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Synthesis of 6-tetrazolyl-substituted sulfocoumarins acting as highly potent and selective inhibitors of the tumor-associated carbonic anhydrase isoforms IX and XII

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ABSTRACT

A series of 6-substituted sulfocoumarins incorporating substituted-1,2,3,4-tetrazol-5-yl moieties were synthesized by reaction of 6-iodo-sulfocoumarin and the corresponding tetrazole via the CH activation reaction. The new sulfocoumarins incorporating alkyl and substituted aryl moieties at the 1-position of the tetrazole, were investigated for the inhibition of four human (h) carbonic anhydrase (hCA, EC 4.2.1.1) isoforms, the cytosolic hCA I and II; and the transmembrane, tumor-associated hCA IX and XII. The tetrazole-substituted sulfocoumarins did not inhibit the ubiquitous, off-target cytosolic isoforms (K_Is >10 μM) but showed effective inhibition against the two transmembrane CAs, with K_Is ranging from 6.5 to 68.6 nM against hCA IX, and between 4.3 and 59.8 nM against hCA XII. As hCA IX and XII are validated anti-tumor targets, such prodrug, isoform-selective inhibitors as the sulfocoumarins reported here, may be useful for identifying suitable drug candidates for clinical trials.

1. Introduction

Our groups recently identified[1] the sulfocoumarins (1,2-benzoxathiine 2,2-dioxides) as a new class of prodrug-type inhibitors of the metallo-enzyme carbonic anhydrase (CA, EC 4.2.1.1).[2,3] Similar to the coumarins,[4–8] the compounds used as lead molecules in their drug design, the sulfocoumarins undergo a hydrolysis mediated by the zinc hydroxide species of the enzyme, with generation of the inhibitory species. In the case of the sulfocoumarins this species is a 2-hydroxyphenyl-ω-ethenylsulfonic acid derivative (as demonstrated by means of X-ray crystallography)[1a] whereas for the coumarins, 2-hydroxy-cinnamic acid derivatives are formed through the active-site mediated hydrolysis.[4–8]

At least three features are remarkable regarding the CA inhibition by these types of derivatives: (i) The inhibition mechanism of coumarins/sulfocoumarins is very different compared to that of the classical CA inhibitors (CAIs) of the sulfonamide/sulfamate/sulfamide type,[2,9] which coordinate to the metal ion within the enzyme active site, substituting the nucleophile (water molecule or hydroxide ion) used in the catalytic process. In fact, coumarins (i.e., their hydrolysis products) bind around 8 Å away from the zinc ion, at the entrance of the active site cavity, occluding it, and making interactions with amino acid residues in the most variable region of the active site of the CA isoforms known to date (15 mammalian CA isoforms are known).[2,4–8] On the other hand, the hydrolyzed sulfocoumarins bind more internally within the active site, but again in a different binding site compared to the sulfonamides.[1] By means of kinetic studies and X-ray crystallography we showed that the 2-hydroxyphenyl-ω-ethenylsulfonic acid formed from the original sulfocoumarin is anchored to the zinc-coordinated water molecule/hydroxide ion by means of a hydrogen bond. In addition, the scaffold of the inhibitor participates in other favorable interactions with amino acid residues and water molecules from the middle of the active site cavity.

(ii) Coumarins and sulfocoumarins are the most isoform-selective CAIs known to date.[1,4–8] This highly desired feature was explained by considering the inhibition mechanism of these compounds, briefly outlined above. Indeed, the hydroxy-cinnamic acids/2-hydroxyphenyl-ω-ethenylsulfonic acids formed after the hydrolysis of the coumarin/sulfocoumarin bind in regions of the CA active site which differ between the 15 CA isoforms known to date in humans,[2] affording the possibility that the inhibitor interacts in a specific manner with residues which are found in some but not in other CA isoforms.[1–4] For example, we have reported many CA IX/XII selective coumarin inhibitors,[4–8] compounds showing low

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nanomolar inhibition of these transmembrane, tumor-associated isoforms CA IX and XII,[10,11] but at the same time not inhibiting at all the cytosolic, off-target isoforms hCA I and II, which are ubiquitous and which are potently inhibited by many sulfonamide CAIs. This is one of the reasons why the sulfonamide CAIs show a rather large number of undesired side effects.[12,13] Furthermore, several coumarins showing isoform specificity for the inhibition of hCA XIII, hCA XIV as well as several other isoforms were also reported.[4]

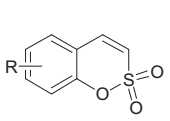
(iii) The substitution pattern at the coumarin/sulfocoumarin ring strongly influences the potency and selectivity against mammalian CA isoforms of these classes of inhibitors. For example, 7,8-disubstituted- but not 6,7-disubstituted coumarins were shown to selectively inhibit the transmembrane, tumor-associated isoforms hCA IX and XII over the cytosolic ones hCA I and II in the low nanomolar/subnanomolar range.[5a] For the sulfocoumarin derivatives, we reported that the 6-substituted compounds[1a] incorporating a range of simple (halogeno, OH, amino, alkyl, etc) or more complex (substituted 1,2,3-triazoles) substituents act as low nanomolar hCA IX/XII inhibitors, but do not inhibit hCA I and II up to the 50 μM range. On the contrary, the 7-substituted derivatives[1b] were shown to act as low nanomolar hCA II inhibitors, showed some inhibition of hCA VA, but were not inhibiting hCA I, hCA IX and XII significantly. All these facts demonstrate that the sulfocoumarins constitute a very interesting class of CAIs with potential of applications in a range of disorders in which various CA isoforms are involved, among which glaucoma,[14] obesity[15] and cancer.[10,11,16]

Continuing our interest in the design and investigation of non-sulfonamide CAIs, we report here the synthesis and inhibition data against four physiologically relevant isoforms (hCA I, II, IX and XII) of a series of sulfocoumarins incorporating 1,2,3,4-tetrazolyl moieties which have been prepared by an original method.

2. Results and discussion

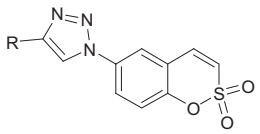
2.1. Chemistry

In previous work from our laboratories[1] we have investigated 6-substituted sulfocoumarins incorporating simple substituents (of type **A**) as well as compounds obtained via click-chemistry, of type B, which possess 1,2,3-triazole moieties in the 6-position of the heterocyclic ring, observing that the most potent/selective inhibition of hCA IX and XII was achieved with the last type of derivatives, **B**.[1a]



A

R = OH, Br, NO_2, NH_2

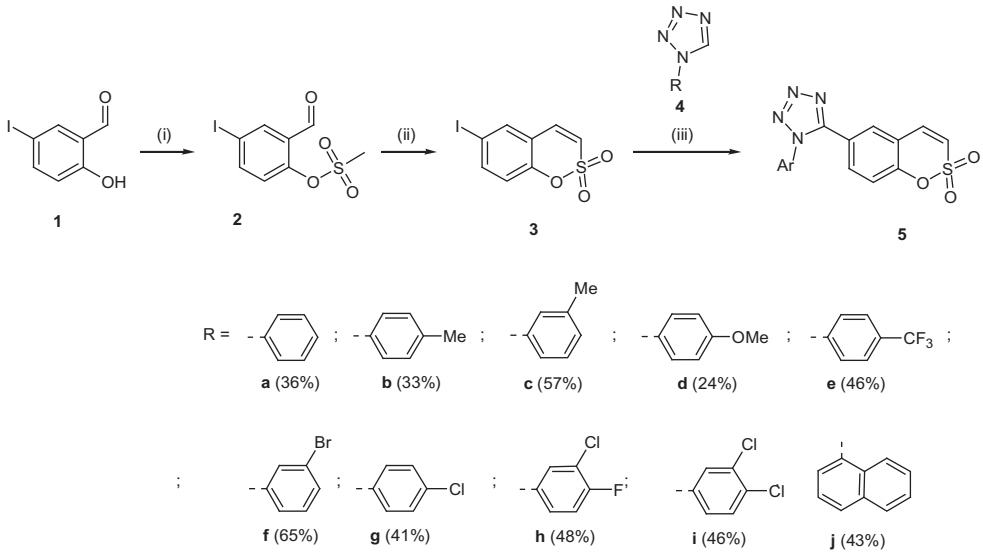


B

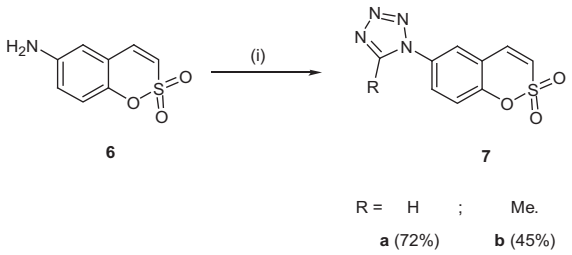
R = Ph, substituted aryl, COOMe, etc.

The rationale of the present study was to replace the 1,2,3-triazole ring present in derivatives **B** by the isosteric 1,2,3,4-tetrazole ring, which affords the exploration of alternative synthetic pathways as well as a different chemical space (due to the fact that the orientation of the R moiety in compounds **B** and **5**, **7**–see later in the text–are different). Thus we have prepared the key intermediate 6-iodo-sulfocoumarin **3**, by the procedure reported earlier for the synthesis of compounds of type **A** (Scheme 1).[17,18] Reaction of 2-hydroxy-5-iodobenzaldehyde **1** with methanesulfonyl chloride afforded the methanesulfonate **2** which was cyclized to **3** by the procedure reported earlier.[17,18] The iodo-sulfocoumarin **3** was then reacted with 1-substituted-1,2,3,4-tetrazoles **4**[19–23] in conditions in which the CH bond in position **5** of the tetrazole ring is activated (i.e., Cs_2CO_3, CuI, $Pd(OAc)_2$ and tri(2-furyl)phosphine),[24] leading to the desired derivatives **5** with acceptable yields (see Section 4 for details)–Scheme 1. The R moieties present in derivatives **5** (and originating from the substituted tetrazoles **4**, reported in the literature[19–23]) were as variable as possible, that is phenyl, mono- or di-substituted phenyls (with alkyl, alkoxy and halogens in various positions of the ring) and 1-naphthyl (Scheme 1), in order to highlight the structure–activity relationship (SAR) for the interaction of these compounds with the various CA isoforms (see later in the paper).

As the procedure employed in Scheme 1 did not work for R = H or alkyl, we designed an alternative synthesis for obtaining the unsubstituted tetrazole-sulfocoumarin **7a** and the corresponding methyl derivative **7b** (Scheme 2). 6-Amino-sulfocoumarin **6** (reported earlier by some of us)[17] was reacted with sodium azide, followed by cyclization of the intermediate with triethyl orthoformate or triethyl orthoacetate (Scheme 2) leading thus to derivatives **7** in acceptable yields. The new compounds **5a**–**5j** and



Scheme 1. Reagents and conditions: (i) MsCl, NEt_3, CH_2Cl_2, rt, 2 h, 99%; (ii) (a) DBU, CH_2Cl_2, 0 °C, 2 h, (b) $POCl_3$, Py, rt, 3 h, 71%; (iii) $Pd(OAc)_2$, tri(2-furyl)phosphine, CuI, Cs_2CO_3, toluene, 40 °C, 20 h.



Scheme 2. Reagents and conditions: (i) NaN_3, $RC(OEt)_3$, AcOH, 100 °C, 4 h.

7a,**7b** were characterized by spectroscopic techniques (IR, ^1H and ^{13}C NMR), high resolution mass spectroscopy (HRMS) as well as elemental analysis, which confirmed their structures (see Section 4 for details).

2.2. CA inhibition

Sulfocoumarins **5a**–**5j** and **7a**,**7b** were screened for the inhibition of four human (h) CA isoforms, the cytosolic, widespread hCA I and II (offtargets in this case) as well as the transmembrane, tumor-associated hCA IX and XII (anticancer drug targets).[4,10,11,16] Table 1 shows inhibition data of sulfocoumarins **5a**–**5j** and **7a**,**7b** (as well as the sulfonamide acetazolamide **AAZ**, as standard) against hCA I, II, IX and XII, after a period of 6 h of incubation of the enzyme and inhibitor solutions.[25] It should be mentioned that assaying the inhibition with the usual 15 min incubation period (as for the sulfonamides)[9] leads to the measurement of a very weak inhibition (data not shown). This was also the case with the coumarins.[4–8] For this reason, a 6 h incubation time has been used for assaying all sulfocoumarins as CAIs. The following SAR should be noted regarding the inhibition data of Table 1:

(i) The cytosolic isoforms hCA I and II were not inhibited by the sulfocoumarins **5a**–**5j** and **7a**, **7b**, which showed inhibition constants >10 μM. It should be observed that acetazolamide, the sulfonamide CAI in clinical use, is a low nanomolar inhibitor of hCA II, and also significantly inhibits hCA I (K_I of 250 nM), Table 1. The fact that the new sulfocoumarins **5a**–**5j** and **7a**, **7b** reported here are not inhibitors of hCA I and II is not surprising, considering the fact that the structurally related triazole-substituted sulfocoumarins **B** reported earlier,[1a] showed the same behavior.

(ii) The transmembrane, tumor-associated hCA IX was effectively inhibited by the new sulfocoumarins **5a**–**5j** and **7a**, **7b** reported here, with inhibition constants ranging between 6.5 and 68.6 nM. The least effective compounds in the series were the unsubstituted tetrazole **7a** and the methyl derivative **7b**, together with the *m*-bromophenyl derivative **5f** (K_Is in the range of 56.8–68.6 nM). Slightly more effective than these compopunds were the two sulfocoumarins **5c** and **5e**, with K_Is of 27.1–39.3 nM (Table 1). They incorporate the *m*-tolyl and 4-trifluoromethylphenyl moieties as R groups at the tetrazole ring. Thus, the unsubstituted tetrazole **7a**, the methyl-substituted derivative **7b** (R = Me) and the aryl-substituted ones with *meta*-methyl and 4-trifluoromethyl substituents at the phenyl ring (**5c** and **5e**) were the least effective hCA IX inhibitors in this series. However, the remaining derivatives, that is **5a**, **5b**, **5d**, **5g**–**5i**, incorporating phenyl, 1-naphthyl, and 4-substituted- or 3,4-disubstituted phenyl groups, were highly effective, low nanomolar hCA IX inhibitors, with inhibition constants ranging between 6.5 and 9.1 nM. Thus there are many substitution patterns leading to highly effective hCA IX inhibitors in this series of tetrazole-substituted sulfocoumarins. These last compounds were around 3–4 times more effective hCA IX inhibitors compared to acetazolamide (Table 1).

(iii) The second transmembrane isoform, hCA XII, was also highly inhibited by the new sulfocoumarins reported here, with inhibition constants ranging between 4.3 and 59.8 nM. Only one compound, **5c** with a *meta*-tolyl substituent, showed a medium potency inhibitory power against this isoform, with a K_I of 59.8 nM. All the other compounds were low nanomolar inhibitors with K_Is in the range of 4.3–13.5 nM (Table 1). Thus, most substitution patterns present in these sulfocoumarins, that is both aliphatic and aromatic R groups, lead to highly effective hCA XII inhibitors. It should be also noted that acetazolamide is a very effective hCA XII inhibitor, but as mentioned earlier, it is a promiscuos CAI, inhibiting significantly most of the 15 CA isoforms found in mammals.[2–4]

Table 1
hCA I, II, IX and XII inhibition data with sulfocoumarins 5 and 7, by a stopped-flow CO_2 hydrase assay[25]

Compound	K_I (nM)*			
	hCA I	hCA II	hCA IX	hCA XII
7a	>10,000	>10,000	68.6	6.4
7b	>10,000	>10,000	65.9	8.8
5a	>10,000	>10,000	8.5	7.1
5b	>10,000	>10,000	9.1	6.1
5c	>10,000	>10,000	27.1	59.8
5d	>10,000	>10,000	8.7	6.3
5e	>10,000	>10,000	39.3	8.1
5f	>10,000	>10,000	56.8	7.3
5g	>10,000	>10,000	8.8	4.3
5h	>10,000	>10,000	6.9	5.4
5i	>10,000	>10,000	8.2	13.5
5j	>10,000	>10,000	6.5	5.7
AAZ*	250	12	25	5.7

Acetazolamide (AAZ) was used as a standard inhibitor.
* Mean from 3 different assays, errors in the range of ±10% of the reported values (data not shown).

3. Conclusions

We report here a series of 5-(2,2-dioxido-1,2-benzoxathiin-6-yl)-1-aryl/alkyl-1*H*-tetrazoles, obtained by an original synthetic procedure involving the cyclization of 2-formyl-4-iodophenyl methanesulfonate to the corresponding 6-iodo-sulfocoumarin, which was then reacted in conditions of CH activation with 1-substituted 1,2,3,4-tetrazoles, leading to the title compounds. The new sulfocoumarins incorporating alkyl and substituted aryl moieties at the 1-position of the tetrazole, were investigated for the inhibition of four hCA isoforms with medicinal chemistry applications, the cytosolic hCA I and II, and the transmembrane, tumor-associated hCA IX and XII. The tetrazole-substituted sulfocoumarins did not inhibit the ubiquitous, offtarget cytosolic isoforms hCA I and II (K_Is >10 μM) but showed effective inhibition against the two transmembrane CAs, with K_Is ranging from 6.5 to 68.6 nM against hCA IX, and between 4.3 and 59.8 nM against hCA XII. As hCA IX and XII are validated anti-tumor targets, such prodrug, isoform-selective inhibitors as the sulfocoumarins reported here, may be useful for identifying suitable drug candidates for clinical trials.

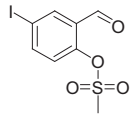
4. Experimental

4.1. Chemistry

Reagents and starting materials were obtained from commercial sources (Sigma–Aldrich) and used as received. Compound **1** was sythesized according literature procedure.[18] The solvents were purified and dried by standard procedures prior to use; petroleum ether of boiling range 40–60 °C was used. Flash chromatography was carried out using Merck silica gel (230–400 mesh). Thin-layer chromatography was performed on silica gel, spots were visualized with UV light (254 and 365 nM). Melting points were determined

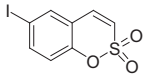
on an OptiMelt automated melting point system. IR spectra were measured on a Shimadzu FTIR IR Prestige-21 spectrometer. NMR spectra were recorded on Varian Mercury (400 MHz) spectrometer with chemical shifts values (δ) in ppm relative to TMS using the residual DMSO-d_6 signal as an internal standard. Elemental analyses were performed on a Carlo Erba CHNSeO EA-1108 apparatus.

4.1.1. 2-Formyl-4-iodophenyl methanesulfonate 2[18]



To a solution of 2-hydroxy-5-iodobenzaldehyde **1** (3.32 g, 17.4 mmol) in dry CH_2Cl_2 (30 mL) triethylamine (3.63 mL, 26.1 mmol) was added. The mixture was cooled to 0 °C and mesyl chloride (2.17 mL, 28.0 mmol) was added. The reaction was stirred at room temperature for 4 h. The mixture was diluted with H_2O (150 mL) and extracted with EtOAc (3 × 80 mL). The organic phase was washed with brine and dried over Na_2SO_4, and the solvent was evaporated to yield a yellow oil (5.61 g, 99%). IR (neat, cm^{-1}) ν_{max}: 1694 (C=O),1369 (S=O), 1356 (S=O), 1183 (S=O), 1165 (S=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 3.59 (s, 3H), 7.35–7.38 (m, 1H), 8.13–8.17 (m, 2H), 10.12 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 37.8, 93.3, 126.1, 130.7, 137.4, 144.2, 149.3, 187.6. HRMS (ESI) $[M+Na]^+$: m/z Calcd for ($C_8H_7INaO_4S$) 348.9007. Found 348.9015.

4.1.2. 6-Iodo-1,2-benzoxathiine 2,2-dioxide 3

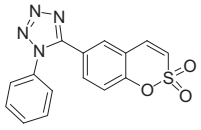


A solution of the above formyl-iodophenyl-mesylate **2** (3.00 g, 9.20 mmol) in dry CH_2Cl_2 (30 mL) was cooled to 0 °C and DBU (1.38 mL, 9.20 mmol) was added. The reaction was stirred at 0 °C for 2 h under argon atmosphere and poured into a mixture of ice and 10% HCl and extracted with Et_2O (3 × 100 mL). The organic phase was dried over Na_2SO_4, and the solvent was evaporated. The crude mixture was dissolved in pyridine (30 mL), cooled to 0 °C and $POCl_3$ (1.72 mL, 18.4 mmol) was added. The mixture was stirred at room temperature for 3 h then poured into a mixture of ice and water. The resulting precipitate was filtered off yielding a yellow solid (2.00 g, 71%). Mp 140–141 °C. IR (neat, cm^{-1}) ν_{max}: 1369 (S=O), 1171 (S=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.27 (d, 1H, J = 8.7 Hz), 7.58 (d, 1H, J = 10.4 Hz), 7.65 (d, 1H, J = 10.4 Hz), 7.91 (dd, 1H, J = 8.7, 2.3 Hz), 8.13 (d, 1H, J = 2.3 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 90.5, 120.7, 120.9, 123.5, 135.4, 138.0, 140.7, 150.5. Anal. Calcd for $C_8H_5IO_3S$ (308.09): C, 31.19; H, 1.64. Found: C, 31.46; H, 1.69.

4.2. General procedure for the synthesis of tetrazoles 5 via C–H activation

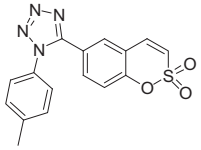
Compound **3** (0.200 g, 0.649 mmol), the appropriate tetrazole (2 equiv, 1.30 mmol), Cs_2CO_3 (0.233 g, 0.714 mmol), CuI (0.124 g, 0.649 mmol), $Pd(OAc)_2$ (0.0146 g, 0.0649 mmol) and tri(2-furyl) phosphine (0.030 g, 0.130 mmol)were suspended in dry toluene (3 mL). The mixture was stirred 40 °C under argon for 20 h, then EtOAc (20 mL) was added and the mixture was filtered through celite. Celite was washed with EtOAc (50 mL). The filtrate was evaporated. The mixture was purified by silica gel chromatography (petroleum ether/EtOAc 2:1) an recrystallized from EtOH.

4.2.1. 5-(2,2-Dioxido-1,2-benzoxathiin-6-yl)-1-phenyl-1*H*-tetrazole 5a



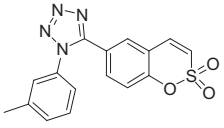
Obtained from 1-phenyl-1,2,3,4-tetrazole **4a**[19] (0.190 g, 1.30 mmol) as yellow crystalline solid (0.076 g, 36%). Mp 189–190 °C. IR (KBr, cm^{-1}) ν_{max}: 1370 (S=O), 1178 (S=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.49–7.56 (m, 2H), 7.58–7.68 (m, 6H), 7.78 (d, 1H, J = 10.4 Hz), 8.09–8.12 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 119.2, 119.3, 121.6, 123.6, 126.0, 130.0, 130.8, 131.0, 132.5, 133.8, 135.9, 152.2, 152.4. Anal. Calcd for $C_{15}H_{10}N_4O_3S$ (326.33): C, 55.21; H, 3.09; N, 17.17. Found: C, 55.25; H, 3.09; N, 17.08.

4.2.2. 5-(2,2-Dioxido-1,2-benzoxathiin-6-yl)-1-(4-methylphenyl)-1*H*-tetrazole 5b



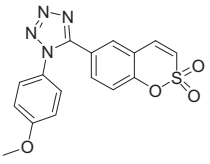
Obtained from 1-(*p*-tolyl)-1,2,3,4-tetrazole **4b**[19,20] (0.208 g, 1.30 mmol) as light brown solid (0.073 g, 33%). Mp 186–187 °C. IR (neat, cm^{-1}) ν_{max}: 1372 (S=O), 1177 (S=O), 1143 (S=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.40 (s, 3H), 7.38–7.43 (m, 2H), 7.46–7.49 (m, 2H), 7.50–7.56 (m, 2H), 7.65 (d, 1H, J = 10.4 Hz), 7.79 (d, 1H, J = 10.4 Hz), 8.12 (d, 1H, J = 1.9 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.8, 119.2, 119.3, 121.7, 123.6, 125.7, 130.4, 131.0, 131.3, 132.4, 135.9, 140.7, 152.1, 152.3. HRMS (ESI) $[M+H]^+$: m/z Calcd for ($C_{16}H_{13}N_4O_3S$) 341.0708. Found 341.0717.

4.2.3. 5-(2,2-Dioxido-1,2-benzoxathiin-6-yl)-1-(3-methylphenyl)-1*H*-tetrazole 5c



Obtained from tetrazole **4c**[20] (0.208 g, 1.30 mmol) as yellow crystalline solid (0.126 g, 57%). Mp 153–154 °C. IR (neat, cm^{-1}) ν_{max}: 1372 (S=O), 1350 (S=O), 1179 (S=O), 1143 (S=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.37 (s, 3H), 7.33–7.37 (m, 1H), 7.44–7.49 (m, 3H), 7.50–7.56 (m, 2H), 7.65 (d, 1H, J = 10.4 Hz), 7.79 (d, 1H, J = 10.4 Hz), 8.12–8.14 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.7, 119.2, 119.3, 121.6, 123.0, 123.6, 126.3, 129.7, 131.0, 131.5, 132.4, 133.7, 135.9, 139.9, 152.2, 152.3. Anal. Calcd for $C_{16}H_{12}N_4O_3S$ (340.36): C, 56.46; H, 3.55; N, 16.46. Found: C, 56.65; H, 3.53; N, 16.39.

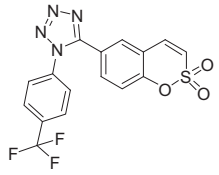
4.2.4. 5-(2,2-Dioxido-1,2-benzoxathiin-6-yl)-1-(4-methoxyphenyl)-1H-tetrazole 5d



Obtained from tetrazole **4d**[19,21] (0.229 g, 1.30 mmol) as white crystalline solid (0.055 g, 24%). Mp 175–176 °C. IR (KBr, cm^{-1})

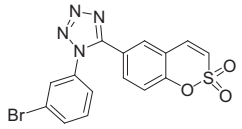
ν_{max}: 1370 (S=O), 1172 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.84 (s, 3H), 7.11–7.16 (m, 2H), 7.51–5.57 (m, 4H), 7.65 (d, 1H, J = 10.4 Hz), 7.80 (d, 1H, J = 10.4 Hz), 8.11 (d, 1H, J = 1.9 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.6, 115.0, 119.2, 119.3, 121.8, 123.6, 126.4, 127.5, 130.9, 132.4, 135.9, 152.1, 152.4, 160.6. Anal. Calcd for $C_{16}H_{12}N_4O_4S$ (356.36): C, 53.93; H, 3.39; N, 15.72. Found: C, 53.78; H, 3.18; N, 15.83.

4.2.5. 5-(2,2-Dioxido-1,2-benzoxathiin-6-yl)-1-[4-(trifluoromethyl)phenyl]-1*H*-tetrazole 5e



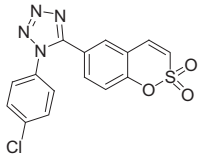
Obtained from tetrazole **4e**[21] (0.278 g, 1.30 mmol) as yellow crystalline solid (0.119 g, 46%). Mp 184–185 °C. IR (neat, cm^{-1}) ν_{max}: 1371 (S=O), 1325 (S=O), 1177 (S=O), 1130 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.56–7.58 (m, 2H), 7.66 (d, 1H, J = 10.4 Hz), 7.82 (d, 1H, J = 10.4 Hz), 7.83–7.88 (m, 2H), 7.99–8.04 (m, 2H), 8.11–8.13 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 119.4, 121.4, 123.5 (q, J = 273.0 Hz), 123.6, 126.7, 127.2 (q, J = 3.6 Hz), 130.7 (q, J = 32.8), 131.2, 132.8, 135.9, 137.0, 152.3, 152.5. Anal. Calcd for $C_{16}H_9F_3N_4O_3S$ (394.33): C, 48.73; H, 2.30; N, 14.21. Found: C, 48.66; H, 2.38; N, 13.98.

4.2.6. 1-(3-Bromophenyl)-5-(2,2-dioxido-1,2-benzoxathiin-6-yl)-1*H*-tetrazole 5f



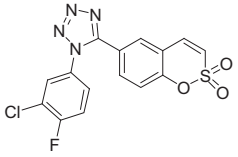
Obtained from tetrazole **4f**[21] (0.293 g, 1.30 mmol) as light yellow crystalline solid (0.171 g, 65%). Mp 197–198 °C. IR (KBr, cm^{-1}) ν_{max}: 1371 (S=O), 1145 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.53–7.59 (m, 3H), 7.60–7.64 (m, 1H), 7.66 (d, 1H, J = 10.4 Hz), 7.82 (d, 1H, J = 10.4 Hz), 7.84–7.88 (m, 1H), 7.96 (t, 1H, J = 2.0 Hz), 8.11–8.13 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 119.3, 119.4, 121.4, 122.2, 123.6, 125.2, 128.8, 131.1, 131.8, 132.6, 133.8, 135.0, 135.9, 152.2, 152.5. Anal. Calcd for $C_{15}H_9BrN_4O_3S$ (405.23): C, 44.46; H, 2.24; N, 13.83. Found: C, 44.49; H, 2.24; N, 13.73.

4.2.7. 1-(4-Chlorophenyl)-5-(2,2-dioxido-1,2-benzoxathiin-6-yl)-1*H*-tetrazole 5g



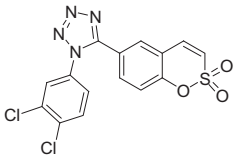
Obtained from tetrazole **4g**[19,20] (0.235 g, 1.30 mmol) as white crystalline solid (0.096 g, 41%). Mp 193.5–194.5 °C. IR (KBr, cm^{-1}) ν_{max}: 1373 (S=O), 1184 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.55–7.57 (m, 2H), 7.63–7.67 (m, 3H), 7.68–7.72 (m, 2H), 7.82 (d, 1H, J = 10.4 Hz), 8.09–8.11 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 119.3, 121.5, 123.6, 127.8, 130.1, 131.1, 132.6, 135.4, 135.9, 152.2, 152.5. Anal. Calcd for $C_{15}H_9ClN_4O_3S$ (360.77): C, 49.94; H, 2.51; N, 15.53. Found: C, 49.80; H, 2.38; N, 15.65.

4.2.8. 1-(3-Chloro-4-fluorophenyl)-5-(2,2-dioxido-1,2-benzoxathiin-6-yl)-1*H*-tetrazole 5h



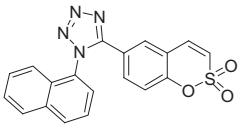
Obtained from tetrazole **4h**[22] (0.258 g, 1.30 mmol) as white crystalline solid (0.118 g, 48%). Mp 174–175 °C. IR (KBr, cm^{-1}) ν_{max}: 1368 (S=O), 1178 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.56 (d, 1H, J = 8.7 Hz), 7.60 (dd, 1H, J = 8.7, 2.1 Hz), 7.66 (d, 1H, J = 10.4 Hz), 7.67–7.70 (m, 2H), 7.83 (d, 1H, J = 10.4 Hz), 8.05–8.10 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 118.3 (d, J = 23.0 Hz), 119.3, 119.4, 120.9 (d, J = 19.2 Hz), 121.3, 123.6, 127.4 (d, J = 8.6 Hz), 128.8, 130.7 (d, J = 3.7 Hz), 131.0, 132.6, 135.9, 152.3, 152.6, 158.4 (d, J = 251.7 Hz). Anal. Calcd for $C_{15}H_8ClFN_4O_3S$ (378.77): C, 47.57; H, 2.13; N, 14.79. Found: C, 47.56; H, 2.06; N, 14.59.

4.2.9. 1-(3,4-Dichlorophenyl)-5-(2,2-dioxido-1,2-benzoxathiin-6-yl)-1*H*-tetrazole 5i



Obtained from tetrazole **4i**[23] (0.280 g, 1.30 mmol) as light yellow crystalline solid (0.119 g, 46%). Mp 199–200 °C. IR (KBr, cm^{-1}) ν_{max}: 1372 (S=O), 1178 (S=O), 1124 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.55–7.64 (m, 3H), 7.66 (d, 1H, J = 10.4 Hz), 7.84 (d, 1H, J = 10.4 Hz), 7.90 (d, 1H, J = 8.6 Hz), 8.08 (d, 1H, J = 2.4 Hz), 8.11–8.13 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 119.3, 119.4, 121.3, 123.6, 126.3, 128.1, 131.1, 131.9, 132.4, 132.7, 133.4, 133.9, 135.9, 152.3, 152.6. Anal. Calcd for $C_{15}H_8Cl_2N_4O_3S$ (395.22): C, 45.58; H, 2.04; N, 14.18. Found: C, 45.46; H, 1.98; N, 14.12.

4.2.10. 5-(2,2-Dioxido-1,2-benzoxathiin-6-yl)-1-(naphthalen-1-yl)-1*H*-tetrazole 5j

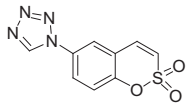


Obtained from tetrazole **4j**[22] (0.255 g, 1.30 mmol) as light pink crystalline solid (0.255 g, 43%). Mp 165–166 °C. IR (neat, cm^{-1}) ν_{max}: 1371 (S=O), 1178 (S=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.23 (d, 1H, J = 8.6 Hz), 7.31 (dd, 1H, J = 8.6, 2.1 Hz), 7.39 (d, 1H, 8.6 Hz), 7.56–7.63 (m, 2H), 7.64–7.70 (m, 1H), 7.71–7.77 (m, 2H), 7.92–7.96 (m, 1H), 8.14–8.20 (m, 2H), 8.30 (d, 1H, J = 8.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 119.3, 119.4, 121.2, 121.3, 123.7, 125.7, 126.4, 127.6, 128.2, 128.7, 128.8, 129.7, 130.6, 131.4, 131.8, 133.7, 135.8, 152.2, 153.6. Anal. Calcd for $C_{19}H_{12}N_4O_3S$ (376.39): C, 60.63; H, 3.21; N, 14.89. Found: C, 60.49; H, 3.04; N, 14.85.

4.3. General procedure for the synthesis of tetrazoles 7

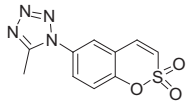
To the appropriate aminoderivative **6**[17] (1.0 equiv), NaN_3 (1,2 equiv) and triethyl orthoformate or triethyl orthoacetate (1.5 equiv) AcOH (3 mL per g amine) was added. The mixture was stirred 100 °C for 4 h, then cooled to room temperature and poured into ice-water. The precipitate was filtered off and dried under vacuum.

4.3.1. 1-(2,2-Dioxido-1,2-benzoxathiin-6-yl)-1*H*-tetrazole 7a



Obtained from compound **6**[17] (2.31 g, 11.7 mmol), NaN_3 (0.914 g; 14.1 mmol) and triethyl orthoformate (2.92 mL, 17.6 mmol). The crude was recrystallized from EtOH to yield **7a** as yellow crystalline solid (2.11 g, 72%). Mp 173.5–174.5 °C. IR (KBr, cm^{-1}) ν_{max}: 1369 (S=O), 1358 (S=O), 1171 (S=O), 1148 (S=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.72 (d, 1H, J = 10.4 Hz), 7.78 (d, 1H, J = 9.0 Hz), 7.83 (d, 1H, J = 10.4 Hz), 8.13 (dd, 1H, J = 9.0, 2.6 Hz), 8.35 (d, 1H, J = 2.6 Hz), 10.11 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 119.9, 120.3, 122.7, 124.2, 125.1, 131.4, 135.6, 142.6, 150.7. HRMS (ESI) $[M+H]^+$: m/z Calcd for ($C_9H_7N_4O_3S$) 251.0239. Found 251.0247.

4.3.2. 1-(2,2-Dioxido-1,2-benzoxathiin-6-yl)-1*H*-tetrazole 7b



Obtained from **6**[17] (0.400 g, 2.03 mmol), NaN_3 (0.159 g; 2.44 mmol) and triethyl orthoacetate (0.56 mL, 3.05 mmol). The crude was recrystallized from EtOH to yield **7b** as yellow crystalline solid (0.239 g, 45%). Mp 209.5–210.5 °C. IR (KBr, cm^{-1}) ν_{max}: 1363 (S=O), 1175 (S=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.60 (s, 3H), 7.72 (d, 1H, J = 10.4 Hz), 7.76 (d, 1H, J = 8.7 Hz), 7.80 (d, 1H, J = 10.4 Hz), 7.95 (dd, 1H, J = 8.7, 2.6 Hz), 8.13 (d, 1H, J = 2.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 9.3, 119.8, 120.2, 124.0, 126.4, 128.7, 131.2, 135.6, 151.2, 152.5. Anal. Calcd for $C_{10}H_8N_4O_3S$ (264.26): C, 45.45; H, 3.05; N, 21.20. Found: C, 45.40; H, 2.99; N, 20.94.

4.4. CA inhibition

An applied photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity.[25] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (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–6 h at room temperature (15 min) or 4 °C (6 h) prior to assay, in order to allow for the formation of the E–I complex. Data from Table 1 were obtained after 6 h incubation of enzyme and inhibitor, as for the sulfocoumarins and coumarins reported earlier.[1,4] The inhibition constants were obtained by non-linear least-squares methods using PRISM 3, as reported earlier,[2] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.[1,4–8]

Acknowledgments

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