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Tellurides bearing benzensulfonamide as carbonic anhydrase inhibitors with potent antitumor activity

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ABSTRACT

We evaluated in vitro a series of telluride containing compounds bearing the benzenesulfonamide group, as effective inhibitors of the physiologically relevant human (h) expressed Carbonic Anhydrase (CA; EC 4.2.1.1) enzymes I, II, IV VII and IX. The potent effects of such compounds against the tumor-associated hCA IX being low nanomolar inhibitors (K_I 2.2 to 2.9 nM) and with good selectivity over the ubiquitous hCA II, gave the possibility to evaluate their lethal effect in vitro against a breast cancer cell line (MDA-MB-231). Among the series, both compounds **3a** and **3g** induced significant toxic effects against tumor cells after 48 h incubation. Under normoxic condition **3a** showed high efficacy killing over 94% of tumor cells at 1 μM, and derivative **3g** reached the tumor cell viability under the 5% at 10 μM. In hypoxic condition, these two compounds showed less effective although retained excellent cancer cell killer. These unusual features make them interesting lead compounds acting as antitumor agents also in tumor types not dependent from hCA IX overexpression.

During the last twenty years, the biological chemistry of tellurium has fueled a renewed interest in this element with a widely range of interesting attempts to exploit its unique properties associated in diagnostics and drugs development [1–3]. Tellurium has metal like proprieties and the development of innovative new materials, such as fluorescent CdTe quantum dots employed in biological detection [4,5], Te-based nanomaterials founded applications in photodynamic therapy (PDT) for tumor growth inhibition interacting with light producing typically reactive oxygen species (ROS) [6–9]. On the other hand, tellurium bio-chemistry is far less recognized than the other chalcogen atoms, although, it generate compounds structurally related to their sulfur or selenium analogues, exhibit different property and reactivity. Historical applications of this element is founded in the treatment of microbial infections prior to the discovery of antibiotics [10,11]. More recently, several organotellurium derivatives exhibited strong antioxidant propriety due to the close connection between the redox chemistry of oxygen, sulfur and selenium, provided the platform for a powerful, tellurium-centered redox catalysis which involves oxygen and sulfur compounds [12,13]. One of this is GPx-like catalytic properties, which are often superior to those of their selenium analogues [14–16]. Interestingly, tellurium compounds may not only weaken the cell's antioxidant defense, but also actively generate ROS, as has been observed for related selenium compounds and this oxidative burst, may explained some of the toxic effects associated with tellurium, including radical generation, mutagenesis, neurotoxicity and interference with DNA repair [17–19]. In this context, the use of organotellurium derivatives as novel antitumor agents gaining attention in medicinal chemistry [20,21] including our group which is involved in the employment of different organotelluride scaffolds as modulators of the Carbonic Anhydrases (CAs, EC 4.2.1.1) [22,23]. These metalloenzymes catalysed the reversible hydration of carbon dioxide to bicarbonate and proton [24]. Focusing on tumorigenicity, this simple reaction contributed in

Abbreviations: CAI(s), carbonic anhydrase inhibitor(s); AAZ, acetazolamide; (h) CA, (human) carbonic anhydras; K_I, inhibition constant.

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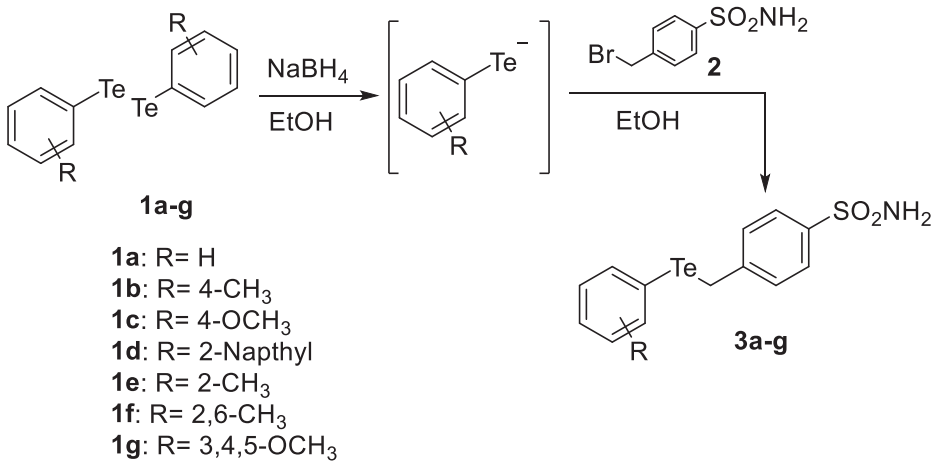

Scheme 1. Synthesis of tellurides bearing benzenesulfonamide moiety **3a-g**.

Table 1
Inhibition data of human CA isoforms I, II, IV, VII and IX with compounds **3a-g** and **AAZ** by a stopped flow CO_2 hydrase assay [31].

	K_I (nM)*					
Cmp	hCA I[a]	hCAII[a]	hCAIV	hCAVII	hCAIX	Selectivity ratio hCA II/IX
3a	209.8	12.2	52.0	8.4	2.4	5.08
3b	24.0	4.4	1.9	0.54	2.2	2.00
3c	1.5	2.0	2.1	0.3	2.6	0.77
3d	2401	182.3	828.2	62.7	2.9	62.86
3e	18.0	0.67	1.8	0.76	2.6	0.26
3f	256.6	3.3	5.1	2.7	2.5	1.32
3g	377.1	13.2	6925	55.4	2.4	5.5
AAZ	250	12.1	74.0	2.5	25.7	0.47

* Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5–10% of the reported values). [a] ref. [30].

hypoxic tumor cell biochemistry acidification showing to induce immunosuppression and to contribute significantly to resistance to chemotherapy and radiotherapy [25–28]. In particular, one of the 15 isoforms present in humans, i.e. the CA IX, was found overexpressed in a wide selection of hypoxic tumors and, for this reason, it was validated as a pharmaceutical target [29].

Compounds **3a-g** are synthesized according to our previous reported procedures [30]. Briefly, diphenylditellurides **1a-g** are reduced with $NaBH_4$ and treated *in situ* with 4-(bromomethyl)benzenesulfonamide (**2**) affording telluride derivatives **3a-g** in excellent yield (Scheme 1).

All tellurides bearing sulfonamide **3a-g** and acetazolamide (**AAZ**) are investigated for their inhibition activity against different human CAs such as I, II, IV, VII and IX by the stopped-flow carbon dioxide hydration assay [31] as outlined in Table 1.

On the basis of the kinetic data reported in Table 1, tellurides bearing benzensulfonamide **3a-g** showed remarkable variation among the different CA isoforms due to the systematically substitutions on the aromatic ring shown to be to be essential for the activity and selectivity. Comparing the inhibition data of compound **3a**, without substituents, was generally less strong CA inhibitor than the corresponding compounds with *para* and *ortho* substituents **3b**, **3c**, **3e** and **3f**. On the other hand, a more bulky scaffold such as naphthalene (**3d**) or trimethoxybenzene (**3f**) showed weakly inhibition against hCA I and hCA IV spanning the kinetic constants in high nanomlar to micromolar range. The cytosolic hCA VII was strong inhibited by several compounds (**3b**, **3c** and **3e**) reaching sub nanomolar range (K_i 0.3 to 0.76 nM). An interesting case is observed for hCA IX, where the different substitutions on the aromatic ring did not affect the potency against this isoform showed the same strong inhibition activity (K_i 2.2 to 2.9 nM). This particular feature has allowed us to obtain some compounds with a good selectivity versus the tumor associate isoform hCA IX such as **3a** and **3f** with a selectivity ratio of 5 and 5.5 respectively and compound **3d** reaching 62.86 times more selective against the tumor isoform.

hCA IX is predominantly founded in tumor cells and showed a rather limited diffusion in normal cells participating significantly in the extracellular acidification with the concomitant alkalization of the cytosol [32]. In this context, two tellurides bearing sulfonamide (**3a** and **3g**) are evaluated against the human adenocarcinoma cell line MDA-MB-231 where the expression of hCA IX is higher than other tumor cell lines

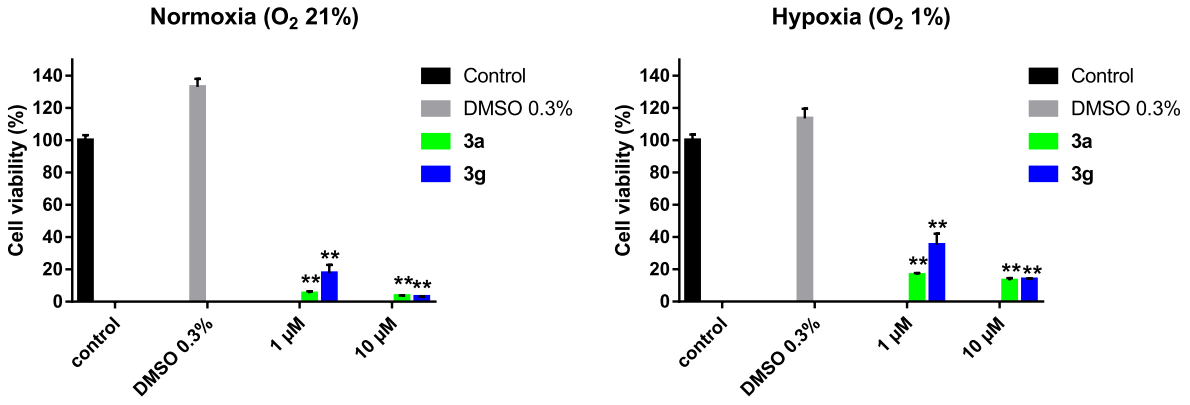

Fig. 1. Effects of telluride derivatives **3a** and **3g** on viability of the human adenocarcinoma cell line MDA-MB231 following 48 h treatment in normoxic (21% O_2,) and hypoxic (1% O_2,) conditions. ** $p < 0.001$ versus control.

and increased with the degree of hypoxia [33]. The selected compounds are tested at 1 and 10 μM concentrations, incubated for 48 h both under normoxic and hypoxic conditions (Fig. 1).

Compound **3a** showed strong activity in normoxia condition, at 1 μM killing over 90% of tumor cells and, increasing the concentration to 10 μM, the cell viability decreased under the 5% threshold. Compound **3g** is observed slightly less powerful than **3a** with a cell viability at 1 μM of 18% but the same effect at 10 μM with a threshold of 3%. In hypoxic condition both compounds decreased their effects against tumor cells showed for compound **3a** a cell viability at 1 μM of 16% and increasing at 10 μM of 13%. On the other hand, compound **3g** at 1 μM killed 65% of tumor cells and at 10 μM killed over the 85% of cells. Although less powerful in hypoxic condition, these compounds showed a strong efficacy in both conditions. This particular feature can be explained by the redox propriety of these compounds modulating the levels of oxidative agents. In this particular condition, however, the interesting data against normoxic conditions on MDA-MB231 showed their effects acts on different pathways and not only against hCA IX, giving the possibility to explore these derivatives against other kinds of tumors.

In conclusion, we explored tellurides bearing sulfonamide **3a-g** as novel and potent carbonic anhydrase IX inhibitors. This excellent potency of inhibition against the tumor associated isoform gives an opportunity to explore them as possible antitumor agents. Two tellurides bearing sulfonamide (**3a** and **3g**) were evaluated against the human adenocarcinoma cell line MDA-MB-231 where the expression of hCA IX is higher than other tumor cell lines and increased with the degree of hypoxia showing strong lethal effects on cancer cells both in normoxic and hypoxic conditions. The less power in hypoxic condition give, in addition, interesting opportunity to explore different tumors where there is no overexpression of hCA IX.

Notes

Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument was used for assaying the CA catalyzed CO_2 hydration activity [31]. 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 [23]. Enzymes concentrations ranged between 5 and 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 [34–38].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128147.

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