



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Chalcogenides-incorporating carbonic anhydrase inhibitors concomitantly reverted oxaliplatin-induced neuropathy and enhanced

Questa è la versione Preprint (Submitted version) della seguente pubblicazione:

Original Citation:

Chalcogenides-incorporating carbonic anhydrase inhibitors concomitantly reverted oxaliplatin-induced neuropathy and enhanced antiproliferative action / Tanini D., Carradori S., Capperucci A., Lupori L., Zara S., Ferraroni M., Ghelardini C., Mannelli L.D.C., Micheli L., Lucarini E., Carta F., Angeli A., Supuran C.T.. - In: EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY. - ISSN 0223-5234. - ELETTRONICO. -

Availability:

The webpage <https://hdl.handle.net/2158/1251025> of the repository was last updated on 2025-02-06T14:39:16Z

Published version:

DOI: 10.1016/j.ejmech.2021.113793

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

Conformità alle politiche dell'editore / Compliance to publisher's policies

Questa versione della pubblicazione è conforme a quanto richiesto dalle politiche dell'editore in materia di copyright.

This version of the publication conforms to the publisher's copyright policies.

La data sopra indicata si riferisce all'ultimo aggiornamento della scheda del Repository FloRe - The above-mentioned date refers to the last update of the record in the Institutional Repository FloRe

(Article begins on next page)

Chalcogenides-incorporating carbonic anhydrase inhibitors concomitantly reverted oxaliplatin-induced neuropathy and enhanced antiproliferative action

Damiano Tanini,^a Simone Carradori,^b Antonella Capperucci,^a Lucrezia Lupori,^a Susi Zara,^b Marta Ferraroni,^a Carla Ghelardini,^c Lorenzo Di Cesare Mannelli,^c Laura Micheli,^c Elena Lucarini,^c Fabrizio Carta,^d Andrea Angeli,^{d,e} Claudiu T. Supuran^d*

^aUniversity of Florence, Department of Chemistry "Ugo Schiff", Via della Lastruccia 3-13, I-50019, Sesto Fiorentino, Italy

^bDepartment of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy

^cPharmacology and Toxicology Section, Department of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), University of Firenze, Firenze, Italy

^dNEUROFARBA Department, Sezione di Scienze Farmaceutiche, University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy

^eCentre of Advanced Research in Bionanoconjugates and Biopolymers Department, "Petru Poni" Institute of Macromolecular Chemistry, 707410, Iasi, Romania

KEYWORDS: carbonic anhydrase; metalloenzymes, inhibitor, neuropathic pain, organochalcogenide, antiproliferative effect.

ABSTRACT

Platinum-based chemotherapy is widely used for the treatment of different tumors but is associated with serious side effects, among which neuropathic pain. Carbonic anhydrase (CA, EC 4.2.1.1) inhibitors have recently been validated as therapeutic agents in neuropathic pain and as antitumor agents. We report the synthesis of new organochalcogenides bearing the benzensulfonamide moiety acting as potent inhibitors of several human CA isoforms and, in particular, against hCA II and VII endowed with potent neuropathic pain attenuating effects. Moreover, in combination with cisplatin or doxorubicin, some of the new CA inhibitors enhanced the effects of the anticancer drugs capability in counteracting breast cancer MCF7 cell viability. The concomitant anti-neuropathic pain effects and antiproliferative of the new chalcogenide-based CA inhibitors represent an innovative approach for the counteraction and management of side effects associated with clinically platinum drugs as antitumor agents.

1. INTRODUCTION

Cancer is one of the most important causes of death, second only to heart diseases, remaining one of the major concerns worldwide [1]. In the last decades, new antitumor drugs improved survival from cancer and in particular metal-based drugs are increasingly used [2-4]. Platinum-based chemotherapeutic agents are the principal drugs for the treatment of different type of tumors, such as gastrointestinal and gynecologic cancers and their use has been increasing during the last two decades [5]. Since 1970s, when the antiproliferative properties of cisplatin were discovered, many efforts were made in order to find new derivatives to improve the oncologists' armamentarium. [6] One of the most clinically used agents nowadays is the third-generation derivative oxaliplatin, which shows a better efficacy and lower toxicity compared to cisplatin and carboplatin. Because of its wide application, the side effects of oxaliplatin have been attentively evaluated and the most

common one is peripheral neuropathy with no existing effective treatments against it, which provokes serious difficulties to the patients for completing a full treatment schedule [7]. The neuropathic pain affects many aspects of daily life and is associated with poor general health, reduction in quality of life where the currently available analgesics such as anti-inflammatory drugs (NSAIDs) and opioids can only alleviate the symptom because of the lack of efficacy and unacceptable side effects [8]. In this context, a new therapeutic target against neuropathic pain has emerged due to the connection between the modulation of Carbonic Anhydrase (CA; EC 4.2.1.1) enzymes and GABA-ergic networks in peripheral/central neurons [9-12]. Initially, the activation of GABAA receptor leads to hyperpolarization by Cl^- influx, on the other hand, prolonged receptor activation leads to a strong bicarbonate (HCO_3^-) efflux dependent depolarization [10]. This condition is aggravated when potassium-chloride (K^+ - Cl^-) cotransporter (KCC2) expression is decreased, thus compromising Cl^- gradients in GABA-responsive neurons [9-11].

In humans 12 of 15 CAs expressed are catalytically active producing HCO_3^- and H^+ by the hydration of CO_2 , an essential molecule in many physiologic processes making CAs crucial enzymes in all kingdoms life [13-16]. For this reason, Carbonic Anhydrase Inhibitors (CAIs) can be used to modulate the HCO_3^- concentration mitigating the deleterious effects on neurons [12].

In this particular contest, our interest has focused on the development of novel organochalcogen CAIs with high affinity to the CA isoforms involved in this pathology such as the physiologically dominant isoform hCA II and hCA VII, the latter being highly expressed in central nervous system (CNS) [17], and the well know antioxidant propriety of organo-chalcogen scaffolds in order to prevent the oxidative stress identified to be one of the responsible for the neuronal damage in different models of neuropathies [18,19]. In addition, we report further their co-administration

with cisplatin on a cancer cell line (MCF7) aimed to investigate their effects, showing great potential for the efficacy and treatment of side effects by platinum-drugs.

2. RESULTS AND DISCUSSION

2.1 DESIGN AND SYNTHESIS

During our studies on the chemistry of chalcogen-containing organic molecules, we developed a series of versatile ring-opening-based procedures for the synthesis of functionalised selenium-, sulfur-, and tellurium-containing small molecules [20-22]. Nucleophilic-ring-opening reactions (NROR) of strained heterocycles with chalcogen-centered nucleophiles represent a rewarding strategy to form new heteroatom-carbon bond and to access densely functionalised systems [23]. In this context, NROR of epoxides and aziridines with sulfur-, selenium-, and tellurium-containing nucleophiles have been successfully applied to the synthesis of a wide array of antioxidants [24,25], carbonic anhydrase inhibitors [26-29] and activators [30]. Considering the biological properties of chalcogen-containing small molecules, we set out to synthesise novel polyfunctionalised sulfur-, selenium-, and tellurium-containing alcohols and to investigate their activity as human CAs inhibitors. Our attention was focused on the synthesis of systems with the general structure reported in **Figure 1**.

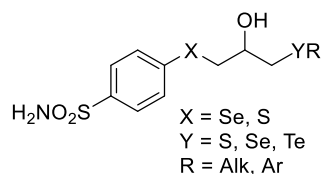
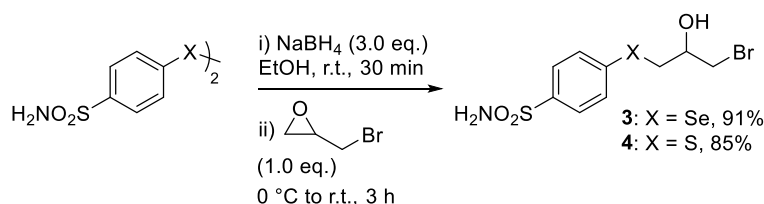


Figure 1. General structure of chalcogen-containing alcohols studied in this work.

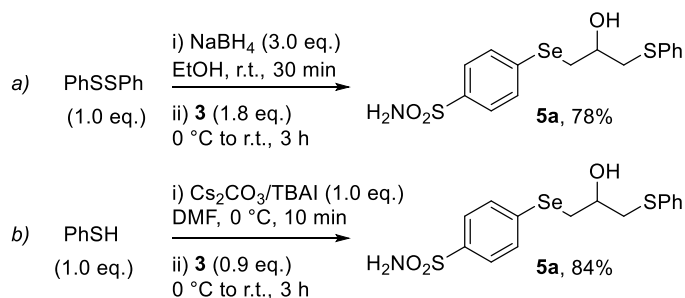
As we previously reported, NROR of epoxides with a suitable selenolate [29] or thiolate [27] bearing the benzenesulfonamide moiety provided rapid access to the corresponding β -hydroxy-

selenides and sulfides. This reaction was also applied to epibromohydrin, leading to selenide **3** and sulfide **4** bearing a brominated chain, which represents an attractive handle for further elaborations (**Scheme 1**).



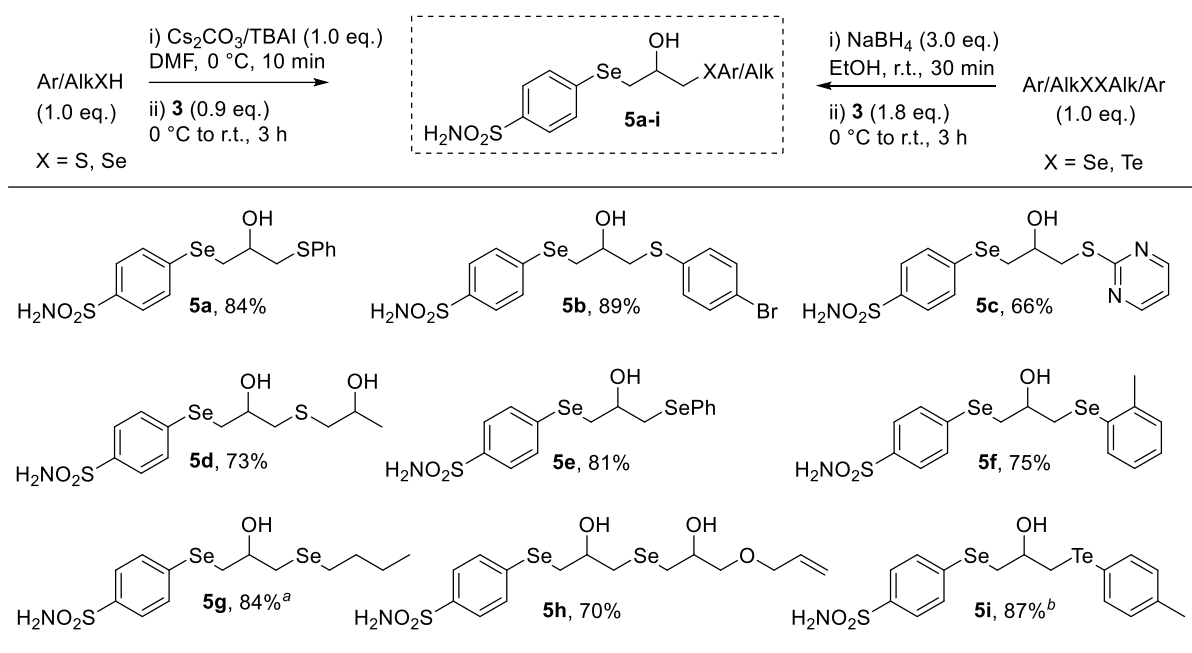
Scheme 1. Synthesis of selenide **3** and sulfide **4** from epibromohydrin.

We sought to employ **3** and **4** as precursors of the target compounds reported in **Figure 1**. In order to establish the optimal reaction conditions, compound **3** was treated with benzenethiolate, easily generated upon reduction of diphenyl disulfide with NaBH_4 . Under these conditions the functionalized alcohol **5a**, bearing both a sulfur- and a selenium-containing moiety, was smoothly achieved in 78% yield through a clean $\text{S}_\text{N}2$ path occurring at the brominated chain. A slightly higher yield (84%) was obtained generating the thiolate from benzenethiol with the $\text{Cs}_2\text{CO}_3/\text{TBAI}$ system in DMF (**Scheme 2**).



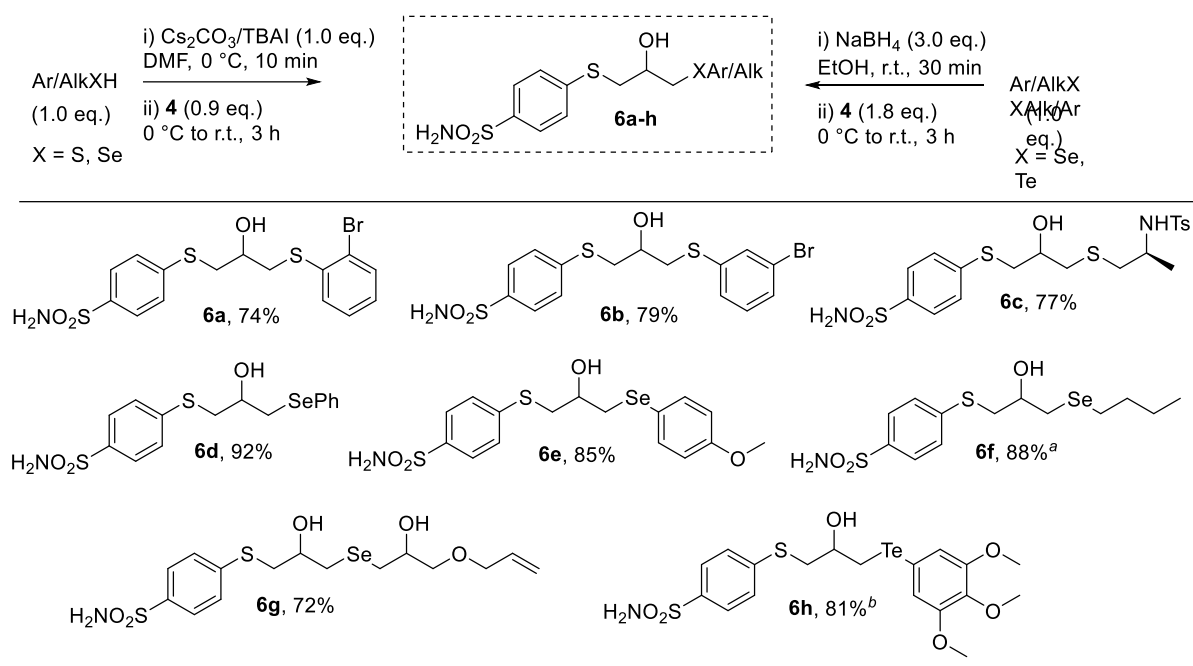
Scheme 2. Synthesis of compound **5a** from **3** and diphenyl disulfide (*reaction a*) or benzenethiol (*reaction b*).

With these conditions in hands, we evaluated whether they could be applied to the synthesis of a series of chalcogen-containing systems with broad structural diversity (**Scheme 3**). The reaction was efficiently extended to 4-bromothiophenol and 2-mercaptopyrimidine, enabling the synthesis of compounds **5b, c**, bearing the benzenesulfonamide moiety and the selenium atom. The S-, and Se-containing diol **5d** was also successfully achieved upon reaction of **3** with 1-mercapto-2-propanol under the standard conditions. In order to investigate the effect of the nature of the chalcogen atom on the CA inhibitor activity, we enlarged the scope of the reaction to include selenols. Exploiting the good nucleophilic character of selenols [31,32], both aromatic [33] and aliphatic [21] selenols were efficiently employed and led to bis-selenides **5e-h** in good yield (**Scheme 3**). Notably, polyfunctionalised systems such as the allyl glycidyl ether-derived diol **5h** could also be prepared by using this approach. Furthermore, the γ -telluro- β -hydroxy selenide **5i** was synthesised by treating *p*-tolyltellurolate with **3** (**Scheme 3**). Taking into account the poor stability of tellurols, the tellurolate was generated *in situ* upon reduction of the corresponding ditelluride with NaBH₄.



Scheme 3. Synthesis of compounds **5a-i** from **3**. Isolated yields are reported. ^aSelenolate obtained upon reduction of the corresponding diselenide. ^bTelluroate obtained upon reduction of the corresponding ditelluride. See experimental section for details.

On the basis of these results, the protocol was applied to the brominated hydroxysulfide **4**, bearing the benzenesulfonamide moiety. Both *S*-aryl- and *S*-alkyl glycerol-derived bis-sulfides **6a-c** were smoothly obtained from the corresponding thiols. Compound **6c**, bearing a tosyl-protected amino group, was synthesized using the L-alanine-derived enantioenriched thiol [34]. Similarly, the reaction was amenable to a series of differently substituted selenols, easily converted into the corresponding γ -seleno- β -hydroxy sulfides **6d-g**, bearing the benzenesulfonamide moiety. The allyl glycidyl ether-derived diol **6g**, which represents the sulfur-containing isoster of the bis-selenide **5h**, was also achieved.



Scheme 4. Synthesis of compounds **6a-h** from **4**. Isolated yields are reported. ^aSelenolate obtained upon reduction of the corresponding diselenide. ^bTellurolate obtained upon reduction of the corresponding ditelluride. See experimental section for details.

2.2 CARBONIC ANHYDRASE INHIBITION

All compounds **5a-i** and **6a-h** were tested *in vitro* for their inhibitory activity against the human CA isoforms I, II IV, VII and IX by means of the stopped-flow carbon dioxide hydration assay [35]. Their activities were compared to the clinically CA inhibitor acetazolamide (**AAZ**) and provided in **Table 1**.

Table 1. Inhibition data of human CA isoforms I, II, IV, VII and IX with compounds **5a-i**, **6a-h** and **AAZ** by a stopped flow CO_2 hydrase assay [35].

K_i (nM)*

Cmp	hCA I	hCA II	hCA IV	hCA VII	hCA IX
5a	7.7	3.1	7.2	8.5	288.3
5b	65.4	6.5	94.0	56.7	171.6
5c	7.3	3.3	71.1	8.7	31.0
5d	46.1	8.8	517.0	23.9	275.5
5e	98.1	5.0	7.5	9.5	84.5
5f	7.2	4.0	90.4	9.3	29.4
5g	5.7	4.8	6.3	28.8	207.5
5h	9.7	5.0	158.4	8.5	156.6
5i	63.5	7.9	43.3	9.3	152.4
6a	218.8	6.8	751.4	29.1	174.6
6b	96.3	8.1	334.9	9.3	134.7
6c	218.0	6.6	1365	35.6	192.9
6d	46.1	6.2	53.8	9.4	29.2
6e	12.4	4.5	54.8	45.8	155.6
6f	6.1	4.2	13.7	9.5	163.7
6g	52.7	7.1	390.4	58.5	241.2
6h	675.2	81.9	3931	37.6	187.2
AAZ	250.0	12.8	74.0	2.5	25.7

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

The inhibition profile against hCA II, for all compounds here tested, showed high potency in the low nanomolar range with a K_i spanning among 3.1 and 8.8 nM except for compound **6h** with a K_i of 81.9. Their potency appears to depend mostly on the linker as for the presence of the different chalcogen atom on the β -hydroxy moiety. In particular, compound **5a** showed a K_i of 3.1 nM and its analog **6d**, where the two chalcogen atoms have been inverted showed halved activity with a K_i of 6.2 nM. When selenium is replaced with a bigger atom as tellurium (**5i**), the potency decreases (K_i 7.9 nM). In addition, the atom on β -hydroxy moiety was observed as a key point to modulate the potency against this isoform and the substituents on the aromatic ring also participate in the modulation of potency showing, in general, less activity compared to compounds without them such as **5a** and **5c**. Taken together these points could explain the less potency of compound **6h** where are present tellurium atom and three methoxy groups as ring substituents (K_i 81.9 nM).

Common observations could be made for the second ubiquitous isoform hCA I. Indeed, compound **5a** showed better inhibition potency than **6d**, as previously reported, and the replacement of sulfur atom with a second selenium (**5e**) resulted detrimental for the activity. Also in this case the bulky trimethoxy substitution of compound **6h** drastically decreased the potency in the high nanomolar range (K_i 675.2 nM). As regards the membrane bound isoform hCA IV, on the other hand, the inhibition potency did not show to be affected by the nature of chalcogen atom on β -hydroxy moiety observing for compounds **5a** and **5e** the same activity (K_i 7.2 and 7.5 nM, respectively). Ring substitutions on derivative **5a** lead to decrease the potency as observed for hCA I and II. An interest point was observed for compounds **5d** and **5h** where the presence of second β -hydroxy moiety proved to be detrimental for the activity reaching the high nanomolar range (K_i 517.0 and 158.4 nM, respectively). On the other hand, after removing this moiety as for **5g**, the activity increased to 6.3 nM proving to be a crucial point in the scaffold to modulate the activity for this isoform. From the general point of view, the replacement of selenium atom bearing benzensulfonamide with sulfur limited the potency as for compounds **5e** and **6d**, where this substitution leads to decrease the activity seven times (K_i 7.5 to 53.8 nM, respectively). The last cytosolic isoform here reported, hCA VII, is effectively inhibited by all compounds spanning a K_i among 8.5 to 56.7 nM. Unlike the other isoforms, the replacement of chalcogenide atom such as for compounds **5a**, **5e** and **6d** did not show significant difference in potency inhibition observing a constant of inhibition around 8.5-9.5 nM. The tumor associated isoform hCA IX is inhibited in the medium nanomolar range (K_i 29.2-288.3 nM) by the majority of compounds. The interesting point for this isoform is shown by compounds **5a** and **6d**. Indeed, inverting the two chalcogen atoms in the β -hydroxy scaffold this time the potency increased around ten times (K_i 288.3 to 29.2

nM). However, these compounds showed a favourable selectivity ratio for hCA II and VII in terms of potency of inhibition proving to be good candidates to explore their efficacy on pain relief.

2.3 X-RAY STUDIES

To further explore the interactions of these series with hCA II, one of the isoforms most inhibited by them and involved on pain relief, we provided an X-ray crystallographic study. The results are summarized in ESI. The compounds chosen were **5g**, **6d**, and **6h** in order to understand primarily if the different type of chalcogenide atom on the β -hydroxy moiety can modulate the interactions with this isoform. The three crystal structures were solved at 1.43 Å of resolution and we observed two inhibitor molecules in the complexes. Inside the active site, after the initial rounds of refinement, the Fo-Fc map showed a clear density for the compounds (**Figure S1**), in particular for the sulfonamide moiety which interacts, as expected, directly with the zinc ion in the active sites. In the complex CAII/**6d** we observed two molecules bound to the protein, one inside the active site and responsible for the activity, the second bound in a task (N-terminal) and not involved in the mechanism of inhibition as outlined in **Figure 2**.

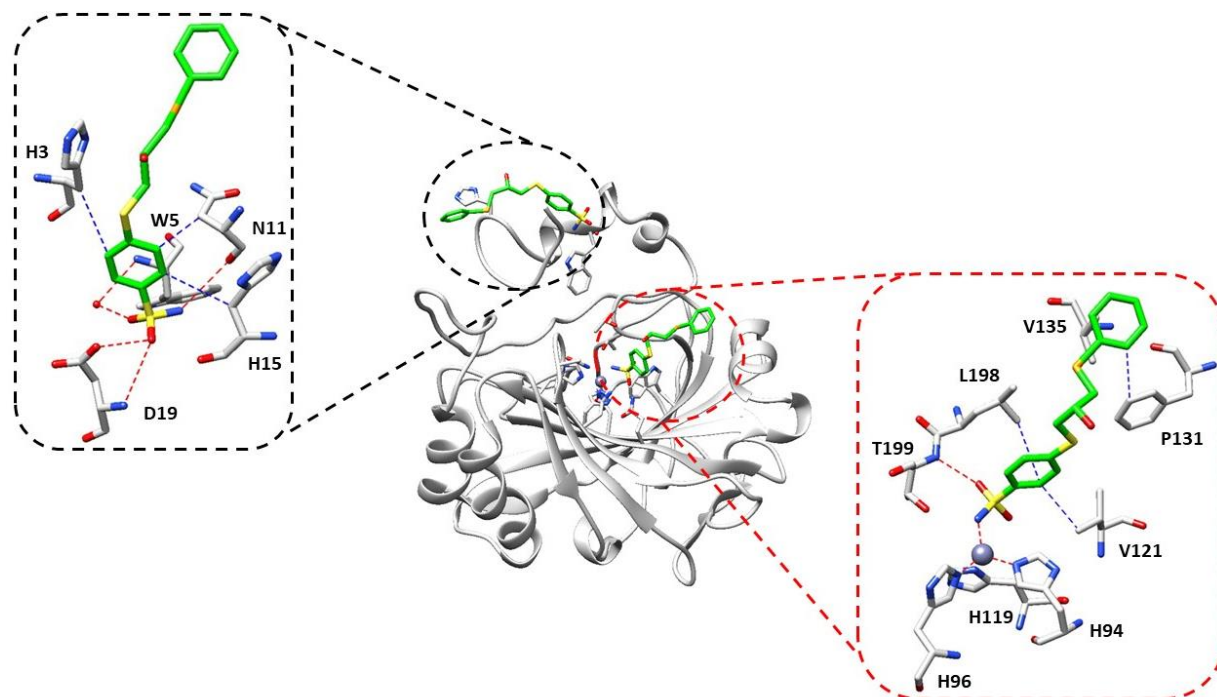


Figure 2: X-ray crystal structures of hCA II bound with compound **6d** (PDB: 7OK8). Residues involved in the binding of inhibitors are also shown and the grey sphere represents the zinc atom in the active site of the proteins.

For the first molecule we observed the traditional interactions between the benzenesulfonamide moiety and the side chains of protein in the active site such as the hydrogen bond between Thr199 and an oxygen of sulfonamide group and, in addition, hydrophobic interaction with Val121 and Leu198 [36]. The tail of compound **6d** is oriented in a hydrophobic pocket by interactions with Phe131 and Val135. The second molecule is anchored at the protein by several hydrogen bonds with the sulfonamide group. In more detail, the nitrogen of sulfonamide forms an H-bond with Asn11, one of its oxygens established two hydrogen bonds with Asp19 and the second one is involved in a water bridge with Trp5. Hydrophobic interactions were observed between the aromatic ring of benzenesulfonamide moiety and the side chains of His3 and His15. The

benzenesulfonamide group of **5g** and **6h** interacts, in similar manner, with the side chains of residues Val121, Leu198 and Thr199 in the active site (**Figure 3**).

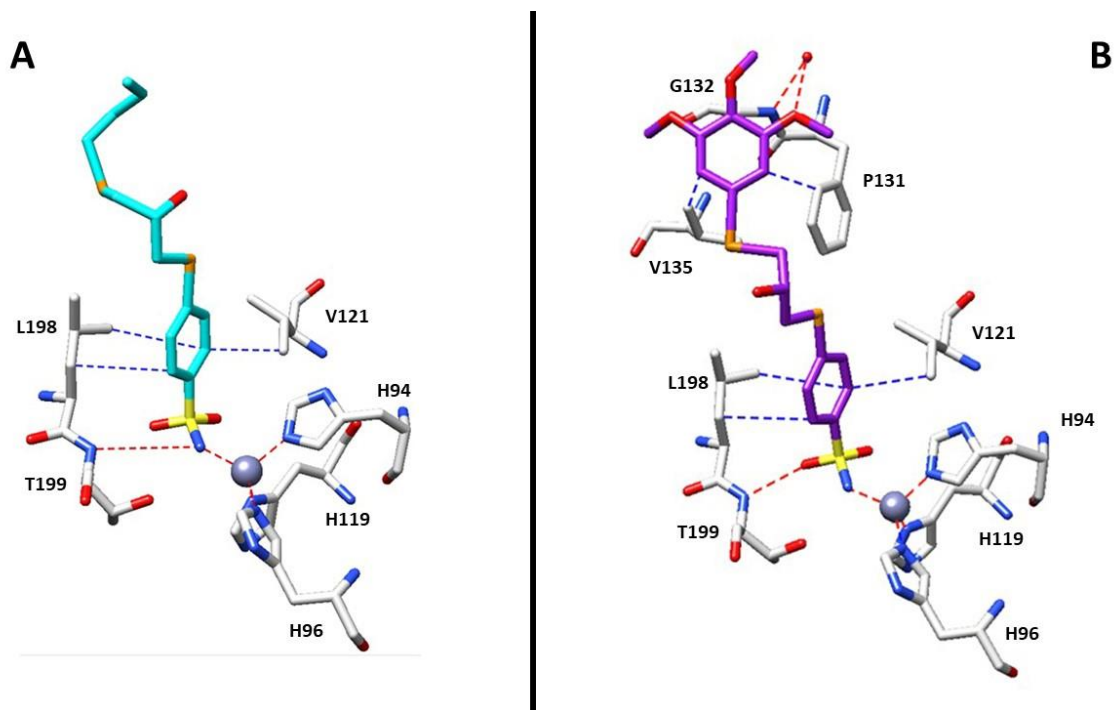


Figure 3: X-ray crystal structures of hCA II bound with compounds **5g** (A, PDB: 7ORQ) and **6h** (B, PDB: 7ORP). Residues involved in the binding of inhibitors are also shown and the grey sphere represents the zinc atom in the active site of the proteins.

The short and aliphatic tail of compound **5g** did not show significant interactions in the active site, on the other hand, the longest tail of compound **6h** is located in the same position of derivative **6d** making the same hydrophobic interactions with Phe131 and Val135. From the general point of view, we did not observe significant differences in the location of inhibitors inside the active site when the chalcogenide atom is replaced as in all three complexes the inhibitors are placed in the same hydrophobic pocket interacting with Phe131, a key residue of hCA II (**Figure S2**).

2.4 IN VIVO NEUROPATHIC PAIN STUDY

The series, here reported, showed potent and selective inhibition against two of the most involved isoforms in neuropathic pain response hCA II and VII. In addition, organoselenides have interesting antioxidant effects that enhanced the pain relievers in a mouse model of neuropathic pain induced by oxaliplatin [37,38]. In this context, the novel compounds were an interesting candidate for the treatment of neuropathic pain and we selected compounds **5b**, **5e**, and **6d** in order to investigate their pain relief propriety. These compounds are investigated in comparison to acetazolamide (AAZ) and the results are summarized below in **Figure 5**.

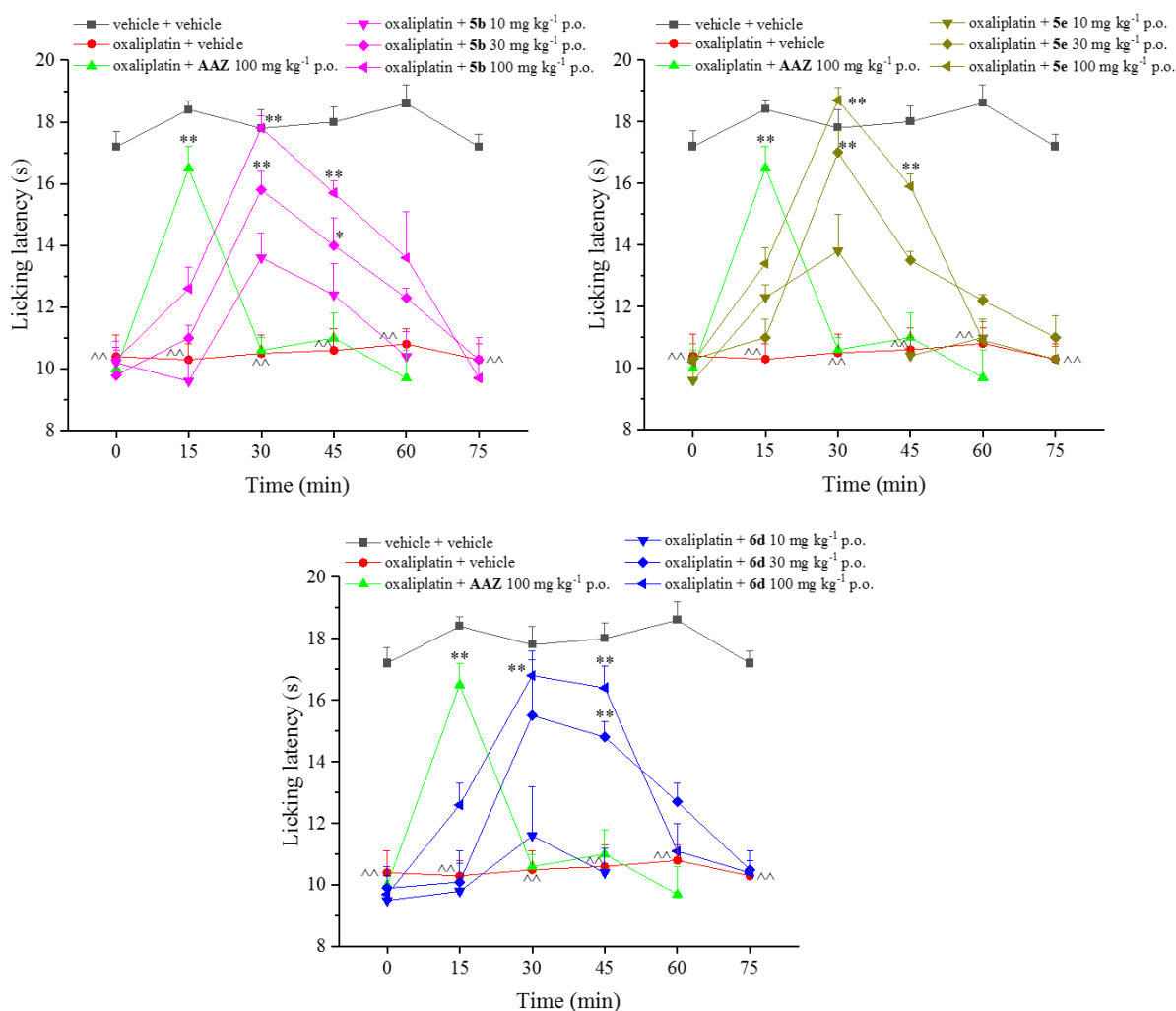


Figure 5. Effect of acute administration of **5b**, **5e**, **6d**, and **AAZ** on oxaliplatin-induced neuropathic pain in the mouse. Pain-related behavior (i.e. licking of the hind paw) were observed and the time (seconds) of the first sign was recorded. $^{^^}p<0.01$ vs vehicle + vehicle treated animals; $^{*}p<0.05$ and $^{**}p<0.01$ vs oxaliplatin + vehicle treated animals. Each value represents the mean of 8 mice performed in 2 different experimental sets.

All three compounds showed a dose dependent pain-relieving effect reaching, at dose of $100\text{ mg}\cdot\text{kg}^{-1}$, the highest anti-hypersensitivity properties with a peak 30 min after treatments and the mouse pain threshold lasted up to 45 min. On the other hand, **AAZ** showed a more rapid effect reaching the high peak at 15 min and rapidly reversed in 30 min. This data turn be interesting because our compounds (**5b**, **5e**, and **6d**) showed a longer duration of action compared to the standard drug making them an excellent starting point for developing new derivatives with better pain relief profile. More in detail, compound **5b** showed anti-hypersensitivity properties at $10\text{ mg}\cdot\text{kg}^{-1}$, exhibited similar biological effects to **AAZ** already at $30\text{ mg}\cdot\text{kg}^{-1}$ and at $100\text{ mg}\cdot\text{kg}^{-1}$ completely restore the pain threshold. The addition of second selenium atom in the scaffold (**5e**) enhanced the pain relief effect. Indeed, this compound at $30\text{ mg}\cdot\text{kg}^{-1}$ surpassed the value of **AAZ** and, at dose of $100\text{ mg}\cdot\text{kg}^{-1}$, a value over the threshold limit reported for the untreated mice used as control. On the other hand, compound **6d** with selenium atom bearing the last β -hydroxy moiety is observed to be the less active among the three compounds tested proving the importance of selenium atom location in the scaffold. This time, **6d** exhibited similar biological effects to **AAZ** only at $100\text{ mg}\cdot\text{kg}^{-1}$ and showed a longer duration of action than the two previously compound mentioned above. To summarize, the novel series, compared to **AAZ**, showed a better pain relief profile characterized by a longer duration of action with a peak of efficacy at 30 min.

2.5 *IN VITRO* TOXICITY

In view of the results of these compounds on pain relief and the appreciable hCA IX inhibition in the medium nanomolar range, we have additionally studied their capability in counteracting cell viability against the MCF7 breast adenocarcinoma cells. In previous experiments CA inhibitors have shown their anticancer effects inhibiting the overexpression of tumor-associated hCA IX [39-42]. In addition, CA IX is commonly recognized to promote tumor resistance mechanisms to different chemotherapeutic treatments, knowing that this isozyme expression can be considered as a clinically relevant prognostic feature in patients with invasive breast cancer [43].

For this reason, compounds **5a**, **6h**, and **6f** were administered to the breast adenocarcinoma cell line MCF7 in order to establish their capability to affect cell viability alone and in association with more commonly used in clinic anticancer drugs such as cisplatin and doxorubicin. Firstly, cisplatin and the newly synthesized compounds were administered to MCF7 cells in order to calculate the IC_{50} for each molecule. Before compounds administration, a pre-treatment of 48 h with $CoCl_2$ 100 μM was applied to cell culture to induce hypoxic conditions. MCF7 were then treated for 72 h with cisplatin, doxorubicin and compounds **5a**, **6h**, and **6f** choosing doses ranging from 0.048 μM to 10000 μM . Under hypoxic conditions, the IC_{50} of cisplatin was approximatively detectable between 150 and 200 μM (**Figure S3**). This result is in line with what evidenced by García-Venzor *et al.* who demonstrated cisplatin resistance in MCF7 and MDA-MB-231 cell lines following induction of hypoxia. Hypoxic environment can induce a complex interplay of changes in the cellular gene expression including long non-coding RNAs (lncRNAs) such as lncMat2B. lncRNAs are associated with antioxidant effects against ROS production and an increased repair of platinum-based adducts in DNA [44].

On the other hand, it was not possible to establish an IC_{50} for compounds **5a** and **6f** because, even at the highest concentration, the MCF7 viability was higher than control sample. Conversely, after 72 h of treatment, for compound **6h** the IC_{50} was detected at 125 μ M (**Figure S4**). Secondly, combinations of cisplatin and the newly synthesized molecules were administered to MCF7 cells under hypoxic conditions. Considering that cisplatin IC_{50} was identified between 150 and 200 μ M, it was administered alone and in combination with the three novel compounds choosing a subtoxic dose (100 μ M). The same dose was also chosen for **5a**, **6h**, and **6f**. Moreover, we decided to evaluate the MCF7 viability also combining doxorubicin, a drug of choice for treatment of breast cancer characterized by a different mechanism of action respect to cisplatin, with the newly synthesized molecules. For doxorubicin, the administered dose was also a subtoxic dose (2.5 μ M), as already calculated and tested in our previous paper [42]. From the cell-based in vitro experiments, a significant reduction in cell viability percentage is detected in cisplatin+**6h**, doxorubicin, doxorubicin+**5a**, doxorubicin+**6f**, and doxorubicin+**6h** compared to control sample. Furthermore, MCF7 percentage viability after administration of both cisplatin+**6f** and cisplatin+**6h** is significantly decreased respect to that recorded in presence of cisplatin alone, whereas there is not a significant viability reduction in cells treated with cisplatin+**5a** respect to cisplatin alone. When doxorubicin+**6h** is administered, a statistically significant decrease in MCF7 percent viability is disclosed respect to MCF7 treated with doxorubicin alone, while there is not an appreciable viability reduction in MCF7 treated with doxorubicin+**5a** and doxorubicin+**6f** respect to MCF7 treated with doxorubicin (**Figure 4**).

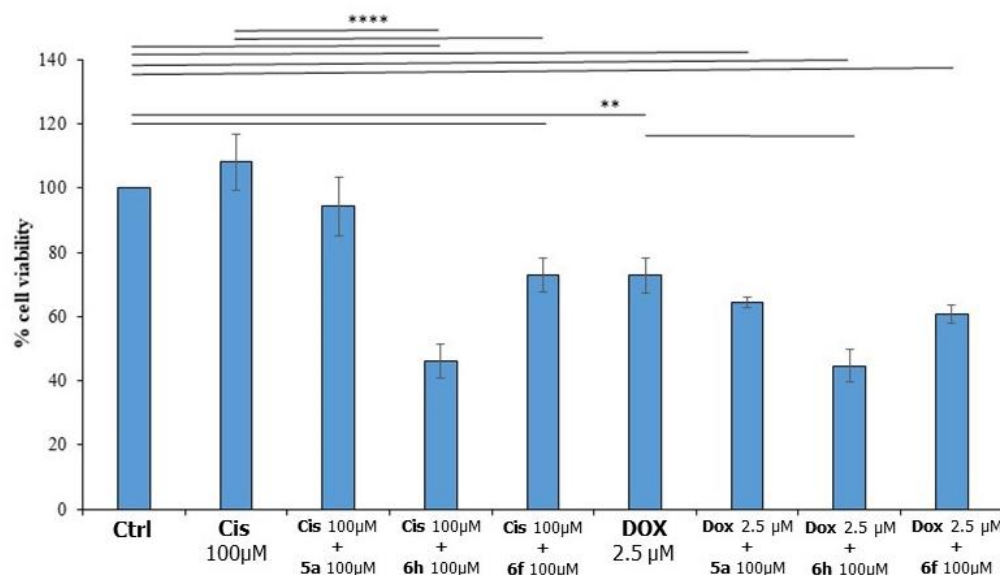


Figure 4. MTT cell viability assay on MCF7 cell line. Histogram represents the viability percentage of MCF7 cells exposed to combinations of cisplatin (**Cis**) 100 µM and doxorubicin (**Dox**) 2.5 µM with compounds **5a**, **6h**, and **6f**, all administered at 100 µM for 72 h and under hypoxic conditions. Proliferation was assessed using MTT assay and normalized to control cells treated with DMSO (0.1% as final concentration) + CoCl₂ 100 µM. The most representative of three separate experiments is shown. Data are presented as the mean ± standard deviation. **** $p < 0.0001$; ** $0.001 < p < 0.01$

As a result of all this, compounds **5a**, **6h**, and **6f** could be used in association with two distinct chemotherapeutic agents limiting the doses (and possibly most of the side-effects such as neuropathic pain) of the current therapy. Their versatile synergistic effects were demonstrated disregarding the mechanism of action of the clinically used drug in combination and this aspect is of paramount importance when tumor progression and poor response are driven and sustained by hypoxia. Furthermore, in the case of cisplatin, we also demonstrated that selenium-containing

compounds are not able to chemically quench platinum-based drugs and interfere with their mechanism of action, conversely, showed synergistic effects [45, 46].

3. CONCLUSION

In summary, we reported the synthesis of novel series of organochalcogenides bearing the benzensulfonamide moiety and possessing excellent inhibitory activity against hCA II and VII, which are the enzymatic isoforms deeply involved in neuropathic pain associated symptoms. In order to determine the binding mode of these novel organochalcogenides, we solved the crystal structures of **5g**, **6d**, and **6h** in adduct with hCA II. More importantly, a selection of our compounds in combination with cisplatin or doxorubicin enhanced their cytotoxic effects on MCF7 breast cancer cell line which resulted increased when compared to single administration of both drugs. The combined antiproliferative and lowering neuropathic pain properties of our organochalcogenides give new perspectives to metal-based antitumoral drugs for safer and more effective employment in clinics.

4 EXPERIMENTAL SECTION

4.1 General

All commercial materials were purchased and used as received, without further purification. Solvents were dried using a solvent purification system (Pure-Solv™). Flash column chromatography purifications were performed with Silica gel 60 (230-400 mesh). ¹H and ¹³C NMR spectra were recorded in CDCl₃ or CD₃OD using Varian Mercury 400, Bruker 400 Ultrashield spectrometers operating at 400 MHz or Varian Gemini 200 MHz for ¹H and 100 MHz or 50 MHz for ¹³C. NMR signals were referenced to nondeuterated residual solvent signals (CDCl₃: 7.26 ppm for ¹H, 77.0 ppm for ¹³C; CD₃OD: 3.31 ppm for ¹H, 49.0 ppm for ¹³C). ⁷⁷Se NMR and ¹²⁵Te NMR spectra were recorded using a Bruker 400 Ultrashield spectrometer, operating at 76 MHz and 126

MHz, respectively. Diphenyl diselenide (PhSe)₂ was used as an external reference for ⁷⁷Se NMR (δ = 461 ppm). Diphenyl ditelluride (PhTe)₂ was used as an external reference for ¹²⁵Te NMR (δ = 420 ppm). Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in Hertz (Hz), rounded to the nearest 0.1 Hz. ¹H NMR data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, ap d = apparent doublet, m = multiplet, dd = doublet of doublet, bs = broad singlet, bd = broad doublet, ecc.), coupling constant (J) or line separation (ls), and assignment. Where reported, NMR assignments are made according to spin systems, using, where appropriate, 2D NMR experiments (COSY, HSQC, HMBC) to assist the assignment. HRMS (ESI) were determined with a GCT Premier Waters instrument. Compounds **3** and **4** were synthesised according to previously reported procedures. β -Hydroxy-selenols [21] and β -hydroxy [47] or β -amino-thiols [34] were synthesised from the corresponding epoxides and aziridines following a reported procedure. Aryl-selenols [33] were prepared through a reported procedure from the corresponding diselenides [48].

4.2 General Procedure for the synthesis of compounds **5** and **6** from thiols or selenols

A solution of thiol or selenol (0.14 mmol, 1.0 eq.) in dry DMF (2 mL) was cooled under inert atmosphere at 0 °C, and treated with Cs₂CO₃ (0.14 mmol, 1.0 eq.), TBAI (0.14 mmol, 1.0 eq.) and then with the electrophile (0.13 mmol, 0.9 eq.). The reaction was allowed to warm to room temperature and stirred for 3 h. Then, the mixture was diluted with diethyl ether (10 mL), washed with water (3 x 5 mL), brine (5 mL) and dried over Na₂SO₄. The solvent was evaporated under vacuum and the crude product was purified by flash column chromatography.

Synthesis of 4-((2-hydroxy-3-(phenylthio)propyl)selenanyl)benzenesulfonamide (**5a**)

Following the General Procedure, bromide **3** (50 mg, 0.13 mmol) and benzenethiol (16 mg, 0.14 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 2:3), **5a**

as a pale yellowish solid (44 mg, 84%). **¹H NMR** (CDCl₃, 200 MHz) δ (ppm): 2.45 (1H, bs, OH), 2.99-3.28 (4H, m), 3.82-3.94 (1H, m), 5.26 (2H, bs, NH₂), 7.19-7.36 (5H, m), 7.49 (2H, ap d, J = 8.5 Hz), 7.69 (2H, ap d, J = 8.5 Hz). **¹³C NMR** (CDCl₃, 50 MHz) δ (ppm): 33.4, 40.7, 68.7, 126.8, 127.0, 129.2, 130.0, 131.3, 137.3, 140.0. **HRMS** (ESI) calc. C₁₅H₁₈NO₃S₂Se [M +H]⁺ 403.9893, found 403.9902.

Synthesis of 4-((3-((4-bromophenyl)thio)-2-hydroxypropyl)selanyl)benzenesulfonamide (**5b**)

Following the General Procedure, bromide **3** (50 mg, 0.13 mmol) and 4-bromobenzenethiol (27 mg, 0.14 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 1:1), **5b** as a white solid (56 mg, 89%). **¹H NMR** (CDCl₃, 400 MHz) δ (ppm): 2.68 (1H, bs, OH), 3.06 (1H, dd, J = 7.3, 13.7 Hz), 3.13 (1H, dd, J = 7.1, 12.9 Hz), 3.17 (1H, dd, J = 4.8, 13.7 Hz), 3.25 (1H, dd, J = 4.7, 12.9 Hz), 3.84-3.90 (1H, m, CHOH), 4.82 (1H, bs, NH₂), 7.22 (2H, ap d, J = 8.4 Hz), 7.40 (2H, ap d, J = 8.4 Hz), 7.56 (2H, ap d, J = 8.6 Hz), 7.76 (2H, ap d, J = 8.6 Hz). **¹³C NMR** (CDCl₃, 100 MHz) δ (ppm): 33.5, 40.7, 68.6, 120.8, 127.0, 131.5, 132.2, 134.0, 137.0, 140.2. **⁷⁷Se NMR** (CDCl₃, 76 MHz) δ (ppm): 264.5. **HRMS** (ESI) calc. C₁₅H₁₆BrNNaO₃S₂Se [M +Na]⁺ 503.8818, found 503.8822.

Synthesis of 4-((2-hydroxy-3-(pyrimidin-2-ylthio)propyl)selanyl)benzenesulfonamide (**5c**)

Following the General Procedure, bromide **3** (40 mg, 0.11 mmol) and pyrimidine-2-thiol (14 mg, 0.12 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 2:3), **5c** as a colourless solid (30 mg, 66%). **¹H NMR** (CD₃OD, 400 MHz) δ (ppm): 3.17 (1H, dd, J = 7.0, 12.8 Hz), 3.29-3.35 (2H, m), 3.48 (1H, dd, J = 5.2, 13.9 Hz), 4.05-4.14 (1H, m, CHOH), 7.11 (1H, t, J = 5.0 Hz), 7.63 (2H, ap d, J = 8.3 Hz), 7.72 (2H, ap d, J = 8.3 Hz), 8.51 (2H, d, J = 5.0 Hz). **¹³C NMR** (CD₃OD, 100 MHz) δ (ppm): 32.8, 36.5, 69.4, 116.8, 126.1, 130.8, 137.0, 141.4,

157.3, 171.4. 33.5, 40.7, 68.6, 120.8, 127.0, 131.5, 132.2, 134.0, 137.0, 140.2. **MS** (ESI) calc. $C_{13}H_{15}N_3NaO_3S_2Se$ $[M+Na]^+$ 428.0, found 427.9.

Synthesis of 4-((2-hydroxy-3-((2-hydroxypropyl)thio)propyl)selenyl)benzenesulfonamide (5d)

Following the General Procedure, bromide **3** (75 mg, 0.2 mmol) and 1-mercaptopropan-2-ol (20 mg, 0.22 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 2:3), **5d** (equimolar mixture of diastereoisomers) as a pale yellowish solid (59 mg, 66%). **¹H NMR** (CD_3OD , 400 MHz) δ (ppm): 1.20 (6H, d, $J = 6.2$ Hz), 2.58-2.60 (4H, m), 2.73 (2H, dd, $J = 6.5$, 13.6 Hz), 2.82 (2H, dd, $J = 5.5$, 13.7 Hz), 3.13-3.19 (2H, m), 3.28-3.32 (2H, m), 3.80-3.88 (2H, m, $CHOH$), 3.90-3.95 (2H, m, $CHOH$), 7.64-7.76 (4H, m), 7.75-7.77 (4H, m). **¹³C NMR** (CD_3OD , 100 MHz) δ (ppm): 21.1, 32.66, 32.68, 38.66, 38.73, 40.96, 41.02, 66.8, 66.9, 70.15, 70.22, 126.2, 130.71, 130.73, 137.1, 141.4. **⁷⁷Se NMR** (CD_3OD , 76 MHz) δ (ppm): 267.9, 268.3. **HRMS** (ESI) calc. $C_{12}H_{19}NNaO_4S_2Se$ $[M+Na]^+$ 407.9818, found 407.9831.

Synthesis of 4-((2-hydroxy-3-(phenylselenyl)propyl)selenyl)benzenesulfonamide (5e)

Following the General Procedure, bromide **3** (50 mg, 0.13 mmol) and benzeneselenol (22 mg, 0.14 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 1:2), **5e** as a pale yellowish solid (47 mg, 81%). **¹H NMR** (CD_3OD , 400 MHz) δ (ppm): 3.06-3.19 (3H, m), 3.27-3.34 (1H, m), 3.89, 3.95 (1H, m, $CHOH$), 7.22-7.24 (3H, m), 7.46-7.49 (2H, m), 7.54-7.56 (2H, m), 7.71-7.73 (2H, m). **¹³C NMR** (CD_3OD , 100 MHz) δ (ppm): 33.1, 33.7, 69.9, 126.2, 126.7, 128.8, 129.8, 130.8, 132.3, 136.9, 141.4. **⁷⁷Se NMR** (CD_3OD , 76 MHz) δ (ppm): 267.7. **MS** (ESI) calc. $C_{15}H_{17}NNaO_3SSe_2$ $[M+Na]^+$ 473.9, found 473.6.

Synthesis of 4-((2-hydroxy-3-(*o*-tolylselenyl)propyl)selenyl)benzenesulfonamide (5f)

Following the General Procedure, bromide **3** (50 mg, 0.13 mmol) and 2-methylbenzeneselenol (22 mg, 0.14 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 2:3), **5f** as a pale yellowish solid (45 mg, 75%). **¹H NMR** (CDCl₃, 400 MHz) δ (ppm): 2.41 (3H, s), 2.86 (1H, bs, OH), 3.03 (1H, dd, J = 7.5, 12.7 Hz), 3.11-3.16 (2H, m), 3.24 (1H, dd, J = 4.7, 12.7 Hz), 3.86-3.94 (1H, m, CHOH), 5.10 (2H, bs, NH₂), 7.07 (1H, t, J = 7.3 Hz), 7.14-7.20 (2H, m), 7.42 (1H, d, J = 7.6 Hz), 7.52 (2H, ap d, J = 8.4 Hz), 7.71 (2H, ap d, J = 8.4 Hz). **¹³C NMR** (CDCl₃, 100 MHz) δ (ppm): 23.1, 34.5, 34.6, 69.7, 127.3, 127.4, 128.1, 130.4, 130.9, 132.0, 132.9, 137.9, 140.3, 140.6. **HRMS** (ESI) calc. C₁₆H₁₉NNaO₃SSe₂ [M +Na]⁺ 487.9314, found 487.9306.

Synthesis of 4-((3-(butylselanyl)-2-hydroxypropyl)selanyl)benzenesulfonamide (**5g**)

A suspension of dibutyl diselenide (40 mg, 0.15 mmol) in EtOH (1 mL) was treated with NaBH₄ (17 mg, 0.44 mmol) and maintained under stirring for 30 minutes. The resulting colourless solution (butyl selenolate) was treated with the bromide **3** (100 mg, 0.27 mmol) and the mixture was stirred at room temperature for additional 3 h. Afterwards, the reaction was quenched with saturated *aq.* NH₄Cl (2 mL) and then EtOAc (5 mL) was added. The organic phase was collected and the aqueous phase was extracted with EtOAc (2 x 5 mL). The combined organic phases were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by flash column chromatography (petroleum ether/EtOAc 3:2) to yield **5g** as a pale yellowish solid (97 mg, 84%). **¹H NMR** (CDCl₃, 400 MHz) δ (ppm): 0.89 (3H, t, J = 7.3 Hz), 1.33-1.42 (2H, m), 1.58-1.65 (2H, m), 2.57 (2H, t, J = 7.5 Hz, CH₂CH₂Se), 2.70 (1H, dd, J = 7.6, 12.8 Hz, 1H, CH_aH_bSe), 2.84 (1H, dd, J = 4.6, 12.8 Hz, CH_aH_bSe), 3.14 (1H, dd, J = 7.2, 12.7 Hz, CH_aH_bSe), 3.24 (1H, dd, J = 4.8, 12.7 Hz, CH_aH_bSe), 3.87-3.93 (1H, CHOH), 5.32 (2H, bs, NH₂), 7.55-7.57 (2H, m), 7.74 (2H, ap d, J = 7.4 Hz). **¹³C NMR** (CDCl₃, 100 MHz) δ (ppm): 13.6, 22.9, 24.8, 31.3

(CH₂CH₂Se), 32.6 (CH₂SeⁿBu), 33.8 (ArSeCH₂), 69.3 (CHOH), 126.8, 131.2, 137.6, 139.9. ⁷⁷Se NMR (CDCl₃, 76 MHz) δ (ppm): 109.9 (ⁿBuSe), 268.6 (ArSe).

Synthesis of 4-((3-((3-(allyloxy)-2-hydroxypropyl)selenyl)-2-hydroxypropyl)selenyl)benzenesulfonamide (5h)

Following the General Procedure, bromide **3** (40 mg, 0.11 mmol) and 1-(allyloxy)-3-hydroselenopropan-2-ol (23 mg, 0.12 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 2:3), **5h** (equimolar mixture of diastereoisomers) as a yellowish solid (38 mg, 70%). ¹H NMR (CD₃OD, 400 MHz) δ (ppm): 2.67-2.90 (8H, m), 3.17 (2H, dd, *J* = 6.8, 12.5 Hz), 3.32 (2H, dd, *J* = 5.1, 12.6, Hz), 3.40-3.53 (4H, m, CH₂O), 3.81-3.95 (4H, m, CHOH), 3.96-4.02 (4H, m, CH₂CH=CH₂), 5.12-5.33 (4H, m, CH₂CH=CH₂), 5.81-6.01 (2H, m, CH₂CH=CH₂), 7.62-7.68 (2H, m), 7.73-7.78 (2H, m). ¹³C NMR (CD₃OD, 100 MHz) δ (ppm): 27.9, 28.0, 30.8, 33.2, 70.2, 70.6, 71.8, 73.0, 115.9, 126.2, 130.7, 134.7, 137.1, 141.4. MS (ESI) calc. C₁₆H₁₉NNaO₄S₂Se [M+Na]⁺ 456.0, found 456.2. MS (ESI) calc. C₁₅H₂₃NNaO₅SSe₂ [M+Na]⁺ 512.0, found 511.8.

Synthesis of 4-((2-hydroxy-3-(*p*-tolyltellanyl)propyl)selenyl)benzenesulfonamide (5i)

A suspension of 1,2-di-*p*-tolyltellane (50 mg, 0.11 mmol) in EtOH (1 mL) was treated with NaBH₄ (13 mg, 0.34 mmol) and maintained under stirring for 30 minutes. The resulting colourless solution (*p*-tolyl tellurolate) was treated with the bromide **3** (74 mg, 0.20 mmol) and the mixture was stirred at room temperature for additional 3 h. Afterwards, the reaction was quenched with saturated *aq.* NH₄Cl (2 mL) and then EtOAc (5 mL) was added. The organic phase was collected and the aqueous phase was extracted with EtOAc (2 x 5 mL). The combined organic phases were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by flash column chromatography (petroleum ether/EtOAc 2:3), to yield **5i** as a pale orange solid (89

mg, 87%). **¹H NMR** (CDCl₃, 400 MHz) δ (ppm): 2.33 (3H, s), 2.72 (1H, bs, OH), 3.06 (1H, dd, J = 7.2, 12.3 Hz), 3.10-3.15 (2H, m), 3.25 (1H, dd, J = 4.4, 12.7 Hz), 3.88-3.97 (1H, m, CHOH), 5.02 (2H, bs, NH₂), 7.02 (2H, ap d, J = 7.6 Hz), 7.53 (2H, ap d, J = 8.2 Hz), 7.62 (2H, ap d, J = 7.6 Hz), 7.73 (2H, ap d, J = 8.2 Hz). **¹³C NMR** (CDCl₃, 100 MHz) δ (ppm): 17.5, 21.2, 35.2, 70.2, 106.8, 126.9, 130.4, 131.4, 137.4, 138.3, 139.0, 140.0. **HRMS** (ESI) calc. C₁₆H₁₉NNaO₃SSeTe [M+H]⁺ 537.9211, found 537.9192.

Synthesis of 4-((3-((2-bromophenyl)thio)-2-hydroxypropyl)thio)benzenesulfonamide (6a)

Following the General Procedure, bromide **4** (45 mg, 0.14 mmol) and 2-bromobenzenethiol (29 mg, 0.16 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 1:1), **6a** as a white solid (45 mg, 74%). **¹H NMR** (CDCl₃, 200 MHz) δ (ppm): 3.03-3.31 (5H, m, CH₂S overlapped with bs, OH), 3.85-3.97 (1H, m, CHOH), 5.24 (2H, bs, NH₂), 7.00-7.11 (1H, m), 7.14-7.34 (2H, m), 7.39-7.58 (3H, m), 7.70 (2H, ap d, J = 8.4 Hz). **¹³C NMR** (CDCl₃, 50 MHz) δ (ppm): 33.5, 40.0, 68.6, 126.1, 126.9, 128.0, 129.7, 131.4, 132.6, 133.3, 137.0, 138.4, 140.1. **HRMS** (ESI) calc. C₁₅H₁₆BrNNaO₃S₃ [M+Na]⁺ 455.9373, found 455.9361.

Synthesis of 4-((3-((3-bromophenyl)thio)-2-hydroxypropyl)thio)benzenesulfonamide (6b)

Following the General Procedure, bromide **4** (45 mg, 0.14 mmol) and 3-bromobenzenethiol (29 mg, 0.16 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 2:3), **6b** as a white solid (48 mg, 79%). **¹H NMR** (CDCl₃, 400 MHz) δ (ppm): 2.81 (1H, bs, OH), 3.07 (1H, dd, J = 7.3, 13.8 Hz, CH_aH_bS), 3.12 (1H, dd, J = 7.2, 13.0 Hz, CH_mH_nS), 3.21 (1H, dd, J = 4.9, 13.8 Hz, CH_aH_bS), 3.27 (1H, dd, J = 4.8, 13.0 Hz, CH_mH_nS), 3.79-3.95 (1H, m, CHOH), 4.97 (2H, bs, NH₂), 7.13 (1H, ap t, J = 7.8 Hz), 7.23-7.36 (2H, m), 7.48 (1H, ap t, J = 1.8 Hz), 7.56 (2H, ap d, J = 8.7 Hz), 7.75 (2H, ap d, J = 8.7 Hz). **¹³C NMR** (CDCl₃, 100 MHz) δ (ppm): 33.5,

40.3, 68.6, 123.0, 127.0, 128.1, 129.8, 130.5, 131.5, 131.9, 137.0, 137.3, 140.2. **HRMS** (ESI) calc. $C_{15}H_{16}BrNNaO_3S_3$ $[M+Na]^+$ 455.9373, found 455.9386.

Synthesis of *N*-((2*S*)-1-((2-hydroxy-3-((4-sulfamoylphenyl)thio)propyl)thio)propan-2-yl)-4-methylbenzenesulfonamide (6c)

Following the General Procedure, bromide **4** (50 mg, 0.15 mmol) and (*S*)-*N*-(1-mercaptopropan-2-yl)-4-methylbenzenesulfonamide (41 mg, 0.17 mmol) gave, after purification by flash column chromatography ($CHCl_3/MeOH$ 14:1), **6c** (equimolar mixture of diastereoisomers) as a yellowish solid (57 mg, 77%). **1H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 1.06 (3H, d, $J = 6.6$ Hz), 1.07 (3H, d, $J = 6.6$ Hz), 2.42 (6H, s), 2.52-2.66 (6H, m), 2.72 (1H, dd, $J = 4.4, 7.3$ Hz), 2.75 (1H, dd, $J = 5.4, 8.3$ Hz), 3.05 (2H, bs, OH), 3.04-3.10 (2H, m, partially overlapped with bs of OH signal), 3.17-3.22 (2H, m), 3.36-3.48 (2H, m, CHNH), 3.79-3.91 (2H, m, CHOH), 4.96-5.35 (6H, m, NH_2 and NHTs), 7.28-7.30 (4H, m), 7.60-7.63 (4H, m), 7.72-7.74 (4H, m), 7.78-7.81 (4H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 20.4, 21.5, 33.6, 39.6, 40.0, 40.2, 49.3, 49.4, 69.2, 69.4, 126.98, 127.0, 129.8, 131.70, 131.74, 136.8, 136.9, 137.4, 140.3, 143.6. **HRMS** (ESI) calc. $C_{19}H_{27}N_2O_5S_4$ $[M+H]^+$ 491.0803, found 491.0795.

Synthesis of 4-((2-hydroxy-3-(phenylselanyl)propyl)thio)benzenesulfonamide (6d)

Following the General Procedure, bromide **4** (45 mg, 0.14 mmol) and benzeneselenol (25 mg, 0.16 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 2:3), **6d** as a pale yellowish solid (52 mg, 92%). **1H NMR** (CD_3OD , 400 MHz) δ (ppm): 3.06-3.19 (3H, m), 3.32 (1H, dd, $J = 4.9, 13.6$ Hz), 3.86-3.92 (1H, m, CHOH), 7.21-7.24 (3H, m), 7.37-7.39 (2H, m), 7.48-7.50 (2H, m), 7.72-7.75 (2H, m). **^{13}C NMR** (CD_3OD , 100 MHz) δ (ppm): 33.2, 38.0, 69.2, 126.2, 126.7, 127.0, 128.8, 129.8, 132.3, 140.2, 142.8. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 258.5. **HRMS** (ESI) calc. $C_{15}H_{17}NNaO_3S_2Se$ $[M+Na]^+$ 425.9713, found 425.9716.

Synthesis of 4-((2-hydroxy-3-((4-methoxyphenyl)selenanyl)propyl)thio)benzenesulfonamide (6e)

Following the General Procedure, bromide **4** (50 mg, 0.15 mmol) and 4-methoxybenzeneselenol (32 mg, 0.17 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 1:1), **6e** as a pale yellowish solid (55 mg, 85%). ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 2.67 (1H, bs, OH), 2.93 (1H, dd, *J* = 7.8, 12.8 Hz), 3.06 (1H, dd, *J* = 5.3, 7.6 Hz), 3.10 (1H, dd, *J* = 5.3, 7.6 Hz), 3.20 (1H, dd, *J* = 4.7, 12.8 Hz), 3.73-3.78 (1H, m, CHOH), 3.80 (3H, s), 5.08 (2H, bs, NH₂), 6.76-6.78 (2H, m), 7.42-7.44 (2H, m), 7.48-7.50 (2H, m), 7.69-7.71 (2H, m). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 33.6, 36.0, 55.4, 68.6, 115.0, 126.8, 131.4, 131.5, 135.9, 140.0, 159.5. MS (ESI) calc. C₁₆H₁₉NNaO₄S₂Se [*M*+Na]⁺ 456.0, found 456.2.

Synthesis of 4-((3-(butylselenanyl)-2-hydroxypropyl)thio)benzenesulfonamide (6f)

A suspension of dibutyl diselenide (40 mg, 0.15 mmol) in EtOH (1 mL) was treated with NaBH₄ (17 mg, 0.44 mmol) and maintained under stirring for 30 minutes. The resulting colourless solution (butyl selenolate) was treated with the bromide **4** (88 mg, 0.27 mmol) and the mixture was stirred at room temperature for additional 3 h. Afterwards, the reaction was quenched with saturated *aq.* NH₄Cl (2 mL) and then EtOAc (5 mL) was added. The organic phase was collected and the aqueous phase was extracted with EtOAc (2 x 5 mL). The combined organic phases were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by flash column chromatography (petroleum ether/EtOAc 1:1) to yield **6f** as a yellowish solid (91 mg, 88%). ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 0.89 (3H, t, *J* = 7.3 Hz), 1.33-1.42 (2H, m), 1.58-1.66 (2H, m), 2.59 (2H, t, *J* = 7.5 Hz, CH₂CH₂Se), 2.71 (1H, dd, *J* = 7.4, 12.8 Hz), 2.85 (1H, dd, *J* = 4.8, 12.8 Hz), 3.14 (1H, dd, *J* = 7.0, 13.6 Hz, overlapped with 1H, bs of CHOH), 3.24 (1H, dd, *J* = 4.9, 13.6 Hz), 3.87-3.91 (1H, m, CHOH), 5.24 (2H, bs, NH₂), 7.38 (2H, ap d, *J* = 8.5 Hz), 7.76

(2H, ap d, $J = 8.5$ Hz). ^{13}C NMR (CDCl_3 , 100 MHz) δ (ppm): 13.6, 22.9, 24.9, 30.8, 32.6, 38.5, 68.9, 126.9, 127.4, 138.8, 143.3. HRMS (ESI) calc. $\text{C}_{13}\text{H}_{22}\text{NO}_3\text{S}_2\text{Se}$ $[M+\text{H}]^+$ 384.0206, found 384.0225.

Synthesis of 4-((3-((3-(allyloxy)-2-hydroxypropyl)selenanyl)-2-hydroxypropyl)thio)benzenesulfonamide (6g)

Following the General Procedure, bromide **4** (50 mg, 0.15 mmol) and 1-(allyloxy)-3-hydroselenopropan-2-ol (33 mg, 0.17 mmol) gave, after purification by flash column chromatography (petroleum ether/EtOAc 2:3), **6g** (equimolar mixture of diastereoisomers) as a yellowish solid (48 mg, 72%). ^1H NMR (CD_3OD , 400 MHz) δ (ppm): 2.66-2.73 (2H, m), 2.78-2.84 (4H, m), 2.87-2.92 (2H, m), 3.13-3.19 (2H, m), 3.33-3.36 (2H, m), 3.45-3.52 (4H, m, CH_2O), 3.86-3.92 (2H, m, CHOH), 3.92-3.96 (2H, m, CHOH), 4.00-4.02 (4H, m, $\text{CH}_2\text{CH}=\text{CH}_2$), 5.15-5.30 (4H, m, $\text{CH}_2\text{CH}=\text{CH}_2$), 5.86-5.96 (2H, m, $\text{CH}_2\text{CH}=\text{CH}_2$), 7.48-7.51 (4H, m), 7.78-7.81 (4H, m). ^{13}C NMR (CD_3OD , 100 MHz) δ (ppm): 28.0, 28.1, 30.2, 30.3, 38.1, 69.90, 69.92, 70.2, 70.3, 71.8, 73.0, 115.9, 126.2, 127.0, 134.7, 140.3, 143.0. HRMS (ESI) calc. $\text{C}_{15}\text{H}_{23}\text{NNaO}_5\text{S}_2\text{Se}$ $[M+\text{Na}]^+$ 464.0081, found 464.0074.

Synthesis of 4-((2-hydroxy-3-((3,4,5-trimethoxyphenyl)tellanyl)propyl)thio)benzenesulfonamide (6h)

A suspension of 1,2-bis(3,4,5-trimethoxyphenyl)ditellane (60 mg, 0.10 mmol) in EtOH (1 mL) was treated with NaBH_4 (12 mg, 0.31 mmol) and maintained under stirring for 30 minutes. The resulting colourless solution (3,4,5-trimethoxyphenyl-tellurolate) was treated with the bromide **4** (59 mg, 0.18 mmol) and the mixture was stirred at room temperature for additional 3 h. Afterwards, the reaction was quenched with saturated *aq.* NH_4Cl (2 mL) and then EtOAc (5 mL) was added. The organic phase was collected and the aqueous phase was extracted with EtOAc (2 x 5 mL). The

combined organic phases were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by flash column chromatography (petroleum ether/EtOAc 1:3), to yield **6h** as a pinkish solid (79 mg, 81%). ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 2.84 (1H, bs, OH), 2.96 (1H, dd, *J* = 8.1, 12.6 Hz), 3.09 (1H, dd, *J* = 7.4, 12.6 Hz), 3.20 (1H, dd, *J* = 4.6, 7.5 Hz), 3.26 (dd, *J* = 5.0, 7.5 Hz), 3.69-3.79 (1H, m, CHOH), 3.78 (9H, s), 5.29 (2H, bs, NH₂), 6.90 (2H, s), 7.41 (2H, ap d, *J* = 8.4 Hz), 7.67 (2H, ap d, *J* = 8.4 Hz). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 17.3, 34.5, 56.3, 61.0, 69.2, 103.5, 116.7, 126.7, 131.4, 136.0, 138.1, 140.5, 153.2. ⁷⁷Se NMR (CDCl₃, 76 MHz) δ (ppm): 271.5. ¹²⁵Te NMR (CDCl₃, 126 MHz) δ (ppm): 457.9. MS (ESI) calc. C₁₈H₂₃NNaO₆S₂Te [*M*+Na]⁺ 566.0, found 565.9.

4.3 Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument was used for assaying the CA catalyzed CO₂ hydration activity [35]. Phenol red (at a concentration of 0.2 mM) was used as an indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.4) as a buffer, and 20 mM Na₂SO₄ (for maintaining constant ionic strength), following the initial rates of the CA-catalyzed CO₂ hydration reaction for a period of 10–100 s. The CO₂ concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants [49]. Enzymes concentrations in the assay system ranged between 5-12 nM. For each inhibitor, at least six traces of the initial 5–10% of the reaction were 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 the 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 the assay, 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 and represent the mean from at least three different determinations. All CA isoforms were recombinant proteins obtained in house, as reported earlier [50-53].

4.4 *In vitro* assays

MCF7 cell culture

MCF7 human breast adenocarcinoma cells (ATCC[®] HTB-22TM) were cultured in DMEM high glucose medium supplemented with 10% of foetal bovine serum (FBS), 1% of penicillin/streptomycin and 1% of l-glutamine (all purchased by EuroClone, Milan, Italy). Cell culture was maintained within an incubator at 37 °C in humidified atmosphere with 5% CO₂.

Hypoxia induction and MCF7 treatment

MCF7 were seeded at cell density of 10000/well into a 96-well tissue culture plate and cultured for 24 h. After on day of culture, medium was removed and replaced by fresh one containing CoCl₂ 100 µM to induce hypoxia, as elsewhere already reported [54]. CoCl₂ treatment was maintained for 48 h. After that, cisplatin, **5a**, **6f** and **6h**, previously solubilized in DMSO (DMSO final concentration established at 0.1% in all tested samples), were administered to MCF7 with concentrations ranging from 0.048 to 10000 µM, for 72 h maintaining CoCl₂ 100 µM within the culture medium, in order to calculate their IC₅₀. Then, combinations of **5a**, **6f** and **6h** with cisplatin 100 µM and doxorubicin 2.5 µM were also administered in presence of CoCl₂ 100 µM for 72 h.

MTT assay

The metabolic activity of MCF7 was evaluated after 72 h of treatment by means of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich, Milan, Italy) test. After 72 h of treatment, the medium was replaced by a fresh one containing 0.5 mg/mL of MTT and incubated for 5 h at 37 °C. Then, the medium containing MTT was discarded, replaced by an equal

volume of DMSO and incubated for 30 min at 37 °C to solubilize formazan salts and then read at 570 nm through a microplate reader (Multiskan GO, Thermo Scientific, MA, USA). Values obtained in the absence of cells were considered as background. Viability was normalized to control cells treated with DMSO 0.1% and CoCl₂ 100 µM.

4.5 Crystallization and X-ray data collection

Crystals of hCA II/inhibitor 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. The complexes were prepared soaking the hCA II native crystals in the mother liquor solution containing the inhibitors at concentration of 10 mM for two days. The crystals were flash-frozen at 100 K 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 XRD2 beamline at Elettra Synchrotron (Trieste, Italy) with a wavelength of 1.000 Å and a DECTRIS Pilatus 6M detector. Data were integrated and scaled using the program XDS [55]. Data processing statistics were shown in supporting information.

4.6 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 [56]. 5% of the unique reflections were selected randomly and excluded from the refinement data set for the purpose of R_{free} calculations. The initial |F_o - F_c| difference electron density maps unambiguously showed the inhibitor molecules. The inhibitor was introduced in the model with 1.0 occupancy. Refinements proceeded using normal protocols of positional, isotropic atomic displacement parameters alternating with manual building of the models using COOT [57]. The quality of the

final models were assessed with COOT and RAMPAGE [58]. Crystal parameters and refinement data are summarized in Electronic Supplementary Information (ESI). Atomic coordinates were deposited in the Protein Data Bank (PDB accession code: 7OK8; 7ORP; 7ORQ). Graphical representations were generated with Chimera [59].

4.7 *In vivo* Assay

Animals. Male CD-1 albino mice (Envigo, Varese, Italy) weighing approximately 22–25 g at the beginning of the experimental procedure, were used. Animals were housed in Ce.S.A.L (Centro Stabulazione Animali da Laboratorio, University of Florence) and used at least 1 week after their arrival. Ten mice were housed per cage (size 26 × 41 cm). Animals were fed a standard laboratory diet and tap water ad libitum, and kept at 23 ± 1 °C with a 12 h light/dark cycle, light at 7 a.m. All animal manipulations were carried out according to the Directive 2010/63/EU of the European parliament and of the European Union council (22 September 2010) on the protection of animals used for scientific purposes. The ethical policy of the University of Florence complies with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication No. 85-23, revised 1996; University of Florence assurance number: A5278-01). Formal approval to conduct the experiments described was obtained from the Animal Subjects Review Board of the University of Florence. Experiments involving animals have been reported according to ARRIVE guideline [60]. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Oxaliplatin-induced neuropathic pain model and pharmacological treatments

Oxaliplatin (2.4 mg kg⁻¹) was dissolved in 5% glucose solution and i.p. administered for 5 consecutive days every week for 14 days [61]. Starting from day 14, compounds were suspended in 1% carboxymethylcellulose sodium salt (CMC) and per os (p.o.) acutely administered. Pain-

related behavior (i.e. lifting and licking of the hind paw) was observed and the time (seconds) of the first sign was recorded. Control animals were treated with an equivalent volume of vehicles.

Cold plate test. Thermal allodynia was assessed using the Cold-plate test. With minimal animal-handler interaction, mice were taken from home-cages and placed onto the surface of the cold-plate (Ugo Basile, Varese, Italy) maintained at a constant temperature of 4 ± 1 °C. Ambulation was restricted by a cylindrical plexiglas chamber (diameter: 10 cm, height: 15 cm), with open top. A timer controlled by foot peddle began timing response latency from the moment the mouse was placed onto the cold-plate. Pain-related behaviour (licking of the hind paw) was observed and the time (seconds) of the first sign was recorded. The cut-off time of the latency of paw lifting or licking was set at 30 s. Behavioural tests were carried out before and after (15, 30, 45, 60 and 75 min) compound's injection [62].

Statistical analysis. Behavioural measurements were performed on 12 mice for each treatment carried out in 2 different experimental sets. Results were expressed as mean $\pm$ S.E.M. The analysis of variance of behavioural data was performed by one-way ANOVA, a Bonferroni's significant difference procedure was used as post-hoc comparison. *p* values of less than 0.05 or 0.01 were considered significant. Investigators were blind to all experimental procedures. Data were analysed using the "Origin 9" software (OriginLab, Northampton, USA).

ASSOCIATED CONTENT

¹H, ¹³C NMR spectra of compounds; Summary of Data Collection and Atomic Model, Refinement Statistics for hCA II; Figures S1-S4.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +39 055 457 3666. E-mail: andrea.angeli@unifi.it

Author Contributions

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

Funding Sources

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CNCS–UEFISCDI, project number PN-III-P4-ID-PCCF-2016–0050, within PNCDI II.

ABBREVIATIONS

CA, Carbonic Anhydrase; CAI, Carbonic Anhydrase Inhibitors; TLC, thin layer chromatography; NROR, Nucleophilic-ring-opening reactions; CNS, central nervous system.

REFERENCES

1. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 68 (2018) 394-424.
2. Jin, S., Guo, Y., Guo, Z., Wang, X. Monofunctional Platinum(II) Anticancer Agents. *Pharmaceuticals (Basel).* 14 (2021) 133.
3. Silva, M.J.S.A., Gois, P.M.P., Gasser, G. Unveiling the Potential of Transition Metal Complexes for Medicine: Translational in Situ Activation of Metal-Based Drugs from Bench to in Vivo Applications. *Chembiochem.* 2021. doi: 10.1002/cbic.202100015.
4. de Almeida, A., Bonsignore, R. Fluorescent metal-based complexes as cancer probes. *Bioorg Med Chem Lett.* 30 (2020) 127219.
5. Simpson, D., Dunn, C., Curran, M., Goa, K.L. Oxaliplatin: a review of its use in combination therapy for advanced metastatic colorectal cancer. *Drugs.* 63 (2003) 2127-2156.

6. Kelland, L. The resurgence of platinum-based cancer chemotherapy. *Nat Rev Cancer*. 7 (2007) 573-584.
7. Saif, M.W., Reardon, J. Management of oxaliplatin-induced peripheral neuropathy. *Ther. Clin. Risk Manag.* 1 (2005) 249–258.
8. Sisignano, M., Baron, R., Scholich, K., Geisslinger, G. Mechanism-based treatment for chemotherapy-induced peripheral neuropathic pain. *Nat Rev Neurol*. 10 (2014) 694-707.
9. Asiedu, M., Ossipov, M.H., Kaila, K., Price, T.J. Acetazolamide and midazolam act synergistically to inhibit neuropathic pain. *Pain* 148 (2010) 302-308.
10. Asiedu, M.N., Mejia, G.L., Hübner, C.A., Kaila, K., Price, T.J. Inhibition of carbonic anhydrase augments GABA_A receptor-mediated analgesia via a spinal mechanism of action. *J Pain* 15 (2014) 395-406.
11. Angeli, A., Di Cesare Mannelli, L., Ghelardini, C., Peat, T.S., Bartolucci, G., Menicatti, M., Carta, F., Supuran, C.T. Benzensulfonamides bearing spirohydantoin moieties act as potent inhibitors of human carbonic anhydrases II and VII and show neuropathic pain attenuating effects. *Eur J Med Chem*. 177 (2019) 188-197.
12. Angeli, A., Di Cesare Mannelli, L., Lucarini, E., Peat, T.S., Ghelardini, C., Supuran, C.T. Design, synthesis and X-ray crystallography of selenides bearing benzenesulfonamide moiety with neuropathic pain modulating effects. *Eur J Med Chem*. 154 (2018) 210-219.
13. Supuran, C.T. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Discov*. 7 (2008) 168-181.
14. Supuran, C.T. Structure and function of carbonic anhydrases. *Biochem J*. 473 (2016) 2023-2032.

15. Mishra, C.B., Tiwari, M., Supuran, C.T. Progress in the development of human carbonic anhydrase inhibitors and their pharmacological applications: Where are we today? *Med Res Rev.* 40 (2020) 2485-2565.
16. Supuran, C.T. Carbonic anhydrase inhibition and the management of neuropathic pain. *Expert Rev Neurother.* 16 (2016) 961-968.
17. Halmi, P., Parkkila, S., Honkaniemi, J. Expression of carbonic anhydrases II, IV, VII, VIII and XII in rat brain after kainic acid induced status epilepticus. *Neurochem Int* 48 (2006) 24-30.
18. G. Negi, A. Kumar, R.P. Joshi, S.S. Sharma, Oxidative stress and Nrf2 in the pathophysiology of diabetic neuropathy: old perspective with a new angle. *Biochem. Biophys. Res. Commun.* 408 (2011) 1–5.
19. G. Negi, A. Kumar, S.S. Sharma, Melatonin modulates neuroinflammation and Oxidative stress in experimental diabetic neuropathy: effects on NF-kB and Nrf2 cascades. *J. Pineal Res.* 50 (2011) 124–131.
20. Tanini, D.; Ricci, L.; Capperucci, A. Rongalite-Promoted *on Water* Synthesis of Functionalised Tellurides and Ditellurides. *Adv. Synth. Catal.* 362 (2020) 1323–1332.
21. Tanini, D.; Tiberi, C.; Gellini, C.; Salvi, P.R.; Capperucci, A. A Straightforward access to stable β -functionalized alkyl selenols. *Adv. Synth. Catal.* 360 (2018) 3367–3375.
22. Tanini, D.; Grechi, A.; Dei, S.; Teodori, E.; Capperucci, A. An easy one-step procedure for the synthesis of novel β -functionalised tellurides. *Tetrahedron* 73 (2017) 5646–5653.
23. Tanini, D.; Capperucci, A. Ring opening reactions of heterocycles with selenium and tellurium nucleophiles. *New J. Chem.* 43 (2019) 11451–11468.

24. Tanini, D.; Lupori, B.; Malevolti, G.; Ambrosi, M.; Lo Nostro, P.; Capperucci, A. Direct biocatalysed synthesis of first sulfur-, selenium- and tellurium- containing L-ascorbyl hybrid derivatives with radical trapping and GPx-like properties. *Chem. Commun.* 55 (2019) 5705–5708.
25. Capperucci, A.; Coronello, M.; Salvini, F.; Tanini, D.; Dei, S.; Teodori, E.; Giovannelli, L. Synthesis of functionalised organochalcogenides and in vitro evaluation of their antioxidant activity. *Bioorg. Chem.* 110 (2021) 104812.
26. Tanini, D.; Ricci, L.; Capperucci, A.; Di Cesare Mannelli, L.; Ghelardini, C.; Peat, T.S.; Carta, F.; Angeli, A.; Supuran, C.T. Synthesis of novel tellurides bearing benzenesulfonamide moiety as carbonic anhydrase inhibitors with antitumor activity. *Eur. J. Med. Chem.* 181 (2019) 111586.
27. Angeli, A.; Tanini, D.; Capperucci, A.; Malevolti, G.; Turco, F.; Ferraroni, M.; Supuran, C. T. Synthesis of different thio-scaffolds bearing sulfonamide with subnanomolar carbonic anhydrase II and IX inhibitory properties and X-ray investigations for their inhibitory mechanism. *Bioorg. Chem.* 81 (2018) 642–648.
28. Angeli, A.; Tanini, D.; Capperucci, A.; Supuran, C.T. First evaluation of organotellurium derivatives as carbonic anhydrase I, II, IV, VII and IX inhibitors. *Bioorg. Chem.* 76, 2018, 268–272.
29. Angeli, A.; Tanini, D.; Capperucci, A.; Supuran, C. T. Synthesis of Novel Selenides Bearing Benzenesulfonamide Moieties as Carbonic Anhydrase I, II, IV, VII, and IX Inhibitors. *ACS Med. Chem. Lett.* 8 (2017) 1213-1217.
30. Tanini, D.; Capperucci, A.; Supuran, C.T.; Angeli, A. Sulfur, selenium and tellurium containing amines act as effective carbonic anhydrase activators. *Bioorg. Chem.* 87 (2019) 516–522.
31. Angeli, A.; Carta, F.; Donnini, S.; Capperucci, A.; Ferraroni, M.; Tanini, D.; Supuran, C.T. Selenolesterase enzyme activity of carbonic anhydrases. *Chem. Commun.* 56 (2020) 4444–4447.

32. Tanini, D.; Scarpelli, S.; Ermini, E.; Capperucci, A. Seleno-Michael reaction of stable functionalised alkyl selenols: A versatile tool for the synthesis of acyclic and cyclic unsymmetrical alkyl and vinyl selenides. *Adv. Synth. Catal.* 361 (2019) 2337–2346.
33. Angeli, A.; Tanini, D.; Nocentini, A.; Capperucci, A.; Ferraroni, M.; Gratteri, P.; Supuran, C. T. Selenols: a new class of carbonic anhydrase inhibitors. *Chem. Commun.*, 55 (2019) 648-651.
34. Tanini, D.; Borgogni, C.; Capperucci, A. Mild and selective silicon-mediated access to enantioenriched 1,2-mercaptoamines and β -amino arylchalcogenides. *New J. Chem.* 43 (2019) 6388–6393.
35. Khalifah, R.G. The carbon dioxide hydration activity of carbonic anhydrase. I. Stop flow kinetic studies on the native human isoenzymes B and C. *J. Biol. Chem.* 246 (1971) 2561-2573.
36. Berrino E, Angeli A, Zhdanov DD, Kiryukhina AP, Milaneschi A, De Luca A, Bozdog M, Carradori S, Selleri S, Bartolucci G, Peat TS, Ferraroni M, Supuran CT, Carta F. Azidothymidine "Clicked" into 1,2,3-Triazoles: First Report on Carbonic Anhydrase-Telomerase Dual-Hybrid Inhibitors. *J Med Chem.* 63 (2020) 7392-7409.
37. Angeli, A., Di Cesare Mannelli, L., Ghelardini, C., Peat, T.S., Bartolucci, G., Menicatti, M., Carta, F., Supuran, C.T. Benzenesulfonamides bearing spirohydantoin moieties act as potent inhibitors of human carbonic anhydrases II and VII and show neuropathic pain attenuating effects. *Eur J Med Chem.* 177 (2019) 188-197.
38. Angeli, A., Di Cesare Mannelli, L., Lucarini, E., Peat, T.S., Ghelardini, C., Supuran, C.T. Design, synthesis and X-ray crystallography of selenides bearing benzenesulfonamide moiety with neuropathic pain modulating effects. *Eur J Med Chem.* 154 (2018) 210-219.

39. Pacchiano, F., Carta, F., McDonald, P.C., Lou, Y., Vullo, D., Scozzafava, A., Dedhar, S., Supuran, C.T. Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis. *J Med Chem.* 54 (2011) 1896-902.
40. Lou, Y., McDonald, P.C., Oloumi, A., Chia, S., Ostlund, C., Ahmadi, A., Kyle, A., Auf dem Keller, U., Leung, S., Huntsman, D., Clarke, B., Sutherland, B.W., Waterhouse, D., Bally, M., Roskelley, C., Overall, C.M., Minchinton, A., Pacchiano, F., Carta, F., Scozzafava, A., Touisni, N., Winum, J.Y., Supuran, C.T., Dedhar, S. Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors. *Cancer Res.* 71 (2011) 3364-3376.
41. Boyd, N.H., Walker, K., Fried, J., Hackney, J.R., McDonald, P.C., Benavides, G.A., Spina, R., Audia, A., Scott, S.E., Libby, C.J., Tran, A.N., Bevensee, M.O., Griguer, C., Nozell, S., Gillespie, G.Y., Nabors, B., Bhat, K.P., Bar, E.E., Darley-Usmar, V., Xu, B., Gordon, E., Cooper, S.J., Dedhar, S., Hjelmeland, A.B. Addition of carbonic anhydrase 9 inhibitor SLC-0111 to temozolomide treatment delays glioblastoma growth in vivo. *JCI Insight.* 2 (2017) e92928.
42. D'Ascenzio, M., Secci, D., Carradori, S., Zara, S., Guglielmi, P., Cirilli, R., Pierini, M., Poli, G., Tuccinardi, T., Angeli, A., Supuran, C.T. 1,3-Dipolar Cycloaddition, HPLC Enantioseparation, and Docking Studies of Saccharin/Isoxazole and Saccharin/Isoxazoline Derivatives as Selective Carbonic Anhydrase IX and XII Inhibitors. *J Med Chem.* 63 (2020) 2470-2488.
43. Pastornická A, Rybářová S, Drahošová S, Mihalik J, Krehel'ová A, Pavliuk-Karachevtseva A, Hodorová I. Influence of Paclitaxel and Doxorubicin Therapy of β III-Tubulin, Carbonic Anhydrase IX, and Survivin in Chemically Induced Breast Cancer in Female Rat. *Int J Mol Sci.* 22 (2021) 6363.

44. García-Venzor A, Mandujano-Tinoco EA, Ruiz-Silvestre A, Sánchez JM, Lizarraga F, Zampedri C, Melendez-Zajgla J, Maldonado V. IncMat2B regulated by severe hypoxia induces cisplatin resistance by increasing DNA damage repair and tumor-initiating population in breast cancer cells. *Carcinogenesis*. 41 (2020) 1485-1497.
45. Zhao, P., Li, M., Chen, Y., He, C., Zhang, X., Fan, T., Yang, T., Lu, Y., Lee, R.J., Ma, X., Luo, J., Xiang, G. Selenium-doped calcium carbonate nanoparticles loaded with cisplatin enhance efficiency and reduce side effects. *Int J Pharm*. 570 (2019) 118638.
46. Spengler, G., Gajdács, M., Maré, M.A., Domínguez-Álvarez, E., Sanmartín, C. Organoselenium Compounds as Novel Adjuvants of Chemotherapy Drugs-A Promising Approach to Fight Cancer Drug Resistance. *Molecules*. 24 (2019) 336.
47. Obijalska, E.; Pawelec, M.; Mlostoń, G.; Capperucci, A.; Tanini, D.; Heimgartner, H. A Remarkable Influence of a Trifluoromethyl Group on the Reactions of β -Mercaptoalcohols with Fluorinated α -Bromoanones. *Eur. J. Org. Chem.* (2018) 3716-3723.
48. Singh, D.; Deobald, A. M.; Camargo, L. R. S.; Tabarelli, G.; Rodrigues, O. E. D.; Braga, A. L. An Efficient One-Pot Synthesis of Symmetrical Diselenides or Ditellurides from Halides with CuO Nanopowder/ Se^0 or Te^0 /Base. *Org. Lett.* 12 (2010) 3288-3291.
49. C.T. Supuran. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Discov*. 7 (2008) 168-181.
50. Angeli, A., Etxebeste-Mitxelorena, M., Sanmartín, C., Espuelas, S., Moreno, E., Azqueta, A., Parkkila, S., Carta, F., Supuran, C.T. Tellurides Bearing Sulfonamides as Novel Inhibitors of Leishmanial Carbonic Anhydrase with Potent Antileishmanial Activity. *J Med Chem*. 63 (2020) 4306-4314.

51. Abdel-Aziz, A.A., Angeli, A., El-Azab, A.S., Hammouda, M.E.A., El-Sherbeny, M.A., Supuran, C.T. Synthesis and anti-inflammatory activity of sulfonamides and carboxylates incorporating trimellitimides: Dual cyclooxygenase/carbonic anhydrase inhibitory actions. *Bioorg Chem.* 84 (2019) 260-268.
52. Mocan, A., Carradori, S., Locatelli, M., Secci, D., Cesa, S., Mollica, A., Riga, S., Angeli, A., Supuran, C.T., Celia, C., Di Marzio, L. Bioactive isoflavones from *Pueraria lobata* root and starch: Different extraction techniques and carbonic anhydrase inhibition. *Food Chem Toxicol.* 112 (2018) 441-447.
53. Angeli, A., Trallori, E., Carta, F., Di Cesare Mannelli, L., Ghelardini, C., Supuran, C.T. Heterocoumarins Are Selective Carbonic Anhydrase IX and XII Inhibitors with Cytotoxic Effects against Cancer Cells Lines. *ACS Med Chem Lett.* 9 (2018) 947-951.
54. Wu, D., Yotnda, P. Induction and testing of hypoxia in cell culture. *J Vis Exp.* 54 (2011) 2899.
55. Leslie, A.G.W., Powell, H.R. Procesing diffraction data with mosflm. In: Read RJ, Sussman JL (eds) *Evolving methods for macromolecular crystallography*, vol 245, NATO Science series, Springer, Dordrecht, 2007, pp. 41-51.
56. Murshudov, G.N., Vagin, A.A., Dodson, E.J. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr D Biol Crystallogr.* 53 (1997) 240-255.
57. Emsley, P., Lohkamp, B., Scott, W., Cowtan, K. Features and development of Coot. *Acta Crystallogr D Biol Crystallogr.* 66 (2010) 486-501.
58. Lovell, S.C., Davis, I.W., Arendall III, W.B., de Bakker, P.I.W., Word, J.M., Prisant, M.G., Richardson, J.S., Richardson, D.C., Structure validation by $C\alpha$ geometry: ϕ, ψ and $C\beta$ deviation, *Proteins*, 50 (2003) 437-450.

59. Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., UCSF Chimera—a visualization system for exploratory research and analysis, *J. Comput. Chem.*, 25 (2004) 1605-1612.
60. McGrath, J.C., Lilley, E. Implementing guidelines on reporting research using animals (ARRIVE etc.): new requirements for publication in BJP. *Br J Pharmacol.* 172 (2015) 3189-3193.
61. Micheli, L., Di Cesare Mannelli, L., Rizzi, A., Guerrini, R., Trapella, C., Calò, G., Ghelardini, C. Intrathecal administration of nociceptin/orphanin FQ receptor agonists in rats: A strategy to relieve chemotherapy-induced neuropathic hypersensitivity. *Eur J Pharmacol.* 766 (2015) 155-162.
62. Pacini, A., Micheli, L., Maresca, M., Branca, J.J., McIntosh, J.M., Ghelardini, C., Di Cesare Mannelli, L. The $\alpha 9\alpha 10$ nicotinic receptor antagonist α -conotoxin RgIA prevents neuropathic pain induced by oxaliplatin treatment. *Exp Neurol.* 282 (2016) 37-48.