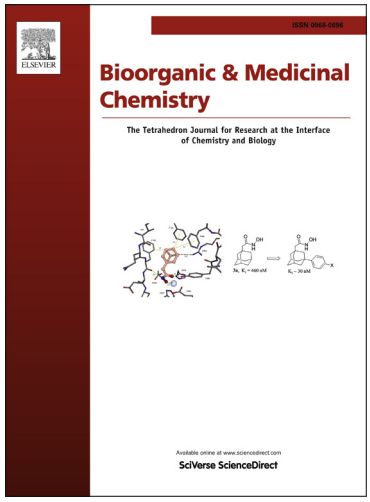

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7-Substituted-sulfocoumarins are isoform-selective, potent carbonic anhydrase II inhibitors

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Abstract: A series of 7-substituted sulfocoumarins and 3,4-dihydrosulfocoumarins was obtained by cyclization of the methanesulfonate of 2,4-dihydroxy- or 2-hydroxy-4-methoxybenzaldehyde, followed by derivatization reactions. The new compounds incorporate a range of substituents in position 7 of the heterocyclic ring (hydroxyl, methoxy, carboxylic and alkylsulfonate ester). The compounds were tsted for the inhibition of the zinc enzyme human (h) carbonic anhydrase (hCA, EC 4.2.1.1). Unlike the 6-substituted sulfocoumarins which were potent hCA IX and XII inhibitors and ineffective hCA I and II inhibitors, compounds from this series showed low nanomolar hCA II inhibitory properties, and inhibited the mitochondrial isoform hCA VA with K_Is in the range of 91 – 9960 nM, but were ineffective as hCA I, IX and XII inhibitors. The structure activity relationship for this class of inhibitors was rather clear, with the nature of the 7-substituent strongly influencing hCA VA inhibition, whereas the nature of these groups were less relevant for hCA II inhibition (all reported compounds were highly effective hCA II inhibitors, with K_Is in the range of 1.5 – 8.4 nM). Since both hCA II and hCA VA are important drug targets (hCA II for antiglaucoma agents; hCA VA for antiobesity drugs), these isoform-selective inhibitors reported here may be considered of interest for various biomedical applications.

Keywords: carbonic anhydrase; sulfocoumarin; coumarin; sulfonamide; isoform-selective inhibitor

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1. Introduction

Sulfocoumarins (1,2-benzoxathiine 2,2-dioxides) were recently reported to act as efficient inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1) and to possess a novel mechanism of inhibition.[1] Similar to coumarins,[2-4] which were used as lead compounds for their design as CA inhibitors (CAIs) – Chart 1, the sulfocoumarins undergo a CA-mediated hydrolysis leading to 2-hydroxyphenyl-ω-ethenylsulfonic acids which thereafter bind to the zinc-coordinated water molecule from the enzyme active site,[1] as shown in Fig. 1. In this last step of the inhibition mechanism, sulfocoumarins act diversely from the isostructural coumarins, which generate 2-hydroxy-cinnamic acid derivatives, which neither coordinate to the zinc ion (as the sulfonamide inhibitors),[1] nor anchor to the water molecule coordinated to it, but occlude the entrance to the enzyme active site.[2-4]

Fig. 1 and Chart 1 here

It should be noted that the sulfocoumarins represent just the last chemotype shown to possess significant CA inhibitory properties, rationally designed by considering the coumarins as lead molecules (Chart 1). Indeed, in the last several years a rather large number of new classes of CAIs were reported starting from coumarins as leads, among which the thiocoumarins, 2-thioxo-coumarins, coumarin oximes, 5-/6-membered /thio)lactones, etc.[4-6] Many of these new classes of CAIs showed a high degree of selectivity for inhibiting various mammalian CA isoforms, of the 16 presently known.[7,8] Indeed, the main problem of the sulfonamide/sulfamate clinically used CAIs is represented by the fact that most of them indiscriminately inhibit most of these isoforms, leading thus to deleterious side effects. [7-9] The sulfonamide/sulfamates CAIs are used for the treatment of glaucoma, obesity, epilepsy, and as diuretics. [7-9] Recently, significant antitumor and antimetastatic effects were reported for many CAIs specifically targeting the tumor-associated isoforms CA IX and XII.[10,11] It should be stressed here that these very different pharmacological applications of the CAIs are due to the fact that different isoforms are responsible for diverse biological functions: CA II and XII are targeted by the antiglaucoma such drugs, CA II, IV and XIV for the diuretic ones, CA VII and XIV for the antiepileptic ones, and as mentioned above, CA IX and XII for the antitumor/antimetastatic effects.[7-11] It is thus highly desirable to develop selective inhibitors for all these isoforms, which has been a challenge for a long period, when only sulfonamides (and their bioisosteres) were known as effective classes of CAIs. However, this situation changed dramatically in the last several years, with the report of a rather large number of new chemotypes acting as efficient and isoform-selective CAIs. In addition to the coumarins and the compounds developed

from them shown in Chart 1, one can mention the polyamines,[12] the phenols[13] and the dithiocarbamates[14] as novel, interesting classes of CAIs. Many of these compounds possess inhibition mechanisms distinct of those of the sulfonamides (or coumarins), as determined by X-ray crystallography of enzyme-inhibitor adducts.[2,7,12-14]

In the previous work[1] we have primarily explored sulfocoumarins possessing various substituents in the 6 position of the heterocyclic ring. As for the coumarins,[1-4] also for sulfocoumarins the substitution pattern and especially the position of the substituent on the heterocyclic ring system, are the main factors influencing CA inhibitory properties. In this paper we report the synthesis and explore CA inhibition with a series of sulfocoumarins possessing various substituents in the 7 position of the ring.

2. Results and Discussion

2.1. Chemistry. In a recent paper we investigated a rather large series of sulfocoumarins incorporating simple functionalities in the 6 position of the ring, such as hydroxyl, methanesulfonyl, benzyloxy, bromo, nitro, amino and azido, as well as the 5,6-benzo- and 6,8-dichloro-substituted derivatives. By using click chemistry on 6-azido-sulfocoumarin which was reacted with various alkynes, a large series of 6-substituted-1,2,3-triazolo-sulfocoumarins were also obtained.[1] These derivatives (most of which were 6-substituted-sulfocoumarins, except for the two compounds with a diverse substitution pattern mentioned above)[1] were generally ineffective as inhibitors of the cytosolic isoforms hCA I and II, but some of them were highly potent inhibitors of the transmembrane, tumnor-associated isoforms hCA IX and XII.[1] The high resolution X-ray crystal structure of a mutant hCA II (in which some residues present in the hCA IX active site were introduced into hCA II) in complex with 6-bromosulfocoumarin also allowed us to understand the inhibition mechanism by this new class of CAIs, which has been discussed in the Introduction (see Fig. 1). Considering the very interesting inhibition profile of the four investigated CA isoforms (CA I, II, IX and XII) with these 6-substituted sulfocoumarins, it appeared of interest to further explore other substitution patterns on this ring system. In this paper we report two structural modifications in the sulfocoumarin scaffold: (i) a series of compounds were prepared which incorporate various moieties in the 7 position of the sulfocoumarin ring (as compounds possessing this substitution pattern were not yet investigated as CAIs), and (ii) we have also explored what are the consequences on the CA inhibitory effects if the double bond between the C3 and C4 atoms is

reduced in the sulfocoumarin scaffold (i.e., we investigated the CA inhibitory properties of 3,4-dihydrosulfocoumarins substituted in the 7 position of the heterocyclic ring).

Schemes 1-3 here

The general strategy of Zalubovskis's group[1,15] for the preparation of 6-substituted sulfocoumarins was applied by us for preparing 7-substituted such derivatives (Scheme 1-3). Reaction of 2,4-dihydroxybenzaldehyde with one equivalent of benzyl bromide afforded the benzyl derivative **1** which was treated with methanesulfonyl chloride followed by cyclization of the sulfonate **2**, in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), with formation of the racemic 4-hydroxy-7-benzyloxy-3,4-dihydrosulfocoumarin **3** (Scheme 1). The alcohol **3** was dehydrated in the presence of mesityl chloride leading to 7-benzyloxy-sulfocoumarin **4**, which by hydrogenation, depending on the pressure of H_2 used, was transformed in the benzyloxy-dihydrosulfocoumarin derivative **5** or the debenzylated dihydrosulfocoumarin **6** which has been transformed to the corresponding methanesulfonate **6a** by reaction with the sulfonyl chloride (Scheme 1). The key intermediate **3** was deprotected at the 7 position by hydrogenation, with formation of the 7-hydroxy derivative **7**, which by dehydration (in the same conditions as mentioned above for the transformation of **3** to **4**) and alkylsulfonylation of the phenol group led to **8**. Hydrolysis of the methylsulfonate ester **8** in the presence of tetraethylammonium hydroxide led to the formation of 7-hydroxysulfocoumarin **9** (Scheme 1). A similar strategy has been applied by using 2-hydroxy-4-methoxy benzaldehyde, which led after several steps, to the formation of the 7-methoxy-sulfocoumarin **12** and the corresponding dihydro derivative **13** (Scheme 2).

In order to create chemical diversity, the 7-hydroxy-3,4-dihydrosulfocoumarin **6** and 7-hydroxy-sulfocoumarin **9** were acylated with alkyl- ar arylalkyl acyl chlorides, leading to the esters **14-16** and **17-19**, respectively (Scheme 3).

Table 1 here

2.2. CA inhibition. Sulfocoumarins and their derivatives of types **3-19** reported here were assayed as inhibitors of five physiologically relevant CA isoforms, the cytosolic hCA I and II (h = human isoform), the mitochondrial hCA VA, and the transmembrane, tumor-associated hCA IX and XII (Table 1). The sulfonamide in clinical use acetazolamide (**AAZ**) has been used as standard in these measurements, for comparison reasons. The following should be noted on the CA inhibitory properties of the compounds **3-19** reported here:

(i) Three of the investigated isoforms, i.e., hCA I, IX and XII, were not significantly inhibited by compounds **3-19** up to 50 μM concentration of inhibitor in the assay system. These data are not very much intriguing regarding hCA I, which was also not inhibited by the 6-substituted sulfocoumarins reported earlier.[1] However, the 6-substututed sulfocoumarins were highly efficient hCA IX and XII inhibitors,[1] unlike the 7-substituted compounds **3-19** reported here, which are not inhibiting these isozymes (Table 1). This is, we think, a highly interesting result, confirming the fact that the position of the substituents in the sulfocoumarin ring (as in the case of the coumarins)[2-4] is the main factor influencing the CA inhibitory properties of these compounds. In fact, the nature of these substituents seemed to be rather irrelevant, as all these derivatives were ineffective as hCA I, IX and XII inhibitors, irrespective of the fact that they possesses small and compact (OH, OMe) or bulkier (4-chlorophenylacetyl; 2-bromophenylacetyl, etc.) moieties in the 7 position of the ring.

(ii) Although the 6-substututed sulfocoumarins reported earlier were also ineffective as hCA II inhibitors,[1] the 7-substituted compounds **3-19** reported here were excellent, low nanomolar inhibitors of this isoform, with inhibition constants in the range of 1.5 – 8.4 nM (Table 1) being thus much more effective compared to acetazolamide, a clinically used sulfonamide CAI. It should be mentioned that this type of behavior, i.e., important differences in the CA inhibitory properties against various CA isoforms of regioisomers, has also been reported by us earlier for coumarin CAIs. For example, disubstituted coumarins incorporating ether and acetyl/propionyl moieties in positions 6,7- and 7,8- of the heterocyclic ring differed very much in their inhibition profiles against hCA I, II, IX and XII.[3a] Whereas 6,7-disubstituted series showed ineffective inhibition for the transmembrane tumor-associated isoforms CA IX and XII (and micromolar affinity for hCA I and II), the corresponding isomeric 7,8-disubstituted coumarins showed nanomolar/subnanomolar inhibition of CA IX/XII (but were ineffective as hCA I and II inhibitors). [3a] Interestingly, the structure-activity relationship (SAR) for the inhibition of hCA II with compounds 3-19 is very flat, with all these derivatives possessing inhibition constants in a limited range (1.5 – 8.4 nM). Thus, the nature of the substituent in position 7 has an influence (but not a very important one) on the hCA II inhibitory properties. For example, The least "effective" hCA II inhibitors detected here, compounds **6** and **9**, possess a HO moiety in position 7 of the heterocyclic ring, and they showed K_Is of 7.6 – 8.4 nM (being extremely effective as hCA II inhibitors). Derivatives of **6** (such as **14-16**) or **9** (such as **17-19**) possessing bulkier moieties at the 7 position (of the ester type) were more effective hCA II inhibitors compared to the parent phenols **6** and **9**, with K_Is in the range of 1.5 – 3.4 nM. There were however not big differences of activity between the more compact acetyl esters (**14** and **17**) and the bulkier phenyl-substituted acetates **15, 16** and **18, 19**, respectively (Table 1). The same may be observed for the methoxy derivatives **12** and **13** (Table 1), which do not differ

significantly in their hCA II inhibitory properties. The other structural element differentiating compounds **3-19** reported here, was the presence or the absence of the double bond between carbons 3 and 4 of the heterocyclic ring. It may be observed that among the least effective hCA II inhibitors (**6** and **9**), one compound is saturated (**6**) whereas the second one has the double bond (**9**). Comparing the saturated (**14-16**) and aromatic (**17-19**) esters one may again see irrelevant differences of activity between the two series of compounds. We may thus conclude that the presence or the absence of the C_3-C_4 double bond does not significantly influence the hCA II inhibitory properties of these compounds. Probably both the sulfocoumarins as well as their dihydro derivatives possess a similar mechanism of hCA II inhibition, as depicted in Fig. 1.

(iii) The mitochondrial isoform CA VA, involved in biosynthetic reactions, and thus a target for anti-obesity agents,[17] was also inhibited by compounds **3-19** reported here, with K_Is in the range of 91 nM – 9.96 μM (Table 1). The best hCA VA inhibitors were compounds **13, 15** and **16** (K_Is in the range of 91 – 206 nM). They all incorporate the 3,4-dihydrosulfocoumarin scaffold, as well as a methoxy, 4-chlorophenylacetyl and 2-bromophenylacetayl moiety in position 7 of the heterocycle. It is interesting to note that the corresponding aromatic compounds, **12, 18** and **19**, are much weaker hCA VA inhibitors (K_Is in the range of 3910 – 9960 nM, Table 1). Thus, a very simple feature vin the scaffold of these compounds drastically influences affinity for the mitochondrial isoform. The remaining compounds investigated here were medium potency – weak hCA VA inhibitors, with inhibition constants in the range of 327 – 5090 nM (Table 1). Compound **13**, the best hCA VA inhibitor detected here, showed an inhibition profile against this isoform similar to that of acetazolamide **AAZ**.

3. Conclusions

We report here a series of 7-substituted sulfocoumarins and 3,4-dihydrosulfocoumarins, obtained by cyclization of the methanesulfonate of 2,4-dihydroxy- or 2-hydroxy-4-methoxybenzaldehyde, possessing a range of substituents in position 7 of the heterocyclic ring. Unlike the 6-substituted sulfocoumarins investigated earlier, which were potent hCA IX and XII inhibitors and ineffective hCA I and II inhibitors, compounds from this series showed low nanomolar hCA II inhibitory properties, inhibited the mitochondrial isoform hCA VA with K_Is in the range of 91 – 9960 nM, and were ineffective as hCA I, IX and XII inhibitors. The structure activity relationship for this class of CAIs was rather well defined (at least for hCA II and VA), with the nature of the 7-substituent

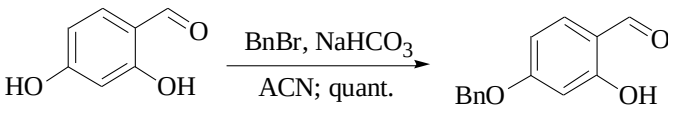
strongly influencing hCA VA inhibition, whereas the nature of these groups were less relevant for hCA II inhibition (all the reported compounds were highly effective hCA II inhibitors, with K_Is in the range of 1.5 – 8.4 nM). Since both hCA II and hCA VA are important drug targets (hCA II for obtaining antiglaucoma agents; hCA VA for antiobesity drugs), these isoform-selective CAIs reported here may be considered of interest for various biomedical applications.

4. Experimental

4.1. Chemistry

Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, Alfa Aesar and TCI. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringes techniques to transfer solutions. Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR, DEPT-135, DEPT-90, HSQC, HMBC) 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; sept, septet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double of doublet,appt, aparent triplet, appq, aparent quartet, brt broad triplet. The assignment of exchangeable protons (O*H* and 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 ethylacetate/*n*-hexane were used as eluents. Melting points (m.p.) were carried out in open capillary tubes and are uncorrected.

Synthesis of 4-benzyloxy-2-hydroxy-benzaldehyde **1**



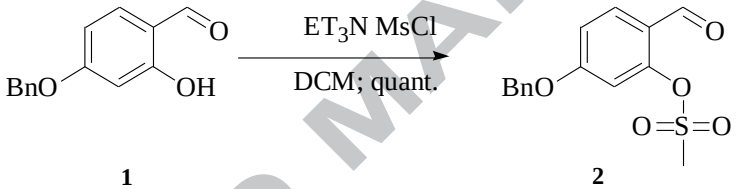
1

2,4-Dihydroxybenzaldehyde (3.0 g, 1.0 eq) was dissolved in acetonitrile (220 ml) and treated with $NaHCO_3$ (2.2 eq) for 45 min at r.t. followed by the addition of benzylbromide (1.1 eq). The reaction

mixture was stirred under reflux O.N. and then quenched with H_2O (100 ml) and extracted with ethyl acetate (3 x 25 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the title compound **12** as a white solid that was used as it is.

4-Benzyloxy-2-hydroxy-benzaldehyde **1**: quantitative; silica gel TLC R_f 0.65 (Ethyl acetate/*n*-hexane 20 % *v/v*); δ_H (400 MHz, DMSO-d_6): 5.21 (s, 2H), 6.60 (d, *J* 2.2, 1H), 6.68 (dd, *J* 2.2, 8.6, 1H), 7.44 (m, 5H), 7.67 (d, *J* 8.6, 1H), 10.5 (s, 1H); δ_C (100 MHz, DMSO-d_6) 70.6, 102.6, 108.9, 117.2, 128.7, 129.0, 129.4, 133.2, 137.1, 163.9, 165.9, 192.0. Experimental data in agreement with reported data.[18]

Synthesis of methanesulfonic acid 5-benzyloxy-2-formyl-phenyl ester **2**

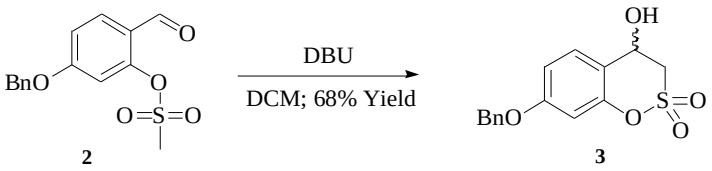


1 **2**

4-Benzyloxy-2-hydroxy-benzaldehyde **1** (5.61 g, 1.0 eq) was dissolved in dry DCM (250 ml) followed by addition of triethylamine (1.2 eq) and methanesulfonylchloride (1.6 eq). The reaction was stirred at r.t. until starting material was consumed (TLC monitoring) and then quenched with a 1.0 M hydrochloric acid aqueous solution (100 ml) and extracted with ethyl acetate (3 x 25 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the title compound **2** as a white solid that was used as it is.

Methanesulfonic acid 5-benzyloxy-2-formyl-phenyl ester **2**: quantitative yield; silica gel TLC R_f 0.35 (Ethyl acetate/*n*-hexane 30 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.61 (s, 3H), 5.30 (s, 2H), 7.22 (d, *J* 2.4, 1H), 7.25 (dd, *J* 2.4, 8.6, 1H), 7.45 (m, 5H), 7.92 (d, *J* 8.6, 1H), 10.11 (s, 1H); δ_C (100 MHz, DMSO-d_6) 30.8, 71.3, 110.7, 115.5, 123.6, 129.0, 129.2, 129.5, 131.5, 136.7, 152.5, 165.0, 188.0. Experimental data in agreement with reported data.[15]

Synthesis of 7-benzyloxy-2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-4-ol **3**

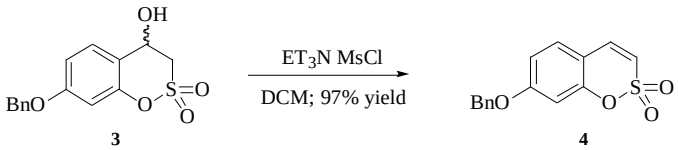


2 3

Methanesulfonic acid 5-benzyloxy-2-formyl-phenyl ester **2** (7.82 g, 1.0 eq) was dissolved in DCM (320 ml) and then the solution was cooled down to 0°C and DBU (1.8 eq) was added drop-wise. The reaction was stirred at r.t. until consumption of the starting material (TLC monitoring) and then quenched with a 1.0 M hydrochloric acid aqueous solution (100 ml) and extracted with ethyl acetate (3 x 25 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with 30 % EtOAc in *n*-hexane to afford the title compound **3** as a white solid.

7-Benzyloxy-2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-4-ol **3**: 68% yield; m.p. 105-107°C; silica gel TLC R_f 0.35 (Ethyl acetate/*n*-hexane 30 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.65 (dd, *J* 9.1, 13.6, 1H), 4.26 (dd, *J* 6.2, 13.6, 1H), 5.09 (m, 1H), 5.19 (s, 2H), 6.30 (d, *J* 7.0, 1H), 6.86 (d, *J* 2.5, 1H), 7.01 (dd, *J* 2.5, 8.7, 1H), 7.44 (m, 6H), 9.70 (s, 1H); δ_C (100 MHz, DMSO-d_6) 52.3, 65.2, 70.5, 104.7, 114.0, 119.1, 128.7, 128.9, 129.4, 130.7, 137.5, 150.3, 160.1; Elemental analysis: calc: C 58.81, H 4.61, S 10.47; found: C 58.16, H 4.79, S 11.44. Experimental data in agreement with reported data.[15]

Synthesis of 7-benzyloxy-benzo[e][1,2]oxathiine 2,2-dioxide **4**

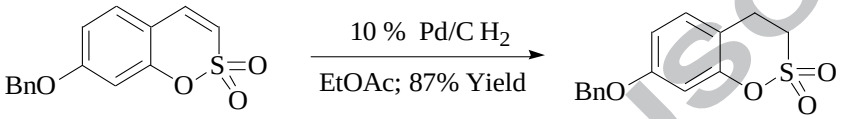


3 4

7-Benzyloxy-2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-4-ol **3** (1.2 g, 1.0 eq) was dissolved in dry DCM (50 ml) and treated with triethylamine (2.0 eq) then the solution was cooled down to 0°C and methanesulfonyl chloride (1.6 eq) was added drop-wise. The reaction was stirred at r.t. until complete consumption of starting material (TLC monitoring) and then quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml) and extracted with ethyl acetate (3 x 10 ml). The combined organic layers were washed with brine (2 x 10 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the title compound **4** as a white solid that was used as it is.

7-Benzyloxy-benzo[e][1,2]oxathiine 2,2-dioxide **4**: 97% yield; m.p. 118-119°C; silica gel TLC R_f 0.39 (Ethyl acetate/*n*-hexane 30 % *v/v*); δ_H (400 MHz, DMSO-d_6): 5.25 (s, 2H), 7.09 (dd, *J* 2.5 8.6, 1H), 7.19 (d, *J* 2.5, 1H), 7.34 (d, *J* 10.3, 1H), 7.45 (m, 5H), 7.65 (d, *J* 2.5, 1H), 7.67 (m, 1H); δ_C (100 MHz, DMSO-d_6) 71.0, 105.6, 113.0, 114.4, 120.1, 128.9, 129.1, 129.5, 132.1, 137.0, 137.4, 153.2, 162.3; Elemental analysis: calc: C 62.49, H 4.20, S 11.12; found: C 61.52, H 4.18, S 11.75.

Synthesis of 7-benzyloxy-3,4-dihydro-benzo[e][1,2]oxathiine 2,2-dioxide **5**

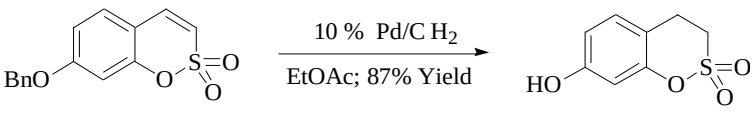


4 **5**

A solution of 7-benzyloxy-benzo[e][1,2]oxathiine 2,2-dioxide **4** (0.15 g, 1.0 eq) in 20 ml of ethyl acetate was hydrogenated for 1 h at 20 Psi using 10 % Pd/C (0,05g) as catalyst. The mixture filtered through Celite®, the solvent was eliminated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with 50 % EtOAc in *n*-hexane to afford the title compound **5** as a white solid.

7-Benzyloxy-3,4-dihydro-benzo[e][1,2]oxathiine 2,2-dioxide **5**: 87% yield; m.p. 120-121°C; silica gel TLC R_f 0.52 (Ethyl acetate/*n*-hexane 30 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.28 (t, *J* 6.8, 2H), 3.87 (t, *J* 6.8, 2H), 5.15 (s, 2H), 6.82 (d, *J* 2.5, 1H), 6.93 (dd, *J* 2.5, 8.6, 1H), 7.29 (d, *J* 8.6, 1H), 7.43 (m, 5H); δ_C (100 MHz, DMSO-d_6) 26.3, 44.8, 70.5, 105.2, 113.7, 113.7, 128.6, 128.8, 129.4, 131.4, 137.5, 152.6, 159.0; Elemental analysis: calc: C 62.05, H 4.86, S 11.04; found: C 61.80, H 4.11, S 11.20. Experimental data in agreement with reported data.[18]

Synthesis of 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-ol **6**

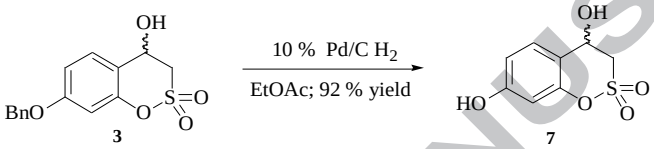


4 **6**

A solution of 7-benzyloxy-benzo[e][1,2]oxathiine 2,2-dioxide **4** (0.10 g, 1.0 eq) in 20 ml of ethyl acetate was hydrogenated O.N. at 75 Psi using 10 % Pd/C (0,05g) as catalyst. The mixture was filtered through Celite®, the solvent was eliminated under *vacuo* to give the title compound **6** as a white solid and was used as it is.

2,2-Dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-ol **6**: 87% yield; m.p. 149-151°C; silica gel TLC R_f 0.14 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.28 (t, *J* 6.8, 2H), 3.83 (t, *J* 6.8, 2H), 6.47 (d, *J* 2.5, 1H), 6.67 (dd, *J* 2.5, 8.4, 1H), 7.29 (d, *J* 8.4, 1H), 9.87 (s, 1H); δ_C (100 MHz, DMSO-d_6) 26.3, 45.0, 105.5, 111.7, 113.9, 131.4, 152.6, 158.3; Elemental analysis: calc: C 47.99, H 4.03, S 16.02; found: C 47.54, H 4.14, S 16.41. Experimental data in agreement with reported data.[18]

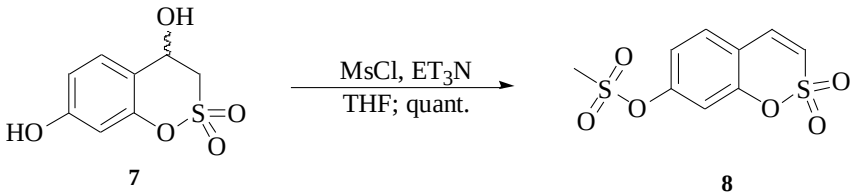
Synthesis of 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiine-4,7-diol **7**



A solution of 7-benzyloxy-2,2-dioxo-3,4-dihydro-2*H*-22λ^6-benzo[e][1,2]oxathiin-4-ol **3** (0.20 g, 1.0 eq) in 20 ml of ethyl acetate was hydrogenated O.N. at 60 Psi using 10 % Pd/C (0,05g) as catalyst. The mixture was filtered through Celite®, the solvent was eliminated under *vacuo* to to give a residue that was purified by silica gel column chromatography eluting with 50 % EtOAc in *n*-hexane to afford the title compound **7** as a white solid.

2,2-Dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiine-4,7-diol **7**: 92 % yield; m.p. 142-143°C; silica gel TLC R_f 0.26 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.60 (dd, *J* 9.1, 13.7 , 1H), 4.22 (dd, *J* 6.1, 13.7, 1H), 5.05 (m, 1H), 6.21 (d, *J* 7.1, 1H), 6.50 (d, *J* 2.4, 1H), 6.76 (dd, *J* 2.4, 8.6, 1H), 7.42 (d, *J* 8.6, 1H), 10.05 (s, 1H); δ_C (100 MHz, DMSO-d_6) 52.4, 65.2, 105.0, 114.1, 117.3, 130.7, 150.3, 159.4; Elemental analysis: calc: C 44.44, H 3.73, S 14.83; found: C 44.30, H 3.66, S 14.62. Experimental data in agreement with reported data.[18]

Synthesis of methanesulfonic acid 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **8**

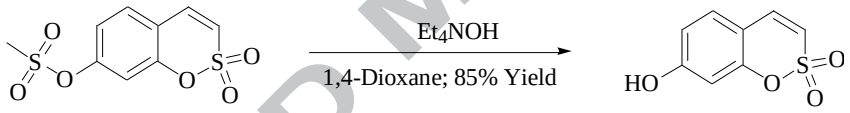


2,2-Dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiine-4,7-diol **7** (0.1 g, 1.0 eq) was dissolved in THF (5.0 ml), then the solution was cooled down to 0°C and treated drop-wise with

methanesulfonylchloride (1.6 eq). The reaction was stirred at r.t until consumption of the starting material (TLC monitoring) and then quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml) and extracted with ethyl acetate (3 x 10 ml). The combined organic layers were washed with brine (2 x 10 ml) dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the title compound **8** as a white solid and was used as it is.

2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **8**: quantitative yield; m.p. 127-128°C; silica gel TLC R_f 0.35 (Ethyl acetate/*n*-hexane 40 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.54 (s, 3H), 7.47 (dd, *J* 2.4, 8.6, 1H), 7.61 (d, *J* 2.4, 1H), 7.63 (d, *J* 10.4, 1H), 7.80 (d, *J* 10.4 Hz, 1H), 7.90 (d, *J* 8.6, 1H); δ_C (100 MHz, DMSO-d_6) 39.0, 114.1, 119.0, 121.5, 123.6, 132.7, 136.8, 151.8, 152.3; Elemental analysis: calc: C 39.12, H 2.92, S 23.21; found: C 39.15, H 3.02, S 26.41. Experimental data in agreement with reported data.[15]

Synthesis of 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-ol **9**

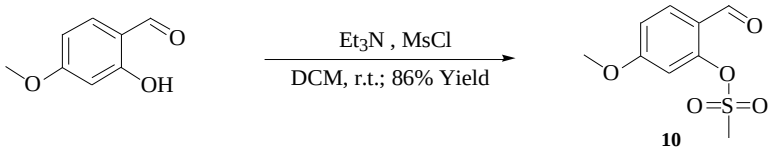


8 **9**

2,2-Dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **8** (0.13 g, 1.0 eq) was dissolved in 1,4-dioxane (5.0 ml) and treated with a 20 % aqueous solution of tetraethylamonium hydroxide (2.0 eq). The solution was stirred at r.t. until consumption of the starting material (TLC monitoring), then quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml) and extracted with ethyl acetate (3 x 10 ml). The combined organic layers were washed with brine (2 x 10 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the title compound **9** as a white solid and was used as it is.

2,2-Dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-ol **9**: 85% yield; m.p. 157-159°C; silica gel TLC R_f 0.43 (Ethyl acetate/*n*-hexane 40 % *v/v*); δ_H (400 MHz, DMSO-d_6): 6.77 (d, *J* 2.4, 1H), 6.83 (dd, *J* 2.4, 8.5, 1H), 7.23 (d, *J* 10.3, 1H), 7.54 (d, *J* 8.5, 1H), 7.60 (d, *J* 10.3, 1H), 10.79 (s, 1H); δ_C (100 MHz, DMSO-d_6) 105.9, 111.5, 114.6, 119.0, 132.3, 137.7, 153.3, 162.1; Elemental analysis: calc: C 48.48, H 3.05, S 16.18; found: C 48.60, H 3.16, S 17.00. Experimental data in agreement with reported data.[19]

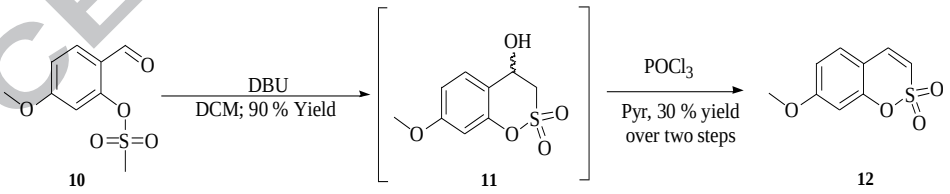
Synthesis of methanesulfonic acid 2-formyl-5-methoxy-phenyl ester **10**



2-Hydroxy-4-methoxybenzaldehyde (1.0 g, 1.0 eq) and triethylamine (1.2 eq) were dissolved in dry DCM (40.0 ml) followed by addition of methanesulfonyl chloride (1.6 eq) at 0°C. The reaction was stirred at r.t. under a nitrogen atmosphere until starting material was consumed (TLC monitoring) then quenched with H_2O (30 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give a residue that was purified silica gel column chromatography eluting with 20 % EtOAc in *n*-hexane to afford the title compound **10** as a white solid.

Methanesulfonic acid 2-formyl-5-methoxy-phenyl ester **10**: 86 % yield; m.p. 120-122°C silica gel TLC R_f 0.05 (Ethyl acetate/*n*-hexane 20 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.62 (s, 3H), 3.94 (s, 3H), 7.12 (d, *J* 2.4, 1H), 7.17 (dd, *J* 2.4, 8.6, 1H), 10.12 (s, 1H); δ_C (100 MHz, DMSO-d_6) 38.8, 57.2, 109.9, 114.8, 123.4, 131.5, 152.5, 165.9, 188.1; Elemental analysis: calc: C 46.95, H 4.38, S 13.93; found: C 47.53, H 4.21, S 12.04.

Synthesis of 7-methoxy-benzo[e][1,2]oxathiine 2,2-dioxide **12**

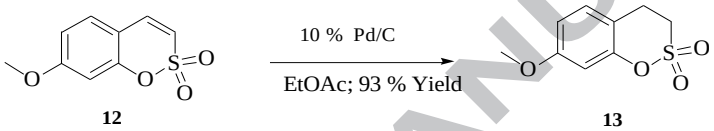


Methanesulfonic acid 2-formyl-5-methoxy-phenyl ester **10** (1.24 g, 1.0 eq) was dissolved in dry DCM (25 ml) and then DBU (1.0 eq) was added. The solution was cooled down to 0°C and mesylchloride (1.6 eq) was added drop-wise. The reaction was stirred at r.t. under a nitrogen atmosphere for 2 hrs and then quenched with a 10% aqueous hydrochloric acid solution (30 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the intermediate **11** which was dissolved in pyridine (20 ml) and the solution was treated at 0°C with phosphorus oxychloride (1.5 eq). The reaction mixture was stirred at r.t. for 3 hrs at r.t. and then

quenched with slush (30 g). The precipitate formed was collected by filtration, dried under *vacuo* and recrystallized from EtOH to afford the title compound **12** as a light brown solid.

7-Methoxy-benzo[e][1,2]oxathiine 2,2-dioxide **12**: 30 % yield over 2 steps; m.p. 111-112°C; silica gel TLC R_f 0.16 (Ethyl acetate/*n*-hexane 40 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.89 (s, 3H), 6.84 (dd, *J* 2.4, 8.7, 1H), 7.29 (d, *J* 2.4, 1H), 7.33 (d, *J* 10.2, 1H), 7.66 (m, 2H); δ_C (100 MHz, DMSO-d_6) 57.0, 104.7, 112.8, 113.8, 120.0, 132.0, 137.4, 153.3, 163.3; Elemental analysis: calc: C 50.94, H 3.80, S 15.11; found: C 50.61, H 3.69, S 16.99.

Synthesis of 7-methoxy-3,4-dihydro-benzo[e][1,2]oxathiine 2,2-dioxide **13**

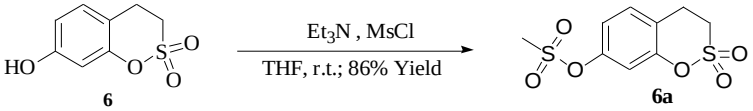


12 **13**

A solution of 7-methoxy-benzo[e][1,2]oxathiine 2,2-dioxide **12** (0.10 g, 1.0 eq) in 20 ml of ethyl acetate was hydrogenated for 2 hrs at 40 Psi using 10 % Pd/C (0,05g) as catalyst. The mixture was filtered through Celite®, the solvent was eliminated under *vacuo* to give the title compound **13** as white solid and was used as it is.

7-Methoxy-3,4-dihydro-benzo[e][1,2]oxathiine 2,2-dioxide **13**: 93 %yield; m.p. 160-161°C; silica gel TLC R_f 0.54 (Ethyl acetate/*n*-hexane 40 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.32 (t, *J* 6.8, 2H), 3.79 (s, 3H), 3.87 (t, *J* 6.8, 2H), 6.74 (d, *J* 2.6, 1H), 6.84 (dd, *J* 2.6, 8.5, 1H), 7.29 (d, *J* 8.6, 1H); δ_C (100 MHz, DMSO-d_6) 26.4, 44.9, 56.5, 104.3, 113.0, 113.5, 131.4, 152.7, 160.1; Elemental analysis: calc: C 50.46, H 4.70, S 14.97; found: C 49.45, H 4.64, S 15.75.

Synthesis of methanesulfonic acid 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **6a**



6 **6a**

2,2-Dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-ol **6** or 2,2-dioxo-2*H*-2λ^6-benzo [e][1,2]oxathiin-7-ol **9** (0.1 g, 1.0 eq) was dissolved in THF (10.0 ml) and treated with triethylamine (1.5 eq). The solution was cooled down to 0°C and treated with methanesulfonyl chloride (1.6 eq). The reaction was stirred at r.t. until starting material was consumed (TLC monitoring) then

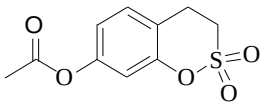
quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml) extracted with ethyl acetate (3 x 10 ml). The combined organic layers were washed with brine (2 x 10 ml) dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the title compound **6a** as a white solid and was used as it is.

Methanesulfonic acid 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **6a**: 86 % yield; m.p. 106-107°C; silica gel TLC R_f 0.19 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.44 (t, *J* 7.0, 2H), 3.46 (s, 3H), 3.98 (t, *J* 7.0, 2H), 7.25 (d, *J* 2.4, 1H), 7.27 (dd, *J* 2.4, 8.2, 1H), 7.52 (d, *J* 8.2, 1H); δ_C (100 MHz, DMSO-d_6) 26.8, 38.5, 44.5, 113.6, 120.8, 121.4, 132.2, 149.1, 152.4; Elemental analysis: calc: C 38.84, H 3.62, S 23.04; found: C 38.73, H 3.80, S 24.41.

Synthesis of 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-acyl (**14-16**) and 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-acyl (**17-19**) derivatives.

2,2-Dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-ol **6** or 2,2-dioxo-2*H*-2λ^6-benzo [e][1,2]oxathiin-7-ol **9** (0.1 g, 1.0 eq) was dissolved in THF (10.0 ml) and treated with triethylamine (1.5 eq). The solution was cooled down to 0°C and treated with the corresponding acyl halide (1.0 eq). The reaction was stirred at r.t. until starting material was consumed (TLC monitoring) then quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml) extracted with ethyl acetate (3 x 10 ml). The combined organic layers were washed with brine (2 x 10 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with the appropriate concentration of EtOAc in *n*-hexane to afford the title compounds

Synthesis of acetic acid 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **14**



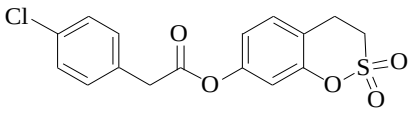
14

The title compound **14** was obtained according to the procedure described above and used as it is.

Acetic acid 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **14**: quantitative; m.p. 99-101°C; silica gel TLC R_f 0.55 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6):

2.30 (s, 3H), 3.41 (t, *J* 6.6, 2H), 3.94 (t, *J* 6.6, 2H), 7.02 (d, *J* 2.0, 1H), 7.05 (dd, *J* 2.3, 8.4, 1H), 7.43 (d, *J* 8.4, 1H); δ_C (100 MHz, DMSO-d_6) 21.7, 26.8, 44.6, 113.2, 119.5, 120.0, 131.5, 150.8, 152.2, 169.9; Elemental analysis: calc: C 49.58, H 4.16, S 13.24; found: C 49.32, H 4.15, S 13.72.

Synthesis of (4-chloro-phenyl)-acetic acid 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **15**

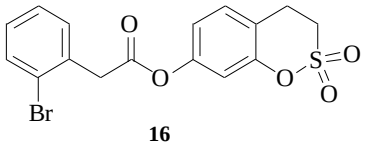


15

The title compound **15** was obtained according to the procedure described above and used as it is.

(4-Chloro-phenyl)-acetic acid 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **15**: quantitative; m.p. 97-99°C; silica gel TLC R_f 0.59 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.40 (t, *J* 6.8, 2H), 3.95 (t, *J* 6.8, 2H), 4.04 (s, 2H), 7.04 (s, 1H), 7.05 (dd, *J* 2.3, 8.4, 1H), 7.45 (m, 5H); δ_C (100 MHz, DMSO-d_6) 26.9, 44.7, 113.2, 119.7 119.8, 120.0, 129.1, 129.4, 131.7, 132.6, 133.8, 150.9, 152.3, 170.8; Elemental analysis: calc: C 54.47, H 3.71, S 9.09; found: C 54.25, H 3.67, S 8.92.

Synthesis of (2-bromo-phenyl)-acetic acid 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **16**



16

The title compound **16** was obtained according to the procedure described above after purification by silica gel column chromatography eluting with 50% EtOAc in *n*-hexane.

(2-Bromo-phenyl)-acetic acid 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **16**: 67 % yield; m.p. 114-116°C; silica gel TLC R_f 0.53 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.40 (t, *J* 6.8, 2H), 3.94 (t, *J* 6.8, 2H), 4.15 (s, 2H), 7.01 (d, *J* 2.3, 1H), 7.06 (dd, *J* 2.3, 8.5, 1H), 7.31 (m, 1H), 7.44 (m, 2H), 7.55 (dd, *J* 1.6, 8.0, 1H), 7.70 (dd, *J* 1.6, 8.0, 1H); δ_C (100 MHz, DMSO-d_6) 26.8, 41.8, 44.6, 112.9, 119.8, 125.6, 128.9, 129.2, 130.5, 131.7, 133.2, 133.35,

133.39, 150.7, 152.2, 169.8; Elemental analysis: calc: C 48.38, H 3.30, S 8.07; found: C 47.49, H 3.26, S 7.60.

Synthesis of acetic acid 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **17**

The title compound **17** was obtained according to the procedure described above and used as it is.

Acetic acid 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **17**: 62 % yield; m.p. 57-59°C; silica gel TLC R_f 0.50 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6): 2.34 (s, 3H), 7.27 (dd, *J* 2.2, 8.4, 1H), 7.41 (d, *J* 2.2, 1H), 7.56 (d, *J* 10.4, 1H), 7.76 (d, *J* 10.4, 1H), 7.80 (d, *J* 8.4, 1H); δ_C (100 MHz, DMSO-d_6) 21.9, 113.8, 117.7, 121.3, 123.0, 132.1, 137.2, 152.2, 153.8, 169.8; Elemental analysis: calc: C 50.00, H 3.36, S 13.35; found: C 50.91, H 4.60, S 11.43.

Sinthesis of (4-chloro-phenyl)-acetic acid 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **18**

The title compound **18** was obtained according to the procedure described above after purification by silica gel column chromatography eluting with 50% EtOAc in *n*-hexane.

(4-Chloro-phenyl)-acetic acid 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **18**: 53 % yield; m.p. 106-108°C; silica gel TLC R_f 0.61 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6): 4.07 (s, 2H), 7.28 (dd, *J* 2.2, 8.4, 1H), 7.44 (d, *J* 2.2, 1H), 7.47 (s, 4H), 7.57 (d, *J* 10.4, 1H), 7.78 (d, *J* 10.4, 1H), 7.81 (d, *J* 8.4, 1H); δ_C (100 MHz, DMSO-d_6) 39.6, 113.6, 117.7, 121.0, 122.9, 129.3, 132.0, 132.5, 132. 8, 133.5, 137.0, 152.1, 153.6, 170.4; Elemental analysis: calc: C 54.78, H 3.16, S 9.14; found: C 53.75, H 3.29, S 8.18.

Synthesis of (2-bromo-phenyl)-acetic acid 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **19**

The title compound **19** was obtained according to the procedure described above after purification by silica gel column chromatography eluting with 40% EtOAc in *n*-hexane.

(2-Bromo-phenyl)-acetic acid 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-yl ester **19**: 50 % yield; m.p. 132-135°C; silica gel TLC R_f 0.73 (Ethyl acetate/*n*-hexane 40 % *v/v*); δ_H (400 MHz, DMSO-d_6): 4.20 (s, 2H), 7.28 (dd, *J* 2.3, 8.4, 1H), 7.32 (m, 1H), 7.43 (m, 2H), 7.57 (d, *J* 10.4, 2H), 7.71 (m, 1H), 7.77 (d, *J* 10.4, 1H), 7.82 (d, *J* 8.4, 1H); δ_C (100 MHz, DMSO-d_6) 41.8, 113.4, 117.8,

120.9, 123.0, 125.5, 128.9, 130.5, 132.1, 133.3, 133.4, 134.6, 137.0, 152.1, 153.5, 169.5; Elemental analysis: calc: C 48.62, H 2.81, S 8.11; found: C 47.51, H 2.90, S 7.47.

4.2. CA inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity.[16] 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) 0r 4 °C (6h) prior to assay, in order to allow for the formation of the E-I complex. Data from table 1 were obtained after 6 hours incubation of enzyme and inhibitor, as for the sulfocoumarins and coumarins reported earlier.[1-3] 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.[20,21]

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Table 1. hCA I, II, VA, IX and XII inhibition data with sulfocoumarins and their derivatives of types **3-19** synthesized in this work, by a stopped-flow CO_2 hydrase assay.[16] The sulfonamide inhibitor acetazolamide (**AAZ**, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide) was used as standard.

Compound	Ki (nM)*				
	hCA I	hCA II	hCA VA	hCA IX	hCA XII
3	>10000	2.3	591	>10000	>10000
4	>10000	2.5	835	>10000	>10000
5	>10000	2.0	2150	>10000	>10000
6	>10000	7.6	327	>10000	>10000
6a	>10000	1.8	517	>10000	>10000
7	>10000	1.6	5100	>10000	>10000
8	>10000	2.1	5090	>10000	>10000
9	>10000	8.4	788	>10000	>10000
12	>10000	1.5	9960	>10000	>10000
13	>10000	2.4	91	>10000	>10000
14	>10000	1.5	1675	>10000	>10000
15	>10000	2.2	206	>10000	>10000
16	>10000	3.4	141	>10000	>10000
17	>10000	2.2	716	>10000	>10000
18	>10000	2.6	3910	>10000	>10000
19	>10000	1.9	7060	>10000	>10000
AAZ	250	12	64	25	5.7

* Errors in the range of ± 10 of the reported values, from 3 different assays.

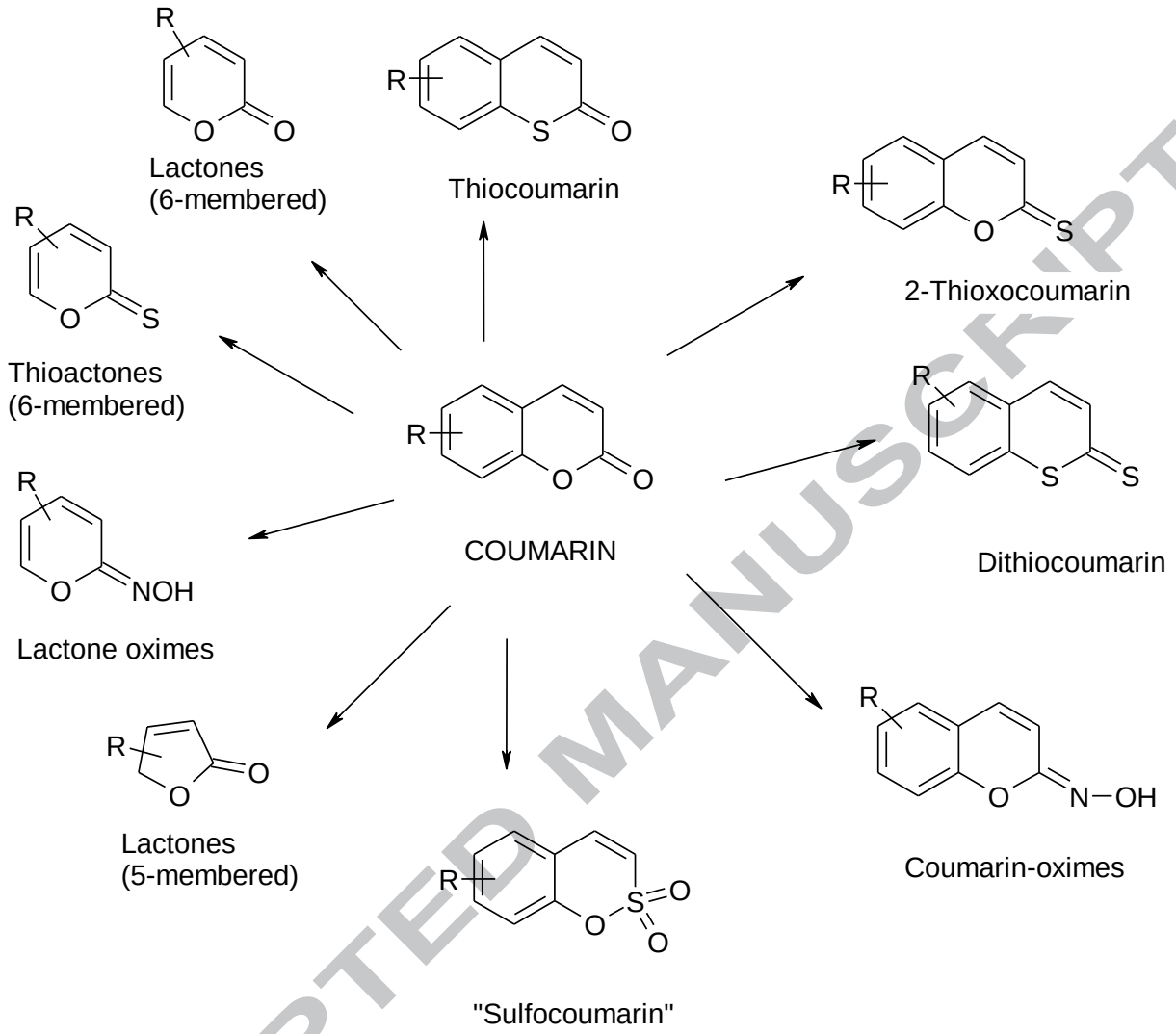
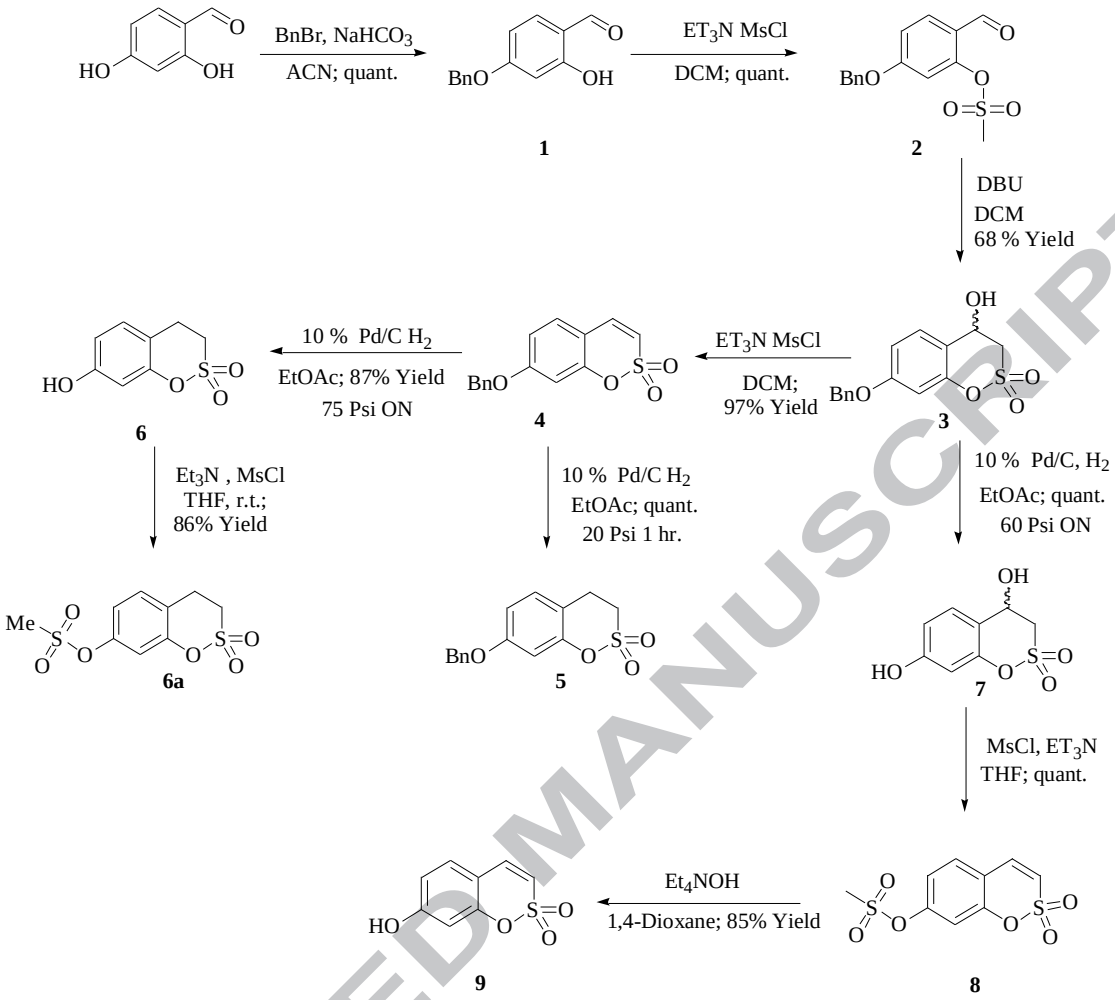
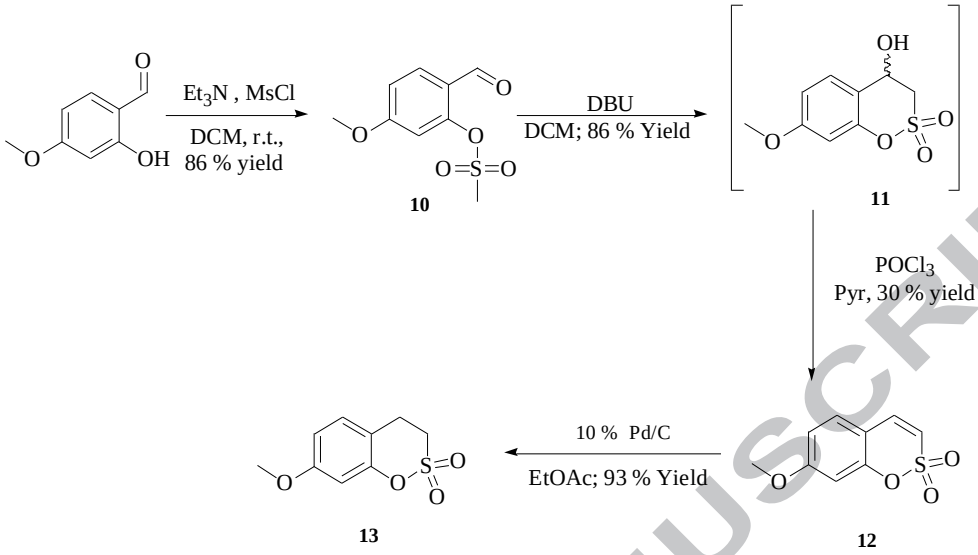


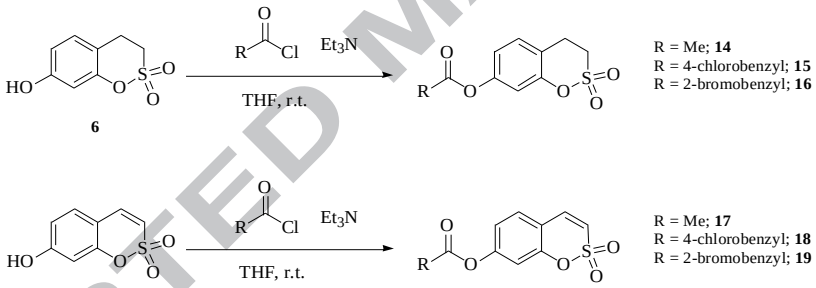
Chart 1: The various classes of CAIs developed by using the coumarins as lead molecules.



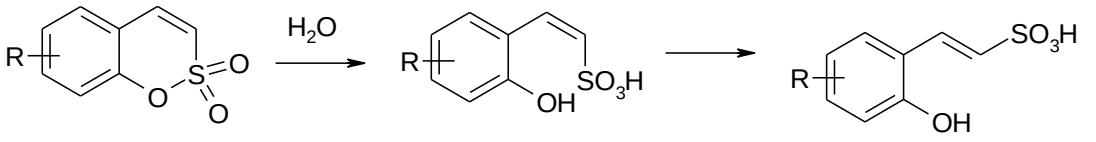
Scheme 1: Synthesis of sulfocoumarins **3-9**.



Scheme 2: Synthesis of sulfocoumarins **12** and **13**.



Scheme 3: Synthesis of 2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-acyl (**14-16**) and 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-7-acyl (**17-19**) derivatives.

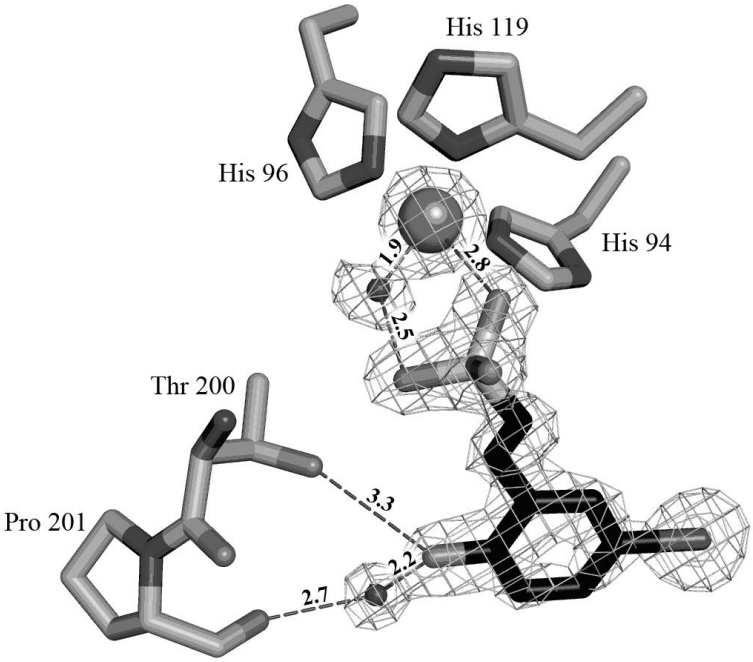


Sulfocoumarin

Reaction intermediated (not isolated)

Hydrolyzed sulfocoumarin

A

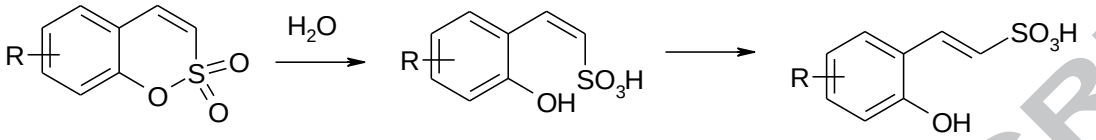


B

Fig. 1: CA inhibition mechanism by sulfocoumarins. A. The sulfocoumarin undergoes an enzyme-mediated hydrolysis with formation of the *trans*-2-hydroxy-phenyl-ω-ethenylsulfonic acid. B. The sulfonic acid binds to a mutant CA II active site, by anchoring of the sulfonic acid group to the zinc-coordinated water molecule. The Zn(II) ion (central larger sphere), its three His ligands (His94, 96 and 119), water molecule coordinated to the zinc (small sphere) as well as active site residues Thr200 and Pro201 involved in the binding of the hydrolyzed sulfocoumarin are shown, as determined by X-ray crystallography (PDB file 4bcw).[1]

7-Substituted-sulfocoumarins are isoform-selective, potent carbonic anhydrase II inhibitors

Muhammet Tanc, Fabrizio Carta, Murat Bozdag, Andrea Scozzafava, and Claudiu T. Supuran*[b]



Sulfocoumarin

Reaction intermediated (not isolated)

Hydrolyzed sulfocoumarin

K_Is (hCA II) = 1.5 – 8.4 nM

K_Is (hCA I, hCA IX, hCA XII) > 10 μM