leishmaniasis. ACS Med. Chem. Lett. 10 (2018) 413-418.

[25] A. Nocentini, D. Moi, G. Balboni, S. Salvadori, V. Onnis, C.T. Supuran. Synthesis and biological evaluation of novel pyrazoline-based aromatic sulfamates with potent carbonic anhydrase isoforms II, IV and IX inhibitory efficacy. Bioorg. Chem. 77 (2018) 633-639.

[26] D. Moi, A. Nocentini, A. Deplano, G. Balboni, C. T. Supuran, V. Onnis. Structure-activity relationship with pyrazoline-based aromatic sulfamates as carbonic anhydrase isoforms I, II, IX and XII inhibitors: synthesis and biological evaluation. Eur. J. Med. Chem. 182 (2019) 111638.

[27] S. Ruiz-Carmona, D. Alvarez-Garcia, N. Foloppe, A.B. Garmendia-Doval, S. Juhos, P. Schmidtke, X. Barril, R.E. Hubbard, S.D. Morley. rDock: a fast, versatile and open source program for docking ligands to proteins and nucleic acids. PLoS Comput. Biol. 10 (2014) e1003571.

[28] S. Doerr, T. Giorgino, G. Martínez-Rosell, J.M. Damas, G. De Fabritiis, Highthroughput automated preparation and simulation of membrane proteins with HTMD. J. Chem. Theory Comput. 13 (2017) 4003-4011.

[29] RDKit, Cheminformatics and machine learning software. http://www.rdkit. Org (2013)

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: