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Letter

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Discovery of new selenoureido analogs of 4-(4-fluorophenylureido)benzenesulfonamide as carbonic anhydrase inhibitors

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KEYWORDS: Carbonic Anhydrase Inhibitors (CAIs), Metalloenzymes, Glutathione Peroxidase, selenoureido, bioisosterism

ABSTRACT: A series of benzenesulfonamides bearing the selenourea moieties were obtained considering the ureido-sulfonamide **SLC-0111**, in Phase I clinical trials as antitumor agent, as a lead molecule. All compounds showed interesting inhibition potencies against the physiologically relevant human (h) carbonic anhydrase (hCAs, EC 4.2.1.1) isoforms I, II, IV and IX. The most flexible analogues in the series **14-19** showed low nanomolar inhibition constants against hCA I, II and IX. We assessed on selected compounds the *in vitro* antioxidant properties, binding modes, as well as the ex-vivo evaluation on human prostate (PC3), breast (MDA-MB-231) and colon-rectal (HT-29) cancer cell lines both in normoxic and hypoxic conditions.

Isosteric replacement is a commonly used approach in medicinal chemistry. It consists in the introduction, within selected lead compounds, of structural modifications (named bioisosteric groups or elements) able to retain the desired biological activity.[1,2] Such an experimental approach can be highly reliable when optimizing pharmacological activities, pharmacokinetics and/or selectivity enhancement for a specific biological target.[1,2] We recently applied the isosteric replacement to the compound in figure 1A, (**SLC-0111**),[3] which successfully ended Phase I clinical programs for the treatment of patients with advanced hypoxic tumors over-expressing the isoform IX of the human (h) metalloenzyme Carbonic Anhydrases (CAs, EC 4.2.1.1).[4-7]

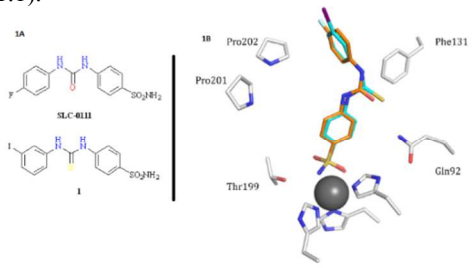


Figure 1A. Structures of the ureido benzenesulfonamide **SLC-0111** and its thioureido analog **1**. **1B** The hCA II–**1** complex (cyan) superposed with the hCA II–**SLC-0111** complex (orange). Active site residues involved in the binding of inhibitors are labeled, the zinc ion is shown as a gray sphere.[3]

These enzymes are highly efficient in reversibly catalyzing the hydration of carbon dioxide into carbonic acid. Such a chemical transformation is deeply involved in various cellular patho/physiological events, which among others include cancerogenesis and progression.[8,9]

Divalent isosteric substitution of the oxygen in **SLC-0111** with the sulfur atom instead (compound **1** in figure 1A) did not significantly affect the *in vitro* inhibition profile on hCAs I, II, IX and XII.[3] Rationalization of these data was offered by the superposition of the X-ray co-crystallographic adducts of **1** and **SLC-0111** with hCA II (Figure 1B).[3] Only a slight distortion of the two structures was observed, with the closer orientation of the C=S moiety in **1** towards the Phe131 residue when compared to the C=O in **SLC-0111**. The *meta* iodophenyl tail didn't show any difference when compared to the parent **SLC-0111** *para* fluorophenyl tail.[3]

The insertion of selenium instead of oxygen or sulfur is an isosteric replacement widely employed within the medicinal chemistry field.[1] Since selenium is an essential element for the human physiology,[10,11] all the isosteric replacements based on this element are particularly valuable or with a high potential for obtaining interesting biological effects.[1] Various diseases in which oxidative stress is a major factor are strictly associated with selenium deficiency or are endemic in areas with low concentrations of Se.[10-12] Tremendous efforts have been directed towards the synthesis of stable organoselenium compounds such as Ebselen, L-Se-Cys or L-Se-Met (figure 2) that

may serve as antioxidant adjuvants in Alzheimer's, Parkinson's, autoimmune diseases, myocardial infarction, atherosclerosis and tumors as the most important.[10,12]

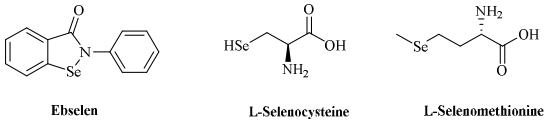
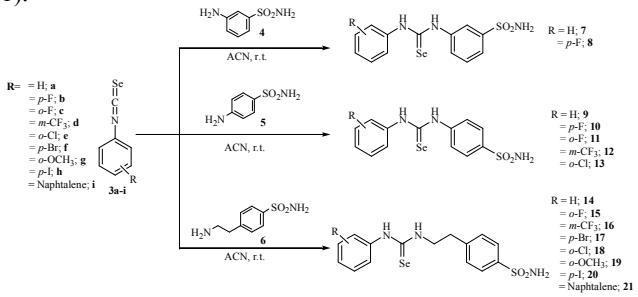


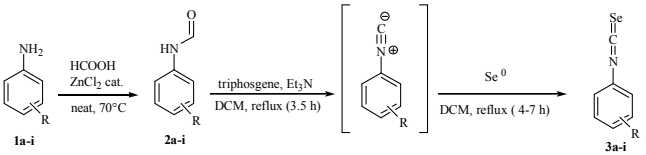
Figure 2. Selenium containing small molecules currently used in clinic.[10]

All the pathologies above listed are commonly characterized by an over-production of Reactive Oxygen Species (ROS), which rapidly determine a work overload/depletion of the human antioxidant enzymatic pool, which is mainly constituted by the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione *S*-transferase (GST) and glutathione (GSH)[10,13] An excess of unbuffered ROS determines, at the molecular level, irreversible damages to cellular structures such as proteins, lipids, and nucleic acids and thus fosters the diseases outcome.[13-15] In this context we considered of particular interest to synthesize a series of **SLC-0111** congeners according to the divalent isosteric replacement approach, by means of introduction within the ureido moiety of the ROS scavenger element selenium. The selenoureido containing compounds **7-21** were obtained by standard coupling reactions of the aromatic isoselenocyanates **3a-i** with commercially available benzenesulfonamides **4-6** in acetonitrile (ACN) as solvent (Scheme 1).[6]



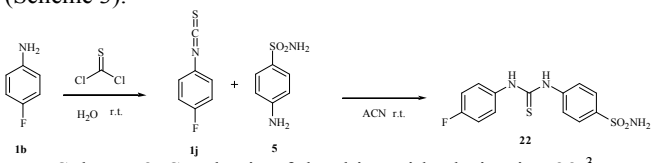
Scheme 1. General synthetic procedure for compounds **7-21**.

The proper isoselenocyanates **3a-i** were obtained in a two step synthetic procedure (scheme 2) which involved: *i*) *N*-formylation in high yields of the commercially available aromatic amines **1a-i** using catalytic zinc (II) chloride and formic acid under neat conditions;[16] *ii*) conversion of the formylanilines **2a-i** to the corresponding isoselenocyanates **3a-i** using the modified Barton's procedure.[17]



Scheme 2. General synthetic scheme of compounds **3a-i**.

Noteworthy that the dehydration of formylanilines **2a-i** was successfully conducted by the use of the safer and handy solid triphosgene,[18,19] and the *in situ* obtained isocyanides (not isolated) were treated with an excess of selenium (0) powder to afford the desired isoselenocyanates **3a-i**.[20] In order to asses a proper SAR, the synthesis of the **SLC-0111** thioureido analog **22** was also carried out and according to standard procedures (Scheme 3).[3]



Scheme 3. Synthesis of the thioureido derivative **22.**[3]

All synthesized compounds, **7-22**, were tested *in vitro* for their inhibitory properties against the physiological relevant hCA isoforms (I, II, IV and IX)[3,7-9] by means of the stopped-flow carbon dioxide hydration assay,[21] and their activities were compared to the standard carbonic anhydrase inhibitor (CAI) acetazolamide (**AAZ**) (Table 1).

Table 1. hCA I, II, IV and IX inhibition data of compounds **7-22** and **SLC-0111** by a Stopped-Flow, CO_2 Hydrase Assay.[21]

	K_I (nM)*			
Compound	**hCA I**	**hCA II**	**hCA IV**	**hCA IX****
7	483.8	343.2	908.7	329.7
8	435.3	388.9	765.9	97.8
9	132.5	54.3	8627	78.9
10	152.3	66.3	7557	63.0
11	5.9	6.3	902.1	5.8
12	32.7	6.1	734.4	15.9
13	7.9	4.0	268.8	3.9
14	6.7	5.5	1782	5.3
15	44.1	7.9	898.2	8.7
16	8.5	4.4	928.9	5.8
17	51.7	1.8	1409	4.8
18	8.3	3.5	731.2	4.8
19	6.0	4.5	765.9	3.1
20	267.4	57.6	6760	54.9
21	501.7	91.2	5423	93.1
22	35.0	14.2	4797	32.1
SLC-0111	5080	960.0	286.0	45.0
AAZ	250	12.1	74	25.8

*Means from three different assays. Errors were within ±5−10% of the reported values (data not shown). **Catalytic domain.

The following SARs for the hCA isoforms considered are reported:

i) The ubiquitous cytosolic hCA I was inhibited by all compounds with K_Is spanning between 5.9 and 501.7 nM. Within the series reported, the compounds bearing the sulfonamide moiety at position 3 of the ring, compounds **7** and **8**, or the bulky naphthyl tail moiety **21,** resulted the least active with K_I values respectively 1.9, 1.7 and 2.0 fold higher when compared to the standard CAI Acetazolamide (**AAZ**) (Table 1). Relocation of the primary sulfonamide moiety in compound **7** from the *meta* to the *para* position, to afford **9**, resulted in a significant enhancement of the inhibitory potency (K_Is of 483.8 and 132.5 nM respectively). The introduction in **9** of the *para*-fluoro moiety, compound **10**, slightly enhanced the K_I value of 1.15 fold. It is noteworthy that the inhibition potency was greatly improved when the fluoro was introduced in the *ortho* position (compound **11** K_I 5.9 nM). The nature of the halide didn't affect the inhibition potency against the hCA I as the introduction in *ortho* of the chloro atom instead of the fluoro, to afford **13**, determined only a slight increase of the K_I

value to 7.9 nM. Conversely the introduction of the trifluoromethyl group in the *meta* position spoiled the inhibition value to 32.7 nM. Among the ethylamino benzenesulfonamides **14-21**, the phenyl and the *ortho* methoxy substituted derivatives **14** and **19** resulted the most active in the series, with K_Is of 6.7 and 6.0 nM respectively. Interestingly the *meta* trifluoromethyl and the *ortho* chloro substituted derivatives **16** and **18** slightly differed for their inhibition potencies (K_Is 8.5 and 8.3 nM) respectively. Among the phenyl halide derivatives, the *ortho* fluoro **15** and the *para* bromo **17** resulted similar potencies in inhibiting the hCA I with K_Is of 44.1 and 51.7 nM respectively. Substitution of the bromine with the iodine, as in compound **20,** was detrimental for the inhibition activity (K_I 267.4 nM). The bioisosteric substitution in **SLC-0111** of the oxygen within the ureido moiety with a sulfur or selenium atom instead (compounds **22** and **10** respectively) resulted in an increase of the inhibition potency against the hCA I isoform of 145.1 and 33.4 fold respectively (table 1).

ii) The cytosolic and highly efficient hCA II isoform, was effectively inhibited by all compounds synthesized (K_I comprised between 3.5 and 388.9 nM), and in analogy to the hCA I the metanilamide derivatives **7** and **8** were the least potent (K_I 343.2 and 388.9 nM respectively). Among the sulfanilamide series, the introduction of the *para*-fluoro moiety within the simple phenyl ring, conversion of **9** to **10**, slightly spoiled the inhibition potency (K_Is 54.3 and 66.3 nM respectively). Placement of the fluoro atom in the *ortho* position, compound **11**, resulted in a 10.5 fold increase of the inhibition potency, which was retained when the *meta*-trifluoromethyl group was introduced instead (compound **12** K_I 6.1 nM) or even reinforced when a *ortho*-chloro moiety was placed (compound **13** K_I 4.0 nM). Among the ethylaminobenzene sulfonamide series **14-21**, all compounds were quite effective in inhibiting the hCA II isoform (K_Is spanning between 1.8 and 7.9 nM), with the only exceptions represented by the bulky *para*-iodo **20** and the naphtyl derivative **21** (K_Is 57.6 and 91.2 nM respectively). As for the hCA II, the bioisosteric substitution of the oxygen atom within the ureido group in **SLC-0111** (K_I 960 nM) resulted in enhancement of the inhibition potency. As reported in table 1, K_Is of 66.3 and 14.2 nM were obtained when the selenium, compound **10** or the sulfur, compound **22**, were introduced.

iii) The membrane bound hCA IV was the least inhibited among the enzymatic isoforms herein considered, and showed K_Is spanning between 8627 and 268.8 nM. The introduction in **7** of the fluoro atom in *para* position, to afford **8**, resulted in a 1.2 fold enhancement of the inhibition potency. In analogy, a 1.1 fold inhibition potency increase was reported when the *para*-fluoro substitution was operated in **9** to afford **10** (K_Is of 8627 and 7557 nM respectively), and it was further improved when a *meta*-trifluoromethyl (K_I 734.4 nM) or a *ortho*-chloro moiety (K_I 268.8 nM) were introduced. Among the ethylaminobenzene sulfonamide series the introduction within the phenyl tail of **14** in *ortho* position either of a fluoro **15**, chloro **18**, methoxy **19** or a *meta*-trifluoromethyl moiety, as in **16**, resulted in a significant enhancement of the inhibition potency with K_Is of 1782, 898.2, 731.2, 765.9 and 928.9 nM respectively (table 1). The introduction in **14** of the *para* bromo moiety determined only a slight reduction of the inhibition activity against the hCA IV. The bulky *para*-iodo **20** and naphthyl **21** were the least effective in the series (K_Is 6760 and 5423 nM respectively). The effects of the divalent isosteric substitution in **SLC-0111** were detrimental for the inhibition properties against the hCA IV isoform. As reported in table 1, the parent **SLC-0111** showed a K_I value of 286 nM, the introduction of the sulfur and the selenium atom within the ureido moiety enhanced the K_I value 16.8 and 26.4 fold, respectively.

iv) The transmembrane and tumor associated hCA IX was effectively inhibited by the compounds herein reported and showed K_I values comprised between 3.1 and 329.7 nM. In particular, the introduction of the *para*-fluoro moiety in **7**, to afford **8**, resulted in a 3.4 fold decrease of the inhibition value. A slight lower potency increase (1.3 fold) was observed when the *para*-fluoro moiety was introduced within the sulfanilamide series (conversion of **9** to **10**). In analogy to the other enzymes herein considered, the inhibition data showed strictly related to the fluoro regioisomerism. Kinetic data in table 1 account for a 10.9 fold potency increase when the fluoro moiety was shifted from the *para* to the *ortho* position (conversion of **10** to **11**). Interestingly, the replacement of the fluoro atom in **11** with the chloro instead, to afford **13**, resulted in an increase of the inhibition potency (K_Is of 5.8 and 3.9 nM respectively). The introduction of the *meta*-trifluoromethyl moiety within the simple phenyl tail on compound **9** to afford **12**, was beneficial for the inhibition potency which showed a 5.0 fold increase (K_Is of 78.9 and 15.9 nM for **9** and **12** respectively). With the exception of the bulky *para*-iodo **20** and naphthyl derivative **21** (K_Is 54.9 and 93.1 nM respectively), all compounds within the ethylbenzenesulfonamide series were quite effective in inhibiting the hCA IX and showed K_Is spanning between 3.1 and 8.7 nM, thus far more active when compared to the standard CAI **AAZ** (K_I 25.8 nM). In particular, the introduction in **14** of the *ortho*-fluoro moiety to afford **15**, slightly spoiled the inhibition potency (K_I of 5.3 and 8.7 nM respectively). Conversely the introduction within the phenyl tail in **14** of a *meta*-trifluoromethyl **16**, or *para*-bromo **17**, *ortho*-chloro **18** and *ortho*-methoxy **19** moiety, clearly resulted in enhancement of the inhibition potency (K_Is of 5.8, 4.8, 4.8 and 3.1 nM respectively). SAR relative to the isosteric substitution of the oxygen within the **SLC-0111** ureido moiety accounted for an increase of the inhibition potency when the sulfur was introduced (compound **22**; K_I 32.1 nM). Conversely, the K_I value was 1.4 fold decreased when the selenium was introduced instead (compound **10**; K_I 63 nM).

In general, the divalent bioisosteric replacement of the ureido oxygen on **SLC-0111** with a sulfur or selenium, compounds **22** and **10** respectively, determined powerful enhancements of the inhibition potencies against the hCA I and II isoforms (Table 1). Any modification at the ureido moiety resulted in a suppression of the inhibition activity against the membrane associated hCA IV (K_Is of 4797 and 7557 for **22** and **10** respectively). Interestingly, the sulfur derivative **22** resulted in only a slight increase of the inhibition potency against hCA IX (K_Is of 45.0 and 32.1 nM for **SLC-0111** and **22** respectively). Conversely, the introduction of the selenium, as in **10**, was detrimental for the activity (K_I 63.0 nM). Although the inhibition potency of the selenium derivative **10** was only 1.4 fold less potent when compared to the parent **SLC-0111**, it is worth mentioning that the selectivity ratio hCA II/hCA IX of this compound was heavily spoiled (21.3 for **SLC-0111** and 1.1 for **10**).

The antioxidant activity of the selenoureido congener of **SLC-0111** (compound **10**) was evaluated according to literature procedures in catalyzing the reaction between hydrogen peroxide (H_2O_2) and two different thiols such as dithiothreitol

(DTT)[22-25] and glutathione (GSH).[26,27] A preliminary investigation of the GPx-like activity of compound **10** was carried out following the oxidation of DTT in CD_3OD by means of ^1H NMR. A control experiment was performed in the absence of catalyst. The catalytic activity of $(PhSe)_2$ against DTT^{red} under these conditions was also determined, to compare the activity of the title compound with commonly used standard materials for the GPx assays.[28-30] As depicted in **Figure 3**, when the reaction was performed in the absence of catalyst, 98% of DTT^{red} remained unreacted after 60 min. Conversely, DTT oxidation was complete within 35 min when 10% of the substituted selenourea **10** was added. Under these conditions, the time required to halve the initial concentration of DTT^{red} (T_{50}) was 8 min. Interestingly, according to this test, **10** proved to behave as a better catalyst than $(PhSe)_2$, in that it exhibited a longer reaction time and higher T_{50} values. Compounds **SLC-0111** and **22**, did not catalyze the DTT oxidation under the studied conditions, being the kinetic profile comparable with that of the control experiment.

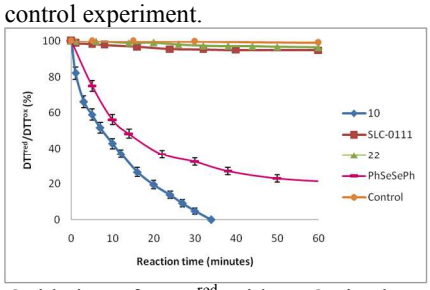


Figure 3: Oxidation of DTT^{red} with H_2O_2 in the presence of compounds **10**, **SLC-0111** and **22**. Reaction conditions: $[DTT^{red}]_0$=0.14 mM, $[H_2O_2]_0$=0.14 mM, [catalyst]=0.014 mM), CD_3OD (1.1 mL). ^1H NMR spectra were measured at variable reaction time at 25 °C. The relative populations of DTT^{red} and DTT^{ox} were determined by integration of the ^1H NMR signals. In the control experiment the reaction was run with no catalyst. Reported are the mean ± SD values of three separate experiments.

The GPx-like properties of **10** were further confirmed by using the nicotinamide adenine dinucleotide phosphate (NADPH)-coupled assay. In this experiment GSH was used as a substrate and H_2O_2 as an oxidant in the presence of NADPH and glutathione reductase (GR).[26,27] The reaction was carried out in H_2O and its progress was monitored by UV spectroscopy, measuring the absorbance decreasing at 340 nm due to the consumption of NADPH (Figure 4).

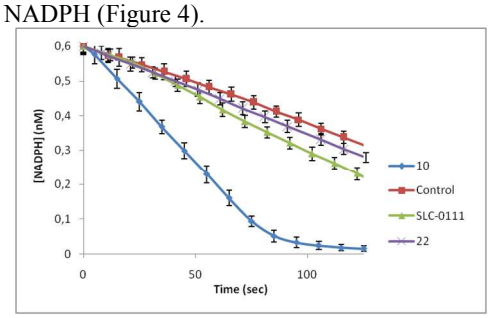


Figure 4: NADPH-coupled GPx assay for compounds **10**, **SLC-0111** and **22**. Reaction conditions: $[NADPH]_0$=0.6 mM, $[GSH]_0$=2.0 mM, $[H_2O_2]_0$=5 mM, [GR]=8 units/mL, [catalyst]=0.2 mM in pH 7.4 phosphate buffer at room temperature. Reported are the mean ± SD values of three separate experiments.

As above reported the NADPH was completely consumed within 100 s in the presence of the selenium containing compound **10**. Conversely the NADPH consumption rate was only slightly higher with respect to the control experiment (no catalyst used) when the sulfur- and oxygen-containing analogues **22** and **SLC-0111** were used instead.

The water soluble selenoureido containing compounds **10**, **15** and **20** were evaluated for their viability effects on human prostate (PC3), breast (MDA-MB-231) and colon cancer (HT-29) cells lines at 30, 100 and 300 μM concentration, incubated for 48 h both under normoxic and hypoxic conditions and using the CA inhibitor **SLC-0111** as a reference compound (Figure 4).

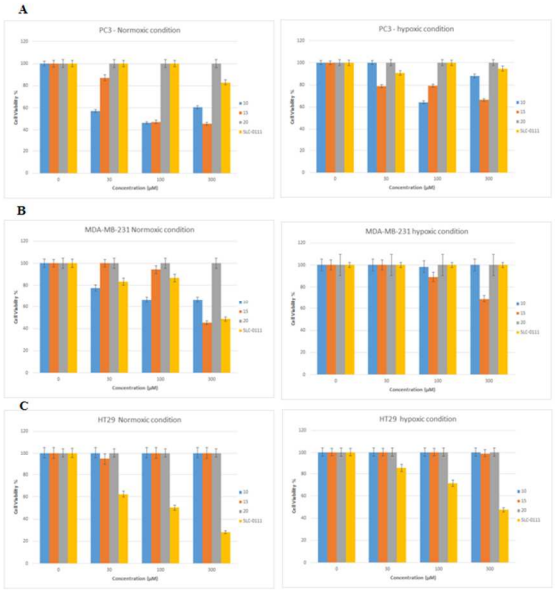


Figure 4A-C. PC3, MDA-MB-231 and HT-29 were plated at $1 \cdot 10^4$/well, incubation was allowed for 48 h in normoxic (20% O_2) and hypoxic conditions (0.1% O_2). Compounds **10**, **15** and **20** in comparison with **SLC-0111** were tested in the 30 – 300 μM concentration range. Control condition was arbitrarily set as 100% and values are expressed as the mean ± S.E.M. of three experiments.

In PC3 cells the selenoureido derivative of **SLC-0111** (compounds **10)** and the its longer *ortho* fluoro derivative **15** significantly reduced cell viability in normoxia (20 % Oxygen), whereas their effects resulted lower when the experiments were carried out under hypoxic conditions (0.1 % Oxygen). Compound **20** was ineffective in all set of experiments, whereas the reference ureido derivative **SLC-0111** induced modest mortality and only at the highest concentration (300 μM) in normoxia (figure 4A). Interestingly**,** in MDA-MB-231 cells, compound **10** was effective in normoxia at 30 μM and with a profile comparable to the reference **SLC-0111**. The elongated derivative **15** was successful in reducing cells viability in both conditions of oxygenation but only at the highest concentration (300 μM). Finally compound **20** was ineffective on the breast cancer cell lines (Figure 4B). As for colon cancer HT-29 cells, all compounds considered (**10**, **15** and **20**) were ineffective in inducing mortality, whereas the reference compounds **SLC-0111** strongly reduced cell viability at all concentrations in normoxic and hypoxic conditions (Figure 4C).

The binding modes of both selenium and sulfur analogues of **SLC-0111** (compound **10** and **22** respectively) were determined by means of their X-ray co-crystallographic adducts with the hCA II. (Figure 5)

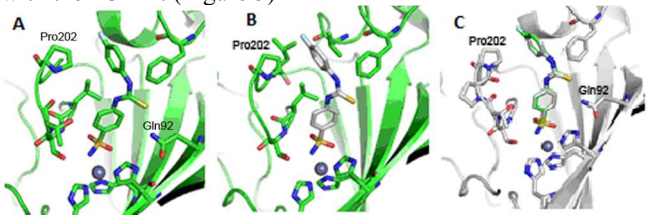


Figure 5. A) Active site region of the hCA II−**10** complex (PDB 5WEX); **B)** hCA II−**22** complex (PDB code: 5ULN); superposition of the hCA II−**10** and **22**

The difference (Fo−Fc) electron density maps of the hCA II−**10** complex revealed a well ordered structure of the benzenesulfonamide section, which became weaker in electron density for the seleno ureido and *para*-fluorobenzene moieties. Instead the hCA II−**22** complex showed clear electron density all through the molecule, thus suggesting a better ordered structure within the enzymatic cleft (Figures S1 and S2 respectively in Supporting Information). In both cases **10** and **22** showed almost identical allocations within the enzyme cavity, with average distances between the two structures (RMSD) of just 0.12 Å across the whole protein (Figure 5). The compounds were buried within the enzyme active site, being coordinated to the Zn (II) ion through the sulfonamide group and orientated towards the hydrophobic half of the pocket. The selenium and sulfur atoms are within 3 Å of the Gln92 sidechain and can make hydrogen bond to the protein, The 4-fluorophenyl tail of **10** and **22** overlays close extends further into the hydrophobic side of the catalytic cleft, with the fluorine closest to Pro202 (within 3.5 Å).

We also determined the binding mode of the longer selenoureido derivative **14** (Figure 6).

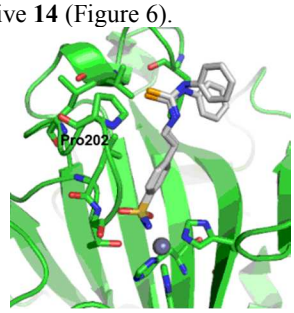


Figure 6. Active site region of the hCA II−**14** complex (PDB code: 5UMC).

The difference (Fo−Fc) electron density maps (Supporting Information, Figure S3) showed that **14** is buried within the enzyme cavity, towards the hydrophobic region of the cleft, where it establishes multiple van der Waals interaction with Phe131, Val135, Leu198, Leu204 and Pro202. The sulfonamide moiety is tightly coordinated to the Zn(II) ion by means of the canonical interactions of all CAs-sulfonamide compounds.[3,31] Conversely to the sulfonamide containing head section, the tail fragment of **14** was somewhat disordered. The *N*-phenyl moiety was modelled with two different conformations in this structure due to the diffuse electron density present in this region (figure 6).

In conclusion we report for the first time the synthesis of a series of derivatives **7-21** a rarely investigated chemotype in the CA field, as congeners of the CAI inhibitor **SLC-0111**. All compounds were obtained according to the bioisosteric replacement approach and its effects on the inhibition potency were examined. The antioxidant activity of the selenium containing CAI **10** was assessed by means of the DTT and GSH oxidation tests. Furthermore the binding modes of compounds **10**, **14** and **22** within the hCA II were determined. Finally we reported a preliminary cytotoxic assay of selected compounds on prostate, breast and colon cell lines. The obtained results suggested that multiple mechanisms of action, not yet identified, may take place and are responsible to exert the compounds cytotoxic effects. In the whole this study clearly opens new perspectives within the CA dependent diseases

ASSOCIATED CONTENT

Supporting Information

Supporting information is available free of charge on the ACS Publications website. Synthetic procedures, characterization of compounds, in vitro kinetic procedure, antioxidant activity procedure, cells viability procedure, X-ray statistics (Table S1), Difference Electron Density Map of Compound **10, 22** and **14** with hCA II (Figures S1-3).

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no conflict of interest.

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ABBREVIATIONS

CAI(s), carbonic anhydrase inhibitor(s); AAZ, acetazolamide; (h)CA, (human) carbonic anhydrase; K_I, inhibition constant; DTT, dithiothreitol; GSH, glutathione.

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For Table of Contents Use Only

Lay Summary: This article reports the synthesis, *in vitro* kinetic evaluation, binding modes, antioxidative properties and preliminary citotoxicity studies of a series of benzenesulfonamides bearing the selenoureido moiety and developed as congeners of the SLC-0111 compound, which recently ended Phase I clinical programs for the treatment of patients with advanced hypoxic tumors over-expressing the isoform IX of the human (h) metalloenzyme Carbonic Anhydrases (CAs, EC 4.2.1.1).

TOC

Discovery of new selenoureido analogs of 4-(4-fluorophenylureido)benzenesulfonamide) as carbonic anhydrase inhibitors

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