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Syntesis of thio- and seleno-acetamides bearing benzenesulfonamide as potent inhibitors of human carbonic anhydrase II and XII

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ABSTRACT

A novel series of thio- and seleno-acetamides bearing benzenesulfonamide were synthetized and tested as human carbonic anhydrase inhibitors. These compounds were tested for the inhibition of four human (h) isoforms, hCA I, II, IX, and XII, involved in pathologies such as glaucoma (CA II and XII) or cancer (CA IX/XII). Several derivatives showed potent inhibition activity in low nanomolar range such as **3a**, **4a**, **7a** and **8a**. Furthermore, based on the tail approach we explain the interesting and selective inhibition profile of compound such as **5a** and **9a,** which were more selective for hCA I, **9b** which was selective for hCA II, **3f** selective for hCA IX and finally, **3e** and **4b** selective for hCA XII, over the other three isoforms. They are interesting leads for the development of more effective and isoform-selective inhibitors.

1. Introduction

Organo-chalcogenide scaffolds are topics of intense research in many fields of medicinal chemistry in the last decades [1–3]. Selenium and sulfur have been demonstrated to play an important role in biological systems as part of the active site in many proteins [4,5] and their organo-derivatives can be employed as antioxidant [6], antimicrobial [7], and antitumor agents [8]. These therapeutic agents also act as modulators on a variety of enzyme such as nitric oxide synthase (NOS) [9], inosine monophosphate dehydrogenase (IMDPH) [10], lipoxygenases (LOX) [11] and more recently, as carbonic anhydrase (CA, E.C. 4.2.1.1) inhibitors [12]. CAs are metalloenzymes present in all life kingdom, catalysing the reversible reaction of the hydration of carbon dioxide to bicarbonate and protons [13]. This reaction plays an important role in many physiological and pathological processes associated with pH regulation, ion transport, fluid secretion and biosynthetic reactions [13–16].

Only α-CAs have been reported in vertebrates and, in humans, 15 CA isoforms are known, with 12 of them being catalytically active and three (CA VIII, X, and XI) devoid of activity, but still playing significant functions in pathologic processes [13–16]. CA inhibitors (CAIs) are clinically used since 1954 in different pathologies such as diuretics [17], antiglaucoma agents [18], antiepileptics [19], and more recently were validated as novel antitumor agents [20]. A major challenge is the discovery of isoform-selective inhibitors and new deverivatives, with different or complementary pharmacophoric functions to those already available, possibly showing synergistic effects with other drugs. For this reason, we continued to investigate the organochalcogenide derivatives as human (h) CA inhibitors (CAIs).

2. Chemistry

During our studies on the reactivity of chalcogen-containing nucleophiles [21–23] we developed novel procedures to access variously functionalised sulfur-, selenium-, and tellurium-containing organic molecules [24–26]. Recently, these routes have been applied to the synthesis of new chalcogenated catalytic antioxidants [6,27,28], enzyme inhibitors [12,29–31], and enzyme activators [32].

On the basis of our previous results, and in order to enlarge the library of chalcogen-containing hCA inhibitors and to evaluate the effect of different functional groups on the inhibitor activity, we focused our attention onto the study of substituted 2-thio- and 2-seleno-acetamides bearing the benzenesulfonamide moiety. We sought to employ 2-chloroacetamides **2a–c**, obtained according to a reported procedures [33], as valuable precursors for the synthesis of the polyfunctionalised target compounds (see Fig. 1).

We began our studies by focusing on the reaction of benzenethiol **1a** with 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a**. In order to search

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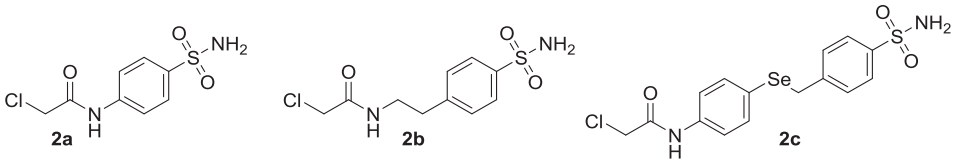
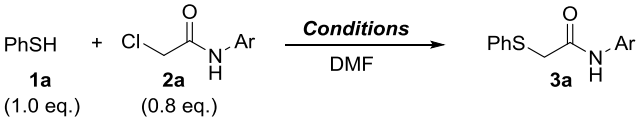
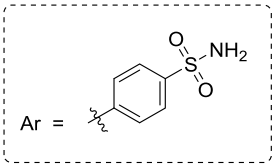


Fig. 1. Substituted 2-chloroacetamides **2a–c** bearing the benzenesulfonamide moiety used in this work.



Entry	Conditions	Yield (%)
1	Cs_2CO_3 (1.0 eq.), TBAI (1.0 eq.), 0°C to r.t.	--
2	Cs_2CO_3 (1.0 eq.), TBAI (1.0 eq.), r.t.	--
3	Cs_2CO_3 (2.0 eq.), TBAI (2.0 eq.), 0°C to r.t.	26
4	KOH (1.0 eq.), 0°C to r.t.	13
5	KOH (2.2 eq.), 0°C to r.t.	89



Scheme 1. Optimisation of the reaction conditions for the synthesis of **3a** from benzenethiol **1a** and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a.**

for suitable reaction conditions, on the basis of previous results on the reactivity of thiols and selenols with different electrophiles, we initially evaluated the use of the Cs_2CO_3/TBAI (tetrabutylammonium iodide) base/phase transfer catalyst system (Scheme 1, entries 1 and 2). However, the desired product **3a** was not formed in the presence of 1.0 eq. of Cs_2CO_3 at different reaction temperatures. This result was probably due to the acid-base reaction occurring between CO_3^{2-} and the amide proton of **2a**. Indeed, when the reaction was performed using 2.0 eq. of Cs_2CO_3 (Scheme 1, entry 3), the desired sulfur-containing amide **3a** was formed, although in a rather low yield. Thus, a stronger base such as KOH was evaluated in order to improve the yield of the reaction. We found that, although the use of 1.0 eq. of KOH led to the formation of **3a** only in poor yield (Scheme 1, entry 4), an excess (2.2 eq.) of KOH (Scheme 1, entry 5) allowed to achieve the desired 2-(phenylthio)acetamide **3a** in excellent yield.

Having established suitable conditions to access the desired 2-(phenylthio)acetamide **3a** bearing the benzenesulfonamide moiety, we next evaluated the possibility to apply this procedure to the synthesis of differently substituted sulfur-containing *N*-(4-sulfamoylphenyl)acetamides (Scheme 2). Thus, *p*-bromothiophenol **1b** and *o*-bromothiophenol **1c** were efficiently reacted with **2a** to afford the corresponding 2-(arylthio)acetamides **3b,c** in good yields. The reaction was also extended to alkyl thiols such as nonanethiol **1d**, 1-mercapto-2-propanol **1e** [34] and the natural aminoacid-derivative L-cysteine ethyl ester **1f**, enabling the synthesis of functionalised 2-(alkylthio)acetamides **3d–f**. Furthermore, in order to enlarge the library of these novel chalcogen-containing molecules as potential CA inhibitors, we then varied the electrophilic partner involved in such reaction (Scheme 2). Thus, sulfur-containing *N*-phenethylacetamides **4a**,**b** and *N*-(4-(benzylselanyl) phenyl)acetamides **5a**,**b** were obtained from the corresponding chlorides **2b**,**c** and thiols **1a**,**b**,**d**. In this context, it is worthwhile remembering that derivatives **3d** and **4b**, bearing a long hydrophobic carbon chain and a polar head, could be employed as novel amphiphilic CA inhibitors (Scheme 2).

Having developed a convenient procedure to access sulfur-containing acetamide derivatives bearing a benzenesulfonamide moiety, we focused our attention on the synthesis of their selenium-containing analogues. Taking into account the low stability of selenols, we sought to employ diselenides as precursors of selenolate anions. Thus, diphenyl diselenide **6a** was treated with $NaBH_4$ in dry ethanol to afford the corresponding benzeneselenolate which was *in situ* reacted with the 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (1.8 eq.). Pleasingly, under these reaction conditions, the desired 2-(phenylseleno)acetamide **7a** was smoothly formed in good yields (Scheme 3).

Having obtained the selenium-containing acetamide with the benzenesulfonamide moiety **7a**, we extended this *one pot* procedure to differently substituted diselenides. Thus, diselenides **6b–d**, bearing *p*-methyl-, *o*-methyl-, and *p*-(*N*,*N*-dimethylamino)-substituted aromatic rings, were efficiently converted into the corresponding 2-(arylseleno) acetamides **7b–d** in good yields under the same reaction conditions (Scheme 4).

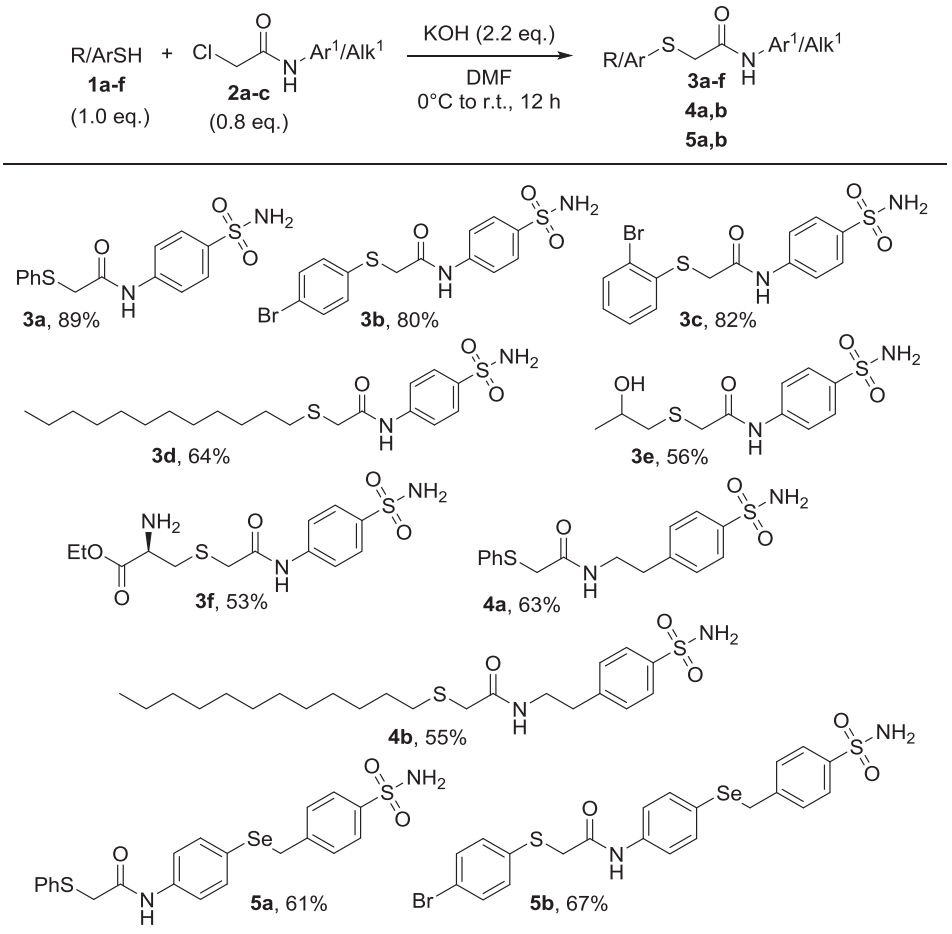
To further enlarge the scope of this procedure, we also investigated the reactivity of chlorides **2b**,**c** with variously substituted diselenides. The reaction proved to be efficient, allowing the synthesis of organoselenides **8a–c** from diselenides **6a–c**, bearing phenyl, *p*-(*N*,*N*-dimethylamino)phenyl, and *p*-(methoxy)phenyl groups. Furthermore, also dialkyl diselenides could be successfully employed in this transformation. Thus, the enantioenriched diselenide **6f** [35], synthesised from L-valine and the natural-product-derived compound **6g** [35], obtained from limonene, were smoothly converted into the corresponding selenium-containing functionalised acetamides **8d** and **8e** (Scheme 4). Finally, 2-chloro-*N*-(4-((4-sulfamoylbenzyl)selanyl)phenyl)acetamide **2c** and diselenides **6a,b,d,e** gave *N*-(4-(benzylselanyl)phenyl)acetamides **9a–d**, bearing two selenated moieties onto the same molecular skeleton (Scheme 4). Interestingly, compounds **7d**, **8b**, add **9c** having a (*N*,*N*-dimethyl)phenyl group onto the selenium atom, could be also achieved by using the corresponding selenocyanate in the presence of 2 eq. of $NaBH_4$ (Scheme 4, note *a*).

3. Carbonic anhydrase inhibition

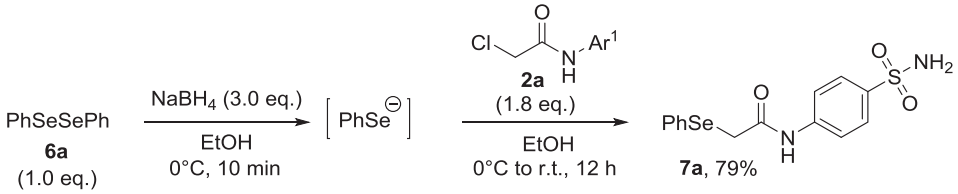
All compounds, here reported, were tested *in vitro* for their inhibitory activity against the physiologically relevant hCA isoforms I, II, IX and XII by means of the stopped-flow carbon dioxide hydration assay [36]. Their activities were compared to the standard CA inhibitor acetazolamide (**AAZ**).

We have investigated the novel series of chalcogenide-containing acetamide derivatives bearing a benzenesulfonamide moiety for their interaction with four hCAs of pharmacologic interest, using a period of 15 min of incubation of the enzyme and inhibitor solutions [37–39]. The following structure-activity-relationship (SAR) may be noted regarding the inhibition data of Table 1:

i) The cytosolic hCA I was weakly inhibited by derivatives **3a–f** (K_I



Scheme 2. Synthesis of sulfur-containing acetamides bearing the benzenesulfonamide moiety. Yields refer to isolated products.

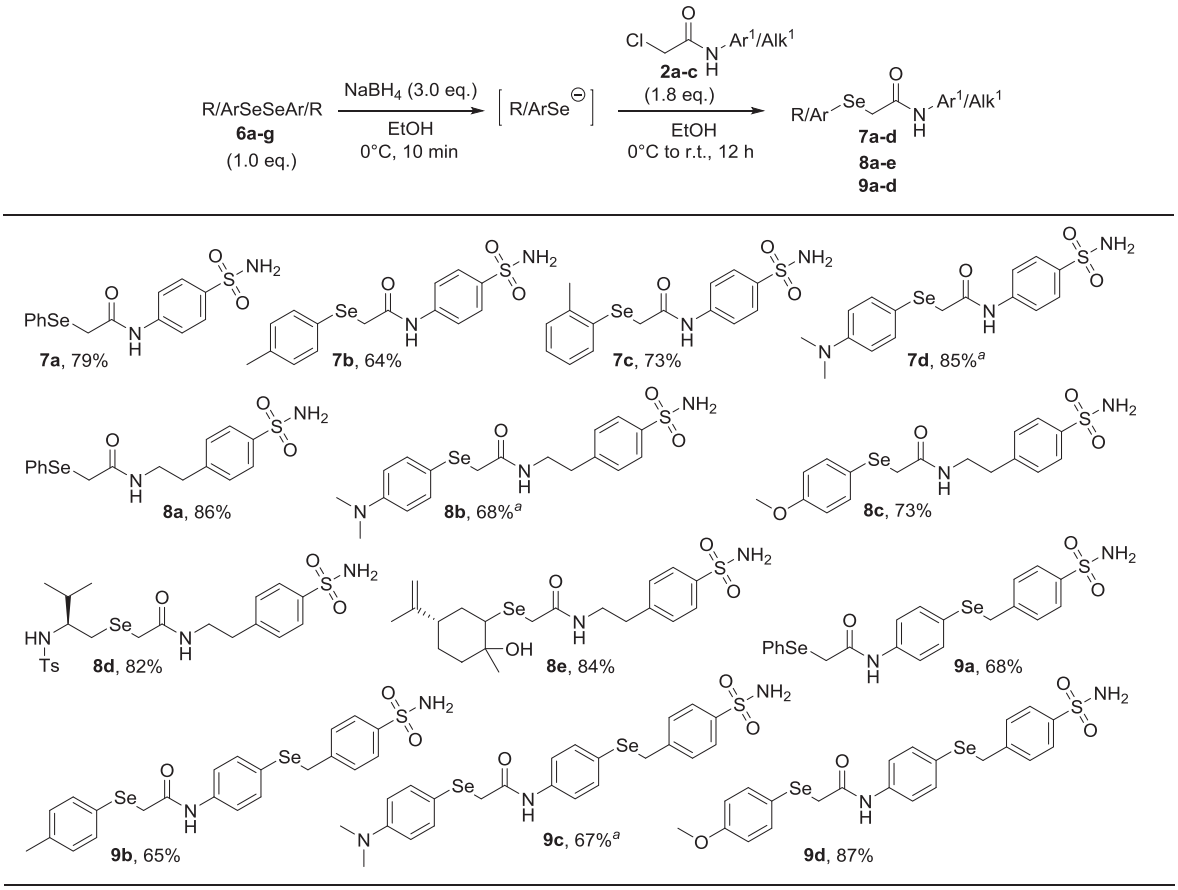


Scheme 3. Synthesis of 2-(phenylseleno)acetamide **7a.**

202.6–6049 nM) and the addition of ethyl linker among benzenesulfonamide scaffold and sulfur-tail of compounds **4a–b** did not increase the potency, which remained still in the micromolar range (K_I 1033 and 4880 nM). On the other hand, compounds **5a–b** with organoseleno moieties increased considerably the inhibition efficacy, now in the medium nanomolar range (K_I 75.2 and 68.6 nM). The same compounds, in which the replacement of sulfur with selenium was performed (**7a–d**), did not show significant changes in the range of inhibition activity (K_I 181.3–6979 nM). Contrarily, this replacement prove to be incisive for derivative **8a,** leading to a 16 fold increase in potency compared to the thio-analog **4a** (K_I 64.2 nM vs. 1033 nM).

ii) The dominant cytosolic human isofom hCA II, was effective inhibited by almost all derivatives here reported, except for compounds with high lipophilic tails such as **3d** and **4b** (as well compound **9c**). Moreover, the inhibition profile of derivatives **3a–f** varied according to the different moiety of the tail. Alkylic scaffolds such as those present in **3e** and **3f** proved to be less effective than aromatic moieties, decreasing the potency to the medium to high nanomolar range. An interesting inhibition pattern was observed for derivatives with organoseleno linker (**5a–b** and **9a–d**), which showed a weak inhibition for this isoform, in the micromolar range but they displayed nanomolar potency for hCA I, as mentioned above.

iii) The transmembrane tumor-associated hCA IX was weakly inhibited by derivatives **5a–b** and **9a–d** incorporating the organoseleno linker, which is not efficacious against this isoform. Moreover, like for the cytosolic hCA II, highly lipophilic moieties such as those present in **3d** and **4b** proved to be deleterious for the activity. An interesting inhibition profile was observed for compounds **3b** and **3c** showing the importance of the substituent position on the thiophenol scaffold. Bromine atom in *ortho* position (**3c**) increased the activity 4 and 6 times compared to derivatives having Br in the *para* position (**3b**) and having H instead of Br (**3a**), respectively. The addition of an ethyl linker in compound **4a** increased the potency over 60 fold compared to **3a**. The replacement of sulphur with selenium, like for hCA II, increased the activity (e.g., for compound **7a** than **3a**). On the other hand, substituents on aromatic ring in derivatives **7a–d** lead to a decrease of the inhibition potency (K_I 3.5 nM of **7a** to K_I 320.5 nM of **7c**). This was also observed for



Scheme 4. Synthesis of selenium-containing acetamides bearing the benzenesulfonamide moiety. Yields refer to isolated products. [a]Comparable yields were observed when the reaction was performed using *p-N,N*-dimethylamino-benzeneselenocyanate instead of the diselenide **6d**.

Table 1
Inhibition data of human CA isoforms I, II, IX and XII with compounds **3a–f, 4a–b, 5a–b, 7a–d, 8a–e, 9a–d** and **AAZ** by a stopped flow CO_2 hydrase assay [36].

K_I (nM)*				
Cmp	**hCA I**	**hCAII**	**hCA IX**	**hCA XII**
3a	6049	6.1	272.1	29.2
3b	3089	18.7	200.2	59.6
3c	3782	5.3	45.3	8.6
3d	202.6	> 10000	3794	246.6
3e	2335	49.9	100.3	9.2
3f	743.2	255.2	4.8	27.6
4a	1033	3.9	4.2	9.3
4b	4880	> 10000	3468	390.7
5a	75.2	3071	3892	587.2
5b	68.6	715.2	1129	87.5
7a	2724	2.9	3.5	1.8
7b	181.3	3.4	32.9	8.2
7c	724.4	3.4	320.5	19.8
7d	6979	48.9	46.9	35.8
8a	64.2	4.9	4.7	3.8
8b	1031	46.5	43.6	9.4
8c	66.3	4.7	200.6	8.0
8d	108.8	420.2	470.7	85.7
8e	944.1	4.9	309.4	8.6
9a	425.6	5870	> 10000	> 10000
9b	547.6	64.9	3494	557.0
9c	719.1	> 10000	> 10000	835.6
9d	68.5	1833	3818	59.5
AAZ	250	12.1	25.8	5.7

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

derivatives **8a–c**.

iv) Like the other transmembrane isoform, hCA XII, was weakly inhibited by derivatives **5a–b** and **9a–d** proving the organoseleno linker to be more effective for potent inhibitors of the cytosolic hCA I and II, but not for the transmembrane isoforms. Contrarily, the inhibition constant value for the highly lipophilic derivative **4b** (K_I 390.7 nM), was the best among the investigated isoforms, making this derivative isoform hCA XII selective. Finally, the replacement of sulfur with selenium enhanced the inhibition potency of compounds **7a** and **8a** compared to **3a** and **4a**.

4. Conclusions

In conclusion, we have synthetized a novel series of thio- and seleno-acetamides bearing benzenesulfonamide as inhibitors on four human carbonic anhydrases such as hCA I, II, IX, XII. These isoforms are drug targets for different pathologies such as glaucoma (hCA I, II) or hypoxic tumors (hCA IX, XII). Excellent inhibitory activity was observed against hCA II and hCA XII with different inhibitors, active in the low nanomolar range (e.g., **3a**, **4a**, **7a** and **8a**). Furthermore, based on the tail approach, we discovered and explained the interesting and selective inhibition profile of compound such as **5a** and **9a**, which were more selective for hCA I, **9b** more selective for hCA II, **3f** more selective for hCA IX and finally, **3e** and **4b** more selective for hCA XII. All these findings make the reported derivatives interesting leads for the development of more effective and more isoform-selective inhibitors.

5. Experimental part

5.1. General

All reactions were carried out in an oven-dried glassware under inert atmosphere (N_2). Ethanol was dried using a solvent purification system (Pure-Solv™). All commercial materials were used as received without further purification. Flash column chromatography purifications were performed with Silica gel 60 (230–400 mesh). Thin layer chromatography was performed with TLC plates Silica gel 60 F_{254}. NMR spectra were recorded in DMSO-d_6 with Mercury 400, and Bruker 400 Ultrashield spectrometers operating at 400 MHz (for ^{1}H), 100 MHz (for ^{13}C) and 76 MHz (for ^{77}Se). NMR signals were referenced to non-deuterated residual solvent signals (2.50 ppm for ^{1}H, 40.5 ppm for ^{13}C). $(PhSe)_2$ was used as an external reference for ^{77}Se NMR (δ = 461 ppm). ^{1}H 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), and assignment. Mercaptoalcohol **1e** [34], dialkyl diselenides [35], and diaryl diselenides [40] were prepared according literature reported procedures.

5.2. General procedure for the synthesis of 2-arylthio- and 2-alkylhio-acetamides 3,4 and 5

KOH (0.44 mmol, 2.2 eq.) was added to a solution of thiol **1a–f** (0.20 mmol, 1.0 eq.) in dry DMF (2 mL) at 0 °C under inert atmosphere (N_2). After 30 min, the suitable substituted 2-chloroacetamide **2a–c** (0.16 mmol, 0.8 eq.) was slowly added and the reaction mixture was allowed to warm to room temperature and stirred for 4–8 h, until complete consumption of the starting material (**2a–c**) was observed by TLC. The reaction was quenched by addition of saturated aq. NH_4Cl (2 mL) and diluted with EtOAc (8 mL), The layers were separated and the organic layer was washed with brine (5 mL) and with H_2O (2 × 5 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under vacuum. The crude material was precipitted from EtOAc/petroleum ether to yield substituted 2-thioacetamides **3**,**4**,**5**.

5.2.1. 2-(Phenylthio)-N-(4-sulfamoylphenyl)acetamide (3a)

Following the general procedure, benzenethiol **1a** (23 mg, 0.21 mmol) and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (43 mg, 0.17 mmol) gave 2-((4-bromophenyl)thio)-*N*-(4-sulfamoylphenyl)acetamide **3a.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (49 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.90 (2H, s, CH_2S), 7.19–7.23 (1H, m), 7.25 (2H, bs, SO_2NH_2), 7.33 (2H, ap t, J = 7.7 Hz), 7.41 (2H, ap d, J = 7.7 Hz), 7.72 (2H, ap d, J = 8.8 Hz), 7.77 (2H, ap d, J = 8.8 Hz), 10.52 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 37.6, 118.7, 126.1, 126.7, 128.3, 129.0, 135.6, 138.6, 141.7, 167.4. HRMS (ESI) calc. $C_{14}H_{14}N_2NaO_3S_2$ $[M+Na]^+$ 345.0344, found 345.0352.

5.2.2. 2-((4-Bromophenyl)thio)-N-(4-sulfamoylphenyl)acetamide (3b)

Following the general procedure, 4-bromobenzenethiol **1b** (40 mg, 0.21 mmol) and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (43 mg, 0.17 mmol) gave 2-((4-bromophenyl)thio)-*N*-(4-sulfamoylphenyl)acetamide **3b.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (55 mg, 80%). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.91 (2H, s, CH_2S), 7.27 (2H, bs, SO_2NH_2), 7.36 (2H, ap d, J = 8.6 Hz), 7.51 (2H, ap d, J = 8.6 Hz), 7.71 (2H, ap d, J = 8.9 Hz), 7.78 (2H, ap d, J = 8.9 Hz), 10.55 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 37.5, 118.7 119.1, 126.7, 130.2, 131.7, 135.2, 138.6, 141.6, 167.1. HRMS (ESI) calc. $C_{14}H_{13}BrN_2NaO_3S_2$ $[M+Na]^+$ 422.9449, found 422.9435.

5.2.3. 2-((2-Bromophenyl)thio)-N-(4-sulfamoylbenzyl)acetamide (3c)

Following the general procedure, 2-bromobenzenethiol **1c** (40 mg, 0.21 mmol) and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (43 mg, 0.17 mmol) gave 2-((2-bromophenyl)thio)-*N*-(4-sulfamoylbenzyl)acetamide **3c.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (58 mg, 82%). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.00 (2H, s, CH_2S), 7.08–7.17 (1H, m), 7.27 (2H, bs, SO_2NH_2), 7.35–7.49 (2H, m), 7.63 (1H, dd, J = 1.0, 7.9 Hz), 7.72 (2H, ap d, J = 9.1 Hz), 7.78 (2H, ap d, J = 9.1 Hz), 10.66 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 36.8, 118.7, 121.3, 126.7, 126.9, 127.5, 128.2, 132.6, 137.0, 138.7, 141.5, 166.7. HRMS (ESI) calc. $C_{14}H_{13}BrN_2NaO_3S_2$ $[M+Na]^+$ 422.9449, found 422.9438.

5.2.4. 2-(Dodecylthio)-N-(4-sulfamoylphenyl)acetamide (3d)

Following the general procedure, dodecane-1-thiol **1d** (40 mg, 0.20 mmol) and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (41 mg, 0.16 mmol) gave 2-(dodecylthio)-*N*-(4-sulfamoylphenyl)acetamide **3d.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (45 mg, 64%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.86 (3H, t, J = 6.5 Hz), 1.24 (18H, bs), 1.51–1.56 (2H, m), 2.62 (2H, t, J = 7.2 Hz, CH_2S), 3.31 (2H, s, SCH_2CO), 7.25 (2H, bs, SO_2NH_2), 7.73 (2H, ap d, J = 8.9 Hz), 7.76 (2H, ap d, J = 8.9 Hz), 10.41 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 13.9, 22.0, 28.1, 28.5, 28.7, 28.86, 28.92, 28.94, 31.2, 31.7, 35.6, 118.6, 126.7, 138.4, 141.9, 168.7. MS (ESI) 437.6 [M + Na].

5.2.5. 2-((2-Hydroxypropyl)thio)-N-(4-sulfamoylphenyl)acetamide (3e)

Following the general procedure, 1-mercaptopropan-2-ol **1e** (20 mg, 0.22 mmol) and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (45 mg, 0.18 mmol) gave 2-((2-hydroxypropyl)thio)-*N*-(4-sulfamoylphenyl) acetamide **3e.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (31 mg, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.12 (3H, d, J = 6.2 Hz), 2.61 (1H, dd, J = 5.9, 13.1 Hz, C**H$_a$**H$_b$S), 2.68 (1H, dd, J = 6.3, 13.1 Hz, CH$_a$**H$_b$**S), 3.36 (2H, s, CH_2S), 3.75–3.81 (1H, m, C**H**OH), 4.82 (1H, d, J = 4.2 Hz, OH), 7.25 (2H, bs, SO_2NH_2), 7.73 (2H, ap d, J = 9 Hz), 7.76 (2H, ap d, J = 9 Hz), 10.41 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 22.6, 36.3, 40.7, 65.7, 118.6, 126.7, 138.4, 141.9, 168.8. HRMS (ESI) calc. $C_{11}H_{17}N_2O_4S_2$ $[M+H]^+$ 305.0630, found 305.0642.

5.2.6. Ethyl S-(2-oxo-2-((4-sulfamoylphenyl)amino)ethyl)-L-cysteinate (3f)

Following the general procedure, ethyl L-cysteinate **1f** (30 mg, 0.16 mmol) and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (33 mg, 0.13 mmol) gave ethyl *S*-(2-oxo-2-((4-sulfamoylphenyl)amino)ethyl)-L-cysteinate **3f.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (29 mg, 53%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.17 (3H, t, J = 7.1 Hz), 2.82 (1H, dd, J = 6.8, 13.4 Hz, C**H$_a$**H$_b$S), 2.90 (1H, dd, J = 5.9, 13.4 Hz, CH$_a$**H$_b$**S), 3.38 (2H, s, CH_2S), 3.57–3.62 (1H, m, C**H**NH_2), 4.08 (2H, q, J = 7.1 Hz), 7.25 (2H, bs, SO_2NH_2), 7.73 (2H, ap d, J = 9.2 Hz), 7.76 (2H, ap d, J = 9.2 Hz), 10.61 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 14.1, 36.2, 36.9, 54.4, 60.3, 118.7, 126.7, 138.5, 141.9, 168.7, 173.8. HRMS (ESI) calc. $C_{13}H_{19}N_3NaO_5S_2$ $[M+Na]^+$ 384.0664, found 884.0675.

5.2.7. 2-(Phenylthio)-N-(4-sulfamoylphenethyl)acetamide (4a)

Following the general procedure, benzenethiol **1a** (36 mg, 0.32 mmol) and 2-chloro-*N*-(4-sulfamoylphenethyl)acetamide **2b** (76 mg, 0.27 mmol) gave 2-(phenylthio)-*N*-(4-sulfamoylphenethyl) acetamide **4a.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (60 mg, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.76 (2H, t, J = 7.0 Hz), 3.30–3.35 (2H, m, CH_2N), 3.63 (2H, s, CH_2S), 7.17–7.23 (1H, m), 7.29–7.26 (6H, m), 7.33 (2H, bs, SO_2NH_2), 7.72 (2H, ap d, J = 8.2 Hz), 8.21 (1H, t, J = 5.5 Hz, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 34.6, 36.3, 40.1, 125.7, 125.8, 127.8, 129.0, 129.1, 136.1, 142.0, 143.5, 167.8. MS (ESI) 351.2 $[M+H]^+$.

*5.2.8. 2-(Dodecylthio)-N-(4-sulfamoylphenethyl)acetamide **(4b)***

Following the general procedure, dodecane-1-thiol **1d** (40 mg, 0.20 mmol) and 2-chloro-*N*-(4-sulfamoylphenethyl)acetamide **2b** (46 mg, 0.17 mmol) gave 2-(dodecylthio)-*N*-(4-sulfamoylphenethyl) acetamide **4b.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (41 mg, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.85 (3H, t, J = 6.2 Hz), 1.19–1.23 (18H, m), 1.46–1.53 (2H, m), 2.46–2.50 (2H, m, overlapped with DMSO residual signal), 2.79 (2H, t, J = 7.0 Hz), 3.05 (2H, s), 3.29–3.34 (2H, m, partially overlapped with H_2O), 7.29 (2H, bs, SO_2NH_2), 7.40 (2H, ap d, J = 8.0 Hz), 7.74 (2H, ap d, J = 8.0 Hz), 8.06 (1H, bt, J = 5.5 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 13.9, 22.1, 28.1, 28.5, 28.6, 28.7, 28.9, 29.0, 29.01, 31.3, 31.6, 34.5, 34.7, 40.0, 125.6, 129.1, 142.1, 143.5, 169.0. MS (ESI) 464.8 $[M+Na]^+$.

*5.2.9. 2-(Phenylthio)-N-(4-((4-sulfamoylbenzyl)selanyl)phenyl)acetamide **(5a)***

Following the general procedure, benzenethiol **1a** (19 mg, 0.17 mmol) and 2-chloro-*N*-(4-((4-sulfamoylbenzyl)selanyl)phenyl) acetamide **2c** (55 mg, 0.14 mmol) gave 2-(phenylthio)-*N*-(4-((4-sulfamoylbenzyl)selanyl)phenyl)acetamide **5a.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (42 mg, 61%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.83 (2H, s), 4.20 (2H, s), 7.16–7.22 (1H, m), 7.26 (2H, bs, SO_2NH_2), 7.29–7.42 (8H, m), 7.46–7.49 (2H, m), 7.66 (2H, ap d, J = 8.3 Hz), 10.24 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 30.8, 37.9, 120.3, 123.5, 126.1, 126.5, 128.5, 129.5, 134.0, 136.2, 138.7, 142.7, 143.9, 167.4. HRMS (ESI) calc. $C_{21}H_{20}N_2NaO_3S_2Se$ $[M+Na]^+$ 514.9978, found 514.9987.

*5.2.10. 2-((4-Bromophenyl)thio)-N-(4-((4-sulfamoylbenzyl)selanyl) phenyl)acetamide **(5b)***

Following the general procedure, 4-bromobenzenethiol **1b** (20 mg, 0.11 mmol) and 2-chloro-*N*-(4-((4-sulfamoylbenzyl)selanyl)phenyl) acetamide **2c** (38 mg, 0.09 mmol) gave 2-((4-bromophenyl)thio)-*N*-(4-((4-sulfamoylbenzyl)selanyl)phenyl)acetamide **5b.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (35 mg, 67%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.85 (2H, s), 4.20 (2H, s), 7.26 (2H, bs, SO_2NH_2), 7.32–7.39 (6H, m), 7.46 (2H, ap d, J = 8.6 Hz), 7.50 (2H, ap d, J = 8.4 Hz), 7.66 (2H, ap d, J = 8.2 Hz), 10.25 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 30.8, 37.8, 119.4, 120.3, 123.6, 126.1, 129.5, 130.5, 132.2, 134.0, 136.0, 138.6, 142.8, 143.9, 167.1. HRMS (ESI) calc. $C_{21}H_{19}BrN_2NaO_3S_2Se$ $[M+Na]^+$ 592.9083, found 529.9065.

5.3. General procedure for the synthesis of 2-arylseleno- and 2-alkylseleno-acetamides 7, 8 and 9

$NaBH_4$ (0.60 mmol, 3.0 eq.) was portion-wise added to a solution of diselenide **6a–g** (0.20 mmol, 1.0 eq.) in EtOH (2 mL) at 0 °C under inert atmosphere (N_2). After 30 min, the suitable substituted 2-chloroacetamide **2a–c** (0.36 mmol, 1.8 eq.) was slowly added and the reaction mixture allowed to warm to room temperature and stirred for 4–8 h, until complete consumption of the starting material (**2a–c**) 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 × 5 mL), dried over Na2SO4, filtered and concentrated under vacuum. The crude material was purified by precipitation from EtOAc/petroleum ether to yield substituted 2-selenoacetamides **7**, **8**, **9**.

*5.3.1. 2-(Phenylselanyl)-N-(4-sulfamoylphenyl)acetamide **(7a)***

Following the general procedure, 1,2-diphenyldiselane **6a** (22 mg, 0.07 mmol) and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (30 mg, 0.12 mmol) gave 2-(phenylselanyl)-*N*-(4-sulfamoylphenyl)acetamide **7a.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (35 mg, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.79 (2H, s, CH_2Se), 7.26 (2H, bs, SO_2NH_2), 7.28–7.34 (3H, m) 7.56–7.59 (2H, m), 7.70 (2H, ap d, J = 8.9 Hz), 7.76 (2H, ap d, J = 8.9 Hz), 10.50 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 30.1, 118.6, 126.7, 127.1, 129.2, 129.8, 131.7, 138.5, 141.8, 168.6. ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 317.7. HRMS (ESI) calc. $C_{14}H_{14}N_2NaO_3SSe$ $[M+Na]^+$ 392.9788, found 392.9777.

*5.3.2. N-(4-Sulfamoylphenyl)-2-(p-tolylselanyl)acetamide **(7b)***

Following the general procedure, 1,2-di-*p*-tolyldiselane **6b** (33 mg, 0.10 mmol) and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (41 mg, 0.16 mmol) gave *N*-(4-sulfamoylphenyl)-2-(*p*-tolylselanyl)acetamide **7b.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (39 mg, 64%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.28 (3H, s), 3.72 (2H, s, CH_2Se), 7.13 (2H, ap d, J = 7.9 Hz), 7.24 (2H, bs, SO_2NH_2), 7.47 (2H, ap d, J = 7.9 Hz), 7.69 (2H, ap d, J = 8.8 Hz), 7.76 (2H, ap d, J = 8.8 Hz), 10.42 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 20.5, 30.4, 118.5, 125.8, 126.7, 129.8, 132.4, 136.8, 138.4, 141.8, 168.6. ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 312.7. MS (ESI) 384.7 $[M+H]^+$.

*5.3.3. N-(4-Sulfamoylphenyl)-2-(o-tolylselanyl)acetamide **(7c)***

Following the general procedure, 1,2-di-*o*-tolyldiselane **6c** (30 mg, 0.9 mmol) and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (37 mg, 0.15 mmol) gave *N*-(4-sulfamoylphenyl)-2-(*o*-tolylselanyl)acetamide **7c.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (42 mg, 73%). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 2.38 (3H, s), 3.81 (2H, s, CH_2Se), 7.17–7.27 (3H,m), 7.30 (2H, bs, SO_2NH_2), 7.61–7.67 (1H, m), 7.73 (2H, bs, J = 8.9 Hz), 7.81 (2H, bs, J = 8.9 Hz), 10.55 (1H, bs, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 20.5, 30.8, 109.5, 117.6, 118.1, 120.8, 121.6, 122.7, 129.3, 129.4, 132.7, 159.3. HRMS (ESI) calc. $C_{15}H_{16}N_2NaO_3SSe$ $[M+Na]^+$ 406.9945, found 406.9961.

*5.3.4. 2-((4-(Dimethylamino)phenyl)selanyl)-N-(4-sulfamoylphenyl) acetamide **(7d)***

Following the general procedure, 4,4′-diselanediylbis(*N*,*N*-dimethylaniline) **6d** (34 mg, 0.08 mmol) and 2-chloro-*N*-(4-sulfamoylphenyl)acetamide **2a** (35 mg, 0.14 mmol) gave 2-((4-(dimethylamino) phenyl)selanyl)-*N*-(4-sulfamoylphenyl)acetamide **7d.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (49 mg, 85%). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 2.93 (6H, s), 3.59 (2H, s, CH_2Se), 6.68 (2H, ap d, J = 8.9 Hz), 7.29 (2H, bs, SO_2NH_2), 7.44 (2H, ap d, J = 8.9 Hz), 7.72 (2H, ap d, J = 8.8 Hz), 7.80 (2H, ap d, J = 8.8 Hz), 10.36 (1H, bs, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 32.2, 40.4, 113.4, 113.5, 119.1, 127.2, 136.1, 138.9, 142.5, 150.8, 169.4. HRMS (ESI) calc. $C_{16}H_{19}N_3NaO_3SSe$ $[M+Na]^+$ 436.0210, found 436.0196.

*5.3.5. 2-(Phenylselanyl)-N-(4-sulfamoylphenethyl)acetamide **(8a)***

Following the general procedure, 1,2-diphenyldiselane **6a** (16 mg, 0.05 mmol) and 2-chloro-*N*-(4-sulfamoylphenethyl)acetamide **2b** (21 mg, 0.08 mmol) gave 2-(phenylselanyl)-*N*-(4-sulfamoylphenethyl) acetamide **8a.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (27 mg, 86%). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 2.79 (2H, t, J = 7.0 Hz), 3.29–3.35 (2H, m), 3.59 (2H, s, CH_2Se), 7.24–7.37 (5H, m), 7.41 (2H, ap d, J = 8.5 Hz), 7.52–7.60 (2H, m), 7.77 (2H, ap d, J = 8.5), 8.23 (1H, t, J = 6.7 Hz). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 29.1, 34.6, 40.0, 125.6, 126.7, 129.0, 129.1, 131.3, 142.0, 143.5, 168.9. ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 305.3. MS (ESI) 421.4 $[M+Na]^+$.

*5.3.6. 2-((4-(Dimethylamino)phenyl)selanyl)-N-(4-sulfamoylphenethyl) acetamide **(8b)***

Following the general procedure, 4,4′-diselanediylbis(*N*,*N*-dimethylaniline) **6d** (34 mg, 0.08 mmol) and 2-chloro-*N*-(4-sulfamoylphenethyl)acetamide **2b** (39 mg, 0.14 mmol) gave 2-((4-

(dimethylamino)phenyl)selanyl)-*N*-(4-sulfamoylphenethyl)acetamide **8b.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (42 mg, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.74 (2H, t, J = 6.9 Hz), 2.89 (6H, s), 3.26–3.33 (2H, m), 6.64 (2H, ap, d ,J = 8.5 Hz), 7.29 (2H, bs, SO_2NH_2), 7.34–7.38 (4H, m), 7.74 (2H, ap d, J = 8.0 Hz), 8.01 (1H, bt, J = 4.4 Hz, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 30.9, 34.7, 39.9, 40.0, 112.9, 113.7, 125.6, 129.1, 135.2, 142.0, 143.6, 150.1, 169.2. ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 291.3. HRMS (ESI) calc. $C_{18}H_{23}N_3NaO_3SSe$ $[M+Na]^+$ 464.0523, found 464.0536.

5.3.7. 2-((4-Methoxyphenyl)selanyl)-N-(4-sulfamoylphenethyl)acetamide (8c)

Following the general procedure, 1,2-bis(4-methoxyphenyl)diselane **6e** (18 mg, 0.05 mmol) and 2-chloro-*N*-(4-sulfamoylphenethyl)acetamide **2b** (22 mg, 0.08 mmol) gave 2-((4-methoxyphenyl)selanyl)-*N*-(4-sulfamoylphenethyl)acetamide **8c.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (25 mg, 73%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.72 (2H, t, J = 6.9 Hz), 3.24–3.29 (2H, m), 3.40 (2H, s, CH2Se), 3.72 (3H, s), 6.86 (2H, ap d, J = 8.5 Hz) 7.28 (2H, bs, SO_2NH_2), 7.35 (2H, ap d, J = 8.0 Hz), 7.44 (2H, ap d, J = 8.5 Hz), 7.71 (2H, ap d, J = 8.5 Hz), 8.09 (1H, bt, J = 4.7 Hz, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 30.3, 34.7, 40.0, 55.2, 114.9, 125.7, 129.1, 134.4, 134.7, 142.1, 143.6, 159.0, 169.1. MS (ESI) 428.8 $[M+H]^+$.

5.3.8. (S)-2-((3-Methyl-2-((4-methylphenyl)sulfonamido)butyl)selanyl)-N-(4-sulfamoylphenethyl)acetamide (8d)

Following the general procedure, *N*,*N*′-((2*S*,2′*S*)-diselanediylbis(3-methylbutane-2,1-diyl))bis(4-methylbenzene-sulfonamide) **6f** (38 mg, 0.06 mmol) and 2-chloro-*N*-(4-sulfamoylphenethyl)acetamide **2b** (28 mg, 0.1 mmol) gave (*S*)-2-((3-methyl-2-((4-methylphenyl)sulfonamido)butyl)selanyl)-*N*-(4-sulfamoylphenethyl)acetamide **8d.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (46 mg, 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.68 (3H, d, J = 8.0 Hz), 0.70 (3H, d, J = 8.2 Hz), 1.73–1.83(1H, m), 2.35 (3H, s), 2.49–2.53 (1H, m), 2.68–2.74 (1H, m), 2.75 (2H, t, J = 6.9 Hz), 2.90 (2H, ap s), 3.14–3.20 (1H, m), 2.24–2.29 (2H, m), 7.28 (2H, bs, SO_2NH_2), 7.34 (2H, ap d, J = 8.1 Hz), 7.38 (2H, ap d, J = 8.1 Hz), 7.56 (1H, bd, J = 8.1 Hz), 7.68 (2H, ap d, J = 8.1 Hz), 7.72 (2H, ap d, J = 8.1), 8.01 (1H, bt, J = 5.3 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 17.6, 19.1, 21.4, 25.4, 27.7, 31.0, 35.2, 40.5, 59.1, 126.1, 126.9, 129.6, 129.8, 139.6, 142.5, 142.7, 144.0, 170.3. MS (ESI) 584.6 $[M+Na]^+$.

5.3.9. 2-(((5S)-2-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexyl)selanyl)-N-(4-sulfamoylphenethyl)acetamide (8e)

Following the general procedure, (4*S*, 4′*S*)-2,2′-diselanediylbis(1-methyl-4-(prop-1-en-2-yl)cyclohexan-1-ol) **6 g** (39 mg, 0.08 mmol) and 2-chloro-*N*-(4-sulfamoylphenethyl)acetamide **2b** (40 mg, 0.14 mmol) gave **8e.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (56 mg, 84%). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 1.32 (3H, s), 1.45–1.63 (4H, m), 1.72 (3H, s), 2.13–2.29 (3H, m), 2.81 (2H, t, J = 6.9 Hz), 3.11–3.19 (1H, m), 3.28–3.32 (2H, m), 3.71–3.86 (2H, m), 4.55 (1H, bs), 4.69–4.80 (2H, m). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 21.5, 26.4, 30.4, 33.5, 33.7, 35.4, 35.6, 39.4, 50.6, 56.4, 70.4, 109.5, 126.2, 129.6, 142.6, 144.1, 149.6, 170.5. MS (ESI) 474.8 $[M+H]^+$.

5.3.10. 2-(Phenylselanyl)-N-(4-((4-sulfamoylbenzyl)selanyl)phenyl)acetamide (9a)

Following the general procedure, 1,2-diphenyldiselane **6a** (19 mg, 0.06 mmol) and 2-chloro-*N*-(4-((4-sulfamoylbenzyl)selanyl)phenyl) acetamide **2c** (42 mg, 0.10 mmol) gave 2-(phenylselanyl)-*N*-(4-((4-sulfamoylbenzyl)selanyl)phenyl)acetamide **9a**. The crude material was solubilized in EtOAc and precipitate from petroleum ether (37 mg, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.73 (2H, s), 4.19 (2H, s) 7.30–7.32 (5H, m), 7.34–7.38 (4H, m), 7.45 (2H, ap d, J = 8.6 Hz), 7.55 (2H, ap d, J = 8.2 Hz), 7.66 (2H, ap d, J = 8.2 Hz), 10.21 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 30.1, 30.4, 119.7, 123.0, 125.7, 127.0, 129.1, 129.2, 130.9, 131.6, 133.6, 138.4, 142.3, 143.5, 168.1. ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 316.6, 374.8. HRMS (ESI) calc. $C_{21}H_{20}N_2NaO_3SSe_2$ $[M+Na]^+$ 562.9423, found 562.9414.

5.3.11. N-(4-((4-Sulfamoylbenzyl)selanyl)phenyl)-2-(p-tolylselanyl)acetamide (9b)

Following the general procedure, 1,2-di-*p*-tolyldiselane **6b** (14 mg, 0.04 mmol) and 2-chloro-*N*-(4-((4-sulfamoylbenzyl)selanyl)phenyl) acetamide **2c** (30 mg, 0.07 mmol) gave *N*-(4-((4-sulfamoylbenzyl)selanyl)phenyl)-2-(p-tolylselanyl)acetamide **9b.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (25 mg, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.27 (3H, s), 3.68 (2H, s), 4.21 (2H, s), 7.12 (2H, ap d, J = 7.9 Hz), 7.27 (2H, bs, SO_2NH_2), 7.36–7.40 (4H, m), 7.44–7.47 (4H, m), 7.67 (2H, ap d, J = 8.2 Hz), 10.17 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.1, 30.7, 30.8, 120.1, 123.3, 126.1, 126.4, 129.5, 130.3, 132.6, 134.0, 137.1, 138.8, 142.6, 144.0, 168.7. HRMS (ESI) calc. $C_{22}H_{22}N_2NaO_3SSe_2$ $[M+Na]^+$ 576.9579, found 576.9602.

5.3.12. 2-((4-(dimethylamino)phenyl)selanyl)-N-(4-((4-sulfamoylbenzyl)selanyl)phenyl)acetamide (9c)

Following the general procedure, 4,4′-diselanediylbis(*N*,*N*-dimethylaniline) **6d** (17 mg, 0.04 mmol) and 2-chloro-*N*-(4-((4-sulfamoylbenzyl)selanyl)phenyl)acetamide **2c** (28 mg, 0.07 mmol) gave **9c.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (26 mg, 67%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.87 (6H, s), 3.49 (2H, s), 4.20 (2H, s), 6.62 (2H, ap d, J = 8.3 Hz), 7.26 (2H, bs, SO_2NH_2), 7.35–7.39 (6H, m), 7.44 (2H, ap d, J = 8.3 Hz), 7.66 (2H, ap d, J = 8.0 Hz), 10.03 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 30.7, 32.0, 40.3, 113.3, 113.5, 120.2, 123.2, 126.0, 129.5, 133.9, 136.0, 138.7, 142.5, 144.0, 150.6, 169.0. ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 304.2, 373.3. MS (ESI) 606.4 $[M+Na]^+$.

5.3.13. 2-((4-Methoxyphenyl)selanyl)-N-(4-((4-sulfamoylbenzyl)selanyl)phenyl)acetamide (9d)

Following the general procedure, 1,2-bis(4-methoxyphenyl)diselane **6e** (13 mg, 0.04 mmol) and 2-chloro-*N*-(4-((4-sulfamoylbenzyl)selanyl) phenyl)acetamide **2c** (27 mg, 0.06 mmol) gave **9d.** The crude material was solubilized in EtOAc and precipitate from petroleum ether (30 mg, 87%). ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.66 (2H, s), 3.77 (3H, s, MeO), 4.26 (2H, s), 6.93 (2H, ap d, J = 8.8 Hz), 7.34 (2H, bs, SO_2NH_2), 7.40–7.58 (8H, m), 7.72 (2H, ap d, J = 8.1 Hz), 10.18 (1H, bs, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 30.4, 31.2, 55.2, 114.9, 119.8, 122.9, 125.6, 129.0, 133.6, 135.0, 138.4, 142.3, 143.4, 159.2, 168.2. MS (ESI) 592.6 $[M+Na]^+$.

5.4. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity [36]. 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 [37–39], and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier [37–39].

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