First evaluation of organotellurium derivatives as carbonic anhydrase I, II, IV, VII and IX inhibitors

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Abstract. A series of tellurides was evaluated as carbonic anhydrase (CA, EC 4.2.1.1) inhibitors against the human (h) carbonic anhydrase isoforms hCA I, II, IV, VII and IX, involved in a variety of diseases, including glaucoma, retinitis pigmentosa, epilepsy, arthritis and tumors. These compounds, which are the first tellurium-containing derivatives acting as inhibitors of carbonic anhydrase enzymes, showed effective inhibition against all isoforms investigated and some of them were selective for inhibiting the cytosolic or the membrane-bound CAs. Thus, these carbonic anhydrase inhibitors are interesting leads for the development of isoform-selective inhibitors.

Keywords: carbonic anhydrase; inhibitor, metalloenzymes, tellurium, telluride

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1. Introduction

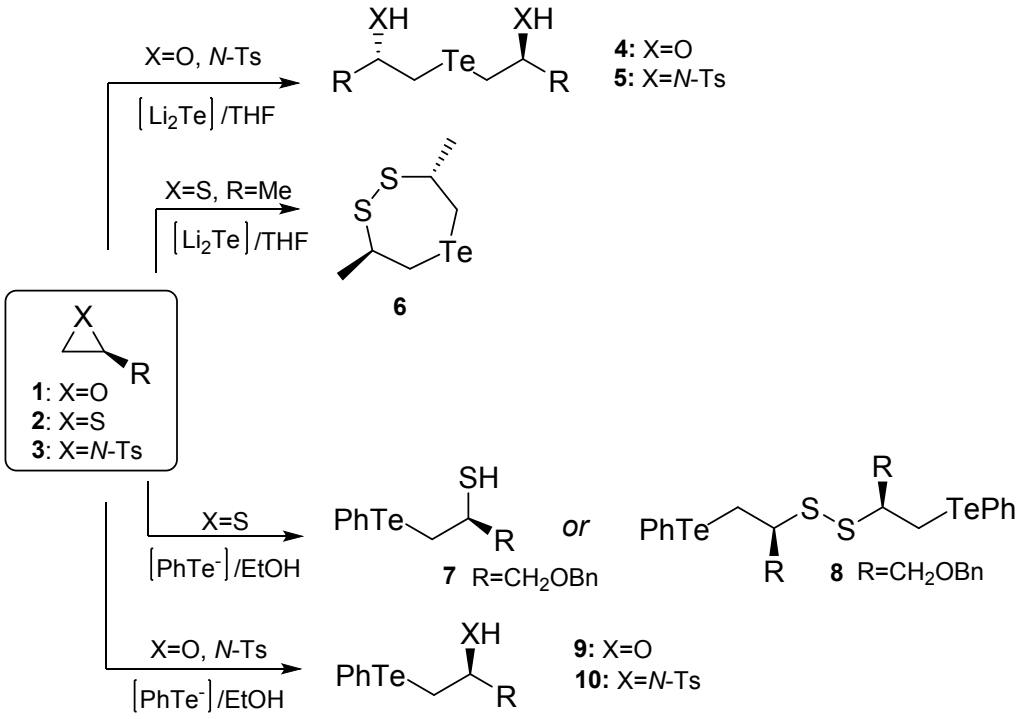
Tellurium is a rare element and, unlike the other group VI members (e.g., O, S, and Se), which have many biological applications, this element has not any known such function.[1]During the last decades, inorganic tellurium derivatives were employed for the development of innovative materials, such as fluorescent CdTe quantum dots as probes in biological detection,[2,3] telluride clusters, nanoparticles and nanotubes, which found potential applications in the electronics and medicine fields.[4-6] At the same time, a range of organotellurium compounds were developed, being shown that they possess a range of unique properties such as interesting activity against pathogenic microorganisms,[7,8] inhibition of cancer cells growth,[9-11] potent caspase and cathepsin inhibitory propieties[12] and antioxidant activity, which are often superior to those of the selenium analogues.[13,14] In this particular context, we should mention carbonic anhydrase (CAs, EC 4.2.1.1) an enzyme that plays an important role in many physiological and pathological processes associated with pH control, ion transport, fluid secretion and biosynthetic reactions.[19-21] Recent studies from our group[15] reported that some of the many CA isoforms known to date, more precisely, the human (h) isoform hCA VII, was involved in the oxidative stress defence processes, interfering with the generation of reactive oxygen species (ROS).[16–17] Furthermore, isoform hCA IX, which is overexpressed in a wide range of hypoxic tumors, where it participates to the survival, proliferation, and metastasis processes, was recently investigated by our groups for its interaction with selenium-containing CA inhibitors (CAIs).[18] For these reasons we decided to also investigate various Te(II) derivatives as human (h) CA inhibitors (CAIs).

2. Results and discussion

2.1. Chemistry

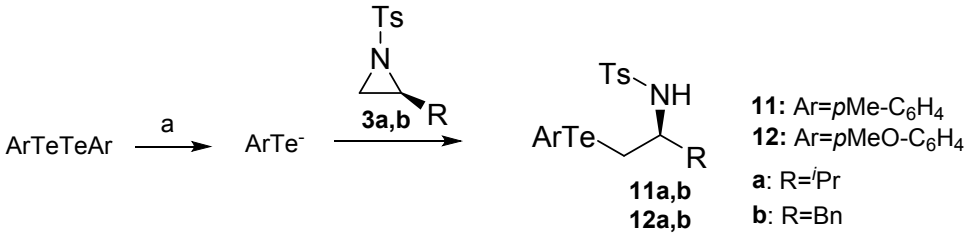
Our long dated interest in the study of chalcogen-containing compounds[22] led us to disclose synthetic routes towards organoselenides[23] and, more recently, organotellurides[24] through the ring opening of

strained heterocycles with selenium and tellurium nucleophilic species. Some of these novel organic chalcogenides exhibited interesting antioxidant properties.[25] With the aim to evaluate the CA inhibitor activity of a series of Te-containing compounds, a wide variety of functionalized dialkyl and aryl-alkyl tellurides has thus been obtained following the **Scheme 1**. Dialkyl substituted β -hydroxy- and β -amino-tellurides **4** and **5** were synthesised by treating Li_2Te with epoxides **1** and aziridines **3**, respectively. Through the same route, dithiatellurepane **6** was achieved from thiirane **2**. β -Phenyltelluro thiol **7** and the corresponding disulfide **8** were selectively obtained from thiirane **2** and diphenyl ditelluride. Reaction of $PhTe^-$, generated through reduction of PhTeTePh, with epoxides and aziridines led smoothly to the formation of alcohols **9** and amines **10.**



Scheme 1: Synthesis of β-functionalized tellurides. Li_2Te is generated in situ from elemental Te (0.5 eq.) and $LiEt_3BH$ (1.0 eq.). $PhTe^-$ is generated in situ from PhTeTePh (0.5 eq.) and $NaBH_4$ (1.5 eq.).

Following a similar synthetic approach, novel chiral *N*-Tosyl β -aryltelluro amines were synthesised from diaryl ditellurides and enantioenriched aziridines, obtained from natural aminoacids, through a high regioselective and enantiospecific ring opening route (**Scheme 2**).



Scheme 2: Synthesis of β-aryltelluro amines. Reagent and conditions: a) ArTeTeAr (0.5 eq.) , $NaBH_4$ (1.5 eq.), EtOH, 0°C.

The synthesised Te-containing CA inhibitors candidates are reported in **Chart 1**.

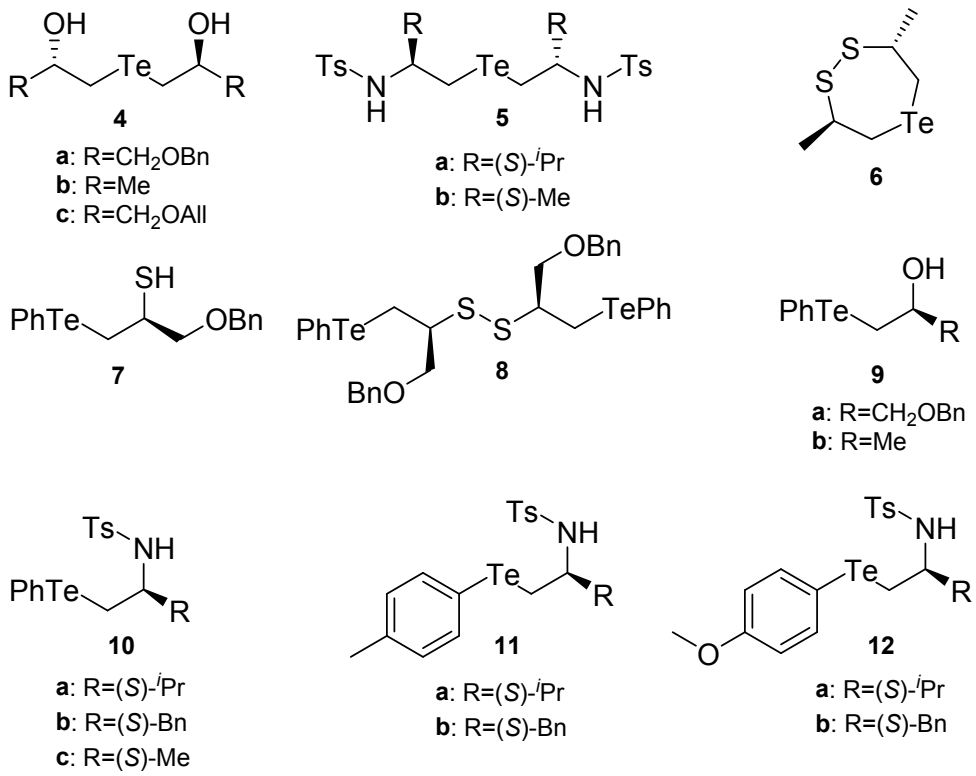


Chart 1: Tellurium-containing chemotypes investigated as CA inhibitors in this work

2.2. Carbonic anhydrase inhibition

All compounds **4a-12b** were tested in vitro for their inhibitory activity against the physiologically relevant hCA isoforms I, II, IV, VII and IX by means of the stopped-flow carbon dioxide hydration assay[26] and their activities were compared to the standard CAI acetazolamide (**AAZ**) (**Table 1**).

Table 1. Inhibition data of human CA isoforms I, II, IV, VII and IX with compounds **4a-12b** and **AAZ** by a stopped flow CO_2 hydrase assay.[26]

	K_I (μM)*				
Cmp	hCA I	hCA II	hCA IV	hCA VII	hCA IX
4a	>100	>100	>100	>100	>100
4b	>100	>100	>100	>100	>100
4c	>100	>100	>100	>100	>100
5a	0.71	0.03	60.8	0.62	3.4
5b	18.0	0.09	>100	5.3	19.7
6	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100
8	>100	>100	>100	>100	>100
9a	>100	>100	>100	>100	>100
9b	>100	>100	>100	>100	>100
10a	27.3	35.8	85.8	3.2	1.5
10b	>100	56.8	>100	4.8	11.0
10c	76.5	61.6	>100	0.05	>100
11a	>100	41.6	>100	5.0	0.7
11b	>100	31.4	>100	3.2	2.0
12a	>100	56.3	>100	4.1	2.3
12b	>100	56.1	>100	4.9	11.7
AAZ	0.25	0.012	0.074	0.006	0.025

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

We have investigated a range of telluride and ditelluride derivatives for their interaction with the five hCA here considered, after a period of 15 min of incubation of the enzyme and inhibitor solutions[26–28]. The following structure activity relationship (SAR) may be noted regarding the inhibition data of **Table 1**:

Cytosolic isoforms (hCA I, hCA II and hCA VII) were inhibited by isopropyl substituted amino telluride **5a** in nanomolar range with particulary efficacy against hCA II (K_i 0.03 μM). Indeed, membrane isoforms here considered was inhibited by **5a** in low micromolar range for transmembrane hCA IX (K_i 3.4 μM) and medium micromolar range for hCA IV (K_i 60.4 μM). The inhibition potency decreased when isopropyl moiety was replaced with methyl group (**5b**). On the other hand, the isopropyl moiety increased the selectivity versus hCA II with an activity 200 folds greater than against hCA I and nearly 60 times greater thans against hCA VII. Furthermore, this substitution caused a loss of activity for membrane isoform hCA IV. Dithiatellurepane **6**, β hydroxy tellurides (**4a-c**), β phenyltelluro substituted thiol **7**, disulfide **8** and alcohols **9a-b** did not inhibit the human isoforms here considered. β phenyl telluro amine **10a** showed a good selectivity for hCA IX. This compound was 20 times more potent than two dominant cytosolic isoforms (hCA I hCA II) and 2 folds more active compared to hCA VII. An interesting inhibition profile was observed for compounds **11a** and **12a**, where the introduction of para substituents in the aryl telluro scaffolds led to a loss of activity for hCA I and hCA IV. Moreover, for compound **11a**, CH_3 group in position 4 of the aryl telluride, increased 7 times the selectivity for hCA IX compared to hCA VII. The benzyl moiety in compounds **10b** and **12b** moved the selectivity for hCA VII and caused a loss of inhibitory power against hCA I and hCA IV. Another interesting point, was the inhibitory activity of **10c**. Methyl moiety increased over 1000 folds the selectivity for hCA VII among the other cytosolic isoforms (hCA I and hCA II), and led to loss the activity for the membrane isoforms hCA IV and hCA IX.

3. Conclusions

We have investigated a serie of tellurides as inhibitors on five α-carbonic anhydrases (CAs, EC 4.2.1.1) of pharmacologic relevance, *i.e.*, hCA I, II, IV, VII and IX. These isoforms are drug targets for antiglaucoma (hCA I, II and IV), antiepileptic (hCA VII) or antitumor (hCA IX) agents. In this contest, the investigated organotellurium compounds showed inhibitory action and in same case selectivity against the cytosolic over membrane-bound isoforms for compounds **5a-b** and **10c**. On the other hand, compounds **10a**, **11a-b** and **12a** showed selectivity against the membrane tumor-associated hCA IX over cytosolic isoforms. In the future, it will be interesting to test organotellurium compounds as anti-infectives against key enzymes that are expressed by pathogenic bacteria, such as the β- and γ-carbonic anhydrases from *Burkholderia pseudomallei*[29] and *Francisella tularensis*[30] the etiological agents of meliodosis and tularemia, respectively.

4. Experimental Part

4.1. General

All reactions were carried out in an oven-dried glassware under inert atmosphere (N_2). All commercial materials were used as received without further purification. Flash column chromatography purifications were performed on Silica gel 60 (230-400 mesh). Thin layer chromatography was performed on TLC plates Silica gel 60 F254. NMR spectra were recorded in $CDCl_3$ with Varian Gemini 200, Mercury 400, and Bruker 400 Ultrashield spectrometers operating at 200 and 400 MHz (for ^{1}H), 50 and 100 MHz (for ^{13}C), and 126 MHz (for ^{125}Te). NMR signals were referenced to nondeuterated residual solvent signals (7.26 ppm for 1H, 77.0 ppm for 13C). $(PhTe)_2$ was used as an external reference for ^{125}Te NMR (δ= 420 ppm). ^{1}H NMR data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, ap d = apparent doublet, m = multiplet, dd = doublet of doublet, ecc.), coupling constant (*J*) or line separation (ls), and assignment.

4.1.1 General procedure for the synthesis of β-hydroxy tellurides (4), β-amino tellurides (5) and dithiatellurepane (6).

A suspension of Li_2Te in THF, generated from elemental tellurium (0.5 mmol, 1.0 eq.) and $LiEt_3BH$ (1.0mmol, 2.0 eq.), was treated with the electrophile (epoxide, aziridine or thiirane - 1.0 mmol, 2.0 eq.) and the reaction was stirred for 12 h at ambient temperature. Afterwards, the mixture was diluted with Et_2O (10 mL), filtered through a short plug of celite, washed with NH_4Cl and then with H_2O (2x 5 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was then purified by flash chromatography to yield β-functionalised tellurides.

4.1.2 Procedure for the synthesis of β -phenyltelluro thiol (7)

$NaBH_4$ (28 mg, 0.75 mmol, 3.0 eq.) was portionwise added to a solution of diphenyl ditelluride (102 mg, 0.25 mmol, 1.0 eq.) in EtOH (2 mL) at 0 °C under inert atmosphere (N_2). After 30 min, the thiirane **2** (0.5 mmol, 2.0 eq.) was slowly added and the reaction mixture was stirred at 0 °C and the reaction progress was monitored by TLC. When the starting thiirane had completely reacted (monitored by TLC), 2 mL of a 50% aqueous solution of citric acid were added and the organic phase was extracted with Et_2O (2 x 5 mL), washed with brine (1 x 5 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield β -phenyltelluro thiol **7**.

4.1.3 Procedure for the synthesis of β -phenyltelluro disulfide (8)

$PhTe^-$, generated as described for the synthesis of thiol **7**, was reacted with thiirane **2** at 0 °C and the reaction mixture was allowed to warm to ambient temperature before leaving to react 14 h at the same temperature. Then, 2 mL of H_2O were added and the organic phase was extracted with Et_2O (2 x 5 mL), washed with brine (1 x 5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by flash chromatography to yield β -phenyltellurodisulfides **8**.

4.1.4 General Procedure for the synthesis of β-phenyltelluro alcohols (9) and *N*-Ts protected β-aryltelluro amines (10-12).

$NaBH_4$ (28 mg, 0.75 mmol, 3.0 eq.) was portionwise added to a solution of diaryl ditelluride (0.25 mmol, 1.0 eq.) in EtOH (2 mL) at 0°C under inert atmosphere (N_2). After 30 min, the electrophile (epoxide or aziridine - 0.45 mmol, 1.8 eq.) was slowly added and 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 saturated aq. NH_4Cl (2 mL) and diluted with Et_2O (5 mL). The layers were separated and the organic layer was washed with H_2O (3 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The crude material was purified by flash chromatography to yield β -phenyltelluro alcohols (**9**) or β -aryltelluro amines (**10-12**).

4.1.5 (*S*)-4-methyl-*N*-(3-methyl-1-(phenyltellanyl)butan-2-yl)benzenesulfonamide (10a)

Following the general procedure, diphenyl ditelluride (51 mg, 0.125 mmol) and (*S*)-2-isopropyl-1-tosylaziridine (54 mg, 0.23 mmol) gave after flash chromatography (petroleum ether/Et_2O 7:1) **10a** (80 mg, 78%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ(ppm): 0.74 (3H, d, *J*=6.7 Hz), 0.80 (3H, d, *J*=6.8 Hz), 1.78-1.90 (1H, m), 2.38 (3H, s, CH_3), 2.72 (1H, dd, *J*=6.5, 12.1 Hz, C**H$_a$**H$_b$Te), 3.10 (1H, dd, *J*=4.6, 12.1 Hz, CH$_a$**H$_b$**Te), 3.18-3.20 (1H, m, C**H**NH), 4.75 (1H, d, *J*=8.8 Hz, NH), 7.17-7.20 (4H, m), 7.29-7.33 (1H, m), 7.61-7.63 (4H, m). **^{13}C NMR** (100 MHz, $CDCl_3$) δ(ppm): 14.6 (CH_2Te), 17.5, 19.2, 32.3, 59.3, 110.9 (TeC), 127.0, 128.0, 129.3, 129.6, 137.8, 138.8, 143.2. MS (ESI positive) *m/z* (%): 468 $[M+Na]^+$, (100). Elemental analysis: $C_{18}H_{23}NO_2STe$ Calcd. C 48.58%, H 5.21%, N 3.15%. Found: C 48.49%, H 5.23%, N 3.17%.

4.1.6 (*S*)-4-methyl-*N*-(1-phenyl-3-(phenyltellanyl)propan-2-yl)benzenesulfonamide (10b)

Following the general procedure, diphenyl ditelluride (51 mg, 0.125 mmol) and (*S*)-2-benzyl-1-tosylaziridine (65 mg, 0.23 mmol) gave after flash chromatography (petroleum ether/Et_2O 3:1) **10b** (105 mg, 93%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ(ppm): 2.38 (3H, s, CH_3), 2.74 (1H, dd, *J*=6.8, 13.8 Hz), 2.84-2.89 (2H, m), 3.12 (1H, dd, *J*=4.3, 12.4 Hz), 3.51-3.59 (1H, m, C**H**NH), 4.78 (1H, d, *J*=7.5 Hz, NH), 6.90-6.92 (2H, m), 7.10-7.22 (7H, m), 7.29-7.33 (1H, m), 7.45 (2H, ap d, ls=8.3 Hz), 7.65-7.67 (2H, m). **^{13}C NMR** (100 MHz, $CDCl_3$) δ(ppm): 16.1 (CH_2Te), 21.5, 42.0, 55.3, 111.1 (TeC), 126.7, 127.0, 128.0, 128.6, 129.2, 129.4, 129.5, 136.5, 137.0, 138.6, 143.1. **^{125}Te NMR** (126 MHz, $CDCl_3$) δ(ppm) : 386.4. MS (ESI positive) *m/z* (%): 516 $[M+Na]^+$, (100). Elemental analysis: $C_{23}H_{23}NO_2STe$ Calcd. C 53.59%, H 4.70%, N 2.84%. Found: C 53.47%, H 4.73%, N 2.86%.

4.1.7 (*S*)-4-methyl-*N*-(1-(phenyltellanyl)propan-2-yl)benzenesulfonamide (10c)

Following the general procedure, diphenyl ditelluride (51 mg, 0.125 mmol) and (*S*)-2-methyl-1-tosylaziridine (47 mg, 0.23 mmol) gave after flash chromatography (petroleum ether/Et_2O 2:1) **10c** (78 mg, 81%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ(ppm): 1.16 (3H, d, *J*=6.5 Hz), 2.43 (3H, s, CH_3), 2.87 (1H, dd, *J*=6.6, 12.4 Hz, C**H$_a$**H$_b$Te), 3.06 (1H, dd, *J*=4.7, 12.4 Hz, CH$_a$**H$_b$**Te), 3.48-3.71 (1H, m, C**H**NH), 4.69 (1H, d, *J*=7.7 Hz, NH), 7.18-7.34 (5H, m), 7.64-7.69 (4H, m). **^{13}C NMR** (100 MHz, $CDCl_3$) δ(ppm): 18.6 (CH_2Te), 21.5, 22.9, 50.1, 111.0 (TeC), 127.0, 128.0, 128.8, 129.4, 129.7, 138.6, 143.4. MS (ESI positive) *m/z* (%): 440 $[M+Na]^+$, (100). Elemental analysis: $C_{16}H_{19}NO_2STe$ Calcd. C 46.09%, H 4.59%, N 3.36%. Found: C 46.16%, H 4.56%, N 3.35%.

4.1.8 (*S*)-4-methyl-*N*-(3-methyl-1-(p-tolyltellanyl)butan-2-yl)benzenesulfonamide (11a)

Following the general procedure, 1,2-di-*p*-tolylditellane (55 mg, 0.125 mmol) and (*S*)-2-isopropyl-1-tosylaziridine (54 mg, 0.23 mmol) gave after flash chromatography (petroleum ether/EtOAc 5:1) **11a** (76 mg, 72%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ(ppm): 0.74 (3H, d, *J*=6.7 Hz, CH_3), 0.8 (3H, d, *J*=6.8 Hz, CH_3),

1.8-1.9 (1H, m), 2.36 (3H, s, CH_3), 2.39 (3H, s, CH_3), 2.66 (1H, dd, *J*=6.6, 12.2 Hz, C$\mathbf{H_a}H_b$Te), 3.05 (1H, dd, *J*=4.6, 12.2 Hz, $CH_a\mathbf{H_b}$Te), 3.11-3.17 (1H, m, C**H**NH), 4.75 (1H, d, *J*=8.9 Hz, NH), 7.0 (2H, ap d, ls=7.9 Hz), 7.17 (2H, ap d, ls=8 Hz), 7.52 (2H, ap d, ls=7.9 Hz), 7.62 (2H, ap d, ls=8 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$) δ(ppm): 14.5 (CH_2Te), 17.5, 19.2, 21.2, 21.5, 32.3, 59.3, 106.7 (TeC), 127.0, 129.5, 130.2, 137.8, 138.1, 139.2, 143.1. MS (ESI positive) *m/z* (%): 482 $[M+Na]^+$, (100). Elemental analysis: $C_{19}H_{25}NO_2STe$ Calcd. C 49.71%, H 5.49%, N 3.05%. Found: C 49.65%, H 5.51%, N 3.06%.

4.1.9 (*S*)-4-methyl-*N*-(1-phenyl-3-(p-tolyltellanyl)propan-2-yl)benzenesulfonamide (11b)

Following the general procedure, 1,2-di-*p*-tolylditellane (55 mg, 0.125 mmol) and (*S*)-2-benzyl-1-tosylaziridine (65 mg, 0.23 mmol) gave after flash chromatography (petroleum ether/EtOAc 5:1) **11b** (101 mg, 87%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ(ppm): 2.36 (3H, s, CH_3), 2.39 (3H, s, CH_3), 2.74 (1H, dd, *J*=6.8, 13.8 Hz), 2.79-2.88 (3H, m), 3.08 (1H, dd, *J*=4.3, 12.4 Hz), 3.47-3.57 (1H, m, C**H**NH), 4.72 (1H, d, *J*=7.5 Hz, NH), 6.90-6.92 (2H, m), 7.02 (2H, d, ls=8.0 Hz), 7.11 (2H, d, ls=8.3 Hz), 7.12-7.17 (3H, m), 7.44 (2H, d, ls=8.3 Hz), 7.56 (2H, d, ls=8.0 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$) δ(ppm): 16.2 (CH_2Te), 21.2, 21.5, 41.7, 55.3, 106.9 (TeC), 126.7, 127.0, 128.6, 129.2, 129.5, 130.3, 136.6, 137.0, 138.1, 139.0, 143.1. **^{125}Te NMR** (126 MHz, $CDCl_3$) δ(ppm): 371.3. MS (ESI positive) *m/z* (%): 530 $[M+Na]^+$, (100). Elemental analysis: $C_{23}H_{25}NO_2STe$ Calcd. C 54.48%, H 4.97%, N 2.76%. Found: C 54.52%, H 4.95%, N 2.75%.

4.1.10 (*S*)-*N*-(1-((4-methoxyphenyl)tellanyl)-3-methylbutan-2-yl)-4-methylbenzenesulfonamide (12a)

Following the general procedure, 1,2-bis(4-methoxyphenyl)ditellane (59 mg, 0.125 mmol) and (*S*)-2-isopropyl-1-tosylaziridine (54 mg, 0.23 mmol) gave after flash chromatography (petroleum ether/EtOAc 5:1) **12a** (81 mg, 74%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ(ppm): 0.73 (3H, d, *J*=6.7 Hz), 0.79 (3H, d, *J*=6.8 Hz), 1.81-1.89 (1H, m), 2.39 (3H, s, CH_3), 2.63 (1H, dd, *J*=6.7, 12.2 Hz, C$\mathbf{H_a}H_b$Te), 3.02 (1H, dd, *J*=4.6,

12.2 Hz, CH_aH_bTe), 3.1-3.15 (1H, m, C**H**NH), 3.81 (3H, s, OCH_3), 4.72 (1H, d, *J*=8.9 Hz, NH), 6.74 (2H, d, ls=8.7 Hz), 7.18 (2H, d, ls=8 Hz), 7.57 (2H, d, ls=8.7 Hz), 7.61 (2H, d, ls=8 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$) δ(ppm): 14.8 (CH_2Te), 17.5, 19.2, 21.5, 32.2, 55.2, 59.4, 99.8 (TeC), 115.3, 127.0, 129.5, 137.9, 141.3, 149.1. **^{125}Te NMR** (126 MHz, $CDCl_3$) δ(ppm): 363.27. MS (ESI positive) *m/z* (%): 498 $[M+Na]^+$, (100). Elemental analysis: $C_{19}H_{25}NO_3STe$ Calcd. C 48.04%, H 5.30%, N 2.95%. Found: C 48.09%, H 5.28%, N 2.96%.

4.1.11 (*S*)-*N*-(1-((4-methoxyphenyl)tellanyl)-3-phenylpropan-2-yl)-4-methylbenzenesulfonamide 12b

Following the general procedure, 1,2-bis(4-methoxyphenyl)ditellane (30 mg, 0.063 mmol) and (*S*)-2-benzyl-1-tosylaziridine (32 mg, 0.113 mmol) gave after flash chromatography (petroleum ether/EtOAc 5:1) **12b** (54 mg, 91%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ(ppm): 2.39 (3H, s, CH_3); 2.74-2.76 (2H, m); 2.79 (1H, dd, *J*=6.5, 13.8 Hz); 3.05 (1H, dd, *J*=4.3, 12.4 Hz); 3.47-3.55 (1H, m, C**H**NH); 3.82 (3H, s, OCH_3); 4.75 (1H, d, *J*=8 Hz, NH); 6.76 (2H, ap.d, ls=8.8 Hz); 6.79-6.91 (2H, m); 7.11-7.17 (5H, m); 7.43 (2H, d, ls=8.3 Hz); 7.62 (2H, ap.d, ls=8.8 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$) δ(ppm): 16.4 (CH_2Te); 21.5; 41.8; 55.18; 55.23; 100.0 (TeC); 115.4; 126.7; 127.0; 128.6; 129.2; 129.5; 136.6; 137.0; 141.1; 143.1. **^{125}Te NMR** (126 MHz, $CDCl_3$) δ(ppm): 369.51. MS (ESI positive) *m/z* (%): 546 $[M+Na]^+$, (100). Elemental analysis: $C_{23}H_{25}NO_3STe$ Calcd. C 52.81%, H 4.82%, N 2.68%. Found: C 52.75%, H 4.84%, N 2.69%.

4.2. Carbonic anhydrase inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[26] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mMHepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration

reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier,[27,28] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.[27,28]

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