Continued Structural Exploration of Sulfocoumarin as Selective Inhibitor of Tumor-Associated Human Carbonic Anhydrases IX and XII

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Abstract: A series of new 3- and 7-substituted sulfocoumarins was obtained by several cyclization reactions and subsequent derivatization for screening as prodrug inhibitors of the human (h) cancer-associated carbonic anhydrases (CAs) IX and XII. All products were ineffective inhibitors against the off-target hCA I and II, whilst hCAs IX and XII were inhibited with inhibition constants (K\textsubscript{I}s) spanning from low nanomolar to the high micromolar range, according to the sulfocoumarin derivatization pattern. In particular, sulfocoumarin 15 turned out to be the most potent and selective inhibitor herein reported (hCA I and II: K\textsubscript{I} > 100 µM; hCA IX: K\textsubscript{I} = 22.9 nM; hCA XII: K\textsubscript{I} = 19.2 nM). Considering that hCA IX and XII 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.

Keywords: carbonic anhydrase; cancer; sulfocoumarin

1. Introduction

Carbonic anhydrases are a ubiquitous metalloenzyme family present in all living organisms. Until now, eight genetically distinct CA subfamilies have been identified [1–3]. In mammals, 16 CA isoforms belonging to α-CAs are characterized, and in humans 15 CA (hCA) isoforms are expressed, showing differences in kinetic properties, subcellular localization and tissue distributions [4,5]. Twelve such isoforms are catalytically active (CAs I–IV, VA, VB, VI, VII, IX, XII and XIV), whereas the remaining three isoforms (VIII, X, XI), called CA-related proteins (CARPs), have no activity. hCAs can be further categorized into four different subsets depending on their subcellular localization as cytosolic (hCA I, II, III, VII, VIII, X, XI, XIII), mitochondrial (hCA VA and VB), secreted (hCA VI), and membrane-bound (hCA IV, IX, XII, and XIV) [5–7]. Four distinct CA inhibition mechanisms have been reported and detailed to date with both kinetic and X-crystallographic studies [8,9]. They include (a) the metal ion binders (anions, sulfonamides and their bioisosteres, dithiocarbamates, xanthates, etc.); (b) compounds that anchor to the zinc-coordinated water molecule/hydroxide ion (phenols, carboxylates, polyamines); (c) compounds occluding the active site entrance, such as coumarins and their isosteres (sulfocoumarins); and (d) compounds binding out of the active site, such as an aromatic carboxylic acid derivative [8–10]. Coumarins, such as 1 (natural product isolated from the Australian plant Leionema ellipticum) and 2 (the simple unsubstituted coumarin), were discovered as a new chemotype that can inhibit the metalloenzyme carbonic anhydrase a decade ago [11–13]. Many differently substituted coumarins were subsequently screened for their inhibitory activity against all the 13 catalytically active mammalian CA isoforms, CA I-VII, IX, XII-XV [14–18]. Many of these isoforms are established drug targets for designing agents with various applications, such as diuretics, antiglaucoma drugs, anticonvulsants, antidiabetes agents or antitumor drugs/cancer diagnostic tools [17,18]. To explain the inhibitor mechanism
of coumarins 1 and 2, they were cococrystallized with human CA II, and the electron density data showed the presence of the hydrolyzed derivatives 4 and 5, respectively (Figure 1). The most notable aspect of this inhibition mechanism is the fact that the 4 and 5 occlude the enzyme active site binding at the entrance of the cavity.

![Figure 1](image_url)

**Figure 1.** Coumarins 1 and 2, and sulfocoumarin 3 and their CA-mediated hydrolysis to the CAI active species 4, 5 and 6, respectively.

Over the last several years, a rather large number of new classes of CAIs were reported, starting from coumarins as the lead, among which are thio coumarins, sulfocoumarins, 2-thioxo-coumarins, coumarin oximes, 5-/6-membered/thio)lactones, etc. [19–28]; coumarins and sulfocoumarins were also shown to be isoform-selective CA inhibitors. Particularly, in 2012, the sulfocoumarins were identified as CAIs using kinetic and X-ray crystallographic studies, in which it has been also revealed that although structurally related to the coumarins, sulfocoumarins possess a different CA inhibition mechanism (Figure 2) [29]. As reported in Figure 1 for compound 3, sulfocoumarin were hydrolyzed by α-CAs to give trans-2-hydroxyphenyl-ω-ethenylsulfonic acid. Afterwards, the formed sulfonic acid binds to the CA II active site by anchoring the SO₃H group to the zinc-coordinated water molecule/hydrxide ion (Figure 2) [30]. After this discovery, many more derivatives were synthesized and analyzed for their interaction with different CA isoforms [31–37].

![Figure 2](image_url)

**Figure 2.** A ribbon view of the active site of hCA II in adduct with (A) Compound 4 (cyan, PDB 5B8L), derived from the CA-mediated hydrolysis of coumarin 1; (B) Compound 5 (magenta, PDB 3F8E), derived from the CA-mediated hydrolysis of coumarin 2. (C) Compound 6 (green, PDB 4BCW), derived from the CA-mediated hydrolysis of sulfocoumarin 3. The Zn(II) is shown as a grey sphere that is bound to the protein His ligands (labels not shown). Water molecules are represented as red spheres. H-bonds are represented as black dashed lines.

The substitution pattern, and especially the position of the substituent on the heterocyclic ring system of the sulfocoumarin, are the main factors influencing CA inhibitory
properties [30]. In this paper, we expanded the structure-activity relationships of the sulfocoumarin scaffold describing the synthesis and the evaluation of more than 30 sulfocoumarin belonging to two different classes: (a) 7-benzyloxsulfocoumarin and (b) 3-amidosulfocoumarin, obtained for the first time in this work.

2. Results
2.1. Chemistry

Due to the difficulties in designing and synthesizing selective sulfonamide inhibitors against each isoform, such as SLC-0111 (Figure 3) [38], a potent and selective zinc binder CAI against hCA IX and XII, scientists opted for the development of novel chemotypes among which are sulfocoumarins, the preferred CAI scaffold adopted in this project.

![Figure 3. The structure of SLC-0111, a specific hCA IX and XII inhibitor studied in phase Ib/II clinical trials for the treatment of hypoxic tumors.](image)

The general strategy of Zalubovskis group [16,29], for the preparation of 6-substituted sulfocoumarins and validated by Nocentini et al. [36], in 2015, for the synthesis of 7-substituted such derivatives, was applied in this manuscript to extend the structure-activity relationships, whereas the general strategy of Liu’s group [39], for the designed of 3-substituted sulfocoumarins, was validated in this project in order to synthesize 3-amido derivatives for the first time. To start, 2-hydroxy-4-methoxybenzaldehyde 7 or 2′-hydroxy-4′-methoxycetophenon 8 were synthesize with the sulfocoumarin scaffold 9 and 10. After that, their phenol moiety was released in the presence of BBr3 in dry DCM to obtain 11 and 12 in high yield and high purity. Finally, a nucleophilic substitution was performed in the presence of different benzyl bromides with K2CO3 as a base in dry DMF at RT to give compounds 13–22 (Scheme 1).

![Scheme 1. A synthetic approach for obtaining products 13–22. Reagents and conditions: (a) Mesityl chloride, Et3N, dry DCM, 0 °C to RT, 2 h; (b) DBU, dry DCM, 0 °C to RT, o.n.; (c) dry Py, dry DCM, 0 °C to RT, o.n.; (d) BBr3, dry DCM, −10 °C to RT, o.n.; (e) appropriate benzyl bromide, K2CO3, dry DMF, RT, o.n.](image)

Compound 23 (2-bromobenzenesulfonyl chloride) was reacted with 3-methoxyphenol in dry DCM in the presence of Et3N as a base to obtain intermediate 24; after that, a cyclization was performed in dry DMA with Pd(OAc)2 as a catalyst at 150 °C to give the sulfocoumarin 25. To prepare the next reaction, the methoxy group was released in the presence of BBr3 in dry DCM between −10 °C and RT to synthesize intermediate 26, and then a nucleophilic substitution was performed in dry DMF at RT with K2CO3 as a base to give us compounds 27–31 (Scheme 2).
A synthetic approach to obtain products 27–31. Reagents and conditions: (a) 3-methoxyphenol, Et₃N, dry DCM, 0 °C to RT, 3 h; (b) KOAc, Pd(OAc)₂, dry DMA, 150 °C, 4 h; (c) BBr₃, dry DCM, −10 °C to RT, o.n.; (d) appropriate benzyl bromide, K₂CO₃, dry DMF, RT, o.n.

A cyclization reaction was performed between starting materials 32, or salicylaldehyde, or 33, 4-methoxysalicylaldehyde, and ethyl 2-chlorosulfonylacetate in DCE at 90 °C in the presence of dry pyridine as a base; after that, the obtained ethyl esters (34, 35) were hydrolyzed in EtOH and NaOH 5M at reflux to release the carboxylic acid moiety of compounds 36 and 37. Finally, a coupling reaction was performed in dry DMF between appropriate anilines and intermediates 36 and 37 to give us products 38–47 with an amide as linker (Scheme 3).

Scheme 2. A synthetic approach to obtain products 27–31. Reagents and conditions: (a) ethyl 2-bromobenzenesulfonyl chloride) was reacted with 3-methoxyphenol, or methoxysalicylaldehyde, or salicylaldehyde, or 3-methoxysalicylaldehyde, and ethyl 2-chlorosulfonylacetate in DCE at 90 °C and RT to synthesize products 28–31. Reagents and conditions: (a) 3-methoxyphenol, Et₃N, dry DCM, 0 °C to RT, 3 h; (b) KOAc, Pd(OAc)₂, dry DMA, 150 °C, 4 h; (c) BBr₃, dry DCM, −10 °C to RT, o.n.; (d) appropriate benzyl bromide, K₂CO₃, dry DMF, RT, o.n.

Scheme 3. A synthetic approach to obtain products 38–47. Reagents and conditions: (a) ethyl 2-chlorosulfonyl acetate, dry Py, DCE, 90 °C, 2 h; (b) NaOH 5M, EtOH, reflux, 30'; (c) appropriate aniline, PyBOP, DIPEA, dry DMF, 0 °C to RT, o.n.

2.2. Carbonic Anhydrase Inhibition

Sulfcoumarins 13–22, 27–31, 38–47 were screened in vitro for the inhibition of four physiologically relevant hCA isoforms, the cytosolic hCA I and II and the trans-membrane tumor-associated hCA IX and XII [4–6,8,40–43]; acetazolamide (AAZ) was used as standard CAI. CA I is the main off-target isoform for most therapeutic applications of CAIs, whilst CA II is considered off-target in many pathologies to reduce side effects resulting from systematic CA inhibition as much as possible. Table 1 shows the inhibition data obtained after a period of incubation of 6 h of the enzyme and inhibitors. Noteworthily, the assay inhibition performed within the usual 15 min incubation period (as for the sulfonamides) led to the very weak inhibition constants (data not shown) [43]. For this reason, we herein report a 6 h incubation time instead. The following structure-activity relationship (SAR) can be gathered from the inhibition data reported in Table 1.
Table 1. Inhibition data of hCA isoforms I, II, IX and XII with sulfocoumarins 13–22, 27–31, 38–47 and the standard sulfonamide inhibitor AAZ by a stopped-flow CO₂ hydrase assay [44].

| Cmpd | R₁ | R₂ | Kᵢ (nM) a,b | CA I | CA II | CA IX | CA XII |
|------|----|----|-------------|------|-------|-------|--------|
| 9    | H  | OCH₃ | >100,000 | 15,360 | 74.9  | 61.4  |
| 10   | CH₃| OCH₃ | >100,000 | 8814  | 97.2  | 73.9  |
| 11   | H  | H    | 16,538   | 2896  | 55.8  | 42.5  |
| 12   | CH₃| H    | 9961     | 3857  | 81.7  | 55.0  |
| 13   | H  | H    | >100,000 | >100,000 | 55.6  | 28.3  |
| 14   | H  | 4-F  | >100,000 | >100,000 | 22.9  | 19.2  |
| 15   | H  | 3-F  | >100,000 | >100,000 | 28.4  | 46.0  |
| 16   | H  | 4-NO₂| >100,000 | >100,000 | 69.8  | 94.7  |
| 17   | H  | 3-NO₂| >100,000 | >100,000 | 40.2  | 77.5  |
| 18   | CH₃| H    | >100,000 | >100,000 | 49.8  | 8.6   |
| 19   | CH₃| 4-F  | >100,000 | >100,000 | 14.3  | 41.7  |
| 20   | CH₃| 3-F  | >100,000 | >100,000 | 33.2  | 25.7  |
| 21   | CH₃| 4-NO₂| >100,000 | >100,000 | 89.7  | 100.8 |
| 22   | CH₃| 3-NO₂| >100,000 | >100,000 | 61.4  | 136.0 |
| 25   | OCH₃| -   | >100,000 | >100,000 | 1286  | 965.8 |
| 26   | H  | -    | 42650    | 8997  | 558.9 | 414.2 |
| 27   | H  | -    | >100,000 | >100,000 | 680.5 | 910.7 |
| 28   | 4-F | -    | >100,000 | >100,000 | 754.9 | 653.8 |
| 29   | 3-F | -    | >100,000 | >100,000 | 615.1 | 566.4 |
| 30   | 4-NO₂| -   | >100,000 | >100,000 | 964.7 | 1056 |
| 31   | 3-NO₂| -   | >100,000 | >100,000 | 898.1 | 1369 |
| 34   | H  | CH₂CH₃| >100,000 | >100,000 | 1631  | 2594 |
| 35   | OCH₃| CH₂CH₃| >100,000 | >100,000 | 1085  | 1528 |
| 36   | H  | H    | 4625     | 996.5 | 499.7 | 563.7 |
| 37   | OCH₃| H    | 7139     | 728.1 | 853.7 | 408.2 |
| 38   | H  | H    | >100,000 | >100,000 | 3288  | 2301 |
| 39   | H  | 4-Br | >100,000 | >100,000 | 34,350 | 58,980 |
| 40   | H  | 3-Br | >100,000 | >100,000 | 40,840 | 78,320 |
| 41   | H  | 4-CH₂O₃ | >100,000 | >100,000 | 8231  | 8550 |
| 42   | H  | 3-CH₂O₃ | >100,000 | >100,000 | 5424  | 3380 |
| 43   | OCH₃| H    | >100,000 | >100,000 | 2429  | 1594 |
| 44   | OCH₃| 4-Br | >100,000 | >100,000 | 51,460 | 42,520 |
| 45   | OCH₃| 3-Br | >100,000 | >100,000 | 64,380 | 76,540 |
| 46   | OCH₃| 4-CH₂O₃| >100,000 | >100,000 | 4367  | 6547 |
| 47   | CH₃| 3-CH₂O₃ | >100,000 | >100,000 | 6558  | 2469 |
| AAZ  | -  | -    | 250      | 12.0  | 25.0  | 5.7   |

a. Mean from 3 different assays by a stopped flow technique (errors in the range of 5–10% of the reported values);
b. 6 h incubation.

The following structure-activity relationships (SAR) should be noted:
- According to the previous reports [31–33,35], isoform hCA I was not inhibited by a large number of substituted sulfocoumarins; however, on the other side, the simplest sulfocoumarin as 11, 12, 26, 36 and 37 showed a weak inhibitory potency. It is likely that compounds 36 (K_I = 4625 nM) and 37 (K_I = 7139 nM) have a low micromolar inhibition due to the presence of the carboxylic acid moiety, which may work by binding to the H_2O molecule coordinated with Zn^{2+} in the active site [45,46].

- Similar to hCA I, for hCA II we did not observe any inhibition by all the substituted sulfocoumarines, except for 9–12, 26, 36 and 37. Compounds 11 and 12 showed a low micromolar inhibition, respectively K_I = 2896 nM and K_I = 3857 nM, whilst analogs 9 and 10 have the K_I in the medium micromolar range, which can likely be associated to the presence of a free phenol moiety on products 11 and 12, which is protected in 9 and 10.

- The target hCA IX resulted in being the most inhibited isoforms by sulfocoumarins reported here. Considering the three different types of products, type 1 shows the most efficient inhibition against this isozyme, with K_I values in the low-medium nanomolar range, between 14.3 nM and 97.2 nM. In detail, the first-in-class compound in term of inhibition potency is the number 19, which shows a fluorine atom in para position on the benzyloxy moiety and a methyl group as R1 substituent (K_I = 14.3 nM), whilst the elimination of the benzyloxy moiety (10) gives us the less effective compound (K_I = 97.2 nM). Interestingly, the general behaviour of these compounds owned on type 1 was that, indeed, the insertion of the methyl group as an R1 substituent produced a worsening of the K_I values; in fact, it was demonstrated by products 16 (K_I = 68.9 nM) and 17 (K_I = 40.2 nM) compared to the corresponding 21 (K_I = 89.7 nM) and 22 (K_I = 61.4 nM). This rule seems to be broken only by derivatives 18 (K_I = 49.8 nM) and 19 (K_I = 14.3 nM), which resulted in better inhibitors with respect to analogs 13 (K_I = 55.6 nM) and 14 (K_I = 22.9 nM). Sulfocoumarines belonging to type 2, even with a benzyloxy moiety, suffered from the addition of a further aromatic ring on the scaffold that improves the steric hindrance on the sulfonate ring, which induced a worsening of the K_I values. Indeed, the inhibition constants are in the high nanomolar range, between 615.1 nM and 1286 nM. The best compound result to be 29 (K_I = 615.1 nM) with a fluorine atom in meta position on the benzyloxy moiety. The movement of the R1 group from meta to para position produces a worsening of the K_I values. Examples are 29 (K_I = 615.1 nM) and 31 (K_I = 898.1 nM), respectively, with 28 (K_I = 754.9 nM) and 30 (K_I = 964.7 nM). Interestingly, it is a significant decline of the inhibition data of the sulfocoumarines belonging to type 3, with an amide as linker on position 3 of the sulfonate ring. It is likely that the presence of bulky groups with the CAI portion results in a worsening of the K_I values, in a micromolar range between 499.7 nM and 64380 nM. The most potent compounds of this class are 43 (K_I = 2429 nM) and the simplest sulfocoumarines 34 (K_I = 1631 nM), 35 (K_I = 1085 nM), 36 (K_I = 499.7 nM) and 37 (K_I = 853.7 nM) showed an ester or carboxylic acid moieties in position 3. Sulfocoumarines with a bromine atom in a meta position as the R2 group (40, 45) resulted in the worst values of inhibition, respectively K_I = 40,840 nM and 64,380 nM. The hCA XII resulted in becoming the second most efficiently inhibited isoform by sulfocoumarines, with it being particularly possible to observe a similar trend of the K_I values as already shown for the hCA IX. Considering products belonging to type 1, they show the most potent inhibition for this isoform, between 8.6 nM and 136.0 nM. The presence of a methyl group in R1 position produced an improvement of the K_I values for derivatives 18 (K_I = 8.6 nM), without substituent on the benzyloxy moiety, and 20 (K_I = 25.7 nM), with fluorine in the meta position. For all the other products, a worsening of Ki values, such as for 14 (K_I = 19.2 nM) and 19 (K_I = 41.7 nM), or for 11 (K_I = 42.5 nM) and 12 (K_I = 55.0 nM), was observed. On the other side, the movement of the fluorine atom from meta to para position (19) resulted in a K_I value of 41.7 nM. Regarding the nitro group, compound 17 (K_I = 77.5 nM) with R2 group in a meta position show an improvement in term of inhibition than its analogue 16 (K_I = 94.7 nM) with R2 group in a para position. For sulfocoumarines 18–22, the fluorine atom resulted in being better than
nitro group as a substituent on the benzyloxy moiety; indeed, 19 ($K_I = 41.7$ nM) and 20 ($K_I = 25.7$ nM) are strongest inhibitors in term of potency than 21 ($K_I = 100.8$ nM) and 22 ($K_I = 136.0$ nM). For compounds belonging to type 2, the addition of an aromatic ring on the sulfocoumarin scaffold determines a worsening of the $K_I$ values in the medium–high nanomolar range, between 414.2 nM and 1369 nM. The presence of a bulky group such as the nitro group leads to a worsening in terms of inhibition potency, particularly products 30 and 31 have $K_I$ of 1056 nM and 1369 nM. The simplest sulfocoumarin with a free phenol moiety, 26, showed the best inhibition for type 2 series, 414.2 nM. Sulfocoumarins belonging to type 3 that present a further enhancement of the steric hindrance on the sulfonate ring, have $K_I$ values in the range of micromolar ($K_I = 408.2–78,320$ nM). Compound 40 and 45, with a bromine atom in the meta position, show the worst inhibition potencies on hCA XII, respectively 78,320 nM and 76,540 nM; on the other side, sulfocoumarins with the less bulky group in R$_2$ position result in being a good inhibitor on this isoform, while, indeed, 38 and 43 with a hydrogen atom show $K_I$ values in the range of low micromolar—respectively, 2301 nM and 1594 nM. The best inhibitors belonging to the type 3 series are compounds 36 ($K_I = 563.7$ nM) and 37 ($K_I = 408.2$ nM), likely thanks to the free carbonylic acid moiety.

3. Materials and Methods

3.1. Chemistry

Anhydrous solvents and all reagents were purchased from Merck, Fluorochem 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 (1H-NMR, 13C-NMR,) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d$_6$. Chemical shifts are reported in parts per million (ppm), and the coupling constants (J) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; bs, broad singlet; dd, doublet of doublets. The assignment of exchangeable protons was confirmed by the addition of D$_2$O. Analytical thin-layer chromatography (TLC) was carried out on Sigma Aldrich silica gel F-254 plates. Flash chromatography purifications were performed on Sigma Aldrich Silica gel 60 (230–400 mesh ASTM) as the stationary phase and ethyl acetate/n-hexane or MeOH/DCM were used as eluents. Melting points (mp) were measured in open capillary tubes with a Gallenkamp MPD350.BM3.5 appara-tus and are uncorrected. The solvents used in mass spectrometry analysis were acetone, acetonitrile (Chromasolv grade), purchased from Sigma-Aldrich (Milan, Italy), and mQ water 18 MΩ cm, obtained from Millipore’s Simplicity system (Milan, Italy). The HPLC-MS and MS/MS analysis was carried out using a Varian 500-MS ion trap system (Palo Alto, CA, USA) equipped by two Prostar 210 pumps, a Prostar 410 autosampler and an Electro spray source (ESI) operating in negative ions. Stock solutions of analytes were prepared in acetone at 1.0 mg mL$^{-1}$ and stored at 4 °C. Working solutions of each analyte were freshly prepared by diluting stock solutions in a mixture of mQ water:acetonitrile 1:1 (v/v) up to a concentration of 1.0 µg mL$^{-1}$. The mass spectra of each analyte were acquired by introducing, via syringe pump at 10 µL min$^{-1}$, the working solution. Raw data were collected and processed by Varian Workstation Vers. 6.8 software.

General synthetic procedure for 7-methoxybenzo[e][1,2]oxathiine 2,2-dioxide (9) and 7-methoxy-4-methylbenzo[e][1,2]oxathiine 2,2-dioxide (10)

Et$_3$N (1.5 equiv.) and Mesyl chloride (1.5 equiv.) were added slowly to a solution of 7 or 8 (3 g, 1 equiv.) in dry DCM (20 mL) at 0 °C under nitrogen atmosphere. The solution was stirred for 2 h at RT. Slush was added to the mixture and the reaction mixture was extracted in DCM (3 × 25 mL), dried with Na$_2$SO$_4$, filtered and evaporated to give us a pale yellow oil. DBU (1.2 equiv.) was added dropwise to a solution of the oil in dry DCM (20 mL) at 0 °C under nitrogen atmosphere. The solution was stirred o.n. at RT. Slush was added slowly and the reaction mixture was extracted in DCM (3 × 25 mL). The collected organic phases were dried with Na$_2$SO$_4$, filtered and evaporated under vacuum to give a brown
oil. PCl₃ (1.5 equiv.) was added dropwise to a solution of the oil in dry Py (3 mL) at 0 °C under nitrogen atmosphere. The solution was stirred for 4 h at RT. Slush and HCl 2M were added to pH = 4 and the reaction mixture was extracted in DCM (3 × 15 mL). The collected organic phases were washed with K₂CO₃ s.s. (2 × 10 mL), dried with Na₂SO₄, filtered and evaporated under vacuum to give products 24 as white powder in high yield and purity.

Yield 96%; m.p. 163–166 °C; silica gel TLC Rf 0.45 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d₆): 8.08 (d, J = 8.4 Hz, 1H, Ar-H), 7.99 (d, J = 8.4 Hz, 1H, Ar-H), 7.75 (dd, J = 7.4 Hz, 1H, Ar-H), 7.65 (dd, J = 7.4 Hz, 1H, Ar-H), 7.33 (dd, J = 7.1 Hz, 1H, Ar-H), 6.93 (d, J = 7.1 Hz, 1H, Ar-H), 6.70 (m, 2H, Ar-H), 3.73 (s, 3H, CH₃); δC (400 MHz, DMSO-d₆): 162.0, 152.7, 154.3, 142.7, 126.8, 119.3, 107.6, 100.3, 97.9, 93.7, 86.3, 85.8, 77.4, 61.4, 55.8, 19.9.

Synthetic procedure for 3-methoxyphenyl 2-bromobenzenesulfonate (24)

Potassium acetate (2 equiv.) and Pd(OAc)₂ (0.01 equiv.) were added to a solution of 24 (1.2 g, 1 equiv.) in dry DMA (5 mL) under nitrogen atmosphere. The solution was heated at 150 °C for 4 h. The resulting mixture was cooled in ice bath and slush was added. The suspension was extracted in EtOAc (3 × 20 mL), and the collected organic phases were washed with brine (3 × 50 mL), dried with Na₂SO₄, filtered and evaporated under vacuum to give 25 as red oil. Product was purified by silica gel chromatography column (MeOH/DCM: from 0.5% to 1% v/v) to obtain a white powder.

Yield 93%; m.p. 180–183 °C; silica gel TLC Rf 0.45 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d₆): 8.25 (dd, J = 8.3 Hz, 2H, Ar-H), 8.07 (dd, J = 8.3 Hz, 2H, Ar-H), 7.95 (m, 1H, Ar-H), 7.72 (m, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 7.15 (dd, J = 8.3 Hz, 1H, Ar-H), 3.92 (s, 3H, CH₃); δC (400 MHz, DMSO-d₆): 160.9, 157.2, 142.4, 134.4, 133.0, 130.3, 128.9, 128.6, 122.7, 119.4, 102.5, 55.8.

General synthetic procedure for 7-hydroxybenzene[1,2]oxathiine 2,2-dioxide (11), 7-hydroxy-4-methylbenzene[1,2]oxathiine 2,2-dioxide (12) and 3-hydroxydibenzo[c,e][1,2]oxathiine 6,6-dioxide (26)

BBr₃ (5 equiv.) was added dropwise and slowly to a solution of 9, 10 or 25 (2 g, 1 equiv.) in dry DCM (15 mL) at −20 °C under nitrogen atmosphere. The solution was stirred 24 h at RT. Ice was added slowly to the solution and the precipitate was filtered. The resulting powder was dissolved in K₂CO₃ s.s. (15 mL) and was filtered off. The H₂O-phase was acidified with HCl 6 M to pH = 4 and the product was extracted in EtOAc (3 × 20 mL), dried with Na₂SO₄, filtered and evaporated under vacuum to give products 11, 12 and 26 as white powder in good yield and high purity.
7-Hydroxybenzo[e][1,2]oxathiine 2,2-dioxide (11)

Compound 11 was obtained according to the general procedure reported earlier with 9 as starting material. Yield 72%; m.p. 157–159 °C; silica gel TLC Rf 0.41 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d6): 10.81 (s, 1H, exchange with D2O, OH); 7.59 (d, J = 9.8 Hz, 1H, Ar-H); 7.52 (d, J = 8.9 Hz, 1H, Ar-H); 7.22 (d, J = 8.7 Hz, 1H, Ar-H); 6.82 (dd, J = 8.9 Hz, 1H, Ar-H); 6.75 (d, J = 1.4 Hz, 1H, Ar-H); δC (400 MHz, DMSO-d6): 159.8, 155.5, 133.4, 131.2, 126.7, 109.1, 108.4, 106.5.

7-Hydroxy-4-methylbenzo[e][1,2]oxathiine 2,2-dioxide (12)

Compound 12 was obtained according to the general procedure reported earlier with 10 as starting material. Yield 70%; m.p. 172–175 °C; silica gel TLC Rf 0.38 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d6): 10.84 (s, 1H, exchange with D2O, OH); 7.63 (d, J = 8.7 Hz, 1H, Ar-H); 7.14 (s, 1H, Ar-H), 6.87 (d, J = 8.7 Hz, 1H, Ar-H); 6.77 (s, 1H, Ar-H), 2.34 (s, 3H, CH3); δC (400 MHz, DMSO-d6): 159.0, 155.1, 142.7, 127.2, 119.3, 108.4, 107.9, 106.2, 19.9.

3-Hydroxydibenz[e,c][1,2]oxathiine 6,6-dioxide (26)

Compound 26 was obtained according to the general procedure reported earlier with 25 as starting material. Yield 86%; m.p. 181–182 °C; silica gel TLC Rf 0.44 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d6): 10.68 (s, 1H, exchange with D2O, OH); 8.19 (d, J = 7.9 Hz, 1H, Ar-H), 8.12 (d, J = 7.9 Hz, 1H, Ar-H), 8.0 (d, J = 7.4 Hz, 1H, Ar-H), 7.92 (dd, J = 7.4 Hz, 1H, Ar-H); 7.68 (dd, J = 7.4 Hz, 1H, Ar-H), 6.97 (d, J = 7.4 Hz, 1H, Ar-H); 6.88 (s, 1H, Ar-H); δC (400 MHz, DMSO-d6): 158.8, 157.6, 142.4, 134.4, 134.4, 133.0, 130.3, 130.7, 128.6, 122.3, 119.8, 109.0, 104.1.

General synthetic procedure for products 13–22, 27–31

K2CO3 (1.05 equiv.) and the appropriate benzyl bromide (0.95 equiv.) were added to a solution of 11, 12 or 26 (0.15 g, 1 equiv.) in dry DMF (2 mL) under nitrogen atmosphere. The reaction mixture was stirred o.n. at RT. The reaction mixture was quenched with slush and the H2O-phase was extracted in EtOAc (3 × 25 mL). The organic phases were washed with NaOH 5M (3 × 10 mL) and brine (3 × 50 mL), then organic phase was dried with Na2SO4, filtered and evaporated under vacuum to obtain products as white powder in high yield and purity.

7-(Benzyloxy)benzo[e][1,2]oxathiine 2,2-dioxide (13)

Compound 13 was obtained according to the general procedure reported earlier with benzyl bromide as starting material. Yield 74%; m.p. 165–167 °C; silica gel TLC Rf 0.45 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d6): 7.65 (d, J = 9.1 Hz, 2H, Ar-H); 7.41 (m, 6H, Ar-H); 7.18 (s, 1H, Ar-H); 7.08 (d, J = 8.5 Hz, 1H, Ar-H); 5.23 (s, 2H, CH2); δC (400 MHz, DMSO-d6): 162.4, 153.3, 137.3, 137.1, 131.2, 129.6, 129.9, 129.0, 120.3, 114.5, 113.1, 105.5, 71.1; m/z (ESI negative): 286.9 [M − H]−, m/z (ESI positive): 289.0 [M + H]+.

7-(4-Fluorobenzyloxy)benzo[e][1,2]oxathiine 2,2-dioxide (14)

Compound 14 was obtained according to the general procedure reported earlier with 4-fluorobenzyl bromide as starting material. Yield 77%; m.p. 197–199 °C; silica gel TLC Rf 0.39 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d6): 7.68 (s, 1H, Ar-H), 7.66 (d, J = 2.5 Hz, 1H, Ar-H); 7.58 (m, 2H, Ar-H), 7.34 (d, J = 10.3 Hz, 1H, Ar-H), 7.28 (dd, J = 8.9 Hz, 2H, Ar-H), 7.20 (d, J = 2.3 Hz, 1H, Ar-H), 7.10 (dd, J = 8.9 2.3 Hz, 1H, Ar-H); 5.24 (s, 2H, CH2); δC (400 MHz, DMSO-d6): 164.2 (d, Jf = 244.0 Hz), 162.3, 153.3, 137.5, 133.4, 132.2, 131.4 (d, Jf = 8.4 Hz), 120.3, 116.5 (d, Jf = 21.5 Hz), 114.5, 113.1, 105.7, 70.4; m/z (ESI negative): 304.9 [M − H]−, m/z (ESI positive): 307.0 [M + H]+.

7-(3-Fluorobenzyloxy)benzo[e][1,2]oxathiine 2,2-dioxide (15)

Compound 15 was obtained according to the general procedure reported earlier with 3-fluorobenzyl bromide as starting material. Yield 76%; m.p. 180–183 °C; silica gel TLC Rf 0.38 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d6): 7.68 (m, 2H, Ar-H), 7.51 (m, 2H, Ar-H), 7.36 (m, 3H, Ar-H), 7.21 (s, 1H, Ar-H), 7.12 (d, J = 7.1 Hz, 1H, Ar-H); 5.29 (s, 2H, CH2); δC (400 MHz, DMSO-d6): 164.4 (d, Jf = 241.1 Hz), 62.1, 153.3, 140.0 (d, Jf = 7.3 Hz), 137.5, 132.2, 131.6 (d, Jf = 7.6 Hz), 124.8 (d, Jf = 2.4 Hz), 120.4, 116.0 (d, Jf = 20.4 Hz), 115.6 (d, Jf = 21.7 Hz), 114.5, 113.3, 105.7, 70.2; m/z (ESI negative): 304.9 [M − H]−, m/z (ESI positive): 307.0 [M + H]+.
7-((4-Nitrobenzyl)oxy)benzo[e][1,2]oxathiine 2,2-dioxide (16)

Compound 16 was obtained according to the general procedure reported earlier with 4-nitrobenzyl bromide as starting material. Yield 82%; m.p. 215–217 °C; silica gel TLC Rf 0.48 (EtOAc/Hexane 50% v/v); δ_H (400 MHz, DMSO-d_6): 8.32 (d, J = 8.6 Hz, 2H, Ar-H), 7.78 (d, J = 8.6 Hz, 2H, Ar-H), 7.70 (d, J = 8.4 Hz, 1H, Ar-H), 7.68 (d, J = 10.0 Hz, 1H, Ar-H), 7.36 (d, J = 10.0 Hz, 1H, Ar-H), 7.23 (d, J = 2.3 Hz, 1H, Ar-H), 7.14 (dd, J = 8.4 Hz, 2H, Ar-H), 5.29 (s, 2H, CH_2); δ_C (400 MHz, DMSO-d_6): 161.9, 153.3, 148.2, 144.9, 137.5, 132.3, 129.5, 124.7, 120.5, 114.5, 113.4, 105.8, 69.8; m/z (ESI negative): 331.9 [M − H]^−, m/z (ESI positive): 334.0 [M + H]^+.

7-((3-nitrobenzyl)oxy)benzo[e][1,2]oxathiine 2,2-dioxide (17)

Compound 17 was obtained according to the general procedure reported earlier with 3-nitrobenzyl bromide as starting material. Yield 81%; m.p. 200–202 °C; silica gel TLC Rf 0.51 (EtOAc/Hexane 50% v/v); δ_H (400 MHz, DMSO-d_6): 8.37 (s, 1H, Ar-H) 8.2 (d, J = 8.1 Hz, 1H, Ar-H), 7.96 (d, J = 8.1 Hz, 1H, Ar-H), 7.71 (m, 3H, Ar-H), 7.34 (d, J = 10.3 Hz, 1H, Ar-H), 7.22 (d, J = 2.8 Hz, 1H, Ar-H), 7.12 (dd, J = 8.3 Hz, 2H, Ar-H), 5.40 (s, 2H, CH_2); δ_C (400 MHz, DMSO-d_6): 162.0, 153.3, 148.9, 139.5, 135.7, 135.4, 133.2, 121.4, 123.4, 120.5, 114.5, 113.4, 105.8, 69.8; m/z (ESI negative): 332.0 [M − H]^−, m/z (ESI positive): 334.0 [M + H]^+.

7-(benzoxyl)-4-methylbenzo[e][1,2]oxathiine 2,2-dioxide (18)

Compound 18 was obtained according to the general procedure reported earlier with benzyloxyl bromide as starting material. Yield 83%; m.p. 174–175 °C; silica gel TLC Rf 0.41 (EtOAc/Hexane 50% v/v); δ_H (400 MHz, DMSO-d_6): 7.73 (d, J = 9.0 Hz, 1H, Ar-H), 7.51 (d, J = 7.1 Hz, 2H, Ar-H), 7.45 (dd, J = 7.1 Hz, 2H, Ar-H), 7.40 (q, J = 7.1 Hz, 1H, Ar-H), 7.23 (s, 1H, Ar-H), 7.19 (d, J = 2.1 Hz, 1H, Ar-H), 7.12 (dd, J = 9.0 Hz, 2H, Ar-H), 5.27 (s, 2H, CH_2), 2.37 (s, 3H, CH_3); δ_C (400 MHz, DMSO-d_6): 162.3, 152.4, 146.6, 137.1, 129.6, 129.3, 129.2, 129.0, 117.3, 114.7, 114.3, 105.7, 71.1, 20.0; m/z (ESI negative): 309.9 [M − H]^−, m/z (ESI positive): 310.0 [M + H]^+.

7-(4-fluorobenzyl)oxy)-4-methylbenzo[e][1,2]oxathiine 2,2-dioxide (19)

Compound 19 was obtained according to the general procedure reported earlier with 4-fluorobenzyl bromide as starting material. Yield 73%; m.p. 195–198 °C; silica gel TLC Rf 0.43 (EtOAc/Hexane 50% v/v); δ_H (400 MHz, DMSO-d_6): 7.74 (d, J = 8.9 Hz, 1H, Ar-H) 7.57 (m, 2H, Ar-H), 7.28 (m, 3H, Ar-H), 7.24 (s, 1H, Ar-H), 7.19 (d, J = 2.1 Hz, 1H, Ar-H), 7.12 (dd, J = 8.8 Hz, 1H, Ar-H), 5.25 (s, 2H, CH_2), 2.37 (s, 3H, CH_3); δ_C (400 MHz, DMSO-d_6): 164.27 (d, J^1 = 214.7 Hz), 162.2, 152.4, 164.5, 133.4 (d, J^1 = 2.5 Hz), 131.3 (d, J^1 = 8.8 Hz), 129.3, 117.4, 116.5 (d, J^1 = 20.7 Hz), 114.7, 114.3, 105.7, 70.3, 20.0; m/z (ESI negative): 318.9 [M − H]^−, m/z (ESI positive): 321.0 [M + H]^+.

7-(3-fluorobenzyl)oxy)-4-methylbenzo[e][1,2]oxathiine 2,2-dioxide (20)

Compound 20 was obtained according to the general procedure reported earlier with 3-fluorobenzyl bromide as starting material. Yield 75%; m.p. 214–215 °C; silica gel TLC Rf 0.55 (EtOAc/Hexane 50% v/v); δ_H (400 MHz, DMSO-d_6): 7.74 (d, J = 9.0 Hz, 1H, Ar-H), 7.50 (m, 1H, Ar-H), 7.35 (m, 2H, Ar-H), 7.25 (m, 2H, Ar-H), 7.20 (d, J = 2.2 Hz, 1H, Ar-H), 7.13 (dd, J = 8.3 Hz, 2H, Ar-H), 5.30 (s, 2H, CH_2), 2.37 (s, 3H, CH_3); δ_H (400 MHz, DMSO-d_6): 164.5 (d, J^1 = 168.2 Hz), 162.0, 152.4, 164.5, 140.1 (d, J^1 = 7.9 Hz), 131.6 (d, J^1 = 9.6 Hz), 129.4, 124.8 (d, J^1 = 2.5 Hz), 117.4, 116.0 (d, J^1 = 19.6 Hz), 115.6 (d, J^1 = 20.5), 114.8, 114.3, 105.8, 70.2, 20.0; m/z (ESI negative): 318.9 [M − H]^−, m/z (ESI positive): 321.0 [M + H]^+.

7-(4-nitrobenzyl)oxy)-4-methylbenzo[e][1,2]oxathiine 2,2-dioxide (21)

Compound 21 was obtained according to the general procedure reported earlier with 4-nitrobenzyl bromide as starting material. Yield 64%; m.p. 247–249 °C; silica gel TLC Rf 0.44 (EtOAc/Hexane 50% v/v); δ_H (400 MHz, DMSO-d_6): 8.27 (d, J = 8.9 Hz, 2H, Ar-H), 7.37 (d, J = 8.9 Hz, 2H, Ar-H), 7.70 (d, J = 8.4 Hz, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 7.14 (d, J = 2.2 Hz, 1H, Ar-H), 7.10 (dd, J = 8.4 Hz, 2H, Ar-H), 5.41 (s, 2H, CH_2), 2.32 (s, 3H, CH_3); δ_C (400 MHz, DMSO-d_6): 161.8, 152.4, 148.2, 146.5, 145.0, 132.8, 129.5, 124.7, 117.5,
115.0, 114.3, 105.8, 69.8, 20.0; m/z (ESI negative): 345.9 [M − H]−, m/z (ESI positive): 348.0 [M + H]+.

7-((3-nitrobenzyl)oxy)-4-methylbenzo[e][1,2]oxathiine 2,2-dioxide (22)

Compound 22 was obtained according to the general procedure reported earlier with 3-nitrobenzyl bromide as starting material. Yield 89%; m.p. 239−241 °C; silica gel TLC Rf 0.57 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d6): 8.34 (s, 1H, Ar-H), 8.21 (d, J = 8.1 Hz, 1H, Ar-H), 7.93 (d, J = 8.1 Hz, 1H, Ar-H), 7.73 (dd, J = 8.1 Hz, 1H, Ar-H), 7.71 (d, J = 8.3 Hz, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 7.19 (d, J = 2.1 Hz, 1H, Ar-H), 7.12 (dd, J = 8.3 2.1 Hz, 1H, Ar-H), 5.39 (s, 2H, CH2), 2.35 (s, 3H, CH3); δC (400 MHz, DMSO-d6): 161.9, 152.4, 148.9, 146.5, 139.5, 135.3, 131.2, 129.1, 124.1, 123.3, 117.5, 115.0, 114.3, 105.8, 69.7, 20.0; m/z (ESI negative): 345.9 [M − H]−, m/z (ESI positive): 348.0 [M + H]+.

3-(benzoxyl) dibenzo[c,e][1,2]oxathiine 6,6-dioxide (27)

Compound 27 was obtained according to the general procedure earlier reported with benzyl bromide as starting material. Yield 51%; m.p. 232−235 °C; silica gel TLC Rf 0.69 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d6): 8.20 (dd, J = 6.6 Hz, 2H, Ar-H), 8.02 (d, J = 7.0 Hz, 1H, Ar-H), 7.90 (dd, J = 7.0 Hz, 1H, Ar-H), 7.67 (dd, J = 7.0 Hz, 1H, Ar-H), 7.49 (d, J = 6.6 Hz, 2H, Ar-H), 7.39 (m, 3H, Ar-H), 7.24 (s, 1H, Ar-H), 7.17 (d, J = 8.8 Hz, 1H, Ar-H), 5.39 (s, 2H, CH2); δC (400 MHz, DMSO-d6): 161.8, 151.1, 137.2, 135.7, 132.0, 131.1, 129.8, 129.5, 129.2, 129.0, 128.1, 126.1, 124.7, 115.4, 114.6, 106.7, 71.6; m/z (ESI negative): 336.9 [M − H]−, m/z (ESI positive): 339.0 [M + H]+.

3-(4-fluorobenzyloxy)dibenzo[c,e][1,2]oxathiine 6,6-dioxide (28)

Compound 28 was obtained according to the general procedure reported earlier with 4-fluorobenzyl bromide as starting material. Yield 49%; m.p. 268−269 °C; silica gel TLC Rf 0.81 (EtOAc/Hexane 50% v/v): 8.26 (m, 2H, Ar-H), 8.07 (d, J = 7.3 Hz, 1H, Ar-H), 7.96 (m, 1H, Ar-H), 7.72 (dd, J = 7.3 Hz, 1H, Ar-H), 7.60 (m, 2H, Ar-H), 7.30 (m, 2H, Ar-H), 7.27 (d, J = 2.3 Hz, 1H, Ar-H), 7.22 (dd, J = 8.4 2.3 Hz, 1H, Ar-H), 5.26 (s, 2H, CH2); δH (400 MHz, DMSO-d6): 163.1 (d, J1 = 186.4 Hz), 161.6, 151.1, 135.7, 133.4 (d, J4 = 3.7 Hz), 132.0, 131.4 (d, J3 = 8.6 Hz), 131.1, 129.8, 182.1, 126.1, 124.7, 116.5 (d, J2 = 21.5 Hz), 115.4, 114.6, 106.7, 70.4; m/z (ESI negative): 354.9 [M − H]−, m/z (ESI positive): 357.1 [M + H]+.

3-(3-fluorobenzyloxy)dibenzo[c,e][1,2]oxathiine 6,6-dioxide (29)

Compound 29 was obtained according to the general procedure reported earlier with 3-fluorobenzyl bromide as starting material. Yield 76%; m.p. 249−252 °C; silica gel TLC Rf 0.77 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d6): 8.27 (d, J = 2.5 Hz, 1H, Ar-H), 8.25 (d, J = 3.3 Hz, 1H, Ar-H), 8.07 (d, J = 7.7 Hz, 1H, Ar-H), 7.95 (dd, J = 7.7 Hz, 1H, Ar-H), 7.72 (dd, J = 7.7 Hz, 1H, Ar-H), 7.51 (q, J = 7.7 Hz, 1H, Ar-H), 7.38 (m, 2H, Ar-H), 7.30 (d, J = 2.5 Hz, 1H, Ar-H), 7.23 (m, 2H, Ar-H), 5.31 (s, 2H, CH2); δC (400 MHz, DMSO-d6): 164.5 (d, J1 = 243.9 Hz), 161.5, 151.2, 140.2 (d, J3 = 7.3 Hz), 135.7, 132.0, 131.6 (d, J4 = 8.6 Hz), 131.1, 129.9, 128.2, 126.2, 124.9 (d, J2 = 2.6 Hz), 124.7, 116.0 (d, J3 = 20.4 Hz), 115.7 (d, J2 = 21.3 Hz), 115.4, 114.8, 106.8, 70.2; m/z (ESI negative): 354.9 [M − H]−, m/z (ESI positive): 357.0 [M + H]+.

3-(4-nitrobenzyloxy)dibenzo[c,e][1,2]oxathiine 6,6-dioxide (30)

Compound 30 was obtained according to the general procedure reported earlier with 4-nitrobenzyl bromide as starting material. Yield 72%; m.p. 264−266 °C; silica gel TLC Rf 0.43 (EtOAc/Hexane 50% v/v); δH (400 MHz, DMSO-d6): 8.28 (d, J = 8.1 Hz, 2H, Ar-H), 8.23 (d, J = 8.8 Hz, 2H, Ar-H), 8.03 (d, J = 7.7 Hz, 1H, Ar-H), 7.91 (dd, J = 7.7 Hz, 1H, Ar-H), 7.76 (d, J = 8.1 Hz, 2H, Ar-H), 7.68 (dd, J = 7.7 Hz, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 7.20 (d, J = 8.8 Hz, 1H, Ar-H), 5.42 (s, 2H, CH2); δC (400 MHz, DMSO-d6): 161.3, 151.1, 148.2, 145.1, 135.7, 131.9, 131.1, 130.0, 129.5, 128.3, 126.2, 124.8, 124.7, 115.4, 115.0, 106.8, 69.8; m/z (ESI negative): 382.0 [M − H]−, m/z (ESI positive): 383.9 [M + H]+.

3-(3-nitrobenzyloxy)dibenzo[c,e][1,2]oxathiine 6,6-dioxide (31)

Compound 31 was obtained according to the general procedure reported earlier with 3-nitrobenzyl bromide as the starting material. Yield 70%; m.p. 248−251 °C; silica gel TLC Rf 0.56 (EtOAc/Hexane 50% v/v): 8.36 (s, 1H, Ar-H), 8.23 (s, J = 8.6 Hz, 3H, Ar-H), 8.03 (d, J
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= 7.7 Hz, 1H, Ar-H), 7.96 (d, J = 7.7 Hz, 1H, Ar-H), 7.91 (dd, J = 7.7 Hz, 1H, Ar-H), 7.73 (dd, J = 7.7 Hz, 1H, Ar-H), 7.68 (dd, J = 7.7 Hz, 1H, Ar-H), 7.29 (d, J = 2.3 Hz, 1H, Ar-H), 7.22 (dd, J = 8.6 2.3 Hz, 1H, Ar-H), 5.41 (s, 2H, CH2); δ1H (400 MHz, DMSO-d6); δC (400 MHz, DMSO-d6); 161.3, 151.1, 148.9, 139.6, 135.7, 135.4, 131.9, 131.2, 131.1, 129.9, 128.3, 126.2, 124.7, 124.1, 123.4, 115.4, 114.9, 106.8, 69.7; m/z (ESI negative): 382.0 [M – H]−, m/z (ESI positive): 384.0 [M + H]+.

**General synthetic procedure for ethyl benzo[e][1,2]oxathiine-3-carboxylate 2,2-dioxide (34)**

A solution of ethyl 2-(chlorosulfonyl)acetate (1.5 equiv.) in DCE (5 mL), prepared as reported by Liu et al.,3 was added dropwise in a sealed tube to a solution of 32 or 33 (1 g, 1 equiv.) and dry Py (2 equiv.) in DCE (3 mL). The resulting suspension was stirred at 90 °C for 4 h. Slush and HCl were added to the reaction mixture and the suspension was extracted in EtOAc (3 × 25 mL). The collected organic phase was washed with K2CO3 s.s. (3 × 20 mL), dried with Na2SO4, filtered and evaporated to give product 34 as pale yellow powders in good yield and high purity.

**ethyl benzo[e][1,2]oxathiine-3-carboxylate 2,2-dioxide (34)**

Compound 34 was obtained according to the general procedure reported earlier with salicyl aldehyde (32) as starting material. Yield 43%; m.p. 111–113 °C; silica gel TLC Rf 0.54 (EtOAc/Hexane 50% v/v); δ1H (400 MHz, DMSO-d6); 8.59 (s, 1H, Ar-H), 8.02 (d, J = 7.6 Hz, 1H, Ar-H), 7.79 (dd, J = 7.6 Hz, 1H, Ar-H), 7.59 (m, 2H, Ar-H), 4.43 (q, J = 6.5 Hz, 2H, CH2), 1.37 (dd, J = 6.5 Hz, 3H, CH3); δC (400 MHz, CDCl3-d3); 159.7, 152.4, 142.2, 131.0, 126.3, 118.9, 118.8, 63.1, 14.1.

**ethyl 7-methoxybenzo[e][1,2]oxathiine-3-carboxylate 2,2-dioxide (35)**

Compound 35 was obtained according to the general procedure reported earlier with 4-methoxysalicylaldehyde (33) as starting material. Yield 41%; m.p. 167–169 °C; silica gel TLC Rf 0.49 (EtOAc/Hexane 50% v/v); δ1H (400 MHz, DMSO-d6); 8.51 (s, 1H, Ar-H), 7.94 (d, J = 8.6 Hz, 1H, Ar-H), 7.22 (d, J = 2.2 Hz, 1H, Ar-H), 7.12 (dd, J = 8.6 2.2 Hz, 1H, Ar-H), 4.40 (q, J = 6.5 Hz, 2H, CH2), 3.95 (s, 3H, CH3); 1.35 (dd, J = 6.5 Hz, 3H, CH3); δC (400 MHz, CDCl3-d3); 164.8, 161.0, 154.4, 142.4, 132.4, 123.8, 113.4, 111.9, 103.8, 62.8, 56.1, 14.1.

**General synthetic procedure for benzo[e][1,2]oxathiine-3-carboxylic acid 2,2-dioxide (36) and 7-methoxybenzo[e][1,2]oxathiine-3-carboxylic acid 2,2-dioxide (37)**

NaOH 5M (7 equiv.) was added to a solution of appropriate ethyl esters 34 or 35 (1g, 1 equiv.) in EtOH (20 mL), and the suspension was heated at reflux for 1h. The reaction mixture was cooled and slushed, and HCl 6M were added to pH = 2. The resulting precipitate was filtered and washed with H2O to give us compounds 36 and 37 as a white powder in high yield and purity.

**benzo[e][1,2]oxathiine-3-carboxylic acid 2,2-dioxide (36)**

Compound 36 was obtained according to the general procedure reported earlier with 34 as starting material. Yield 81%; m.p. 105–107 °C; silica gel TLC Rf 0.04 (MeOH/DCM 5% v/v); δ1H (400 MHz, DMSO-d6); 14.57 (s, 1H, exchange with D2O, COOH), 8.50 (s, 1H, Ar-H), 7.98 (d, J = 7.4 Hz, 1H, Ar-H), 7.71 (dd, J = 7.4 Hz, 1H, Ar-H), 7.55 (m, 2H, Ar-H); δC (400 MHz, CDCl3-d3); 169.1, 159.4, 142.1, 132.5, 126.7, 118.8, 118.5.

**7-methoxybenzo[e][1,2]oxathiine-3-carboxylic acid 2,2-dioxide (37)**

Compound 37 was obtained according to the general procedure reported earlier with 35 as starting material. Yield 73%; m.p. 150–151 °C; silica gel TLC Rf 0.01 (MeOH/DCM 5% v/v); δ1H (400 MHz, DMSO-d6); 14.31 (s, 1H, exchange with D2O, COOH), 8.41 (s, 1H, Ar-H), 7.89 (d, J = 8 Hz, 1H, Ar-H), 7.18 (d, J = 2.1 Hz, 1H, Ar-H), 7.10 (dd, J = 8 2.1 Hz, 1H, Ar-H), 3.94 (s, 3H, CH3); δC (400 MHz, CDCl3-d3); 170.0, 157.6, 154.7, 140.6, 132.0, 123.1, 113.4, 111.8, 103.9, 56.0.

**General synthetic procedure for products 38–47**

PyBOP (1.2 equiv.) and appropriate aniline (1.2 equiv.) were added at 0 °C to a solution of 36 or 37 (0.2 g, 1 equiv.) in dry DMF (2 mL) under nitrogen atmosphere. DIPEA (3 equiv.) were added dropwise at 0 °C and the resulting solution was stirred o.n. at RT. The reaction mixture was quenched with slush and HCl 6M and the H2O-phase was extracted in EtOAc.
(3 × 30 mL). The collected organic phases were washed with HCl 1M (3 × 20 mL), NaHCO₃ (2 × 20 mL) and brine (3 × 20 mL), then the organic phase was dried with Na₂SO₄, filtered and evaporated under vacuum to obtain. All the products were purified by silica gel chromatography (MeOH/DCM 0.5% v/v).

**N-phenylbenz[e][1,2]oxathiine-3-carboxamide 2,2-dioxide (38)**

Compound 38 was obtained according to the general procedure reported earlier with aniline as starting material. Yield 60%; m.p. 192–195 °C; silica gel TLC Rf 0.82 (MeOH/DCM 5% v/v); δH (400 MHz, DMSO-d₆); 10.85 (s, 1H, exchange with D₂O, CONH), 8.35 (s, 1H, Ar-H), 7.92 (d, J = 8.0 Hz, 1H, Ar-H), 7.74 (m, 3H, Ar-H), 7.58 (m, 2H, Ar-H), 7.44 (dd, J = 7.3 Hz, 2H, Ar-H), 7.21 (dd, J = 7.3 Hz, 1H, Ar-H); δC (400 MHz, DMSO-d₆): 159.0, 152.1, 139.1, 138.5, 135.2, 132.4, 132.3, 130.0, 127.9, 125.6, 121.1, 120.0, 119.8; m/z (ESI negative): 299.9 [M − H]⁻, m/z (ESI positive): 302.0 [M + H]⁺.

**N-(4-bromophenyl)benz[e][1,2]oxathiine-3-carboxamide 2,2-dioxide (39)**

Compound 39 was obtained according to the general procedure reported earlier with 4-bromoaniline as starting material. Yield 21%; m.p. 249–252 °C; silica gel TLC Rf 0.88 (MeOH/DCM 5% v/v); δH (400 MHz, DMSO-d₆); 10.96 (s, 1H, exchange with D₂O, CONH), 8.36 (s, 1H, Ar-H), 7.92 (dd, J = 7.7 1.4 Hz, 1H, Ar-H), 7.76 (ddd, J = 7.7 1.4 Hz, 1H, Ar-H), 7.69 (d, J = 8.8 Hz, 2H Ar-H), 7.62 (d, J = 8.8 Hz, 2H, Ar-H), 7.58 (m, 2H, Ar-H); δC (400 MHz, DMSO-d₆): 159.1, 152.2, 138.9, 138.5, 135.4, 132.8, 132.3, 132.2, 128.0, 123.0, 119.9, 119.8, 117.4; m/z (ESI negative): 379.9 [M − H]⁻, m/z (ESI positive): 381.9 [M + H]⁺.

**N-(4-methoxyphenyl)benz[e][1,2]oxathiine-3-carboxamide 2,2-dioxide (40)**

Compound 40 was obtained according to the general procedure reported earlier with 3-bromoaniline as starting material. Yield 38%; m.p. 228–229 °C; silica gel TLC Rf 0.90 (MeOH/DCM 5% v/v); δH (400 MHz, DMSO-d₆); 10.96 (s, 1H, exchange with D₂O, CONH), 8.36 (s, 1H, Ar-H), 7.91 (dd, J = 7.9 1.7 Hz, 1H Ar-H), 7.77 (ddd, J = 7.9 1.7 Hz, 1H, Ar-H), 7.67 (m, 1H, Ar-H), 7.58 (m, 2H, Ar-H), 7.41 (d, J = 5.5 Hz, 2H, Ar-H); δC (400 MHz, DMSO-d₆): 159.2, 152.2, 140.7, 139.0, 135.4, 132.3, 132.1, 132.0, 128.2, 128.0, 123.5, 122.6, 119.9, 119.8, 118.2; m/z (ESI negative): 379.9 [M − H]⁻, m/z (ESI positive): 381.9 [M + H]⁺.

**N-(3-bromophenyl)benz[e][1,2]oxathiine-3-carboxamide 2,2-dioxide (41)**

Compound 41 was obtained according to the general procedure reported earlier with 4-methoxyaniline as starting material. Yield 37%; m.p. 231–233 °C; silica gel TLC Rf 0.82 (MeOH/DCM 5% v/v); δH (400 MHz, DMSO-d₆); 10.70 (s, 1H, exchange with D₂O, CONH), 8.30 (s, 1H, Ar-H), 7.90 (dd, J = 7.6 1.8 Hz, 1H, Ar-H), 7.75 (ddd, J = 7.6 1.8 Hz, 1H, Ar-H), 7.63 (d, J = 9.0 Hz, 2H, Ar-H), 7.56 (m, 2H, Ar-H), 7.00 (d, J = 9.0 Hz, 2H, Ar-H), 3.80 (s, 3H, CH₃); δC (400 MHz, DMSO-d₆): 158.6, 157.1, 152.1, 138.1, 135.4, 132.3, 132.1, 130.1, 128.0, 123.5, 122.6, 119.9, 115.1, 56.3; m/z (ESI negative): 329.9 [M − H]⁻, m/z (ESI positive): 332.0 [M + H]⁺.

**N-(3-methoxyphenyl)benz[e][1,2]oxathiine-3-carboxamide 2,2-dioxide (42)**

Compound 42 was obtained according to the general procedure reported earlier with 3-methoxyaniline as starting material. Yield 31%; m.p. 219–221 °C; silica gel TLC Rf 0.94 (MeOH/DCM 5% v/v); δH (400 MHz, DMSO-d₆); 10.79 (s, 1H, exchange with D₂O, CONH), 8.34 (s, 1H, Ar-H), 7.91 (dd, J = 7.5 1.3 Hz, 1H, Ar-H), 7.76 (ddd, J = 7.5 1.3 Hz, 1H, Ar-H), 7.57 (m, 2H, Ar-H), 7.33 (m, 3H, Ar-H), 6.79 (dd, J = 8.4 1.8 Hz, 1H, Ar-H), 3.81 (s, 3H, CH₃); δC (400 MHz, DMSO-d₆): 160.6, 159.0, 152.1, 140.3, 138.6, 135.3, 132.4, 132.3, 130.8, 128.0, 119.9, 119.8, 113.3, 111.1, 106.7, 56.1; m/z (ESI negative): 329.9 [M − H]⁻, m/z (ESI positive): 332.0 [M + H]⁺.

**7-methoxy-N-phenylbenz[e][1,2]oxathiine-3-carboxamide 2,2-dioxide (43)**

Compound 43 was obtained according to the general procedure reported earlier with aniline as starting material. Yield 50%; m.p. 241–244 °C; silica gel TLC Rf 0.76 (MeOH/DCM 5% v/v); δH (400 MHz, DMSO-d₆); 10.71 (s, 1H, exchange with D₂O, CONH), 8.29 (s, 1H, Ar-H), 7.83 (d, J = 8.4 Hz, 1H, Ar-H), 7.71 (d, J = 7.1 Hz, 2H, Ar-H), 7.43 (dd, J = 7.1 Hz, 2H, Ar-H), 7.17 (m, 3H, Ar-H), 3.94 (s, 3H, CH₃); δC (400 MHz, DMSO-d₆): 164.9, 159.3, 154.1,
139.3, 138.9, 133.6, 129.9, 129.0, 125.4, 121.0, 114.7, 112.7, 105.1, 57.4; m/z (ESI negative): 329.9 [M − H]−, m/z (ESI positive): 332.0 [M + H]+.

N-(4-bromophenyl)-7-methoxybenzo[e][1,2]oxathiine-3-carboxamide 2,2-dioxide (44)

Compound 44 was obtained according to the general procedure reported earlier with 4-bromoaniline as starting material. Yield 38%; m.p. 272–275 °C; silica gel TLC Rf 0.92 (MeOH/DCM 5% v/v); δ_H (400 MHz, DMSO-d_6): 10.83 (s, 1H, exchange with D_2O, CONH); 8.31 (s, 1H, Ar-H), 7.83 (d, J = 8.4 Hz, 1H, Ar-H), 7.68 (d, J = 9.1 Hz, 2H, Ar-H), 7.61 (d, J = 9.1 Hz, 2H, Ar-H), 7.22 (d, J = 2.2 Hz, 1H, Ar-H), 7.12 (dd, J = 8.4 2.2 Hz, 1H, Ar-H), 3.94 (s, 3H, CH_3); δ_C (400 MHz, DMSO-d_6): 165.0, 159.4, 154.1, 139.2, 138.7, 133.7, 132.8, 128.8, 122.9, 117.2, 114.8, 112.7, 105.2, 57.4; m/z (ESI negative): 407.9 [M − H]−, m/z (ESI positive): 411.9 [M + H]+.

N-(3-bromophenyl)-7-methoxybenzo[e][1,2]oxathiine-3-carboxamide 2,2-dioxide (45)

Compound 45 was obtained according to the general procedure reported earlier with 3-bromoaniline as starting material. Yield 32%; m.p. 218–220 °C; silica gel TLC Rf 0.87 (MeOH/DCM 5% v/v); δ_H (400 MHz, DMSO-d_6): 10.80 (s, 1H, exchange with D_2O, CONH), 8.26 (s, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 7.78 (d, J = 8.3 Hz, 1H, Ar-H), 7.62 (d, J = 4.5 Hz, 1H, Ar-H), 7.35 (m, 2H, Ar-H), 7.18 (d, J = 2.2 Hz, 1H, Ar-H), 7.08 (dd, J = 8.3 2.2 Hz, 1H, Ar-H), 3.90 (s, 3H, CH_3); δ_C (400 MHz, DMSO-d_6): 165.1, 159.6, 154.1, 140.8, 139.4, 133.7, 132.0, 128.6, 128.0, 123.4, 122.6, 119.8, 114.8, 112.6, 105.2, 57.4; m/z (ESI negative): 409.9 [M − H]−, m/z (ESI positive): 411.9 [M + H]+.

N-(4-methoxyphenyl)-7-methoxybenzo[e][1,2]oxathiine-3-carboxamide 2,2-dioxide (46)

Compound 46 was obtained according to the general procedure reported earlier with 4-methoxyaniline as starting material. Yield 7%; m.p. 280–282 °C; silica gel TLC Rf 0.95 (MeOH/DCM 5% v/v); δ_H (400 MHz, DMSO-d_6): 8.48 (s, 1H, exchange with D_2O, CONH), 8.24 (s, 1H, Ar-H), 7.82 (d, J = 8.2 Hz, 1H, Ar-H), 7.62 (d, J = 8.8 Hz, 2H, Ar-H), 7.21 (d, J = 2.2 Hz, 1H, Ar-H), 7.12 (dd, J = 8.2 2.2 Hz, 1H, Ar-H), 6.99 (d, J = 8.8 Hz, 2H, Ar-H), 3.97 (s, 3H, CH_3), 3.82 (s, 3H, CH_3); δ_H (400 MHz, DMSO-d_6); δ_C (400 MHz, DMSO-d_6): 164.8, 158.9, 157.1, 154.0, 138.4, 133.5, 132.2, 129.2, 122.6, 115.1, 114.7, 112.8, 105.1, 57.4, 56.3; m/z (ESI negative): 359.9 [M − H]−, m/z (ESI positive): 362.0 [M + H]+.

N-(3-methoxyphenyl)-7-methoxybenzo[e][1,2]oxathiine-3-carboxamide 2,2-dioxide (47)

Compound 47 was obtained according to the general procedure reported earlier with 3-methoxyaniline as starting material. Yield 41%; m.p. 222–225 °C; silica gel TLC Rf 0.79 (MeOH/DCM 5% v/v); δ_H (400 MHz, DMSO-d_6): 10.69 (s, 1H, exchange with D_2O, CONH), 8.27 (s, 1H, Ar-H), 7.37 (dd, J = 2.0 Hz, 1H, Ar-H), 7.32 (dd, J = 8.0 Hz, 1H, Ar-H), 7.27 (d, J = 8.0 Hz, 1H, Ar-H), 7.22 (d, J = 2.1 Hz, 1H, Ar-H), 7.12 (dd, J = 8.6 2.1 Hz, 1H, Ar-H), 6.77 (dd, J = 8.0 2.0 Hz, 1H, Ar-H), 3.94 (s, 3H, CH_3), 3.80 (s, 3H, CH_3); δ_C (400 MHz, DMSO-d_6): 164.9, 160.6, 159.3, 154.1, 144.6, 140.4, 138.9, 133.6, 130.7, 129.0, 114.7, 113.2, 111.0, 106.7, 105.1, 57.4, 56.1; m/z (ESI negative): 359.9 [M − H]−, m/z (ESI positive): 362.0 [M + H]+.

3.2. Carbonic Anhydrase Inhibition

An Applied Photophysics stopped-flow instrument was used for assaying the CA catalyzed CO_2 hydration activity [44]. 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 were used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of 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 6h 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 and represent the
mean from at least three different determinations. The enzyme concentrations were in the range 5–16 nM. All CA isoforms were recombinant ones obtained in-house [47,48].

4. Conclusions

We report here a series of 7-substituted and 3-substituted sulfocoumarins, obtained by cyclization of 2-hydroxy-4-methoxybenzaldehyde (7), 2′-hydroxy-4′-methoxyacetophenon (8), 2-bromobenzensulfonfyl chloride (23), salicylaldehyde (32) or 4-methoxysalicylaldehyde (33) and possessing different substituents in position 3 or 7 of the heterocyclic ring. 7-Substituted sulfocoumarins resulted in being good inhibitors of hCA IX and XII, whilst 3-substituted sulfocoumarins showed a worsening in terms of inhibition potency on this isoform. A common feature of all the synthesized products was the absence of inhibition on hCA I and II. The most potent products belong to Type 1 group, probably due to the absence of the bulky group on the heterocyclic ring; particularly, sulfocoumarin 15 showed a nanomolar inhibition on hCA IX and XII and turned out to be the most potent inhibitor achieved (hCA IX: $K_I = 22.9$ nM; hCA XII: $K_I = 19.2$ nM). The structure-activity relationship for this class of CAIs has been expanded considering the synthesis of 3-substituted sulfocoumarines for the first time. The observed isoform-selective inhibition displayed here may be considered to be of interest for various biomedical applications.

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