

Accepted Manuscript

Synthesis and carbonic anhydrase I, II, IX and XII inhibitory activity of sulfamates incorporating piperazinyl-ureido moieties

Cenzo Congiu, Valentina Onnis, Alessandro Deplano, Gianfranco Balboni, Mariangela Ceruso, Claudiu T. Supuran

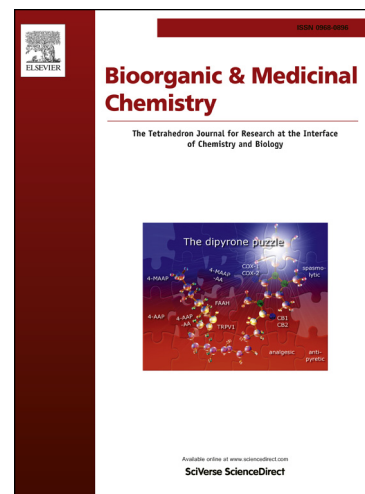
PII: S0968-0896(15)00608-2
DOI: <http://dx.doi.org/10.1016/j.bmc.2015.07.024>
Reference: BMC 12453

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 17 June 2015
Revised Date: 13 July 2015
Accepted Date: 14 July 2015

Please cite this article as: Congiu, C., Onnis, V., Deplano, A., Balboni, G., Ceruso, M., Supuran, C.T., Synthesis and carbonic anhydrase I, II, IX and XII inhibitory activity of sulfamates incorporating piperazinyl-ureido moieties, *Bioorganic & Medicinal Chemistry* (2015), doi: <http://dx.doi.org/10.1016/j.bmc.2015.07.024>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Synthesis and carbonic anhydrase I, II, IX and XII inhibitory activity of sulfamates incorporating piperazinyl-ureido moieties

Cenzo Congiu,^{a,*} Valentina Onnis,^a Alessandro Deplano,^a Gianfranco Balboni,^a Mariangela Ceruso,^b and Claudiu T. Supuran^{b,c,*}

^aDepartment of Life and Environmental Sciences, Unit of Pharmaceutical, Pharmacological and Nutraceutical Sciences, University of Cagliari, via Ospedale 72, Cagliari, I-09124, Italy.

^bUniversità degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, 50019 Sesto Fiorentino (Florence); Italy.

^cUniversità degli Studi di Firenze, NEUROFARBA Department, Section of Pharmaceutical and Nutraceutical Chemistry, Via Ugo Schiff 6, 50019 Sesto Fiorentino (Florence); Italy.

Abstract: A series of sulfamates were synthesized using as lead compound SLC-0111, a sulfonamide carbonic anhydrase (CA, EC 4.2.1.1) inhibitor in Phase I clinical trials. The new derivatives incorporated ureido moieties as spacers between the benzene sulfamate fragment which binds the zinc ion from the active site, and the tail of the inhibitor, but the urea moieties were part of a substituted piperazine ring system. The derivatives (and some of their phenol precursors) were tested for the inhibition of the cytosolic, hCA I and II (off-target isoforms) and the trans-membrane, tumor-associated hCA IX and XII enzymes (anticancer drug targets). Generally hCA I was not effectively inhibited, whereas many low nanomolar inhibitors were evidenced against hCA II (K_i s in the range of 1.0 – 94.4 nM) IX (K_i s in the range of 0.91 – 36.9 nM), and XII (K_i s in the range of 1.0 – 84.5 nM). The best substitution fragments at the piperazine ring included the following moieties: 3-methylphenyl, 2,3-dimethylphenyl, 4-methoxyphenyl, 6-arylpyrimidine-2-yl.

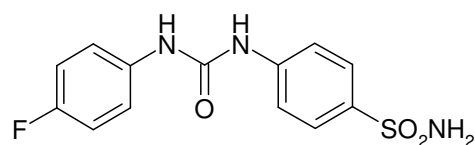
Keywords: carbonic anhydrase; sulfamate; inhibitor; piperazine; tumor-associated isoform

*Correspondence authors. Tel +39-070-675-8630; Fax: +39-070-675-8612; E-mail: ccongiu@unica.it (CC); Phone: +39-055-4573005; Fax: +39-055-4573385; E-mail: claudiu.supuran@unifi.it (CTS).

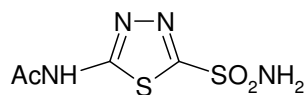
1. Introduction

The carbonic anhydrases (CAs, EC 4.2.1.1) represent a superfamily of widespread enzymes which catalyze a crucial biochemical reaction, the reversible hydration of CO₂ to bicarbonate and protons.¹⁻⁴ These three simple ions/molecules acting as substrates/reaction products in this process are involved in a range of physiologic processes related to pH regulation, electrolyte secretion, chemosensing, biosynthetic reactions involving carboxylating enzymes (gluconeogenesis, lipogenesis, ureagenesis, etc.), and as thus, their formation is tightly controlled in organisms all over the phylogenetic tree.¹⁻⁴ This is probably the reason why at least six unrelated genetic families encode for CAs (the α , β , γ , δ , ζ and therecently proposed group of η -CAs).⁵⁻⁸ Furthermore, in most organisms investigated in detail, a large number of isoforms of the CA family are present, which possess specialized functions in various tissues/organs, also having a diversified catalytic activity, and susceptibility to be inhibited or activated by various modulators of activity.¹⁻⁴ For example, in humans 15 α -CA isoforms were described, hCA I-hCA VA, hCA VB-hCA XIV, whereas the non-primate mammals have an additional isoform hCA XV. In mammals the isoform hCA XVII has been lost, but it is however present in other vertebrates.⁹ Such a multitude of CA isoforms present in these organisms and their diverse subcellular localization (as there are cytosolic, mitochondrial, trans-membrane and secreted CA isoforms)^{1-4,9} prove that the high amounts of CO₂ generated in metabolic processes must be efficiently converted to bicarbonate (plus protons), and indeed, some α -CAs are among the most effective catalysts known in nature.¹⁰

There are some forms of cancer in which some CA isoforms were observed to be overexpressed, such as CA IX and XII and more rarely CA II.¹¹ CA II is also involved in other pathologies such as acute mountain sickness (AMS) and apparently, atherosclerosis and osteoporosis.¹² Many of the hCAs are drug targets for diuretics, anticonvulsants, antiglaucoma agents, and antitumor/antimetastatic drugs.^{1-4,13} Recently a sulfonamide CA inhibitor (CAI) developed by our group, SLC-0111¹⁴ entered Phase I clinical trials for the treatment of patients with advanced solid, metastatic tumors overexpressing CA IX/XII.¹⁴ Unlike the prototypical sulfonamide CAI acetazolamide (AAZ), SLC-0111 is a selective inhibitor for the trans-membrane, tumor-associated isoforms hCA IX/XII over the cytosolic widespread isoforms hCA I and II.¹⁴



SLC-0111



AAZ

One of the main features of SLC-0111 and the series of compounds to which it belongs,^{14a} is the presence of the ureido functionality as linker between the benzenesulfonamide fragment (which binds to the metal ion from the CA active site) and the tail of the inhibitor. By means of X-ray crystallography (for SLC-0111 and a series of other four of its congeners complexed to hCA II), we demonstrated that the reasons for the isoform selectivity is the ureido linker, which allows a great flexibility to the tails present in these molecules and

the possibility for the inhibitor to adopt a variety of orientations when complexed within the enzyme active site.^{14a,d} These tails bind towards the entrance of the CA active site cavity, which is the most diverse region among the many isoforms with medicinal chemistry applications, such as for example CA I, II, IX and XII. As few CAIs incorporating ureido linkers were reported so far, in this paper we report a novel such series. A series of sulfamates incorporating the ureido linker mentioned above and substituted piperazinyl moieties were prepared and assayed for the inhibition of the two widespread cytosolic isoforms hCA I and II, as well as the transmembrane, tumor-associated ones hCA IX and XII.

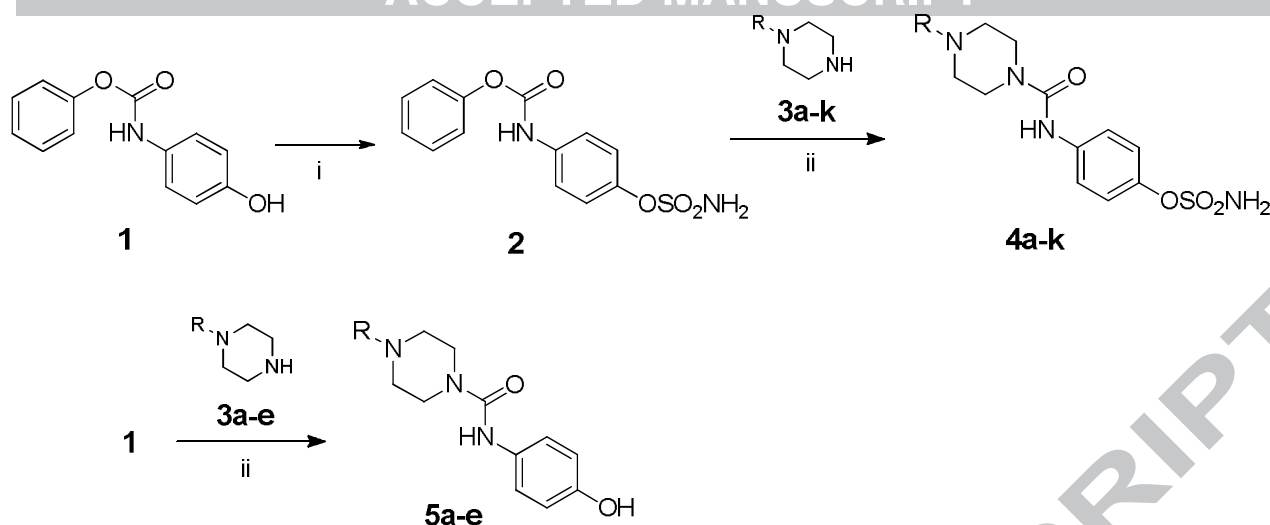
2. Results and Discussion

2.1. Chemistry.

The rationale for the drug design of the compounds reported here is a rather simple one. We used SLC-0111 as lead molecule, as this compound is a low nanomolar, CA IX/XII-selective inhibitor (over CA I and II), with a significant antitumor activity in animal models of hypoxic tumors, and it also shows a good bioavailability.^{13,14}

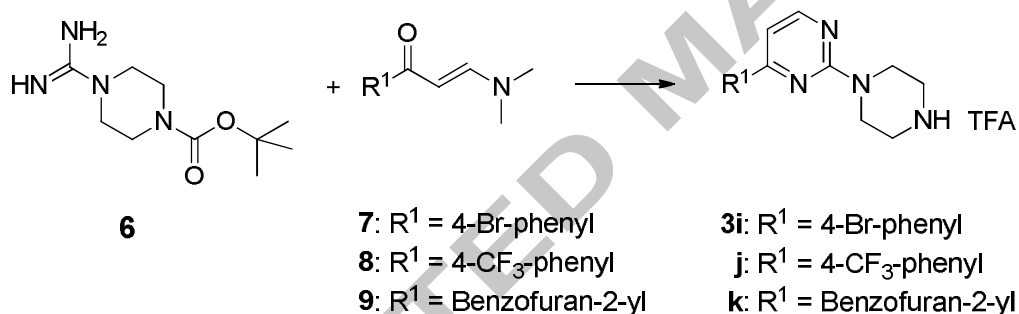
We report here a series of compounds possessing two major modifications compared to the lead SLC-0111: (i) the sulfonamide zinc-binding group (ZBG) from the lead was replaced by the isosteric sulfamate¹⁵ one in the compounds, and (ii) the left part of the ureido moiety from the lead SLC-0111 was incorporated into a more rigid heterocyclic system, i.e., the substituted piperazine one. We incorporated into the scaffolds of the new sulfamates a large number of aromatic/heterocyclic moieties (substituting the second nitrogen atom from the piperazine ring) in order to generate chemical diversity and also because the terminal part of the inhibitor, far from the ZBG, usually interacts with the entrance of the CA active site cavity.¹⁻⁴ As that is the region with most diverse amino acid residues among the different α -CAs, selective inhibition may be achieved through particular interactions between the tail of the inhibitor and this region of the active site, as demonstrated by a large number of X-ray crystallographic studies of enzyme-inhibitor complexes.^{1,3,4}

The synthesis of the new compounds is reported in Schemes 1 and 2. Sulfamoylation of phenyl carbamate **1**¹⁶ upon treatment with sulfamoyl chloride¹⁷ in *N,N*-dimethylacetamide (DMA) solution furnished the key intermediate **2** in 82% yield. Coupling of **2** with 1-substituted piperazines **3a-k** in DMSO solution gave the piperazinyl urea derivatives **4a-k** in 42-93% yields. In a similar manner, the phenyl carbamate **1** reacted with piperazines **3a-e** to produce the phenolic derivatives **5a-e** in 56-88% yields (Scheme 1). The pyrimidinyl-piperazines **3i-k** were synthesized by heterocyclization of 4-Boc-piperazine-1-carboxamide **6**¹⁸ with 3-(dimethylamino)propanones **7-9** in boiling *n*-propanol, followed by trifluoroacetic acid (TFA)-mediated deprotection in dichloromethane (DCM) solution (Scheme 2). Indeed, phenols were also reported to act as CAIs, although their mechanism of action is different compared to the sulfonamides, as they anchor to the zinc-coordinated water molecule.¹⁹



3-5a: R = benzyl	e: R = 3-Me-phenyl	i: R = 4-(4-Br-phenyl)pyrimidin-2-yl
b: R = phenyl	f: R = 2,3-Me ₂ -phenyl	j: R = 4-(4-CF ₃ -phenyl)pyrimidin-2-yl
c: R = 4-F-phenyl	g: R = 3-MeO-phenyl	k: R = 4-benzofuran-2-ylpyrimidin-2-yl
d: R = 4-Cl-phenyl	h: R = 4-MeO-phenyl	

Scheme 1. Reagents and conditions: i) Sulfamoyl chloride, DMA, 0 °C to r.t., overnight; ii) DMSO, r.t., 18h.



7: R ¹ = 4-Br-phenyl	3i: R ¹ = 4-Br-phenyl
8: R ¹ = 4-CF ₃ -phenyl	j: R ¹ = 4-CF ₃ -phenyl
9: R ¹ = Benzofuran-2-yl	k: R ¹ = Benzofuran-2-yl

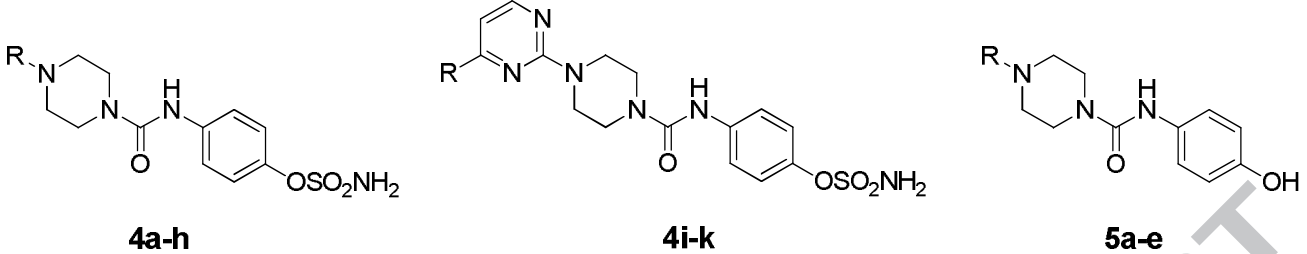
Scheme 2. Reagents and conditions: i) 1-PrOH, reflux, 6h; ii) TFA, DCM, r.t., overnight.

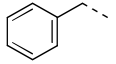
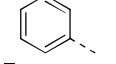
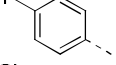
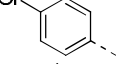
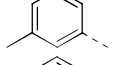
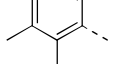
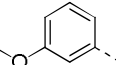
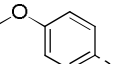
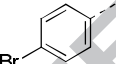

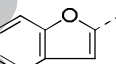
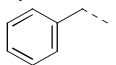
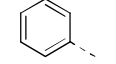
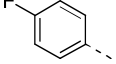
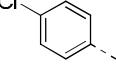
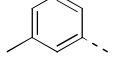
2.2. CA inhibition

Sulfamates **4a-4k** and phenols **5a-5e** were screened²⁰ for the inhibition of four human (h) CA isoforms involved in important physiologic/pathologic processes, i.e., the cytosolic, hCA I and II (off-targets in this case) and the trans-membrane, tumor-associated hCA IX and XII (anticancer drug targets).¹¹⁻¹³ Table 1 shows inhibition data of the derivatives reported here and the sulfonamide acetazolamide **AAZ** (as standard inhibitor) against hCA I, II, IX and XII, after a period of 15 min of incubation of the enzyme and inhibitor solutions.²⁰⁻²³ The following structure activity relationship (SAR) may be noted regarding the inhibition data of Table 1:

(i) The slow cytosolic isoform hCA I was effectively inhibited by one sulfamate (**4c**, K_I of 9.4 nM), rather well inhibited by other three sulfamates from the new series (**4f**, **4g** and **4k**, with inhibition constants ranging between 63.5 and 88.1 nM, Table 1) whereas the remaining sulfamates were much weaker inhibitors (K_s in the

Table 1: Inhibition data of human (h) CA isoforms hCA I, II, IX and XII with sulfamates **4a-k** and phenols **5a-e**, and the standard sulfonamide inhibitor acetazolamide (**AAZ**) by a stopped flow CO₂hydrase assay.²⁰



Compound	R	K _I (nM)*			
		hCA I	hCA II	hCA IX	hCA XII
4a		316.5	11.7	10.5	1.0
4b		896.8	71.9	11.1	1.0
4c		9.4	18.2	61.5	64.7
4d		581.3	16.9	10.4	84.5
4e		851.5	15.9	0.91	35.8
4f		63.5	11.1	32.3	1.0
4g		88.1	11.2	34.1	37.0
4h		282.4	9.1	114.1	1.1
4i		277.2	1.2	113.6	1.0
4j		290.9	1.1	24.3	1.0
4k		70.2	1.0	6.7	1.0
5a		>10000	94.4	112.9	7.9
5b		300.4	81.6	120.7	7.1
5c		799.8	5935.6	36.9	6.3
5d		>10000	519.5	80.5	8.6
5e		5296.1	1427.7	549.2	9.5
AAZ	-	250	12	25	5.7

* Mean from 3 different assays (errors were in the range of \pm 5-10 % of the reported values).

range of 277.2 – 896 nM. It may be observed that the effective hCA I inhibitors incorporate 4-fluorophenyl-, 2,3-dimethylphenyl- and 3-methoxyphenyl moieties at the 4-nitrogen atom of the piperazine ring, whereas

small differences in the nature of the R moiety or the substitution pattern (e.g., F from **4c** replaced by H or Cl, as in **4b** and **4d**) lead to dramatic changes in the inhibition constant of the inhibitor, proving probably that our working hypothesis (i.e., interaction of the tail from the inhibitor molecule with the amino acid residues at the entrance of the active site) is correct. The phenols **5** were also poorly active (**5b** and **5c** with K_{IS} in the range of 300.4 – 799.8 nM) or inactive ($K_{IS} > 5 \mu\text{M}$, for **5a**, **5d** and **5e**, Table 1). Acetazolamide (AAZ) is a medium potency hCA I inhibitor with a K_I of 250 nM.

(ii) hCA II, the dominant physiologic isoform,¹ was potently inhibited by all sulfamates**4**, with K_{IS} in the range of 1.0 – 71.9 nM, in the same activity range as AAZ (K_I of 12 nM). Thus, the SAR is quite flat in this case, although three compounds (**4i**, **4j** and **4k**) have inhibition constants of 1.0 – 1.2 nM being thus an order of magnitude more effective inhibitors compared to the remaining ones. All of them incorporate the long tail based on the 6-substituted-pyrimidine scaffold. It is thus obvious that the compounds with this elongated scaffold make excellent contacts with the rim of the active site entrance of hCA II, leading thus to low nanomolar inhibition. The remaining sulfamates (**4a-4g**) incorporating benzyl or substituted phenyl moieties at the piperazine ring also showed a rather compact inhibitory power with K_{IS} in the range of 11.1 – 18.2 nM, except for **4b**, which was the weakest inhibitor in the series, with a K_I of 71.9 nM. In fact this derivative does not have substituents on the phenyl ring, which as it may be observed, is detrimental for the hCA II inhibition. Two of the reported phenols (**5a** and **5b**) had $K_{IS} < 100$ nM whereas the remaining ones were ineffective as hCA II inhibitors (Table 1).

(iii) hCA IX, the tumor-associated isoform, was also effectively inhibited by many of the sulfamates reported here. Indeed, compounds **4a**, **4b**, **4d-4g**, **4j**, **4k** and **5c** showed K_{IS} in the range of 0.91 – 36.9 nM, being thus more effective or in the same range of activity as AAZ, a compound for which a notable anticancer activity was reported in some animal models of hypoxic tumors.²⁴ It may be noted that for the sulfamates the substitution patterns at the piperazine ring leading to the best hCA IX inhibition included the 3-methylphenyl moiety (in **4e**), the benzofuran-pyrimidinyl (in sulfamate**4k**) as well as the 4-fluorophenyl in the phenol **5c**. For the pair **4c/5c**, it is noteworthy that the phenol shows a better hCA IX inhibition profile compared to the sulfamate, whereas for all other sulfamate/phenol pairs, the sulfamate was a much more potent enzyme inhibitor compared to the corresponding phenol (Table 1). The remaining sulfamates/phenols (**4c**, **4h**, **4i**, **5a**, **5b**, **5d**) showed moderate hCA IX inhibitory activity with K_{IS} in the range of 61.5 – 120.7 nM. The weakest inhibitor in the series of investigated compounds was the phenol **5e**, with a K_I of 549.2 nM (Table 1).

(iv) A very interesting inhibition profile with the sulfamates/phenols investigated here was observed for the second transmembrane isoform, hCA XII. Indeed, many sulfamates (**4a**, **4b**, **4f**, **4h-4k**) had a very potent inhibitory effect with K_{IS} varying very little (the range of 1.0 – 1.1 nM) whereas the remaining ones (**4c-4e** and **4g**) were slightly less potent (K_{IS} in the range of 35.8 – 84.5 nM) Thus, most of the substitution patterns present in these sulfamates are beneficial for obtaining very effective hCA XII inhibitors (e.g., benzyl, phenyl, 2,3-dimethylphenyl, 4-methoxyphenyl, substituted-pyrimidinyl). The phenols on the other hand were also highly effective hCA XII inhibitors, with K_{IS} in the range of 6.3 – 9.5 nM (the same as AAZ). Basically hCA XII was the most sensitive isoform to be inhibited by the compounds reported here.

(v) The compounds reported here do not show a marked isoform-selectivity effect (as compared to the SLC-0111 series used as lead).^{14a} However many of the new sulfamates/phenols showed excellent hCA II and XII inhibitory effects (in the low nanomolar range) and good selectivity for these two isoforms over hCA I and IX, which is a very interesting profile not observed earlier with any other class of CAIs. As hCA II and XII are the two isoforms mainly involved in glaucoma,^{4,12} it would be of interest to test some of these derivatives in vivo for antiglaucoma effects.

3. Conclusions

A series of sulfamates were synthesized using SLC-0111, a sulfonamide CAI in Phase I clinical trials, as lead compound. The new derivatives incorporated ureido moieties as spacers between the benzene sulfamate fragment which binds the zinc ion from the active site, and the tail of the inhibitor, but the urea moieties were part of a substituted piperazine ring system. The derivatives (and some of their phenol precursors) were tested for the inhibition of the cytosolic, hCA I and II (off-target isoforms) and the transmembrane, tumor-associated hCA IX and XII enzymes (anticancer drug targets). Generally hCA I was not effectively inhibited, whereas many low nanomolar inhibitors were evidenced against hCA II (K_{iS} in the range of 1.0 – 94.4 nM) IX (K_{iS} in the range of 0.91 – 36.9 nM), and XII (K_{iS} in the range of 1.0 – 84.5 nM). The best substitution fragments at the piperazine ring included the following moieties: 3-methylphenyl, 2,3-dimethylphenyl, 4-methoxyphenyl, 6-arylpyrimidine-2-yl.

4. ExperimentalSection

4.1. Chemistry.

Unless otherwise noted, all solvents, including anhydrous solvents and chemicals, were purchased from Aldrich Co. and/or Alfa Aesar, and used without further purification. Melting points were recorded on a Stuart Scientific melting point SMP1 apparatus and are uncorrected. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing Finnigan MAT 95 instrument with BE geometry. Proton nuclear magnetic resonance spectra were recorded on a Varian Inova 500 spectrometer at 500 MHz, in DMSO- d_6 as the solvent, and TMS as the internal standard. Chemical shifts are expressed in ppm relative to tetramethylsilane. Splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). Infrared spectra were run on Bruker Vector 22 spectrophotometer. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F₂₅₄ Merck plates). Developed plates were visualized by a Spectroline ENF 260C/F UV apparatus. Concentration and evaporation of the solvent after reaction or extraction were carried out on a rotary evaporator (BüchiRotavapor) operating at reduced pressure. Elemental analyses were carried out with a Carlo Erba model 1106 elemental analyzer, and all values were within 0.4% of the calculated values, which indicates >95% purity of the tested compounds.

4-(Phenoxy carbonyl)aminophenylsulfamate (2). To a stirred ice-cooled solution of phenyl (4-hydroxyphenyl)carbamate (**1**) (4.6 g, 20 mmol) in anhydrous DMA (50 mL), freshly prepared sulfamoyl chloride (60 mmol) in DMA (10 mL) was added dropwise in 30 min. The reaction mixture was stirred at room temperature overnight, then water (100 mL) was added; the mixture was stirred for additional 2 h, the formed solid filtered off, and vacuum dried to give sulfamate **2** in good purity to be used in the next step without further purification. 5.01 g, 82% yield, mp 165-168 °C. ESI-MS (m/z): 309 ($M + H$)⁺. ¹HNMR: δ 7.23 (m, 4H), 7.43 (m, 3H), 7.55 (m, 2H), 7.91 (s, 2H), 10.32 (s, 1H); IR (Nujol) 3383, 3336, 3242, 1726 cm⁻¹.

Preparation of 1-(4-arylpyrimidin-2-yl)piperazines 3i-k. Typical procedure: 1-(4-(4-bromophenyl)pyrimidin-2-yl)piperazine (3i). A solution of 1-(4-bromophenyl)-3-(dimethylamino)prop-2-en-1-one²⁵ (**7**, 0.51 g, 2 mmol) and 4-(*tert*-butoxycarbonyl)piperazine-1-carboxamide (**6**, 0.46 g, 2 mmol) in anhydrous 1-PrOH (5 mL) was refluxed 6 h. The solvent was removed under reduced pressure, the residue was suspended in dichloromethane (10 mL) and treated with trifluoroacetic acid (5 mL). The mixture was stirred at room temperature overnight; after evaporation of the solvent, the residue was treated with *iso*-propyl ether, the formed solid was filtered off, and dried in vacuum, to give **3i** as trifluoroacetate salt in good purity to be used in the next step without further purification. 0.64 g, 74% yield, mp 218-221 °C. ESI-MS (m/z): 320 ($M + H$)⁺. ¹HNMR: δ 3.17 (m, 4H), 4.07 (m, 4H), 7.34 (d, $J = 4.7$ Hz, 1H), 7.72 (d, $J = 6.0$ Hz, 2H), 8.12 (d, $J = 6.0$ Hz, 2H), 8.52 (d, $J = 4.7$ Hz, 1H), 9.52 (br, 2H); IR (Nujol) 2860, 1669, 1580 cm⁻¹.

1-(4-(4-Trifluoromethylphenyl)pyrimidin-2-yl)piperazine (3j). Obtained from 1-(4-trifluoromethylphenyl)-3-(dimethylamino)prop-2-en-1-one²⁶ (**8**) following the typical procedure as **3i**. 0.56 g, 67%, mp 176-178 °C. ESI-MS (m/z): 309 ($M + H$)⁺. ¹HNMR: δ 3.24 (m, 4H), 4.07 (m, 4H), 7.75 (d, $J = 4.6$ Hz, 1H), 7.89 (d, $J = 5.8$ Hz, 2H), 8.37 (d, $J = 5.8$ Hz, 2H), 8.60 (d, $J = 4.6$ Hz, 1H), 9.08 (br, 2H); IR (Nujol) 2840, 1662, 1584 cm⁻¹.

1-(4-(Benzofuran-2-yl)pyrimidin-2-yl)piperazine (3k). Obtained from 1-benzofuran-2-yl-3-(dimethylamino)prop-2-en-1-one²⁷ (**9**) following the typical procedure as **3i**. 0.66 g, 84%, mp 176-178 °C. ESI-MS (m/z): 281 ($M + H$)⁺. ¹HNMR: δ 3.21 (m, 4H), 4.07 (m, 4H), 7.24 (d, $J = 4.9$ Hz, 1H), 7.34 (m, 1H), 7.45 (m, 1H), 7.70 (m, 1H), 7.78 (m, 1H), 7.82 (s, 1H), 8.58 (d, $J = 4.9$ Hz, 1H), 9.33 (br, 2H); IR (Nujol) 2886, 1678, 1575 cm⁻¹.

General procedure for the preparation of 4-(piperazinocarbonyl)aminophenylsulfamates 4a-k, and 4-(piperazinocarbonyl)aminophenols 5a-e. A mixture of 1-substituted piperazine **3a-k** (1 mmol) and 4-(phenoxy carbonyl)aminophenylsulfamate (**2**, 0.31 g, 1 mmol) or phenyl (4-hydroxyphenyl)carbamate (**1**, 0.23 g, 1 mmol) in anhydrous DMSO (5 mL) was stirred at room temperature for 18 h. The reaction mixture was then diluted with water (10 mL); the formed solid was collected and washed with water, air dried, and crystallized from EtOH to give **4a-k**, and **5a-e**.

4-(4-Benzylpiperazinocarbonyl)aminophenylsulfamate(4a). Following the general procedure, the title compound was obtained from **3a**. 0.28 g, 73%, mp 168-170 °C. ESI-MS (m/z): 391 ($M + H$)⁺. ¹HNMR: δ 2.37 (m, 4H), 3.43 (m, 4H), 3.50 (s, 2H), 7.13 (d, $J = 7.5$ Hz, 2H), 7.25 (m, 2H), 7.31 (m, 3H), 7.47 (d, $J = 7.5$ Hz, 2H), 7.73 (br s, 2H), 8.58 (s, 1H); IR (Nujol) 3419, 3323, 1649 cm^{-1} . Anal.Calcd for $\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_4\text{S}$: C, 55.37; H, 5.68; N, 14.35. Found: C, 55.30; H, 5.63; N, 14.31.

4-(4-Phenylpiperazinocarbonyl)aminophenylsulfamate(4b). Following the general procedure, the title compound was obtained from **3b**. 0.31 g, 83%, mp 196-198 °C. ESI-MS (m/z): 377 ($M + H$)⁺. ¹HNMR: δ 3.15 (m, 4H), 3.59 (m, 4H), 6.80 (m, 2H), 6.98 (d, $J = 7.0$ Hz, 2H), 7.15 (m, 2H), 7.23 (m, 3H), 7.84 (br s, 2H), 8.71 (s, 1H); IR (Nujol) 3411, 3306, 1642 cm^{-1} . Anal.Calcd for $\text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_4\text{S}$: C, 54.24; H, 5.36; N, 14.88. Found: C, 54.19; H, 5.30; N, 14.81.

4-(4-(4-Fluorophenyl)piperazinocarbonyl)aminophenylsulfamate (4c). Following the general procedure, the title compound was obtained from **3c**. 0.25 g, 64%, mp 188-190 °C. ESI-MS (m/z): 395 ($M + H$)⁺. ¹HNMR: δ 3.10 (m, 4H), 3.59 (m, 4H), 7.00 (m, 2H), 7.06 (m, 2H), 7.14 (d, $J = 8.8$ Hz, 2H), 7.51 (d, $J = 8.8$ Hz, 2H), 7.86 (s, 2H), 8.70 (s, 1H); IR (Nujol) 3368, 3188, 1661 cm^{-1} . Anal.Calcd for $\text{C}_{17}\text{H}_{19}\text{FN}_4\text{O}_4\text{S}$: C, 51.77; H, 4.86; N, 14.20. Found: C, 51.72; H, 4.85; N, 14.14.

4-(4-(4-Chlorophenyl)piperazinocarbonyl)aminophenylsulfamate (4d). Following the general procedure, the title compound was obtained from **3d**. 0.38 g, 93%, mp 192-194 °C. ESI-MS (m/z): 412 ($M + H$)⁺. ¹HNMR: δ 3.16 (m, 4H), 3.58 (m, 4H), 6.98 (m, 2H), 7.14 (d, $J = 7.3$ Hz, 2H), 7.23 (m, 2H), 7.50 (d, $J = 7.3$ Hz, 2H), 7.86 (s, 2H), 8.70 (s, 1H); IR (Nujol) 3418, 3256, 1640 cm^{-1} . Anal.Calcd for $\text{C}_{17}\text{H}_{19}\text{ClN}_4\text{O}_4\text{S}$: C, 49.69; H, 4.66; N, 13.64. Found: C, 49.61; H, 4.60; N, 13.58.

4-(4-(3-Methylphenyl)piperazinocarbonyl)aminophenylsulfamate (4e). Following the general procedure, the title compound was obtained from **3e**. 0.30 g, 78%, mp 202-204 °C. ESI-MS (m/z): 391 ($M + H$)⁺. ¹HNMR: δ 2.26 (s, 3H), 3.14 (m, 4H), 3.58 (m, 4H), 6.63 (m, 1H), 6.98 (m, 2H), 7.14 (m, 3H), 7.51 (d, $J = 6.3$ Hz, 2H), 7.72 (br s, 2H), 8.71 (s, 1H); IR (Nujol) 3373, 3208, 1658 cm^{-1} . Anal.Calcd for $\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_4\text{S}$: C, 55.37; H, 5.68; N, 14.35. Found: C, 55.32; H, 5.64; N, 14.27.

4-(4-(2,3-Dimethylphenyl)piperazinocarbonyl)aminophenylsulfamate (4f). Following the general procedure, the title compound was obtained from **3f**. 0.17 g, 42%, mp 176-178 °C. ESI-MS (m/z): 405 ($M + H$)⁺. ¹HNMR: δ 2.19 (s, 3H), 2.21 (s, 3H), 2.80 (m, 4H), 3.60 (m, 4H), 6.89 (m, 2H), 7.04 (m, 1H), 7.14 (d, $J = 8.8$ Hz, 2H), 7.51 (d, $J = 8.8$ Hz, 2H), 7.86 (s, 2H), 8.68 (s, 1H); IR (Nujol) 3353, 3196, 3097, 1648 cm^{-1} . Anal.Calcd for $\text{C}_{19}\text{H}_{24}\text{N}_4\text{O}_4\text{S}$: C, 56.42; H, 5.98; N, 13.85. Found: C, 56.37; H, 5.94; N, 13.77.

4-(4-(3-Methoxyphenyl)piperazinocarbonyl)aminophenylsulfamate (4g). Following the general procedure, the title compound was obtained from **3g**. 0.24 g, 59%, mp 193-195 °C. ESI-MS (m/z): 407 ($M + H$)⁺. ¹HNMR: δ 3.15 (m, 4H), 3.57 (m, 4H), 3.71 (s, 3H), 6.39 (m, 1H), 6.49 (s, 1H); 6.56 (m, 1H), 7.14 (m, 3H), 7.51 (d, $J = 8.8$ Hz, 2H), 7.80 (br s, 2H), 8.70 (s, 1H); IR (Nujol) 3383, 3179, 1657 cm^{-1} . Anal.Calcd for $\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_5\text{S}$: C, 53.19; H, 5.46; N, 13.78. Found: C, 53.14; H, 5.40; N, 13.72.

4-(4-(4-Methoxyphenyl)piperazinocarbonyl)aminophenylsulfamate (4h). Following the general procedure, the title compound was obtained from **3h**. 0.32 g, 80%, mp 190-192 °C. ESI-MS (m/z): 407 ($M + H$)⁺. ¹HNMR: δ 3.02 (m, 4H), 3.58 (m, 4H), 3.68 (s, 3H), 6.83 (d, $J = 8.8$ Hz, 2H), 6.93 (s, 2H); 7.14 (d, $J = 8.8$ Hz, 2H), 7.50 (d, $J = 8.8$ Hz, 2H), 7.86 (s, 2H), 8.69 (s, 1H); IR (Nujol) 3406, 3284, 1638 cm^{-1} . Anal.Calcd for $\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_5\text{S}$: C, 53.19; H, 5.46; N, 13.78. Found: C, 53.12; H, 5.41; N, 13.70.

4-(4-(4-(4-Bromophenyl)pyrimidin-2-yl)piperazinocarbonyl)aminophenyl sulfamate (4i). Following the general procedure, the title compound was obtained from **3i** in presence of equimolar amounts of DIPEA. 0.41 g, 76%, mp 176-178 °C. ESI-MS (m/z): 534 ($M + H$)⁺. ¹HNMR: δ 3.58 (m, 4H), 3.89 (m, 4H), 7.15 (d, $J = 8.8$ Hz, 2H), 7.26 (d, $J = 4.9$ Hz, 1H), 7.52 (d, $J = 8.8$ Hz, 2H), 7.71 (d, $J = 8.3$ Hz, 2H), 7.86 (br s, 2H), 8.10 (d, $J = 8.3$ Hz, 2H), 8.48 (d, $J = 4.9$ Hz, 1H), 8.72 (s, 1H); IR (Nujol) 3357, 3264, 1644 cm^{-1} . Anal.Calcd for $\text{C}_{21}\text{H}_{21}\text{BrN}_6\text{O}_4\text{S}$: C, 47.29; H, 3.97; N, 15.76. Found: C, 47.23; H, 3.94; N, 15.69.

4-(4-(4-(4-Trifluoromethylphenyl)pyrimidin-2-yl)piperazinocarbonyl)aminophenyl sulfamate (4j). Following the general procedure, the title compound was obtained from **3j** in presence of equimolar amounts of DIPEA. 0.34 g, 66%, mp 152-154 °C. ESI-MS (m/z): 523 ($M + H$)⁺. ¹HNMR: δ 3.59 (m, 4H), 3.89 (m, 4H), 7.15 (d, $J = 8.8$ Hz, 2H), 7.32 (m, 1H), 7.52 (d, $J = 8.8$ Hz, 2H), 7.80 (br s, 2H), 7.88 (d, $J = 7.3$ Hz, 2H), 8.34 (d, $J = 7.3$ Hz, 2H), 8.54 (m, 1H), 8.73 (s, 1H); IR (Nujol) 3407, 3319, 1645 cm^{-1} . Anal.Calcd for $\text{C}_{22}\text{H}_{21}\text{F}_3\text{N}_6\text{O}_4\text{S}$: C, 50.57; H, 4.05; N, 16.08. Found: C, 50.50; H, 4.04; N, 16.00.

4-(4-(4-Benzofuran-2-ylpyrimidin-2-yl)piperazinocarbonyl)aminophenyl sulfamate (4k). Following the general procedure, the title compound was obtained from **3k** in presence of equimolar amounts of DIPEA. 0.36 g, 73%, mp 146-148 °C. ESI-MS (m/z): 495 ($M + H$)⁺. ¹HNMR: δ 3.59 (m, 4H), 3.89 (m, 4H), 7.17 (m, 3H), 7.33 (m, 1H), 7.44 (m, 1H), 7.52 (d, $J = 8.8$ Hz, 2H), 7.70 (m, 1H), 7.78 (m, 2H), 7.87 (s, 2H), 8.54 (d, $J = 4.9$ Hz, 1H), 8.74 (s, 1H); IR (Nujol) 3410, 3321, 3103, 1648 cm^{-1} . Anal.Calcd for $\text{C}_{23}\text{H}_{22}\text{N}_6\text{O}_5\text{S}$: C, 55.86; H, 4.48; N, 16.99. Found: C, 55.79; H, 4.45; N, 16.92.

4-(4-Benzylpiperazinocarbonyl)aminophenol(5a). Following the general procedure, the title compound was obtained from **3a**. 0.23 g, 74%, mp 182-184 °C. ESI-MS (m/z): 312 ($M + H$)⁺. ¹HNMR: δ 2.35 (m, 4H), 3.39 (m, 4H), 3.49 (s, 2H), 6.60 (d, $J = 8.0$ Hz, 2H), 7.15 (d, $J = 8.0$ Hz, 2H), 7.25 (m, 1H), 7.31 (m, 4H), 8.17 (s, 1H),

8.95 (s, 1H); IR (Nujol) 3414, 3263, 1634 cm^{-1} . Anal.Calcd for $\text{C}_{18}\text{H}_{21}\text{N}_3\text{O}_2$: C, 69.43; H, 6.80; N, 13.49.

Found: C, 69.36; H, 6.73; N, 13.42.

4-(4-Phenylpiperazinocarbonyl)aminophenol (5b). Following the general procedure, the title compound was obtained from **3b**. 0.24 g, 82%, mp 228-230 °C.ESI-MS (m/z): 298 ($M + H$)⁺. ¹HNMR: δ 3.13 (m, 4H), 3.55 (m, 4H), 6.63 (d, $J = 8.5$ Hz, 2H), 6.80 (m, 1H), 6.97 (d, $J = 8.5$ Hz, 2H), 7.20 (m, 4H), 8.31 (s, 1H), 8.97 (s, 1H); IR (Nujol) 3436, 3178, 1649 cm^{-1} . Anal.Calcd for $\text{C}_{17}\text{H}_{19}\text{N}_3\text{O}_2$: C, 68.67; H, 6.44; N, 14.13. Found: C, 68.62; H, 6.42; N, 14.08.

4-(4-(4-Fluorophenyl)piperazinocarbonyl)aminophenol (5c). Following the general procedure, the title compound was obtained from **3c**. 0.28 g, 88%, mp 208-210 °C.ESI-MS (m/z): 316 ($M + H$)⁺. ¹HNMR: δ 3.07 (m, 4H), 3.54 (m, 4H), 6.63 (d, $J = 8.5$ Hz, 2H), 6.98 (m, 2H), 7.05 (m, 2H), 7.19 (d, $J = 8.5$ Hz, 2H), 8.30 (s, 1H), 8.97 (s, 1H); IR (Nujol) 3455, 3201, 1652 cm^{-1} . Anal.Calcd for $\text{C}_{17}\text{H}_{18}\text{FN}_3\text{O}_2$: C, 64.75; H, 5.75; N, 13.33. Found: C, 64.68; H, 5.70; N, 13.27.

4-(4-(4-Chlorophenyl)piperazinocarbonyl)aminophenol (5d). Following the general procedure, the title compound was obtained from **3d**. 0.18 g, 56%, mp 225-227 °C.ESI-MS (m/z): 333 ($M + H$)⁺. ¹HNMR: δ 3.14 (m, 4H), 3.54 (m, 4H), 6.61 (d, $J = 8.0$ Hz, 2H), 6.98 (d, $J = 8.5$ Hz, 2H), 7.19 (d, $J = 8.0$ Hz, 2H), 7.23 (d, $J = 8.5$ Hz, 2H), 8.31 (s, 1H), 8.97 (s, 1H); IR (Nujol) 3432, 3242, 1654 cm^{-1} . Anal.Calcd for $\text{C}_{17}\text{H}_{18}\text{ClN}_3\text{O}_2$: C, 61.54; H, 5.47; N, 12.66. Found: C, 61.49; H, 5.42; N, 12.58.

4-(4-(3-Methylphenyl)piperazinocarbonyl)aminophenol (5e). Following the general procedure, the title compound was obtained from **3e**. 0.24 g, 79%, mp 208-210 °C.ESI-MS (m/z): 312 ($M + H$)⁺. ¹HNMR: δ 2.25 (s, 3H), 3.11 (m, 4H), 3.54 (m, 4H), 6.63 (m, 3H), 6.79 (m, 2H), 7.10 (m, 1H), 7.19 (d, $J = 8.5$ Hz, 2H), 8.30 (s, 1H), 8.97 (s, 1H); IR (Nujol) 3316, 3298, 1636 cm^{-1} . Anal.Calcd for $\text{C}_{18}\text{H}_{21}\text{N}_3\text{O}_2$: C, 69.43; H, 6.80; N, 13.49. Found: C, 69.36; H, 6.78; N, 13.42.

4.2. CA inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.²⁰ Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10-100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5-10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and

dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation, as reported earlier,²¹⁻²³ and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.²¹⁻²³

Acknowledgments: This work was financed in part by a Marie Curie ITN FP7 project (Dynano).

ACCEPTED MANUSCRIPT

References

1. a) De Simone, G.; Alterio, V.; Supuran, C.T. *Expert Opin. Drug Discov.* **2013**, *8*, 793; b) Masini, E.; Carta, F.; Scozzafava, A.; Supuran, C.T. *Expert Opin. Ther. Pat.* **2013**, *23*, 705; c) Briganti, F.; Pierattelli, R.; Scozzafava, A.; Supuran, C.T. *Eur. J. Med. Chem.* **1996**, *31*, 1001.
2. a) Arechederra, R.L.; Waheed, A.; Sly, W.S.; Supuran, C.T.; Minter, S.D. *Bioorg. Med. Chem.* **2013**, *21*, 1544; b) Scozzafava, A.; Supuran, C.T.; Carta, F. *Expert Opin. Ther. Pat.* **2013**, *23*, 725; c) Harju, A. K.; Bootorabi, F.; Kuuslahti, M.; Supuran, C. T.; Parkkila, S. *J Enzyme Inhib Med Chem* **2013**, *28*, 231; d) Capasso, C.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 379.
3. a) Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C.T.; De Simone, G. *Chem. Rev.* **2012**, *112*, 4421; b) Supuran, C.T. *Nat. Rev. Drug Discov.* **2008**, *7*, 168; c) Neri, D.; Supuran, C.T. *Nat. Rev. Drug Discov.* **2011**, *10*, 767; d) Aggarwal, M.; Kondeti, B.; McKenna, R. *Bioorg. Med. Chem.* **2013**, *21*, 1526.
4. a) Aggarwal, M.; McKenna, R. *Expert. Opin. Ther. Pat.* **2012**, *22*, 903; b) Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 759; c) Supuran, C.T. *Expert Opin. Ther. Pat.* **2013**, *23*, 677; d) Supuran, C.T. *Bioorg. Med. Chem.* **2013**, *21*, 1377; e) Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 229.
5. a) Tripp, B. C.; Bell, C. B., 3rd; Cruz, F.; Krebs, C.; Ferry, J. G. *J. Biol. Chem.* **2004**, *279*, 6683; b) Zimmerman, S. A.; Ferry, J. G. *Curr. Pharm. Des.* **2008**, *14*, 716
6. a) Syrjanen, L.; Tolvanen, M.; Hilvo, M.; Olatubosun, A.; Innocenti, A.; Scozzafava, A.; Leppiniemi, J.; Niederhauser, B.; Hytonen, V. P.; Gorr, T. A.; Parkkila, S.; Supuran, C. T. *BMC Biochem.* **2010**, *11*, 28; b) Parkkila, S.; Syrjanen, L.; Scozzafava, A.; Supuran, C.T. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2797; c) Syrjanen, L.; Vermelho, A. B.; de Almeida Rodrigues, I.; Corte-Real, S.; Salonen, T.; Pan, P.; Vullo, D.; Parkkila, S.; Capasso, C.; Supuran, C. T. *J. Med. Chem.* **2013**, *56*, 7372; d) Capasso, C.; Supuran, C.T. *Expert Opin. Ther. Pat.* **2013**, *23*, 693.
7. a) Xu, Y.; Feng, L.; Jeffrey, P. D.; Shi, Y.; Morel, F. M. *Nature* **2008**, *452*, 56; b) Lane, T. W.; Saito, M. A.; George, G. N.; Pickering, I. J.; Prince, R. C.; Morel, F. M. *Nature* **2005**, *435*, 42; c) Vullo, D.; Del Prete, S.; Osman, S.M.; De Luca, V.; Scozzafava, A.; AlOthman, Z.; Supuran, C.T.; Capasso, C. *Bioorg Med. Chem. Lett.* **2014**, *24*, 275.
8. a) Del Prete, S.; Vullo, D.; Fisher, G.M.; Andrews, K.T.; Poulsen, S.A.; Capasso, C.; Supuran, C.T. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4389; b) Vullo, D.; Del Prete, S.; Fisher, G.M.; Andrews, K.T.; Poulsen, S.A.; Capasso, C.; Supuran, C.T. *Bioorg. Med. Chem.* **2015**, *23*, 526.
9. Tolvanen, M.E.; Ortutay, C.; Barker, H.R.; Aspatwar, A.; Patrikainen, M.; Parkkila, S. *Bioorg. Med. Chem.* **2013**, *21*, 1503.
10. De Luca, V.; Vullo, D.; Scozzafava, A.; Carginale, V.; Rossi, M.; Supuran, C.T.; Capasso, C. *Bioorg. Med. Chem.* **2013**, 1465.
11. a) Krall, N.; Pretto, F.; Decurtins, W.; Bernardes, G. J. L.; Supuran, C. T.; Neri, D., *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 4231; b) Monti, S.M.; Supuran, C.T.; De Simone, G. *Expert Opin. Ther. Pat.* **2013**, *23*, 737; c) Ebbesen, P.; Pettersen, E.O.; Gorr, T.A.; Jobst, G.; Williams, K.; Kienninger, J.; Wenger, R.H.; Pastorekova,

S.; Dubois, L.; Lambin, P.; Wouters, B.G.; Supuran, C.T.; Poellinger, L.; Ratcliffe, P.; Kanopka, A.; Görlach, A.; Gasmann, M.; Harris, A.L.; Maxwell, P.; Scozzafava, A. *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, (Supplement 1), 1; d) 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; e) Chohan, Z.H.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 259; f) Borrás, J.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C.T. *Bioorg. Med. Chem.* **1999**, *7*, 2397.

12. a) Supuran, C.T.; McKenna, R. Carbonic anhydrase inhibitors drug design, in *Carbonic Anhydrase: Mechanism, regulation, Links to Disease, and Industrial Applications*, McKenna R, Frost S, Eds., Springer Verlag, Heidelberg, **2014**, pp. 291-323 (*Subcell. Biochem.* **2014**, *75*, 291); b) Supuran, C.T.; Clare, B.W. *Eur. J. Med. Chem.* **1999**, *34*, 41; c) Chohan, Z.H.; Supuran, C.T.; Scozzafava, A. *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 303; d) Chohan, Z.H.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2002**, *17*, 261.

13. See more at ClinicalTrials.gov: Safety Study of SLC-0111 in Subjects With Advanced Solid Tumours - Full Text View - ClinicalTrials.gov.mht.

14. 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) McDonald, P.C.; Winum, J.Y.; Supuran, C.T.; Dedhar, S. *Oncotarget*, **2012**, *3*, 84; c) Nishimori, I.; Minakuchi, T.; Morimoto, K.; Sano, S.; Onishi, S.; Takeuchi, H.; Vullo, D.; Scozzafava, A.; Supuran, C.T. *J. Med. Chem.* **2006**, *49*, 2117; d) Pacchiano, F.; Aggarwal, M.; Avvaru, B.S.; Robbins, A.H.; Scozzafava, A.; McKenna, R.; Supuran, C.T. *Chem. Comm.* **2010**, *46*, 8371.

15. a) Winum, J.Y.; Scozzafava, A.; Montero, J.L.; Supuran, C.T. *Med. Res. Rev.* **2005**, *25*, 186; b) Abbate, F.; Winum, J.Y.; Potter, B.V.L.; Casini, A.; Montero, J.L.; Scozzafava, A.; Supuran, C.T. *Bioorg. Med. Chem.Lett.* **2004**, *14*, 231; c) De Monte, C.; Carradori, S.; Secci, D.; D'Ascenzio, M.; Vullo, D.; Ceruso, M.; Supuran, C.T. *Eur. J. Med. Chem.* **2014**, *84*, 240.

16. Mack, C. H.; McGregor, H. H., Jr.; Hobart, S. R. *J. Chem. Eng. Data*, **1969**, *14*, 258.

17. Okada, M.; Iwashita, S.; Koizumi, N. *Tetrahedron Lett.* **2000**, *41*, 705.

18. Coffen, D. L.; Dillon, M. P.; Ford, A. P. D. W.; Li, Z.; Williams, T. J. *US Patent* **2002**, 6355641 B1.

19. a) Innocenti, A.; Vullo, D.; Scozzafava, A.; Supuran, C.T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1583; b) Innocenti, A.; Vullo, D.; Scozzafava, A.; Supuran, C.T. *Bioorg. Med. Chem.* **2008**, *16*, 7424; c) Şentürk, M.; Gülçin, I.; Daştan, A.; Küfrevioğlu, Ö.I.; Supuran, C.T. *Bioorg. Med. Chem.* **2009**, *17*, 3207; d) Innocenti, A.; Öztürk Sarıkaya, S.B.; Gülçin, I.; Supuran, C.T. *Bioorg. Med. Chem.* **2010**, *18*, 2159; e) Mastrolorenzo, A.; Rusconi, S.; Scozzafava, A.; Barbaro, G.; Supuran, C.T. *Curr. Med. Chem.* **2007**, *14*, 2734.

20. Khalifah, R.G. *J. Biol. Chem.* **1971**, *246*, 2561.

21. a) Maresca, A.; Carta, F.; Vullo, D.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 407; b) Ekinçi, D.; Kurbanoglu, N.I.; Salamci, E.; Senturk, M.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 845; c) Ekinçi, D.; Karagoz, L.; Ekinçi, 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.; Ekinçi, D.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 294.

22. 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) Moya, A.; Tambutté, S.; Bertucci, A.; Tambutté, E.; Lotto, S.; Vullo, D.; Supuran, C.T.; Allemand, D.; Zoccola, D. *J. Biol. Chem.* **2008**, *283*, 25475; d) Nishimori, I.; Onishi, S.; Takeuchi, H.; Supuran, C.T. *Curr. Pharm. Des.* **2008**, *14*, 622.

23. a) Maresca, A.; Vullo, D.; Scozzafava, A.; Supuran, C.T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 388; b) Koz, O.; Ekinçi, 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.; Gregaň, 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) Scozzafava, A.; Menabuoni, L.; Mincione, F.; Mincione, G.; Supuran, C.T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 575.

24. 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.

25. Lin, Y.-I.; Lang, S. A. Jr. *J. Org. Chem.* **1980**, *45*, 4857.

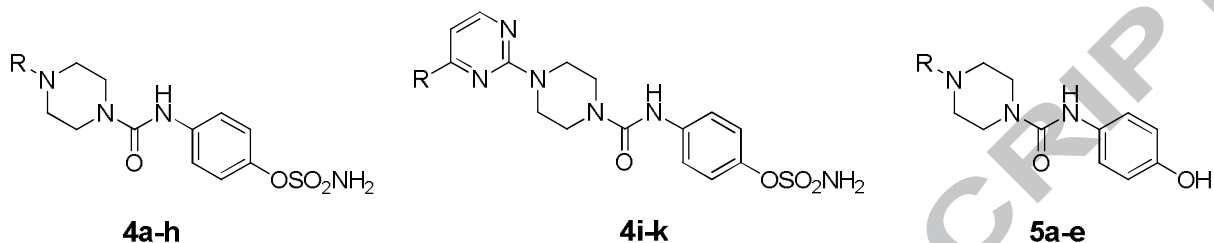
26. Ingham, R. J.; Riva, E.; Nikbin, N.; Baxendale, I. R.; Ley, S. V. *Org. Lett.* **2012**, *14*, 3920.

27. Saleh, T. S.; Al-Omar, M. A.; Abdel-Aziz, H. A. *Lett. Org. Chem.* **2010**, *7*, 483.

Synthesis and carbonic anhydrase I, II, IX and XII inhibitory activity of sulfamates incorporating piperazinyl-ureido moieties

Cenzo Congiu,* Valentina Onnis, Alessandro Deplano, Gianfranco Balboni, Mariangela Ceruso, and Claudiu T.

Supuran*



R = Ph, benzyl, substituted aryl; etc

K_I (hCA I) = 9.4 – 896.8 nM; K_I (hCA II) = 1.0 – 1427 nM; K_I (hCA IX) = 0.91 – 120.7 nM; K_I (hCA XII) = 1.0 – 84.5 nM;