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Design, synthesis and evaluation of *N*-substituted saccharin derivatives as selective inhibitors of tumor-associated carbonic anhydrase XII

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ABSTRACT

A series of *N*-alkylated saccharin derivatives were synthesized and tested for the inhibition of four different isoforms of human carbonic anhydrase (CA, EC 4. 2.1.1): the transmembrane tumor-associated CA IX and XII, and the cytosolic CA I and II. Most of the reported derivatives inhibited CA XII in the nanomolar/low micromolar range, hCA IX with K_Is ranging between 11 and 390 nM, whereas they were inactive against both CA I (K_Is >50 μM) and II (K_Is ranging between 39.1 nM and 50 μM). Since CA I and II are off-targets of antitumor carbonic anhydrase inhibitors (CAIs), the obtained results represent an encouraging achievement for the development of new anticancer candidates without the common side effects of non-selective CAIs. Moreover, the lack of an explicit zinc binding function on these inhibitors opens the way towards the exploration of novel mechanisms of inhibition that could explain the high selectivity of these compounds for the inhibition of the transmembrane, tumor-associated isoforms over the cytosolic ones.

1. Introduction

Solid tumors are often surrounded by a dynamic microenvironment characterized by acidic pH and low levels of oxygen, glucose and other nutrients.[1] In order to survive these harsh conditions cancer cells acquire various adaptive features which have been proposed to be responsible for the development of invasive and metastatic phenotypes.[2–4] In fact, we know that cancer is not only a genetic disorder but also a disease of dysregulated metabolism. As a direct result of regional hypoxia, cancer cells fulfill their need for high levels of ATP by switching their metabolism from aerobic respiration to fermentative glycolysis, a phenomenon also known as Warburg effect.[5,6]

Metabolic reprogramming represents one of the hallmarks of pre-malignant lesions and it is the cause of a pH dysregulation which coupled with poor vascularization determines the formation of a cytostatic and/or lethal microenvironment.[7] In general, cancer cells try to compensate these changes in internal pH by enhancing the expression of a series of proteins and membrane transporters such as H^+-ATPases, the Na^+-H^+ exchanger NHE1, the monocarboxylate-H^+ efflux co-transporters MCT1 and MCT4, and carbonic anhydrases (CA) IX and XII.[8–10] Therefore, targeting these pH modulating proteins is an effective approach to treat recurrent, metastatic and drug resistant tumors.[9–13]

CA IX and XII are overexpressed in multiple primary and metastatic cancer cell-lines and nowadays represent well-established targets both for tumor imaging, as diagnostic markers, as well as for the treatment of tumors expressing them.[14–19] As a result, a considerable amount of literature has been produced in the last few years in the search for effective, potent and selective inhibitors of the fifteen reported isoforms of carbonic anhydrase,[20–24] with particular attention to the cancer related ones, CA IX and XII.[25,26] However, the differences between the active sites of different CA isoforms are minimal, subtle and this often results in the inhibition of both target and off-target isoforms of CA.[23,26,27]

Although a wide variety of CAIs has been described in the past,[22,28,29] one of the most common approaches to design small molecules targeting this family of metalloenzymes consists of inserting zinc binding moieties into the structure of the inhibitor:

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0968-0896/

the sulfonamide group is one of the most important and widely used moieties.[22,30–33] In all of the crystallographic/molecular modelling studies reported to date the primary sulfonamide derivatives have been shown to bind to the catalytic zinc ion in the active site of CA in their deprotonated form.[34,35] However, it has recently been demonstrated that even secondary/tertiary sulfonamides are able to selectively inhibit the cancer related isoforms of CA IX and XII, suggesting a different mechanism of action compared to the classical, primary sulfonamides.[36,37]

Herein we report the synthesis of a new series of tertiary sulfonamides obtained by functionalizing the sulfonamide nitrogen of saccharin, a renowned sweetener characterized by a really safe toxicological profile and a peculiar ability of inhibiting carbonic anhydrase isoforms in the high nanomolar/low micromolar range.[38,39]

2. Chemistry

Compounds **1**–**20** and **11a** were synthesized according to the general procedure reported in Scheme 1. Saccharin (1.0 equiv) was deprotonated using freshly grinded anhydrous potassium carbonate and the corresponding salt was then reacted with an electrophile (2–4 equiv) by stirring the reaction mixture in *N*,*N*-dimethylformamide at 80 °C overnight. Compound **21** was synthesized via a 1-3 dipolar cycloaddition of **2** to the dipole originated by the reaction of **27** with triethylamine in ethyl acetate. Compound **27** was obtained via a two-step synthetic route: commercially available 4-nitrobenzaldehyde was reacted with hydroxylamine hydrochloride and triethylamine to give the corresponding 4-nitrobenzaldoxime **26** in a 1:10 *cis*:*trans* mixture.[40] Compound **26** underwent a chlorination reaction using *N*-chlorosuccinimide and catalytic hydrochloric acid in *N*,*N*-dimethylformamide to give **27** in good yield.[41] Compound **22** was synthesized by reacting **5** with hydroxylamine hydrochloride and triethylamine in methanol at 80 °C to give the corresponding oxime in a 1:4 *cis*:*trans* mixture. Compound **23** was obtained by refluxing derivative **6** in concentrated HCl. The reaction of **23** with methylamine in the presence of carbonyl diimidazole (CDI) in *N*,*N*-dimethylformamide at room temperature gave **24** in moderate yield. Opening of the tensioned lactam ring of compound **8** using sodium borohydride (12 equiv) in methanol gave the corresponding secondary sulfonamide **25**. All synthesized compounds have been fully characterized by analytical and spectral data (see Section 5.2 for details).

Compounds **PH010918**, **S763217** and **S780197** were purchased by Sigma–Aldrich® Italy and used in the biological assays without further characterization and/or purification.

3. Results and discussion

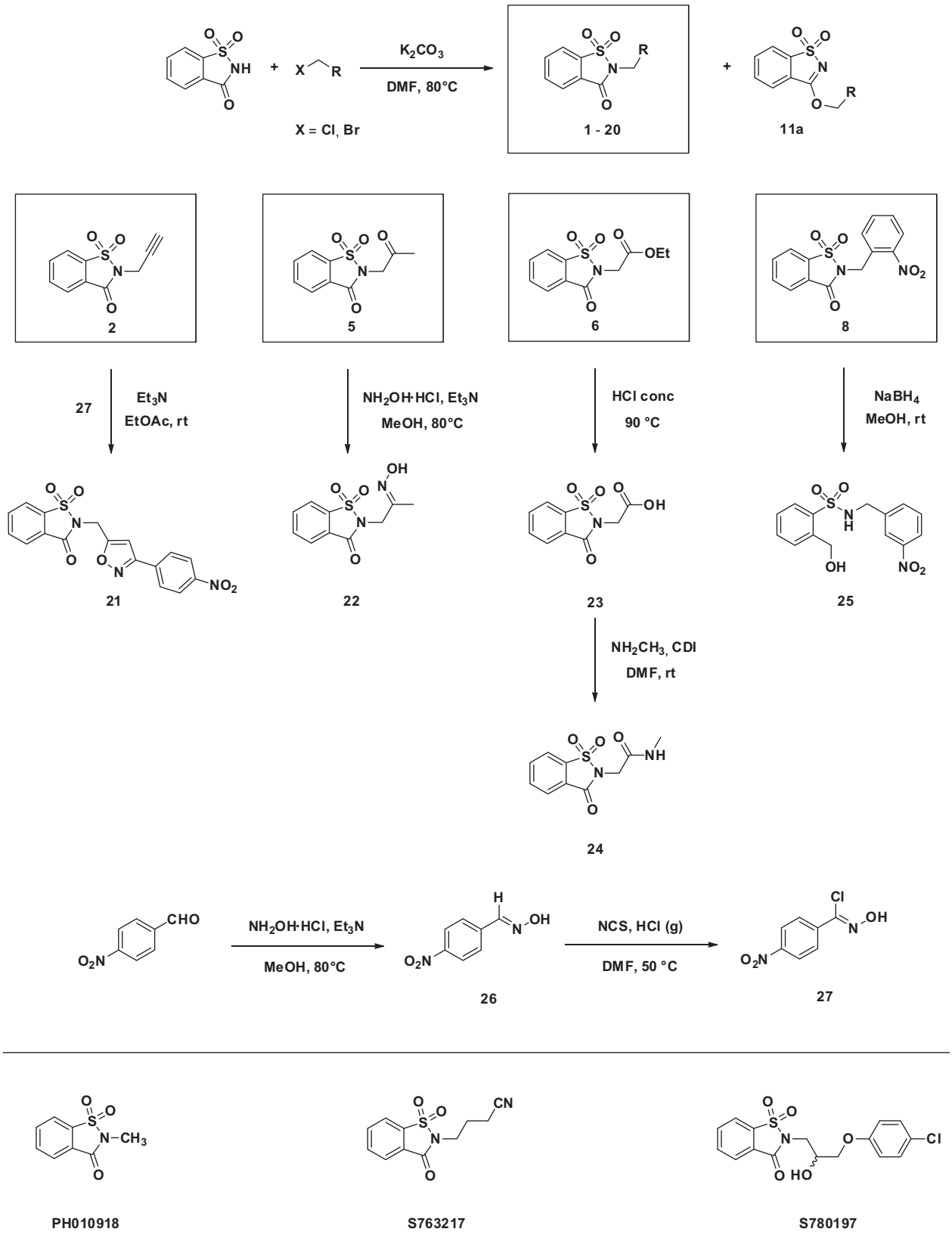
All the synthesized (**1**–**25** and **11a**) and purchased (**PH010918**, **S763217** and **S780197**) compounds were tested in order to evaluate their biological activity against the cancer related isoforms of carbonic anhydrase CA IX and XII, as well as against their corresponding off-targets CA I and II (Tables 1 and 2).

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity. Phenol red (0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5, for α-CAs) as buffer, and 20 mM $NaClO_4$ (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. For the determination of the kinetic parameters and inhibition constants, the CO_2 concentrations ranged from 1.7 to 17 mM. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (1 μM) were prepared in distilled–deionized water and dilutions up to 0.1 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex or for the eventual active site mediated hydrolysis of the inhibitor. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng–Prusoff equation,[42] and represent the mean from at least three different determinations. All recombinant CA isoforms were obtained in-house as previously reported.

An exhaustive SAR for this class of putative CAIs can be defined as follows:

(i) All of the tested compounds proved to be inactive (K_I >50 μM) against hCA I, one of the most common off-target isoforms of anticancer CAIs. In fact, hCA I is ubiquitously expressed in the body, and can be found in high concentrations in the blood and in the gastro-intestinal tract. hCA II was also poorly inhibited (K_I >50 μM) by most of the compounds reported here, such as **1**, **12**, **15**–**21**, **23** and **25**, whereas some of them were nanomolar inhibitors (**2**–**5**, **7**–**11**, **13**, **22** and **24** showed inhibition constants in the range of 36.7–158 nM). Few other derivatives were micromolar hCA II inhibitors (**6**, **14**, **PH010918**, **S763217** and **S780197** showed K_Is in the range of 0.46–2.6 μM).

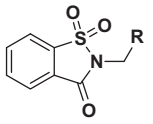
(ii) All the investigated compounds showed to inhibit hCA XII in the low micromolar/nanomolar range, with the only exception of compounds **11a**, **PH010918** and **S763217** which were not active at concentrations higher than 50 μM. The introduction of small unsaturated carbon chains on the sulfonamidic nitrogen of saccharin determined an increase of activity of the obtained compounds against CA XII (see compounds **2**–**4** compared to saccharin in Table 1), although no selectivity was obtained against the off target isoform CAII. In particular, compound **4** showed to be more active against CA II (K_I = 36.7 nM) than against the cancer related isoforms CA IX and XII (K_I = 97 nM and 0.21 μM, respectively). When the unsaturated substituent was a cyano group (compound **1**) the obtained derivative showed a slight loss of activity against CA IX and XII (K_I = 0.29 μM and 0.71 μM, respectively), together with a promising gain of selectivity (K_I >50 μM towards CA I and II). However, the introduction of a 3 methylene long spacer between the sulfonamidic nucleus of saccharin and the cyano group (**PH010918**) caused a dramatic loss of activity against CA XII (K_I >50 μM) and selectivity against CA II (K_I = 1.26 μM). Saccharin derivatization at the sulfonamidic nitrogen with various electron poor benzyl substituents gave derivatives **7**–**15**. Among these compounds, those in which the aromatic ring was substituted with an *ortho*/*meta* nitro group (compounds **8**–**9**) or bromine (compounds **10**–**11**) displayed the highest activity against CA XII (21.1 nM < K_I < 28.1 nM) despite their low selectivity (39.1 nM < K_I < 86.8 nM towards CA II). Moreover, compounds **8** and **9** showed to be good inhibitors even of the other cancer related isoform CA IX (K_I = 91 nM and 11 nM, respectively). On the other hand, the exchange of bromine with fluorine or chlorine on the benzyl ring determined a significative loss of activity of the obtained compounds against both CA IX and CA XII (see compounds **12**–**15**). The use of bulky naphthyl or phthalyl substituents had a similar outcome (see compounds **16** and **17**), although these compounds showed a promising selectivity against CA XII with reference to all of the other tested isoforms: CA I, II and IX (K_I >50 μM), whereas compound **15** inhibited both CA IX and XII. A similar behavior



Scheme 1. Synthesis of compounds **1**–**27**.

was observed by introducing an extra carbonyl between the methylene and the aromatic ring of the benzyl group. Benzoyl derivatives **18**–**20** showed to inhibit CA XII only in the low micromolar range (K_I = 1.78 μM, 0.97 μM and 2.01 μM, respectively), and they were not active against CA I, II and IX at concentrations higher than 50 μM. Analogous results were obtained for the cycloaddition product **21** (K_I CA XII = 0.24 μM, K_ICA IX = 0.31 μM, K_I CA I/II >50 μM), which

Table 1
Inhibitory activity of derivatives **1**–**25**, **PH010918**, **S763217**, and **S780197** against selected hCA isoforms by a stopped-flow CO_2 hydrase assay



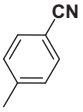
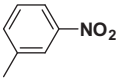
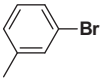
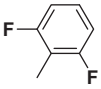
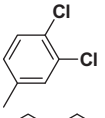
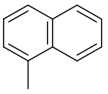
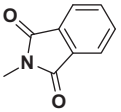
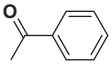
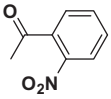
Compound	R	K_I (nM)			
		hCA I	hCA II	hCA IX	hCA XII
1		>50,000	>50,000	290	710
2		>50,000	158	210	570
3		>50,000	86	140	94
4		>50,000	36.7	97	210
5		>50,000	42	150	440
6		>50,000	2210	330	76.5
7		>50,000	47.6	240	150
8		>50,000	40.9	91	28.1
9		>50,000	39.1	11	24.6
10		>50,000	43.5	>50,000	25.1
11		>50,000	86.8	360	21.1
12		>50,000	>50,000	>50,000	2520
13		>50,000	91.0	380	2690
14		>50,000	460	>50,000	1310
15		>50,000	>50,000	390	2760
16		>50,000	>50,000	>50,000	2540
17		>50,000	>50,000	>50,000	13,410
18		>50,000	>50,000	>50,000	1780
19		>50,000	>50,000	>50,000	970

Table 1 (*continued*)

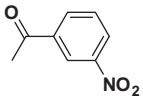
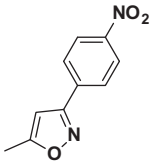
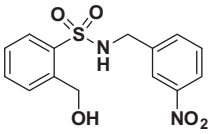
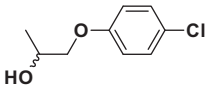
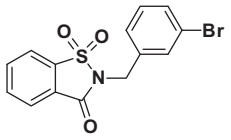
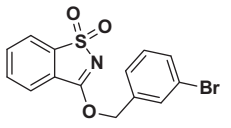
Compound	R	K_I (nM)			
		hCA I	hCA II	hCA IX	hCA XII
20		>50,000	>50,000	>50,000	2010
21		>50,000	>50,000	310	240
22		>50,000	41.4	270	240
23		>50,000	>50,000	310	41.9
24		>50,000	38.1	200	28.4
25		>50,000	>50,000	>50,000	250
PH010918	H	>50,000	2600	400	>50,000
S763217		>50,000	1260	290	>50,000
S780197		>50,000	1,800	230	2910
	Saccharin	18,540	5950	103	633
	AAZ (acetazolamide)	250	12	25	6

Table 2
Inhibitory activity of derivatives **11** (*N*-alkylated saccharin) and **11a** (*O*-alkylated saccharin) against selected hCA isoforms by a stopped-flow CO_2 hydrase assay

Compound		K_I (nM)			
		hCA I	hCA II	hCA IX	hCA XII
11		>50,000	86.8	360	21.1
11a		>50,000	>50,000	>50,000	>50,000

showed to be a selective inhibitor of the cancer related isoforms of CA. However, the best results of activity and selectivity were obtained when saccharin was derivatized using a carboxylic moiety: ethyl ester **6** and its hydrolyzed product **23** showed to inhibit CA XII at nanomolar concentrations (K_I = 76.5 nM and 41.9 nM, respectively), with derivative **23** being the most selective compound of the entire series (ratio K_I CA I-II/K_I CA XII >1193). Because of the well-known hydrolytic activity of some CA isoenzymes, **6** could be considered a prodrug, which might be converted into **23** inside the active site, thus resulting in enzyme inhibition. However, the conversion of **23** into its corresponding *N*-methylamide **24** did not cause any loss of activity against CA XII (K_I = 28.4 nM), although it determined a significative loss of selectivity against CA II: ratio K_I CA II/K_I CA XII = 1.34). On the contrary, the exchange of the carboxylic moiety with a carbonyl/oxime caused a switch of selectivity of compounds **5** and **22** towards CA II.

(iii) hCA IX was also inhibited by some of the saccharin derivatives reported here. Inactive compounds were **10**, **12**, **14**, **16**–**20** and **25** (K_I >50 μM). Derivative **9** was an effective hCA IX inhibitor, with an inhibition constant of 11.0 nM (better than acetazolamide), whereas the remaining compounds were medium potency inhibitors, with inhibition

constants ranging between 91 and 400 nM. Small modifications in the structures of these inhibitors led to drastic changes in their hCA IX inhibitory activity: for example, nitro-substituted regioisomers **8** and **9** differ in their affinity for hCA IX by a factor of 8.3. The same behaviour was observed for the pair of regioisomers **10** and **11** (incorporating bromine on the phenyl ring) or **12** and **13** (incorporating fluorine), although in these cases the *ortho*-substituted derivatives were totally inactive and the *meta*-substituted ones showed moderate hCA IX inhibition (Table 1).

(iv) Lastly, a consideration must be done regarding the importance of *N*-substitution of saccharin against *O*-substitution in order to obtain potent and selective CAIs. Alkylation of saccharin under basic conditions is known to happen both at the nitrogen and at the oxygen of the lactam ring, giving a mixture of products.[43] However, the products of the reported saccharin derivatization were mostly only *N*-alkylated derivatives, which are believed to be the thermodynamic products of the reaction.[44] Only in one case (compounds **11** and **11a**) both products were isolated and tested against the four different isoforms of CAs reported before (Table 2). It is clear from the biological results that only *N*-alkylated compounds were able to inhibit CA.

4. Conclusions

In conclusion, a new series of heterogeneous saccharin derivatives were synthesized and tested against four human CAs: the two cancer-related isoforms CA IX and XII, and the most common off-targets of antitumoral CAIs, CA I and II. All investigated compounds showed no affinity for CA I, while they were all able to inhibit CA XII in the low micromolar/nanomolar range. Few of the new compounds were not active as CA I and II inhibitors, showing effective inhibition of CA XII. These compounds represent a promising start for the development of new selective inhibitors of this less investigated isoform of CA as novel anticancer agents based on the toxicologically safe scaffold of saccharin. However, further studies will be needed to determine whether these compounds directly bind to the active site of the enzyme, which is highly conserved amongst the different isozymes, or outside the active site, since they have lost the zinc binding moiety. An in-depth study of the mechanism of binding will give more information about the origin of the high selectivity displayed by this new series of inhibitors. However, at this moment the inhibition mechanism with this type of compounds is not known. We hypothesize that these compounds may bind within the coumarin binding site at the entrance of the cavity or even further away from the metal ion.

5. Experimental protocols

5.1. General

Solvents were used as supplied without further purification. Where mixtures of solvents are specified, the stated ratios are volume:volume. Unless otherwise indicated, all aqueous solutions used were saturated. 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, 200–425 mesh particle size). Analytical thin-layer chromatography was carried out on Sigma–Aldrich® silica gel on TLA aluminium foils with fluorescent indicator 254 nm. 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). Chemical shifts are quoted in ppm, based on appearance rather than interpretation, and are referenced to the residual non deuterated solvent peak. Infra-red spectra were recorded on a Bruker Tensor 27 FTIR spectrometer equipped with an attenuated total reflectance attachment with internal calibration. Absorbtion 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 ChemBioDraw Ultra® 12.0 following IUPAC conventions.

5.2. Chemistry

General procedure for the synthesis of compounds **1**–**20**: freshly grinded anhydrous potassium carbonate (1.1 equiv) was added to a stirring solution of saccharin (1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. Up to 4.0 equiv of the corresponding electrophile were added portionwise and the reaction stirred at 80 °C for 24–72 h. The reaction was poured on ice and the resulting solid was purified by filtration or column chromatography on silica gel.

5.2.1. 2-(1,1-Dioxido-3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetonitrile (1)

1.66 g of potassium carbonate and 0.18 g of potassium iodide (10 mol %) were added to a stirring solution of saccharin (2.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide. 2.8 mL of chloroacetonitrile (4.0 equiv) were added portionwise and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered to give title compound as a light brown solid (2.0 g, 82% yield); mp 130–134 °C; IR ν_{max} 3104 (ν C_{sp2}-H), 2260 (ν CN), 1744 (ν C=O), 1336 (ν_{as} S=O), 1251 (ν C-N), 1165 (ν_s S=O), 728 (δ C_{sp2}-H), 676 (δ C_{sp2}-H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.11 (2H, s, CH_2), 8.05 (1H, t, J = 7.6 Hz, CH_{Ar}), 8.12 (1H, t, J = 7.6 Hz, CH_{Ar}), 8.19 (1H, d, J = 7.6 Hz, CH_{Ar}), 8.40 (1H, d, J = 7.6 Hz, CH_{Ar}). ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.9 (CH_2), 115.35 (CN), 122.5 (Ar), 126.1 (Ar), 126.2 (Ar), 136.1 (Ar), 136.9 (Ar), 137.2 (Ar), 158.4 (C=O).

5.2.2. 2-(2-Propyn-1-yl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (2)

1.86 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (2.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 5.0 mL of a 80% solution of propargyl bromide in toluene (4.0 equiv) were added portionwise and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the aqueous phase extracted with dichloromethane. The organics were reunited, dried over sodium sulfate and concentrated in vacuo. Column chromatography on silica gel (ethyl acetate–petroleum ether 1:1) gave title compound as a white solid (1.44 g, 60% yield); mp 112–116 °C; IR ν_{max} 3274 (ν C_{sp}-H), 3093 (ν C_{sp2}-H), 1737 (ν C=O), 1332 (ν_{as} S=O), 1259 (ν C-N), 1178 (ν_s S=O), 749 (δ C_{sp2}-H) cm^{-1}; ^{1}H NMR-cis (400 MHz, DMSO-d_6) δ 3.39 (s, 1H, C≡H), 4.57 (s, 2H, CH_2), 7.99 (dt, 1H, J_1 = 7.6 Hz, J_2 = 0.8 Hz, CH_{Ar}), 8.05 (dt, 1H, J_1 = 7.6 Hz, J_2 = 0.8 Hz, CH_{Ar}), 8.11 (d, 1H, J = 7.6 Hz, CH_{Ar}), 8.31 (d, 1H, J = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 27.9 (CH_2), 75.7 (C≡CH), 77.5 (C≡CH), 122.2 (Ar), 125.7 (Ar), 126.4 (Ar), 135.8 (Ar), 136.6 (Ar), 137.4 (Ar), 158.4 (C=O).

5.2.3. 2-Allylbenzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (3)

0.83 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 1.1 mL of allyl bromide (4.0 equiv) were added portionwise and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered to give title compound as a white solid

(0.95 g, 76% yield); mp 73–75 °C; IR ν_{max} 3274 (ν C_{sp}-H), 3094 (ν C_{sp2}-H), 1731 (ν C=O), 1331 (ν_{as} S=O), 1262 (ν C-N), 1180 (νs S=O), 751 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 4.34 (s, 1H CH_2), 5.25 (d, 1H, J = 10.0 Hz, =CH), 5.36 (d, 1H, J = 16.8 Hz, =CH), 5.88–5.95 (m, 1H, CH=), 8.00 (t, 1H, J = 7.6 Hz, CHAr), 8.06 (t, 1H, J = 7.6 Hz, CH_{Ar}), 8.11 (d, 1H, J = 7.6 Hz, CH_{Ar}), 8.32 (d, 1H, J = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 41.0 (CH_2), 119.0 (=CH_2), 122.2 (Ar), 125.6 (Ar), 126.7 (Ar), 131.7 (CH=), 135.7 (Ar), 136.3 (Ar), 137.4 (Ar), 158.7 (C=O).

5.2.4. 2-(2-Buten-1-yl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (4)

0.83 g of anhydrous potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 4-Bromo-2-butene (0.62 mL, 1.1 equiv, 1:5 *cis*/*trans* mixture) was added and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered to give title compound as a white solid (0.51 g, 40% yield, 1:5 *cis*/*trans* mixture); mp 72–75 °C; IR ν_{max} 3098 (ν C_{sp2}-H), 1742 (ν C=O), 1724 (ν C=O), 1324 (ν_{as} S=O), 1265 (ν C-N), 1172 (ν_s S=O), 749 (δ C_{sp2}-H), 674 (δ C_{sp2}-H) cm^{-1}; ^1H NMR-trans (400 MHz, DMSO-d_6) δ 1.66 (d, 3H, J = 6.4 Hz, CH_3), 4.27 (d, 2H, J = 6.0 Hz, CH_2), 5.51-5.59 (m, 1H, CH=), 5.80–5.87 (m, 1H, CH=), 7.99 (t, 1H, J = 7.6 Hz, CH_{Ar}), 8.05 (t, 1H, J = 7.6 Hz, CH_{Ar}), 8.10 (d, 1H, J = 7.6 Hz, CH_{Ar}) 8.30 (d, 1H, J = 7.6 Hz, CH_{Ar}); ^1H NMR-cis (400 MHz, DMSO-d_6) δ 1.76 (d, 0.6H, J = 5.2 Hz, CH_3), 4.37 (d, 0.4H, J = 6.8 Hz, CH_2), 5.71–5.73 (m, 0.2H, CH=), 6.03–6.08 (m, 0.2H, CH=); ^{13}C NMR-trans (101 MHz, DMSO-*d*6) δ 17.8 (CH_3), 122.0 (Ar), 124.4 (CH=), 125.5 (Ar), 126.7 (Ar), 130.7 (CH=), 135.7 (Ar), 136.3 (Ar), 137.4 (Ar), 158.7 (C=O), (CH_2 signal missing due to overlap with DMSO-d_6); ^{13}C NMR-cis (101 MHz, DMSO-d_6) δ 15.0 (CH_3), 123.6 (CH=), 130.5 (CH=).

5.2.5. 2-(2-Oxopropyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (5)

0.83 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 2-Chloroacetone (1.0 mL, 2.2 equiv) was added portionwise and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered to give title compound as a pink solid (1.17 g, 89% yield); mp 118–120 °C; IR ν_{max} 3098 (ν C_{sp2}-H), 1742 (ν C=O), 1724 (ν C=O), 1324 (ν_{as} S=O), 1265 (ν C-N), 1172 (ν_s S=O), 749 (δ C_{sp2}-H), 674 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.24 (s, 3H, CH_3), 4.77 (s, 2H, CH_2), 8.03 (dt, 1H, J_1 = 7.6 Hz, J_2 = 0.8 Hz, CH_{Ar}), 8.09 (dt, 1H, J_1 = 7.6 Hz, J_2 = 0.8 Hz, CH_{Ar}), 8.13 (d, 1H, J = 7.6 Hz, CH_{Ar}), 8.33 (d, 1H, J = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 27.2 (CH_3), 47.2 (CH_2), 122.2 (Ar), 125.6 (Ar), 126.6 (Ar), 135.8 (Ar), 136.4 (Ar), 137.5 (Ar), 159.0 (C=O), 199.7 (C=O).

5.2.6. Ethyl 2-(1,1-dioxido-3-oxobenzo[*d*]isothiazol-2(3*H*)-yl) acetate (6)

0.45 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (0.5 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 0.67 mL of α-bromoethyl acetate (2.0 equiv) were added and the reaction stirred at 80 °C overnight. The reaction was poured on ice and the aqueous phase was extracted with ethyl acetate (3 × 50 mL). The organics were reunited, washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane-ethyl acetate 1:1) to give title compound as a white solid (0.53 g, 70% yield); mp 86–89 °C; ^1H NMR (DMSO-d_6, 400 MHz) δ 1.21 (3H, t, J = 7.2 Hz, CH_3), 4.17 (2H, q, J = 7.2 Hz, CH_2), 4.62 (2H, s, CH_2), 8.04 (1H, t, J = 7.6 Hz, CH_{Ar}), 8.10 (1H, t, J = 7.6 Hz, CH_{Ar}), 8.16 (1H, d, J = 7.6 Hz, CH_{Ar}), 8.37 (1H, d, J = 7.6 Hz, CH_{Ar}).[45]

5.2.7. 2-(4-Cyanobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (7)

0.83 g of anhydrous potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 4-Cyanobenzyl bromide (1.17 g, 1.1 equiv) was added and the reaction stirred at 80 °C for 72 h. The reaction was poured on ice and the resulting suspension was filtered. Purification via column chromatography on silica gel (ethyl acetate–petroleum ether 1:1) gave title compound as a white solid (1.14 g, 71% yield); mp 178–180 °C; IR ν_{max} 3096 (ν C_{sp2}-H), 2228 (ν CN), 1724 (ν C=O), 1334 (ν_{as} S=O), 1255 (ν C-N), 1181 (ν_s S=O), 752 (δ C_{sp2}-H), 673 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5.00 (2H, s, CH_2), 7.62 (d, 2H, J = 8.4 Hz, 2 × CH_{Ar}), 7.85 (d, 2H, J = 8.4 Hz, 2 × CH_{Ar}), 8.02 (dt, 1H, J_1 = 7.6 Hz, J_2 = 0.8 Hz, CH_{Ar}), 8.09 (dt, 1H, J_1 = 7.6 Hz, J_2 = 0.8 Hz, CH_{Ar}), 8.13 (d, 1H, J = 7.6 Hz, CH_{Ar}), 8.36 (d, 1H, J = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 41.6 (CH_2), 111.0 (CN), 119.1 (Ar), 122.2 (Ar), 125.8 (Ar), 126.7 (Ar), 129.0 (Ar), 132.9 (Ar), 135.8 (Ar), 136.4 (Ar), 137.3 (Ar), 141.4 (Ar), 159.1 (C=O).

5.2.8. 2-(2-Nitrobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (8)

0.45 g of potassium carbonate (1.1 equiv) and potassium iodide (0.5 g, 10 mol %) were added to a stirring solution of saccharin (0.5 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 2-Nitrobenzyl chloride (0.57 g, 1.1 equiv) was added and the reaction stirred at 80 °C for 24 h. The reaction was poured on ice and the resulting suspension was filtered. Purification via column chromatography on silica gel (ethyl acetate–*n*-hexane 1:2) gave title compound as a light brown solid (0.75 g, 78% yield); mp 173–178 °C; IR ν_{max} 3086 (ν C_{sp2}-H), 1721 (ν C=O), 1527 (ν_{as} N-O), 1333 (ν_{as} S=O), 1302 (ν_s N-O), 1260 (ν C-N), 1161 (ν_s S=O), 723 (δ C_{sp2}-H), 670 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5.33 (2H, s, CH_2), 7.63 (1H, t, J = 8.24 Hz, CH_{Ar}), 7.64 (1H, d, J = 8.36 Hz, CH_{Ar}), 7.76 (1H, dt, J_1 = 7.78 Hz, J_2 = 1.16 Hz, CH_{Ar}), 8.03 (1H, dt, J_1 = 7.54 Hz, J_2 = 1.16 Hz, CH_{Ar}), 8.09 (1H, dt, J_1 = 7.54 Hz, J_2 = 1.16 Hz, CH_{Ar}), 8.16 (2H, m, 2 × CH_{Ar}), 8.36 (1H, d, J = 7.52 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 122.2 (Ar), 125.6 (Ar), 125.8 (Ar), 126.7 (Ar), 129.8 (Ar), 130.0 (Ar), 130.3 (Ar), 134.7 (Ar), 135.8 (Ar), 136.45 (Ar), 137.3 (Ar), 148.2 (Ar), 159.3 (C=O); (CH_2 signal missing due to overlap with DMSO-d_6).

5.2.9. 2-(3-Nitrobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (9)

0.83 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 20 mL of *N*,*N*-dimethylformamide at room temperature. 3-Nitrobenzyl bromide (1.46 g, 1.1 equiv) was added and the reaction stirred at 80 °C for 72 h. The reaction was poured on ice and the resulting suspension filtered. Purification via column chromatography on silica gel (ethyl acetate–*n*-hexane 1:1) gave title compound as a light orange solid (0.70 g, 37% yield); mp 179–181 °C; IR ν_{max} 3091 (ν C_{sp2}-H), 1736 (ν C=O), 1529 (ν_{as} N-O), 1326 (ν_{as} S=O), 1294 (ν_s N-O), 1263 (ν C-N), 1165 (ν_s S=O), 738 (δ C_{sp2}-H), 672 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) δ 4.83 (2H, s, CH_2), 7.41 (1H, t, J = 8.0 Hz, CH_{Ar}), 7.69 (1H, t, J = 6.8 Hz, CH_{Ar}), 7.71–7.78 (2H, m, 2 × CH_{Ar}), 7.81 (1H, d, J = 7.6 Hz, CH_{Ar}), 7.91 (1H, d, J = 7.2 Hz, CH_{Ar}), 8.01 (1H, d, J = 7.2 Hz, CH_{Ar}), 8.18 (s, 1H, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 41.6 (CH_2), 121.1 (Ar), 123.2 (Ar), 123.4 (Ar), 125.3 (Ar), 127.0 (Ar), 129.8 (Ar), 134.6 (Ar), 134.7 (Ar), 135.3 (Ar), 136.9 (Ar), 137.6 (Ar), 148.1 (Ar), 159.9 (C=O).

5.2.10. 2-(2-Bromobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-ioxide (10)

0.83 g of potassium carbonate (1.1 equiv) was added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 2-Bromobenzyl bromide (1.37 g, 1.0 equiv) was added and the reaction stirred at 80 °C overnight. The reaction was poured on ice and the resulting suspension was filtered to give title compound as a white solid (0.90 g, 46% yield); mp 160–161 °C; IR ν_{max} 1731 (ν C=O), 1335 (ν_{as} S=O), 1260 (ν C-N), 1161 (ν_s S=O), 747 (ν C_{sp2}-Br), 724 (δ $_{csp2}$-H), 674 (δ C_{sp2}-H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.98 (s, 2H, CH_2), 7.30 (d, 1H, J = 7.6 Hz, CH_{Ar}), 7.39 (t, 1H, J = 7.6 Hz, CH_{Ar}), 7.44 (t, 2H, J = 7.6 Hz, CH_{Ar}), 7.68 (d, 1H, J = 7.6 Hz, CH_{Ar}), 8.03 (t, 1H, J = 7.6 Hz, CH_{Ar}), 8.09 (t, 1H, J = 7.6 Hz, CH_{Ar}), 8.16 (d, 1H, J = 7.2 Hz, CH_{Ar}), 8.35 (d, 1H, J = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 42.5 (CH_2), 122.1 (Ar), 122.7 (Ar), 125.8 (Ar), 126.7 (Ar), 128.5 (Ar), 129.7 (Ar), 130.4 (Ar), 133.2 (Ar), 134.0 (Ar), 135.8 (Ar), 136.5 (Ar), 137.3 (Ar), 159.1 (C=O).

5.2.11. 2-(3-Bromobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (11) and 3-((3-bromobenzyl)oxy)benzo[*d*]isothiazole 1,1-dioxide (11a)

0.83 g of potassium carbonate (1.1 equiv) was added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 3-Bromobenzyl bromide (1.5 g, 1.1 equiv) was added and the reaction stirred at 80 °C for 72 h. The reaction was poured on ice and the aqueous phase was extracted with ethyl acetate (3 × 50 mL). The organics were reunited, dried over sodium sulfate, and evaporated in vacuo. Purification via column chromatography on silica gel (ethyl acetate–petroleum ether 2:1) gave compound **11** as a white solid (1.65 g, 86% yield); mp 92–95 °C; IR ν_{max} 2900 (ν C_{sp2}-H), 1721 (ν C=O), 1331 (ν_{as} S=O), 1266 (ν C-N), 1181 (ν C_{sp2}-Br), 678 (δ C_{sp2}-H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.95 (2H, s, CH_2), 7.33 (t, 1H, J = 7.6 Hz, CH_{Ar}), 7.45 (d, 1H, J = 6.8 Hz, CH_{Ar}), 7.50 (d, 1H, J = 6.8 Hz, CH_{Ar}), 7.65 (s, 1H, CH_{Ar}), 8.00 (t, 1H, J = 7.2 Hz, CH_{Ar}), 8.06 (t, 1H, J = 7.2 Hz, CH_{Ar}), 8.11 (d, 1H, J = 7.2 Hz, CH_{Ar}), 8.34 (d, 1H, J = 7.2 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 41.35 (CH_2), 122.1 (Ar), 125.7 (Ar), 126.7 (Ar), 127.4 (Ar), 131.0 (Ar), 131.2 (Ar), 135.8 (Ar), 136.4 (Ar), 137.2 (Ar), 138.4 (Ar), 159.1 (C=O) (two aromatic carbon missing due to signals overlapping); and compound **11a** as a light violet solid (0.007 g; 1% yield); mp 82–84 °C; IR ν_{max} 2927 (ν C_{sp2}-H), 1633 (ν C=N), 1337 (ν_{as} S=O), 1282 (ν C-O), 1151 (ν_s S=O), 1061 (ν C_{sp2}-Br), 678 (δ C_{sp2}-H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (2H, s, CH_2), 7.39–7.41 (m, 1H, CH_{Ar}), 7.44–7.46 (m, 1H, CH_{Ar}), 7.52–7.58 (m, 3H, CH_{Ar}), 7.59–7.60 (m, 1H, CH_{Ar}), 8.03 (s, 1H, CH_{Ar}), 8.11–8.13 (m, 1H, CH_{Ar}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 66.82 (CH_2), 122.5 (Ar), 126.9 (Ar), 128.6 (Ar), 129.2 (Ar), 130.2 (Ar), 130.7 (Ar), 131.2 (Ar), 131.3 (Ar), 131.4 (Ar), 131.7 (Ar), 137.7 (Ar), 140.3 (Ar), 167.7 (C=N).

5.2.12. 2-(2-Fluorobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (12)

0.83 g of potassium carbonate (1.1 equiv) was added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 2-Fluorobenzyl chloride (0.867 g, 1.1 equiv) was added and the reaction stirred at 80 °C for 72 h. The reaction was poured on ice and the resulting suspension was filtered to give title compound as a white solid (0.14 g, 8% yield); mp 90–92 °C; IR ν_{max} 3091 (ν C_{sp2}-H), 1731 (ν C=O), 1334 (ν_{as} S=O), 1262 (ν C-N), 1157 (ν_s S=O), 1177 (ν C-F), 749 (ν C_{sp2}-H), 674 (δ C_{sp2}-H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.98 (2H, s, CH_2), 7.20 (dt, 1H, J_1 = 7.6 Hz, J_2 = 1.2 Hz, CH_{Ar}), 7.24 (m, 1H, J_1 = 10.3 Hz, J_2 = 8.4 Hz, J_3 = 1.2 Hz, CH_{Ar}), 7.49 (m, 1H, CH_{Ar}), 7.47 (dt, 1H, J_1 = 7.6 Hz, J_2 = 1.6 Hz, CH_{Ar}), 8.02 (t, 1H, J = 7.2 Hz, CH_{Ar}), 8.08 (t, 1H, J = 7.6 Hz, CH_{Ar}), 8.14 (d, 1H, J = 7.2 Hz, CH_{Ar}), 8.34 (d, 1H, J = 7.2 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 36.0 (d, J = 4.8 Hz, CH_2), 115.8 (d, J = 21.1 Hz, Ar), 122.1 (Ar), 122.4 (d, J = 14.1 Hz, Ar), 125.1 (d, J = 3.0 Hz, Ar), 125.7 (Ar), 126.6 (Ar), 130.7 (d, J = 14.1 Hz, Ar), 130.8 (d, J = 2.0 Hz, Ar), 135.8 (Ar), 136.4 (Ar), 137.3 (Ar), 158.9 (C=O), 160.6 (d, J = 247.5 Hz, Ar).

5.2.13. 2-(3-Fluorobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (13)

0.83 g of potassium carbonate (1.1 equiv) was added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 3-Fluorobenzyl chloride (0.97 g, 1.1 equiv) was added and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice to give and the resulting suspension was filtered to give title compound as a white solid (0.54 g, 34% yield); mp 105–107 °C; IR ν_{max} 3075 (ν C_{sp2}-H), 1737 (ν C=O), 1330 (ν_{as} S=O), 1265 (ν C-N), 1181 (ν C-F), 754 (δ C_{sp2}-H), 675 (δ C_{sp2}-H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.96 (2H, s, CH_2), 7.15 (t, 1H, J = 7.6 Hz, CH_{Ar}), 7.27 (m, 2H, 2 × CH_{Ar}), 7.41 (q, 1H, J = 6.8 Hz, CH_{Ar}), 8.01 (t, 1H, J = 7.6 Hz, CH_{Ar}), 8.07 (t, 1H, J = 7.2 Hz, CH_{Ar}), 8.13 (d, 1H, J = 7.2 Hz, CH_{Ar}), 8.35 (d, 1H, J = 7.2 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 41.4 (CH_2), 115.1 (d, J = 22.2 Hz, Ar), 115.4 (d, J = 20.2 Hz, Ar), 122.1 (Ar), 124.3 (d, J = 4.0 Hz, Ar), 125.7 (Ar), 126.7 (Ar), 131.0 (d, J = 8.1 Hz, Ar), 135.8 (Ar), 136.4 (Ar), 137.3 (Ar), 138.5 (d, J = 7.1 Hz, Ar), 159.1 (C=O), 162.6 (d, J = 244.4 Hz, Ar).

5.2.14. 2-(2,6-Difluorobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (14)

0.45 g of anhydrous potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (0.5 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 2,6-Difluorobenzyl bromide (0.68 g, 1.1 equiv) was added and the reaction stirred at 80 °C overnight. The reaction was poured on ice and the resulting suspension was filtered to give title compound as a white solid (0.75 g, 81% yield); mp 173–175 °C; IR ν_{max} 1734 (ν C=O), 1339 (ν_{as} S=O), 1295 (ν C_{sp2}-F), 1253 (ν C-N), 1159 (ν_s S=O), 754 (δ C_{sp2}-H), 674 (δ C_{sp2}-H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.01 (2H, s, CH_2), 7.15 (t, 2H, J = 8.0 Hz, 2 × CH_{Ar}), 7.48 (t, 1H, J = 7.2 Hz, CH_{Ar}), 8.00–8.05 (m, 2H, 2 × CH_{Ar}), 8.15 (d, 1H, J = 7.2 Hz, CH_{Ar}), 8.28 (d, 1H, J = 7.2 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 30.3 (m, CH_2), 110.8 (t, J = 19.2 Hz, Ar), 112.1 (d, J = 25.3 Hz, Ar), 121.9 (Ar), 125.8 (Ar), 126.4 (Ar), 131.8 (t, J = 11.1 Hz, Ar), 135.8 (Ar), 136.5 (Ar), 137.2 (Ar), 158.2 (C=O), 161.5 (d, J = 258.4 Hz, Ar).

5.2.15. 2-(3,4-Dichlorobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (15)

0.83 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 3,4-Dichlorobenzyl chloride (0.83 mL, 1.1 equiv) was added and the reaction stirred at 80 °C for 48 h. The reaction was poured on ice and the resulting suspension was filtered to give title compound as a white solid (0.86 g, 46% yield); mp 135–137 °C; IR ν_{max} 3079 (ν C_{sp2}-H), 1727 (ν C=O), 1323 (ν_{as} S=O), 1264 (ν C-N), 1182 (ν_s S=O), 750 (δ C_{sp2}-H), 675 (δ C_{sp2}-H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.97 (2H, s, CH_2), 7.43 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz, CH_{Ar}), 7.63 (d, 1H, J = 8.4 Hz, CH_{Ar}), 7.71 (d, 1H, J = 2.0 Hz, CH_{Ar}), 8.01 (t, 1H, J = 7.6 Hz, CH_{Ar}), 8.07 (t, 1H, J = 7.6 Hz, CH_{Ar}), 8.12 (d, 1H, J = 7.6 Hz, CH_{Ar}), 8.34 (d, 1H, J = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 40.8 (CH_2), 122.1 (Ar), 125.7 (Ar), 126.7 (Ar), 128.7 (Ar), 130.4 (Ar), 131.0 (Ar), 131.2 (Ar), 131.5 (Ar), 135.8 (Ar), 136.4 (Ar), 136.9 (Ar), 137.3 (Ar), 159.1 (C=O).

5.2.16. 2-(Naphthalen-1-ylmethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (16)

0.83 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. 1-(Chloromethyl)naphthalene (0.9 mL, 1.1 equiv) was added and the reaction stirred at 80 °C for 72 h. The reaction was poured on ice. The aqueous phase was extracted with ethyl acetate (3 × 50 mL), the organics were reunited, dried over sodium sulfate and evaporated in vacuo. Purification by column chromatography on silica gel (ethyl acetate–petroleum ether 1:2) gave title compound as a white solid (0.33 g, 19% yield); mp 142–145 °C; IR ν_{max} 3083 (ν C_{sp2}-H), 1725 (ν C=O), 1335 (ν_{as} S=O), 1298 (ν C-N), 1171 (ν_s S=O), 748 (δ C_{sp2}-H), 675 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5.43 (s, 2H, CH_2), 7.49–7.63 (m, 4H, 4 × CH_{Ar}), 7.93 (d, 1H, *J* = 7.6 Hz, CH_{Ar}), 7.99–8.09 (m, 3H, 3 × CH_{Ar}), 8.19 (d, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.22 (d, 1H, *J* = 8.0 Hz, CH_{Ar}), 8.33 (d, 1H, *J* = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 122.1 (Ar), 123.5 (Ar), 128.8 (Ar), 125.9 (Ar), 126.5 (Ar), 126.6 (Ar), 126.7 (Ar), 127.1 (Ar), 129.1 (Ar), 129.2 (Ar), 130.5 (Ar), 130.9 (Ar), 133.7 (Ar), 135.8 (Ar), 136.4 (Ar), 137.5 (Ar), 159.3 (C=O); (CH_2 signal missing due to overlap with DMSO-d_6).

5.2.17. 2-((1,1-Dioxido-3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)methyl)isoindoline-1,3-dione (17)

0.45 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (500 mg, 1.0 equiv) in 20 mL of *N*,*N*-dimethylformamide at room temperature. *N*-(Chloromethyl)phthalimide (0.54 g, 1.0 equiv) was added and the reaction stirred at 80 °C for 72 h. The reaction was poured on ice. The aqueous phase was extracted with ethyl acetate (3 × 50 mL), the organics were reunited, dried over sodium sulfate and evaporated in vacuo. Purification via column chromatography on silica gel (ethyl acetate–*n*-hexane 2:1) gave title compound as a white solid (0.61 mg, 66% yield); mp 284–287 °C; IR ν_{max} 1725 (ν C=O), 1337 (ν_{as} S=O), 1252 (ν C-N), 1166 (ν_s S=O), 727 (δ C_{sp2}-H), 677 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5.66 (2H, s, CH_2), 7.90 (2H, m, 2 × CH_{Ar}), 7.97 (m, 2H, 2 × CH_{Ar}), 8.02 (1H, t, *J* = 7.6 Hz, CH_{Ar}), 8.07 (1H, t, *J* = 7.6 Hz, CH_{Ar}), 8.19 (1H, d, *J* = 7.6 Hz, CH_{Ar}), 8.28 (1H, d, *J* = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 41.8 (CH_2), 122.0 (Ar),124.1 (Ar), 126.0 (Ar), 126.15 (Ar), 131.6 (Ar), 135.6 (Ar), 135.9 (Ar), 136.7 (Ar), 137.1 (Ar), 157.75 (C=O), 166.9 (C=O).

5.2.18. 2-(2-Oxo-2-phenylethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (18)

0.83 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. α-Bromoacetophenone (1.19 g, 1.1 equiv) was added and the reaction stirred at 80 °C overnight. The reaction was poured on ice and the resulting suspension was filtered to give title compound as a yellow solid (1.46 g, 89% yield); mp 192–194 °C; IR ν_{max} 3092 (ν C_{sp2}-H), 1735 (ν C=O), 1698 (ν C=O), 1332 (νas S=O), 1296 (ν C-N), 1180 (ν_s S=O), 744 (δ C_{sp2}-H), 673 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5.48 (2H, s, CH_2), 7.60 (t, 2H, *J* = 7.6 Hz, 2 × CH_{Ar}), 7.74 (t, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.05 (dt, 1H, J_1 = 7.6 Hz, J_2 = 0.8 Hz, CH_{Ar}), 8.09–8.13 (m, 2H, 2 × CH_{Ar}), 8.17 (d, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.36 (d, 1H, *J* = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 45.3 (CH_2), 122.2 (Ar), 125.6 (Ar), 126.7 (Ar), 128.9 (Ar), 129.4 (Ar), 134.3 (Ar), 134.8 (Ar), 135.9 (Ar), 136.5 (Ar), 137.7 (Ar), 159.3 (C=O), 190.6 (C=O).

5.2.19. 2-(2-(2-Nitrophenyl)-2-oxoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (19)

0.83 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. α-Bromo-2-nitroacetophenone (1.46 g, 1.1 equiv) was added and the reaction stirred at 80 °C overnight. The reaction was poured on ice and the resulting suspension was filtered. Purification via column chromatography on silica gel (ethyl acetate–petroleum ether 1:1) gave title compound as an orange solid (0.10 g, 5% yield); mp 182–187 °C; IR ν_{max} 3103 (ν C_{sp2}-H), 1739 (ν C=O), 1728 (ν C=O), 1337 (ν_{as} S=O), 1312 (ν C-N), 1186 (ν_s S=O), 752 (δ C_{sp2}-H), 675 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5.30 (2H, s, CH_2), 7.86 (t, 1H, *J* = 6.8 Hz, CH_{Ar}), 7.91–7.95 (m, 2H, 2 × CH_{Ar}), 8.05 (t, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.11 (t, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.18–8.20 (m, 2H, 2 × CH_{Ar}), 8.38 (d, 1H, *J* = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 46.7 (CH_2), 119.6 (Ar), 122.3 (Ar), 124.9 (Ar), 125.7 (Ar), 126.4 (Ar), 129.4 (Ar), 133.3 (Ar), 134.7 (Ar), 135.9 (Ar), 136.6 (Ar), 137.6 (Ar), 146.7 (Ar), 159.1 (C=O), 193.3 (C=O).

5.2.20. 2-(2-(3-Nitrophenyl)-2-oxoethyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (20)

0.83 g of potassium carbonate (1.1 equiv) were added to a stirring solution of saccharin (1.0 g, 1.0 equiv) in 10 mL of *N*,*N*-dimethylformamide at room temperature. α-Bromo-3-nitroacetophenone (1.46 g, 1.1 equiv) was added and the reaction stirred at 80 °C overnight. The reaction was poured on ice to give and the resulting suspension was filtered. Purification via column chromatography on silica gel (ethyl acetate–petroleum ether 1:1) gave title compound as an orange solid (0.7 g, 37% yield); mp 183–186 °C; IR ν_{max} 3083 (ν C_{sp2}-H), 1731 (ν C=O), 1709 (ν C=O), 1336 (ν_{as} S=O), 1317 (ν C-N), 1181 (ν_s S=O), 754 (δ C_{sp2}-H), 670 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5.30 (2H, s, CH_2), 7.91 (t, 1H, *J* = 8.0 Hz, CH_{Ar}), 8.07 (t, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.11 (t, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.18 (d, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.39 (d, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.53 (d, 1H, *J* = 8.0 Hz, CH_{Ar}), 8.56 (d, 1H, *J* = 8.0 Hz, CH_{Ar}), 8.82 (s, 1H, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 45.5 (CH_2), 122.3 (Ar), 123.4 (Ar), 125.7 (Ar), 126.6 (Ar), 128.9 (Ar), 131.2 (Ar), 135.1 (Ar), 135.4 (Ar), 135.9 (Ar),136.6 (Ar),137.7 (Ar),148.6 (Ar), 159.2 (C=O), 189.9 (C=O).

5.2.21. 2-((3-(4-Nitrophenyl)isoxazol-5-yl)methyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (21)

2-(1-Propyn-3-yl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide **2** (0.5 g, 1.0 equiv) and *p*-nitrohydroxyiminoacyl chloride (0.57 g, 1.25 equiv) were dissolved in 20 mL of ethyl acetate. 1.0 mL of a solution of 0.2 mL of triethylamine (1.25 equiv) in ethyl acetate was added dropwise over 30 min. A white suspension was formed and the reaction stirred overnight at room temperature. The reaction was quenched with 20 mL of water and diluted with 10 mL of dichloromethane. The resulting suspension was filtered to give title compound as a light yellow solid (0.35 g, 40% yield); mp 260–262 °C; IR ν_{max} 3146 (ν C_{sp2}-H), 1721 (ν C=O), 1598 (ν C=N), 1511 (ν N-O), 1334 (ν_{as} S=O), 1303 (ν N-O), 1268 (ν C-N), 1184 (ν_s S=O), 755 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5.24 (s, 2H, CH_2), 7.31 (s, 1H, C(4)H-isoxazole), 8.02 (t, 1H, *J* = 7.2 Hz, CH_{Ar}), 8.08 (t, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.13 (d, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.17 (d, 2H, *J* = 8.0 Hz, 2 × CH_{Ar}), 8.32 (d, 2H, *J* = 8.0 Hz, 2 × CH_{Ar}), 8.36 (d, 1H, *J* = 7.2 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 33.8 (CH_2), 102.8 (C(4)-isoxazole) 122.3 (Ar), 124.8 (2 × Ar), 125.9 (Ar), 126.7 (Ar), 128.4 (2 × Ar), 134.6 (Ar), 135.9 (Ar) 136.6 (Ar), 137.3 (Ar), 148.9 (Ar), 156.1 (C(5)-isoxazole), 158.7 (C=O), 168.3 (C(3)-isoxazole).

5.2.22. 2-(2-(Hydroxyimino)propyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (22)

2-(2-Oxopropyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide **5** (0.5 g, 1.0 equiv) was added to a stirring solution of hydroxylamine hydrochloride (0.22 g, 1.5 equiv) and triethylamine (0.34 mL, 2.2 equiv) in 5.0 mL of anhydrous methanol. The reaction was

stirred overnight at 80 °C. Methanol was removed in vacuo and the residue triturated with ice-cold water to give title compound as a light brown solid (66 mg, 12.5% yield, 1:4 *cis*/*trans* mixture); mp 139–140 °C; IR ν_{max} 3202 (ν O-H), 3069 (ν C_{sp2}-H), 1736 (ν C=O), 1620 (ν C=N) 1328 (ν_{as} S=O), 1250 (ν C-N), 1143 (ν_s S=O), 746 (δ C_{sp2}-H), 677 (δ C_{sp2}-H) cm^{-1}; ^1H NMR-trans (400 MHz,$CDCl_3$) δ 1.97 (s, 3H, CH_3), 4.48 (s, 2H, CH_2), 7.87–7.95 (m, 3H, 3 × CH_{Ar}), 8.67 (d, 1H, *J* = 7.6 Hz, CH_{Ar}); ^1H NMR-trans (400 MHz,$CDCl_3$) δ 1.97 (s, 3H, CH_3), 4.48 (s, 2H, CH_2), 7.87–7.95 (m, 3H, 3 × CH_{Ar}), 8.11 (d, 1H, *J* = 7.6 Hz, CH_{Ar}), 8.67 (br s, 1H, OH); ^1H NMR-cis (400 MHz,$CDCl_3$) δ 1.93 (s, 0.7H, CH_3), 4.48 (s, 0.5H, CH_2); ^{13}C NMR-trans (101 MHz, $CDCl_3$) δ 11.8 (CH_3), 42.2 (CH_2), 121.1 (Ar), 125.5 (Ar), 127.0 (Ar), 134.5 (Ar), 135.1 (Ar), 137.7 (Ar), 152.0 (C=N), 159.0 (C=O); ^{13}C NMR-cis (101 MHz, $CDCl_3$) δ 16.8 (CH_3), 35.8 (CH_2).

5.2.23. 2-(1,1-Dioxido-3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetic acid (23)

Ethyl 2-(1,1-dioxido-3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetate **6** (500 mg, 1.0 equiv) was suspended in 12 mL of concentrated HCl. The reaction mixture was heated up to 90 °C and stirring was carried out overnight. Dilution of the concentrated acid with water gave a white suspension which was filtered to give title compound as a white solid (387 mg, 84% yield); mp 200–203 °C; IR ν_{max} 3393 (ν O-H), 1728 (ν C=O), 1336 (ν_{as} S=O), 1257 (ν C-N), 1190 (ν_s S=O), 747 (δ C_{sp2}-H), 681 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 4.49 (2H, s, CH_2), 8.03 (1H, t, *J* = 7.6 Hz, CH_{Ar}), 8.09 (1H, t, *J* = 7.6 Hz, CH_{Ar}), 8.15 (1H, d, *J* = 7.6 Hz, CH_{Ar}), 8.34 (1H, d, *J* = 7.6 Hz, CH_{Ar}), 13.34 (1H, bs, D_2O exch., COOH); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 32.3 (CH_2), 122.2 (Ar), 125.65 (Ar), 126.5 (Ar), 135.8 (Ar), 136.5 (Ar), 137.5 (Ar), 159.0 (C=O), 167.95 (C=O).

5.2.24. *N*-Methyl-2-(1,1-dioxido-3-oxobenzo[*d*]isothiazol-2(3*H*)-yl) acetylamide (24)

Carbonyl diimidazole (202 mg, 1.5 equiv) and 0.62 mL of a 2 M solution of methylamine in THF (1.5 equiv) were added to a stirring solution of 2-(1,1-dioxido-3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetic acid **23** (200 mg, 1.0 equiv) in 5 mL of anhydrous DMF at room temperature. After 4 h the reaction was diluted with dichloromethane (20 mL) and washed with a saturated solution of sodium bicarbonate. The organics were dried over sodium sulfate and evaporated in vacuo. Purification via column chromatography on silica gel (ethyl acetate–*n*-hexane 2:1) gave title compound as a white solid (87 mg, 41% yield); mp 204–206 °C; IR ν_{max} 3297 (ν N-H), 3094 (ν C_{sp2}-H), 1737 (ν C=O), 1664 (ν C=O), 1333 (ν_{as} S=O), 1256 (ν C-N), 1187 (ν_s S=O), 753 (δ C_{sp2}-H), 672 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.62 (d, 3H, *J* = 4.4 Hz, CH_3), 4.29 (2H, s, CH_2), 8.02 (1H, t, *J* = 7.2 Hz, CH_{Ar}), 8.08 (1H, t, *J* = 7.2 Hz, CH_{Ar}), 8.13 (1H, d, *J* = 7.6 Hz, CH_{Ar}), 8.17 (bd, 1H, *J* = 4.0 Hz, NH), 8.34 (1H, d, *J* = 7.6 Hz, CH_{Ar}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.15 (CH_3), 122.2 (Ar), 125.5 (Ar), 127.1 (Ar), 135.7 (Ar), 136.2 (Ar), 137.3 (Ar), 159.1 (C=O), 165.5 (C=O); (CH_2 signal missing due to overlap with DMSO-d_6).

5.2.25. 2-(Hydroxymethyl)-*N*-(2-nitrobenzyl)benzenesulfonamide (25)

(2-Nitrobenzyl)benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide **8** (0.50 g, 1.0 equiv) was suspended in 20 mL of anhydrous methanol at room temperature. $NaBH_4$ (540 mg, 9.0 equiv) was added portionwise and the reaction monitored by TLC. After 2 h the reaction was quenched with water and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The organics were reunited, dried over sodium sulfate, and concentrated in vacuo to give title compound as a light brown solid (0.19 g, 37% yield); mp 103–105 °C; IR ν_{max} 3498 (ν O-H), 3238 (ν N-H), 2973 (ν C_{sp2}-H), 1522 (ν N-O), 1327 (ν_{as} S=O), 1165 (ν_s S=O), 698 (δ C_{sp2}-H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 4.34 (d, 2H, *J* = 5.6 Hz, CH_2), 4.88 (s, 2H, CH_2), 5.45 (br s, 1H, NH), 7.39 (t, 1H, *J* = 7.2 Hz, CH_{Ar}), 7.50 (t, 1H, *J* = 7.6 Hz, CH_{Ar}), 7.60–7.68 (m, 3H, 3 × CH_{Ar}), 7.74–7.79 (m, 2H, 2 × CH_{Ar}), 7.96 (d, 1H, *J* = 8.4 Hz, CH_{Ar}), 8.29 (br s, 1H, OH); ^{13}C NMR (101 MHz, DMSO-d_6) δ 43.4 (CH_2), 60.0 (CH_2), 125.1 (Ar), 127.3 (Ar), 128.2 (Ar), 128.5 (Ar), 129.1 (Ar), 130.7 (Ar), 133.0 (Ar), 133.4 (Ar), 134.1 (Ar), 137.0 (Ar), 141.6 (Ar), 148.1 (Ar); LRMS (ESI^+) m/z 345.17 $(M+Na)^+$, calcd for $C_{14}H_{14}N_2O_5S$ 345.05.

5.2.26. 4-Nitrobenzaldehyde oxime (26)

In an oven-dried flask hydroxylamine hydrochloride (1.37 g, 1.5 equiv) and triethylamine (2.1 mL, 2.25 equiv) were dissolved in 10 mL of anhydrous methanol and stirred for 5 min at room temperature. 2.0 g of 4-nitrobenzaldehyde (1.0 equiv) were added and the reaction mixture was stirred at 80 °C for 4 h. After complete consumption of starting material, the reaction was cooled down to room temperature and the solvent removed in vacuo. 20 mL of ice cold water were added to the residue and the resulting suspension was kept in an ice bath for 15 min. The obtained solid was filtered and washed with petroleum ether/diethyl ether to give title compound as white solid (2.10 g, 97% yield); mp 99–102 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.83 (d, 2H, *J* = 7.6 Hz, C(2)H and C(6)H), 8.22 (d, 2H, *J* = 7.6 Hz, C(3)H and C(5)H), 8.28 (s, 1H, CH=N), 11.83 (s, 1H, OH).[40]

5.2.27. *N*-Hydroxy-4-nitrobenzimidoyl chloride (27)

In an oven-dried flask 4-nitrobenzaldheyde oxime **26** (1.86 g, 1.0 equiv) was dissolved in 10 mL of anhydrous *N*,*N*-dimethylformamide. 0.3 g of *N*-chlorosuccinimide (0.2 equiv) were added and the reaction stirred for 10 min at room temperature. The solution was purged with 20 mL of HCl_g. The reaction was stirred for 10 additional minutes and then heated up to 50 °C. 1.20 g of *N*-chlorosuccinimide were added portionwise (0.2 equiv at the time) over half an hour. The reaction mixture was stirred at 50 °C overnight. The progression of the reaction was monitored using iodine starch paper. When the iodine starch paper was not turning brown anymore, the reaction was quenched with 4 volumes of ice cold water. The aqueous phase was extracted with diethyl ether (3 × 50 mL), the reunited organics were dried over sodium sulfate and evaporated in vacuo to give title compound as light yellow solid (1.92 g, 85% yield); mp 107–110 °C; IR ν_{max} 3293 (ν O-H), 2868 (ν C_{sp2}-H), 1523 (ν C=N), 1349 (ν N-O), 1240 (ν C-N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.00 (d, 2H, *J* = 8.0 Hz, 2 × CH_{Ar}), 8.26 (d, 2H, *J* = 8.0 Hz, 2 × CH_{Ar}), 12.95 (s, 1H, OH).[41]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.01.056.

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