# First in class dual targeting compounds for the management of seizures in glucose transporter type 1 deficiency syndrome

| | |
|---|---|
| Journal: | *Journal of Medicinal Chemistry* |
| Manuscript ID | Draft |
| Manuscript Type: | Article |
| Date Submitted by the Author: | n/a |
| Complete List of Authors: | Angeli, Andrea; University of Florence, Neurofarba department<br>Ferraroni, Marta; Università di Firenze, Dipartimento di Chimica<br>Granchi, Carlotta; Universita degli Studi di Pisa, Farmacia<br>Minutolo, Filippo; Universita degli Studi di Pisa, Farmacia<br>Chen, Xiaozhuo; Ohio University, Biomedical Sciences<br>Shriwas, Pratik; Ohio University, Biomedical Sciences<br>Russo, Emilio; Magna Graecia Univ Catanzaro<br>Leo, Antonio; Magna Graecia Univ Catanzaro<br>Selleri, Silvia; University of Firenze, Pharmaceutical Sciences<br>Carta, Fabrizio; University of Florence, Neurofarba Dept.<br>Supuran, Claudiu; Università degli Studi di Firenze, Dipartimento Neurofarba |
| | |

# First in class dual targeting compounds for the management of seizures in glucose transporter type 1 deficiency syndrome

Andrea Angeli,[1*] Marta Ferraroni,[2] Carlotta Granchi,[3] Filippo Minutolo,[3] Xiaozhuo Chen,[4] Pratik Shriwas,[4] Emilio Russo, [5] Antonio Leo, [5] Silvia Selleri,[1] Fabrizio Carta[1*] and Claudiu T. Supuran [1]

[1] NEUROFARBA Department, Sezione di Scienze Farmaceutiche e Nutraceutiche, University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Florence, Italy.

[2] University of Florence, Department of Chemistry "Ugo Schiff", Via della Lastruccia 3-13, I-50019, Sesto Fiorentino, Italy.

[3] Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126 Pisa (Italy).

[4] Edison Biotechnology Institute, Department of Biological Sciences, Molecular and Cellular Biology Program, Ohio University, Athens, OH 45701 (USA).

[5] University of Catanzaro, School of Medicine, Science of Health Department, FAS@UMG Research Center, 88100 Catanzaro, Italy.

**Abstract.** The genetic disorder glucose transporter type 1 deficiency syndrome (GLUT1-DS) heavily affects the main intake of energy in tissues and determines the most relevant outcomes at the central nervous system (CNS) district, which is highly dependent of glucose. Herein we designed and developed a set of compounds able to either enhance the GLUT1 glucose intake and to inhibit the Carbonic Anhydrase (CA; EC 4.2.1.1) enzymes with the aim to reduce the occurrence

of uncontrolled seizures on *in vivo* induced maximal electroshock seizures (MES) model. Our findings are in sustainment of an unprecedently reported pharmacological approach for the management of GLUT1-DS associated diseases.

## Introduction.

Since glucose is the main source of energy and carbon for eukaryotic cells, a fine tunable transportation machinery based on glucose transporter (GLUT) proteins regulates the intake of such a compound according to its concentration gradient across membranes, also in response to specific metabolic needs of each tissutal district [1-9]. To date, humans do express 14 isoforms of GLUTs which differ each other for their tissutal and/or subcellular distribution as well as for substrate affinity, are all encoded by the SLC2A genes [10-12] and grouped into 3 classes by means of primary sequence alignments [13]. Each GLUT isoform is constituted by 12 transmembrane helices with amino acid sequence homologies comprised between 28 and 65% when compared GLUT1 [9, 12, 13]. Biologically relevant isoforms account for GLUT1 which is widely and abundantly expressed among all tissues and it is responsible for basal glucose uptake in many cell types [10-12]. GLUT1 is typically present in tissues that have a high demand of glucose, such as the central nervous system (CNS), red blood cells (RBCs) and placenta [11, 12]. GLUT2 is involved in glucose sensing and its uptake within liver, pancreas and small intestine cells. GLUT3 is essentially expressed in neurons and is implicated in translocation of glucose across the blood-brain barrier (BBB). Other GLUT isoforms have more limited expression patterns. For example,

GLUT4 is present in muscle and adipose tissues and it is regulated by insulin, while GLUT5 is primarily expressed in the small intestine cells and it is responsible for fructose intake [8-13].

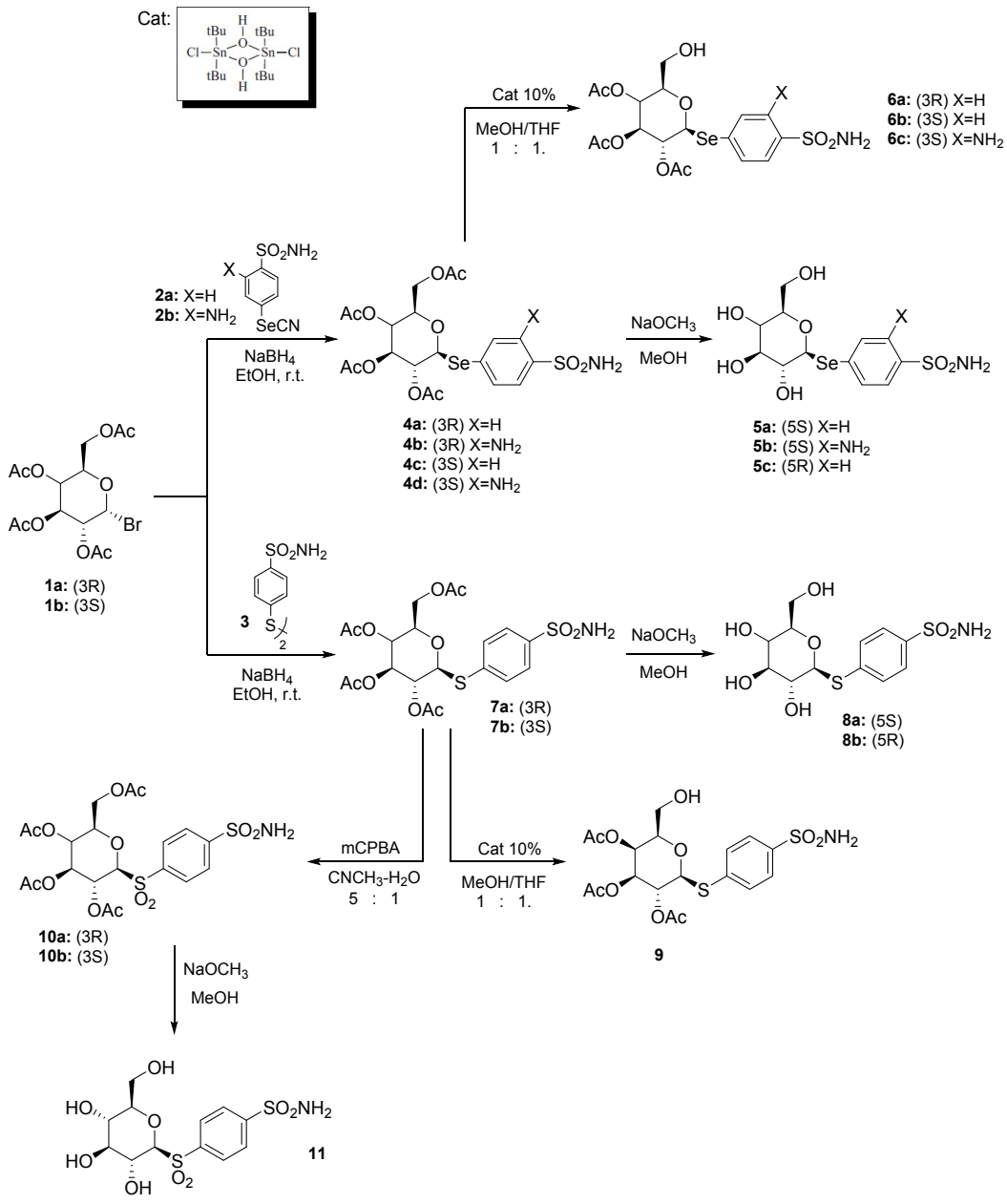
Among all isoforms, GLUT1 is the most investigated [14] since its implications at physiological level are particularly relevant [15-19]. For the purposes of this study, we focused on the glucose transporter type 1 deficiency syndrome (GLUT1-DS), which is properly classified as a mutational based genetic disorder at the SLC2A1 genes resulting in aberrant expression of the transporter, and thus affecting its ability to intake glucose [20]. Since the first report on GLUT1-DS in 1991 [21], the number of diagnosed clinical cases steadily grown up to the 2020 [20]. GLUT1-DS usually is diagnosed in childhood, but it is seldom identified in adulthood as well [20]. Overall, such a disease leads to shortage of energy of the tissues and the brain is the most affected one. Large arrays of symptoms are associated to GLUT1-DS such as fatigue, weakness, headaches, movement disorders (i.e. ataxia, dystonia, spasticity), cognitive impairments and drug resistant seizures [20]. The treatment typically involves a ketogenic diet, with the intent to provide alternative energy source other than glucose and the association of drugs for the management of associated symptoms. The use of antiseizure medications (ASMs) such as valproate or even better the inhibitors of the Carbonic Anhydrase (CA; EC 4.2.1.1) enzymes topiramate and acetazolamide are particularly effective in controlling seizures [22-24]. However, the response to ASMs may vary widely among individuals, thus requiring *ad-hoc* pharmacological treatments which require constant medical supervision.

In this context, we considered worth investigating compounds of low molecular weight able to interact either with GLUT transporters as well as the humans expressed CAs with the aim to study any effect *in vitro* on the targets which could possibly be considered for biomedical purposes.

## RESULTS AND DISCUSSION

### Design and Synthesis of Compounds.

We envisage to explore whether compounds bearing the prototypic CA inhibiting moiety (i.e. sulfonamide) and a GLUT1 substrate such as the D-glucose and D-galactose [25] may be beneficial to modulate uncontrolled seizures associated to GLUT1-DS affected patients. The carbohydrate bearing sulfonamide derivatives **4a-d, 5a-c, 6a-c, 7a, b, 8a, b, 9, 10a, b, 11, 15a, b, 16a, b, 18a-c** and **19a-c** reported in this study were all derived from the commercially available monosaccharides α-D-glucose **1a** and α-D-galactose **1b** according to the synthetic routes in **Schemes 1** and **2**.




**Scheme 1**. Synthesis of glycosyl compounds **4a-11.**

The freshly obtained intermediates **2a**, **b** and **3** [26] were reduced with $NaBH_4$ to the corresponding selenolate or thiolate respectively, which in turn were trapped *in situ* by means of the bromo monosaccharides **1a** and **b** to afford **4a-d** and **7a, b** in good yields (**Scheme 1**).

As expected, the synthetic process towards **4a-d** and **7a, b** proved highly enantioselective, as the β-isomers were obtained exclusively. The stereochemistry at the anomeric centers in **4a-d** and **7a, b** were readily assessed by means of the coupling constant of the affected protons. In **Figure 1** is reported the $^{1}$H-NMR spectra superposition of bromo-α-D-glucose **1a** with **4a** as representative example. Significant changes in coupling constant magnitudes were in agreement to reported data in the literature on similar compounds [27].

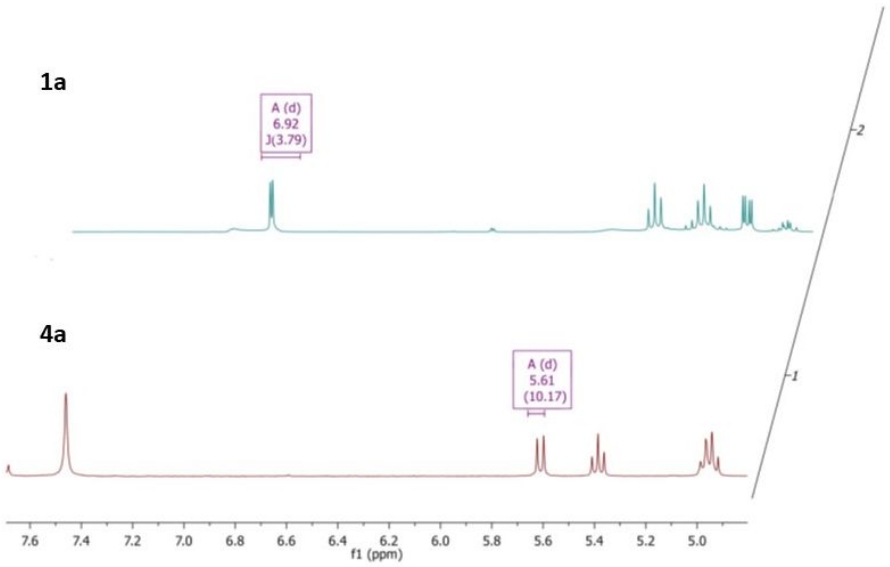



**Figure 1.** Coupling constant of $H_3$ ($J_{1,2}$) for compound **1a** and **4a**.

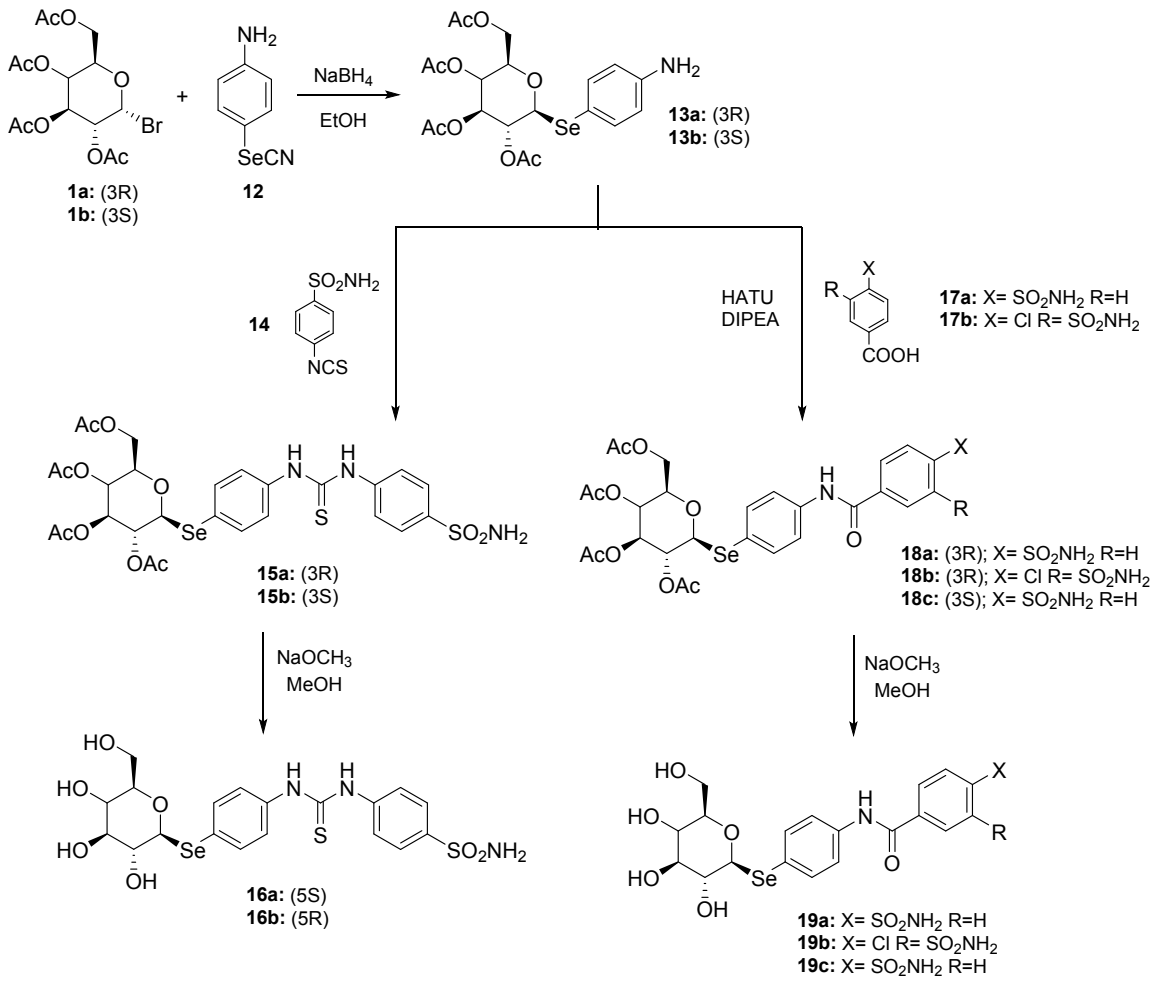
Regioselective deacetylation of **4a**, **4c** and **4d** to afford primary alcohols **6a-c** respectively, was carried out by using the neutral organotin dimer bis(di-*tert*-butylchlorohydroxytin) at 10 mol % in a 1/1 MeOH/THF solution [28]. Unfortunately, this procedure was ineffective on **4b** and any

attempt to force the reaction conditions (i.e. temperature and/or reaction times) resulted in complex mixtures difficult to purify.

Full deacetylation of **4a-c** and **7a, b** was accomplished by using standard reaction conditions such as sodium methoxide in methanol to afford **5a-c** and **8a, b** respectively in good yields (**Scheme 1**). In the case of **4d** we encountered the same synthetic difficulties previously discussed for **4b**, thus suggesting the incompatibility of the aniline moiety for such cleavage procedures at least in such specific substrates.

Oxidation of thioglycosides **7a** and **b** to the corresponding sulfones **10a** and **b** was successfully achieved by means of *meta*-chloroperbenzoic acid (*m*-CPBA) in acetonitrile/water 5/1. Quite interestingly, full deacetylation on **10a** and **10b** was partially satisfactory only on the former which afforded **11** in 50% yield. As for the galactosyl containing moiety we did observe its complete decomposition although exposed to mildest reaction conditions (i.e. temperature and/or dilution variations). Noteworthy was the outcome of the primary alcohol deprotection reaction when applied to **7a** and **7b** as it was effective (i.e. 54 % yield) only on the galactosyl substrate (**Scheme 1**).

We envisioned to obtain elongated derivatives by inserting a *para*-disubstituted aryl tether between the glycosyl tail and the primary sulfonamide warhead (**Scheme 2**). For such purpose, **13a** and **b** were readily obtained by making use of the chemistry reported for the functionalization of the commercially available bromo-α-D-glycosyls **1a** and **1b**. Thus, the aniline moiety in **13a** and **13b** was trapped by the isothiocyanate **14** [29] to afford the flexible [30, 31] thioureido derivatives **15a** and **15b** which in turn were subjected to full deprotection of the glycosyl tails (i.e. **16a** and **16b**). As alternative we explored the conformationally restricted amides **18a-c** and their fully deprotected derivatives **19a-c** (**Scheme 2**).




**Scheme 2**. Synthesis of glycosyl compounds **15a, b, 16a, b, 18a-c** and **19a-c**.

All the final compounds here reported were purified by silica gel column chromatography using appropriate eluting mixtures followed by trituration or recrystallization as needed. In addition, they were fully characterized by means of $^{1}$H-NMR, $^{13}$C-NMR, $^{19}$F-NMR, $^{77}$Se-NMR, and elemental analyses and account for a purity grade ≥ 95%.

## *In vitro* Carbonic Anhydrase inhibition.

*In vitro* inhibition profiles of compounds **4a-d, 5a-c, 6a-c, 7a, b, 8a, b, 9, 10a, b, 11, 15a, b, 16a, b, 18a-c** and **19a-c** and the reference drug acetazolamide (**AAZ**) and topiramate (TPR) on the physiologically and catalytically active hCA isoforms (i.e. I-VII, IX, XII and murine (m) XIII) were determined through the stopped-flow $CO_2$ hydrase assay [32] and are reported in **Table 1**.

**Table 1.** Inhibition data of **4a-d, 5a-c, 6a-c, 7a, b, 8a, b, 9, 10a, b, 11, 15a, b, 16a, b, 18a-c** and **19a-c** and the reference drug acetazolamide (**AAZ**) and topiramate (**TPR**) on hCA isoforms I-VII, IX, XII and XIII [#] by the stopped flow $CO_2$ hydrase assay [32].

| | $K_I$ (nM)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cmp** | **hCA I** | **hCA II** | **hCA IV** | **hCA VA** | **hCA VB** | **hCA VI** | **hCA VII** | **hCA IX** | **hCA XII** | **mCA XIII** |
| **4a** | 80.3 | 41.4 | 4118 | 595.9 | 706.8 | 58.4 | 14.6 | 219.0 | 944.3 | 273.5 |
| **4b** | >10000 | 6292 | >10000 | 2541 | >10000 | 4946 | >10000 | >10000 | >10000 | 6694 |
| **4c** | 95.8 | 4.1 | 344.3 | 87.2 | 6.6 | 8.3 | 0.5 | 205.5 | 803.6 | 70.4 |
| **4d** | 9596 | 6317 | >10000 | 6571 | 406.0 | >10000 | >10000 | >10000 | >10000 | >10000 |
| **5a** | 540.9 | 13.8 | 647.0 | 325.0 | 87.3 | 8.3 | 26.3 | 54.4 | 589.0 | 568.4 |
| **5b** | 7682 | 4014 | >10000 | 2301 | >10000 | 2477 | >10000 | >10000 | 2562 | 7040 |
| **5c** | 647.0 | 53.7 | 580.2 | 426.7 | 8.7 | 7.3 | 30.3 | 180.2 | 82.4 | 74.6 |
| **6a** | 78.4 | 3.7 | 67.1 | 735.8 | 5.5 | 350.0 | 4.1 | 29.9 | 5.9 | 9.6 |
| **6b** | 86.2 | 17.2 | 146.3 | 96.8 | 2.6 | 6.1 | 0.8 | 219.6 | 42.3 | 634.6 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **6c** | 6816 | 6664 | 7276 | 235.7 | 172.3 | >10000 | >10000 | >10000 | >10000 | >10000 |
| **7a** | 69.6 | 6.6 | 1949 | 91.1 | 503.1 | 93.2 | 19.2 | 96.5 | 895.7 | 25.2 |
| **7b** | 50.8 | 4.9 | 1684 | 70.4 | 84.8 | 629.6 | 0.2 | 103.1 | 89.5 | 8.7 |
| **8a** | 622.2 | 7.5 | 564.8 | 72.6 | 503.8 | 84.7 | 51.6 | 31.2 | 90.0 | 90.0 |
| **8b** | 353.2 | 47.9 | 467.1 | 39.7 | 18.9 | 775.0 | 4.8 | 28.6 | 31.0 | 869.7 |
| **9** | 84.4 | 27.6 | 385.4 | 478.4 | 5.8 | 501.7 | 0.9 | 94.8 | 491.3 | 9.2 |
| **10a** | 502.4 | 24.2 | 586.9 | 20.9 | 70.4 | 8.8 | 0.3 | 143.0 | 8.4 | 22.4 |
| **10b** | 522.1 | 60.5 | 748.3 | 6229 | 6.0 | 47.8 | 0.7 | 25.7 | 138.9 | 289.5 |
| **11** | 168.8 | 18.5 | 82.2 | 336.5 | 4.2 | 30.9 | 2.2 | 47.9 | 358.2 | 900.9 |
| **15a** | 55.1 | 6.3 | 555.1 | 254.5 | 8.9 | 58.5 | 1.8 | 1184 | 55.8 | 861.5 |
| **15b** | 48.1 | 6.6 | 361.8 | 481.8 | 4.7 | 68.7 | 2.0 | 10.7 | 34.2 | 55.7 |
| **16a** | 71.4 | 5.2 | 282.8 | 70.5 | 7.6 | 49.3 | 4.4 | 166.3 | 19.8 | 658.6 |
| **16b** | 41.5 | 4.5 | 77.7 | 358.2 | 6.2 | 9.6 | 0.9 | 198.2 | 9.1 | 19.5 |
| **18a** | 85.2 | 5.1 | 378.2 | 90.6 | 8.7 | 30.5 | 2.8 | 1594 | 9.4 | 382.4 |
| **18b** | >10000 | 213.8 | 1573 | 347.8 | 40.9 | 4626 | 87.3 | 1419 | >10000 | >10000 |
| **18c** | 51.4 | 4.0 | 30.8 | 57.4 | 4.0 | 593.8 | 0.7 | 233.1 | 5.6 | 737.5 |
| **19a** | 48.0 | 1.7 | 347.7 | 119.5 | 88.0 | 37.6 | 9.6 | 385.9 | 90.5 | 40.7 |
| **19b** | 637.2 | 14.8 | 77.7 | 215.5 | 21.9 | 41.5 | 29.7 | 44.5 | 88.7 | 8.4 |
| **19c** | 46.4 | 6.1 | 8.9 | 45.1 | 3.0 | 484.8 | 0.9 | 245.7 | 7.2 | 65.2 |
| **AAZ** | 250.0 | 12.1 | 74.0 | 63.0 | 54.0 | 11.0 | 2.5 | 25.8 | 5.7 | 17.0 |
| **TPR** | 250 | 10.0 | 4900 | 63.0 | 30.0 | 45.0 | 0.9 | 58.0 | 3.8 | 47.0 |

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

[#] Inhibition data of **4a-d, 5a-c, 6a-c, 7a, b, 8a, b, 9, 10a, b, 11, 15a, b, 16a, b, 18a-c** and **19a-c** and the reference drug acetazolamide (**AAZ**) on the hCA III isoform were reported in Supporting Information **Table S1**

Based on reported data in **Table 1**, structure-activity relationship (SAR) considerations can be drawn:

*i)* As for the hCA I isoform, the seleno containing glucose derivative **4a** resulted in a medium nanomolar inhibitor ($K_I$ of 80.3 nM). Full deprotection of the glucose moiety to afford **5a** determined 6.7-fold enhancement of the $K_I$ value (i.e. $K_I$s of 80.3 and 540.9 nM for **4a** and **5a** respectively). Interestingly the introduction of the amine moiety at 2-position of the benzenesulfonamide CAI warhead in **4a** to give **4b** suppressed any inhibition activity ($K_I$ > 10000 nM) which was restored when the acetyl moieties were all removed ($K_I$ of 7682 nM for **5b**). Regioselective deprotection of the primary alcohol in **4a** to afford **6a** didn't significantly affect the inhibition potency ($K_I$s of 80.3 and 78.4 nM for **4a** and **6a** respectively). Elongation of **4a** by means of the thioureido phenyl moiety as in **15a** determined a 1.5-fold enhancement of the potency which was slightly spoiled (i.e. 1.3-fold compared to **15a**) after removal of the acetyl protecting moieties ($K_I$s of 55.1 and 71.4 nM for **15a** and **16a** respectively). As expected, the conformationally restricted amide **18a** was less potent hCA I inhibitor when compared to **15a** ($K_I$s of 55.1 and 85.2 nM for **15a** and **18** respectively). Interestingly full acetyl deprotection of **18a** determined considerable gain of the inhibition potency ($K_I$ of **19a** 48.0 nM). Compound **18b** bearing a substituted metanilamide warhead was ineffective hCA I inhibitor and it became effective at medium nanomolar concentrations after removal of the protecting acetyl groups (i.e. **19b** $K_I$ of 637.2 nM). A slight (i.e. 1.2-fold) inhibition increase was observed when the seleno ether in **4a**

was replaced with the thiol moiety instead (i.e. compound **7a**). The opposite trend although with a similar magnitude (i.e. 1.5 eq) was observed for the glucose deprotected derivatives **5a**/**8a** ($K_I$s of 540.9 and 622.2 nM for **5a** and **8a** respectively). The oxidation of the sulfur in **7a** to afford **10a** determined 7.2-fold increase of the $K_I$ value (i.e. $K_I$ of 502.4 nM) which was reduced up to 3.0-fold after full deprotection of the glucosyl alcohols was carried out (i.e. $K_I$ of **11** 168.8 nM). The galactosyl derivatives **4c** and **4d** reported medium-high nanomolar $K_I$ values (i.e. 95.8 and 9596 nM for **4c** and **4d** respectively). Interestingly full deprotection of the sugar moiety in **4c** to afford **5c** determined increase of the $K_I$ value matching with the one observed for the glucosyl derivatives **4a**/**5a** (i.e. 6.7-fold). On the contrary the removal of the protection on the primary alcohol in **4c** and **4d** to afford derivatives **6b** and **6c** determined improvement of the inhibition potencies (i.e. $K_I$s of 86.2 and 6816 nM for **6b** and **6c** respectively). Again, the elongation of **4b** by means of the thioureido (**15b**) and amide (**18c**) induced high improvements of the inhibition potency against the hCA I isoform. Specifically, introduction of the thioureido linker restored the inhibition potency of the cognate precursor **4b** ($K_I$ of 48.1 nM for **15b**), which was further increased after full deprotection of the sugar moiety ($K_I$ of 41.5 nM for **16b**). A similar trend was observed for the amide containing series, being **19c** 1.1-fold more effective when compared to the fully protected galactose precursor **18c** ($K_I$s of 51.4 and 46.4 nM for **18c** and **19c** respectively). A 1.9-fold inhibition potency increase was observed when the selenium ether moiety in compound **4c** was replaced with the sulfur isostere instead ($K_I$s of 95.8 and 50.8 nM for **4c** and **7b** respectively). Identical kinetic trend was observed for the fully deprotected galactosyl derivative **8b** which was 1.8-fold more potent when compared to its isosteric analogue **5c** ($K_I$s of 647 and 353.2 nM for **5c** and **8b** respectively). Manipulation of 7**b** by means of sulfur oxidation to afford **10b** and

regioselective deprotection of the primary alcohol (**9**) was detrimental for the inhibition potency ($K_I$s of 522.1 and 84.4 nM for **10b** and **9** respectively).

*ii)* A general overlook at the kinetic values in **Table 1** for the hCA II isoform showed the compounds tested being more effective inhibitors when compared to the hCA I. Modifications at the glucosyl tail in **4a** ($K_I$ of 41.4 nM) to afford the fully- and the mono-deprotected **5a** and **6a** derivatives respectively induced significant gain of the inhibition potency ($K_I$s of 13.8 and 3.7 nM for **5a** and **6a** respectively). The introduction within the CAI warhead of the amine moiety as in **4b** and **5b** spoiled the inhibition potency against the hCA II isoform, which was 151.9- and 290.9-fold weaker when compared to their cognate precursors **4a** and **5a** respectively. Considerable increase of the hCA II inhibition potency of the glucosyl derivative **4a** was obtained by means of its elongation to afford the derivatives containing the thioureido (**15a**; $K_I$ of 6.3 nM) and the amide (**18a**; $K_I$ of 5.1 nM) moieties respectively. Deprotection of the glucosyl moieties in **15a** and **18a** to afford **16a** and **19a**, resulted in further reduction of the associated $K_I$ values up to 5.2 and 1.7 nM respectively (**Table 1**). Similarly, removal of the acetyl groups in **18b** to afford the derivative **19b** was beneficial for the inhibition potency too which was enhanced up to 14.4-fold ($K_I$s of 213.8 and 14.8 nM for **18b** and **19b** respectively). Strong isosteric effect was observed for the sulfur containing derivatives **7a** and **8a** when compared to their selenium cognates **4a** and **5a**. Data in **Table 1** accounted for the full protected glycosyl thioether **7a** being 6.3-fold more effective hCA II inhibitor when compared to its selenium cognate **4a** ($K_I$s of 41.4 and 6.6 nM for **4a** and **7a** respectively). In analogy, compound **8a** also was more potent than its bioisotere **5a** although with reduced magnitude (i.e. 1.8-fold; $K_I$s of 13.8 and 7.5 nM for **5a** and **8a** respectively). Manipulation of the sulfur tether in **7a** by means of *m*-CPBA promoted oxidation to afford **10a** was detrimental for the inhibition potency which was reduced up to 3.7-fold (i.e. $K_I$s of 6.6 and 24.2 nM for **7a** and

**10a** respectively). Conversely, slight restoration of the potency (1.3-fold) was obtained after removal of the acetyl groups on the glucosyl moiety in **10a** (i.e. $K_I$ of 18.5 nM for compound **11**). The galactosyl derivative **4c** was very effective inhibitor of the hCA II isoform with a $K_I$ value of 4.1 nM. Interestingly, full removal of the acetyl groups to afford **5c** determined enhancement of the $K_I$ value up to 13.1-fold. Greater increase of the inhibition value ($K_I$ of 6317 nM) was observed for the aniline containing derivative **4d** which retained the inhibition potency after removal of the primary alcohol protection (i.e. $K_I$ of **6c** 6664 nM). Conversely the same chemical transformation applied to **4c** to afford **6b** determined a 4.2-fold decrease of the inhibition potency (i.e. $K_I$s of 4.1 and 17.2 nM for **4c** and **6b** respectively). The elongation strategy applied to the galactosyl derivative **4c** to afford the thiouredo derivative **15b** and the amide containing **18c** didn't result in relevant changes of the hCA II inhibition performances (i.e. $K_I$s of 4.1, 6.6 and 4.0 nM for compounds **4c**, **15b** and **18c** respectively). Full deprotection of the galactosyl moiety in **15b** resulted in a slight increase of the inhibition potency (i.e. $K_I$ of 4.5 nM for **16b**) whereas the same transformation in **18c** to afford **19c** determined opposite effects although with the same magnitude ($K_I$s of 4.0 and 6.1 nM for **18c** and **19c** respectively). The galactosyl derivatives **4c** and **7c** showed close matching $K_I$ values of 4.1 and 4.9 nM respectively. In analogy the full deprotected derivatives **7b** and **5c** didn't show appreciable differences (i.e. $K_I$s of 53.7 and 47.9 nM for **5c** and **7b** respectively), thus demonstrating the Se/S isosteric replacement being trivial on the *in vitro* inhibition against the hCA II isozyme. On the contrary selective deprotection of the primary alcohol in **7b** to afford **9** determined a 5.6-fold decrease of the inhibition potency against the hCA II isoform (i.e. $K_I$ of 27.6 nM for compound **9**). Oxidation of the sulfur tether in **7b** to afford the derivative **10b** resulted in important increase (i.e. 9.2-fold) of the $K_I$ value up to 60.5 nM, which

was reduced of 3.3-fold after the galactosyl moiety was fully deprotected to afford the compound **11** (i.e. $K_I$ of 18.5 nM for compound **11**).

*iii)* All compounds considered in this study reported $K_I$ values for the hCA IV ranging in the medium-high nanomolar range. Deprotections of the glucosyl moiety in **4a** ($K_I$ of 4118 nM) to either afford **5a** ($K_I$ of 647.0 nM) and **6a** ($K_I$ of 67.1 nM) was beneficial for the inhibition potency against the hCA IV. On the contrary the aniline containing moiety **4b** and **5b** was ineffective ($K_I$s > 10000 nM). Elongation of **4a** by means of the arylthioureido spacer to afford **15a** enhanced the inhibition potency up to 7.4-fold ($K_I$s of 4118 and 555.1 nM for **4a** and **15a** respectively). Superior enhancement of the inhibition potency (i.e. 10.9-fold) was obtained with the introduction of the amide moiety as in compound **18a** ($K_I$ of 378.2 nM). Full deprotection of the glycosyl tails in **15a** and **18a** further increased the compound's potency against the hCA IV isoform. As reported in **Table 1**, **16a** showed a $K_I$ value of 282.8 nM and **19a** a $K_I$ of 347.7 nM, thus 2.0- and 1.1-fold respectively more potent when compared to their full protected cognate precursors. Of interest is the metanilamide containing derivative **18b** as the deprotection of its sugar moiety to afford **19b** greatly enhanced the inhibition potency on the hCA IV up to 20.2-fold and thus registered a $K_I$ value close matching with the reference **AAZ** (i.e. $K_I$s of 77.7 and 74.0 nM for **19b** and **AAZ** respectively). A strong isosteric effect was reported for the sulfur containing derivative **7a** when compared to its counterpart **4a** being their $K_I$ values separated by a 2.1-fold factor (i.e. $K_I$s of 1949 and 4118 nM for **7a** and **4a** respectively). Reduced differences (1.1-fold) were observed for the fully deprotected derivatives **8a**/**5a** being the sulfur containing **8a** affective in inhibiting the hCA IV isoform at 564.8 nM whereas its selenium counterpart **5a** was at 647.0 nM. Again, oxidation of the thioether in **7a** to afford the derivative **10a** determined effective reduction of the $K_I$ value (i.e. 3.3-fold), which was further reduced upon full removal of the acetyl moieties to afford the

derivative **11** (i.e. $K_I$ of 82.2 nM). Among the galactosyl containing series the fully protected derivative **4c** was a medium nanomolar hCA IV inhibitor (i.e. $K_I$ of 344.3 nM). Removal of the acetyl groups in **4c** to afford **5c** spoiled the inhibition potency of 1.7-fold (i.e. $K_I$ of 580.2 nM for **5c**), whereas modification of the CAI warhead as in **4d** suppressed any activity for the hCA IV (i.e. $K_I$ > 10000 nM). Regioselective deprotection of the glucosyl tail in **4c** and **4d** to afford derivatives **6b** and **6c** was beneficial for the inhibition potency. Specifically, **6b** was 2.4-fold more effective inhibitor of the hCA IV isoform whereas with compound **6c** restoration of the activity was achieved (i.e. $K_I$s of > 10000 and 7276 nM for **4d** and **6c** respectively). Elongation of the derivative **4c** by means of the phenylthioureido linker to afford **15b** determined slight increase of the $K_I$ value (i.e. $K_I$ of 361.8 nM for **15b**). Full deprotection of the galactosyl moiety determined significant improvement of the inhibition potency up to 4.7-fold, thus close matching with the glucosyl derivative **19b** and the reference drug **AAZ** (i.e. $K_I$ of 77.7 nM for **16b**). Very interesting results were obtained for the amide containing derivative **18c** which reported a $K_I$ value of 30.8 nM, thus 2.4-fold more effective that the reference drug **AAZ** for the hCA IV isoform. Furthermore, removal of the acetyl protecting groups in **18c** to afford the derivative **19c** resulted in enhancement of the inhibition potency (i.e. 3.5-fold). Noteworthy is the $K_I$ value of **19c** which was in the low nanomolar range (i.e. $K_I$ of 8.9 nM). Isosteric substitution of the selenium tether in **4c** with the sulfur instead (as in compound **7b**) resulted in a 4-9-fold reduction of the inhibition potency (i.e. $K_I$s of 344.3 and 1684 nM for **4c** and **7b** respectively). Removal of the galactosyl protective groups to afford **8b** was beneficial for the inhibition against the hCA IV (i.e. $K_I$ of 467.1 nM). Comparison between **5c** and **8b**, which differ for the S/Se tether, showed the latter being slightly more potent (i.e. 1.2-fold; $K_I$s of 580.2 and 467.1 nM for **5c** and **8b** respectively). Major effects on the in vitro enzymatic assay was obtained when the primary alcohol in **7b** was removed

to afford the derivative **9** (i.e. $K_I$s of 1684 and 385.4 nM for **7b** and **9** respectively). In addition, strong effect in the inhibition potency was observed after oxidation of the sulfur tether in **7b** to afford the derivative **10b** (i.e. $K_I$s of 1684 and 748.3 nM for **7b** and **10b** respectively). Further enhancement of the inhibition potency was observed after removal of the protecting groups in **10b** to afford the derivative **11** (i.e. $K_I$ of 82.2 nM).

*iv)* As for the constitutively expressed hCA VA the glucosyl derivative **4a** reported a medium nanomolar inhibition potency ($K_I$ of 595.9 nM) which was reinforced upon removal of the acetyl groups up to 2.0-fold (i.e. $K_I$ of 325.0 nM for **5a**). Regioselective deprotection of the primary alcohol in **4a** to afford **6a** spoiled the inhibition potency, being such derivative 1.2-fold less effective (i.e. $K_I$s of 595.9 and 735.8 nM for **4a** and **6a** respectively). Introduction within the CAI benzenesulfonamide head of the aniline group as in **4b** set the $K_I$ value at 2541 nM, which was reduced of 1.1-fold after full deprotection of the glucosyl tail as in **5b** (i.e. $K_I$ of 2301 nM). Elongation of the glucosyl compound **4a** by means of the aryl thioureido moiety as in **15a** enhanced the inhibition potency of 2.3-fold, which was further improved upon removal of the glucosyl protecting moieties to afford **16a** (i.e. $K_I$ of 70.5 nM). As for the amide containing derivatives **18a**, **b** a 6.6- and 2.0-fold respectively potency increase was observed (i.e. $K_I$s of 90.6 and 347.8 nM for **18a** and **18b**). Removal of the acetyl groups to afford compounds **19a** and **19b** determined a 1.3-fold increase for the former and 1.6-fold decrease of the inhibition potency for the latter (i.e. $K_I$s of 119.5 and 215.5 nM for **19a** and **19b** respectively). A strong isosteric effect was obtained for both the thioether containing moiety **7a** and **8a** when compared to their selenium counterpart **4a** and **5a** respectively. Data in **Table 1** accounted for $K_I$ values of 595.9 and 91.1 nM for **4a** and **7a** respectively. $K_I$s of 325.0 and 72.6 nM were obtained for the isosteric couple **5a**/**8a**. Oxidation of the thioether linker in **7a** to afford the derivative **10a** strongly reduced the $K_I$ value up to 20.9

nM, thus 3.0-fold lower when compared to the reference drug **AAZ** (i.e. $K_I$ of 63 nM for **AAZ**). Successive deprotection of the glucosyl moiety as in **11** heavily spoiled the inhibition potency against the hCA VA which gave a $K_I$ value of 336.5 nM. As for the galactosyl containing series the derivative **4c** was a medium nanomolar hCA VA inhibitor being its associated $K_I$ value of 87.2 nM. Full deprotection of the galactosyl moiety to afford the derivative **5c** determined a 4.9-fold increase of the $K_I$ value (i.e. $K_I$ of 426.7 nM for **5c**). The inhibition activity was partially restored when mono-deprotection of the primary alcohol as in **6b** was operated thus determining a $K_I$ value of 96.8 nM. Introduction of the aniline moiety was detrimental for the inhibition of the hCA VA as the derivative **4d** was a low micromolar inhibitor with a $K_I$ value of 6.6 μM. Mono-deprotection of the galactosyl tail allowed to partially gain inhibition potency as the $K_I$ of **6c** was 27.9-fold lower when compared to the fully protected galactosyl derivative **4d**. Introduction within **4c** of the phenylthioureido moiety as in **15b** determined a significant increase of the inhibition value which was of 481.8 nM. A slight increase of the inhibition potency (i.e. 1.3-fold) was observed when the glycosyl tail in **15b** was deprotected to afford the derivative **16b**. As for the phenylamide derivative **18c** a $K_I$ inhibition value of 57.4 nM was observed, which was 1.3-lower when the acetyl protecting groups were removed (i.e. $K_I$ of 45.1 nM for **19c**). The sulfur containing **7a** derivative reported a 6.5-fold enhancement of the inhibition potency when compared to its isosteric analogue **4a**. The same trend was reported for the fully deprotected isosteric pair **5a** and **8a** with the latter being 4.5-fold more effective hCA VA inhibitor (i.e. $K_I$s of 325.0 and 82.6 nM for **5a** and **8a** respectively). Among the galactosyl containing derivatives small differences of inhibition potency were observed between **7b/4c** (i.e. 1.2-fold), whereas for **8b/5c** the former compound was 10.7-fold more potent in inhibiting the hCA VA isozyme (i.e. $K_I$s of 39.7 and 426.7 nM for **8b** and **5c** respectively). Mono-deprotection of the primary alcohol in **7b** afforded the derivative **9** which reported a $K_I$

value of 478.4 nM thus 4.9-fold less potent when compared to its selenium containing congener **6b** (i.e. $K_I$ of 96.8 nM).

*v)* Striking differences among the compound series were obtained for the hCA VB enzyme. The glucosyl **4a** was a high nanomolar inhibitor (i.e. $K_I$ of 706.8 nM) and its activity was cancelled after introduction of the aniline moiety to afford **4b** (i.e. $K_I$ >10000 nM). Interestingly full deprotection of the protecting groups in **4a** to afford the derivative **5a** determined important enhancement of the inhibition potency up to 8.1-fold. On the contrary no effects were observed in the case of **4b/5b** (i.e. $K_I$s >10000 nM). Deprotection of the primary alcohol in **4a** determined very important enhancement of the inhibition potency up to 128.5-fold ($K_I$ of 5.5 nM for compound **6a**). Interestingly the elongated derivatives of **4a** (i.e. compounds **15a** and **18a**) also were highly potent inhibitors with $K_I$ values of 8.9 and 8.7 nM for the thioureido and amido containing moieties respectively. Full deprotection of the glucosyl moiety in **15a** and **18a** afforded the corresponding free alcohols **16a** and **19a** with the former being 1.2-fold more effective than its fully protected counterpart ($K_I$ of 7.6 nM for **16a**). Conversely the latter was 10.0-fold less potent when compared to its congener **18a** (i.e. $K_I$ of 88.0 nM for **19a**). As expected, the aniline containing moiety **18b** showed a medium nanomolar inhibition value ($K_I$ of 40.9 nM) and its deprotection to afford the derivative **19b** determined an increase of the inhibition potency up to 1.9-fold. Introduction of the thioether moiety as in compound **7a** set the $K_I$ value to 503.1 nM thus slightly lower (1.4-fold) when compared to its selenium isostere **4a**. Removal of the acetyl groups in **7a** to afford **8a** did not affect the $K_I$ value which matched each other ($K_I$s of 503.1 and 503.8 nM for **7a** and **8a** respectively). A clear isosteric effect was observed between **8a** and its selenium congener **5a** with the latter being 5.8-fold more potent hCA VB inhibitor. In agreement with the previous discussed isoforms the oxidation of the sulfur tether in **7a** to afford **10a** determined an increase of the

inhibition potency up to 70.4 nM. Deprotection of the glycosyl tail in **10a** further enhanced the compound potency which showed $K_I$ value of 4.2 nM (i.e. compound **11**). As for the galactosyl series the derivative **4c** was a very potent inhibitor with a $K_I$ value of 6.6 nM. Such an activity was almost retained after deprotection of the sugar tail (i.e. $K_I$ of 8.7 nM for **5c**). The introduction of the aniline group in **4c** to afford the derivative **4d** spoiled the inhibition potency (i.e. $K_I$ of 406.0 nM for 4d). Interestingly deprotection of the primary alcohol in **4c** to afford the derivative **6b** resulted in a further increase of the inhibition potency with a $K_I$ value of 2.6 nM. In analogy the galactosyl **6c** was 2.4-fold more effective when compared to its fully protected congener **4d**. Elongated molecules as **15b** and **18c** were very effective inhibitors with $K_I$ values in the low nanomolar range with $K_I$ values of 4.7 and 4.0 nM respectively. Removal of the galactosyl protecting groups in both **15b** and **18c** to afford **16b** and **19c** respectively, determined opposite effects on kinetics. Specifically, **16b** showed a $K_I$ value of 6.2 nM, thus 1.3-fold less effective when compared to its precursor **15b**. As for **19c** an increase potency of the same magnitude (i.e. 1.3-fold) was reported ($K_I$s of 4.0 and 3.0 nM for **16b** and **19c** respectively). The presence of the sulfur tether as in compound **7b** determined a $K_I$ value of 84.8 nM thus 12.8-fold higher than its selenium congener **4c**. Removal of the galactosyl protecting groups in **7b** afforded **8b** and reduced the $K_I$ to 18.9 nM which in turn was 2.2-fold less efficient when compared to its selenium counterpart **5c**. Manipulation of **7b** to afford the sulfoxide **10b** or removal of the protection on the primary alcohol (i.e. compound **9**) determined nearly identical reduction of the $K_I$ value up to 5.8 and 6.0 nM respectively.

*vi)* A 7.0-fold increase of the inhibition potency against the hCA VI isozyme was induced after full deprotection of the glucosyl **4a** moiety to afford the derivative **5a** (i.e. $K_I$s of 58.4 and 8.3 nM for **4a** and **5a** respectively). The same trend, although with a reduced magnitude, was obtained for

**4b/5b** with the latter being 2.0-fold more potent (i.e. $K_I$s of 4946 and 2477 nM for **4b** and **5b** respectively). Conversely, significant increase of the $K_I$ value was obtained when **4a** was subjected to selective deprotection of the primary alcohol to afford compound **6a** ($K_I$ of 350.0 nM for **6a**). Interestingly no significant inhibition potency differences were observed on **15a** and its glycosyl deprotected derivative **16a** when compared to their shortest cognate precursor **4a** (i.e. $K_I$ values of 58.4, 58.5 and 49.3 nM for **4a**, **15a** and **16a** respectively). Appreciable $K_I$ differences were obtained among the amide containing moiety **18a** when compared to its shortest derivative **4a** being the latter 1.9-fold more effective inhibitor on the hCA VI isozyme. Removal of the protecting groups on **18a** to afford **19a** (i.e. $K_I$ of 37.6 nM) determined a 1.2-fold increase of the inhibition potency. Interestingly the high nanomolar hCA VI inhibitor **18b** was highly potentiated (i.e. up-to 111.5-fold) upon removal of the acetyl groups to afford the derivative **19b** (i.e. $K_I$ of 41.5 nM). Isosteric induced differences were reported for the sulfur containing derivatives **7a** and **8a** when compared to their corresponding selenium ethers **4a** and **5a** respectively. Data in **Table 1** showed that **7a** and **8a** were 1.6- and 10.2-fold respectively less potent (i.e. $K_I$s of 93.2 and 84.7 nM for **7a** and **8a** respectively). Interestingly oxidation of the sulfur tether in **7a** to afford the derivative **10a** greatly enhanced the inhibition potency up to 8.8 nM. However, removal of the glucosyl protecting groups as in **11** was detrimental for the inhibition potency which was increased up to 3.5-fold (i.e. $K_I$ of 30.9 nM for **11**). As for the galactosyl derivatives, **4c** was reported a very efficient hCA VI inhibitor bearing a $K_I$ of 8.3 nM whereas its fully deprotected derivative **5c** was only slightly more effective (i.e. $K_I$ of 7.3 nM). Modification of the CAI warhead in **4c** to afford **4d** spoiled the inhibition potency (i.e. $K_I$ >10000 nM). Interestingly deprotection of the primary alcohol in **4c** to afford the derivative **6b** determined a 1.4-fold increase of the inhibition potency. The same transformation on **4d** did not affect the $K_I$ value (i.e. $K_I$ > 10000 for **6c**). Elongated molecules such

as **15b** and **18c** weere far less effective inhibitors of the hCA VI isozyme having $K_I$ values 8.3- and 71.5-fold higher when compared to their common cognate **4d** (i.e. $K_I$s of 68.7 and 593.8 nM for **15b** and **18c** respectively). Removal of the galactosyl protecting groups as in **16b** and **19c** was highly beneficial for the inhibition potency being the former 7.2- and the latter 1.22-fold more effective when compared to their precursors **15b** and **18c** respectively.

An important isosteric effect was observed for the galactosyl derivatives when sulfur in **7b** was replaced with the selenium in **4c** instead as the former was 75.9-fold more potent inhibitor of the hCA VI isoform. The hydrolysis of the acetyl groups in **7b** to afford the derivative **8b** resulted in a slight worsening of the inhibition potency ($K_I$s of 629.6 and 775.0 nM for **7b** and **8b** respectively). Also, in this case the sulfur containing moiety **8b** was far less effective hCA VI inhibitor when compared to its selenium congener **5c** (i.e. $K_I$s of 775.0 and 7.3 nM for **8b** and **5c** respectively). Analogous kinetic trend was also reported for the mono-deprotected galactosyl derivative **9** in comparison to its selenium counterpart **6b** being the latter 82.2-fold more potent inhibitor. Finally, oxidation of the sulfur tether in **7b**, drastically reduced the $K_I$ value of 13.2-fold ($K_I$s of 629.6 and 47.8 nM for **7b** and **10b** respectively).

*vii)* The set of compounds afforded a very distinctive kinetic profile for CNS abundantly expressed hCA VII as the structural modifications introduced determined important effects on the associated $K_I$ values (**Table 1**). The glucosyl derivative **4a** was very effective inhibitor showing a $K_I$ value of 14.6 nM. Full deprotection of the glucosyl moiety to afford compound **5a** determined slight reduction of the inhibition potency (i.e. $K_I$ value of 26.3 nM). Conversely regioselective deprotection of the glucosyl primary alcohol to afford **6a** determined a significant increase of the inhibition potency which reported a $K_I$ value of 0.8 nM thus 3.1-fold more potent when compared to the reference **AAZ** (i.e. $K_I$ of 2.5 nM). Modifications at the CAI warhead as in **4b** and **5b**

suppressed the inhibition potency against the hCA VII (i.e. $K_I$s > 10000 nM). The elongation approach afforded quite interesting results. Specifically, the thioureido derivative **15a** showed a $K_I$ value of 1.8 nM thus 8.1-fold more effective when compared to its shorter counterpart **4a**. Removal of the acetyl protecting groups in **15a** to afford **16a** spoiled the potency of 2.4-fold. As for the benzamide containing spacer **18a** a $K_I$ of 2.8 nM was reported, and again removal of the acetyl groups was detrimental for the *in vitro* inhibition potency (i.e. $K_I$ of 9.6 nM for **19a**). Opposite kinetic trend was obtained for the benzamide containing pair **18b**/**19b** being the latter 2.9-fold less effective inhibitor of the hCA VII (i.e. $K_I$s of 87.3 and 29.7 nM for **18b** and **19b** respectively). Slight isosteric effects were observed among the glucose containing pairs **7a** and **4a** being the former 1.3-fold less effective. Identical trend, although with enhanced magnitude, was reported for **8a**/**5a** (i.e. $K_I$s of 26.3 and 51.6 nM for **5a** and **8a** respectively). As for the galactosyl series, the seleno containing arylsulfonylamide **4c** was particularly effective hCA VII inhibitor (i.e. $K_I$ of 0.5 nM). Full deprotection of the galactosyl moiety in **4c** to afford **5c** heavily spoiled the inhibition potency up to 61-fold (i.e. $K_I$s of 0.5 and 30.3 nM for **4c** and **5c** respectively). Conversely the derivative **6b**, which was obtained by means of regioselective deprotection of **4c**, retained most of the inhibition potency against the hCA VII isoform being 1.6-fold less effective thus with an associated $K_I$ value of 0.8 nM. Additional variations within the seleno arylsulfonylamide series such as in **4d** and **6c** determined full suppression of the inhibition potency (i.e. $K_I$s > 10000 nM). Better results were obtained when the elongation approach was applied. For instance, the phenylthioureido **15b** was a low nanomolar hCA VII inhibitor (i.e. $K_I$ value of 2.0 nM) and its activity was further enhanced when the galactosyl tail was subjected to full deprotection to afford **16b** (i.e. $K_I$ of 0.9 nM).

***In vitro* glucose uptake measurement studies**.

The final compounds reported in this study were screened for the effects on glucose uptake mediated by GLUT1 (i.e. compounds **6a**, **c**, **18b, c** and **19a-c** were not included). To this end, a standard glucose uptake assay was carried out as previously described [33, 34]. Briefly, H1299 human non-small cell lung carcinoma (NSCLC) cells were treated with the tested compounds at a fixed concentration (30 μM). After 15 minutes, the cells were incubated for 30 minutes in glucose-free KRP buffer in the presence of 2-deoxy-D-[$^3$H]-glucose. Then, the cells were rinsed with cold PBS and lysed in 0.2 M NaOH. The resulting lysates were moved to scintillation counting vials. The levels of radioactivity found in the cell lysates were measured by liquid scintillation counting. The obtained data are indicated in **Table 2** as percentage uptake of control tests and do not take in consideration any contribution arising from GLUT3 and 4 isoforms which are expressed, although in very lower concentration when compared to GLUT1, from NSCLC cells [33, 34].

**Table 2.** *In vitro* glucose uptake measurement studies.

| Compound | Glucose uptake (%) at 30 μM | Cell Viability (%) at 30 μM |
|---|---|---|
| **4a** | 276.7±1.3 | 98.9±12.7 |
| **4b** | 152±21.2 | 95.8±3.2 |
| **4c** | 118.9±5 | 90.8±2.6 |
| **4d** | 180.9±28.2 | 89.8±4.7 |
| **5a** | 118.8±15.8 | 75.1±13 |
| **5b** | 137.9±20.7 | 64.6±4.8 |
| **5c** | 161.6±34.3 | 83.8±14.6 |
| **6b** | 80.8±10.9 | 81.3±10.3 |

| | | |
|---|---|---|
| **7a** | 143.9±42.1 | 96.9±10.9 |
| **7b** | 117.6±5.3 | 78.6±5.6 |
| **8a** | 183.2±32.9 | 93.6±23.5 |
| **8b** | 111.1±8.4 | 88.4±5.5 |
| **10a** | 239.2±29.3 | 96.6±7.6 |
| **11** | 243.1±38.2 | 96±8.9 |
| **15a** | 136.4±36.3 | 94±5.1 |
| **15b** | 91±16.2 | 86.1±7.4 |
| **16a** | 18±1.4 | 90.1±10.9 |
| **16b** | 101.3±18.1 | 85.6±4.2 |
| **18a** | 115.8±16.7 | 77.4±9.1 |
| **Vehicle treated** | 100±14.6 | 100±7.2 |

Much to our surprise, most compounds either did not affect or even increased the glucose uptake in this assay, thus indicating that they generally do not interfere with GLUT1 activity or, rather, may induce an enhanced request for glucose. On the other hand, only compound **16a** gave a significant inhibition (18 % Glucose uptake) at 30 μM concentration. Compound **16a** is a seleno-glycoside containing a β-glucose portion linked to a diaryl-thiourea moiety displaying a terminal sulfonamide group. It is likely that the glucose portion of this molecule is able to effectively interact with GLUT1 and to inhibit the glucose uptake through this transporter. It is interesting to note that the corresponding β-galactose analogue (**16b**) does not induce any inhibition in this assay, thus confirming the specificity that this transporter has for glucose analogues, as long as the rest of the molecule does not interfere with its interaction.

All these compounds underwent an antiproliferative MTT assay in the same NSCLC H1299 cell lines incubated with 30 μM concentration. The percentage values of viable cells after 48h exposure are reported in **Table 2**. None of the tested compounds exhibited significant antiproliferative effect, with only two compounds (i.e. **5a** and **5b**) displaying a reduction of cell viability at or below 75%. These two compounds are β-selenoglucosides containing an aryl substituent that is directly linked to the selenium atom. This structural feature does not seem to induce any significant effect on cellular glucose uptake, but somehow it slightly affects the proliferation rate of this cancer cell line.

**hCA II X-Ray crystallography.**

The X-ray crystal structure of hCA II in adduct with the inhibitor **8a** was determined at 1.48 Å resolution with the aim to establish the major interactions occurring between the amino acid residues lining the enzyme active site with the ligand (**Figure 2** and **Table S2** in Supporting Information file). Among the series here reported, compound **8a** was assumed as ideal ligand either for its particularly favorable binding affinity for the hCA II ($K_I$ of 7.5 nM) and for its structural features such as compactness and absence of chalcogen elements which all together are beneficial for X-ray investigations. Overall inspection of the refined structure clearly showed fully defined electron density within the hCA II active site and fully compatible with inhibitor **8a** buried deep within the enzymatic cleft, with the primary sulfonamide moiety anchored to the zinc metal ion according to the canonical binding cluster typical of the α-hCAs [35] (**Figure 2** and **Figure S1** in Supporting Information file).

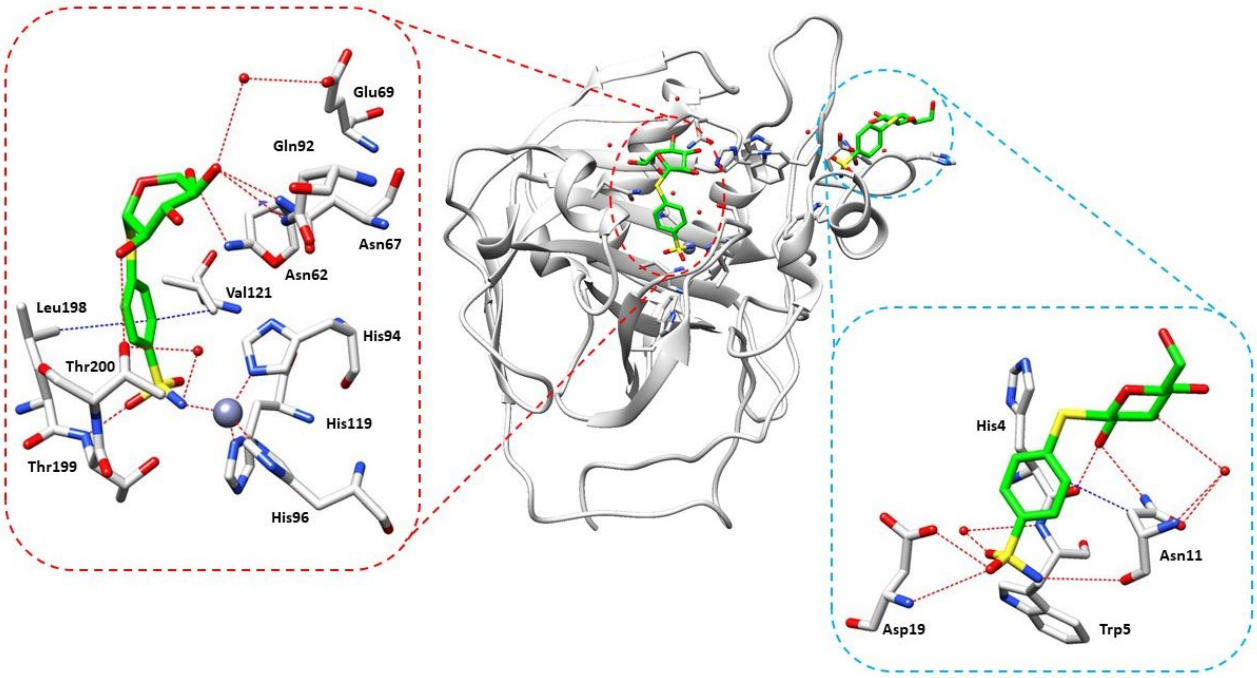



**Figure 2.** X-Ray crystal structure of the hCA II/**8a** complex (PDB: 7ZWB). Van der Waals interactions, hydrogen bonds and water bridges are shown in blue and red colors respectively.

The ligand-enzyme complex was stabilized by several hydrogen bonds and water bridge interactions. For instance, the glucosyl hydroxyl groups were engaged in an intricate network with Asn62, Asn67 and Gln92 which also involved the Glu69 residue by means of a water bridge. The observed peculiar conformational folding of the ligand on the sulfur tether acting as a hinge allowed the hydroxyl group at 3-position of the glucose to interact effectively with Thr200 located at the bottom of the active site.

A second molecule of **8a** was found bound out of the active site, thus located in a region not involved with the enzymatic mechanism (**Figure 2** and **Figure S1** in Supporting Information file). In this case only one valuable hydrophobic interaction between the benzene ring of **8a** and

Asn11 was retrieved. Conversely, several hydrogen bonds contributed to stabilize the adduct. For instance, the primary sulfonamide showed hydrogen bonds with Asp19, Asn11 and through a water bridge with Trp5. The glucosyl moiety interacted with His4 and Asn11 and by means of a water bridge with Asn11.

***In vivo* animal studies.**

We evaluated the effects of selected compounds endowed with variable abilities to induce glucose uptake on *in vivo* induced maximal electroshock seizures (MES) model (**Table 3**). Specifically, we considered the highly effective compound **4a**, the derivative **4b** which had an intermediate potency and the least effective of the series compound **16a**. We initially tested the effects of a 100 mg/kg dose for each of the compounds and then subsequent doses were modulated according to the observed effects in order to build a dose response curve for each compound and to calculate the corresponding $ED_{50}$s (**Table 3**).

**Table 3.** $ED_{50}$ values of compounds **4a**, **4b** and **16a.**

| Compound | $ED_{50}$ (mg/kg)† | $ED_{50}$ (nmol/kg)† |
|---|---|---|
| **4a** | 183.7 (175-197.6) | 324.3 (308.9-348.8) |
| **4b** | 59.81 (54-64.9) | 102.9 (92.9-111.6) |
| **16a** | 132.2 (92.6-192.7) | 241 (168.9-351.3) |

† In brackets are data confidence limits.

All compounds selected were able to reduce the occurrence of tonic extension in the MES model with the following order of potency according to their $ED_{50}$ values: **4b** > **16a** > **4a**. Notably, **4b** was able to completely abolish the occurrence of seizures at the dose of 75 mg/kg without inducing any obvious side effects which were instead clearly noticeable (e.g., ataxia and sedation) at dose of 100 mg/kg. On the other hand, either **4a** and **16a** were not able to abolish completely the induces seizures at the tested doses. In addition, both compounds when administered at higher concentrations induced marked sedation and thus were not useful for the purposes of this study. In detail we found that compound **4a** showed marked toxicity at 150 mg/kg and 20 % of animals died at 300 mg/kg. The derivative **16a** was toxic at 100 mg/kg with 30 % mortality at 200 mg/kg (**Figure 3**).

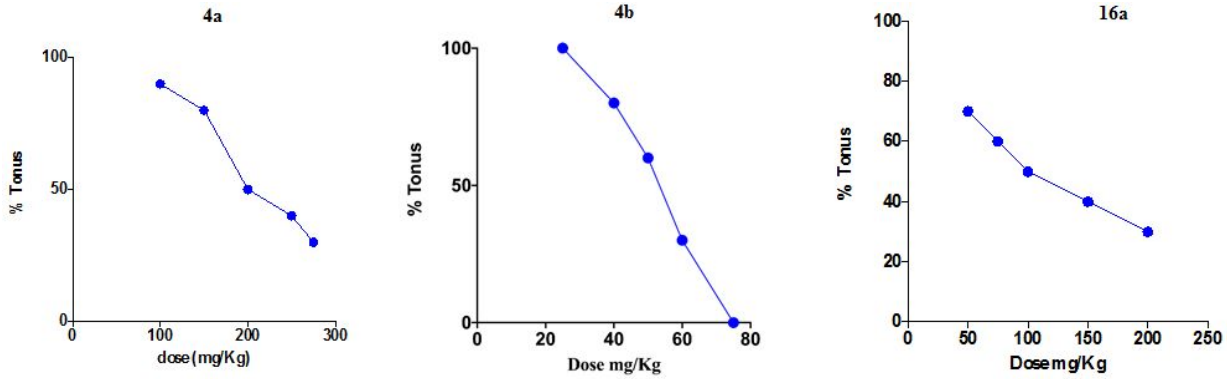



**Figure 3.** Effects of **4a**, **4b** and **16a** on tonic extension in the MES model.

**Conclusions.**

Here we report a set of compounds structurally based on the GLUT1 substrates D-glucose and D-galactose which have been substituted at the anomeric position with a β-oriented arylsulfonamide moiety intended to interact with the hCA isoforms. All compounds have been profiled *in vitro* on the human expressed CAs and the obtained data accounted for quite a variegate inhibition pattern, thus allowing to decipher the main structure activity relationships (SARs) governing either potency

and selectivity for the enzymes considered. The adduct hCA II/**8a** was investigated by means of X-ray crystallography and allowed to determine the binding mode of such an inhibitor which confirmed an extended network of hydrogen bonds contributing to stabilize the complex. It is therefore reasonable to consider that a similar cluster of interactions also occurs among the set of compounds reported featured with high binding affinities.

Surprisingly, the glucose uptake evaluation *in vitro* showed all the compound series (except for **16a**) being effective activators of GLUT1 with values up to 277% at 30 μM concentration in respect to the reference compound glucose. It is noteworthy that no cytotoxicity effects were registered at the same concentration.

Compounds **4a**, **4b** and **16a** endowed with high, intermediate and low GLUT1 activation properties respectively as well as distinct CA inhibition profiles were investigated for their ability to abolish the occurrence of seizures *in vivo* by means of the induced MES model. The glucosyl derivative **4b** was particularly effective in suppressing the seizures at the dose of 75 mg/kg without showing any side effects at the same concentration.

Overall, the results here reported allowed to obtain and develop compounds endowed with dual targeting features on the relevant targets related to GLUT1-DS associated epileptic symptoms. Quite surprisingly we demonstrated that the effectiveness of such compounds mainly relies on the ability to activate the GLUT1 transporter rather than to suppress the progression of uncontrolled electric spikes through a Carbonic Anhydrase mediate pathway [24]. It is worth considering that our study is focused on GLUT1 and does not give evidence for any other transporter isoform which may contribute to the overall glucose uptake enhancement registered. Appropriate measurement experiments are currently ongoing in such a direction.

All this paves the way for an unprecedently reported approach useful for the management of such pathology which lacks effective pharmacological tools.

## EXPERIMENTAL SECTION

### Materials and methods.

Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, VWR and TCI. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere. Nuclear magnetic resonance ($^{1}$H NMR, $^{13}$C NMR, $^{77}$Se NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-$d_6$. Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; bs, broad singlet; dd, double of doubles. The assignment of exchangeable protons (N*H*) was confirmed by the addition of $D_2O$. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck silica gel 60 (230–400 mesh ASTM) as the stationary phase, and ethyl acetate, *n*-hexane, acetonitrile and methanol were used as eluents. The solvents used in MS measurements were acetone, acetonitrile (Chromasolv grade), purchased from Sigma-Aldrich (Milan, Italy), and mQ water 18 MΩ, obtained from Millipore's Simplicity system (Milan, Italy). The mass spectra were obtained using

a Varian 1200L triple quadrupole system (Palo Alto, CA, USA) equipped with electrospray source (ESI) operating in both positive and negative ions. Stock solutions of analytes were prepared in acetone at 1.0 mg $mL^{-1}$ and stored at 4 ˚C. Working solutions of each analyte were freshly prepared by diluting stock solutions in a mixture of mQ $H_2O$/ACN 1/1 (*v/v*) up to a concentration of 1.0 μg $mL^{-1}$ The mass spectra of each analyte were acquired by introducing, via syringe pump at 10/L $min^{-1}$, the working solution. Raw data were collected and processed by Varian Workstation, version 6.8, software. All compounds reported are >95% of purity.

**General Procedure for the synthesis of Compounds 4a-d:**

$NaBH_4$ (3.0 eq.) was portion-wise added to a solution of selenocyanatobenzenesulfonamide derivative **2a, b** (1.0 eq.) in EtOH (2 mL) at 0˚C under inert atmosphere ($N_2$). After 30 min, the Acetobromo sugar **1a, b** (1.0 eq.) was slowly added and the reaction mixture was stirred at room temperature for 2 h, until complete consumption of the starting material was observed by TLC. The reaction was quenched by addition of saturated aq. $NH_4Cl$ (2mL) and diluted with EtOAc (5 mL), The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over $Na_2SO_4$, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield derivatives **4a-c.**

*Synthesis of (2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-((4-sulfamoylphenyl)selanyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(4a):**

Following the General Procedure, Acetobromo-α-D-glucose **1a** (1.53 mmol) and 4-selenocyanatobenzenesulfonamide **2a** (1.53 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **4a** as a white solid (50%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.78 (2H, d, $J$ = 8.58 Hz), 7.75 (2H, d, $J$ = 8.53 Hz), 7.46 (2H, bs), 5.61 (1H, d, $J$ = 10.17 Hz), 5.39 (1H, t, $J$ = 9.44 Hz), 4.99-4.92 (2H, m), 4.20-4.11 (3H, m), 2.06 (3H, s), 2.05 (3H, s), 2.03 (3H, s), 1.98 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.8, 170.4, 170.2, 170.0, 133.8, 133.7, 127.8, 127.0, 80.1, 76.3, 73.6, 71.2, 68.8, 62.8, 21.4, 21.3, 21.2, 21.1. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 410.4. **MS** (ESI negative) *m/z*: 566.2 $[M-H]^-$

*Synthesis of (2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-((3-amino-4-sulfamoylphenyl)selanyl) tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(4b):**

Following the General Procedure, Acetobromo-α-D-glucose **1a** (1.53 mmol) and 2-amino-4-selenocyanatobenzenesulfonamide **2b** (1.53 mmol) gave, after purification by flash column

chromatography (EtOAc/Hex 1:1), **4b** as a white solid (46%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.79 (1H, d, $J$ = 2.01 Hz), 7.42 (1H, dd, $J$ = 8.50, 2.01 Hz), 7.40 (2H, bs), 6.79 (1H, d, $J$ = 8.53 Hz), 6.15 (2H, bs), 5.32 (1H, t, $J$ = 9.42 Hz), 5.20 (1H, d, $J$ = 10.14 Hz), 4.89 (1H, t, $J$ = 9.69 Hz), 4.81 (1H, m), 4.16 (1H, dd, $J$ = 12.20, 5.21 Hz), 4.07-4.00 (2H,m), 2.07 (3H, s), 2.06 (3H, s), 2.01 (3H, s), 1.96 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm):171.0, 170.4, 170.2, 170.0, 147.0, 141.1, 136.3, 125.3, 118.3, 110.3 81.2, 76.2, 73.8, 71.6, 68.8, 62.8. 21.5, 21.4, 21.3, 21.2. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 396.5. **MS** (ESI negative) *m/z*: 581.3 $[M-H]^-$

*Synthesis of (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((4-sulfamoylphenyl)selanyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(4c):**

Following the General Procedure, Acetobromo-α-D-galactose **1b** (1.65 mmol) and 4-selenocyanatobenzenesulfonamide **2a** (1.65 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **4c** as a white solid (66%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.78 (4H, aps), 7.46 (2H, bs), 5.55 (1H, d, $J$ = 10.08 Hz), 5.39 (1H, d, $J$ = 2.81 Hz), 5.28 (1H, dd, $J$ = 9,88, 3.43 Hz), 5.13 (1H, t, $J$ = 9.96 Hz), 4.37 (1H, t, $J$ = 6.25 Hz), 4.11 (2H, d, $J$ = 6.15 Hz), 2.15 (3H, s), 2.07 (3H, s), 2.05 (3H, s), 1.96 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$

(ppm): 170.8, 170.7, 170.3, 170.2, 144.1, 134.3, 133.3, 126.9, 80.7, 75.6, 71.7, 68.6, 62.7, 60.7, 21.4 (X2), 21.3, 21.2. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 413.4. **MS** (ESI positive) *m/z*: 568.1 $[M+H]^+$

*Synthesis of (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((3-amino-4-sulfamoylphenyl)selanyl) tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(4d):**

Following the General Procedure, Acetobromo-α-D-galactose **1b** (1.44 mmol) and 2-amino-4-selenocyanatobenzenesulfonamide **2b** (1.44 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **4d** as a white solid (66%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.81 (1H, d, $J$ = 1.83 Hz), 7.44 (1H, dd, $J$ = 8.48, 1.84 Hz), 7.32 (2H, bs), 6.80 (1H, d, $J$ = 8.52 Hz), 6.12 (2H, bs), 5.31 (1H, d, $J$ = 3.13 Hz), 5.22 (1H, d, $J$ = 9.76, 3.41 Hz), 5.13 (1H, d, $J$ = 10.05 Hz), 5.03 (1H, t, $J$ = 9.88 Hz), 4.22 (1H, t, $J$ = 6.15 Hz), 4.09-4.05 (2H, m), 2.12 (3H, s), 2.09 (3H, s), 2.03 (3H, s), 1.94 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.9, 170.8, 170.3, 170.2, 146.8, 140.8, 136.1, 125.3, 118.2, 110.8, 82.0, 75.3, 71.7, 68.9, 68.5, 62.6, 21.5, 21.4, 21.3, 21.2 **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 450.4. **MS** (ESI positive) *m/z*: 582.2 $[M+H]^+$

**General Procedure for the synthesis of Compounds 5a-c:**

Na (10.0 eq.) was portion wise added to a solution of **4a-c** (1.0 eq.) in MeOH (2 mL) at room temperature under inert atmosphere ($N_2$). The reaction mixture was stirred at room temperature until complete consumption of the starting material was observed by TLC. The reaction was quenched by addition of Amberlist 120 until the pH was acid. The mixture was filtered and concentrated under vacuum to yield the corresponding derivatives **5a-c.**

*Synthesis of 4-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)selanyl)benzenesulfonamide* **(5a):**

Following the General Procedure, Na ( 2.5 mmol) and **4a** ( 0.25 mmol) gave **5a** as a white solid (60%).**$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.77 (2H, d, $J$ = 8.48 Hz), 7.71 (2H, d, $J$ = 8.44 Hz), 7.39 (2H, bs), 5.43 (1H, d, $J$ = 5.86 Hz), 5.18 (1H, d, $J$ = 4.66 Hz), 4.64-4.61 (2H, m), 4.63 (1H, t, $J$ = 5.64 Hz), 3.73 (1H, dd, $J$ = 10.30, 5.14 Hz), 3.49-3.25 (1H, m), 3.23-3-17 (4H, m). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 143.1, 136.5, 132.5, 126.8, 84.9, 83.2 79.1, 74.1, 70.7, 61.9. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 412.2. **MS** (ESI positive) *m/z*: 399.2 $[M+H]^+$

*Synthesis of 2-amino-4-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)selanyl)benzenesulfonamide* **(5b):**

Following the General Procedure, Na (2.5 mmol) and **4b** ( 0.25 mmol) gave **5b** as a white solid (63%).**$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.83 (1H, s), 7.53 (1H, d, $J$ = 8.35 Hz), 7.52 (2H, bs), 7.32 (1H, d $J$ = 8.46 Hz ), 5.04 (6H, bs), 4.62 (1H, d, $J$ = 9.65 Hz), 3.69 (1H, d, $J$ = 11.53 Hz), 3.46 (1H, dd, $J$ = 11.57, 4.51 Hz), 3.18-3.05 (4H, m). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 146.2, 140.7, 135.3, 125.4, 118.2, 112.2, 85.8, 83.0, 79.0, 74.1, 70.7, 62.1. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 393.0. **MS** (ESI positive) *m/z*: 414.3 $[M+H]^+$

*Synthesis of 4-(((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)selanyl)benzenesulfonamide* **(5c):**

Following the General Procedure, Na (2.5 mmol) and **4c** ( 0.25 mmol) gave **5c** as a white solid (98%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.77 (2H, d, $J$ = 8.52 Hz), 7.70 (2H, d, $J$ = 8.49 Hz), 7.38 (2H, bs), 5.27 (1H, d, $J$ = 6.13 Hz), 5.00-4.95 (2H, m), 4.69 (1H, bs), 4.58 (1H, d, $J$ = 4.38 Hz), 3.78. (1H, bs), 3.60-3.53 (5H, m), 3.21 (1H, d, $J$ = 5.25 Hz). **$^{13}$C NMR** (DMSO-$d_6$, 100

MHz) $\delta$ (ppm):142.9, 137.0, 132.2, 126.8, 85.6, 81.5, 75.5, 70.9, 69.4. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 412.3. **MS** (ESI positive) *m/z*: 399.2 $[M+H]^+$

**General Procedure for the synthesis of Compounds 6a-c:**

Tin derivative catalyst (0.1 eq.) was added to a solution of derivative **4a,c,d** (1.0 eq.) in MeOH/THF solution (2 mL) at room temperature under inert atmosphere ($N_2$). The reaction mixture was stirred at room temperature for 18 h until complete consumption of the starting material observed by TLC. The reaction was quenched by addition of $H_2O$. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over $Na_2SO_4$, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield derivatives **6a-c.**

*Synthesis of (2R,3R,4S,5R,6S)-2-(hydroxymethyl)-6-((4-sulfamoylphenyl)selanyl) tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(6a):**

Following the General Procedure, derivative **4a** (0.44 mmol) and tin derivative catalyst (0.044 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **6a** as a white solid (66%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.79 (2H, d, $J$ = 8.66 Hz), 7.76 (2H, d, $J$ =

8.58 Hz), 7.42 (2H, bs), 5.57 (1H, d, *J* = 10.12 Hz), 5.34 (1H, t, *J* = 9.38 Hz), 4.97-4.90 (3H, m), 3.88 (1H, m), 3.59-3.55. (2H, m), 2.03 (3H, s), 2.02 (3H, s), 1.96 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm):170.4, 170.1, 170.0, 144.1, 134.1, 133.5, 126.9, 80.4, 79.7, 74.2, 71.4, 69.1, 60.9, 21.3, 21.2, 21.1. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 408.6. **MS** (ESI negative) *m/z*: 523.4 $[M-H]^-$

*Synthesis of (2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-((4-sulfamoylphenyl)selanyl) tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(6b):**

Following the General Procedure, derivative **4c** (0.44 mmol) and tin derivative catalyst (0.044 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **6b** as a white solid (66%). **$^{1}$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.77 (4H, aps), 7.44 (2H, bs), 5.52 (1H, d, *J* = 9.96 Hz), 5.40 (1H, d, *J* = 3.11 Hz), 5.24 (1H, dd, *J* = 9.88, 3.34 Hz), 5.14 (1H, d, *J* = 9.91 Hz), 5.00 (1H, t, *J* = 5.55 Hz), 4.09-4.04 (2H, m), 3.55-3.49 (1H, m), 2.13 (3H, s), 2.05 (3H, s), 1.94 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm):170.7, 170.4, 170.2, 143.9, 134.6, 133.2, 126.9, 81.1, 78.6, 72.2, 68.8, 68.2, 21.4, 21.4, 21.3. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 412.1. **MS** (ESI negative) *m/z*: 523.3 $[M-H]^-$

*Synthesis of (2S,3R,4S,5S,6R)-2-((3-amino-4-sulfamoylphenyl)selanyl)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(6c):**

Following the General Procedure, derivative **4d** (0.44 mmol) and tin derivative catalyst (0.044 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **6c** as a white solid (64%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.82 (1H, d, $J$ = 1.74 Hz), 7.45 (1H, d, $J$ = 10.33 Hz), 7.30 (2H, bs), 6.79 (1H, d, $J$ = 8.48 Hz), 6.09 (2H, bs), 5.35 (1H, d, $J$ = 3.05 Hz), 5.17 (1H, dd, $J$ = 9.41, 3.31 Hz), 5.11-5.01 (2H, m), 4.89 (1H, t, $J$ = 5.60 Hz), 3.91 (1H, t, $J$ = 6.60 Hz), 3.52-3.48 (2H, m), 2.09 (3H, s), 2.08 (3H, s), 1.93 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.8, 170.4, 170.2, 146.7, 140.7, 135.9, 125.4, 118.2, 111.1, 82.3, 78.2, 72.4, 69.2, 68.1, 59.7, 21.6, 21.5, 21.3. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 399.1. **MS** (ESI negative) *m/z*: 538.4 $[M-H]^-$

**General Procedure for the synthesis of Compounds 7a-b:**

$NaBH_4$ (3.0 eq.) was portion wise added to a solution of disulfide derivative **3** (1.0 eq.) in EtOH (2 mL) at 0˚C under inert atmosphere ($N_2$). After 30 min, the Acetobromo sugar **1a,b** (2.0 eq.) was

slowly added and the reaction mixture was stirred at room temperature for 2 h, until complete consumption of the starting material was observed by TLC. The reaction was quenched by addition of saturated aq. $NH_4Cl$ (2mL) and diluted with EtOAc (5 mL), The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over $Na_2SO_4$, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield derivatives **7a-b.**

*Synthesis of (2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-((4-sulfamoylphenyl)thio)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(7a):**

Following the General Procedure, Acetobromo-α-D-glucose **1a** (2 mmol) and disulfide derivative **3** (1 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **7a** as a white solid (90%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.81 (2H, d, $J$ = 8.32 Hz), 7.63 (2H, d, $J$ = 8.34 Hz), 7.45 (2H, bs), 5.51 (1H, d $J$ = 10.05 Hz ), 5.43 (1H, t, $J$ = 9.41 Hz), 4.99-4.91 (2H, m), 4.24-4.12 (3H, m), 2.07 (3H, s), 2.06 (3H, s), 2.04 (3H, s), 1.99 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.9, 170.4, 170.2, 170.0, 143.7, 138.3, 130.7, 127.1, 83.3, 75.3, 73.7, 70.3, 68.9, 62.8, 21.4, 21.3, 21.2. **MS** (ESI negative) *m/z*: 518.3 $[M-H]^-$

*Synthesis of (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((4-sulfamoylphenyl)thio)tetrahydro-2H-pyran-3,4,5-triyl triacetate* (**7b**):

Following the General Procedure, Acetobromo-α-D-galactose **1b** (2 mmol) and disulfide derivative **3** (1 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **7b** as a white solid (40%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.81 (2H, d, $J$ = 8.55 Hz), 7.65 (2H, d, $J$ = 7.65 Hz), 7.45 (2H, bs), 5.44 (1H, d $J$ = 10.01 Hz), 5.40 (1H, d, $J$ = 3.27 Hz), 5.31 (1H, dd, $J$ = 9.88, 3.45 Hz), 5.11 (1H, t, $J$ = 9.93 Hz), 4.42 (1H, t, $J$ = 6.13 Hz), 4.12 (2H, d, $J$ = 6.16 Hz), 2.17 (3H, s), 2.09 (3H, s), 2.05 (3H, s), 1.97 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.8, 170.7, 170.4, 170.2, 143.5, 138.8, 130.4, 127.0, 83.8, 74.6, 71.9, 68.4, 67.7, 62.7, 21.4 (X2), 21.3, 21.2. **MS** (ESI negative) *m/z*: 518.3 $[M-H]^-$

**General Procedure for the synthesis of Compounds 8a-b:**

Na (10.0 eq.) was portion wise added to a solution of **7a,b** (1.0 eq.) in MeOH (2 mL) at room temperature under inert atmosphere ($N_2$). The reaction mixture was stirred at room temperature until complete consumption of the starting material was observed by TLC. The reaction was

quenched by addition of Amberlist 120 until the pH was acid. The mixture was filtered and concentrated under vacuum to yield the corresponding derivatives **8a-b.**

*Synthesis of 4-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)thio)benzenesulfonamide* **(8a).**

Following the General Procedure, Na (1.8 mmol) and **7a** (0.18 mmol) gave **8a** as a white solid (67%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.75 (2H, d, $J$ = 7.77 Hz), 7.62 (2H, d, $J$ = 7.82 Hz), 7.38 (2H, bs), 5.45 (1H, d $J$ = 5.48 Hz), 5.19 (1H, d, $J$ = 3.66 Hz), 5.07 (1H, d, $J$ = 4.37 Hz), 4.82 (1H, d, $J$ = 9.46 Hz), 4.63 (1H, m), 3.74 (1H, d, $J$ = 7.21 Hz), 3.49-3.38 (2H, m), 3.27-3.15 (4H, m). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 142.3, 141.4, 129.3, 126.9, 86.7, 81.9, 79.1, 73.3, 70.7, 61.8. **MS** (ESI negative) *m/z*: 350.4 $[M-H]^-$.

*Synthesis of 4-(((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)thio)benzenesulfonamide* **(8b).**

Following the General Procedure, Na (1.8 mmol) and **7b** (0.18 mmol) gave **8b** as a white solid (88%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.75 (2H, d, $J$ = 8.59 Hz), 7.62 (2H, d, $J$ = 8.57

Hz), 7.38 (2H, bs), 4.78 (1H, d $J$ = 9.58 Hz), 4.25 (6H, bs), 3.77 (1H, d, $J$ = 2.89 Hz), 3.61-3.52 (2H, m), 3.43-3.41 (1H, m). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 142.1, 141.8, 129.0, 126.9, 87.3, 80.2, 75.6, 70.0, 69.3, 61.5. **MS** (ESI negative) *m/z*: 350.3 $[M-H]^-$

*Synthesis of (2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-((4-sulfamoylphenyl)thio)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(9).**

Tin derivative catalyst (0.1 eq.) was added to a solution of **7b** (1.0 eq.) in MeOH/THF solution (2 mL) at room temperature under inert atmosphere ($N_2$). The reaction mixture was stirred at room temperature for 18 h until complete consumption of the starting material observed by TLC. The reaction was quenched by addition of $H_2O$. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over $Na_2SO_4$, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield derivatives **9** in 54%. **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.79 (2H, d, $J$ = 8.41 Hz), 7.65 (2H, d, $J$ = 8.47 Hz), 7.43 (2H, bs), 5.42-5.40 (2H, m), 5.28 (1H, dd, $J$ = 9.87, 3.35 Hz), 5.12 (1H, t, $J$ = 9.90 Hz), 5.01 (1H, bs), 4.13 (1H, t, $J$ = 6.70 Hz), 3.55-3.51 (1H, m), 2.15 (3H, s), 2.08 (3H, s), 1.95 (3H, s). **$^{13}$C NMR**

(DMSO-$d_6$, 100 MHz) $\delta$ (ppm):170.7, 170.4, 170.3, 143.4, 139.1, 130.2, 127.0, 84.0, 77.5, 72.4, 68.2, 67.9, 59.8, 21.4, 21.3, 21.2. **MS** (ESI negative) *m/z*: 476.5 $[M-H]^-$

**General Procedure for the synthesis of Compounds 10a-b:**

*meta*-Chloroperoxybenzoic acid (m-CPBA) (2 eq.) was added to a solution of derivative **7a,b** (1.0 eq.) in Acetonitrile/$H_2O$ solution (ratio 5:1, 2 mL) at room temperature under inert atmosphere ($N_2$). The reaction mixture was stirred at 0˚C for 1 h until complete consumption of the starting material observed by TLC. The reaction was quenched by addition of $H_2O$. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over $Na_2SO_4$, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield derivatives **10a-b.**

*Synthesis of (2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-((4-sulfamoylphenyl)sulfonyl) tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(10a).**

Following the General Procedure, **7a** (0.4 mmol) and mCPBA (0.8 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 70:30), **10a** as a white solid (57%). **$^1$H NMR**

(DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 8.13 (2H, d, $J$ = 8.46 Hz), 8.05 (2H, d, $J$ = 8.45 Hz), 7.76 (2H, bs), 5.42-5.37 (2H, m), 5.22 (1H, t, $J$ = 9.49 Hz), 4.84 (1H, t, $J$ = 9.31 Hz), 4.10 (2H, d, $J$ = 9.31 Hz), 4.05-4.01 (1H, m), 2.06 (3H, s), 2.01 (3H, s), 1.98 (3H,s), 1.93 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.9, 170.5, 170.2, 169.8, 150.4, 139.2, 131.5, 127.4, 87.7, 75.6, 73.4, 68.2, 67.3, 62.3, 21.5, 21.4, 21.3, 21.2. **MS** (ESI negative) *m/z*: 550.3 $[M-H]^-$

*Synthesis of (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((4-sulfamoylphenyl)sulfonyl) tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(10b).**

Following the General Procedure, **7b** (0.2 mmol) and mCPBA (0.4 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 70:30), **10b** as a white solid (54%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 8.05 (2H, d, $J$ = 8.30 Hz), 7.87 (2H, d, $J$ = 8.22 Hz), 7.59 (2H, bs), 5.46 (1H, d, $J$ = 9.64 Hz), 5.32-5.27 (2H, m), 5.08 (1H, d, $J$ = 9.57 Hz), 4.37 (1H, t, $J$ = 5.92 Hz), 4.07-4.05 (2H, m), 2.12 (3H, s), 2.02 (3H, s), 1.92 (3H,s), 1.64 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.8, 170.7, 170.3, 169.5, 147.3, 149.9, 127.0, 126.5, 92.4, 75.3, 71.9, 68.3, 65.8, 64.3, 21.5, 21.3, 21.2, 21.1. **MS** (ESI negative) *m/z*: 550.5 $[M-H]^-$

*Synthesis of 4-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)sulfonyl)benzenesulfonamide* (11).

Na (1.0 mmol) was added to a solution of **10b** (0.1 mmol) in MeOH (2 mL) at room temperature under inert atmosphere ($N_2$). The reaction mixture was stirred at room temperature until complete consumption of the starting material was observed by TLC. The reaction was quenched by addition of Amberlist 120 until the pH was acid. The mixture was filtered and concentrated under vacuum to yield the corresponding derivative **11** in 50%. **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 8.13 (2H, d, $J$ = 8.34 Hz), 8.06 (2H, d, $J$ = 8.35 Hz), 7.69 (2H, bs), 5.46 (1H, d, $J$ = 4.52 Hz), 5.27 (1H, bs), 5.14 (1H, bs), 4.56 (1H, d, $J$ = 9.31 Hz), 4.51 (1H, m), 3.55-3.21 (6H, m), 3.10 (1H, t, $J$ = 8.69 Hz) . **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 149.4, 141.4, 131.0, 126.9, 92.3, 82.1, 78.2, 70.8, 69.8, 61.2. **MS** (ESI negative) *m/z*: 382.4 $[M-H]^-$

**General Procedure for the synthesis of Compounds 13a-b:**

$NaBH_4$ (3.0 eq.) was portion wise added to a solution of 4-selenocyanatoaniline **12** (1.0 eq.) in EtOH (2 mL) at 0˚C under inert atmosphere ($N_2$). After 30 min, the Acetobromo sugar **1a,b** (1.0 eq.) was slowly added and the reaction mixture was stirred at room temperature for 2 h, until

complete consumption of the starting material was observed by TLC. The reaction was quenched by addition of saturated aq. $NH_4Cl$ (2mL) and diluted with EtOAc (5 mL), The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over $Na_2SO_4$, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield derivatives **13a-b.**

*Synthesis of (2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-((4-aminophenyl)selanyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(13a):**

Following the General Procedure, Acetobromo-α-D-glucose **1a** (2 mmol) and 4-selenocyanatoaniline **12** (2 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **13a** as a light yellow solid (47%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.21 (2H, d, $J$ = 8.52 Hz), 6.53 (2H, d, $J$ = 8.57 Hz), 5.38 (2H, bs), 5.29 (1H, d, $J$ = 9.43 Hz), 5.12 (1H, d, ), 5.14 (1H, $J$ = 10.08 Hz), 4.83 (1H, t, $J$ = 9.80 Hz), 4.76 (1H, m), 4.15-4.11 (2H, m), 4.10-3.98 (1H, m), 2.06 (3H, s), 2.05 (3H, s), 2.01 (3H, s), 1.95 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.8, 170.3, 170.1, 169.8, 150.4, 137.8, 115.1, 110.3, 80.5, 75.9, 73.9, 71.5, 68.8,

62.7, 21.4, 21.2, 21.1. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 388.4. **MS** (ESI negative) *m/z*: 502.4 $[M-H]^-$

*Synthesis of (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((4-aminophenyl)selanyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(13b):**

Following the General Procedure, Acetobromo-α-D-galactose **1b** (2 mmol) and 4-selenocyanatoaniline **12** (2 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **13b** as a light yellow solid (60%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 7.24 (2H, d, $J$ = 8.53 Hz), 6.54 (2H, d, $J$ = 8.59 Hz), 5.37 (2H, bs), 5.30 (1H, dd, $J$ = 3.42, 0.75 Hz), 5.21 (1H, ddd, $J$ = 8.59, 3.47, 1.01 Hz), 5.08-5.01 (2H, m), 4.20 (1H, t, $J$ = 6.47 Hz), 4.10-4.00 (2H, m), 2.11 (3H, s), 2.07 (3H, s), 2.04 (3H, s) 1.94 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.8, 170.7, 170.3, 170.1, 150.3, 137.3, 115.2, 111.5, 82.0, 75.2, 71.8, 69.0, 68.6, 62.6, 21.6, 21.4, 21.3, 21.2. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 392.1. **MS** (ESI negative) *m/z*: 502.3 $[M-H]^-$

**General Procedure for the synthesis of Compounds 15a-b:**

4-Isothiocyanatobenzenesulfonamide **14** (1.0 eq.) was added to a solution of derivative **13,b** (1.0 eq.) in Acetonitrile (2 mL) at room temperature under inert atmosphere ($N_2$) until complete consumption of the starting material was observed by TLC. The reaction was quenched by addition of $H_2O$ and the precipitate was collected to yield derivatives **15a,b.**

*Synthesis of (2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-((4-(3-(4-sulfamoylphenyl)thioureido) phenyl)selanyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(15a):**

Following the General Procedure, derivative **13a** (1 mmol) and 4-isothiocyanatobenzenesulfonamide **14** (1.0 eq.) gave **15a** as a light yellow solid (64%).**$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 10.18 (1H, bs), 10.15 (1H, bs), 7.80 (2H, d, $J$ = 8.72 Hz), 7.72 (2H, d, $J$ = 8.68 Hz), 7.55 (4H, aps), 7.32 (2H, bs), 5.43 (1H, d, $J$ = 10.14 Hz), 5.36 (1H, t, $J$ = 9.41 Hz), 4.95-4.87 (2H, m), 4.19-4.09 (3H, m), 2.06 (3H, s), 2.02 (3H, s), 1.97 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 213.0, 170.8, 170.3, 170.1, 170.0, 143.5, 140.3, 140.1, 135.0, 127.1, 124.6, 123.5, 123.2, 80.4, 76.2, 73.7, 71.5, 68.8, 65.8, 21.4, 21.3, 21.2, 21.1. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 398.5. **MS** (ESI negative) *m/z*: 715.6 $[M-H]^-$

*Synthesis of (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((4-(3-(4-sulfamoylphenyl)thioureido) phenyl)selanyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(15b):**

Following the General Procedure, derivative **13b** (1 mmol) and 4-isothiocyanatobenzenesulfonamide **14** (1.0 eq.) gave **15b** as a light yellow solid (61%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 10.20 (1H, bs), 10.17 (1H, bs), 7.80 (2H, d, $J$ = 8.69 Hz), 7.72 (2H, d, $J$ = 8.70 Hz), 7.57 (2H, d, $J$ = 8.82 Hz), 7.54 (2H, d, $J$ = 8.86 Hz), 7.34 (2H, bs), 5.40-5.29 (2H, m), 5.27 (1H, dd, $J$ = 9.86, 3.36 Hz), 5.13 (1H, d, $J$ = 9.95 Hz), 4.32 (1H, t, $J$ = 6.25 Hz), 4.10-4.07 (2H, m), 2.15 (3H, s), 2.08 (3H, s), 2.05 (3H, s), 1.96 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 180.4, 171.0, 170.9, 170.5, 170.4, 143.6, 140.2, 140.1, 134.5, 127.2, 124.8, 124.2, 123.6, 81.4, 75.5, 71.8, 68.6, 65.9, 62.8, 21.6, 21.5, 21.4, 21.3. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 400.8. **MS** (ESI negative) *m/z*: 715.7 $[M-H]^-$

**General procedure for the synthesis of derivatives 16a,b:**

Na (10 eq.) was added to a solution of **15a,b** (1 eq.) in MeOH (2 mL) at room temperature under inert atmosphere ($N_2$). The reaction mixture was stirred at room temperature until complete consumption of the starting material was observed by TLC. The reaction was quenched by addition

of Amberlist 120 until the pH was acid. The mixture was filtered and concentrated under vacuum to yield the corresponding derivative **16a,b**.

*Synthesis of 4-(3-(4-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)selanyl)phenyl)thioureido)benzenesulfonamide* **(16a):**

Following the General Procedure, Na (1.4 mmol) and **15a** ( 0.14 mmol) gave **16a** as a yellow solid (60%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 10.18 (1H, bs), 10.13 (1H, bs), 7.80 (2H, d, $J$ = 8.70 Hz), 7.73 (2H, d, $J$ = 8.72 Hz), 7.60 (2H, d, $J$ = 8.48 Hz), 7.47 (2H, d, $J$ = 8.49 Hz), 7.32 (2H, bs), 4.84 (1H, d, $J$ = 9.60 Hz), 4.09 (10H, bs). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 180.2, 143.5, 140.0, 139.2, 134.0, 127.1, 125.7, 124.7 123.5, 85.7, 83.1, 79.1, 74.1, 70.7, 62.0. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 398.1. **MS** (ESI negative) *m/z*: 547.5 $[M-H]^-$

*Synthesis of 4-(3-(4-(((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)selanyl)phenyl)thioureido)benzenesulfonamide* **(16b):**

Following the General Procedure, Na ( 2.5 mmol) and **4a** ( 0.25 mmol) gave **5a** as a white solid (60%).**$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 10.17 (1H, bs), 10.12 (1H, bs), 7.79 (2H, d, $J$ =

8.56 Hz), 7.73 (2H, d, *J*= 8.60 Hz), 7.58 (2H, d, *J*= 8.39 Hz), 7.45 (2H, d, *J*= 8.41 Hz), 7.34 (2H, bs), 5.23, (4H, bs), 4.82 (1H, d, *J*= 9.77 Hz), 4.3.75-3.34 (6H, m). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$(ppm): 180.3, 143.6, 140.0, 139.0, 133.6, 127.1, 126.5, 124.9, 123.6, 86.6, 81.4, 75.5, 71.1, 69.3, 61.4. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$(ppm): 398.2. **MS** (ESI negative) *m/z*: 547.7 $[M-H]^-$

**General procedure for the synthesis of derivatives 18a-d:**

To derivative **13a,b** (1 eq) in DMF (3 mL) cooled to 0 ˚C was added carboxylic acid derivative **17a,b** (1 eq) and HATU (1.2 eq). After 10 min at 0 ˚C, DIPEA (2 eq) was added and the reaction stirred at room temperature under inert atmosphere ($N_2$) until complete consumption of the starting material was observed by TLC. The reaction was quenched by addition of saturated $NH_4Cl$ solution and diluted with EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL), dried over $Na_2SO_4$, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield derivatives **18a-d.**

*Synthesis of (2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-((4-(4-sulfamoylbenzamido)phenyl) selanyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(18a):**

Following the General Procedure, **13a** (2.5 mmol) and **17a** (0.25 mmol) gave, after purification by flash column chromatography (MeOH/DCM 1:9), **18a** as a light yellow solid (40%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 10.56 (1H, bs), 8.14 (2H, d, $J$ = 8.42 Hz), 8.00 (2H, d, $J$ = 8.41 Hz), 7.81 (2H, d, $J$ = 8.66 Hz), 7.59-7.58 (4H, m), 5.41-5.33 (2H, m), 4.90-4.84 (2H, m), 4.20-4.06 (3H, m), 2.09 (3H, s), 2.07 (3H, s), 2.02 (3H, s), 1.96 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.9, 170.4, 170.2 170.0, 165.6, 147.5, 140.1, 138.6, 135.8, 129.3, 126.6, 122.1, 121.6, 80.4, 76.2, 73.8, 71.4, 68.8, 62.8, 21.5, 21.4, 21.3, 21.2. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 397.7. **MS** (ESI negative) *m/z*: 685.1 $[M-H]^-$

*Synthesis of (2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-((4-(4-chloro-3-sulfamoylbenzamido) phenyl)selanyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(18b):**

Following the General Procedure, **13a** (0.4 mmol) and **17b** (0.4 mmol) gave, after purification by flash column chromatography (EtOAc/Hex 1:1), **18b** as a light yellow solid (49%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 10.65 (1H, bs), 8.56 (1H, d, $J$ = 2.02 Hz), 8.22 (1H, dd, $J$ = 8.30, 2.05 Hz), 7.88 (1H, d, $J$ = 8.27 Hz), 7.80-7.78 (4H, m), 7.58 (2H, d, $J$ = 8.57 Hz), 5.41-5.32 (2H, m), 4.90-4.85 (2H, m), 4.19-4.08 (3H, m), 2.08 (3H, s), 2.06 (3H, s), 2.02 (3H, s), 1.96 (3H, s).

**$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 170.9, 170.4, 170.2, 170.0, 164.6, 142.2, 139.9, 135.8, 134.6, 134.5, 132.9, 132.6, 129.5, 122.2, 121.8, 80.4, 76.2, 73.8, 71.4, 68.8, 62.8, 21.5, 21.4, 21.3, 21.2. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 398.4. **MS** (ESI negative) *m/z*: 719.1 [M-H]$^-$

*Synthesis of (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-((4-(4-sulfamoylbenzamido)phenyl) selanyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate* **(18c):**

Following the General Procedure, **13b** (0.6 mmol) and **17a** (0.6 mmol) gave, after purification by flash column chromatography (MeOH/DCM 1:9), **18a** as a light yellow solid (42%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 10.57 (1H, bs), 8.14 (2H, d, $J$ = 8.38 Hz), 8.00 (2H, d, $J$ = 8.18 Hz), 7.81 (2H, d, $J$ = 8.68 Hz), 7.62-7.60 (4H, m), 5.37-5.28 (2H, m), 5.26 (1H, d, $J$ = 9.89, 3.27 Hz), 5.09 (1H, t, $J$ = 9.94 Hz), 4.30 (1H, t, $J$ = 5.91 Hz), 4.11-4.10 (2H, m), 2.14 (3H, s), 2.09 (3H, s), 2.07 (3H, s), 1.95 (3H, s). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm):170.9, 170,8, 170.4, 170.3, 165.6, 147.6, 139.8, 138.6, 135.1, 129.4, 126.6, 123.0, 121.7, 81.4, 75.5, 71.8, 68.6, 65.9, 62.8, 21.6, 21.5, 21.4, 21.3. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 400.3. **MS** (ESI negative) *m/z*: 685.0 [M-H]$^-$

**General Procedure for the synthesis of Compounds 19a-c:**

Na (10.0 eq.) was portion wise added to a solution of **18a-c** (1.0 eq.) in MeOH (2 mL) at room temperature under inert atmosphere ($N_2$). The reaction mixture was stirred at room temperature until complete consumption of the starting material was observed by TLC. The reaction was quenched by addition of Amberlist 120 until the pH was acid. The mixture was filtered and concentrated under vacuum to yield the corresponding derivatives **19a-c.**

*Synthesis of 4-sulfamoyl-N-(4-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)selanyl)phenyl)benzamide* **(19a):**

Following the General Procedure, Na (0.6 mmol) and **18a** (0.6 mmol) gave **19a** as a light yellow solid (42%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 10.48 (1H, bs), 8.14 (2H, d, $J$ = 8.31 Hz), 7.99 (2H, d, $J$ = 8.29 Hz), 7.75 (2H, d, $J$ = 8.56 Hz), 7.64 (2H, d, $J$ = 8.53 Hz), 7.56 (2H, bs), 4.96 (7H, bs), 4.82 (1H, d, $J$ = 9.71 Hz), 3.72 (1H, d, $J$ = 11.27 Hz), 3.50-3.45 (1H, m), 3.24-3.11 (4H, m). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 165.4, 147.5, 139.1, 138.7, 134.7, 129.3, 126.6, 124.4, 121.6, 85.6, 83.1, 79.1, 74.1, 70.8, 62.0. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 396.9. **MS** (ESI negative) *m/z*: 517.0 $[M-H]^-$

*Synthesis of 4-chloro-3-sulfamoyl-N-(4-(((2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)selanyl)phenyl)benzamide* **(19b):**

Following the General Procedure, Na (0.6 mmol) and **18b** (0.6 mmol) gave **19b** as a light yellow solid (42%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 10.59 (1H, bs), 8.56 (1H, d, $J$ = 1.91 Hz), 8.21 (1H, dd, $J$ = 8.26, 1.91 Hz), 7.78 (2H, bs), 7.73 (2H, d, $J$ = 8.62 Hz), 7.64 (2H, d, $J$ = 8.60 Hz), 5.04 (6H, bs), 4.82 (1H, d, $J$ = 9.78 Hz), 3.72 (1H, d, $J$ = 10.73 Hz), 3.50-3.47 (1H, m), 3.24-3.11 (4H, m). **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 164.4, 142.1, 138.9, 134.7, 134.4, 133.7, 132.9, 132.6, 129.4, 124.6, 122.2, 121.8, 85.6, 83.1, 79.0, 74.1, 70.8, 62.0. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 397.0. **MS** (ESI negative) *m/z*: 550.9 $[M-H]^-$

*Synthesis of 4-sulfamoyl-N-(4-(((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)selanyl)phenyl)benzamide* **(19c):**

Following the General Procedure, Na (0.6 mmol) and **18c** (0.6 mmol) gave **19c** as a light yellow solid (42%). **$^1$H NMR** (DMSO-$d_6$, 400 MHz) $\delta$ (ppm): 10.59 (1H, bs), 8.13 (2H, d, $J$ = 8.37 Hz), 7.99 (2H, d, $J$ = 8.36 Hz), 7.74 (2H, d, $J$ = 8.62 Hz), 7.62 (2H, d, $J$ = 8.59 Hz), 7.55 (2H, bs), 4.80

(1H, d, $J$ = 9.71 Hz), 4.15 (4H, bs), 3.75 (1H, d, $J$ = 2.73 Hz), 3.56-3.50 (3H, m), 3.45 (1H, t, $J$ = 5.97 Hz), 3.35 (1H, dd, $J$ = 8.97, 3.10 Hz) . **$^{13}$C NMR** (DMSO-$d_6$, 100 MHz) $\delta$ (ppm): 165.4, 147.4, 138.8, 138.7, 134.2, 129.2, 126.6, 125.1, 121.7, 86.5, 81.3, 75.5, 71.0, 69.3, 61.5. **$^{77}$Se NMR** (DMSO-$d_6$, 76 MHz) $\delta$ (ppm): 397.3. **MS** (ESI negative) *m/z*: 517.1 $[M-H]^-$

**Carbonic anhydrase inhibition assay.**

An Applied Photophysics stopped-flow instrument was used for assaying the CA catalyzed $CO_2$ hydration activity [32]. 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 $Na_2SO_4$ (for maintaining constant 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. 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 [36, 37].

*Crystallization and X-ray data collection*

Crystals of hCA II 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 100K using a solution obtained by adding 15% (v/v) glycerol to the mother liquor solution as cryoprotectant. Data on crystals of the complexes were collected using synchrotron radiation at the 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 [38]. Data processing statistics were showed in supporting information.

*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 [39]. 5% of the

unique reflections were selected randomly and excluded from the refinement data set for the purpose of Rfree calculations. The initial |Fo - Fc| difference electron density maps unambiguously showed the inhibitor molecules. The inhibitor was introduced in the model with 1.0 occupancy. Refinements proceeded using normal protocols of positional, isotropic atomic displacement parameters alternating with manual building of the models using COOT [40]. The quality of the final models were assessed with COOT and RAMPAGE [41]. Crystal parameters and refinement data are summarized in Electronic Supplementary Information (ESI). Atomic coordinates were deposited in the Protein Data Bank (PDB accession code: 7ZWB). Graphical representations were generated with Chimera [42].

*Maximal electroshock induced seizures in mice*

For experiments in mice, we used male C57BL/6 mice weighing 22-28 g (8 weeks old, Envigo Italy SRL, Correzzana, Milano, Italy), housed in groups of 8-10 per cage under stable conditions of humidity (60 ± 5%) and temperature (21 ± 2°C). The animals were maintained on a 12 h light and 12 h dark cycle (lights on at 7:00 pm). Food and water were provided ad libitum. All experimental protocols and animal handling procedures were conducted in conformity with international and national law and policies (EU Directive 2010/63/EU for animal experiments, ARRIVE guidelines, and the Basel declaration, including the "3R" concept). The experimental protocols and the procedures reported herein were approved by the Animal Care Committee of the University of Catanzaro, Italy. All efforts were made to minimize animal suffering and to use only

the number of animals necessary to produce reliable scientific data. Maximal electroshock seizures were induced in mice via ear-clip electrodes by a constant supra-threshold current (rectangular 20-ms impulses, 80 mA, 60Hz, 0.2 s duration) [43, 44]. The prevention of the hindlimb tonic extensor was set as the protection endpoint. The dose-response curves were estimated by testing 5 doses and 10 mice per dose for each compound. The calculation of $ED_{50}$ values was statistically performed according to the method of Litchfield and Wilcoxon [45], as previously described [43].

## ASSOCIATED CONTENT

The Supporting Information is available free of charge at https://pubs.acs.orgXXX. SMILES representation for compounds (CSV), $^{1}$H-NMR, $^{13}$C-NMR and $^{77}$Se-NMR spectra of final compounds, Inhibition data of final compounds on hCA isoform III (**Table S1**), Summary of Data Collection and Atomic Model Refinement Statistics for hCA II/**8a** (**Table S2**), Electron density of **8a** bound in and out the hCA II active site (**Figure S1**). Authors will release the atomic coordinates upon article publication.

## AUTHOR INFORMATION

### Corresponding authors

**Dr. Andrea Angeli**. NEUROFARBA Department, Sezione di Scienze Farmaceutiche e Nutraceutiche, Università degli Studi di Firenze, 50019 Sesto Fiorentino (Florence), Italy. E-mail address: andrea.angeli@unifi.it

**Dr. Fabrizio Carta** (FC). NEUROFARBA Department, Sezione di Scienze Farmaceutiche e Nutraceutiche, Università degli Studi di Firenze, 50019 Sesto Fiorentino (Florence), Italy; E-mail address: fabrizio.carta@unifi.it

**Authors**

**Marta Ferraroni**. Dipartimento di Chimica "Ugo Schiff", Università degli Studi di Firenze, Via della Lastruccia 3-13, 50019 Sesto Fiorentino (Florence), Italy.

**Carlotta Granchi**. Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126 Pisa (Italy).

**Filippo Minutolo**. Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126 Pisa (Italy).

**Xiaozhuo Chen**. Edison Biotechnology Institute, Department of Biological Sciences, Molecular and Cellular Biology Program, Ohio University, Athens, OH 45701 (USA).

**Pratik Shriwas**. Edison Biotechnology Institute, Department of Biological Sciences, Molecular and Cellular Biology Program, Ohio University, Athens, OH 45701 (USA).

**Emilio Russo**. University of Catanzaro, School of Medicine, Science of Health Department, FAS@UMG Research Center, Catanzaro, Italy.

**Silvia Selleri**. NEUROFARBA Department, Sezione di Scienze Farmaceutiche e Nutraceutiche, Università degli Studi di Firenze, 50019 Sesto Fiorentino (Florence), Italy

**Claudiu T. Supuran**. NEUROFARBA Department, Sezione di Scienze Farmaceutiche e Nutraceutiche, Università degli Studi di Firenze, 50019 Sesto Fiorentino (Florence), Italy

**Author's Contribution**

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

## Abbreviations

CA, Carbonic Anhydrase; CAI, Carbonic Anhydrase Inhibitors; CNS, central nervous system; GLUT, glucose transporter; GLUT1-DS, glucose transporter type 1 deficiency syndrome;

**KEYWORDS:** carbonic anhydrase inhibitors; metalloenzymes; glucose uptake; epilepsy; glucose transporter type 1 deficiency syndrome.

## References


1. Mueckler, M.; Caruso, C.; Baldwin, S. A.; Panico, M.; Blench, I.; Morris, H. R.; Allard, W. J.; Lienhard, G. E.; Lodish, H. F. Sequence and structure of a human glucose transporter. *Science* **1985**, *229*, 941–945.

2. Thorens, B.; Mueckler, M. Glucose transporters in the 21st century. *Am. J. Physiol. Endocrinol. Metab.* **2010**, *298*, E141–E145.

3. Kasahara, M.; Hinkle, P. C. Reconstitution and purification of the D-glucose transporter from human erythrocytes. *J. Biol. Chem.* **1977**, *252*, 7384–7390.

4. Dick, A. P.; Harik, S. I.; Klip, A.; Walker, D. M. Identification and characterization of the glucose transporter of the blood-brain barrier by cytochalasin B binding and immunological reactivity. *Proc. Natl Acad. Sci. USA*, **1984**, *81*, 7233–7237.

5. Pardridge, W. M.; Boado, R. J.; Farrell, C. R. Brain-type glucose transporter (GLUT-1) is selectively localized to the blood-brain barrier. Studies with quantitative western blotting and in situ hybridization. *J. Biol. Chem.* **1990**, *265*, 18035–18040.

6. Maher, F.; Vannucci, S. J.; Simpson, I. A. Glucose transporter proteins in brain. *FASEB J.* **1994**, *8*, 1003–1011.

7. Klepper, J.; Wang, D.; Fischbarg, J.; Vera, J. C.; Jarjour, I. T.; O'Driscoll, K. R.; De Vivo, D. C. Defective glucose transport across brain tissue barriers: a newly recognized neurological syndrome. *Neurochem. Res.* **1999**, *24*, 587–594.

8. Meireles, P.; Sales-Dias, J.; Andrade, C. M.; Mello-Vieira, J.; Mancio-Silva, L.; Simas, J. P.; Staines, H. M.; Prudêncio, M. GLUT1-mediated glucose uptake plays a crucial role during Plasmodium hepatic infection. *Cell Microbiol.* **2017**, *2*, e12646.

9. Matsumoto, T.; Jimi, S.; Migita, K.; Takamatsu, Y.; Hara, S. Inhibition of glucose transporter 1 induces apoptosis and sensitizes multiple myeloma cells to conventional chemotherapeutic agents. *Leuk. Res.* **2016**, *41*, 103–110.

10. Rungaldier, S.; Oberwagner, W.; Salzer, U.; Csaszar, E.; Prohaska, R. Stomatin interacts with GLUT1/SLC2A1, band 3/SLC4A1, and aquaporin-1 in human erythrocyte membrane domains. *Biochim Biophys Acta* **2013**, *1828*, 956–966.

11. Flier, J. S.; Mueckler, M. M.; Usher, P.; Lodish, H. F. Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. *Science* **1987**, *235*, 1492–1495.

12. Yamamoto, T.; Seino, Y.; Fukumoto, H.; Koh, G.; Yano, H.; Inagaki, N.; Yamada, Y.; Inoue, K.; Manabe, T.; Imura, H. Over-expression of facilitative glucose transporter genes in human cancer. *Biochem. Biophys. Res. Commun.* **1990**, *170*, 223–230.

13. Navale, A. Glucose transporters and their cellular form, role and function. In *Mol. Nutrition: Carbohydrates*. **2019**, 21-34.

14. Deng, D.; Xu, C.; Sun, P.; Wu, J.; Yan, C.; Hu, M.; Yan, N. Crystal structure of the human glucose transporter GLUT1. *Nature* **2014**, *7503*, 121-125.

15. Maiden, M. C.; Davis, E. O.; Baldwin, S. A.; Moore, D. C.; Henderson, P. J. Mammalian and bacterial sugar transport proteins are homologous. *Nature* **1987**, *325*, 641–643.

16. Amann, T.; Maegdefrau, U.; Hartmann, A.; Agaimy, A.; Marienhagen, J.; Weiss, T. S.; Stoeltzing, O.; Warnecke, C.; Schölmerich, J.; Oefner, P. J.; Kreutz, M.; Bosserhoff, A. K.; Hellerbrand, C. GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis. *Am J Pathol* **2009**, *174*, 1544–1552.

17. Pragallapati, S.; Manyam, R. Glucose transporter 1 in health and disease. *J. Oral. Maxillofac. Pathol.* **2019**, *3*, 443.

18. Galochkina, T.; Ng Fuk Chong, M.; Challali, L.; Abbar, S.; Etchebest, C. New insights into GluT1 mechanics during glucose transfer. *Sci Rep*. **2019**, *1*, 998.

19. Zezina, E.; Sercan-Alp, O.; Herrmann, M.; Biesemann, N. Glucose transporter 1 in rheumatoid arthritis and autoimmunity. *Wiley Interdiscip Rev. Syst. Biol. Med*. **2020**, *4*, e1483.

20. Klepper, J.; Akman, C.; Armeno, M.; Auvin, S.; Cervenka, M.; Cross, H. J.; De Giorgis, V.; Della Marina, A.; Engelstad, K.; Heussinger, N.; Kossoff, E. H.; Leen, W. G.; Leiendecker,

B.; Monani, U. R.; Oguni, H.; Neal, E.; Pascual, J. M.; Pearson, T. S.; Pons, R.; Scheffer, I. E.; Veggiotti, P.; Willemsen, M.; Zuberi, S. M.; De Vivo, D. C. Glut1 Deficiency Syndrome (Glut1DS): State of the art in 2020 and recommendations of the international Glut1DS study group. *Epilepsia Open*, **2020**, *3*, 354-365.

21. De Vivo, D. C.; Trifiletti, R. R.; Jacobson, R. I.; Ronen, G. M.; Behmand, R. A.; Harik, S.I. Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. *N. Engl. J. Med*. **1991**, *10*, 703–709.

22. Chambon, R.; Vuillaumier-Barrot, S.; Seta, N.; Wagner. S.; Sarret, C. Partial effectiveness of acetazolamide in a mild form of GLUT1 deficiency: a pediatric observation. *Mov. Disord*. **2013**, *12*, 1749–1751.

23. Tchapyjnikov, D.; Mikati, M. A. Acetazolamide-responsive episodic ataxia without baseline deficits or seizures secondary to GLUT1 deficiency: a case report and review of the literature. *Neurologist*. **2018**, *1*, 17–18.

24. Scozzafava, A.; Supuran, C. T.; Carta, F. Antiobesity carbonic anhydrase inhibitors: a literature and patent review. *Expert Opin Ther Pat*. **2013**, *6*, 725-735.

25. Man-Un Ung, P.; Song, W.; Cheng, L.; Zhao, X.; Hu, H.; Chen, L.; Schlessinger, A. Inhibitor discovery for the human GLUT1 from homology modeling and virtual screening. *ACS Chem. Biol*. 2016, *7*, 1908-1916.

26. Al-Tamimi, A. S.; Etxebeste-Mitxeltorena, M.; Sanmartín, C.; Jiménez-Ruiz, A.; Syrjänen, L.; Parkkila, S.; Selleri, S.; Carta, F.; Angeli, A.; Supuran, C. T. Discovery of new organoselenium compounds as antileishmanial agents. *Bioorg. Chem*. **2019**, *86*, 339-345.

27. Jana, M.; Misra, A. K. Stereoselective synthesis of β-glycosyl thiols and their synthetic applications. *J. Org. Chem*. 2013, *6*, 2680-2686.

28. Orita, A.; Hamada, Y.; Nakano, T.; Toyoshima, S.; Otera, J. Highly efficient deacetylation by use of the neutral organotin catalyst [tBu2SnOH(Cl)]2. *Chemistry* **2001**, *15*, 3321-3327.

29. Karalı, N.; Akdemir, A.; Göktaş, F.; Eraslan, E. P.; Angeli, A.; Kızılırmak, M.; Supuran, C. T. Novel sulfonamide-containing 2-indolinones that selectively inhibit tumor-associated alpha carbonic anhydrases. *Bioorg. Med. Chem.* **2017**, *25*, 3714-3718.

30. Pacchiano, F.; Aggarwal, M.; Avvaru, B. S.; Robbins, A. H.; Scozzafava, A.; McKenna, R.; Supuran, C. T. Selective hydrophobic pocket binding observed within the carbonic anhydrase II active site accommodate different 4-substituted-ureido-benzenesulfonamides and correlate to inhibitor potency. *Chem Commun (Camb).* **2010**, *44*, 8371-8373.

31. 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.* **2011**, *6*, 1896-1902.

32. Kalifah, 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.* **1971**, *246*, 2561–2573.

33. Tuccinardi, T.; Granchi, C.; Iegre, J.; Paterni, I.; Bertini, S.; Macchia, M.; Martinelli, A.; Qian, Y.; Chen, X.; Minutolo, F.; *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6923-6927.

34. Liu, Y.; Cao, Y.; Zhang, W.; Bergmeier, S.; Qian, Y.; Akbar, H.; Colvin, R.; Ding, J.; Tong, L.; Wu, S.; Hines, J.; Chen, X. A small-molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth in vitro and in vivo. *Mol. Cancer Ther.* **2012**, *11*, 1672-1682.

35. Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? *Chem Rev*. **2012**, *8*, 4421-4468.

36. Berrino, E.; Micheli, L.; Carradori, S.; di Cesare Mannelli, L.; Guglielmi, P.; De Luca, A.; Carta, F.; Ghelardini, C.; Secci, D.; Supuran, C. T. Novel insights on CAI-CORM hybrids: evaluation of the CO releasing properties and pain-relieving activity of differently substituted coumarins for the treatment of rheumatoid arthritis. *J. Med. Chem*. **2023**, *3*, 1892-1908.

37. Combs, J.; Bozdag, M.; Cravey, L. D.; Kota, A.; McKenna, R.; Angeli, A.; Carta, F.; Supuran, C. T. New insights into conformationally restricted carbonic anhydrase inhibitors. *Molecules*. **2023**, *2*, 890.

38. 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**, 41-51.

39. Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr D Biol Crystallogr.* **1997***, 53*, 240-255.

40. Emsley, P.; Lohkamp, B.; Scott, W.; Cowtan, K. Features and development of Coot. *Acta Crystallogr D Biol Crystallogr.* **2010**, *66*, 486-501.

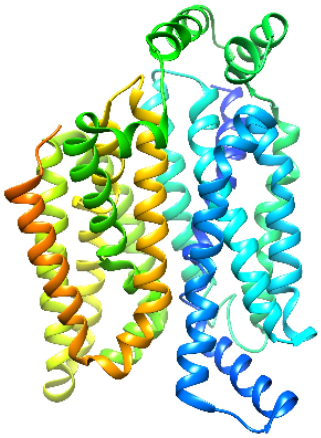
41. Lovell, S. C.; Davis, I. W.; Arendall III, W. B.; de Bakker, P. I. W.; Word, J.M.; Prisant, M. G.; Richardson, J. S.; Richardson, D. C. Structure validation by Cα geometry: ϕ,ψ and Cβ deviation. *Proteins* **2003**, 50, 437-450.

42. 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.,* **2004**, *25*, 1605-1612.

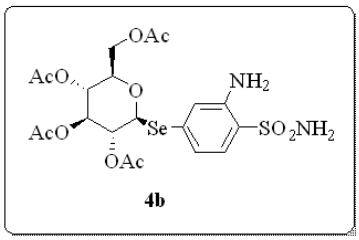
43. Citraro, R.; Scicchitano, F.; De Fazio, S.; Raggio, R.; Mainardi, P.; Perucca, E.; De Sarro, G.; Russo, E. Preclinical activity profile of α-lactoalbumin, a whey protein rich in tryptophan, in rodent models of seizures and epilepsy. *Epilepsy Res*. **2011**, *1-2*, 60-69.

44. Fischer, W.; Kittner, H.; Regenthal, R.; Russo, E.; De Sarro, G. Effects of piracetam alone and in combination with antiepileptic drugs in rodent seizure models. *J. Neural. Transm*. **2004**, *9*, 1121-1139.

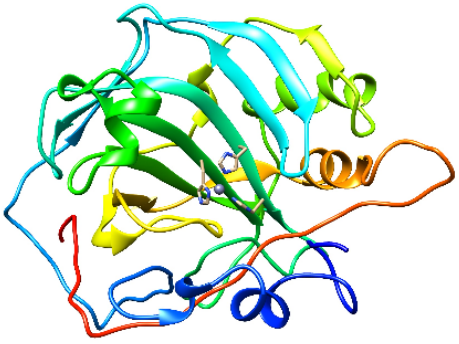
45. Litchfield Jr., J. T.; Wilcoxon, F. A. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther*. **1949**, *96*, 99-113.



GLUT1




4b



CAII