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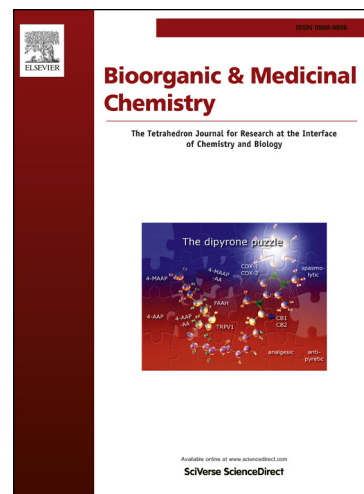
PII: S0968-0896(16)30018-9  
DOI: <http://dx.doi.org/10.1016/j.bmc.2016.01.018>  
Reference: BMC 12761

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 19 November 2015  
Revised Date: 4 January 2016  
Accepted Date: 8 January 2016

Please cite this article as: Barresi, E., Salerno, S., Marini, A.M., Taliani, S., Motta, C.L., Simorini, F., Settimo, F.D., Vullo, D., Supuran, C.T., Sulfonamides incorporating heteropolycyclic scaffolds show potent inhibitory action against carbonic anhydrase isoforms I, II, IX and XII, *Bioorganic & Medicinal Chemistry* (2016), doi: <http://dx.doi.org/10.1016/j.bmc.2016.01.018>

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**Sulfonamides incorporating heteropolycyclic scaffolds show potent inhibitory action against carbonic anhydrase isoforms I, II, IX and XII**

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**Abstract.** Three series of polycyclic compounds possessing either primary sulfonamide or carboxylic acid moieties as zinc-binding groups were investigated as inhibitors of four physiologically relevant CA isoforms, the cytosolic hCA I and II, as well as the transmembrane hCA IX and XII. Most of the new sulfonamides reported here showed excellent inhibitory effects against isoforms hCA II, IX and XII, but no highly isoform-selective inhibition profiles. On the other hand, the carboxylates selectively inhibited hCA IX ( $K_i$ s ranging between 40.8 and 92.7 nM) without inhibiting significantly the other isoforms. Sulfonamides/carboxylates incorporating polycyclic ring systems such as benzothiopyranopyrimidine, pyridothiopyranopyrimidine or dihydrobenzothiopyrano[4,3-c]pyrazole may be considered as interesting candidates for exploring the design of isoform-selective CAIs with various pharmacologic applications.

**Keywords:** carbonic anhydrase; sulfonamide; carboxylic acid; benzothiopyranopyrimidine; pyridothiopyranopyrimidine; dihydrobenzothiopyrano[4,3-c]pyrazole.

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## 1. Introduction

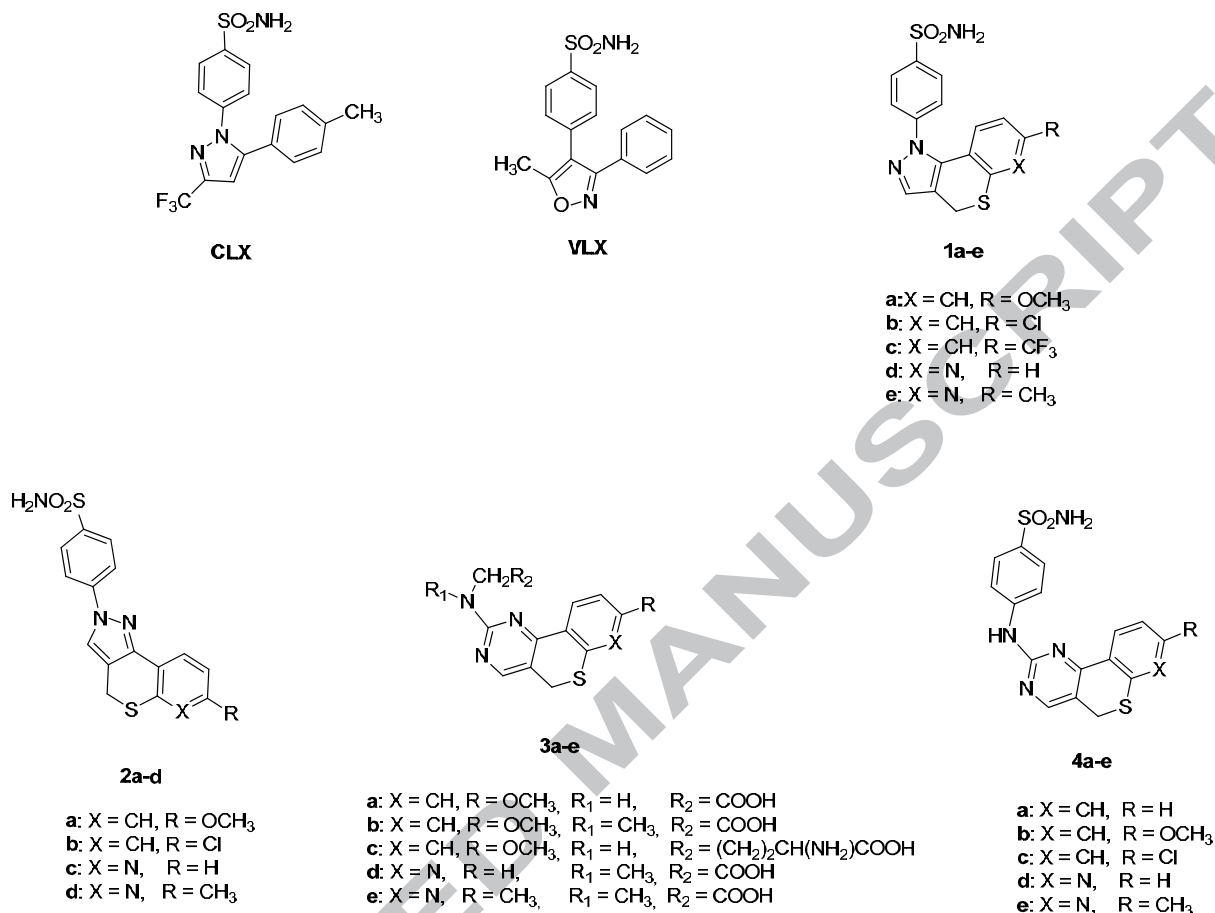
Sulfonamides possessing the general formula  $\text{RSO}_2\text{NH}_2$  constitute the historically most important class of carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs).<sup>1-8</sup> They are in clinical use for more than 70 years as diuretics, antiglaucoma, antiepileptic, and antiobesity agents,<sup>1-7</sup> and, more recently, they have shown applications as antitumor/antimetastatic agents or diagnostic tools, with at least one sulfonamide in Phase I clinical trials for the treatment of advanced, metastatic solid tumors.<sup>8</sup>

Recently,<sup>1</sup> we have disclosed a new class of sulfonamides **1a-e** featuring the classical benzenesulfonamide moiety for binding to the catalytically crucial zinc ion forming the enzyme active site. The head part of these molecules is connected to a pyrazole moiety, further annealed with a bulky heterocyclic ring, benzothiopyrano[4,3-*c*]pyrazole (**1a-c**) and pyridothiopyrano[4,3-*c*]pyrazole (compounds **1d-e**), (Chart 1) in order to explore both alternative chemotypes and the possibility to further enhance the isoform selectivity observed with celecoxib (CLX) and valdecoxib (VLX) as CAIs.<sup>2-8</sup> Actually, compounds **1a-e** may be regarded as geometrically constrained analogues of the two reference leads CLX and VLX.

The most interesting feature of this new class of sulfonamides was their capability to predominantly exert strong inhibition of only human (h) isoforms hCA I and II, as well as of the mycobacterial  $\beta$ -class enzymes (Rv1284, Rv3273, and Rv3588c), whereas their inhibitory activity against hCA III, IV, VA, VB, VI, VII, IX, XII, XIII, and XIV was at least 2 orders of magnitude lower. The combination of X-ray crystal structure of the hCA II- **1e** adduct, and homology modeling allowed us to explain this peculiar inhibition profile, which was also quite different from those of the reference coxibs, CLX and VLX. Thus, the benzenesulfonamides **1a-e** constitute a highly interesting class of compounds which, inhibiting only a restricted number of physiologically relevant CA isoforms among the 12 catalytically active human enzymes, should lead to fewer side effects. In addition, the good inhibition profile of some of the new derivatives (**1d-e**) against mycobacterial CAs is also to be considered as relevant because these enzymes are less inhibited by other classes of sulfonamides.

As a further development of this project, in the present work we aim to study three novel series of compounds in search for selective CAIs. In the first class (compounds **2a-d**, Chart 1), we decided to move the benzenesulfonamide function from position 1 to position 2 of the pyrazole system of benzothiopyrano[4,3-*c*]pyrazole (**2a,b**) and pyridothiopyrano[4,3-*c*]pyrazole (**2c,d**), to assess whether this change of position could lead to selective compounds for some CA isoforms.

Substituents at 7-position (R) were chosen by considering the groups that most contributed to the activity of the above described compounds **1a-e**.<sup>1</sup>



**Chart 1:** Sulfonamide CAIs of types **1-4** discussed in the paper.

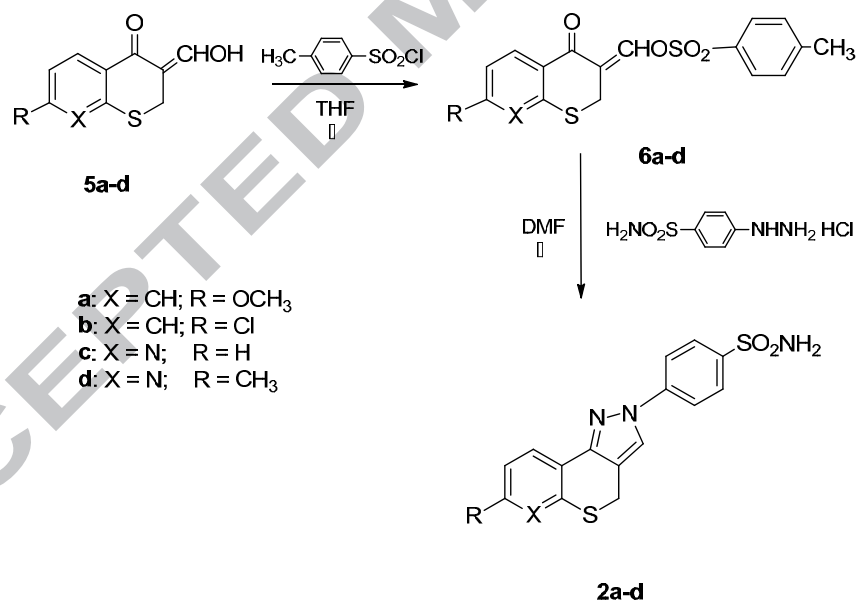
Then, with the aim to identify novel chemotypes to further expand the chemical diversity of CAIs, we searched among our in-house database of heteropolycyclic scaffold-based derivatives.<sup>9-13</sup> Benzothio-pyranopyrimidines (**3a-c**) and pyridothio-pyranopyrimidines (**3d-e**), reported by us earlier,<sup>11,13</sup> attracted our attention due to their structural similarity to the pyrazoles of types **1** and **2**. Thus, in this work, we considered compounds **3a-e**, featuring a carboxylic group. Actually, it was reported that some carboxylates<sup>14-16</sup> share with dithiocarbamates<sup>17-19</sup> the CA inhibition mechanism of sulfonamides and their bioisosteres (sulfamates, sulfamides),<sup>20,21</sup> representing one of the main class of pharmacologically relevant CAIs.<sup>20-23</sup>

Finally, the benzothio-pyranopyrimido and the pyridothio-pyranopyrimido scaffolds were further investigated, by the design of compounds **4a-e** (Chart1), that bear a benzenesulfonamide moiety, typical of the classical CAIs, at the 2-position of the tricyclic system and spaced by an NH linker.

## 2. Results and Discussion

### 2.1. Chemistry.

The preparation of the 2-(*p*-sulfonamidophenyl)substituted-pyrazole derivatives **2a-d** was performed following the synthetic route described in Scheme 1. The starting key intermediates 7-substituted-3-hydroxymethylenebenzothiopyranones **5a-b**<sup>9</sup> or 3-hydroxymethylene-pyridothiopyranones **5c-d**<sup>10,11</sup> were converted into the corresponding *p*-toluenesulfonates **6a-d**. In these intermediates the reactive methine functionality was protected as previously reported by us.<sup>12</sup> The subsequent condensation of **6a-d** with 4-sulfonamidophenylhydrazine hydrochloride, in anhydrous dimethylformamide at room temperature, involved, as a first step, only the carbonyl moiety functionalization at the 4-position, affording the intermediate arylhydrazones.<sup>12</sup> These latter compounds easily cyclized *in situ* by heating the reaction mixture at 100 °C, to afford the desired 2-substituted derivatives **2a-d**. All compounds were purified by flash chromatography and characterized by analytical and spectral data (see Experimental protocols for details) which were in accordance with analogue compounds reported earlier.<sup>1, 9, 12</sup>

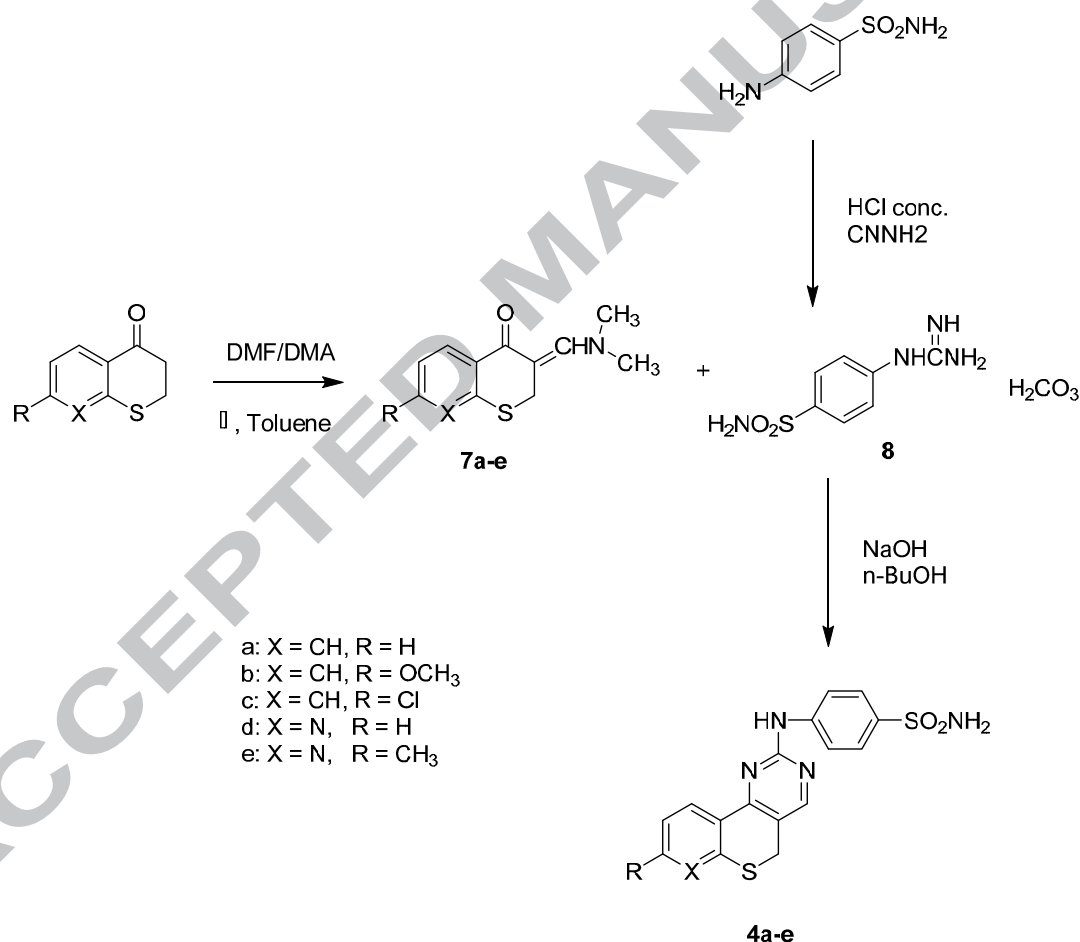


Scheme 1. Preparation of sulfonamides **2a-d**.

The synthetic pathway leading to the target 2-(*p*-benzensulfonamido)-benzothiopyranopyrimidines **4a-c** and 2-(*p*-benzensulfonamido)pyridothiopyranopyrimidines **4d-e** took advantage of the use of the 7-substituted benzothiopyrano or pyridothiopyrano moiety as “synthetic tool”, which demonstrated to be versatile intermediates in many synthetic procedures.<sup>13</sup> The 1,3-bielectrophile

reactivity of the 7-substituted-3-dimethylaminomethylene derivatives **7a-e**<sup>13,24-26</sup> in the reaction with the 4-guanidinobenzenesulfonamide (as bicarbonate salt) **8**<sup>27</sup> in butanol at reflux, in the presence of sodium hydroxide,<sup>28</sup> allowed to easily obtain the desired 2-benzenesulfonamidophenyl substituted pyrimidines **4a-e**, as shown in Scheme 2. The *N*-(4-aminosulfonylphenyl) guanidine carbonate **8** was obtained in good yield by reaction of a 50% cyanamide water solution and a solution of sulfanilamide in the presence of concentrated HCl. The mixture was heated at 100 °C for 20 minutes, as described in the literature.<sup>27</sup>

The purity of the target compounds was assessed by physicochemical methods, analytical and <sup>1</sup>H NMR and <sup>13</sup>C NMR spectral data, which were in agreement with the proposed structures and with other previously reported results. (see Experimental protocols for details).



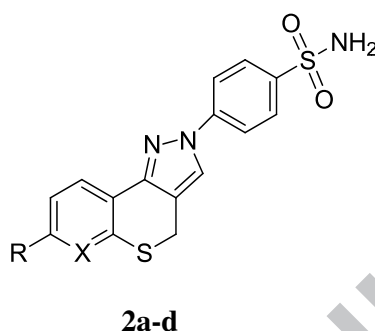
Scheme 2. Synthesis of sulfonamides **4a-e**.

## 2.2. CA inhibition

The compounds **2a-d**, **3a-e** and **4a-e** reported here were investigated for their enzyme inhibitory capacity against four physiologically relevant CA isoforms, the human (h) hCA I, II, IX and XII

(Table 1). Acetazolamide (**AAZ**, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide) was used as standard drug in the assay.<sup>28</sup>

Table 1. Inhibition of isoforms hCA I, II, IX and XII with sulfonamides **2a-d**, by a stopped-flow CO<sub>2</sub> hydrase assay.<sup>28</sup> Acetazolamide (**AAZ**) used as standard inhibitor.



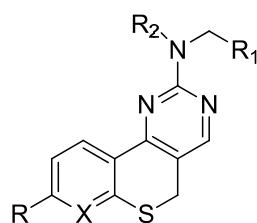
N	X	R	K <sub>I</sub> (nM)*			
			hCA I	hCA II	hCA IX	hCA XII
<b>2a</b>	CH	Cl	41.5	8.5	6.1	68.6
<b>2b</b>	CH	OCH <sub>3</sub>	68.4	7.4	7.4	38.1
<b>2c</b>	N	H	22.5	7.1	6.3	66.7
<b>2d</b>	N	CH <sub>3</sub>	37.1	7.6	7.7	62.5
<b>AAZ</b>	-	-	250	12	25	5.6

\* Mean from 3 different assays. Errors in the range of  $\pm 10$  of the reported values.

The data listed in Table 1 show that benzenesulfonamides **2a-d**, bearing the bulky annealed tricyclic tails, significantly inhibited all four investigated CA isoforms, with inhibition constants in the range of 22.5 - 68.4 nM against hCA I, 7.1 - 8.5 nM against hCA II, 6.1 - 7.7 nM against hCA IX and 38.1 - 68.6 nM against hCA XII, respectively. The structure-activity relationship (SAR) against all four isoforms was rather flat, since the substituent R and the X moiety showed a small influence on the inhibitory activity. The other salient feature was that the isoform-selectivity, observed with compounds **1** reported earlier,<sup>1</sup> is lost for the sulfonamides **2**. This may presumably be attributed to the capacity of the quite bulky scaffold present in the derivatives investigated here, to interact favorably with the active sites of all four CA isoforms.

Thus, as the shift of the benzenesulfonamide moiety from position 1 to position 2 of the pyrazole system (compounds **2a-d**) led to less isoform selective compounds, we decided to continue the study in this area by changing the starting pyrazole with a pyrimidine ring fused on the benzothiopyrano or pyridothiopyrano moieties, thus obtaining compounds **3** and **4**, whose inhibition data are reported in Tables 2 and 3.

Table 2. Inhibition of isoforms hCA I, II, IX and XII with carboxylic acids **3a-e**, by a stopped-flow CO<sub>2</sub> hydrase assay.<sup>28</sup> Acetazolamide (**AAZ**) used as standard inhibitor.



**3a-e**

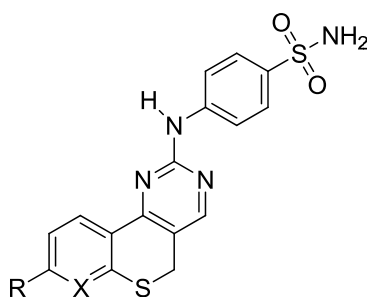
N	X	R	R <sub>1</sub>	R <sub>2</sub>	K <sub>i</sub> (nM)*			
					hCA I	hCA II	hCA IX	hCA XII
<b>3a</b>	CH	OCH <sub>3</sub>	COOH	CH <sub>3</sub>	510	5390	40.8	8390
<b>3b</b>	CH	OCH <sub>3</sub>	COOH	H	871	4220	54.1	5975
<b>3c</b>	CH	OCH <sub>3</sub>	(CH <sub>2</sub> ) <sub>2</sub> CH(NH <sub>2</sub> )COOH	H	>50000	>50000	335	9030
<b>3d</b>	N	H	COOH	CH <sub>3</sub>	440	638	67.8	7170
<b>3e</b>	N	CH <sub>3</sub>	COOH	CH <sub>3</sub>	370	7450	92.7	6185
<b>AAZ</b>	-	-	-		250	12	25	5.6

\* Mean from 3 different assays. Errors in the range of  $\pm 10$  of the reported values.

Data of Table 2 show that the carboxylic acids **3** (except the one with the longer aliphatic chain, **3c**) possess a more interesting inhibition profile against the four CA isoforms compared to sulfonamides **2**. Indeed, compounds **3a,b,d** and **e** behaved as hCA IX-selective inhibitors ( $K_i$ s in that range of 40.8 - 92.7 nM) with much lower affinity for hCA I, II and XII, for which  $K_i$ s in the range of 370 - 9030 nM were measured (Table 2). The lysine derivative **3c** did not significantly inhibit hCA I, II and XII, being a weak hCA IX inhibitor too ( $K_i$  of 335 nM).

Table 3. Inhibition of isoforms hCA I, II, IX and XII with sulfonamides **4a-e**, by a stopped-flow CO<sub>2</sub> hydrase assay.<sup>28</sup> Acetazolamide (**AAZ**) used as standard inhibitor.



**4a-e**

N	X	R	K <sub>I</sub> (nM)*			
			hCA I	hCA II	hCA IX	hCA XII
<b>4a</b>	CH	H	80.5	96.0	7.0	75.1
<b>4b</b>	CH	OCH <sub>3</sub>	66.9	63.2	22.5	48.9
<b>4c</b>	CH	Cl	84.0	218	8.7	15.5
<b>4d</b>	N	H	68.3	9.4	27.6	17.8
<b>4e</b>	N	CH <sub>3</sub>	68.9	8.0	8.1	34.4
<b>AAZ</b>	-	-	250	12	25	5.6

\* Mean from 3 different assays. Errors in the range of  $\pm 10$  of the reported values.

Data of Table 3 show that all CA isoforms investigated here were inhibited by sulfonamides **4a-e**. The slow cytosolic isoform hCA I was inhibited moderately, with K<sub>I</sub>s ranging between 66.9 and 84.0 nM. Again the SAR was rather flat for this isoform, proving that the nature of the X and R fragments of the molecule do not significantly influence the biological activity. This is however not the case for the inhibition of the physiologically dominant isoform hCA II, for which a more diversified inhibitory behavior has been evidenced for these sulfonamides. Indeed, **4d** and **4e** were low nanomolar, highly effective hCA II inhibitors (K<sub>I</sub>s ranging between 8.0 and 9.4 nM), being thus more effective than the standard drug **AAZ**, whereas **4a-c** showed a reduced efficacy, with K<sub>I</sub>s ranging between 63.2 and 218 nM. Thus, the best substitution pattern was that incorporating pyridine and not benzene in the terminal ring of the polycyclic system. The presence of H or Me as R moieties was not highly influential on the hCA II inhibition profile of these compounds.

The transmembrane isoform hCA IX, a validated antitumor target,<sup>8</sup> was also effectively inhibited by sulfonamides **4**, which showed K<sub>I</sub>s ranging between 7.0 and 27.6 nM (Table 3). In this case both benzene- and pyridine in the third ring of the polycyclic system led to effective inhibitors (e.g., compare **4a** and **4e** which show very similar K<sub>I</sub> values). The methoxy moiety (in **4b**) led to an almost three-fold loss of inhibitory efficacy (compared to **4a**) whereas chlorine (in **4c**) was better

tolerated, with the last compound having a similar activity as **4a**. The presence of the methyl moiety in **4e** was this time beneficial for the hCA IX inhibitory effects, with this compound being 3-times more potent compared to **4d**, which has a hydrogen in place of the methyl group (Table 3).

The second transmembrane isoform investigated here, hCA XII, was also effectively inhibited by these sulfonamides, which had  $K_{IS}$  ranging between 15.5 and 75.1 nM. The best inhibitors were the chlorine derivative in the benzene series (**4c**) and the unsubstituted derivative ( $R = H$ ) in the pyridine series (**4d**) with  $K_{IS}$  of 15.5 nM and 17.8 nM, respectively (Table 3).

### 3. Conclusions

Primary sulfonamides **2** and **4** or carboxylic acids **3** incorporating polycyclic ring systems such as benzothiopyranopyrimidine, pyridothiopyranopyrimidine or dihydrobenzothiopyrano[4,3-*c*]pyrazole were investigated as inhibitors of four physiologically relevant CA isoforms, the cytosolic hCA I and II, as well as the transmembrane hCA IX and XII. An excellent inhibitory activity against isoforms hCA II, IX and XII was shown by most of the new sulfonamides **2** and **4**, although with a low selectivity profile among the various isoforms. The carboxylates **3** on the other hand, acted as hCA IX-selective inhibitors ( $K_{IS}$  in that range of 40.8 - 92.7 nM), with much lower affinity for hCA I, II and XII. Thus, sulfonamide or carboxylic acid moieties as zinc-binding groups connected to a pyrazole or a pyrimidine moiety annealed with a bulky heterocyclic ring may be considered as interesting candidates for exploring the design of novel isoform-selective CAIs with various pharmacologic applications. Such a “compromise” between a lower affinity but higher selectivity of enzyme inhibitors might be acceptable, as reported for example in the field of MMP inhibitors,<sup>29a</sup> or for some carboxylate-based CAIs.<sup>29b,c</sup> As a consequence, exploration of diverse scaffolds and zinc-binding groups may lead to inhibitors with the desired properties for the various pharmacologic applications of this class of derivatives.

## 4. Experimental Section

### 4.1. Chemistry

**General.** The uncorrected melting points were determined using a Reichert Köfler hot-stage apparatus. NMR spectra were obtained on a Bruker AVANCE 400 ( $^1H$ , 400 MHz,  $^{13}C$ , 100 MHz) in DMSO- $d_6$  or  $CDCl_3$  (internal standard tetramethylsilane). The coupling constants are given in Hertz. Magnesium sulphate was used as the drying agent. Evaporations were made in vacuo (rotating

evaporator). Analytical TLC have been carried out on Merck 0.2 mm precoated silica gel aluminium sheets (60 F-254). Silica gel 60 (230-400 mesh) was used for column chromatography. Purity of the target inhibitors **2a-d**, **3a-e**, and **4a-e** was determined, using a Shimadzu LC- 20AD SP liquid chromatograph equipped with a DDA Detector at 196 nm(column C18 (250 mm × 4.6 mm, 5 µm, Shim-pack)). The mobile phase, delivered at isocratic flow, consisted of acetonitrile (40-60%) and water (40-60%) and a flow rate of 1.0 mL/ min.

All the compounds showed percent purity values of  $\geq 95\%$ .

Reagents, starting materials, and solvents were purchased from commercial suppliers and used as received. The following intermediates were obtained according to methods previously described: 7-Methoxy-2,3-dihydro-3-hydroxymethylene-1-benzothiopyran-4(4H)-one **5a**<sup>9</sup> and 7-chloro-2,3-dihydro-3-hydroxymethylene-1-benzothiopyran-4(4H)-one **5b**,<sup>9</sup> 2,3-dihydro-3-hydroxymethylene-thiopyrano[2,3-*b*]-pyridin-4(4H)-one **5c**,<sup>10</sup> 7-Methyl-2,3-dihydro-3-hydroxymethylenethiopyrano[2,3-*b*]-pyridin-4(4H)-one **5d**,<sup>11</sup> 3-[(*p*-Methylphenyl)sulfonyl]oxymethylen-2,3-dihydro-benzothiopyran-4(4H)-one **6a**,<sup>9</sup> 3-[(*p*-Methylphenyl)sulfonyl]oxymethylen-2,3-dihydrothiopyrano[2,3-*b*]pyridin-4(4H)-one **6c**<sup>12</sup> and 7-Methyl-3-[(*p*-Methylphenyl)sulfonyl]oxymethylen-2,3-dihydrothiopyrano[2,3-*b*]pyridin-4(4H)-one **6d**,<sup>12</sup> 3-Dimethylaminomethylen-2,3-dihydrobenzo[3',2':5,6]thiopyran-4(4H)-one **7a**,<sup>26</sup> 7-Methoxy-3-dimethylaminomethylen-2,3-dihydrobenzo[3',2':5,6]thiopyran-4(4H)-one **7b**,<sup>13</sup> 7-Chloro-3-dimethylaminomethylen-2,3-dihydrobenzo[3',2':5,6]thiopyran-4(4H)-one **7c**,<sup>26</sup> 2,3-Dihydro-3-dimethylaminomethylenethiopyrano[2,3-*b*]pyridin-4(4H)-one **7d**,<sup>11</sup> 7-Methyl-2,3-Dihydro-3-dimethylaminomethylenethiopyrano[2,3-*b*]pyridin-4(4H)-one **7e**,<sup>11</sup> and *N*-(4-Aminosulfonylphenyl)guanidine carbonate **8**.<sup>27</sup> 2-Glyciny-**3a**,<sup>13</sup> 2-(*N*-methyl)glyciny- **3b**,<sup>13</sup> 2-(5*N*-ornithiny-)-8-methoxy- 5*H*-benzo[3',2':5,6]thiopyrano[4,3-*d*]pyrimidine **3c**,<sup>13</sup> *N*-Methyl-*N*-(5*H*-pyrido[3',2';5,6]thiopyrano[4,3-*d*]pyrimidin-2-yl)glycine **3d**,<sup>11</sup> and *N*-Methyl-*N*-(8-Methyl-5*H*-pyrido[3',2';5,6]thiopyrano[4,3-*d*]pyrimidin-2-yl)glycine **3e**<sup>11</sup> were prepared in accordance with reported procedures.

### 3-[(*p*-Methylphenyl)sulfonyl]chloromethylen-2,3-dihydrobenzothiopyran-4(4H)-one **6b**.

*p*-Toluensulphonylchloride (0.801 g, 4.8 mmol) was added to a solution of **5b** (0.903 g, 2.4mmol) in 8 mL of anhydrous tetrahydrofurane in the presence of potassium carbonate (1.161 g, 9.6 mmol). The reaction mixture was stirred at room temperature, under nitrogen atmosphere, for 15 hours and then refluxed for 3 hours. After cooling, the suspension was concentrated under reduced pressure, and the residue obtained was treated with water and then extracted with dichloromethane. The organic layers were dried and evaporated to give the crude product **6b** which was purified by flash chromatography on a silica gel column (60/0.040-0.063 mm) using petroleum ether 40-60°C/ethyl

acetate 8/2 as the eluting system. Yield 22%, mp 80-82 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 2.50 (s, 3H, CH<sub>3</sub>), 3.83 (s, 2H, CH<sub>2</sub>-S), 7.20 (dd, 1H, Ar-H, *J*<sub>max</sub> = 8.4 Hz, *J*<sub>min</sub> = 2.0 Hz), 7.29 (d, 1H, Ar-H, *J* = 2.0 Hz), 7.43 (m, 2H, Ar-H), 7.67-7.68 (m, 1H, CH), 7.87-7.89 (m, 2H, Ar-H), 8.04 (d, 1H, Ar-H, *J* = 8.4 Hz).

**General Procedure for the synthesis of 7-Substituted-2-(*p*-sulfonamidophenyl)-1,4-dihydrobenzothiopyrano[4,3-*c*]pyrazoles 2a-b and 7-Substituted-2-(*p*-sulfonamidophenyl)-1,4-dihydropyrido[3',2':5,6]thiopyrano[4,3-*c*]pyrazoles 2c-d.**

4-Hydrazinobenzenesulfonamide hydrochloride (0.300g, 1.3 mmol) in 9 ml of *N,N*-dimethylformamide was added dropwise to a solution of the opportune derivative **6a-d** (2.0 mmol) in 6 mL of the same solvent. The reaction mixture was stirred at room temperature for 15 hours, then refluxed for 2 hours. After cooling, the suspension was poured into a saturated aqueous potassium carbonate solution and extracted with dichloromethane. The organic layers were dried and evaporated under reduced pressure to give desired crude pyrazoles **2a-d** which were purified by flash chromatography on a silica gel column (60/0.040-0.063 mm) using petroleum ether 40-60 °C/ethyl acetate in various ratio as the eluting system.

**7-Methoxy-2-(*p*-sulfamidophenyl)-1,4-dihydrobenzothiopyranopyrazole 2a.**

Yield 35%, mp 230-232 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ (ppm) 3.74 (s, 3H, OCH<sub>3</sub>), 3.99 (s, 2H, CH<sub>2</sub>-S), 6.67 (dd, 1H, Ar-H, *J*<sub>max</sub> = 8.8 Hz; *J*<sub>min</sub> = 2.4 Hz), 6.76-6.74 (m, 1H, Ar-H), 7.09 (d, 1H, Ar-H, *J* = 2.4 Hz), 7.53 (s, 2H, NH<sub>2</sub> *exch.*), 7.76 (d, 2H, Ar-H *J* = 8.8 Hz), 7.71 (s, 1H, Ar-H), 7.95 (d, 2H, Ar-H, *J* = 8.8 Hz). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ (ppm) 23.24, 55.87, 112.53, 114.30, 117.06, 118.54, 126.23, 126.30, 127.52, 135.63, 137.87, 137.90, 143.16, 143.98, 159.33.

**7-Chloro-2-(*p*-sulfamidophenyl)-1,4-dihydrobenzothiopyranopyrazole 2b.**

Yield 51%, mp: 210-212 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ (ppm) 4.04 (s, 2H, CH<sub>2</sub>-S), 6.81 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.16 (dd, 1H, Ar-H, *J*<sub>max</sub> = 8.4, *J*<sub>min</sub> = 2.0 Hz), 7.53 (bs, 2H, NH<sub>2</sub> *exch.*), 7.60-7.64 (m, 3H, Ar-H), 7.78 (s, 1H, Ar-H), 7.94-7.96 (m, 2H, Ar-H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ (ppm) 23.12, 118.81, 124.48, 126.21, 126.34, 126.40, 127.63, 128.61, 133.12, 136.29, 137.06, 138.04, 142.81, 144.20.

**2-(*p*-Sulfamidophenyl)-1,4-dihydropyridothiopyranopyrazole 2c.**

Yield 30%, mp 213-215 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ (ppm) 4.19 (s, 2H, CH<sub>2</sub>-S), 7.05-7.07 (m, 2H, Ar-H), 7.54 (bs, 2H, NH<sub>2</sub> *exch.*), 7.61-7.63 (m, 2H, Ar-H), 7.78 (s, 1H, Ar-H), 7.94-7.96 (m, 2H, Ar-H), 8.29-8.30 (m, 1H, Ar-H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ (ppm) 22.90, 118.13, 120.77, 121.69, 126.35, 127.64, 131.52, 136.75, 138.20, 142.64, 144.35, 148.76, 157.44.

**7-Methyl-2-(*p*-sulfamidophenyl)-1,4-dihydropyridothiopyranopyrazole 2d.**

Yield 25%, mp 235-238 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm) 2.38 (s, 3H, CH<sub>3</sub>), 4.15 (s, 2H, CH<sub>2</sub>-S), 6.90 (d, 1H, Ar-H, *J* = 8.0 Hz), 6.95 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.52 (s, 2H, NH<sub>2</sub>*exch.*), 7.59-7.61 (m, 2H, Ar-H), 7.75 (s, 1H, Ar-H), 7.93-7.95 (m, 2H, Ar-H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ (ppm) 22.99, 24.03, 117.41, 118.97, 120.15, 126.28, 127.61, 131.80, 137.00, 138.13, 142.70, 144.29, 156.73, 157.74.

**General procedure for the synthesis of 4-((8-substituted-5*H*-thiochroman[4,3-*d*]pyrimidin-2-yl)amino)benzenesulfonamides 4a-c and 4-((8-substituted-5*H*-pyrido[3',2':5,6]thiopyrano[4,3-*d*]pyrimidin-2-yl)amino)benzenesulfonamides 4d-e.**

*N*-(4-Aminosulfonylphenyl)guanidine carbonate **8** (0.332 g, 1.2 mmol) and NaOH (0.096 g, 2.4 mmol) were added to a solution of the appropriate 3-dimethylaminomethylen-2,3-dihydrobenzo[3',2':5,6]thiopyran-4(4*H*)-one **7a-c** or 2,3-Dihydro-3-dimethylaminomethylenethiopyrano[2,3-*b*]pyridin-4(4*H*)-one **7d-e** (1.2 mmol) in 10 mL of *n*-BuOH. The solution was heated at 120 °C for 4 hours. After cooling, a precipitate was obtained in the case of compounds **4a-c**, which was collected by vacuum filtration. Instead, starting from compounds **7d-e**, it was not observed any formation of precipitate, thus, the solvent of reaction was evaporated under reduced pressure. All compounds were purified by flash chromatography on a silica gel column (60/0.040-0.063 mm) using ethyl acetate/ petroleum ether 40-60 °C in various ratio as the eluting system.

**4-((5*H*-thiochroman[4,3-*d*]pyrimidin-2-yl)amino)benzenesulfonamide 4a.**

Yield 44%, mp 275-278 °C. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm) 4.07 (s, 2H, CH<sub>2</sub>-S), 7.20 (s, 2H, NH<sub>2</sub>*exch.*), 7.41-7.47 (m, 3H, Ar-H), 7.76-7.78 (m, 2H, Ar-H), 7.97-8.00 (m, 2H, Ar-H), 8.32-8.34 (m, 1H, Ar-H), 8.56 (s, 1H, Ar-H), 10.15 (bs, 1H, NH<sub>2</sub>*exch.*). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ (ppm) 26.42, 116.66, 118.21, 126.83, 127.10, 127.67, 128.43, 131.83, 132.40, 136.58, 137.12, 144.16, 157.00, 158.41, 159.43.

**4-((8-Methoxy-5*H*-thiochroman[4,3-*d*]pyrimidin-2-yl)amino)benzenesulfonamide 4b.**

Yield 31%, mp 228-230 °C. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm) 3.85 (s, 3H, -OCH<sub>3</sub>), 4.05 (s, 2H, CH<sub>2</sub>-S), 6.98-7.01 (m, 2H, Ar-H), 7.19 (bs, 2H, NH<sub>2</sub>*exch.*), 7.76 (d, 2H, Ar-H, *J*=8.8 Hz), 7.98 (d, 2H, Ar-H, *J* = 9.2 Hz), 8.27 (d, 1H, Ar-H, *J*= 8.8 Hz), 8.48 (s, 1H, Ar-H), 10.07 (bs, 1H, NH<sub>2</sub>*exch.*). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ (ppm) 26.64, 56.09, 112.68, 113.63, 115.47, 118.10, 125.11, 127.08, 129.36, 136.40, 139.0, 144.26, 156.50, 158.42, 159.33, 161.93.

**4-((8-Chloro-5*H*-thiochroman[4,3-*d*]pyrimidin-2-yl)amino)benzenesulfonamide 4c.**

Yield 45%, mp 268- 270 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm) 4.10 (s, 2H, CH<sub>2</sub>-S), 7.21 (s, 2H, NH<sub>2</sub>*exch.*), 7.47-7.50 (dd, 1H, Ar-H, *J*<sub>max</sub> = 8.6, *J*<sub>min</sub> = 2.4 Hz), 7.60 (d, 1H, Ar-H, *J* = 2.4 Hz), 7.77 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.97 (d, 2H, Ar-H, *J* = 8.8 Hz), 8.30 (d, 1H, Ar-H, *J* = 8.8 Hz),

8.57(s, 1H, Ar-H), 10.19 (s, 1H, NH<sub>exch.</sub>). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ (ppm) 26.40, 116.27, 118.25, 126.94, 127.12, 127.68, 129.20, 131.22, 136.41, 136.67, 139.46, 144.05, 157.24, 157.59, 159.43.

**4-((5*H*-Pyrido[3',2':5,6]thiopyrano[4,3-*d*]pyrimidin-2-yl)amino)benzenesulfonamide 4d.**

Yield 45%, mp 283-285 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm) 4.23 (s, 2H, CH<sub>2</sub>-S), 7.19 (s, 2H, NH<sub>2exch.</sub>), 7.41-7.44 (m, 1H, Ar H), 7.78 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.97 (d, 2H, Ar-H, *J* = 8.8 Hz), 8.52-8.54 (m, 1H, Ar-H), 8.57-8.59 (m, 1H, Ar-H), 8.60 (s, 1H, Ar-H), 10.18 (bs, 1H, NH<sub>exch.</sub>). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ (ppm) 26.31, 115.62, 118.36, 121.84, 127.14, 128.66, 134.75, 136.76, 143.95, 151.90, 157.57, 157.75, 159.41, 160.13.

**4-((8-Methyl-5*H*-pyrido[3',2':5,6]thiopyrano[4,3-*d*]pyrimidin-2-yl)amino)benzenesulfonamide 4e.**

Yield 45%, mp 283-285 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm) 4.20 (s, 2H, CH<sub>2</sub>-S), 7.21 (s, 2H, NH<sub>2exch.</sub>), 7.27 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.96 (d, 2H, Ar-H, *J* = 8.8 Hz), 8.46 (d, 2H, Ar-H, *J* = 8.0 Hz), 8.56 (s, 1H, Ar-H), 10.17 (s, 1H, NH<sub>exch.</sub>). <sup>13</sup>C NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm) 24.42, 26.36, 115.20, 118.27, 121.38, 125.97, 127.13, 135.01, 136.66, 144.01, 157.31, 157.98, 159.36, 159.46, 161.28.

**4.2. CA inhibition.** An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO<sub>2</sub> hydration activity.<sup>28</sup> Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na<sub>2</sub>SO<sub>4</sub> (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO<sub>2</sub> hydration reaction for a period of 10-100 s. The CO<sub>2</sub> concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5-10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation, as reported earlier,<sup>29-31</sup> and represent the mean from at least three different determinations. All CA isofoms were recombinant enzymes obtained in-house as reported earlier.<sup>30-32</sup>

ACCEPTED MANUSCRIPT



## References

1. Marini, A.M.; Maresca, A.; Aggarwal, M.; Orlandini, E.; Nencetti, S.; Da Settimo, F.; Salerno, S.; Simorini, F.; La Motta, C.; Taliani, S.; Nuti, E.; Scozzafava, A.; McKenna, R.; Rossello, A.; Supuran C.T., *J. Med. Chem.* **2012**, *55*, 9619.
2. a) Krishnamurthy, V. M.; Kaufman, G. K.; Urbach, A. M.; Gitlin, I.; Gudiksen, K. L.; Weibel, D. B.; Whitesides, G. M. *Chem. Rev.* **2008**, *108*, 946; b) Supuran, C. T. *Nat. Rev. Drug Discovery* **2008**, *7*, 168; c) Supuran, C.T. *Bioorg. Med. Chem.* **2013**, *21*, 1377; d) Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, in press (doi: 10.3109/14756366.2015.1122001).
3. a) Di Fiore, A.; Pedone, C.; D'Ambrosio, K.; Scozzafava, A.; De Simone, G.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 437; b) Weber, A.; Casini, A.; Heine, A.; Kuhn, D.; Supuran, C. T.; Scozzafava, A.; Klebe, G. *J. Med. Chem.* **2004**, *47*, 550.
4. a) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Mincione, G.; Supuran, C.T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 575; b) Borrás, J.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C.T. *Bioorg. Med. Chem.* **1999**, *7*, 2397; c) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C.T. *J. Med. Chem.* **1999**, *42*, 2641; d) Scozzafava, A.; Briganti, F.; Mincione, G.; Menabuoni, L.; Mincione, F.; Supuran, C.T. *J. Med. Chem.* **1999**, *42*, 3690.
5. a) Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. *Chem. Rev.* **2012**, *112*, 4421; b) Neri, D.; Supuran, C. T. *Nat. Rev. Drug Discovery* **2011**, *10*, 767; c) Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 229; d) Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 759; e) Aggarwal, M.; Boone, C.D.; Kondeti, B.; McKenna, R. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 267.
6. a) Dogne, J. M.; Hanson, J.; Supuran, C. T.; Pratico, D. *Curr. Pharm. Des.* **2006**, *12*, 971; b) Dogne, J. M.; Thiry, A.; Pratico, D.; Masereel, B.; Supuran, C.T. *Curr. Top. Med. Chem.* **2007**, *7*, 885; c) Dogne, J. M.; Supuran, C. T.; Pratico, D. *J. Med. Chem.* **2005**, *48*, 2251; d) Supuran, C.T. *Expert Rev. Neurother.* **2015**, *15*, 851.
7. a) Knaus, E. E.; Innocenti, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5892; b) Supuran, C. T.; Casini, A.; Mastrolorenzo, A.; Scozzafava, A. *Mini. Rev. Med. Chem.* **2004**, *4*, 625; c) Capasso, C.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 379; d) De Simone, G.; Di Fiore, A.; Supuran, C.T. *Curr. Pharm. Des.* **2008**, *14*, 655.
8. a) Pacchiano, F.; Carta, F.; McDonald, P.C.; Lou, Y.; Vullo, D.; Scozzafava, A.; Dedhar, S.; Supuran, C.T. *J. Med. Chem.* **2011**, *54*, 1896; b) Krall, N.; Pretto, F.; Decurtins, W.; Bernardes, G. J. L.; Supuran, C. T.; Neri, D., *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 4231; c) Gieling, R.G.; Parker, C.A.; De Costa, L.A.; Robertson, N.; Harris, A.L.; Stratford, I.J.; Williams, K.J.



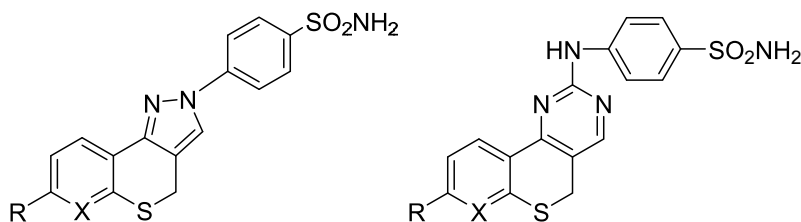
- J. Enzyme Inhib. Med. Chem.* **2013**, 28, 360; d) Dubois, L.; Lieuwes, N.G.; Maresca, A.; Thiry, A.; Supuran, C.T.; Scozzafava, A.; Wouters, B.G.; Lambin, P. *Radiother. Oncol.* **2009**, 92, 423.
9. Dalla Via, L.; Marini, A.M.; Salerno, S.; La Motta, C.; Condello, M.; Arancia, G.; Agostinelli, E.; Toninello, A. *Bioorg. Med. Chem.* **2009**, 17, 326.
  10. Da Settimo, A.; Marini, A. M.; Primofiore, G.; Da Settimo, F.; Salerno, S.; Simorini, F.; Pardi, G.; La Motta, C. and Bertini, D. *J. Heterocyclic Chem.* **2002**, 39, 1001.
  11. Primofiore, G.; Marini, A. M.; Da Settimo, F.; Salerno, S.; Bertini, D.; Dalla Via, L.; Marciani Magno, S. *J. Heterocyclic Chem.* **2003**, 40, 783.
  12. Primofiore, G.; Marini, A. M.; Salerno, S.; Da Settimo, F.; Bertini, D.; Dalla Via, L. *J. Heterocyclic Chem.* **2005**, 42, 1357.
  13. Marini, A. M.; Da Settimo, F.; Salerno, S.; La Motta, C.; Simorini, F.; Taliani, S.; Bertini, D.; Gia, O.; Dalla Via, L. *J. Heterocycl. Chem.*, **2008**, 45, 745.
  14. a) De Simone, G.; Supuran, C. T. *J. Inorg. Biochem.* **2012**, 111, 117; b) Carta, F.; Temperini, C.; Innocenti, A.; Scozzafava, A.; Kaila, K.; Supuran, C.T. *J. Med. Chem.* **2010**, 53, 5511; c) Akbaba, Y.; Akincioglu, A.; Göçer, H.; Göksu, S.; Gülçin, I.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, 29, 35.
  15. a) Parkkila, S.; Vullo, D.; Maresca, A.; Carta, F.; Scozzafava, A.; Supuran, C.T. *Chem. Commun.* **2012**, 48, 3551; b) Temperini, C.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, 20, 474.
  16. Pan, J.; Lau, J.; Mesak, F.; Hundal, N.; Pourghiasian, M.; Liu, Z.; Benard, F.; Dedhar, S.; Supuran, C.T.; Lin, K.S. *J. Enzyme Inhib. Med. Chem.* **2014**, 29, 249; b) Demirdağ, R.; Yerlikaya, E.; Şentürk, M.; Küfrevioğlu, Ö.I.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, 28, 278; c) Sharma, A.; Tiwari, M.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, 29, 292; d) Şentürk, M.; Gülçin, I.; Daştan, A.; Küfrevioğlu, Ö.I.; Supuran, C.T. *Bioorg. Med. Chem.* **2009**, 17, 3207; e) Bayram, E.; Senturk, M.; Kufrevioglu, O.I.; Supuran, C.T. *Bioorg. Med. Chem.* **2008**, 16, 9101.
  17. Carta, F.; Aggarwal, M.; Maresca, A.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *Chem. Commun.* **2012**, 48, 1868.
  18. Monti, S. M.; Maresca, A.; Carta, F.; De Simone, G.; Mulschlegel, F. A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2012**, 22, 859.
  19. Carta, F.; Aggarwal, M.; Maresca, A.; Scozzafava, A.; McKenna, R.; Masini, E.; Supuran, C. T. *J. Med. Chem.* **2012**, 55, 1721.
  20. a) De Simone, G.; Alterio, V.; Supuran, C.T. *Expert Opin. Drug Discov.* **2013**, 8, 793; b) Di Fiore, A.; Maresca, A.; Alterio, V.; Supuran, C. T.; De Simone, G. *Chem. Commun.* **2011**, 47,

- 11636; c) Supuran, C.T.; Clare, B.W. *Eur. J. Med. Chem.* **1999**, *34*, 41; d) Del Prete, S.; Vullo, D.; De Luca, V.; Supuran, C.T.; Capasso, C. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 906.
21. a) Biswas, S.; Aggarwal, M.; Guzel, O.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *Bioorg. Med. Chem.* **2011**, *19*, 3732; b) Biswas, S.; Carta, F.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *Bioorg. Med. Chem.* **2013**, *21*, 2314; c) Bozdog, M.; Pinard, M.; Carta, F.; Masini, E.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *J. Med. Chem.* **2014**, *57*, 9673; d) Carta, F.; Di Cesare Mannelli, L.; Pinard, M.; Ghelardini, C.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *Bioorg. Med. Chem.* **2015**, *23*, 1828; e) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C.T. *J. Med. Chem.* **2000**, *43*, 4542.
22. a) Smith, K. S.; Jakubzick, C.; Whittam, T. S.; Ferry, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 15184; b) Zimmerman, S. A.; Ferry, J. G.; Supuran, C. T. *Curr. Top. Med. Chem.* **2007**, *7*, 901; c) Del Prete, S.; De Luca, V.; Scozzafava, A.; Carginale, V.; Supuran, C.T.; Capasso, C. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 23; d) Del Prete, S.; De Luca, V.; Vullo, D.; Scozzafava, A.; Carginale, V.; Supuran, C.T.; Capasso, C. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 532.
23. a) Nishimori, I.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C.T. *J. Med. Chem.* **2005**, *48*, 7860; b) Durdagi, S.; Scozzafava, G.; Vullo, D.; Sahin, H.; Kolayli, S.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 469; c) Abbate, F.; Supuran, C.T.; Scozzafava, A.; Orioli, P.; Stubbs, M.T.; Klebe, G. *J. Med. Chem.* **2002**, *45*, 3583; d) Briganti, F.; Pierattelli, R.; Scozzafava, A.; Supuran, C.T. *Eur. J. Med. Chem.* **1996**, *31*, 1001; e) Abbate, F.; Winum, J.Y.; Potter, B.V.; Casini, A.; Montero, J.L.; Scozzafava, A.; Supuran, C.T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 231.
24. Bruno, O.; Schenone, S.; Ranise, A.; Bondavalli, F.; Filippelli, W.; Falcone, G.; Motola, G.; Mazzeo, F. *Farmaco* **1999**, *54*, 95.
25. S. Schenone, O. Bruno, A. Ranise, F. Bondavalli, M.L. Cenicola, C. Losasso, M. Carnevale, R. Ottavo, M. D'Antonio, *Farmaco* **1990**, *45*, 1309.
26. Chu, S.L.; Chyan, W.H.; Chang, C.C. *Huaxue Xuebao* **1956**, *22*, 371.
27. Harris, P.A.; Jung, D.K.; Peel, M.R.; Reno, M.J.; Rheault, T.R.; Stanford, J.B.; Stevens, Kirk L.; Veal, J.M.; Badiang, J.G.; *WO Pat.* **2003**, 051886, 20030626.
28. Khalifah, R.J. *J. Biol. Chem.* **1971**, *246*, 2561.
29. a) Santamaria, S.; Nuti, E.; Cercignani, G.; La Regina, G.; Silvestri, R.; Supuran, C.T.; Rossello, A. *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 947; b) Abdel-Aziz, A.A.; El-Azab, A.S.; Ceruso, M.; Supuran, C.T. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5185; c) El-Azab, A.S.; Abdel-Aziz, A.A.; Ayyad, R.R.; Ceruso, M.; Supuran, C.T. *Bioorg. Med. Chem.* **2016**, *24*, 20.

30. a) Maresca, A.; Carta, F.; Vullo, D.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, 28, 407;  
 b) Ekinici, D.; Kurbanoglu, N.I.; Salamci, E.; Senturk, M.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2012**, 27, 845; c) Ekinici, D.; Karagoz, L.; Ekinici, D.; Senturk, M.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, 28, 283; d) Alp, C.; Maresca, A.; Alp, N.A.; Gültekin, M.S.; Ekinici, D.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, 28, 294; e) Bilginer, S.; Unluer, E.; Gul, H.I.; Mete, E.; Isik, S.; Vullo, D.; Beyaztas, S.; Ozensoy-Guler, O.; Capasso, C.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, 29, 495.
31. a) Liu, F.; Martin-Mingot, A.; Lecornué, F.; Maresca, A.; Thibaudeau, S.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2012**, 27, 886; b) Demirdağ, R.; Yerlikaya, E.; Şentürk, M.; Küfrevioğlu, Ö.I.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, 28, 278; c) Singh, S.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, 29, 449; d) Ceruso, M.; Vullo, D.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, 29, 686.
32. a) Maresca, A.; Vullo, D.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, 28, 388; b) Koz, O.; Ekinici, D.; Perrone, A.; Piacente, S.; Alankus-Caliskan, O.; Bedir, E.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, 28, 412; c) Supuran, C.T.; Maresca, A.; Gregañ, F.; Remko, M. *J. Enzyme Inhib. Med. Chem.* **2013**, 28, 289; d) Migliardini, F.; De Luca, V.; Carginale, V.; Rossi, M.; Corbo, P.; Supuran, C. T.; Capasso, C. *J. Enzyme Inhib. Med. Chem.* **2014**, 29, 146; e) Singh, S.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, 29, 877.

**Sulfonamides incorporating heteropolycyclic scaffolds show potent inhibitory action against carbonic anhydrase isoforms I, II, IX and XII**

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$K_{IS}$  (hCA IX) = 6.1 - 92.7 nM