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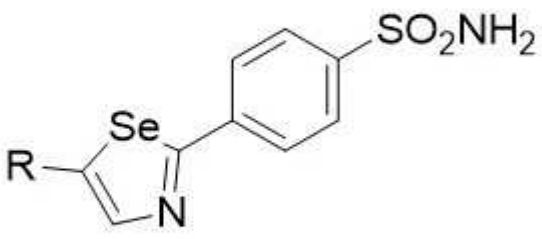

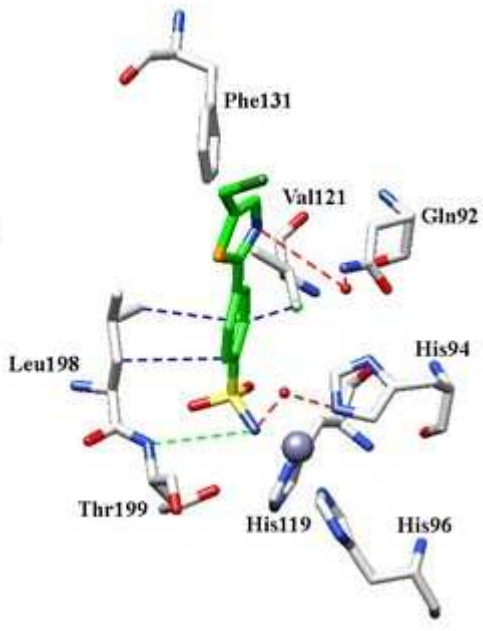

Discovery of new 2, 5-disubstituted 1,3-selenazoles as selective human carbonic anhydrase IX inhibitors with potent anti-tumor activity

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Abstract. A series of disubstituted selenazole derivatives was synthetized and evaluated as carbonic anhydrase (CA, EC 4.2.1.1) inhibitors against the human (h) isoforms hCA I, II, IV, VA, VB and IX, involved in a variety of diseases including glaucoma, retinitis pigmentosa, epilepsy, arthritis and tumors. The investigated compounds showed potent inhibition against the tumor-associated transmembrane hCA IX, with K_Is in the subnanomolar – low nanomolar range, and were evaluated for their effects on cell viability against the human prostate (PC3) and breast (MDA-MB-231) cancer cell lines, showing effective anti-tumor activity. These selenazoles are interesting leads for the development of new, isoform-selective CA IX inhibitors.

Keywords: carbonic anhydrase; inhibitor, metalloenzymes, selenazole, selenium, tumors.

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1.Introduction

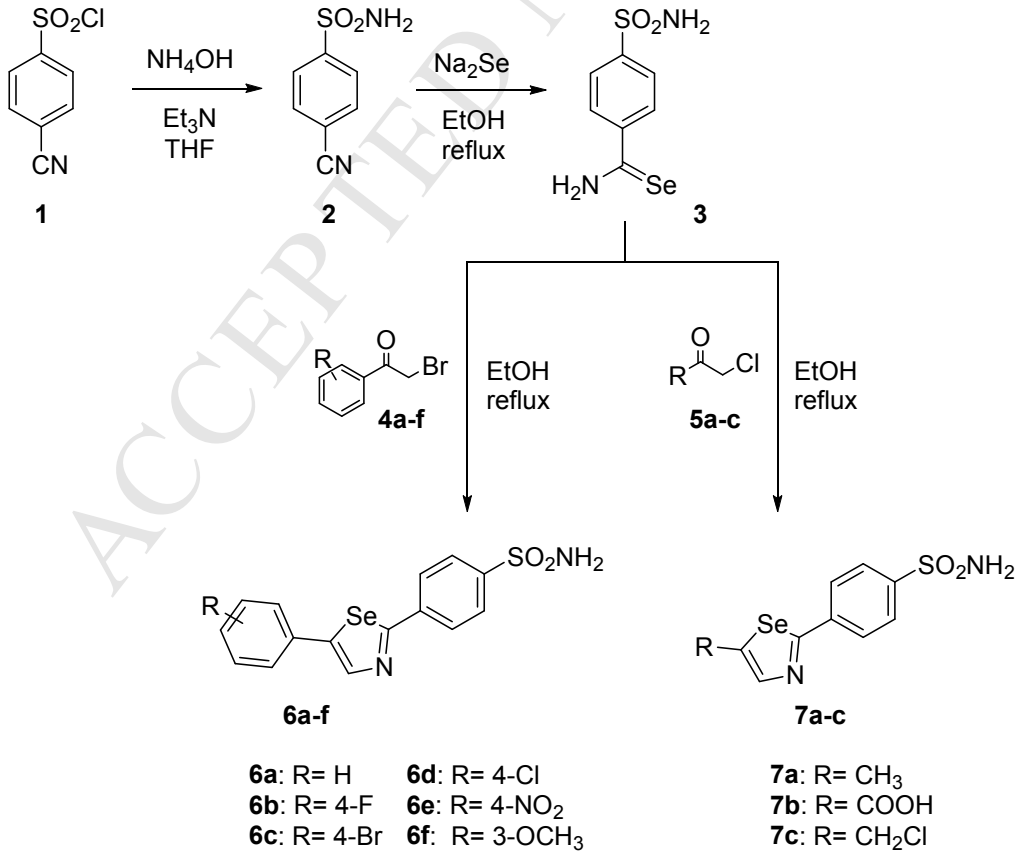
Selenium has a long history of association with human health and disease.[1, 2] Its toxicity is an old and debated issue that for a long time hampered the idea that Se-containing compounds may have a potential as therapeutic agents.[3] Today, the biochemistry and pharmacology of organoselenium compounds are topics of intense research in medicinal chemistry, because it has been demonstrated that such compounds may be used as antioxidants, antitumor, antiviral, antimicrobial, or antihypertensive agents as well as enzyme modulators targeting nitric oxide synthase (NOS),[4] carbonic anhydrase (CA, EC 4.2.2.1)[5] and lipoxygenase (LOX) among others [6]. Some of these enzymes are involved in serious diseases, thus leading to possible applications as therapeutic agents of such derivatives.[7-9] Selenium compounds have proved to be very potent anticarcinogenic agents in different models, such as spontaneous, chemically induced, or transplanted tumours, as well as *ex vivo* cultures.[10-12] The unique redox properties of selenium alter the proliferative potential of epithelial cancers by inducing apoptosis [13], but selenium may also be considered as a double-edged sword in terms of possessing antioxidant as well as pro-oxidant properties that may be beneficial or harmful, depending upon the form and dose being used in a normal or cancerous tissue. Thus, the concept of the U-shape was introduced to reflect the “good” and the “bad” effects of selenium as a function of dose.[14] Despite very promising research, to date, no synthetic organoselenium compounds are in clinical use as anti-cancer agents. The applicability of selenoheterocyclic compounds in tumor control has been demonstrated in compounds incorporating a five-membered ring system.[15,16]

Many tumour types are characterized by an upregulated glucose metabolism, low levels of oxygen (hypoxia) and a dysregulated acid base balance, with the extracellular pH more acidic than the normal values.[17-19] In this particular contest, the metalloenzyme human (h) carbonic anhydrase (CA, EC 4.2.1.1) hCA IX is overexpressed in a wide range of hypoxic tumours as downstream target of the transcription factor hypoxia-inducible factor-1α (HIF-1α) activation.[20,21] hCA IX participates in the survival, proliferation, and metastasis processes of such hypoxic tumors. Indeed, hCA IX targeting has received a lot of interest, and several efforts are being done towards developing novel inhibitors.[22] Among the huge number of sulfonamide, sulfamate, sulfamide and coumarin hCA IX inhibitors reported to date,[23-25] few compounds were investigated in detail in animal tumour models, and only SLC-0111, an ureido-substituted benzenesulfonamide derivative, successfully ended Phase I clinical trials, in 2016, for the treatment of patients with advanced hypoxic tumors overexpressing the hCA IX and the compound is currently scheduled to enter Phase II trials this year.[26]

2. Results and discussion

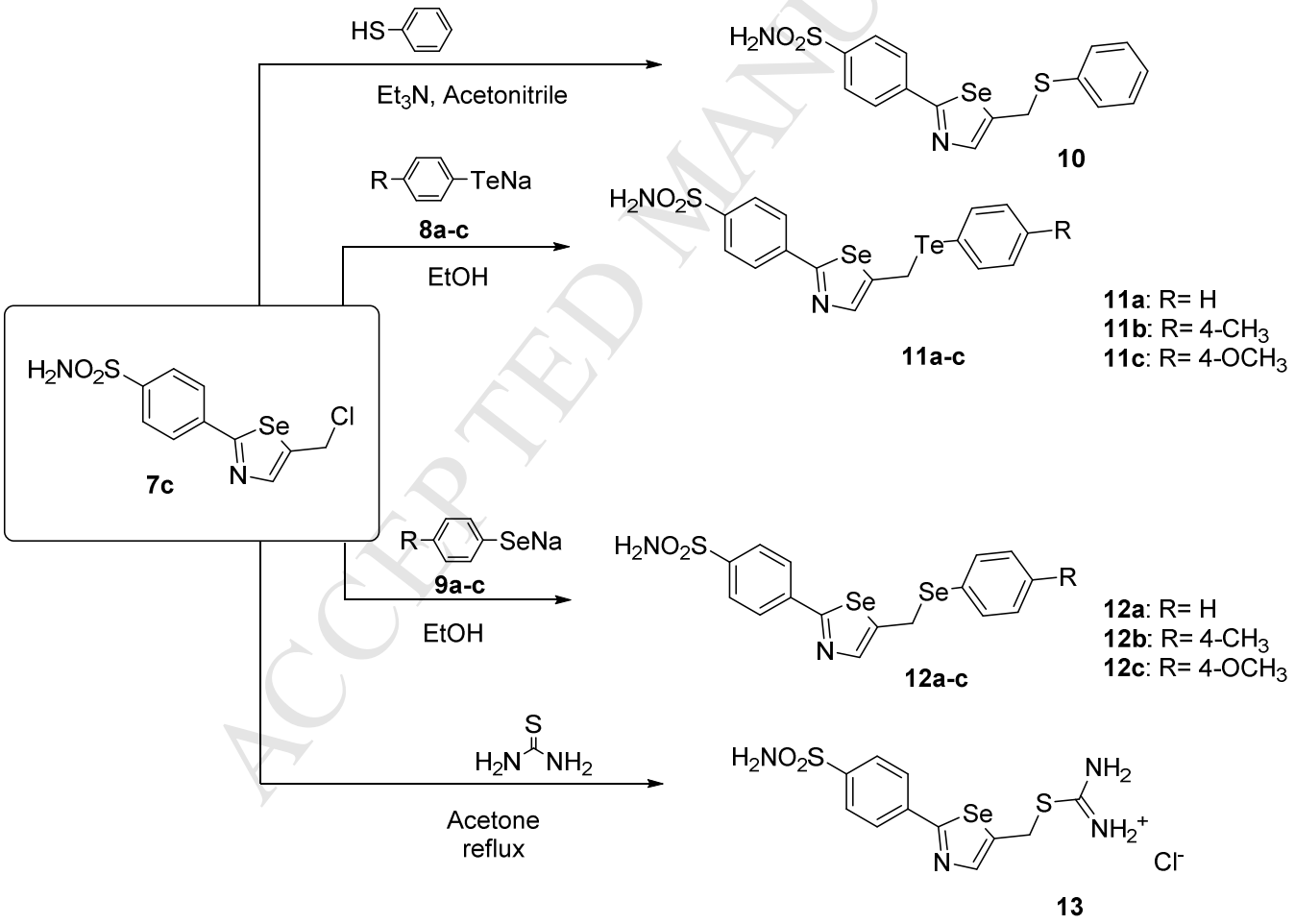
2.1. Chemistry

Given our interest in the study of chalcogen-containing compounds, we have designed and synthetized a series of selenazole derivatives with the aim to identify novel hCA IX selective inhibitors.[1,3] Selenazoles are an important class of heterocycles with significant biological effects and considerable pharmacological relevance.[15,16] Moreover, these five membered selenium heterocycles are easily synthetized from primary selenoamide as starting materials. Thus, our attention focused on the synthesis of primary selenoamide containing sulfonamide moiety, as shown in **Scheme 1**. First step was the synthesis of 4-cyanobenzenesulfonamide (**2**) by reaction of the corresponding sulfonyl chloride (**1**) with an aqueous solution of ammonium hydroxide. Successively, 4-sulfamoylbenzoselenoamide (**3**) was prepared by the reaction of nitrile compound **2** with Na_2Se as selenating reagent in reflux ethanol, for 6h.



Scheme 1: Synthesis of primary selenoamide incorporating a sulfonamide moiety **3** and the corresponding functionalized selenazoles **4a-f** and **5a-c**. Na_2Se was generated in situ from elemental Se (0.5 eq.) and $NaBH_4$ (1.0 eq.).

Finally, the reaction of primary selenobenzamide (**3**) with different α-haloketones incorporating aromatic **4a-f** or aliphatic **5a-c** moieties, in refluxing ethanol, gave various 2, 5-disubstituted 1, 3-selenazoles **6a-f** and **7a-c**. In addition, herein, we report the synthesis of a variety of double-functionalized and ionic 1, 3-selenazoles, by nucleophilic displacement reactions of 4-halomethyl- 1,3-selenazoles **7c** as shown in **Scheme 2**.



Scheme 2: Synthesis of substituted 2,5-selenazoles **10-13.**

Treatment of **7c** with thiophenol and Et_3N in acetonitrile afforded in excellent yields the corresponding functionalized 1,3-selenazoles **10**. A number of 1,3-selenazoles containing aromatic chalcogenide side chains were also prepared (**11a-c** and **12a-c**). The reaction started with the reduction of the appropriate dichalcogenide and $NaBH_4$ to afford the corresponding chalcogenate (**8a-c** and **9a-c**), which was treated *in situ* with 4-halomethyl- 1,3-selenazoles **7c** leading to the corresponding functionalized 1,3-selenazoles **11a-c** and **12a-c** in good yield (**Scheme 2**). Finally, 1, 3-selenazole containing the pharmacologically relevant isothiouronium moiety (**13**) was prepared from **7c** and thiourea.

2.2. Carbonic anhydrase inhibition

All compounds **3**, **6a-f**, **7a-c**, **10**, **11a-c**, **12a-b** and **13** were tested in vitro for their inhibitory activity against the physiologically relevant hCA isoforms I, II, IV, VA, VB and IX by means of the stopped-flow carbon dioxide hydration assay[27] and their activities were compared to the standard CAI acetazolamide (**AAZ**) (**Table 1**).

Table 1. Inhibition data of human CA isoforms I, II, IV, VA, VB and IX with compounds **3**, **6a-f, 7a-c, 10, 11a-c, 12a-c, 13** and **AAZ** by a stopped flow CO_2 hydrase assay.[27]

	K_I (nM)					
Cmp	hCA I	hCA II	hCA IV	hCA VA	hCA VB	hCA IX
3	44.2	84.8	941.1	63.4	552.7	7.6
6a	33.5	29.6	652.3	9.7	703.1	6.5
6b	637.9	85.8	6353.0	75.3	724.2	7.5

6c	206.0	88.6	3098.0	86.6	620.4	0.87
6d	414.1	67.9	873.1	82.2	730.0	8.4
6e	539.7	245.1	9386.0	85.7	757.3	8.2
6f	42.2	70.9	359.5	82.5	808.7	56.3
7a	15.8	47.3	384.7	82.3	600.0	6.9
7b	41.6	77.0	392.4	42.5	460.0	9.0
7c	7.3	73.2	326.2	58.4	90.9	2.8
10	935.0	74.6	6860.0	55.8	550.0	5.4
11a	135.2	79.7	5155.0	56.3	81.0	7.2
11b	8.2	186.7	2695.0	56.8	809.5	2.6
11c	47.7	9.5	853.9	40.0	85.0	0.89
12a	289.1	9.8	751.0	68.3	517.0	9.1
12b	30.3	9.6	203.9	75.6	54.1	7.1
12c	7.1	7.7	120.8	47.1	319.1	0.55
13	14.6	80.4	216.5	57.3	700.0	7.0
AAZ	250.0	12.1	74	63.0	54.0	25.8

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

We have investigated a range of various kinds of 2, 5-disubstituted 1, 3-selenazole derivatives for their interaction with the six hCAs here considered, after a period of 15 min of incubation of the enzyme and inhibitor solutions.[28–31] The following structure activity relationship (SAR) may be noted regarding the inhibition data of **Table 1**:

i) The cytosolic hCA I was inhibited by primary selenoamide **3** in the medium nanomolar range (K_i 44.2 nM). On the other hand, the inhibition profile of selenazole derivatives varied according to the substituent in position 5. The benzene moiety (**6a**) led to a slight increase in the activity (K_i 33.5 nM) and substitutions in positions 3 or 4 led to a decrease of the potency. An interesting inhibition profile was observed for compounds **6b-d** incorporating halogens. The dimension of this substituent proved to be crucial for the inhibition potency. Indeed, replacement of fluorine (**6b**) by bromine (**6c**) caused an increase of activity for hCA I (K_i 637.9 nM vs. K_i 206.0 nM). Moreover, substitution in position 3 (**6f**) led to a better activity than the corresponding compounds with *para* groups (**6b-e**). Aliphatic moieties in position 5 of selenazoles (**7a-c**) increased the efficacy compared to aromatic compounds (**6a-f). 7c** showed an inhibition constant in the low nanomolar range (K_i 7.3 nM). Another interesting point was the further substitution of the side chain in compound **7c** with chalcogen aromatic moiety. Going down along the chalcogen group of the periodic table (from sulphur **10** to tellurium **11a**) the activity increased (K_i 935.0 nM to K_i 135.2 nM) as the chalcogen group element increased from Sulphur (**10**) to tellurium (**11a**). The second dominant cytosolic isoform, hCA II, was inhibited by all these compounds in the medium nanomolar range except for compounds **11c** and **12a-c** which showed low nanomolar inhibition (K_i 7.7 to 9.8 nM). The different moieties in position 5 of the selenazole scaffold did not influence significantly the inhibition constant. On the other hand, the further functionalization of 4-halomethyl-1,3-selenazoles **7c** with different selenites **12a-c** and tellurate **11c** moieties led to an increase of potency near ten folds compared to the other compounds here considered.

ii) Almost all compounds investigated here, possessed low inhibitory activity for the membrane-bound isoform hCA IV with ranges spanning between the high nanomolar to the micromolar

(K_i 120.8 to 9386 nM). Aliphatic substituents in position 5 proved to be better than aromatic ones, with an inhibition constants spanning from K_i 326.2 to 392.4 nM. Indeed, as for the previously discussed isoform hCA II mentioned above, the functionalization of 4-halomethyl-1,3-selenazoles **7c** with selenolate (**12a-c**) and tellurate **11c** moieties increased the efficacy.

iii) An interesting inhibition pattern was observed for the mitochondrial hCA VA and hCA VB isoforms. All compounds here considered, except **12a**, exhibited a preference of inhibition for the isoform hCA VA over hCA VB and, selenazole **6a** showed this preference with an activity 70 folds more potent for hCA VA (K_i 9.7 nM) compared to hCA VB (K_i 703.1 nM). On the other hand, the different moieties in position 5 of the selenazole scaffold did not influence significantly the inhibition potency.

iv) The membrane-bound, tumor-associated, hCA IX, is effectively inhibited by selenazoles in low nanomolar to sub-nanomolar range (K_i 0.55 nM to 9 nM) except for compound **6f** (K_i 56.3 nM). An interesting case was constituted by the isosteric substitution of the halogen atom in the aromatic moiety of **6c-d** and **6f**. The transition from fluorine atom (**6f**) to bromine (**6b**) led to a significant increase of the inhibitory potency (K_i 56.3 to 0.87 nM). The activity was influenced also for the other chalcogen moieties (**11a-c** and **12a-c**), especially for methoxy substituent, which led to a subnanomolar inhibition (K_i 0.89 and 0.55 nM).

2.3 X-ray Crystallographic Results

X-ray crystallography (statistics summarized in ESI) was used to analyse the mode of binding of compound **7c** in complex with hCA II. Structural analysis of the initial F_o-F_c electron density map of the active site showed well defined electron density, fully compatible with the presence of inhibitor **7c** but, electron density for chlorine atom was not present (**Figure 1A**). The sulfonamide moiety coordinates the

catalytic zinc ion of hCA II with a tetrahedral geometry, by means of one nitrogen atom of the sulfonamide group and displacing the zinc bound water molecule/hydroxide ion similarly to other sulfonamide and sulfamide derivatives investigated earlier.[32,33]

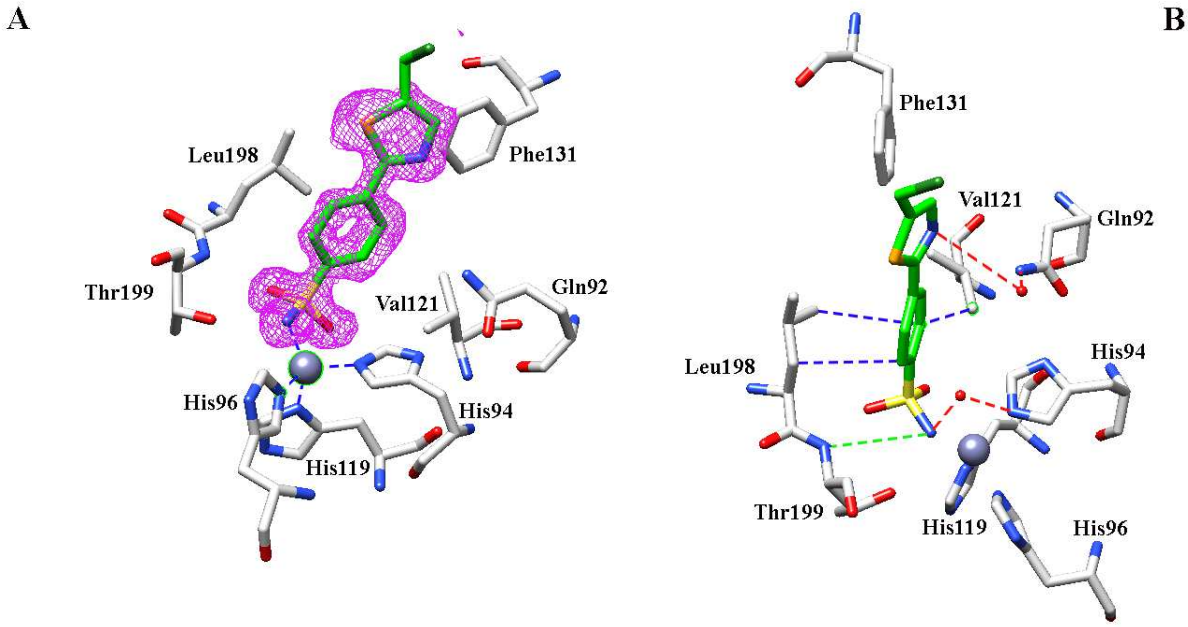


Figure 1. Active site region of hCA II/**7c** adduct (PDB 6H3Q). Inhibitor showing the σA-weighted $|F_o - F_c|$ map (at 2.5 σ) (**A**). Hydrogen bonds, van der Waals interactions and Water Bridges are shown and labelled in green, blue and red respectively (**B**).

The deprotonated nitrogen atom of the sulfonamide moiety forms a hydrogen bond with the NH moiety of Thr199. Furthermore, a hydrogen bond is involved with a water molecule showing a well defined solvent network that contributes to stabilize the inhibitor within the active site (**Figure 1B**). The phenyl ring of inhibitor **7c** is located in the active site channel, where it is involved in a number of hydrophobic interaction with the side chains of residues Val121 and Leu198. The medium potency of inhibition against hCA II (K_i 73 nM) could be explained with the rigid structure conferred by two consecutive aromatic rings; this scaffold prevented the inhibitor from locating its tail in a small hydrophobic pocket delimited by residues Phe131 and which strongly correlates with the inhibition potency against this

isoform. On the other hand, nitrogen of selenazole moiety is involved in a water bridge with residue Gln92 (**Figure 1B**).

2.4 Biological assays

We focused our attention on the *ex vivo* activity of compounds **6e**, **11c** and **12c**, which were evaluated for their effects on cell viability against the human prostate (PC3) and breast (MDA-MB-231) cancer cell lines. All compounds were low/sub nanomolar hCA IX inhibitors, and were used at different concentrations, being incubated for 48 h in both normoxic and hypoxic conditions, when overexpression of high amounts of CA IX occurs.[17] In PC3 cells, selenazole derivative **6e**, with a 4-NO_2 phenyl moiety, reduced the cell viability to 60% at 1µM. Its efficacy increased significantly at 10 µM reducing the viability to 10% (**Figure 2**). In the hypoxic conditions, compound **6e** showed significantly more effects on cytotoxicity which reached 32% at 1µM. At higher concentration the compound was comparable to its effects in normoxic condition. Chalcogenide atom in the side chain of compounds **11c** and **12c** proved to play a crucial role for cytotoxicity. Compound **11c**, whit tellurium atom in the side chain, showed a strong activity against this cancer cell line in normoxic condition with a viability of 23% at 1 µM. On the other hand, in hypoxic condition compound **11c** showed an efficacy two times lower than in the normoxia. Finally, compound **12c**, with selenium in the side chain, proved to be inactive at lower doses against PC3 cell line, but the efficacy increased at 30 and 100 µM showing a cell viability in normoxic condition of 59% and 11% respectively. In hypoxic condition compound **12c** exhibited more cytotoxic effects at low concentration (77% at 10 µM) whereas at higher doses exhibited an efficacy comparable to that in normoxic condition.

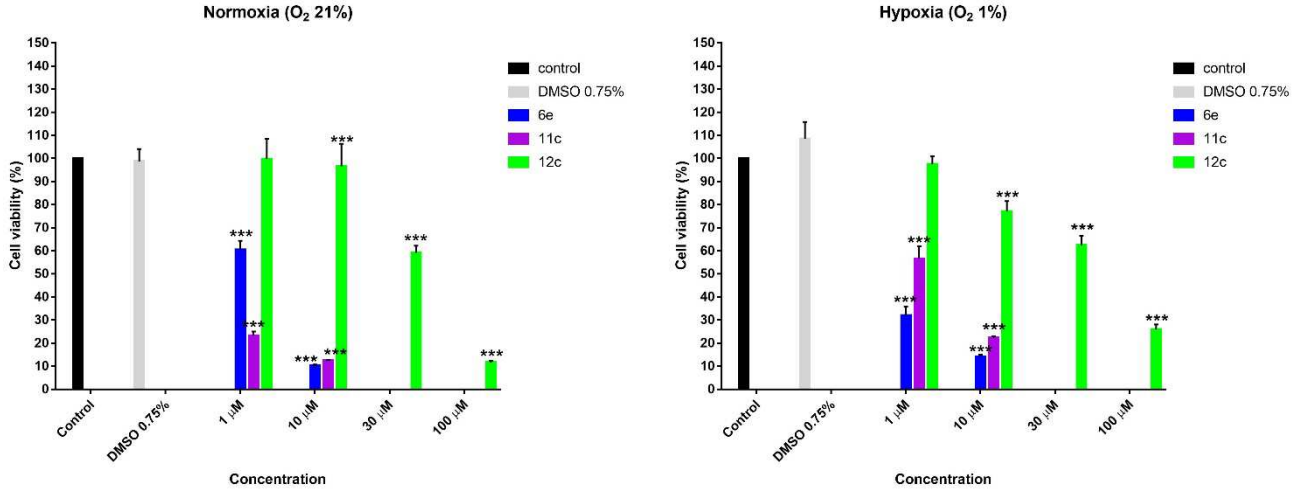


Figure 2. Effects of newly synthetized compounds **6e**, **12c** and **11c** on viability of the human prostatic cancer cell line PC3 following 48h treatment in normoxic and hypoxic (1% O_2) conditions. ***p<0.001 *versus* control.

In the MDA-MB231 cell line, derivative **6e** at the lower concentration showed a weak activity with viability of 73%. On the other hand, at 10 μM the potency against this cancer cell line increased drastically killing over 90% of the cells. Also for MDA-MB231 cell line the different chalcogenide in the side chain of compound **11c** and **12c** played an important role on the viability (**Figure 3**). Indeed, tellurium atom in derivative **11c** exhibited a strong cytotoxicity in normoxic conditions, already at lower concentration (16% at 1 μM). Also this time, the potency decreased over two time in hypoxic condition. Compound **12c** did not show any activity in this *ex vivo* normoxia assay at 1 and 10 μM concentration. A reduced cell viability was observed for this compound only at high concentration (30 μM and 100 μM). In the hypoxic conditions the selenium derivative **12c** did not show any significant activity, only at higher concentration of 100 μM the cell viability arrived at 46%.

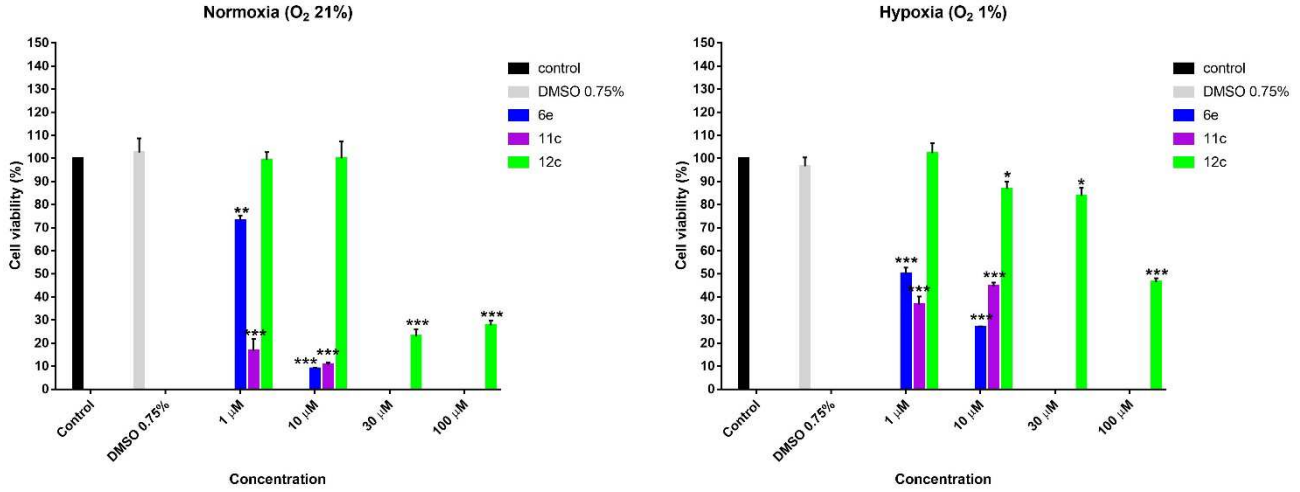


Figure 3. Effects of the newly synthetized compounds **6e**, **12c** and **11c** on viability of the human adenocarcinoma breast cell line MDA-MB231 following 48h treatment in normoxic and hypoxic (1% O_2) conditions. *p<0.05, ** p<0.01, ***p<0.001 *versus* control.

3. Conclusions

We report here a novel series of selenazole derivatives as inhibitors on six α-carbonic anhydrases (CAs, EC 4.2.1.1) of pharmacologic relevance *i.e.*, hCA I, II, IV, VA, VB and IX. Most of the new derivatives were medium potency inhibitors of the ubiquitous cytosolic hCA I and hCA II isoforms, but they showed significant inhibition potency against the tumor-associated transmembrane hCA IX isoform, with inhibition constants spanning from the sub-nanomolar to the low nanomolar range. These compounds exhibited potent effects on cell viability against the human prostate (PC3) and breast (MDA-MB-231) cancer cells lines, possessing thus effective anti-tumor activity. Moreover, we studied the complex of one such selenazole with hCA II by X-ray crystallography, for better understanding the mechanism of binding and the inhibition potency. Thus, this study clearly opens new perspectives in the field of CA-dependent diseases and, the interesting leads detected here may be useful for the development of more potent and hCA IX isoform-selective inhibitors.

4. Experimental Part

4.1. General

Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, Alfa Aesar and TCI. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringes techniques to transfer solutions. Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR, ^{77}Se-NMR, ^{125}Te-NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. $(PhSe)_2$ and $(PhTe)_2$were used as an external references for ^{77}Se NMR (δ = 461 ppm) and ^{125}Te NMR (δ = 420 ppm). Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double of doublets. The assignment of exchangeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck Silica gel 60 (230-400 mesh ASTM) as the stationary phase and ethyl acetate/*n*-hexane were used as eluents. Melting points (mp) were measured in open capillary tubes with a Gallenkamp MPD350.BM3.5 apparatus and are uncorrected. The solvents used in MS measures were acetone, acetonitrile (Chromasolv grade), purchased from Sigma-Aldrich (Milan - Italy), and mQ water 18 MΩ, obtained from Millipore's Simplicity system (Milan-Italy). The mass spectra were obtained using a Varian 1200L triple quadrupole system (Palo Alto, CA, USA) equipped by Electrospray Source (ESI) operating in both positive and negative ions. Stock solutions of analytes were prepared in acetone at 1.0 mg mL^{-1} and stored at 4°C. Working solutions of each analyte were freshly prepared by diluting stock solutions in a mixture of mQ H_2O/ACN 1/1 (*v/v*) up to a concentration of 1.0 μg mL^{-1} The mass spectra of each analyte were acquired by introducing, via syringe pump at 10 μL min^{-1}, of the its working solution. Raw-data were collected and processed by Varian Workstation Vers. 6.8 software.

4.1.1 Procedure for the synthesis of 4-cyanobenzenesulfonamide (2).

4-cyanobenzenesulfonyl chloride **1** (10 mmol) was added to a solution of anhydrous THF (40 mL). An aqueous solution of ammonium hydroxide (30%) (3mL) was added to the mixture at 0°C and stirred at room temperature for 1 h. The mixture was extracted with ethyl acetate, dried with anhydrous Na_2SO_4, and triturated with diethyl ether (1.6 g, 88%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ(ppm): 8.11 (2H, d, *J*=8.58 Hz), 8.02 (2H, d, *J*=8.62 Hz), 7.69 (2H, bs, N**H$_2$**, exchange with D_2O). **^{13}C NMR** (100 MHz, DMSO- d_6) δ(ppm): 148.9, 134.2, 127.4, 118.8, 115.3.

4.1.2 Procedure for the synthesis of 4-sulfamoylbenzoselenoamide (3).

$NaBH_4$ (626 mg, 16.47 mmol, 3.0 eq.) was portion wise added to a solution of elemental selenium (650mg, 8.24 mmol, 1.5 eq.) in EtOH (20 mL) at 0°C under inert atmosphere (N_2). After 30 min, 4-cyanobenzenesulfonamide **2** (1 g, 5.49 mmol, 1.0 eq.) was added and the reaction mixture was stirred at reflux for 6h. When the starting 4-cyanobenzenesulfonamide **2** had completely reacted (monitored by TLC), cooling to room temperature and hydrochloric acid (6N, 2 ml) was added and the solution was stirred for about an hour. The organic phase was extracted with EtOAc, washed with brine (1 x 5 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The crude material was purified by flash chromatography (1:1 hexane/ethyl acetate) to yield 4-sulfamoylbenzoselenoamide (**3**) as orange solid (1.1 g, 76%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ(ppm): 11.05 (1H, bs, N**H**, exchange with D_2O), 10.42 (1H, bs, N**H**, exchange with D_2O), 7.99 (2H, d, *J*=8.65 Hz), 7.85 (2H, d, *J*=8.65 Hz), 7.51 (2H, bs, N**H$_2$**, exchange with D_2O). **^{13}C NMR** (100 MHz, DMSO- d_6) δ(ppm): 203.7, 146.6, 146.3, 128.5, 126.1. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 627.5. MS (ESI negative) *m/z* (%): 263.0 $[M-H]^-$.

4.1.3 General procedure for the synthesis of 2, 5 substitutes 1, 3-selenazoles (6a-f and 7a-c).

An EtOH solution (20 mL) of 4-sulfamoylbenzoselenoamide (**3**) (100 mg, 0.38 mmol) and appropriate ω-halo derivatives **4a-f** or **5a-c** (0.38 mmol, 1 Eq.) was stirred at reflux for 20 min. After cooling, the mixture was poured into H_2O (20 mL), which resulted in the formation of a precipitate. This was filtered off and recrystallized (EtOH) to give the corresponded 2,5 subtitutes 1, 3-selenazoles (**6a-f** and **7a-c**).

4.1.4 4-(5-phenyl-1,3-selenazol-2-yl)benzenesulfonamide (6a)

Following the general procedure, 4-sulfamoylbenzoselenoamide (**3**) (100 mg, 0.38 mmol) and 2-Bromoacetophenone **4a** (76 mg, 0.38 mmol) gave **6a** as white solid (115 mg, 83%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.83 (1H, s), 8.25 (2H, d, *J* = 8.62 Hz), 8.11 (2H, dd, *J* = 8.31, 1.21 Hz), 7.99 (2H, d, *J* = 8.63 Hz), 7.54-7.50 (4H, m), 7.42 (1H, t, *J* = 7.33 Hz). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.6, 156.9, 146.2, 139.2, 135.6, 129.7, 129.0, 128.1, 127.6, 127.3, 123.3; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 738.9. **MS** (ESI negative) *m/z*: 363.1 $[M-H]^-$.

4.1.5 4-(5-(4-fluorophenyl)-1,3-selenazol-2-yl)benzenesulfonamide (6b)

Following the general procedure, 4-sulfamoylbenzoselenoamide (**3**) (100 mg, 0.38 mmol) and 2-Bromo-4'-fluoroacetophenone **4b** (83 mg, 0.38 mmol) gave **6b** as pink solid (93 mg, 64%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.81 (1H, s), 8.25 (2H, d, *J* = 8.42 Hz), 8.15 (2H, dd, *J* = 8.79, 5.54 Hz), 7.99 (2H, d, *J* = 8.41 Hz), 7.54 (2H, bs, N**H$_2$**, exchange with D_2O), 7.35 (2H, t, *J* = 8.86 Hz). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.7, 162.8 (d, *J* = 245.19 Hz), 155.8, 146.3, 139.2, 132.2, 129.4 (d, *J* = 8.25 Hz), 128.1, 127.6, 123.0, 116.6 (d, *J* = 21.55 Hz); **^{19}F-NMR** (376 MHz, DMSO-d_6) δ (ppm): -113.89; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 741.0. **MS** (ESI negative) *m/z*: 381.1 $[M-H]^-$.

4.1.6 4-(5-(4-bromophenyl)-1,3-selenazol-2-yl)benzenesulfonamide (6c)

Following the general procedure, 4-sulfamoylbenzoselenoamide (**3**) (100 mg, 0.38 mmol) and 2-Bromo-4'-bromoacetophenone **4c** (106 mg, 0.38 mmol) gave **6c** as white solid(101 mg, 60%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.90 (1H, s), 8.25 (2H, d, *J* = 8.21 Hz), 8.07 (2H, d, *J* = 8.38 Hz), 7.98 (2H, d, *J* = 8.23 Hz), 7.71 (2H, d, *J* = 8.35 Hz), 7.55 (2H, bs, N**H$_2$**, exchange with D_2O). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.9, 155.6, 146.3, 139.1, 134.8, 132.7, 129.3, 128.1, 127.6, 124.2, 122.1; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 744.8. **MS** (ESI negative) *m/z*: 441.1 $[M-H]^-$.

4.1.7 4-(5-(4-chlorophenyl)-1,3-selenazol-2-yl)benzenesulfonamide (6d)

Following the general procedure, 4-sulfamoylbenzoselenoamide (**3**) (100 mg, 0.38 mmol) and 2-Bromo-4'-chloroacetophenone **4d** (89 mg, 0.38 mmol) gave **6d** as white solid(92 mg, 61%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.89 (1H, s), 8.25 (2H, d, *J* = 8.40 Hz), 8.14 (2H, d, *J* = 8.55 Hz), 7.99 (2H, d, *J* = 8.40 Hz), 7.59-7.54 (4H, m). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.9, 155.6, 146.3, 139.1, 134.4, 133.5, 129.8, 129.1, 128.1, 127.6, 124.1; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 744.2. **MS** (ESI negative) *m/z*: 397.1 $[M-H]^-$.

4.1.8 4-(5-(4-nitrophenyl)-1,3-selenazol-2-yl)benzenesulfonamide (6e)

Following the general procedure, 4-sulfamoylbenzoselenoamide (**3**) (100 mg, 0.38 mmol) and 2-Bromo-4'-nitroacetophenone **4e** (93 mg, 0.38 mmol) gave **6e** as yellow solid(85 mg, 55%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 9.19 (1H, s), 8.38 (4H, aps), 8.28 (2H, d, *J* = 8.48 Hz), 8.00 (2H, d, *J* = 8.53 Hz), 7.56 (2H, bs, N**H$_2$**, exchange with D_2O). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 173.6, 154.6, 147.6, 146.5, 141.5, 138.9, 128.3, 128.2, 127.9, 127.6, 125.2; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 757.4. **MS** (ESI negative) *m/z*: 408.1 $[M-H]^-$.

4.1.9 4-(5-(3-methoxyphenyl)-1,3-selenazol-2-yl)benzenesulfonamide (6f)

Following the general procedure, 4-sulfamoylbenzoselenoamide (**3**) (100 mg, 0.38 mmol) and 2-Bromo-3'-methoxyacetophenone **4f** (87 mg, 0.38 mmol) gave **6f** (120 mg, 80%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ (ppm): 8.86 (1H, s), 8.25 (2H, d, *J* = 8.53 Hz), 7.99 (2H, d, *J* = 8.53 Hz), 7.68-7.66 (2H, m), 7.55 (2H, bs, N**H$_2$**, exchange with D_2O), 7.43 (1H, t, *J* = 7.94 Hz), 7.00 (1H, ddd, *J* = 8.53 Hz), 3.88 (3H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.4, 160.6, 156.7, 146.2, 139.2, 136.9, 130.8, 128.1, 127.6, 123.6, 119.8, 114.5,112.9, 56.1 **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 757.4. **MS** (ESI positive) *m/z*: 395.0 $[M+H]^+$.

4.1.10 4-(5-methyl-1,3-selenazol-2-yl)benzenesulfonamide (7a)

Following the general procedure, 4-sulfamoylbenzoselenoamide (**3**) (100 mg, 0.38 mmol) and Chloroacetone **5a** (31 µl, 0.38 mmol) gave **7a** as yellow solid (70 mg, 61%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.12 (2H, d, *J* = 8.61 Hz), 7.94 (3H, m), 7.50 (2H, bs, N**H$_2$**, exchange with D_2O), 2.49 (3H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.1, 154.8, 145.9, 139.3, 127.8, 127.5, 122.7, 18.9; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 711.9. **MS** (ESI negative) *m/z*: 301.0 $[M-H]^-$.

4.1.11 2-(4-sulfamoylphenyl)-1,3-selenazole-5-carboxylic acid (7b)

Following the general procedure, 4-sulfamoylbenzoselenoamide (**3**) (100 mg, 0.38 mmol) and Chloroacetic acid **5b** (64 mg, 0.38 mmol) gave **7b** as white solid (69 mg, 55%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 13.07 (1H, bs, COO**H**), 9.22 (1H, apd), 8.20 (2H, d, *J* = 8.48 Hz), 7.98 (2H, d, *J* = 8.44 Hz), 7.54 (2H, bs, N**H$_2$**, exchange with D_2O). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 173.6, 173.2, 163.1, 149.1, 146.5, 138.8, 128.3, 127.6; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 763.7. **MS** (ESI negative) *m/z*: 331.0 $[M-H]^-$.

4.1.12 4-(5-(chloromethyl)-1,3-selenazol-2-yl)benzenesulfonamide (7c)

Following the general procedure, 4-sulfamoylbenzoselenoamide (**3**) (100 mg, 0.38 mmol) and 1,3 Chloroacetone **5c** (48 mg, 0.38 mmol) gave **7c** as white solid (89 mg, 70%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.47 (1H, s), 8.16 (2H, d, *J* = 8.62 Hz), 7.96 (2H, d, *J* = 8.62 Hz), 7.53 (2H, bs, N**H$_2$**, exchange with D_2O), 4.93 (2H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 173.8, 154.5, 146.3, 138.9, 128.3, 128.0, 127.6, 42.7; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 728.4. **MS** (ESI negative) *m/z*: 335.0 $[M-H]^-$.

4.1.13 Procedure for the synthesis of 4-(5-((phenylthio)methyl)-1,3-selenazol-2-yl) benzenesulfonamide (10).

An acetonitrile solution (10 mL) of 2,5 substituted 1,3 selenazole (**7c**) (100 mg, 0.298 mmol), thiophenol (36 µL, 1.2 Eq) and Et_3N (79 µL, 2Eq) were stirred overnight at room temperature. The mixture was extracted with ethyl acetate, dried with anhydrous Na_2SO_4 and the crude material was purified by flash chromatography (1:1 hexane/ethyl acetate) to yield compound **10** as white solid (98 mg, 80%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.16 (1H, s), 8.10 (2H, d, *J* = 8.36 Hz), 7.94 (2H, d, *J* = 8.34 Hz), 7.52 (2H, bs, N**H$_2$**, exchange with D_2O), 7.45 (2H, d, *J* = 7.62 Hz), 7.36 (2H, t, *J* = 7.66 Hz), 7.24 (1H, t, *J* = 7.33 Hz), 4.43 (2H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.9, 154.8, 146.1, 139.1, 136.8, 129.9, 129.4, 127.9, 127.5, 126.8, 125.5, 34.4; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 720.9. **MS** (ESI positive) *m/z*: 411.0 $[M+H]^+$.

4.1.14 General procedure for the synthesis of 2, 5 substitutes 1, 3-selenazoles 11a-c and 12a-c.

$NaBH_4$ (43 mg, 1.14 mmol, 3.0 eq.) was portionwise added to a solution of appropriate chalcogenide **8a-c** or **9a-c** (0.5 eq.) in EtOH (10 mL) at room temperature under inert atmosphere (N_2). After 30 min, 2,5 substituted 1,3 selenazole **7c** (127 mg, 0.38 mmol, 1.0 eq.) was slowly added and the reaction mixture was stirred at room temperature for 3 h, until complete consumption of the starting material was

observed by TLC. The reaction was quenched by addition of saturated aq. NH_4Cl (2 mL) and diluted with EtOAc (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The crude material was purified by flash chromatography (hexane/EtOAc 1:1) to yield the corresponded chalcogenide **11a-c** or **12a-c**.

4.1.15 4-(5-((phenyltellanyl)methyl)-1,3-selenazol-2-yl)benzenesulfonamide (11a).

Following the general procedure, 4-(5-(chloromethyl)-1,3-selenazol-2-yl)benzenesulfonamide **7c** (127 mg, 0.38 mmol) and diphenyl ditelluride **8a** (78 mg, 0.19 mmol) gave **11a** as yellow solid (96 mg, 50%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.05 (2H, d, *J* = 8.52 Hz), 7.93 (2H, d, *J* = 8.54 Hz), 7.89 (1H, s), 7.75 (2H, dd, *J* = 1.32, 8.06 Hz), 7.52 (2H, bs, N**H$_2$**, exchange with D_2O), 7.33-7.25 (3H, m), 4.45 (2H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.2, 157.5, 146.0, 139.2, 138.6, 130.1, 128.4, 127.8, 127.5, 122.5, 114.4, 8.0; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 717.6. **^{125}Te NMR** (126 MHz, DMSO-d_6) δ(ppm): 608.3. **MS** (ESI negative) *m/z*: 506.9 $[M-H]^-$.

4.1.16 4-(5-((p-tolyltellanyl)methyl)-1,3-selenazol-2-yl)benzenesulfonamide (11b).

Following the general procedure, 4-(5-(chloromethyl)-1,3-selenazol-2-yl)benzenesulfonamide **7c** (127 mg, 0.38 mmol) and *p*-tolyl ditelluride **8b** (83 mg, 0.19 mmol) gave **11b** as yellow solid (154 mg, 78%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.05 (2H, d, *J* = 8.59 Hz), 7.93 (2H, d, *J* = 8.60 Hz), 7.85 (1H, s), 7.63 (2H, d, *J* = 7.96 Hz), 7.51 (2H, bs, N**H$_2$**, exchange with D_2O), 7.09 (2H, d, *J* = 7.61 Hz), 4.40 (2H, s), 2.32 (3H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.1, 157.6, 146.0, 139.2, 139.1, 138.1, 130.9, 127.8, 127.5, 122.5, 110.1, 21.6, 8.0; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 717.0. **^{125}Te NMR** (126 MHz, DMSO-d_6) δ (ppm): 600.6. **MS** (ESI negative) *m/z*: 520.9 $[M-H]^-$.

4.1.17 4-(5-(((4-methoxyphenyl)tellanyl)methyl)-1,3-selenazol-2-yl)benzenesulfonamide (11c)

Following the general procedure, 4-(5-(chloromethyl)-1,3-selenazol-2-yl)benzenesulfonamide **7c** (127 mg, 0.38 mmol) and 4-methoxyphenyl ditelluride **8c** (89 mg, 0.19 mmol) gave **11c** as yellow solid (153 mg, 75%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.05 (2H, d, *J* = 8.59 Hz), 7.93 (2H, d, *J* = 8.58 Hz), 7.85 (1H, s), 7.78 (1H, s), 7.64 (2H, d, *J* = 8.75 Hz), 7.51 (2H, bs, N**H$_2$**, exchange with D_2O), 6.84 (2H, d, *J* = 8.77 Hz), 4.35 (2H, s), 3.76 (3H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.1, 160.3, 157.2, 146.0, 141.6, 139.2, 127.8, 127.5, 122.4, 116.1, 103.0, 55.9, 8.3; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 716.1. **^{125}Te NMR** (126 MHz, DMSO-d_6) δ (ppm): 600.9. **MS** (ESI negative) *m/z*: 536.9 $[M-H]^-$.

4.1.18 4-(5-((phenylselanyl)methyl)-1,3-selenazol-2-yl)benzenesulfonamide (12a)

Following the general procedure, 4-(5-(chloromethyl)-1,3-selenazol-2-yl)benzenesulfonamide **7c** (127 mg, 0.38 mmol) and phenyl diselenide **9a** (59 mg, 0.19 mmol) gave **12a** as white solid (128 mg, 74%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.09-8.06 (3H, m), 7.94 (2H, d, *J* = 8.41 Hz), 7.57 (2H, dd, *J* = 1.36, 8.01 Hz), 7.56 (2H, bs, N**H$_2$**, exchange with D_2O), 7.36-7.30 (3H, m), 4.41 (2H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.7, 155.8, 146.1, 139.1, 132.9, 131.2, 130.0, 127.9, 127.8, 127.5, 124.8, 27.5; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 719.4, 347.3. **MS** (ESI positive) *m/z*: 459.0 $[M+H]^+$.

4.1.19 4-(5-((p-tolylselanyl)methyl)-1,3-selenazol-2-yl)benzenesulfonamide (12b).

Following the general procedure, 4-(5-(chloromethyl)-1,3-selenazol-2-yl)benzenesulfonamide **7c** (127 mg, 0.38 mmol) and *p*-tolyl diselenide **9b** (65 mg, 0.19 mmol) gave **12b** as white solid (143 mg, 80%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.08 (2H, d, *J* = 8.63 Hz), 8.01 (1H, s), 7.94 (2H, d, *J* = 8.63 Hz), 7.52 (2H, bs, N**H$_2$**, exchange with D_2O), 7.44 (2H, d, *J* = 8.05 Hz), 7.16 (2H, d, *J* = 7.81 Hz), 4.35 (2H, s), 2.31 (3H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.6, 156.0, 146.1, 139.2, 137.4,

133.5, 130.7, 127.8, 127.5, 127.2, 124.7, 27.9, 21.5; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 722.2 **MS** (ESI positive) *m/z*: 473.0 $[M+H]^+$.

4.1.20 4-(5-(((4-methoxyphenyl)selanyl)methyl)-1,3-selenazol-2-yl)benzenesulfonamide (12c).

Following the general procedure, 4-(5-(chloromethyl)-1,3-selenazol-2-yl)benzenesulfonamide **7c** (127 mg, 0.38 mmol) and 4-methoxyphenyl diselenide **9c** (71 mg, 0.19 mmol) gave **12c** as white solid (129 mg, 70%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 8.08 (2H, d, *J* = 8.54 Hz), 7.95-7.92 (3H, m), 7.51 (2H, bs, N**H$_2$**, exchange with D_2O), 7.46 (2H, d, *J* = 8.80 Hz), 6.91 (2H, d, *J* = 8.80 Hz), 4.28 (2H, s), 3.77 (3H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 172.6, 159.9, 156.0, 146.0, 139.2, 136.3, 127.8, 127.5, 124.5, 120.5, 115.7, 56.0, 28.7; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 717.8, 341.4. **MS** (ESI negative) *m/z*: 485.1 $[M-H]^-$.

4.1.21 Procedure for the synthesis of 2-((2-(4-sulfamoylphenyl)-1,3-selenazol-5-yl)methyl) isothiouronium chloride (13)

4-(5-(chloromethyl)-1,3-selenazol-2-yl)benzenesulfonamide **7c** (127 mg, 0.38 mmol) and Thiourea (29mg, 0.38 mmol) were dissolved in dry acetone. The mixture was stirred at reflux for 1h and after cooling, the precipitate was filtered off and washed with cold acetone to yield compound **13** as grey solid (78 mg, 50%). **^{1}H NMR** (400 MHz, DMSO- d_6) δ (ppm): 9.37 (2H, bs, N**H$_2$**, exchange with D_2O), 8.37 (1H, s), 8.14 (2H, d, *J* = 8.41 Hz), 7.96 (2H, d, *J* = 8.40 Hz), 7.56 (2H, bs, N**H$_2$**, exchange with D_2O), 7.13 (2H, bs, N**H$_2$**, exchange with D_2O), 4.71 (2H, s). **^{13}C NMR** (100 MHz, DMSO-d_6) δ (ppm): 174.2, 170.4, 152.3, 146.4, 138.7, 128.0, 127.6, 127.1, 32.0; **^{77}Se NMR** (76 MHz, DMSO-d_6) δ (ppm): 731.5. **MS** (ESI positive) *m/z*: 377.0 $[M+H]^+$.

4.2. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[27] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mMHepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in 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,[28-31] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.[28-31]

4.3. Crystallization and X-ray data collection

Crystals of hCAII were obtained using the hanging drop vapor diffusion method using 24 well Linbro plate. 2 µl of 10 mg/ml solution of hCA II in Tris-HCl 20 mM pH 8.0 were mixed with 2 µl of a solution of 1.5 M sodium citrate, 0.1 M Tris pH 8.0 and were equilibrated against the same solution at 296 K. Crystals of the protein grew in one week. Afterwards hCAII crystals were soaked in 5mM inhibitor solution for 3 days. The crystals were flash-frozen at 100K using a solution obtained by adding 15% (v/v) glycerol to the mother liquor solution as cryoprotectant. Data on crystals of the complexes were collected using synchrotron radiation at the ID23-2 beamline at ESRF (Grenoble, France) with a

wavelength of 0.8731 Å and a PILATUS3 2M Dectris detector. Data were integrated and scaled using the program XDS.[34] Data processing statistics were showed in supporting information.

4.4. Structure determination

The crystal structure of hCA II (PDB accession code: 4FIK) without solvent molecules and other heteroatoms was used to obtain initial phases of the structures using Refmac5.[35] 5% of the unique reflections were selected randomly and excluded from the refinement data set for the purpose of Rfree calculations. The initial |Fo - Fc| difference electron density maps unambiguously showed the inhibitor molecules. The inhibitor was introduced in the model with 1.0 occupancy. Atomic models for inhibitors were calculated and energy minimized using the program JLigand 1.0.40.[36] Refinements proceeded using normal protocols of positional, isotropic atomic displacement parameters alternating with manual building of the models using COOT.[37] Solvent molecules were introduced automatically using the program ARP.[38] The quality of the final models were assessed with COOT and RAMPAGE.[39] Crystal parameters and refinement data are summarized in Electronic Supplementary Information (ESI). Atomic coordinates were deposited in the Protein Data Bank (PDB accession code: 6H3Q). Graphical representations were generated with Chimera.[40]

4.4. Biological Assays.

Human prostate cancer cell line PC3 and human breast cancer cell line MDA-MB-231 were obtained from American Type Culture Collection (Rockville, MD). PC3 and MDA-MB-231 were cultured in DMEM high glucose with 20% FBS in 5% CO_2 atmosphere at 37° C. Media contained 2 mM L-glutamine, 1% essential aminoacid mix, 100 IU ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (Sigma, Milan, Italy). Cells were plated in 960 wells cell culture ($1 \cdot 10^4$/well) and, 24 h after, treated with the tested compounds for 48 h. Low oxygen conditions were acquired in a hypoxic workstation

(Concept 400 anaerobic incubator, Ruskinn Technology Ltd., Bridgend, UK). The atmosphere in the chamber consisted of 1% O_2 (hypoxia), 5% CO_2, and residual N_2. In parallel, normoxic (21% O_2) dishes were incubated in air with 5% CO_2. Cell vitality was assessed via MTT assay. Viability is expressed as % in comparison to the control cells (arbitrarily set 100 % of viable cells). Data are presented as mean ± SEM. One-way ANOVA with a Bonferroni post-hoc test was used to compare the treated samples to the control. A p-value less than 0.05 was considered to indicate a significant difference.

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Highlights

1. Synthesis of 2, 5-disubstituted 1,3-selenazoles as selective human carbonic anhydrase IX inhibitors are reported
2. *In vitro* studies reported potent and selective inhibition activity against hCA IX isoform.
3. Determined X-ray structure of hCA II in complex with one compound in order to obtain ligand−protein interaction
4. *Ex vivo* studies showed potent anti-tumor activity.