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Short communication

Cyclic tertiary sulfamates: Selective inhibition of the tumor-associated carbonic anhydrases IX and XII by *N*- and *O*-substituted acesulfame derivatives☆

Celeste De Monte [a, *], Simone Carradori [a], Daniela Secci [a], Melissa D'Ascenzio [a], Daniela Vullo [b], Mariangela Ceruso [b], Claudiu T. Supuran [b, c, **]

[a] Sapienza University of Rome, Department of Drug Chemistry and Technologies, 00185 Roma, Italy
[b] Università degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica, 50019 Florence, Italy
[c] Università degli Studi di Firenze, Neurofarba Department, Section of Pharmaceutical and Nutraceutical Sciences, Sesto Fiorentino, 50019 Florence, Italy

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ABSTRACT

Carbonic anhydrase (hCA) IX and XII isoforms are over-expressed both in primary and in metastatic cell lines of hypoxic tumors and are innovative targets for cancer diagnosis and treatment. On the basis of the importance of the pharmacophoric sulfamate moiety (bioisostere of the sulfonamide group) present in the structure of recent human CA inhibitors, we designed *N*-alkylated and *O*-alkylated derivatives of acesulfame, a cyclic tertiary sulfamate, assessing the inhibitory activity against the ubiquitous isoforms hCA I and II and the cancer-related isoforms hCA IX and XII. All derivatives were nanomolar inhibitors, with some of them possessing an outstanding selectivity towards the tumor-associated hCA IX and/or hCA XII isoforms.

1. Introduction

Malignancies need new blood vessels to provide nutrients and oxygen, but not all the cancer cells take enough amounts of oxygen and so they become hypoxic [1]. As a consequence of hypoxia, an upregulation of genes involved in anaerobic metabolism and tumor vascularization occurs [2]. Hypoxia-inducible factor-1 (HIF-1) is a protein transcription factor which presents two subunits, the constitutive subunit HIF-1β and the oxygen-regulated subunit HIF-1α, and controls the responses to a hypoxic microenvironment. In normoxia, the hydroxylation of the prolines of HIF-1α isoform is normally required to be recognized by the von Hippel–Lindau (VHL) tumor suppressor protein for the proteasomal degradation of HIF-1α. Under hypoxic conditions, HIF-1α eludes the hydroxylation and the degradation, and forms a dimer with HIF-1β in the nucleus, interacting with co-activators in a transcriptional complex which binds a hypoxia-response element (HRE) in the promoter or enhancer regions of HIF target genes [3] which are involved in angiogenesis, vascular tone, erythropoiesis and in the production of glycolysis enzymes [4]. While in normal tissues glucose is converted to glucose-6-phosphate and then to pyruvate, that is oxidized in the mitochondria to carbon dioxide and water, cancer cells in hypoxia produce energy from glycolysis by lactic acid fermentation in the cytosol (the so-called Warburg effect) [5]. The enhanced generation and export to the extracellular environment of lactic and carbonic acids by glycolysis causes an extracellular acidification: to cope with this unkind condition and to maintain their intracellular pH weakly alkaline, tumor cells use buffer systems, pumps and carbonic anhydrases (CAs) [6], which are zinc metalloenzymes catalyzing the reversible hydration of carbon dioxide to bicarbonate and protons [7]. The known human CA (EC 4.2.1.1) isozymes belong to the α-class CA family present in mammals and the most common ubiquitous isoforms are the cytosolic hCA I and hCA II. On the other hand, CA IX and CA XII isoforms have been validated as targets of antitumor agents [8]. In fact, the bicarbonate ion produced at the extracellular surface

☆ We dedicate this paper to the 60th anniversary of Prof. Marius Andruh, close friend and collaborator of Univ. of Bucharest, Romania.

* Corresponding author.
** Corresponding author. Università degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica, 50019 Florence, Italy.
E-mail addresses: celeste.demonte@uniroma1.it (C. De Monte), claudiu.supuran@unifi.it (C.T. Supuran).

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by CA IX is transported into the cytosol to regulate intracellular pH while the protons produced by CA IX contribute to diminish extracellular pH, causing matrix breakdown and tumor invasion and metastasis. According to these data, CA IX could be used as a biomarker of hypoxic tumors and is an attractive target for anticancer therapy [9]. Moreover, HIF-1 regulated the expression of CA IX [10], that has been found in different forms of cancer such as glioblastoma, colorectal and breast cancer and it is constitutively present in renal carcinoma [11]. CA IX is also expressed in cancer-associated fibroblasts and its activity leads to epithelial–mesenchymal transition in prostate carcinoma cells causing major motility, survival and stemness [12]. It has been demonstrated that the inhibition of CA IX supports anti-VEGF therapy with bevacizumab [13]. Another tumor-related CA isoform is CA XII, which is over-expressed not only in gastric, colorectal and breast cancer but also in T-cell Acute Lymphoblastic Leukemia human samples [14].

Hence, pursuing our interest in the field of anticancer therapy [15–17], we developed new selective tumor-associated CAs inhibitors [18–20] based on the two most important bioactive chemotypes present in literature, the metal-complexing anions and the sulfonamides [21]. Thanks to the encouraging results obtained by the saccharin derivatives, and basing on the evidence that sulfamates act as effective CA IX inhibitors [22,23], we decided to modify the saccharin skeleton endowed with a cyclic tertiary sulfonamide moiety designing a bioisosteric series of *N*-alkylated and *O*-alkylated derivatives of the well known sweetener acesulfame [24], that bears the cyclic tertiary sulfamate group (Fig. 1). Then, we assessed their inhibitory activity against the ubiquitous hCA I and II and the cancer-related hCA IX and XII isoforms demonstrating their outstanding selectivity towards the latter isozymes.

2. Results and discussion

2.1. Chemistry

Twenty-two compounds were synthesized according to the general procedure reported in Scheme 1 based on a procedure published in our previous work [20]. The reaction between potassium acesulfame and the electrophile agents in *N*,*N*-dimethylformamide gave the resulting *N*-alkylated derivatives (**a series**) and/or *O*-alkylated derivatives (**b series**). The formation of a mixture of amide and imidate compounds or the generation of only one of them depended not only on the thermodynamic stability of the products, but also on the nature and on the steric hindrance of the electrophile reactant which could affect the product distribution and selectivity [25]. These considerations could explain the formation of the derivatives belonging to **a series** and/or **b series** starting from the ambident nucleophile potassium acesulfame. All synthesized compounds have been fully characterized by analytical and spectral data (see Section 5.1). Potassium acesulfame was purchased by Sigma–Aldrich® Italy and used in the syntheses and in the biological assays without further purification.

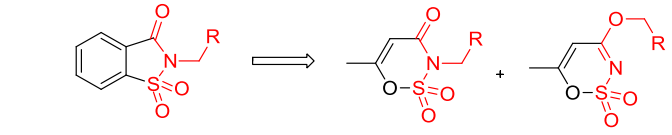
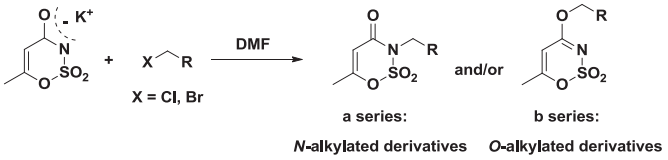


Fig. 1. Chemical evolution of novel CA IX and XII inhibitors.



Scheme 1. Synthesis of acesulfame derivatives (sulfamate inhibitors).

3. Biological evaluation

3.1. Carbonic anhydrase inhibition

All the synthesized compounds of **a series** and **b series** and the purchased potassium acesulfame were assayed to establish their inhibitory effect against the ubiquitous off-target isoforms, CA I and II, and the cancer-related ones, CA IX and XII by a stopped-flow, CO_2 hydrase assay method [26] and the CA inhibition data are summarized in Table 1.

In general, the introduction of an alkyl/benzyl substituent on the parent compound led to better and selective CA inhibitors. A comprehensive structure–activity relationship for this series can be described as follows.

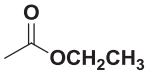
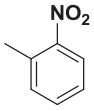
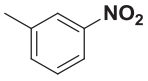
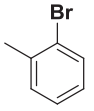
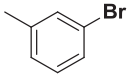
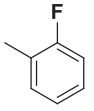
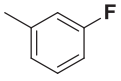
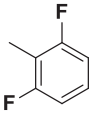
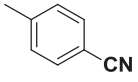
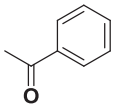
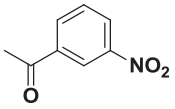
(i) Most of the tested compounds were not active (K_I > 20000 nM) against two of the common off target CA I and CA II isoenzymes. They also were more effective than the starting compound potassium acesulfame and more selective than the reference drug acetazolamide for the inhibition of the tumor-associated isoforms. Although weak inhibitory effects were exerted on CA I by derivatives **3b** and **4b** (K_I = 2900 and 2485 nM, respectively), and on CA II by **4a** (K_I = 2175 nM), these compounds also displayed a considerable activity on the tumor-associated isoforms: **3b**, which contained a CN moiety, possessed a K_I of 47 nM towards CA IX, while the carbonylic derivatives **4a** and **4b** inhibited CA XII (K_I = 159 and 157 nM, respectively). Compound **15a**, presenting the sterically hindered 3′-nitroacetophenone in the alkylating chain, was not selective towards the off-target CA II and the tumor-associated CA IX and XII with K_I values ranging from 16.3 to 25.5 nM, and it was also slightly effective on CA I (1300 nM).

(ii) Compounds **1a**, **1b**, **2b**, **7a**, **8b**, **9a**, **10a**, **13a**, **14a** and **14b** selectively inhibited both the tumor-related isozymes: CA IX was inactivated in the range of 5.7–309 nM, whereas CA XII in the range of 16.5–180 nM. When acesulfame derivatives were functionalized with a phenyl ring bearing a bromine (compounds **8b** and **9a**) or a cyano moiety (compound **13a**), they lost their selectivity against CA IX and XII. On the other hand, even though with slight differences, *O*-alkylated products **1b**, that was functionalized with an allyl moiety, and **2b**, containing a propargyl group, were more active on CA XII (85.8 and 178 nM respectively), while the *O*-alkylated acetophenone derivative **14b** was more selective on CA IX with respect to the corresponding *N*-alkylated **14a** at a greater concentration (**14a** at 5.7 nM, while **14b** at 15.3 nM).

(iii) Outstanding results for the inhibition of CA IX were reached with derivatives **5a** and **5b**, bearing an ethyl ester moiety (K_I = 47 and 43 nM respectively), and **6a** and **6b**, containing a phenyl ring substituted in the *ortho* position with a nitro group (K_I = 41 nM).

(iv) As regards the inhibitory activity against CA XII, it was noteworthy that the *O*-alkylated derivative **7b** was more selective than its counterpart *N*-alkylated **7a**: in fact the former only inhibited this isoform (134 nM), while the latter

Table 1
Inhibitory activity of *N*-alkylated (**a series**) and *O*-alkylated (**b series**) acesulfame derivatives, potassium acesulfame and the reference drug acetazolamide against selected hCA isoforms by a stopped-flow CO_2 hydrase assay.

Compound	R	K_I (nM)			
		CA I	CA II	CA IX	CA XII
1a		>20,000	>20,000	114.4	103
1b		>20,000	>20,000	199	85.8
2b		>20,000	>20,000	252	178
3b		2900	>20,000	47	2900
4a		15,650	2175	>20,000	159
4b		2485	>20,000	>20,000	157
5a		>20,000	>20,000	47	4530
5b		>20,000	>20,000	43	2710
6a		>20,000	>20,000	41	2170
6b		>20,000	>20,000	41	2830
7a		>20,000	>20,000	266.6	177
7b		>20,000	>20,000	>20,000	134
8b		>20,000	>20,000	249	180
9a		>20,000	>20,000	284	170
10a		>20,000	>20,000	285	180
11a		>20,000	>20,000	275	18.3
11b		>20,000	>20,000	>20,000	91.5
12a		>20,000	>20,000	>20,000	170
13a		>20,000	>20,000	309	136
14a		>20,000	>20,000	5.7	16.5
14b		>20,000	>20,000	15.3	126
15a		1300	25.5	16.8	16.3
Potassium acesulfame		>20,000	>20,000	2410	>20,000
Acetazolamide		250	12.1	25.0	5.7

both CA IX and XII as reported above. Furthermore, in order to define a structure–activity relationship, we could state that the presence of the nitro group in the *meta* position rather than in the *ortho* position of the phenyl ring reversed the selectivity of action from CA IX (for derivatives **6a** and **6b**) to CA XII (for **7a** and **7b**). Similarly, compound **11b**, which presented a phenyl ring with a fluorine at C3, had an inhibitory effect exclusively on CA XII with a K_I of 91.5 nM, whereas its *N*-alkylated counterpart **11a** was only weakly more selective on CA XII (18.3 nM) with respect to CA IX (275 nM). Instead, product **10a**, that contained the fluorine at C2 of the aromatic nucleus, was active towards both CA IX and XII (K_I = 285 and 180 nM, respectively). Finally, compound **12a**, presenting two fluorine atoms in the *ortho* positions of the aromatic ring, was an effective inhibitor exclusively of CA XII isozyme (K_I = 170 nM).

These encouraging biological results demonstrated that this series of *N*- and *O*-alkylated acesulfame derivatives could represent an important starting point for the development of new anticancer agents.

4. Conclusions

A new series of twenty-two acesulfame derivatives were designed, synthesized and assayed against four human carbonic anhydrases: CA IX and XII that are the two tumor-associated isozymes, and CA I and II which represent the most common off-targets for the development of selective anticancer CA inhibitors. Most of the compounds had no affinity for hCA I and II, and they all possessed an inhibitory effect selectively on CA IX and/or CA XII in the nanomolar range.

These CAIs should be further studied not only for their pharmacological importance, but also to elucidate their mechanism of action. As we already did for other selective CA XII inhibitors that we synthesized in the past [19], computational investigation with molecular modeling and docking studies could be used to investigate their selective interaction with CA IX and CA XII in order to understand if they could be involved or not in a direct binding with the active site of the enzyme. Therefore, the promising biological *in vitro* results demonstrated that this novel class of sulfamate compounds could represent an innovative and attractive tool for the development of new antitumor agents based on the inhibition of the cancer-related isoforms of human carbonic anhydrase.

5. Experimental protocols

5.1. Chemistry

Solvents were used as supplied without further purification. Where mixtures of solvents are specified, the stated ratios are volume:volume. Reagents were used directly as supplied by

Sigma–Aldrich® Italy. Column chromatography was carried out using Sigma–Aldrich® silica gel (high purity grade, pore size 60 Å, 230–400 mesh particle size). Analytical thin-layer chromatography was carried out on Sigma–Aldrich® silica gel on TLA aluminum foils with fluorescent indicator. Visualization was carried out under ultra-violet irradiation (254 nm). NMR spectra were recorded on a Bruker AV400 (^{1}H: 400 MHz, ^{13}C: 101 MHz). Coupling constants *J* are valued in Hertz (Hz). Chemical shifts are expressed as δ units (parts per millions), based on appearance rather than interpretation, and are referenced to the residual non deuterated solvent peak. IR spectra were recorded on a FT-IR Perkin–Elmer Spectrum One equipped with ATR system. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). All melting points were measured on a Stuart® melting point apparatus SMP1, and are uncorrected. Temperatures are reported in °C. Where given, systematic compound names are those generated by ChemSketch following IUPAC conventions.

5.1.1. General procedure for the synthesis of acesulfame derivatives

Up to 2.5 eq of the corresponding electrophile were added portionwise to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide and the reaction mixture was stirred under nitrogen for 24–72 h at 80 °C, with the exception of derivatives **1a**, **1b** and **2b**, for which the reaction was carried out at room temperature because of the low thermostability of the reagents. Then, the mixture was poured on ice and the resulting product was extracted or filtered and purified by column chromatography on silica gel.

*5.1.1.1. 6-Methyl-3-(prop-2-en-1-yl)-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (**1a**) and 6-methyl-4-(prop-2-en-1-yloxy)-1,2,3-oxathiazine 2,2-dioxide (**1b**).* Allyl bromide (2.5 eq) was added portionwise to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at room temperature for 48 h, poured on ice and extracted with chloroform (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:2) gave compounds **1a** and **1b**.

1a: light yellow oil (48% yield); IR ν_{max} 3093 (ν C_{sp2}–H), 1702 (ν C=O), 1401 (ν_{as} SO_2), 1314 (ν C–N), 1198 (ν_s SO_2), 930 (ν O–S), 908 (δ C_{sp2}–H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.18 (s, 3H, CH_3), 4.40 (s, 2H, CH_2), 5.25 (d, J_{cis} = 10.4 Hz, 1H, =CH_2), 5.33 (d, J_{trans} = 17.2 Hz, 1H, =CH_2), 5.79 (s, 1H, CH =), 5.81–5.88 (m, 1H, CH=); ^{13}C NMR (101 MHz, $CDCl_3$) δ 19.62 (*C*H_3), 45.04 (*C*H_2), 104.39 (*C*H=), 119.79 (*C*H=), 130.55 (*C*H_2=), 159.85 (CH_3*C*=), 161.92 (*C*=O). Anal. Calcd for $C_7H_9NO_4S$: C, 41.37; H, 4.46; N, 6.89. Found: C, 41.19; H, 4.75; N, 6.61.

1b: yellow oil (21% yield); IR ν_{max} 3103 (ν C_{sp2}–H), 1648 (ν C=N), 1553 (ν C=C), 1374 (ν_{as} SO_2), 1271 (ν C–O–C), 1187 (ν_s SO_2), 967 (δ C_{sp2}–H), 932 (ν O–S), 910 (δ C_{sp2}–H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (s, 3H, CH_3), 4.77 (s, 2H, CH_2), 5.30 (d, J_{cis} = 10.4 Hz, 1H, =CH_2), 5.36 (d, J_{trans} = 17.2 Hz, 1H, =CH_2), 5.76 (s, 1H, CH=), 5.89–5.95 (m, 1H, CH=); ^{13}C NMR (101 MHz, $CDCl_3$) δ 20.44 (*C*H_3), 60.30 (*C*H_2), 95.58 (*C*H=), 120.54 (*C*H=), 130.04 (*C*H_2=), 168.77 (CH_3*C*=), 169.01 (*C*=N). Anal. Calcd for $C_7H_9NO_4S$: C, 41.37; H, 4.46; N, 6.89. Found: C, 41.60; H, 4.22; N, 7.07.

*5.1.1.2. 6-Methyl-4-(prop-2-yn-1-yloxy)-1,2,3-oxathiazine 2,2-dioxide (**2b**).* Propargyl bromide (1.1 eq) was added portionwise to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at room temperature for 24 h, poured on ice and extracted with chloroform (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:2) gave compound **2b** as a light yellow oil (73% yield); IR ν_{max} 3291 (ν C_{sp}–H), 3108 (ν C_{sp2}–H), 2131 (ν C≡C), 1650 (ν C=N), 1558 (ν C=C), 1378 (ν_{as} SO_2), 1190 (ν_s SO_2), 1159 (ν C–O–C), 726 (ν O–S) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 3H, CH_3), 2.64 (s, 1H, CH≡), 4.94 (s, 2H, CH_2), 5.84 (s, 1H, CH=); ^{13}C NMR (101 MHz, $CDCl_3$) δ 20.63 (*C*H_3), 56.14 (*C*H_2), 75.48 (*C*H≡), 77.23 (*C*≡CH), 95.31 (*C*H=), 168.52 (CH_3*C*=), 169.46 (*C*=N). Anal. Calcd for $C_7H_7NO_4S$: C, 41.79; H, 3.51; N, 6.96. Found: C, 41.55; H, 3.24; N, 7.12.

*5.1.1.3. [(6-Methyl-2,2-dioxido-1,2,3-oxathiazin-4-yl)oxy]acetonitrile (**3b**).* Chloroacetonitrile (2.0 eq) was added portionwise to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 72 h, poured on ice and extracted with dichloromethane (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:1) gave compound **3b** as a light brown oil (64% yield); IR ν_{max} 2920 (ν C_{sp2}–H), 2350 (ν C≡N), 1649 (ν C=N), 1566 (ν C=C), 1385 (ν_{as} SO_2), 1263 (ν C–O–C), 1194 (ν_s SO_2), 930 (ν O–S), 734 (δ C_{sp2}–H) cm^{-1}; ^{1}H NMR (400 MHz, CD_2Cl_2): δ 2.31 (s, 3H, CH_3), 5.05 (s, 2H, CH_2), 5.94 (s, 1H, CH=); ^{13}C NMR (101 MHz, $CDCl_3$) δ 20.80 (*C*H_3), 51.76 (*C*H_2), 94.60 (*C*H=), 112.79 (*C*N), 167.87 (CH_3*C*=), 170.89 (*C*=N). Anal. Calcd for $C_6H_6N_2O_4S$: C, 35.64; H, 2.99; N, 13.86. Found: C, 35.40; H, 3.17; N, 14.05.

*5.1.1.4. 6-Methyl-3-(2-oxopropyl)-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (**4a**) and 1-[(6-methyl-2,2-dioxido-1,2,3-oxathiazin-4-yl) oxy]propan-2-one (**4b**).* Chloroacetone (1.1 eq) was added portionwise to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 48 h, poured on ice and extracted with chloroform (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:2) gave compounds **4a** and **4b**.

4a: light yellow powder (28% yield); mp 83–86 °C; IR ν_{max} 3103 (ν C_{sp2}–H), 1732 (ν C=O), 1697 (ν C=O), 1395 (ν_{as} SO_2), 1315 (ν C–N), 1197 (ν_s SO_2), 925 (ν O–S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.19 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 4.80 (s, 2H, CH_2), 6.31 (s, 1H, CH=); ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.49 (*C*H_3), 27.14 (CO*C*H_3), 51.46 (*C*H_2), 104.07 (*C*H=), 159.93 (CH_3*C*=), 163.56 (*C*=O), 200.20 (*C*=O). Anal. Calcd for $C_7H_9NO_5S$: C, 38.35; H, 4.14; N, 6.39. Found: C, 38.57; H, 4.39; N, 6.62.

4b: yellow oil (35% yield); IR ν_{max} 3022 (ν C_{sp2}–H), 1742 (ν C=O), 1652 (ν C=N), 1567 (ν C=C), 1386 (ν_{as} SO_2), 1214 (ν C–O–C), 1196 (ν_s SO_2), 745 (ν O–S) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.22 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.96 (s, 2H, CH_2), 5.93 (s, 1H, CH=); ^{13}C NMR (101 MHz, $CDCl_3$) δ 20.66 (*C*H_3), 26.12 (CO*C*H_3), 70.97 (*C*H_2), 95.28 (*C*H=), 168.78 (CH_3*C*=), 169.69 (*C*=N), 198.91 (*C*=O). Anal. Calcd for $C_7H_9NO_5S$: C, 38.35; H, 4.14; N, 6.39. Found: C, 38.10; H, 3.89; N, 6.17.

*5.1.1.5. Ethyl (6-methyl-2,2-dioxido-4-oxo-1,2,3-oxathiazin-3(4H)-yl)acetate (**5a**) and ethyl [(6-methyl-2,2-dioxido-1,2,3-oxathiazin-4-yl)oxy]acetate (**5b**).* α-Bromoethyl acetate (2.0 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 24 h, poured on ice and extracted with dichloromethane (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:1) gave compounds **5a** and **5b**.

5a: light yellow oil (20% yield); IR ν_{max} 3104 (ν C_{sp2}–H), 1751 (ν C=O), 1703 (ν C=O), 1659 (ν C=C), 1320 (ν_{as} SO_2), 1195 (ν C–O), 1175 (ν_s SO_2), 1020 (ν C–N), 925 (ν O–S) cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2): δ 1.31 (t, 3H, J = 7.2 Hz, CH_3), 2.28 (s, 3H, CH_3), 4.26 (q, 2H, J = 7.2 Hz, CH_2), 4.54 (s, 2H, N–CH_2), 5.92 (s, 1H, CH=); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.02 (*C*H_3), 19.85 (*C*H_3), 43.37 (*C*H_2), 62.28 (*C*H_2), 104.34 (*C*H=), 159.80 (CH_3*C*=), 162.52 (*C*=O), 166.22 (*C*=O). Anal. Calcd for $C_8H_{11}NO_6S$: C, 38.55; H, 4.45; N, 5.62. Found: C, 38.21; H, 4.74; N, 5.93.

5b: light yellow oil (51% yield); IR ν_{max} 3113 (ν C_{sp2}–H), 1753 (ν C=O), 1647 (ν C=N), 1557 (ν C=C), 1371 (ν_{as} SO_2), 1185 (ν C–O), 1158 (ν_s SO_2), 1037 (ν C–O–C), 931 (ν O–S) cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2): δ 1.32 (t, 3H, J = 7.2 Hz, CH_3), 2.28 (s, 3H, CH_3), 4.28 (q, 2H, J = 7.2 Hz, CH_2), 4.91 (s, 2H, O–CH_2), 5.97 (s, 1H, CH=); ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.03 (*C*H_3), 20.62 (*C*H_3), 62.05 (*C*H_2), 63.74 (*C*H_2), 95.24 (*C*H=), 165.93 (CH_3*C*=), 168.85 (*C*=O), 169.69 (*C*=N). Anal. Calcd for $C_8H_{11}NO_6S$: C, 38.55; H, 4.45; N, 5.62. Found: C, 38.79; H, 4.22; N, 5.36.

*5.1.1.6. 6-Methyl-3-(2-nitrobenzyl)-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (**6a**) and 6-methyl-4-[(2-nitrobenzyl)oxy]-1,2,3-oxathiazine 2,2-dioxide (**6b**).* 2-Nitrobenzyl chloride (1.1 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 72 h and poured on ice. The resulting suspension was filtered and washed with water, petroleum ether and diethyl ether. Purification by column chromatography on silica gel (diethyl ether:-petroleum ether, 2:1) gave compounds **6a** and **6b**.

6a: light yellow powder (11% yield); mp 100–104 °C; IR ν_{max} 3090 (ν C_{sp2}–H), 1705 (ν C=O), 1527 (ν_{as} N–O), 1394 (ν_{as} SO_2), 1344 (ν_s N–O), 1305 (ν C–N), 1199 (ν_s SO_2), 926 (ν O–S), 725 (δ_o C_{sp2}–H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H, CH_3), 5.39 (s, 2H, CH_2), 6.39 (s, 1H, CH=), 7.46 (d, J = 7.2 Hz, 1H, benzene), 7.63 (t, J = 7.2 Hz, 1H, benzene), 7.80 (t, J = 8.4 Hz, 1H, benzene), 8.13 (d, J = 8.4 Hz, 1H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.53 (*C*H_3), 43.06 (*C*H_2), 104.18 (*C*H=), 125.68 (*C*H-benzene), 128.86 (*C*H-benzene), 129.76 (*C*H-benzene), 130.39 (*C*H-benzene), 134.77 (*C*-benzene), 148.17 (*C*-benzene), 160.33 (CH_3C=), 163.85 (*C*=O). Anal. Calcd for $C_{11}H_{10}N_2O_6S$: C, 44.29; H, 3.38; N, 9.39. Found: C, 44.55; H, 3.11; N, 9.02.

6b: light yellow powder (72% yield); mp 141–144 °C; IR ν_{max} 3112 (ν C_{sp2}–H), 1646 (ν C=N), 1562 (ν C=C), 1527 (ν_{as} N–O), 1356 (ν_s N–O), 1344 (ν_{as} SO_2), 1191 (ν_s SO_2), 1163 (ν C–O–C), 933 (ν O–S), 735 (δ_o C_{sp2}–H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.29 (s, 3H, CH_3), 5.73 (s, 2H, CH_2), 6.45 (s, 1H, CH=), 7.71 (t, J = 7.8 Hz, 1H, benzene), 7.79 (d, J = 7.2 Hz, 1H, benzene), 7.86 (t, J = 7.6 Hz, 1H, benzene), 8.18 (d, J = 8.0 Hz, 1H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 20.36 (*C*H_3), 67.39 (*C*H_2), 95.89 (*C*H=), 125.58 (*C*H-benzene), 129.84 (*C*H-benzene), 130.61 (*C*H-benzene), 130.91 (*C*H-benzene), 134.80 (*C*-benzene), 148.01 (*C*-benzene), 169.75 (CH_3*C*=), 170.41 (*C*=N). Anal. Calcd for $C_{11}H_{10}N_2O_6S$: C, 44.29; H, 3.38; N, 9.39. Found: C, 43.98; H, 3.04; N, 9.67.

*5.1.1.7. 6-Methyl-3-(3-nitrobenzyl)-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (**7a**) and 6-methyl-4-[(3-nitrobenzyl)oxy]-1,2,3-oxathiazine 2,2-dioxide (**7b**).* 3-Nitrobenzyl bromide (1.1 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 72 h, poured on ice and extracted with chloroform (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:2) gave compounds **7a** and **7b**.

7a: white powder (71% yield); mp 93–97 °C; IR ν_{max} 1696 (ν C=O), 1530 (ν_{as} N–O), 1396 (ν_{as} SO_2), 1351 (ν_s N–O), 1303 (ν C–N), 1196 (ν_s SO_2), 923 (ν O–S), 812 (δ_m C_{sp2}–H), 763 (δ_m C_{sp2}–H), 686 (δ_m C_{sp2}–H) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.23 (s, 3H, CH_3), 5.06 (s, 2H, CH_2), 5.89 (s, 1H, CH=), 7.57 (t, J = 7.8 Hz, 1H, benzene), 7.78 (d, J = 7.6 Hz, 1H, benzene), 8.21 (d, J = 8.0 Hz, 1H, benzene), 8.30 (s, 1H, benzene); ^{13}C NMR (101 MHz, $CDCl_3$) δ 19.79 (*C*H_3), 45.20 (*C*H_2), 104.44 (*C*H=), 123.51 (*C*H-benzene), 123.70 (*C*H-benzene), 129.85 (*C*H-benzene), 134.72 (*C*H-benzene), 136.71 (*C*-benzene), 148.44 (*C*-benzene), 160.04 (CH_3*C*=), 162.34 (*C*=O). Anal. Calcd for $C_{11}H_{10}N_2O_6S$: C, 44.29; H, 3.38; N, 9.39. Found: C, 44.46; H, 3.15; N, 9.05.

7b: yellow powder (17% yield); mp 115–123 °C; IR ν_{max} 3120 (ν C_{sp2}–H), 1645 (ν C=N), 1555 (ν C=C), 1529 (ν_{as} N–O), 1353 (ν_s N–O), 1340 (ν_{as} SO_2), 1180 (ν_s SO_2), 1166 (ν C–O–C), 964 (ν O–S), 822 (δ_m C_{sp2}–H), 741 (δ_m C_{sp2}–H), 699 (δ_m C_{sp2}–H) cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) δ 2.28 (s, 3H, CH_3), 5.50 (s, 2H, CH_2), 5.94 (s, 1H, CH=), 7.66 (t, J = 7.8 Hz, 1H, benzene), 7.80 (d, J = 7.6 Hz, 1H, benzene), 8.27 (d, J = 8.4 Hz, 1H, benzene), 8.32 (s, 1H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 20.36 (*C*H_3), 67.39 (*C*H_2), 95.89 (*C*H=), 125.58 (*C*H-benzene), 129.84 (*C*H-benzene), 130.61 (*C*H-benzene), 130.91 (*C*H-benzene), 134.80 (*C*-benzene), 148.01 (*C*-benzene), 169.75 (CH_3*C*=), 170.41 (*C*=N). Anal. Calcd for $C_{11}H_{10}N_2O_6S$: C, 44.29; H, 3.38; N, 9.39. Found: C, 44.07; H, 3.10; N, 9.58.

*5.1.1.8. 4-[(2-Bromobenzyl)oxy]-6-methyl-1,2,3-oxathiazine 2,2-dioxide (**8b**).* 2-Bromobenzyl bromide (1.1 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 24 h, poured on ice and extracted with dichloromethane (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:2) gave compound **8b** as a white powder (81% yield); mp 95–96 °C; IR ν_{max} 1651 (ν C=N), 1556 (ν C=C), 1527 (ν C=C), 1344 (ν_{as} SO_2), 1190 (ν_s SO_2), 1162 (ν C–O–C), 934 (ν O–S), 760 (δ_o C_{sp2}–H) cm^{-1};^1H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H, CH_3), 5.44 (s, 2H, CH_2), 6.42 (s, 1H, CH=), 7.39 (t, J = 7.6 Hz, 1H, benzene), 7.47 (t, J = 7.4 Hz, 1H, benzene), 7.62 (d, J = 7.6 Hz, 1H, benzene), 7.72 (d, J = 8.0 Hz, 1H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 20.34 (*C*H_3), 69.45 (*C*H_2), 95.91 (*C*H=), 124.19 (*C*-benzene), 128.56 (*C*H-benzene), 131.71 (*C*H-benzene), 132.16 (*C*H-benzene), 133.30 (*C*H-benzene), 133.64 (*C*-benzene), 169.86 (CH_3*C*=), 170.34 (*C*=N). Anal. Calcd for $C_{11}H_{10}BrNO_4S$: C, 39.77; H, 3.03; N, 4.22. Found: C, 39.98; H, 3.34; N, 4.01.

*5.1.1.9. 3-(3-Bromobenzyl)-6-methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (**9a**).* 3-Bromobenzyl bromide (1.1 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 72 h, poured on ice and extracted with dichloromethane (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:*n*-hexane, 1:1) gave compound **9a** as a white powder (73% yield); mp 71–76 °C; IR ν_{max} 3089 (ν C_{sp2}–H), 1706 (ν C=O), 1387 (ν_{as} SO_2), 1315 (ν C–N), 1193 (ν_s SO_2), 926 (ν O–S), 870 (δ_m C_{sp2}–H), 791 (δ_m C_{sp2}–H), 696 (δ_m C_{sp2}–H) cm^{-1};^1H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H, CH_3), 5.02 (s, 2H, CH_2), 6.35 (s, 1H, CH=), 7.34–7.37 (m, 2H, benzene), 7.53–7.54 (m, 2H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.47 (*C*H_3), 45.18 (*C*H_2), 104.21 (*C*H=), 122.17 (*C*-benzene), 127.32 (*C*H-benzene), 131.04 (*C*H-benzene), 131.32 (*C*H-benzene), 131.42 (*C*H-benzene), 138.38 (*C*-benzene), 160.36 (CH_3*C*=), 163.59 (*C*=O). Anal. Calcd for $C_{11}H_{10}BrNO_4S$: C, 39.77; H, 3.03; N, 4.22. Found: C, 39.53; H, 3.27; N, 4.44.

*5.1.1.10. 3-(2-Fluorobenzyl)-6-methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (**10a**).* 2-Fluorobenzyl chloride (1.1 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 72 h and poured on ice. The resulting suspension was filtered and washed with water and petroleum ether to give compound **10a** as an orange powder (81% yield); mp 44–45 °C; IR ν_{max} 3097 (ν C_{sp2}–H), 1703 (ν C=O), 1494 (ν C=C), 1395 (ν_{as} SO_2), 1299 (ν C–N), 1192 (ν_s SO_2), 1161 (ν C_{sp2}-F), 922 (ν O–S), 756 (δ_o C_{sp2}–H) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6) δ 2.54 (s, 3H, CH_3), 5.34 (s, 2H, CH_2), 6.61 (s, 1H, CH=), 7.48–7.53 (m, 2H, benzene), 7.63–7.66 (m, 2H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.43 (*C*H_3), 34.58 (*C*H_2), 104.22 (*C*H=), 115.88 (*C*H-benzene), 116.08 (*C*H-benzene), 122.38 (*C*H-benzene), 122.52 (*C*-benzene), 125.05 (*C*H-benzene), 130.71 (*C*-benzene), 160.18 (CH_3*C*=), 163.47 (*C*=O). Anal. Calcd for $C_{11}H_{10}FNO_4S$: C, 48.70; H, 3.72; N, 5.16. Found: C, 48.94; H, 3.55; N, 5.31.

*5.1.1.11. 3-(3-Fluorobenzyl)-6-methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (**11a**) and 4-[(3-fluorobenzyl)oxy]-6-methyl-1,2,3-oxathiazine 2,2-dioxide (**11b**).* 3-Fluorobenzyl chloride (1.1 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 48 h, poured on ice and extracted with dichloromethane (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:3) gave compounds **11a** and **11b**.

11a: yellow oil (41% yield); IR ν_{max} 1703 (ν C=O), 1593 (ν C=C), 1402 (ν_{as} SO_2), 1265 (ν C–N), 1198 (ν_s SO_2), 1167 (ν C_{sp2}-F), 928 (ν O–S), 800 (δ_m C_{sp2}–H), 732 (δ_m C_{sp2}–H), 703 (δ_m C_{sp2}–H) cm^{-1};^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 3H, CH_3), 5.00 (s, 2H, CH_2), 5.87 (s, 1H, CH=), 7.04 (t, J = 8.4 Hz, 1H, benzene), 7.15 (d, J = 9.2 Hz, 1H, benzene), 7.22 (d, J = 7.6 Hz, 1H, benzene), 7.31–7.36 (m, 1H, benzene); ^{13}C NMR (101 MHz, $CDCl_3$) δ 19.76 (*C*H_3), 45.63 (*C*H_2), 104.52 (*C*H=), 115.39 (*C*-benzene), 115.60 (*C*H-benzene), 115.83 (*C*H-benzene), 124.35 (*C*H-benzene), 130.27 (*C*H-benzene), 130.36 (*C*-benzene), 160.09 (CH_3*C*=), 162.01 (*C*=O). Anal. Calcd for $C_{11}H_{10}FNO_4S$: C, 48.70; H, 3.72; N, 5.16. Found: C, 48.91; H, 3.43; N, 5.42.

11b: light yellow powder (29% yield); mp 61–63 °C; IR ν_{max} 3088 (ν C_{sp2}–H), 1651 (ν C=N), 1542 (ν C=C), 1441 (ν C=C), 1351 (ν_{as} SO_2), 1263 (ν C_{sp2}-F), 1193 (ν_s SO_2), 1179 (ν C–O–C), 918 (ν O–S), 868 (δ_m C_{sp2}–H), 785 (δ_m C_{sp2}–H), 690 (δ_m C_{sp2}–H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 6.41 (s, 1H, CH=), 7.24 (t, J = 8.4 Hz, 1H, benzene), 7.34 (t, J = 9.2 Hz, 2H, benzene), 7.45–7.49 (m, 1H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 20.31 (*C*H_3), 69.74 (*C*H_2), 71.86 (*C*H=), 96.09 (*C*H-benzene), 116.03 (*C*-benzene), 125.21 (*C*H-benzene), 131.16 (*C*H-benzene), 137.30 (*C*H-benzene), 162.51 (d, J = 244.82 Hz, *C*-benzene), 169.89 (CH_3*C*=), 170.06 (*C*=N). Anal. Calcd for $C_{11}H_{10}FNO_4S$: C, 48.70; H, 3.72; N, 5.16. Found: C, 48.51; H, 3.99; N, 4.92.

*5.1.1.12. 3-(2,6-Difluorobenzyl)-6-methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (**12a**).* 2,6-Difluorobenzyl chloride (1.1 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 48 h and poured on ice. The resulting suspension was filtered and washed with water and *n*-hexane to give compound **12a** as a white powder (78% yield); mp 65–66 °C; IR ν_{max} 3098 (ν C_{sp2}–H), 1716 (ν C=O), 1470 (ν C=C), 1386 (ν_{as} SO_2), 1316 (ν C–N), 1193 (ν_s SO_2), 1068 (ν C_{sp2}-F), 990 (ν C_{sp2}-F), 918 (ν O–S), 808 (δ_o C_{sp2}–H), 785 (δ_o C_{sp2}–H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.25 (s, 3H, CH_3), 5.10 (s, 2H, CH_2), 6.31 (s, 1H, CH=), 7.12 (t, J = 8.0 Hz, 2H, benzene), 7.45–7.49 (m, 1H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.37 (*C*H_3), 34.59 (*C*H_2), 104.19 (*C*H=), 111.21 (*C*H-benzene), 112.28 (2× *C*H-benzene), 131.70 (*C*-benzene), 159.81 (CH_3*C*=), 161.39 (d, J = 258.36 Hz, 2× *C*-benzene), 163.36 (*C*=O). Anal. Calcd for $C_{11}H_9F_2NO_4S$: C, 45.68; H, 3.14; N, 4.84. Found: C, 45.44; H, 3.32; N, 5.02.

*5.1.1.13. 4-[(6-Methyl-2,2-dioxido-4-oxo-1,2,3-oxathiazin-3(4H)-yl) methyl]benzonitrile (**13a**).* 4-(Bromomethyl)benzonitrile (1.1 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 72 h, poured on ice and extracted with chloroform (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:2) gave compound **13a** as a white powder (82% yield); mp 85–87 °C; IR ν_{max} 3092 (ν C_{sp2}–H), 2235 (ν C≡N), 1694 (ν C=O), 1395 (ν_{as} SO_2), 1311 (ν C–N), 1199 (ν_s SO_2), 926 (ν O–S), 796 (δ_p C_{sp2}–H) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (s, 3H, CH_3), 5.12 (s, 2H, CH_2), 6.36 (s, 1H, CH=), 7.51 (d, J = 7.6 Hz, 2H, benzene), 7.86 (d, J = 7.6 Hz, 2H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.48 (*C*H_3), 45.39 (*C*H_2), 104.16 (*C*H=), 111.27 (*C*-benzene), 119.00 (*C*-benzene), 128.92 (2× *C*H-benzene), 133.08 (2× *C*H-benzene), 141.25 (*C*N), 160.32 (CH_3*C*=), 163.69 (*C*=O). Anal. Calcd for $C_{12}H_{10}N_2O_4S$: C, 51.79; H, 3.62; N, 10.07. Found: C, 51.50; H, 3.36; N, 9.84.

*5.1.1.14. 6-Methyl-3-(2-oxo-2-phenylethyl)-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (**14a**) and 2-[(6-methyl-2,2-dioxido-1,2,3-oxathiazin-4-yl)oxy]-1-phenylethanone (**14b**).* 2-Bromoacetophenone (1.1 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 24 h, poured on ice and extracted with chloroform (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel (ethyl acetate:petroleum ether, 1:2) gave compounds **14a** and **14b**.

14a: light yellow powder (63% yield); mp 109–113 °C; IR ν_{max} 3098 (ν C_{sp2}–H), 1709 (ν C=O), 1693 (ν C=O), 1396 (ν_{as} SO_2), 1318 (ν C–N), 1193 (ν_s SO_2), 920 (ν O–S), 751 (δ C_{sp2}–H), 685 (δ C_{sp2}–H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.33 (s, 3H, CH_3), 5.50 (s, 2H, CH_2), 6.37 (s, 1H, CH=), 7.59 (t, J = 7.6 Hz, 2H, benzene), 7.73 (t, J = 7.2 Hz, 1H, benzene), 8.07 (d, J = 7.6 Hz, 2H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.55 (*C*H_3), 49.64 (*C*H_2), 104.19 (*C*H=), 128.74 (2× *C*H-benzene), 129.44 (2× *C*H-benzene), 134.17 (*C*H-benzene), 134.83 (*C*-benzene), 160.17 (CH_3*C*=), 163.68 (*C*=O), 191.43 (*C*=O). Anal. Calcd for $C_{12}H_{11}NO_5S$: C, 51.24; H, 3.94; N, 4.98. Found: C, 51.02; H, 3.69; N, 4.72.

14b: yellow powder (26% yield); mp 132–134 °C; IR ν_{max} 3118 (ν C_{sp2}–H), 1701 (ν C=O), 1655 (ν C=N), 1560 (ν C=C), 1449 (ν C=C), 1374 (ν_{as} SO_2), 1229 (ν C–O–C), 1193 (ν_s SO_2), 963 (δ C_{sp2}–H), 921 (ν O–S), 738 (δ C_{sp2}–H), 690 (δ C_{sp2}–H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.32 (s, 3H, CH_3), 5.94 (s, 2H, CH_2), 6.55 (s, 1H, CH=), 7.60 (t, J = 7.6 Hz, 2H, benzene), 7.73 (t, J = 7.4 Hz, 1H, benzene), 8.00 (d, J = 7.2 Hz, 2H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 20.36 (*C*H_3), 70.68 (*C*H_2), 95.91 (*C*H=), 128.41 (2× *C*H-benzene), 129.48 (2× *C*H-benzene), 133.85 (*C*H-benzene), 134.79 (*C*-benzene), 170.00 (CH_3*C*=), 170.62 (*C*=N), 191.44 (*C*=O). Anal. Calcd for $C_{12}H_{11}NO_5S$: C, 51.24; H, 3.94; N, 4.98. Found: C, 51.55; H, 4.15; N, 5.17.

*5.1.1.15. 6-Methyl-3-[2-(3-nitrophenyl)-2-oxoethyl]-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide (**15a**).* 2-Bromo-3′-nitroacetophenone (1.1 eq) was added to a stirring solution of potassium acesulfame (1.0 eq) in 10 mL of dry *N*,*N*-dimethylformamide. The reaction mixture was stirred at 80 °C for 24 h, poured on ice and extracted with chloroform (3 × 20 mL). The organics were reunited, dried over sodium sulfate and concentrated *in vacuo*. Purification by column chromatography on silica gel

(ethyl acetate:petroleum ether, 1:2) gave compound **15a** as a light yellow powder (87% yield); mp 145–147 °C; IR ν_{max} 3088 (ν C_{sp2}–H), 1709 (ν C=O), 1693 (ν C=O), 1526 (ν_{as} N–O), 1348 (ν_s N–O), 1320 (ν_{as} SO_2), 1204 (ν_s SO_2), 927 (ν O–S), 821 (δ_m C_{sp2}–H), 792 (δ_m C_{sp2}–H), 670 (δ_m C_{sp2}–H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.33 (s, 3H, CH_3), 5.65 (s, 2H, CH_2), 6.39 (s, 1H, CH=), 7.89–7.91 (m, 1H, benzene), 8.48–8.56 (m, 2H, benzene), 8.77 (s, 1H, benzene); ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.84 (CH_3), 49.73 (CH_2), 104.35 (*C*H=), 123.22 (*C*H-benzene), 129.61 (*C*H-benzene), 131.79 (*C*H-benzene), 135.71 (*C*H-benzene), 148.45 (*C*-benzene), 160.20 (*C*-benzene), 164.12 (CH_3*C*=), 184.95 (*C*=O), 190.82 (*C*=O). Anal. Calcd for $C_{12}H_{10}N_2O_7S$: C, 44.17; H, 3.09; N, 8.59. Found: C, 44.42; H, 3.28; N, 8.84.

5.2. CA inhibition assay

An SX.18MV-R Applied Photophysics (Oxford, UK) stopped-flow instrument has been used to assay the catalytic/inhibition of various CA isozymes as reported by Khalifah [26]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nM, with 10 mM Hepes (pH 7.4) as buffer, 0.1 M Na_2SO_4 or $NaClO_4$ (for maintaining constant the ionic strength; these anions are not inhibitory in the used concentration), following the CA-catalyzed CO_2 hydration reaction for a period of 5–10 s. Saturated CO_2 solutions in water at 25 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 10 mM (in DMSO-water 1:1, v/v) and dilutions up to 0.01 nM done with the assay buffer mentioned above. At least 7 different inhibitor concentrations have been used for measuring the inhibition constant. Inhibitor and enzyme solutions were pre-incubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E–I complex. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. The inhibition constants were obtained by non-linear least squares methods using PRISM 3, from Lineweaver burk plots as reported earlier [27,28] and represent means from at least three different determinations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.014.

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