Discovery of Halogenated Benzothiadiazine Derivatives with Anticancer Activity

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Abstract

Mitochondrial respiratory complex II (CII), also known as succinate dehydrogenase, plays a critical role in mitochondrial metabolism. Known but low potency CII inhibitors are selectively cytotoxic to cancer cells including the benzothiadiazine-based anti-hypoglycemic diazoxide. Herein, we study the structure-activity relationship of benzothiadiazine derivatives for CII inhibition for the first time. A number of more potent derivatives were identified. Cytotoxicity evaluation of the novel derivatives resulted in the identification of compounds with greater anticancer effect than the parent; two benzothiadiazine derivative classes (**24a-d** and **30a, 30c, 30d**) that possess activity to reduce the cell viability of 22Rv1 prostate cancer cells and five novel 7-fluorobenzothiadiazine derivatives which possessed significant cytotoxicity in a cellular model of triple negative breast cancer. No correlation between cytotoxicity and CII inhibition was found, indicating an as yet undefined mechanism of action of this scaffold.

Introduction

Targeting metabolic pathways of cancer has emerged as an appealing strategy for the development of selective antineoplastic agents in drug discovery.[1] This approach may provide a therapeutic advantage that can help overcome drug resistance, enhance the specificity of cancer cell targeting, increase the potency of existing treatments and overcome, or attenuate, adverse effects.[2] Much emerging evidence implicates mitochondrial metabolism as a key driver of tumor growth.[3,4] Mitochondria are crucial not only for ATP production, but also for regulating essential steps of cell apoptosis and reactive oxygen species (ROS) generation;[5,6] contributing to many processes within the cell, most notably in electron transport-linked phosphorylation, central carbon metabolism (CCM), and the biosynthesis of intermediates for cell growth.[4] Mitochondria participate in nearly all aspects of cell function including growth (including aberrant cancerous growth), inflammation, metabolic signaling, cell death, and transformation.[4] Furthermore, cancer cells possess a more hyperpolarized mitochondrial membrane potential than non-cancerous cells,[7,8] with increasing hyperpolarization directly corresponding to more invasive and aggressive cancers.[9] Adenosine triphosphate (ATP) synthesis is crucial to the survival of all cells; however, as cancerous cells are continually dividing, they require more energy than non-cancerous cells.[10] These two facets of cancer cell mitochondria provide for the possibility of selectively targeting mitochondria in cancer cells over healthy cells. Hence, modulation of mitochondrial oxidative metabolism is gaining increased attention as a target for anticancer drug discovery.[11]

Mitochondrial respiratory complex II (CII), or succinate dehydrogenase (SDH), is a well-characterized 124 kDa protein complex located to the inner membrane of mitochondria **(Figure 1)**.[12,13] Recently, it has attracted considerable attention as a therapeutic target.[14] The protein plays a vital role in mitochondrial metabolism, where it catalyzes the oxidation of succinate to fumarate and the reduction of ubiquinone (UQ) to ubiquinol (UQH_2).[5] Mitochondrial complex II connects the tricarboxylic acid cycle (TCA) and the electron transport chain (ETC), while lacking any contribution to maintaining the proton gradient across the mitochondrial inner membrane in comparison to other complexes.[14] In some cell types, inhibition of the ETC has been shown to induce apoptosis, via mechanisms that may include the generation of ROS.[15]

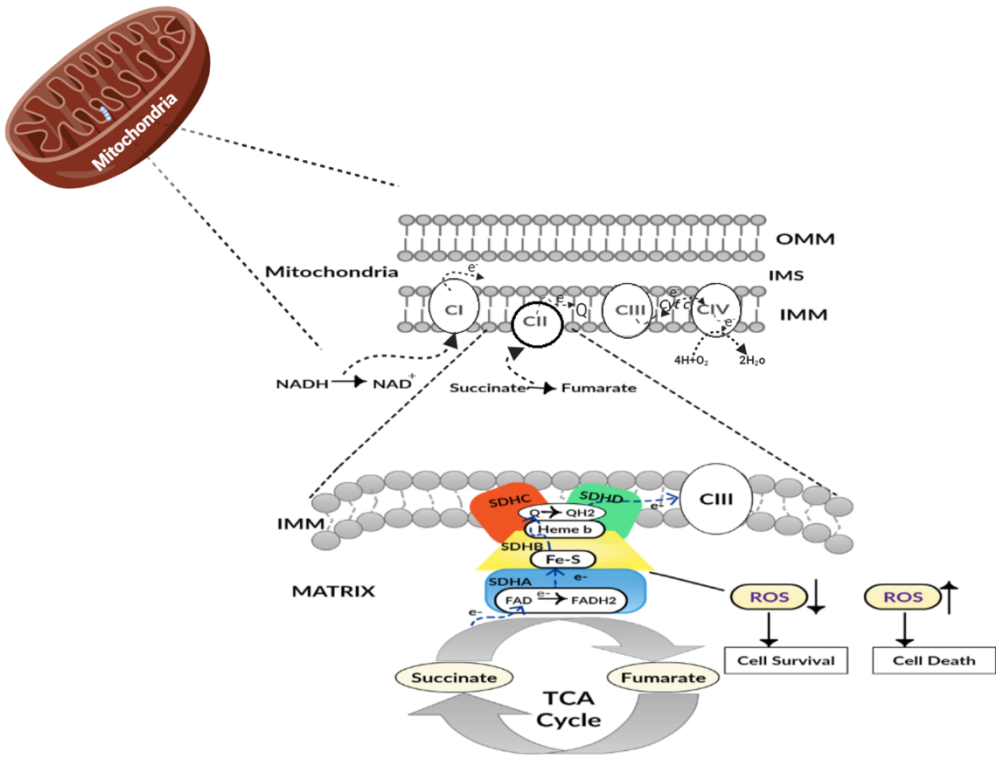


Figure 1: Mitochondria respiratory complex II; succinate dehydrogenase (SDH), is a member of the respiratory chain and TCA cycle, wherein it catalyzes the oxidation of succinate to fumarate. SDHA, B, C, D represent the subunits of SDH.

Many factors make mitochondria an emerging therapeutic target for several human diseases, including myocardial infarction (MI), stroke, and cancer. Complex II activity is responsible for the oxidative stress in stroke and MI due to its role in the generation of pathological ROS. There is significant promise for the development of selective small molecule chemotherapeutics to inhibit glutaminolysis, the primary source of energy for cancer cells, at the level of the mitochondria.[6, 15] Mitochondrial CII is a vital member of the NADH-fumarate reductase system and is involved in the maintenance of mitochondrial energy production in tumor microenvironments under hypoxic conditions.[16] The inhibition of CII leads to the activation of both autophagy and apoptosis in tumor cells, which could be an appropriate strategy for combating drug resistance.[17] Promisingly, known CII inhibitors are selectively cytotoxic to cancer cells while conferring negligible effects on healthy cells.[18, 19] Mutation of CII is rare, which makes it a unique target for drug development. Such mutations are associated only in infrequent and nonaggressive neoplasias such as pheochromocytomas.[20, 21]

Most reported CII inhibitors in the literature **(Figure 2)** exhibit only moderately potent inhibition. The alkylating agent and hexokinase inhibitor 3-Bromopyruvate (**1**, 3BP), was the first identified CII inhibitor, but no

IC_{50} has been reported.[22, 23] Malonate (**2**) with an IC_{50} value of 40 μM, was one of the first identified CII inhibitors, albeit a competitive inhibitor, and is often used as a reference compound.[24] The vitamin E analog α -Tocopheryl succinate (**3**, α-TOS) has a CII IC_{50} value of 42 μM, and is known to induce apoptosis in cancer cells by ROS generation.[25] Mitochondrially targeted vitamin E succinate (**4**, MitoVES) has a CII IC_{50} of 70 μM,[26] although it was 20-50 times more effective in inducing apoptosis in cancer cells than **3**.[27] This is attributed to the introduction of a cationic triphenylphosphonium (TPP) group which acts to target the compound to mitochondria. The inhibition of CII has been shown to be selective to cancerous cells with MitoVES possessing an IC_{50} of 0.5-3 μM for apoptosis induction in cancer cells and approximately 20-60 μM for non-malignant cells.[27] Thenoyltrifluoroacetone (**5**, TFFA), with an IC_{50} value of 30 μM is widely used as a control compound in CII assay kits.[28] The natural product atpenin A5 (**6**, AA5) is a potent and specific CII inhibitor at the ubiquinone binding site (IIQ), with IC_{50} of 3.6-10 nM.[29] We have previously reported Atpenin A5 derivative 16c (**7**) which possess an IC_{50} value of 64 nM and a 'drug-like' ligand-lipophilicity efficiency of 5.62.[30] Compound **7** was shown to induce anti-proliferative activity across multiple prostate cancer cells. Furthermore, Atpenin A5 derivative 16k (**8**) with an IC_{50} of 3.3 nM, was reported from the same study as the most potent CII inhibitor described to date, albeit with limiting lipophilicity.

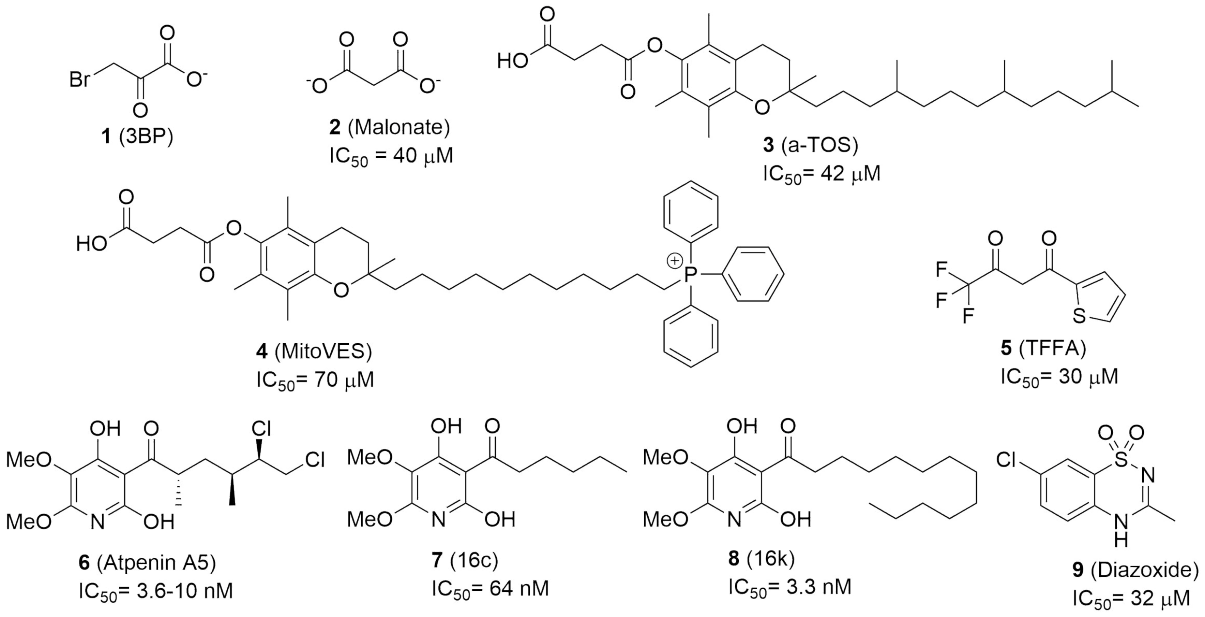


Figure 2. Structures of known complex II inhibitors **1**-**9**.

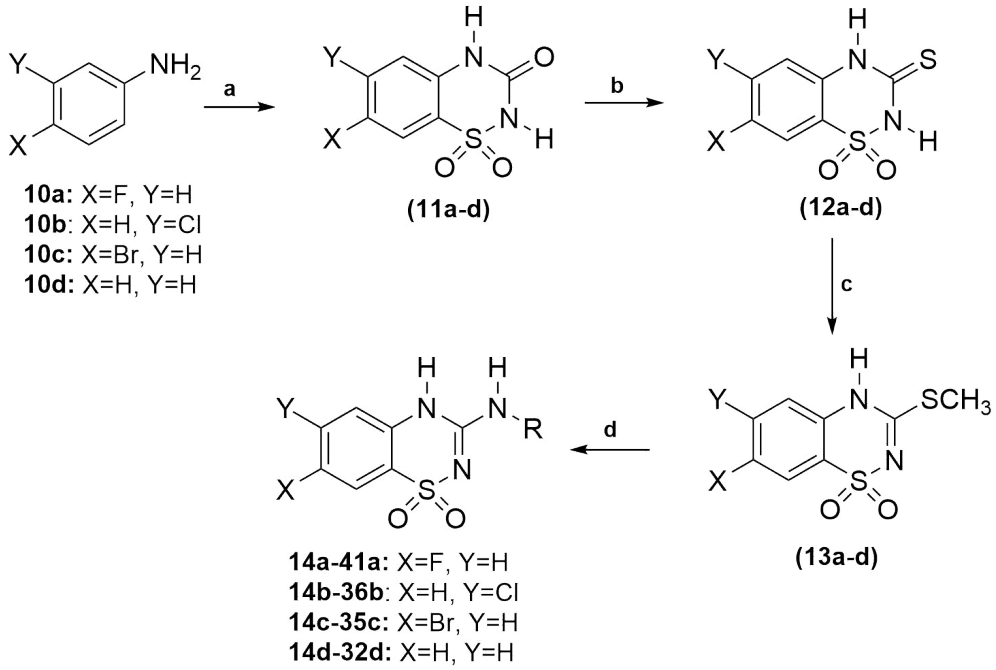
The Food and Drug Administration (FDA) approved clinical vasodilator drug diazoxide (**9**, DZX) has a reported CII IC_{50} value of 32 μM in rat heart mitochondria,[31] and is known to regulate ROS production, protecting normal cells from ischemic damage and also inducing specific cancer cell death.[32] Benefits, as well as drawbacks and a narrow therapeutic window have been observed from DZX administration across different tissues and organelles. In the pancreas, DZX opens K_{ATP} channels, the closing of which is required for glucose-induced insulin secretion (GSIS) in pancreatic beta cells, thus insulin secretion is blocked, and hypoglycemia prevented. In the context of ischemia, DZX can regulate ROS production and confer protection. However, further reports found that high doses of diazoxide (750 μM) led to increased levels of ROS.[33, 34] In cortical neuron mitochondria <200 μM of DZX had no effect on mitochondria, but a 300 μM dose did result in depolarization.[33] Further, a 100 μM concentration of diazoxide was reported to inhibit CII in mouse heart mitochondrial but IC_{50} was not reached.[35] Diazoxide has been shown to be neuroprotective in animal models of Alzheimer's disease,[36, 37] protect neurons from a range of neurotoxic insults, including exposure to amyloid-β peptide (25-35),[38] and was reported to reduce proliferation in both acute leukemic T cells,[39] and triple negative breast cancer (TNBC) MDA-MB-468 cells.[37] One mechanism of action of this observed cytotoxicity was attributed to the downregulation of beta-catenin-mediated Cyclin D1 transcription.[40] No studies have been reported, to the best of our knowledge, that attempt to probe the structure-activity relationship (SAR) of diazoxide for either CII inhibition or antineoplastic activity.

Herein, we report the synthesis of a library of novel diazoxide derivatives to understand structural effect on CII inhibition activity and antineoplastic effect in 22Rv1 prostate cancer cells and the TNBC MDA-MB-468 cell line. In our hands the diazoxide parent compound exhibited no CII inhibition activity at 100 μM (IC_{50} was found to be 1236 μM) which corresponded to no effect to reduce cell viability of either prostate or breast cancer cells at 100 μM up to 72 hours. Several derivatives were identified that possessed enhanced CII inhibition (IC_{50} values 11.88 - 89 μM) over diazoxide, which resulted in increased effect to reduce cell viability in both cancer cell lines. Importantly, 13 diazoxide derivatives were identified that possessed potent activity to reduce either TNBC or prostate cancer cell viability, which represent novel hit compounds for further optimization as potential chemotherapeutics.

Results and Discussion

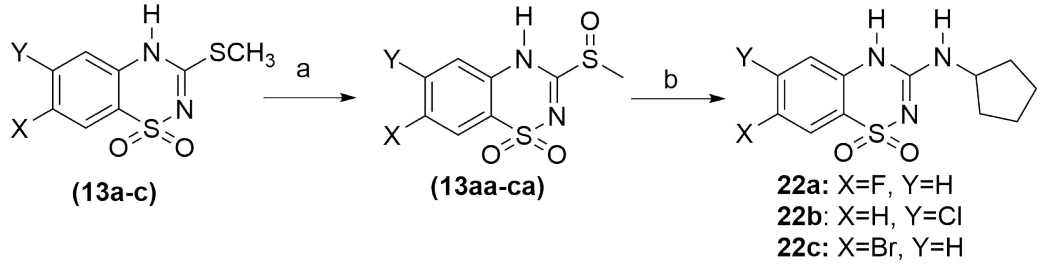
Chemistry

The parent compound diazoxide (7-chloro-3-methyl-2H-1,2,4-benzothiadiazine 1,1-dioxide) (**9**) can be accessed by a number of reported syntheses, our adopted route blends elements of several.[41-44] Additionally, a number of chain derivatives of **9** have been synthesized as K_{ATP} channel activators that are selective to pancreatic B-cells, although no determination of antineoplastic effects of these compounds have been reported.[41] Halogen substituted Diazoxide analogs at the 4- and or 5- position of the phenyl ring were accessed in good yield over four steps **(Scheme 1)** starting from an appropriately substituted commercially available aniline (**10a-d**). Electrophilic substitution of the appropriate aniline with chlorosulfonyl isocyanate in the presence of anhydrous aluminum chloride and nitromethane resulted in ring closure to yield 6- and 7-halo-3-oxo-3,4-dihydro-2H-1,2,4-benzothiadiazine1,1dioxides **(11a-c)**, or 3-oxo-3,4-dihydro-2H-1,2,4-benzothiadiazine1,1dioxides **(11d)** in moderate yield.[41] Subsequently, the 3-oxo compounds **(11a-d)** were converted into the corresponding 3-thioxo derivatives **(12a-d)** by reacting with phosphorus pentasulfide in anhydrous pyridine **(Table 1)**.[42] Methylation of **12a-d** was accomplished with methyl iodide in a solution of sodium bicarbonate to yield the desired 3-methylsulfide intermediates (**13a-d**) in good yield.[43] Nucleophilic substitution of these intermediates with the corresponding primary amine was accomplished with overnight heating at 130 ^{o}C in a sealed vessel to afford the desired diazoxide derivatives **(Tables 2-5)**.



Scheme 1. *Reagents and Conditions:* a) $ClSO_2NCO$, CH_3NO_2, -5 °C, $AlCl_3$, reflux; b) P_2S_5, pyridine, reflux; c) $NaHCO_3$, CH_3I, CH_3OH/H_2O, r.t.; d) RNH_2, sealed tube, 130 °C.

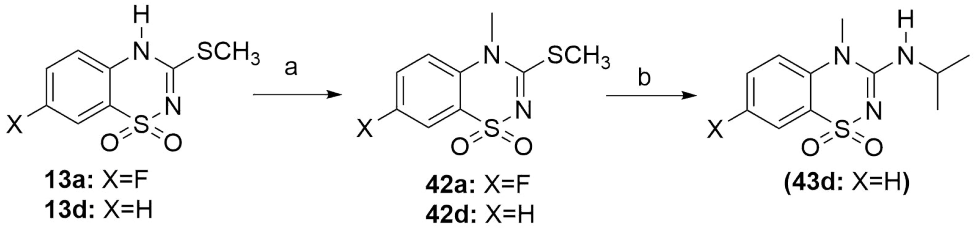
Derivatives possessing a cyclopentamine substituent could not be obtained in sufficient yield from the methylsulfide intermediates (**13a-c**) due to the increased steric bulk. To increase the reactivity of **13a-c** to nucleophilic substitution, the methylsulfides were oxidized to the corresponding methylsulfinyl (**13aa-ca**) **(Scheme 2)**. Subsequently the 3-methylsulfinyl intermediates were reacted with cyclopentamine to yield diazoxide derivatives **22a-c**.[44]



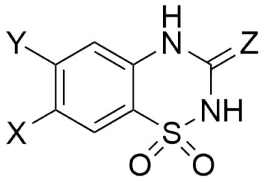
Scheme 2. *Reagents and Conditions:* a) Na_2CO_3, Br_2, H_2O; b) cyclopentamine, 1,4-dioxane, sealed tube, 130 °C.

Access to the *N*-methylated diazoxide derivative (**43d**) was achieved **(Scheme 3)** by exposing methylsulfides **13a** and **13d** to methyliodide in the presence of base to provide corresponding intermediates **42a**

and **42d,** respectively. Subsequent nucleophilic substitution with isopropylamine failed to yield fluoro-substituted derivative **43a**, but did yield 3-(isopropylamino)-4-methyl-4*H*-1,2,4benzothiadiazine 1,1-dioxide (**43d**).



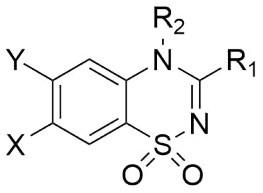
Scheme 3. *Reagents and Conditions:* a) K_2CO_3, CH_3I, CH_3CN/DMF, r.t.; b) isopropylamine, 1,4-dioxane, sealed tube, 130 °C.

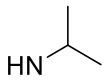
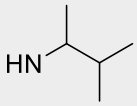
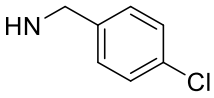
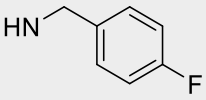
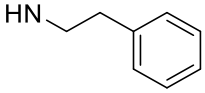
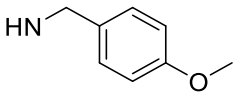
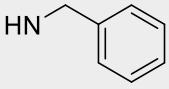
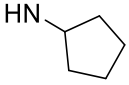
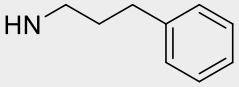
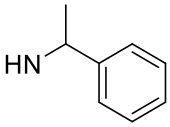


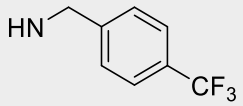
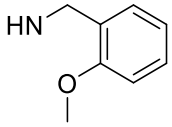
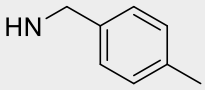
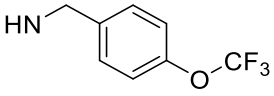
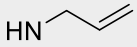
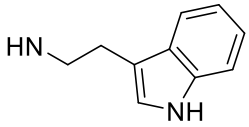
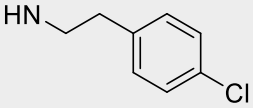
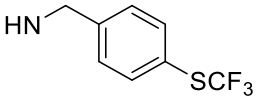
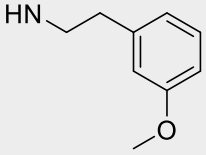
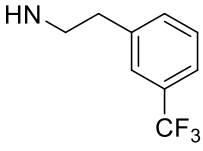
Compound	X	Y	Z	Mw	ClogP[a]	PSA[b]
11a	F	H	O	216.19	0.95	75.27
12a	F	H	S	232.25	0.76	58.2
11b	H	Cl	O	232.64	1.52	75.27
12b	H	Cl	S	248.7	1.33	58.2
11c	Br	H	O	277.09	1.67	75.27
12c	Br	H	S	293.15	1.48	58.2
11d	H	H	O	198.2	0.49	75.27
12d	H	H	S	214.26	0.36	58.2

[a] Calculated by ChemDraw Professional 16.0.
[b] Polar surface area (pH 7.4), calculated by ChemDraw Professional 16.0.

Table 1. Structure, molecular weight, calculated logP, and polar surface area of diazoxide derivatives **11a-d** and **12a-d**.

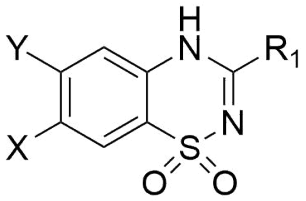


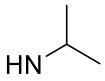
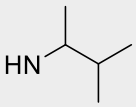
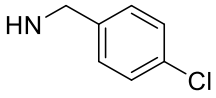
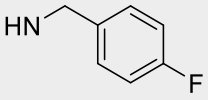
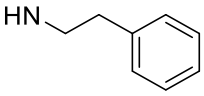
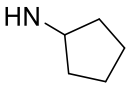
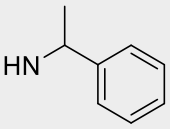
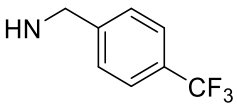
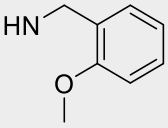
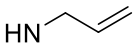
Compound	X	Y	R_1	R_2	Mw	ClogP[a]	PSA[b]
13a	F	H	SCH_3	H	246.27	0.97	58.53
14a	F	H		H	257.28	0.73	70.56
15a	F	H		H	285.34	1.66	70.56
16a	F	H		H	339.77	2.37	70.56
17a	F	H		H	323.32	1.8	70.56
18a	F	H		H	319.35	1.99	70.56
19a	F	H	$NHCH_2CH_3$	H	243.26	0.42	70.56
20a	F	H		H	335.35	1.58	79.79
21a	F	H		H	305.33	1.66	70.56
22a	F	H		H	283.32	1.36	70.56
23a	F	H		H	333.38	2.37	70.56
24a	F	H		H	319.35	1.97	70.56

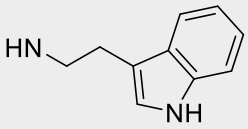
25a	F	H		H	373.33	2.54	70.56
26a	F	H		H	335.35	1.58	79.79
27a	F	H		H	319.35	2.16	70.56
30a	F	H		H	389.32	2.69	79.79
32a	F	H		H	255.27	0.67	70.56
36a	F	H		H	358.39	1.98	82.59
37a	F	H		H	353.80	2.70	70.56
38a	F	H		H	405.39	3.33	70.56
39a	F	H		H	349.38	1.91	79.79
41a	F	H		H	387.35	2.87	70.56
42a	F	H	SCH_3	Me	290.30	1.84	49.74

[a]Calculated by ChemDraw Professional 16.0.
[b]Polar surface area (pH 7.4), calculated by ChemDraw Professional 16.0.

Table 2. Structure, molecular weight, calculated logP, and polar surface area of diazoxide derivatives with 7-fluoro substitution.

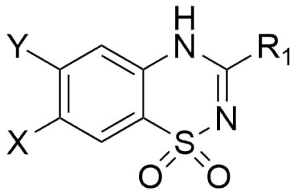


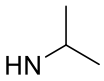
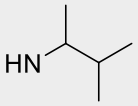
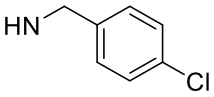
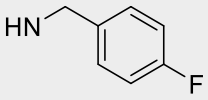
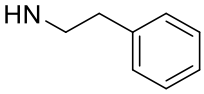
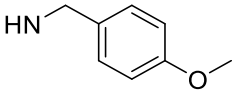
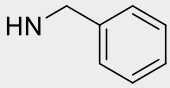
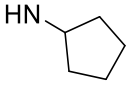
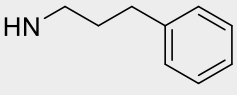
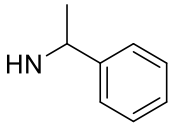
Compound	X	Y	R1	Mw	ClogP[a]	PSA[b]
13b	H	Cl	SCH_3	262.73	1.54	58.53
14b	H	Cl		273.74	1.3	70.56
15b	H	Cl		301.79	2.23	70.56
16b	H	Cl		356.22	2.94	70.56
17b	H	Cl		339.77	2.37	70.56
18b	H	Cl		335.81	2.56	70.56
19b	H	Cl	$NHCH_2CH_3$	259.71	0.99	70.56
22b	H	Cl		299.77	1.93	70.56
24b	H	Cl		335.81	2.54	70.56
25b	H	Cl		389.28	3.11	70.56
26b	H	Cl		351.81	2.15	79.79
32b	H	Cl		271.72	1.24	70.56

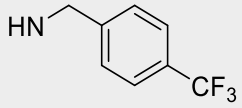
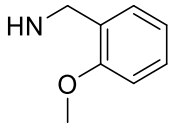
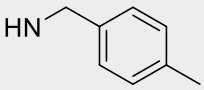
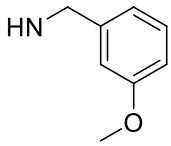
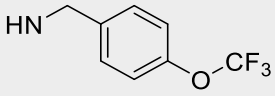
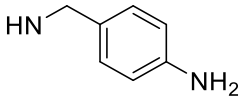
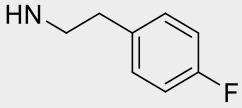
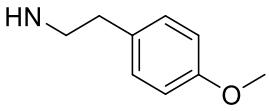
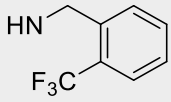
36b	H	Cl		374.84	1.98	82.59

[a]Calculated by ChemDraw Professional 16.0.
[b]Polar surface area (pH 7.4), calculated by ChemDraw Professional 16.0.

Table 3. Structure, molecular weight, calculated logP, and polar surface area of diazoxide derivatives with 6-chloro substitution.

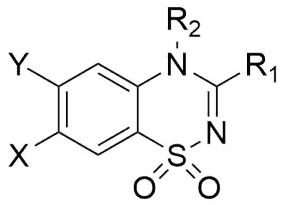


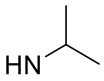
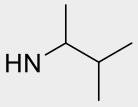
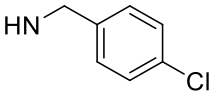
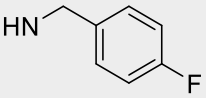
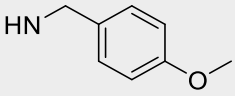
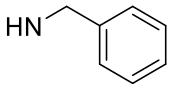
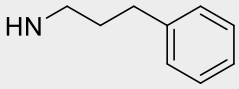
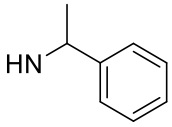
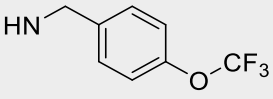
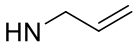
Compound	X	Y	R1	Mw	ClogP[a]	PSA[b]
13c	Br	H	SCH_3	307.18	1.69	58.53
14c	Br	H		318.19	1.45	70.56
15c	Br	H		346.24	2.38	70.56
16c	Br	H		400.68	3.09	70.56
17c	Br	H		384.22	2.52	70.56
18c	Br	H		380.26	2.71	70.56
19c	Br	H	$NHCH_2CH_3$	304.16	1.14	70.56
20c	Br	H		396.26	2.30	79.79
21c	Br	H		366.23	2.38	70.56
22c	Br	H		344.23	2.08	70.56
23c	Br	H		394.29	3.09	70.56
24c	Br	H		380.26	2.69	70.56

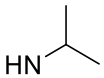
25c	Br	H		434.23	3.26	70.56
26c	Br	H		396.26	2.30	79.79
27c	Br	H		380.26	2.88	70.56
28c	Br	H		396.26	2.30	79.79
30c	Br	H		450.23	3.41	79.79
31c	Br	H		381.25	1.15	96.58
33c	Br	H		398.25	2.85	70.56
34c	Br	H		410.29	2.63	79.79
35c	Br	H		434.23	3.26	70.56

[a]Calculated by ChemDraw Professional 16.0.
[b]Polar surface area (pH 7.4), calculated by ChemDraw Professional 16.0.

Table 4. Structure, molecular weight, calculated logP, and polar surface area of diazoxide derivatives with 7-bromo substitution.



Compound	X	Y	R_1	R_2	Mw	ClogP[a]	PSA[b]
13d	H	H	SCH_3	H	228.28	1.69	58.53
14d	H	H		H	239.29	0.59	70.56
15d	H	H		H	267.35	1.51	70.56
16d	H	H		H	321.78	2.23	70.56
17d	H	H		H	305.33	1.66	70.56
19d	H	H	$NHCH_2CH_3$	H	225.27	0.28	70.56
20d	H	H		H	317.36	1.44	79.79
21d	H	H		H	287.34	1.51	70.56
23d	H	H		H	315.39	2.22	70.56
24d	H	H		H	301.36	1.83	70.56
30d	H	H		H	371.33	2.54	79.79
32d	H	H		H	237.28	0.52	70.56
42d	H	H	SCH_3	Me	242.31	1.69	49.74

43d	H	H		Me	253.32	1.46	61.77

[a]Calculated by ChemDraw Professional 16.0.
[b]Polar surface area (pH 7.4), calculated by ChemDraw Professional 16.0.

Table 5. Structure, molecular weight, calculated logP, and polar surface area of diazoxide derivatives with a saturated ring.

Complex II inhibition assay

Mitochondrial respiratory complex II activity was measured spectrophotometrically using isolated mouse liver mitochondria, with suitable modifications to ensure rapid isolation as previously described.[45] The natural product, and potent CII inhibitor, Atpenin A5 (**6**) IC_{50} = 3.3 nM,[24, 29] was employed as positive control with DMSO as negative control. The parent compound diazoxide (**9**), was found to be inactive in our hands with no inhibition activity at 100 μM and a calculated IC_{50} >1000 μM (**Table 6**) compared with the value of 32 μM reported in the literature.[31] It should be noted that the literature CII IC_{50} was determined in rat heart mitochondria while in this study we employ mouse liver mitochondria which may account for this apparent discrepancy. The positive control compound **6** induced 93% inhibition at 0.1 μM, validating the assay protocol. To unequivocally associate this activity to the parent compound we employed both synthesized and commercially acquired samples of **9**. The general inactivity was confirmed in the prostate and breast cancer cell lines wherein **9** had no effect on cell viability (see below). Literature studies employing **9** mostly employ a concentration much greater than the reported 32 μM IC_{50} value, with some experiments performed up to 750 μM.[31, 33, 34]

Undeterred by the apparent lack of CII inhibition activity of our parent compound (IC_{50} = 1236 μM) we continued to screen derivatives of **9** for CII inhibition. All of the synthesized derivatives were initially screened at 100 μM. Halogen substitution on the benzothiadiazine ring provided increased CII inhibition activity over saturated counterparts. Among 7-fluorobenzothiadiazine substituted derivatives **(Scheme 3A)**, 4-chlorobenzylamine (**16a**) provided 30% inhibition. When the chain length was extended, the 4-chlorophenethyamine derivative (**37a**) induced 34% inhibition. Replacement of the electron-withdrawing chlorine on the side chain benzylamine with an electron-donating methyl substituent to afford 4-methylbenzylamine

derivative **27a**, decreased inhibition to 23%. Replacement with a highly electron withdrawing trifluoromethyl substituent (**25a**) afforded an inactive compound (7% inhibition at 100 μM) while the 2-methoxybenzylamine (**26a**) induced 37% inhibition. The most active derivative of the 7-fluoro series was allylamine **32a**, inducing 38% inhibition, equipotent with **26a**, indicating that a side chain containing an aromatic ring is not required for activity.

When the 7-fluoro substituent on the benzothiadiazine ring was switched to a 6-chloro substituent **(Figure 3B)** the unfunctionalized thiourea derivative (**12b**) exhibited startlingly potent inhibition activity (81% inhibition at 100 μM). However, the chromophoric nature of the compound was determined to interfere with the assay readout, leading to what we postulate to be a false positive. The compound shares structure similarity to a number of promiscuous compounds known as pan-assay interfering compounds (PAINS).[46] The 4-chlorobenzylamine derivative (**16b**) induced 34% inhibition, equipotent with its 7-fluoro benzothiadiazine substituted counterpart **16a**. Cyclopentamine derivative **22b** induced 27% inhibition, substantially more active than the respective 7-fluoro benzothiadiazine substituted compound **22a** (inactive). The H-Indole-3-ethylamine side chain substituted compound (**36b**) induced 25% inhibition. The 7-fluoro benzothiadiazine substituted derivatives with the same side chain; 3-phenylpropylamine (**23a**) induces just 14% inhibition while H-Indole-3-ethylamine (**36a**) is equipotent. The most active derivative from the 6-chloro series possessed a 1-phenylethylamine side chain (**24b**) inducing 51% CII inhibition at 100 μM, possibly indicating a role for the phenyl ring in pi-pi stacking at this position of the molecule. Overall, the 6-chloro substitution pattern on the benzothiadiazine ring provided no appreciable increase in inhibition activity compared to 7-fluoro substitution.

When the 7-fluoro substituent on the benzothiadiazine ring was interchanged with 7-bromo substitution the inhibitory activity of the derivatives notably increased **(Figure 3C)**. The unfunctionalized thiourea derivative **12c** was active, inducing 55% inhibition of CII at 100 μM, compared with 81% with the 6-chloro substituted benzothiadiazine. However, the chromophore of the compound was again found to interfere with the assay read out; the structure again acting in the role of a PAINS. The 4-chlorobenzylamine derivative (**16c**) induced 45% CII inhibition at 100 μM with the 7-bromo substituted benzothiadiazine ring, conferring increased activity over its 7-fluoro (**16b**) and 6-chloro (**16a**) counterparts and in contrast to the inactive unsubstituted derivative **16d**. Equipotent inhibition to **16c** was noted with 3-phenylpropylamine (**23c**), which induced 46% inhibition. Again the 7-bromo substituted benzothiadiazine ring was more active than the 6-chloro substituted 3-phenylpropylamine

(**23a**) and the unsubstituted derivative **23d**, which induced 14% and 0% inhibition respectively. The 1-phenylethylamine derivative (**24c**) induced 55% inhibition of CII at 100 μM, equipotent with its 6-chloro counterpart (**24b**). The most active compound identified in this study outside of the chromophoric false positives, 4-methoxybenzylamine (**20c**), induced 64% inhibition at 100 μM. Derivatives possessing an unsubstituted benzothiadiazine ring **(Figure 3D),** exhibited no inhibition of CII at 100 μM.

A preliminary structure-activity relationship can be derived for CII inhibition activity of this scaffold. Halogen substitution at the 6- or 7- position of the benzothiadiazine ring affords for inhibition activity which is completely absent from the respective saturated derivatives. Of all halogen substituents evaluated herein, 7-bromo represents the most active inhibitors. The side chain derivatives require either aromatic or possibly allyl (in the case of a 7-F substituted benzothiadiazine ring, but interestingly not when combined with 6-Cl substitution) moieties to confer CII inhibition activity. However, no clear chain substituent pattern can be derived beyond 4-CF_3 is deleterious to activity (**25a** and **25c** confer 0% inhibition while **25b** induces only 19% inhibition). Alkyl side chains yield inactive compounds; however, a cyclopentane ring does provide some activity (approximately 25% inhibition).

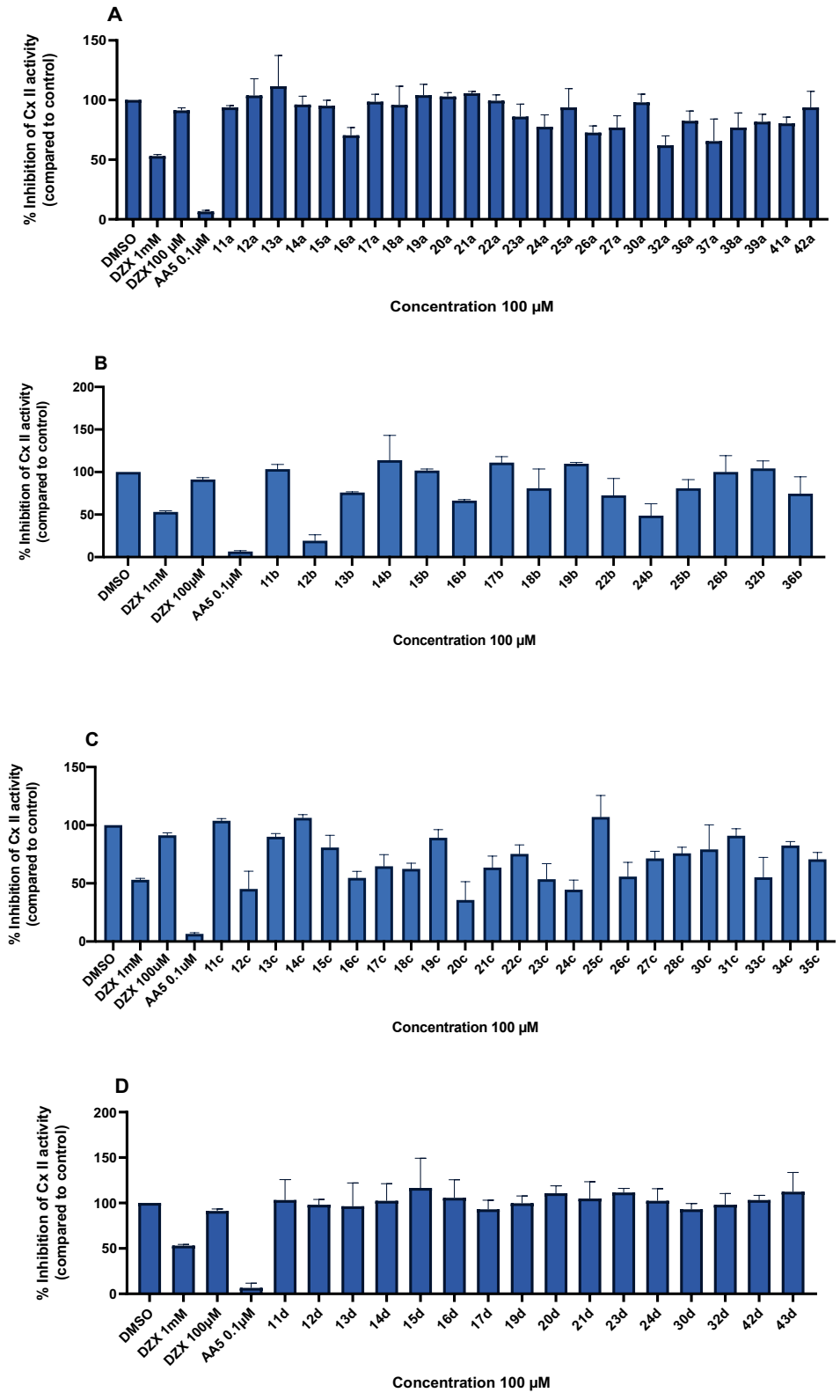


Figure 3. Percentage inhibition of mitochondrial complex II relative to DMSO control at 100 µM concentration of diazoxide derivative. A) Complex II inhibitory activity for diazoxide derivatives with 7-fluoro substitution. B) Complex II inhibitory activity for diazoxide derivatives with 6-chloro substitution. C) Complex II inhibitory activity for diazoxide derivatives with 7-bromo substitution. D) Complex II inhibitory activity for diazoxide derivatives with a saturated ring. Values represent the mean ±SD of n = 4 experiments.

The five most active CII inhibitors at 100 μM (**12b**, 81% inhibition; **12c**, 55%; **20c**, 64%; **24b**, 51% and **24c**, 55%), the parent compound diazoxide (**9**, 9% inhibition at 100 μM) and positive control Atpenin A5 (**6**, 93% inhibition at 0.1 μM) were selected for IC_{50} determination (**Table 6**). The parent compound **9** possessed an IC_{50} = 1236 μM in our hands, greatly reduced over the 32 μM IC_{50} reported in the literature.[31] Positive control compound **6** possessed an IC_{50} = 3.3 nM, in accordance with literature values.[46] The two unfunctionalized sulfonylureas **12b** and **12c** displayed the most potent IC_{50} values of 11.88 and 36.98 μM respectively, as exected from the initial compound screen at 100 μM. However, it should again be noted we expect these compounds to be false positive PAINS. The most active compound identified in the initial screen **20c**, possessed an IC_{50} = 79.68 μM. The 6-chloro substituted 1-phenylethylamine side chain derivative **24b** possessed an IC_{50} = 89 μM and its 7-fluoro counterpart (**24c**) an IC_{50} = 79.82 μM. The obtained IC_{50} values directly correlate with the activity pattern obtained in the initial screen conducted at 100 μM. Several novel diazoxide derivatives have been identified with significantly increased activity to inhibit CII, with the most active compounds conferring >15-fold increased potency over the parent compound.

Compound	Mw	ClogP[a]	PSA[b]	CII IC_{50} (μM)[c]
Diazoxide (**9**)	230.67	1.0	58.53	1236 ±2.5
Atpenin A5 (**6**)	366.24	2.64	88.88	0.0033 ±2
12b	248.7	1.33	58.2	11.88* ±3.3
12c	293.15	1.48	58.2	36.98* ±2.4
20c	396.26	2.30	79.79	79.68 ±4
24b	335.81	2.54	70.56	89.01 ±10.4
24c	380.26	2.69	70.56	79.82 ±4.1

[a]Calculated by ChemDraw Professional 16.0.
[b]Polar surface area (pH 7.4), calculated by ChemDraw Professional 16.0.
[c]Values are the mean ±SD of n=4 experiments.
*Probable PAINS

Table 6. Mitochondrial respiratory complex II IC_{50} values of selected diazoxide derivatives.

Cytotoxicity assay

The cytotoxicity of the diazoxide derivatives at 100 μM concentration was determined in 22Rv1 prostate cancer cells after 48 hrs treatment employing the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay as previously reported.[47, 48] Atpenin A5 derivative **7** (**Figure 2**) which possesses a CII IC_{50} = 64 nM and a 'drug-like' ligand-lipophilicity efficiency of 5.62 was employed as a positive control. This compound has been previously reported by our lab to reduce cell viability of 22Rv1 cells.[30] In this assay, **7** provided a significant inhibitory effect at 20 μM concentration, reducing cell viability by 60%. The parent compound **9**, despite lacking any CII inhibition activity at 100 μM, reduced 22Rv1 prostate cancer cell survival by 12% **(Figure 4)**, possibly due to the aforementioned ability of the compound to down regulate beta-catenin-mediated cyclin D1 transcription.[40]

The 7-flourobenzothiadiazine substituted derivatives generally conferred the least effect on 22Rv1 prostate cancer cell viability of all of the halogen substituted derivatives. The most potent CII inhibitor from this series, allylamine (**32a**) displaying 38% CII inhibition, afforded 24% reduction of cell viability **(Figure 4A)**. However, this derivative was not the most cytotoxic in the 22Rv1 cells; 1-Phenylethylamine (**24a**) which possess 22% CII inhibition affords 34% reduction in cell viability while the 3-Indoleethylamine derivative (**36a**) which possesses 17% CII inhibition activity induces 30% reduction of cell viability in 22Rv1 cells. The 4-chlorobenzylamine **16a** (30% CII inhibition) and 4-chlorophenethylamine homologue **37a** (34% CII inhibition) both proved inactive in 22Rv1 cells.

From the 6-chlorobenzothiadiazine substituted derivatives the most active compound, 1-phenylethylamine (**24b**) (51% CII inhibition at 100 μM, IC_{50} = 89.0 ±10.4 μM) afforded a 50% reduction in 22Rv1 cell viability **(Figure 4B)**. The cyclopentamine derivative (**22b**) which afforded a 34% reduction in cell viability was the next most active of this class. However, 4-chlorobenzylamine **16b**, which was equipotent with **22b** in CII inhibition, afforded just 10% reduction of 22Rv1 cell viability. Unfunctionalized thiourea compound **12b** (CII Inhibition IC_{50} = 11.88 ±3.3 μM) was confirmed as a probable PAINS affording just 12% reduction in 22Rv1 cell viability.

The most active 7-bromobenzothiadiazine substituted derivative, 1-phenylethylamine **24c** (55% CII inhibition at 100 μM, IC_{50} = 79.8 ±4.1 μM) significantly reduced 22Rv1 cell survival by 70% at the same concentration after 48 hrs incubation and is the most potent derivative in the 22Rv1 cell line (IC_{50} = 38.9 ±3.2 μM). Derivatives **30c**, the 4-(trifluoromethoxy)benzylamine (21% CII inhibition) and **23c**, the 3-Phenylpropylamine (47% CII inhibition) reduced cell survival of 22Rv1 cells by 45% and 42% respectively, while the 4-methoxybenzylamine **(20c)** (64% CII inhibition) and 4-(trifluoromethyl)benzylamine **(25c)** (0% CII inhibition) derivatives resulted in 41% and 34% reduced cell viability respectively. Unfunctionalized thiourea **12c** (55% CII Inhibition) was confirmed as a probable PAINS affording just 16% reduction in 22Rv1 cell viability **(Figure 4C)**.

The unsubstituted benzothiadiazine derivatives that possess no significant CII inhibition activity generally afforded no reduction of 22Rv1 cell viability. However, 1-phenylethylamine **(24d)** and 4-(trifluoromethoxy)benzylamine **(30d)** were both equipotent to reduce cell survival of 22Rv1 cells by approximately 30%. These two side chain derivatives display the greatest reduction in 22Rv1 cell viability across all benzothiadiazine derivative classes tested, suggesting the 1-phenethylamine and 4-(trifluoromethoxy)benzylamine contribute to a common pharmacophore. Furthermore, greater cytotoxicity was correlated with increased ClogP. While several novel benzothiadiazine derivatives have been identified that possess significant activity to suppress prostate cancer cell viability, potency to inhibit CII does not correlate to antineoplastic activity. Indeed, the derivatives with the greatest effect to reduce cell viability in 22Rv1 cells **(Figure 4)** possess a range of CII inhibition activity from 0% to 64%. Derivative **24c** possessing an IC_{50} = 38.9 ±3.2 μM in 22Rv1 prostate cancer cells is more potent in this cell line than the clinical agents apalutamide (IC_{50} = 77.0 ±17 μM) and darolutamide (IC_{50} = 46.0 ±10 μM),[47] identifying this scaffold as a hit for further studies.

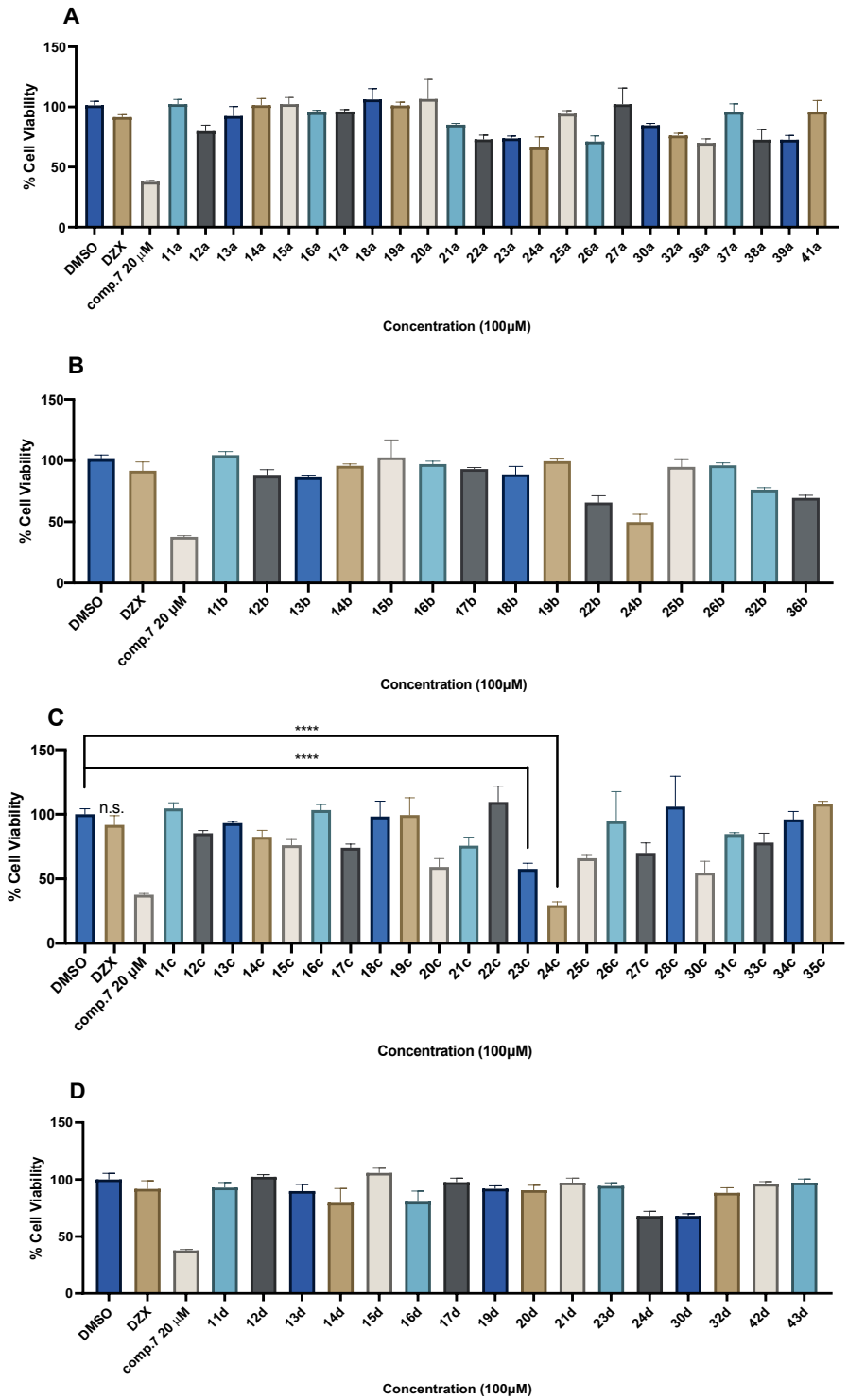


Figure 4. Cytotoxic effect of diazoxide derivatives (100 μM, 48 hr treatment) in 22Rv1 prostate cancer cells. A) Cytotoxic effect of diazoxide derivatives with 7-fluoro substitution. B) Cytotoxic effect of diazoxide derivatives with 6-chloro substitution. C) Cytotoxic effect of diazoxide derivatives with 7-bromo substitution D) Cytotoxic effect of diazoxide derivatives with a saturated ring. Values represent the mean ±SD of n = 3 experiments. Unpaired t test; n.s. = not significant, ****$p < 0.0001$.

Administration of 300 mg/kg of diazoxide to rats bearing hormone-dependent mammary carcinomas was reported to result in 90% inhibition of tumor growth but induced mild reversible diabetes.[49] Additionally, diazoxide has been reported to be cytotoxic in TNBC cells.[37] Based on these studies, we explored the cytotoxic effect of selected diazoxude derivatives in the TNBC MDA-MB-468 cell line. Derivatives were dosed at 10, 50 and 100 μM for 24, 48 and 72 hours and cell viability measured by MTS assay **(Figure 5)**.

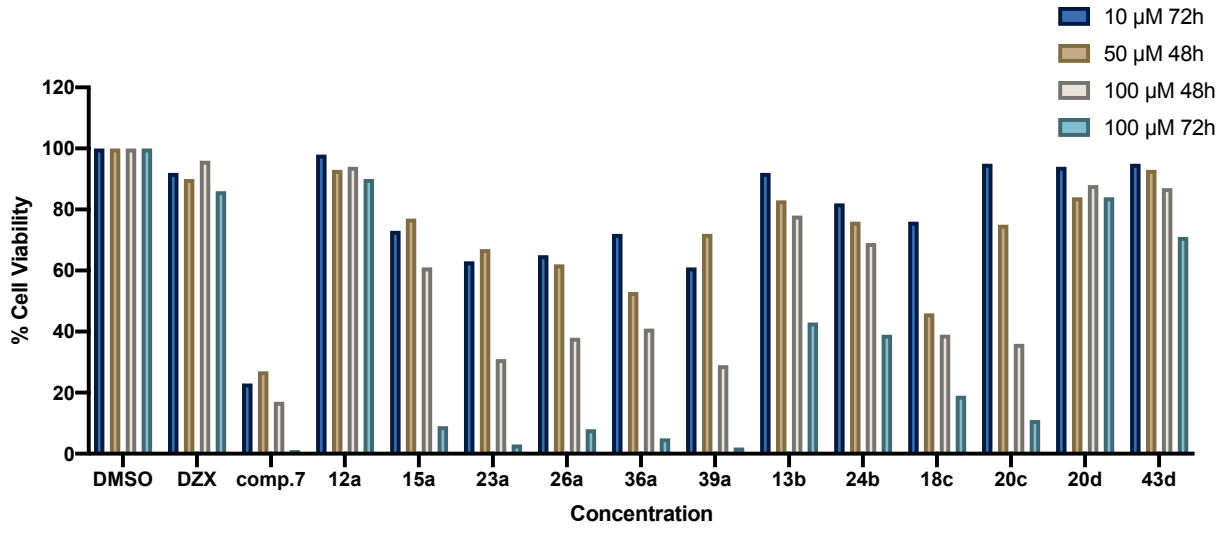


Figure 5. Cytotoxic effect of selected diazoxide derivatives on triple negative breast cancer (TNBC) MDA-MB-468 cells. Values represent the mean of three separate experiments performed in triplicate.

The parent compound diazoxide afforded little activity to reduce TNBC cell viability. Gratifyingly, several derivatives demonstrated marked dose and time-dependant reduction of cell viability; 7-fluorobenzothiadiazine derivatives in particular, including **23a**, **26a**, **36a** and **39a** induced almost complete amelioration of cell viability at 100 μM concentration after 72 hours. Interestingly the 3-methylbutanamine side chain derivative (**15a**) was substantially less active than its aromatic side chain bearing counterparts. The 7-bromobenzothiadiazine derivatives **18c** and **20c** displayed potent activity but generally less so than the 7-fluorobenzothiadiazine derivatives. Unsubstituted thiourea **12a** as well as unsubstituted benzothiadiazine derivatives **20d** and **43d** demonstrated no reduction of cell viability at any time or dose tested. Of the most active compounds in the TNBC cells, with the single exception of **26a**, none show appreciable activty to inhibit CII, pointing to an as yet undetermined target of action for these novel halogen substituted benzothiadiazines.

Exposure of human T leukemic Jurkat cells to 100 μM of diazoxide resulted in significant inhibition of proliferation; however, upon removal of the compound proliferation resumed. The study demonstrated that while diazoxide exposure depolarized the mitochondrial membrane, this was insufficient to modulate cellular energy metabolism. It was found that exposure to diazoxide resulted in reduction of cellular Ca^{2+} influx.[39] Diazoxide has further been reported to inhibit lung cancer cell proliferation by downregulating Cyclin D1 transcription.[40]

Diazoxide has been investigated in one pilot clinical study in breast cancer patients at a dose of 200-300 mg per day. Treatment of nine patients resulted in a 33% response rate conferring stable disease for between 4-8 months either in combination with tamoxifen (two patients) or monotherapy (one patient).[50] The repurposing of DZX as a potential treatment for TNBC has been recently proposed based on a study employing a KinomeScan™ assay of 438 kinases, the three most inhibited at 100 μM were TTK (15%), IRAK1 (9%) and DYRK1A (7%). Dysfunction of all three kinases are known to be associated with various cancers. In this study, as observed herein, the activity of DZX was highly dependent on the cell line employed; no activity was observed in MCF-7 breast cancer cells (IC_{50} = 130 μM) but in MDA-MB-468 TNBC cells an IC_{50} = 0.87 μM was reported for DZX.[37] The potential of repurposing DZX in breast cancer has been advanced previously, with the authors suggesting combination treatment to manage the hyperglycemia 'side effect' of diazoxide in this context.[50] Our studies dispute the use of DZX for direct repurposing as in our hands, DZX is inactive in MDA-MB-468 TNBC cells as well as in a prostate cancer cell line. Through the SAR studies initiated herein, medicinal chemistry modulation of the parent compound has been shown to increase antineoplastic effect significantly and presents the possibility of tuning out the known pharmacophore of K_{ATP} opening activity along with the associated hyperglycemic effect, potentially allowing access to novel treatments for cancer.

In summary, we identify two novel benzothiadiazine derivative classes (**24a-d** and **30a, 30c, 30d**) that possess enhanced activity to reduce the cell viability of 22Rv1 prostate cancer cells and at least five novel 7-fluorobenzothiadiazine derivatives that show significant dose and time-dependent inhibition of MDA-MB-468 triple negative breast cancer cells suitable for further investigation. We demonstrate that the CII inhibition activity of diazoxide derivatives is not responsible for the observed cytotoxicity in either cancer cell line and that the cytotoxicity is selective between TNBC and prostate cancer cells with no derivatives conferring potent cytotoxic effect in both. Thus, indicating that the derivatives are not generally toxic and engage a target not commonly

expressed in MDA-MB-468 or 22Rv1 cells. Studies continue within our lab to identify the target of action of these hit compounds.

Experimental Section

Chemistry

General: All reactions were carried out in oven- or flame-dried glassware under positive nitrogen pressure unless otherwise noted. Reaction progress was monitored by thin-layer chromatography (TLC) carried out on silica gel plates (2.5 cm x 7.5 cm, 200 μm thick, 60 F254) and visualized by using UV (254 nm) or by potassium permanganate or phosphomolybdic acid stain as indictor. Flash column chromatography was performed with silica gel (40-63 μm, 60 Å) or on a Teledyne Isco (CombiFlash R_f 200 UV/Vis). Commercial grade solvents and reagents were purchased from Fisher Scientific (Houston, TX) or Sigma Aldrich (Milwaukee, WI) and were used without further purification except as indicated. Anhydrous solvents were purchased from Across Organics and stored under an atmosphere of dry nitrogen over molecular sieves.

^{1}H and ^{13}C NMR spectra were recorded in the indicated solvent on a Bruker 400 MHz Advance III HD spectrometer at 400 and 100 MHz for ^{1}H and ^{13}C respectively with solvent peak as an internal standard. Multiplicities are indicated by s (single), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), br (broad). Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (*J*), in hertz. High-resolution mass spectroscopy (HRMS) was performed on a TripleTOF 5600 (SCIEX)) using an ESI source conducted at the Texas Tech University Health Sciences Center School of Pharmacy in Dallas, TX. The spectral data was extracted from total ion chromatogram (TIC). High-pressure liquid chromatography was performed on a Gilson HPLC system with 321 pumps and 155 UV/Vis detector using trilution software v2.1 with an ACE Equivalence 3 (C18, 3 μM, 4.6 x 150 mm) column. All samples were determined to possess >95% purity.

7-fluoro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (11a). A solution of chlorosulfonyl isocyanate (2.82 mL, 32.4 mmol) in nitromethane (30 mL) was mixed in a closed dried vessel under nitrogen pressure and cooled at -5 °C (ice and salt bath). To this mixture 4-fluoroaniline (**10a**, 2.6 mL, 27 mmol) was

added slowly. The contents were vigorously stirred for 20 mins followed by the addition of anhydrous $AlCl_3$ (4.7 g, 35.1 mmol) and the mixture was refluxed for 1h. The hot solution was poured onto ice (200 g) and stirred for and additional 30 mins and the resulting precipitate was collected by filtration and washed with water. The crude solid was treated with an aqueous solution of sodium bicarbonate (5 g/100 mL) followed by heating until the solid precipitate was dissolved. The solution was treated with charcoal and was filtered, the filtrate solution was adjusted to pH 1 using 12N HCl. The resulting pure white precipitate was filtered, washed with water, and air dried (3.17 g, 54%) : ^{1}H NMR (400 MHz, DMSO-d6): δ 7.30 (m, 1H), 7.55 (1H, t, *J*=8.7 Hz), 7.68 (1H, dd, *J*=7.5, 2.8 Hz), 11.40 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 109.27, 119.81, 121.88, 123.58, 132.18, 151.67, 156.58, 159.01; HRMS (ESI) m/z calcd for $C_7H_5FN_2O_3S$ $[M+Na]^+$: 238.9902, found: 238.9901.

6-chloro-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine1,1-dioxide (11b). The white compound was obtained from 3-chloroaniline (**10b**, 3.32 mL, 31.35 mmol) by following the experimental conditions described for **11a** (4.5 g, 62%): ^{1}H NMR (400 MHz, DMSO-d6): δ 7.26 (1H, d, *J*=2 Hz), 7.32 (1H, dd, *J*=8.5, 1.8 Hz), 7.80 (1H, d, *J*=8.5 Hz), 11.39 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 116.91, 121.73, 123.96, 124.65, 136.97, 138.54, 151.15; HRMS (ESI) m/z calcd for $C_7H_5ClN_2O_3S$ $[M+Na]^+$: 254.9607, found: 254.9606.

7-bromo-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine1,1-dioxide (11c). The white compound was obtained from 4-bromoaniline (**10c**, 3 g, 17.44 mmol) by following the experimental conditions described for **11a** with the slight modification that the crude material was dissolved in a 1:1 hydromethanolic solution of sodium bicarbonate instead of an aqueous solution of sodium bicarbonate (3.1 g, 64%): ^{1}H NMR (400 MHz, DMSO-d6): δ 7.19 (1H, d, *J*=8.7 Hz), 7.78 (1H, dd, *J*=8.7, 2.2 Hz), 7.91 (1H, d, *J*=2.2 Hz), 11.46 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 115.08, 119.83, 124.34, 124.79, 134.86, 137.00, 151.52; HRMS (ESI) m/z calcd for $C_7H_5BrN_2O_3S$ $[M+Na]^+$: 298.9101, found: 298.9096.

2*H*-benzo[*e*][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide (11d). The white compound was obtained from aniline (**10d**, 4.86 mL, 53.7 mmol) by following the experimental conditions described for **11a** (5.7 g, 53%): ^{1}H NMR (400 MHz, DMSO-d6): δ 7.27 (m, 2H), 7.63 (1H, t, *J*=7.2 Hz), 7.77 (1H, d, *J*=7.6 Hz), 11.27 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 117.47, 122.43, 122.97, 123.89, 134.40, 135.48, 151.08; HRMS (ESI) m/z calcd for $C_7H_6N_2O_3S$ $[M+Na]^+$: 220.9996, found: 220.9998.

7-fluoro-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (12a). A mixture of 7-fluoro-3-oxo-3,4-dihydro-2H-1,2,4-benzothiadiazine1,1-dioxide (**11a**, 2.8 g, 12.95 mmol) and phosphorus pentasulfide (5.47 g, 12.95 mmol) was dissolved in anhydrous pyridine (50 mL) and refluxed under nitrogen pressure overnight. The reaction was allowed to cool and the solvent removed in vacuo, the crude product was dissolved in an aqueous solution of sodium hydroxide (NaOH) (5 g/100 mL). This solution was treated with charcoal and was filtered. The filtrate was acidified to pH 1 using 12N HCl. The precipitated compound was collected by filtration, washed with water and was allowed to air dry. The dried compound was suspended in an aqueous solution of sodium bicarbonate ($NaHCO_3$) (10 g/200 mL) and heated until the solid was dissolved. This solution was treated with charcoal and filtered. The filtrate was adjusted to pH 1 using 12N HCl, and the white precipitate was collected by filtration, washed with water, and air dried. (1.76 g, 58%): ^{1}H NMR (400 MHz, DMSO-d6): δ 7.29 (m, 1H), 7.56 (m, 1H), 7.68 (1H, dd, *J*=7.5, 2.8 Hz), 11.35 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 109.93, 121.01, 122.12, 123.31, 132.56, 158.33, 160.79; HRMS (ESI) m/z calcd for $C_7H_5FN_2O_2S_2$ $[M+Na]^+$: 254.9674, found: 254.9674.

6-chloro-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (12b). The white compound was obtained from **11b** (4.5 g, 19.34 mmol) by following the experimental conditions described for **12a**. (2.7 g, 56%): ^{1}H NMR (400 MHz, DMSO-d6): δ 7.26 (1H, d, *J*=2 Hz), 7.34 (1H, dd, *J*=8.4, 2 Hz), 7.80 (1H, d, *J*=8.4 Hz), 11.33 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 117.76, 121.21, 126.03, 126.95, 137.55, 137.90, 144.72; HRMS (ESI) m/z calcd for $C_7H_5ClN_2O_2S_2$ $[M+Na]^+$: 270.9378, found: 270.9373.

7-bromo-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (12c). The white compound was obtained from **11c** (2.4 g, 8.63 mmol) by following the experimental conditions described for **12a** with the slight modification that the crude material was dissolved in 1:1 hydromethanolic solution of sodium bicarbonate instead of an aqueous solution of sodium bicarbonate by heating the mixture until most of the insoluble material dissolved. Charcoal was added to the suspension and filtered. The filtrate was adjusted to pH 1 with 12 N HCl, and the white precipitate was collected by filtration, washed with water, and air dried (1.35 g, 53%): ^{1}H NMR (400 MHz, DMSO-d6): δ 5.08 (br, 1H), 7.32 (1H, d, *J*=8.8 Hz), 7.85 (1H, d, *J*=8.7 Hz), 7.95 (s, 1H), 11.45 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 118.22, 120.67, 123.82, 126.08, 135.15, 136.82, 157.57; HRMS (ESI) m/z calcd for $C_7H_5BrN_2O_2S_2$ $[M+Na]^+$: 314.8873, found: 314.8863.

2*H*-benzo[*e*][1,2,4]thiadiazine-3(4H)-thione 1,1-dioxide (12d). The white compound was obtained from **11d** (4.9 g, 24.72 mmol) by following the experimental conditions described for **12a** (2.67 g, 50%): ^{1}H NMR (400 MHz, DMSO-d6): δ 7.38 (m, 2H), 7.70 (1H, t, *J*=7.8 Hz), 7.80 (1H, d, *J*=7.4 Hz), 12.12 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 115.52, 121.14, 125.21, 126.83, 136.59, 138.94, 144.16, 144.66; HRMS (ESI) m/z calcd for $C_7H_6N_2O_2S_2$ $[M+Na]^+$: 236.9768, found: 236.9765.

7-Fluoro-3-methylsulfanyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (13a). 7-Fluoro-3-thioxo-3,4dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (**12a**, 2.8 g, 12.06 mmol) was dissolved in a 1:1 hydromethanolic solution of sodium bicarbonate (5 g/ 200 mL). Methyl iodide was added (1.5 mL, 24.12 mmol) and the solution was stirred for 1h. The resulting suspension was adjusted to pH 5 using 6N HCl. The suspension was concentrated under reduced pressure, and the white precipitate was collected by filtration, washed with water, and air dried (1.67 g, 89%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.53 (s, 2H), 7.33 (m, 1H), 7.58 (1H, t, *J*=8.8 Hz), 7.55(1H, dd, *J*=7.5, 2.8 Hz), 12.61 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ d= 13.85, 109.80, 120.06, 121.91, 122.83, 132.77, 157.84,161.61; HRMS (ESI) m/z calcd for $C_8H_7FN_2O_2S_2$ $[M+Na]^+$: 268.9831, found: 268.9832.

6-Chloro-3-methylsulfanyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (13b). The white compound was obtained from **12b** (2.5, 10.05 mmol) by following the experimental conditions described for **13a** (2.23 g, 84%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.52 (s, 2H), 7.26 (1H, d, *J*=2 Hz), 7.34 (1H, dd, *J*=8.5, 2 Hz), 7.80 (1H, d, *J*=8.5 Hz), 12.61 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 13.90, 116.83, 120.76, 126.12, 126.48, 137.20, 137.87,161.88; HRMS (ESI) m/z calcd for $C_8H_7ClN_2O_2S_2$ $[M+Na]^+$: 284.9535, found: 284.9527.

7-Bromo-3-methylsulfanyl-4H-1,2,4-benzothiadiazine 1,1-Dioxide (13c). The white compound was obtained from **12c** (3.73 g, 12.72 mmol) by following the experimental conditions described for **13a** (3.19 g, 81%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.52 (s, 2H), 7.24 (1H, d, *J*=8.8 Hz), 7.83 (1H, dd, *J*=8.7, 2.2 Hz), 7.93 (1H, d, *J*=2.1 Hz), 12.65 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 13.88, 117.22, 119.87, 123.43, 125.97, 135.26, 136.60, 161.75; HRMS (ESI) m/z calcd for $C_8H_7BrN_2O_2S_2$ $[M+Na]^+$: 328.90300, found: 328.90244.

3-(methylthio)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (13d). The white compound was obtained from **12d** (1.92 g, 8.96 mmol) by following the experimental conditions described for **13a** (1.84 g, 90%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.52 (s, 2H), 7.28 (1H, d, *J*=8.7 Hz), 7.41 (1H, t, *J*=7.2 Hz), 7.67 (1H, d, *J*=8.7 Hz), 7.78 (1H, dd,

J=7.9, 2 Hz), 12.51 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 13.98, 117.31, 122.08, 123.85, 126.41, 133.73, 135.99, 161.38; HRMS (ESI) m/z calcd for $C_8H_8N_2O_2S_2$ $[M+Na]^+$: 250.9924, found: 250.9920.

General Procedures for the Synthesis of 3-(Alkylamino)-7-halo-4H-1,2,4-benzothiadiazine 1,1-Dioxides (14a-a41) (14b-36) (14c-35c) (14d-32d).

Method A. The appropriate 3-methylsulfanyl-4H-1,2,4-benzothiadiazine1,1-dioxide **(13a-13d)** (0.25g) and an appropriate alkylamine (0.7 mL) were dissolved in 1,4-dioxane (8 mL) in a sealed vessel and heated for 24h at 130 °C. The solvent and the excess amine were removed in vacuo, and the residue was dissolved in an aqueous 2% w/v solution of NaOH (7 mL). This solution was treated with charcoal and was filtered. The filtrate was adjusted to pH 1 using 6N HCl. The precipitated compound was collected by filtration, washed with water and air dried. The dried compound was suspended in an aqueous solution of sodium bicarbonate $NaHCO_3$ (1 g/40 mL). The alkaline solution was treated with charcoal and filtered; the filtrate was adjusted to pH 4-5 with 6N HCl. The white precipitate was collected by filtration, washed twice with water, and air dried.

Method B. A solution of the appropriate 3-methylsulfanyl-4H-1,2,4-benzothiadiazine1,1-dioxide **(13a-13d)** (0.25g) and the appropriate amine (5mL) was heated in a sealed vessel for 48 hr at 120°C. The solvent and excess amine was removed in vacuo, and the residue was dissolved in an aqueous 2% w/v solution of sodium hydroxide (7 mL). This solution was treated with charcoal and was filtered. The filtrate was adjusted to pH 1 using 6N HCl. The precipitated compound was collected by filtration, washed with water and air dried. The dried compound was suspended in an aqueous solution of sodium bicarbonate $NaHCO_3$ (1 g/40 mL). The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 4-5 with 6N HCl. The white precipitate was collected by filtration, washed twice with water, and air dried.

7-Fluoro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (14a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (79%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.16 (6H, d, *J*=6.3 Hz), 3.91 (m, 1H), 7.09 (s,1H), 7.26 (q, 1H), 7.50 (m, 1H), 10.42 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 22.66, 43.27, 109.18, 119.50, 120.69, 132.79, 150.96, 156.77,159.19; HRMS (ESI) m/z calcd for $C_{10}H_{12}FN_3O_2S$ $[M+Na]^+$: 280.0532, found: 280.0541.

6-Chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (14b). The white compound was obtained from **13b** by following the experimental conditions described for Method **A** (76%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.18 (6H, d, *J*=6.4 Hz), 3.93 (m, 1H), 7.09 (br, 1H), 7.27 (m, 1H), 7.47 (m, 1H), 10.40 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 22.67, 43.26, 109.18, 119.50, 120.68, 132.79, 150.96, 156.77,159.19; HRMS (ESI) m/z calcd for $C_{10}H_{12}ClN_3O_2S$ $[M+Na]^+$: 296.0236, found: 296.0237.

7-Bromo-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (14c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (81%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.16 (6H, d, *J*=6.5 Hz), 3.91 (m, 1H), 7.16 (2H, d, *J*=8.3 Hz), 7.70 (1H, dd, *J*=8.7, 2.1 Hz), 7.76 (1H, d, *J*=2.1 Hz), 10.48 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 22.62, 43.30, 114.97, 119.59, 124.61, 125.34, 135.42, 145.54,150.68; HRMS (ESI) m/z calcd for $C_{10}H_{12}BrN_3O_2S$ $[M+Na]^+$: 339.9731, found: 339.9716.

3-(isopropylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (14d). The white compound was obtained from **13d** by following the experimental conditions described for Method **A** (76%): ^{1}H NMR (400 MHz, DMSO-d6 δ 1.16 (6H, d, *J*=6.5 Hz), 3.93 (m, 1H), 6.97 (s,1H), 7.18 (1H, d, *J*=8.2 Hz), 7.24 (1H, t, *J*=7.8 Hz), 7.54 (1H, t, *J*=8.7 Hz), 7.65 (1H, dd, *J*=7.8, 2 Hz), 10.31 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 22.70, 43.13, 116.96, 123.12, 123.24, 124.10, 132.78, 136.10,150.82; HRMS (ESI) m/z calcd for $C_{10}H_{13}N_3O_2S$ $[M+Na]^+$: 262.0626, found: 262.0629.

7-fluoro-3-((3-methylbutan-2-yl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (15a). The white compound was obtained from **13a** by following the experimental conditions described for Method **B** (67%): ^{1}H NMR (400 MHz, DMSO-d6): δ 0.87 (q, 6H), 1.09 (3H, d, *J*=6.6 Hz), 1.74 (m, 1H), 3.69 (m, 1H), 6.96 (br,1H), 7.24 (br, 1H), 7.49 (m, 2H), 10.32 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 17.47, 18.70, 18.97, 32.70, 52.08, 109.19, 119.39, 120.67, 132.70, 124.10, 151.42, 156.77, 159.19; HRMS (ESI) m/z calcd for $C_{12}H_{16}FN_3O_2S$ $[M+Na]^+$: 308.0844 found: 308.0844.

6-chloro-3-((3-methylbutan-2-yl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (15b). The white compound was obtained from **13b** by following the experimental conditions described for Method **B** (64%): ^{1}H NMR (400 MHz, DMSO-d6): δ 0.90 (q, 6H), 1.08 (3H, d, *J*=6.6 Hz), 1.74 (m, 1H), 3.69 (m, 1H), 6.96 (br,1H), 7.24 (br, 1H), 7.50 (m, 2H), 10.31 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 17.48, 18.70, 18.97, 32.70, 52.08,

109.19, 119.39, 120.43, 132.71, 151.42, 156.77, 159.19; HRMS (ESI) m/z calcd for $C_{12}H_{16}ClN_3O_2S$ $[M+Na]^+$: 324.0550 found: 324.0565.

7-bromo-3-((3-methylbutan-2-yl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (15c). The white compound was obtained from **13c** by following the experimental conditions described for Method **B** (71%): ^{1}H NMR (400 MHz, DMSO-d6): δ 0.89 (q, 6H), 1.08 (3H, d, *J*=6.6 Hz), 1.76 (m, 1H), 3.71 (m, 1H), 6.99 (br,1H), 7.16 (1H, d, *J*=8.7 Hz), 7,72 (1H, dd, *J*=8.7, 1.9 Hz), 7.75 (1H, d, *J*=1.9 Hz), 10.37 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 17.45, 18.71, 18.97, 32.68, 52.11, 114.95, 119.57, 124.68, 125.35, 135.36, 135.52, 151.16; HRMS (ESI) m/z calcd for $C_{12}H_{16}BrN_3O_2S$ $[M+Na]^+$: 368.00443 found: 368.004.

3-((3-methylbutan-2-yl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (15d). The white compound was obtained from **13d** by following the experimental conditions described for Method **B** (73%): ^{1}H NMR (400 MHz, DMSO-d6): δ 0.90 (q, 6H), 1.10 (3H, d, *J*=6.6 Hz), 1.75 (m, 1H), 3.72 (m, 1H), 6.88 (br,1H), 7.16 (1H, d, *J*=7.4 Hz), 7,24 (1H, t, *J*=8.1 Hz), 7.54 (1H, t, *J*=8.2 Hz), 7.66 (1H, dd, *J*=7.8, 2 Hz), 10.23 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 17.47, 18.69, 18.97, 32.68, 51.89, 116.93, 123.17, 123.25, 124.09,132.78,136.04, 151.27; HRMS (ESI) m/z calcd for $C_{12}H_{17}N_3O_2S$ $[M+Na]^+$: 290.0939 found: 290.0947

3-((4-chlorobenzyl)amino)-7-fluoro-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (16a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (73%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.46 (s, 2H), 7.28 (q, 1H), 7.36 (2H, d, *J*=8.5 Hz), 7.41-7.52 (m, 4H), 7.70 (br, 1H), 10.89 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.67, 109.48, 119.62, 120.80, 123.77, 127.64, 128.78, 129.50, 132.07, 132.94, 138.15, 151.82, 156.83, 159.25; HRMS (ESI) m/z calcd for $C_{14}H_{11}ClFN_3O_2S$ $[M+Na]^+$: 362.01422 found: 362.0137.

6-chloro-3-((4-chlorobenzyl)amino)-4*H*-benzo*[e]*[1,2,4]thiadiazine 1,1-dioxide (16b). The white compound was obtained from **13b** by following the experimental conditions described for Method **A** (76%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.47 (2H, d, *J*=5.8 Hz), 7.29 (q, 2H), 7.37 (2H, d, *J*=8.5 Hz), 7.41(2H, d, *J*=8.5 Hz), 7.68 (1H, d, *J*=8.5 Hz), 7.70 (br, 1H) 10.84 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.67, 116.64, 121.84, 124.34, 125.45, 128.80, 129.54, 131.31, 132.11, 137.01, 137.53, 138.00, 151.47; HRMS (ESI) m/z calcd for $C_{14}H_{11}Cl_2N_3O_2S$ $[M+Na]^+$: 377.9846 found: 377.9839.

7-bromo-3-((4-chlorobenzyl)amino)-4*H*-benzo*[e]*[1,2,4]thiadiazine 1,1-dioxide dioxide (16c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (68%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.46 (2H, d, *J*=5.8 Hz), 7.19 (1H, d, *J*=8.7 Hz), 7.36 (2H, d, *J*=8.7 Hz), 7.40 (2H, d, *J*=8.7 Hz), 7.78 (m, 3H), 7.70 (s, 1H), 11.01 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.68, 115.14, 119.63, 124.53, 125.41, 128.80, 129.52, 132.11, 135.45, 135.66, 138.01, 151.52; HRMS (ESI) m/z calcd for $C_{14}H_{11}ClBrN_3O_2S$ $[M+Na]^+$: 421.9341 found: 421.93244.

3-((4-chlorobenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (16d). The white compound was obtained from **13d** by following the experimental conditions described for Method **A** (67%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.47 (2H, d, *J*=5.8 Hz), 7.21 (1H, d, *J*=8.5 Hz), 7.26 (1H, t, *J*=7.8 Hz), 7.37 (2H, d, *J*=8.5 Hz), 7.41 (2H, d, *J*=8.5 Hz), 7.56 (1H, t, *J*=8.3 Hz), 7.61 (br, 1H), 7.65 (1H, dd, *J*=7.8, 2 Hz), 10.85 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.57, 117.02, 119.63, 123.05, 123.30, 124.26, 128.79, 129.51, 132.05, 132.89, 136.13, 138.22, 151.62; HRMS (ESI) m/z calcd for $C_{14}H_{11}ClN_3O_2S$ $[M+Na]^+$: 344.0236 found: 344.0236.

7-fluoro-3-((4-fluorobenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (17a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (81%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.46 (s, 2H), 7.16 (2H, t, *J*=8.8 Hz), 7.27 (q, 1H), 7.38 (m, 2H), 7.45 (1H, t, *J*=8.8 Hz), 7.52 (1H, dd, *J*=7.5, 2.8 Hz), 7.68 (br, 1H), 10.68 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.66, 109.23, 115.48, 119.60, 120.77, 123.80, 129.77, 132.92, 135.20, 151.80, 156.84, 159.26, 160.58, 163.00; HRMS (ESI) m/z calcd for $C_{14}H_{11}F_2N3O_2S$ $[M+Na]^+$: 346.0437 found: 346.0428.

6-chloro-3-((4-fluorobenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (17b). The white compound was obtained from **13b** by following the experimental conditions described for Method **A** (77%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.47 (s, 2H), 7.18 (2H, t, *J*=8.8 Hz), 7.28 (q,1H), 7.39 (q, 2H), 7.46 (1H, t, *J*=8.8 Hz), 7.53 (1H, dd, *J*=7.5, 2.8 Hz), 7.67 (br, 1H), 10.83 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.65, 109.50, 115.69, 119.50, 120.79, 123.86, 129.76, 132.84, 135.19, 151.75, 156.85, 159.27, 160.58, 163.00; HRMS (ESI) m/z calcd for $C_{14}H_{11}ClFN3O_2S$ $[M+Na]^+$: 362.0142 found: 362.0160.

7-bromo-3-((4-fluorobenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (17c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (63%): ^{1}H NMR (400

MHz, DMSO-d6): δ 4.45 (2H, d, *J*=5.8 Hz), 7.18 (3H, d, *J*=8.8 Hz), 7.38 (q, 2H), 7.72 (2H, dd, *J*=8.7, 2.2 Hz), 7.78 (1H, d, *J*=2.2 Hz), 10.94 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.66, 115.12, 115.50, 115.71, 119.63, 124.56, 125.40, 129.77, 135.11, 135.46, 135.65, 151.49, 160.59, 163.00; HRMS (ESI) m/z calcd for $C_{14}H_{11}BrFN3O_2S$ $[M+Na]^+$: 405.963708 found: 405.96021.

3-((4-fluorobenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (17d). The white compound was obtained from 13**d** by following the experimental conditions described for Method **A** (66%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.45 (2H, d, *J*=5.8 Hz), 7.18 (3H, t, *J*=8.8 Hz), 7.26 (2H, d, *J*=7.4 Hz), 7.39 (q, 2H), 7.55 (2H, t, *J*=7.2 Hz), 7.69 (1H, d, *J*=7.8 Hz), 10.77 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.56, 115.49, 115.70, 117.02, 123.07, 123.70, 124.24, 129.77, 132.87, 135.30, 136.13 , 151.59, 160.58, 162.99; HRMS (ESI) m/z calcd for $C_{14}H_{12}FN3O_2S$ $[M+Na]^+$: 328.0531 found: 328.0530.

7-fluoro-3-(phenethylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (18a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (76%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.86 (2H, t, *J*=7.2 Hz), 3.48 (q, 2H), 7.20- 7.34 (m,7H), 7.45 (1H, t, *J*=7.3 Hz), 7.52 (1H, dd, *J*=7.5, 2.8 Hz), 10.55 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 35.08, 43.42, 109.23, 119.47, 120.49, 120.72, 123.79, 126.74, 128.90, 129.15, 132.85, 139.32,151.69, 156.76, 159.21; HRMS (ESI) m/z calcd for $C_{15}H_{14}FN_3O_2S$ $[M+Na]^+$: 342.06884 found: 342.0687.

6-chloro-3-(phenethylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (18b). The white compound was obtained from **13b** by following the experimental conditions described for Method **A** (73%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.86 (2H, t, *J*=7.2 Hz), 3.47 (q, 2H), 7.20- 7.34 (m, 8H), 7.68 (1H, dd, *J*=8.4 Hz), 10.71 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 35, 42.43, 116.52, 121.86, 124.22, 125.43, 126.74, 128.89, 129.15, 132.85, 136.94, 137.52, 139.29, 151.39; HRMS (ESI) m/z calcd for $C_{15}H_{14}ClN_3O_2S$ $[M+Na]^+$: 358.0392 found: 358.0388.

7-bromo-3-(phenethylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (18c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (65%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.86 (2H, t, *J*=7.3 Hz), 3.48 (q, 2H), 7.15- 7.34 (m,7H), 7.70 (1H, dd, *J*=8.7, 2.2 Hz), 7.77 (1H, d, *J*=2.2 Hz), 10.79 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 35.03, 42.42, 115.01, 119.51, 124.52, 125.39,

126.75, 128.90, 129.14, 135.44, 135.58, 139.27,151.43; HRMS (ESI) m/z calcd for $C_{15}H_{14}BrN_3O_2S$ $[M+Na]^+$: 401.98878 found: 401.98571.

3-(ethylamino)-7-fluoro-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (19a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (71%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.12 (3H, t, *J*=7.1 Hz), 3.26 (m, 2H), 7.18 (br,1H), 7.26 (m, 1H), 7.42(m,1H), 7.49 (1H, dd, *J*=7.5, 2.8 Hz), 10.66 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 14.99, 36.03, 109.42, 119.44, 123.82, 132.89, 151.59, 156.78, 159.71; HRMS (ESI) m/z calcd for $C_9H_{10}FN_3O_2S$ $[M+Na]^+$: 266.037547 found: 266.0385.

6-chloro-3-(ethylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (19b). The white compound was obtained from **13b** by following the experimental conditions described for Method **A** (79%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.12 (3H, t, *J*=7.1 Hz), 3.25 (m, 2H), 7.19 (br,1H), 7.27 (m, 1H), 7.42- 7.50 (m, 2H), 10.66 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 15.00, 36.03, 109.43, 119.44, 120.45, 132.88, 151.58, 156.75, 159.17; HRMS (ESI) m/z calcd for $C_9H_{10}ClN_3O_2S$ $[M+Na]^+$: 282.0079 found: 282.0110.

7-bromo-3-(ethylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (19c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (69%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.12 (3H, t, *J*=7.1 Hz), 3.25 (m, 2H), 7.19 (1H, d, *J*=8.5 Hz), 7.23 (br, 1H), 7.71 (1H, dd, *J*=8.7, 2.2 Hz), 7.75 (1H, d, *J*=2.2 Hz), 10.72 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 14.96, 36.05, 114.92, 119.54, 124.60, 125.35, 135.54, 151.33; HRMS (ESI) m/z calcd for $C_9H_{10}BrN_3O_2S$ $[M+Na]^+$: 325.95748 found: 325.95503.

3-(ethylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (19d). The white compound was obtained from **13d** by following the experimental conditions described for Method **A** (74%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.12 (3H, t, *J*=7.1 Hz), 3.25 (m, 2H), 7.07 (br,1H), 7.19 (1H, d, *J*=8 Hz), 7.24 (1H, t, *J*=8 Hz), 7.53 (1H, t, *J*=8.7 Hz), 7.64 (1H, dd, *J*=7.8, 2 Hz), 10.56 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 15.03, 35.93, 116.90, 123.09, 123.25, 124.06, 132.76, 136.21, 151.45; HRMS (ESI) m/z calcd for $C_9H_{11}N_3O_2S$ $[M+Na]^+$: 248.046969 found: 248.0466.

7-fluoro-3-((4-methoxybenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (20a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (82%): ^{1}H NMR (400 MHz, DMSO-d6): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.73 (s, 3H), 4.40 (s, 2H), 6.92 (2H, d, *J*=8.5 Hz), 7.27 (m,

3H), 7.45 (2H, t, *J*=8.7 Hz), 7.51 (1H, dd, *J*=7.5, 2.8 Hz) 7.60 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.85, 55.51, 109.21, 114.25, 119.54, 120.48, 120.72, 123.81, 129.14, 130.83, 133.06, 151.80, 156.77, 158.90, 159.19; HRMS (ESI) m/z calcd for $C_{15}H_{14}FN_3O_3S$ $[M+Na]^+$: 358.063762 found: 358.0616.

7-bromo-3-((4-methoxybenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (20c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (75%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.73 (s, 3H), 4.39 (2H, d, *J*=5.8 Hz), 6.92 (m, 2H), 7.19 (1H, d, *J*=8.7 Hz), 7.26 (2H, d, *J*=8.7 Hz), 7.65 (br, 1H), 7.71 (1H, dd, *J*=8.7, 2.2 Hz), 7.78 (1H, d, *J*=2.2 Hz), 10.85 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.86, 55.54, 114.28, 115.05, 119.59, 124.59, 125.39, 129.17, 130.66, 135.47, 135.61, 151.53, 158.94; HRMS (ESI) m/z calcd for $C_{15}H_{14}BrN_3O_3S$ $[M+Na]^+$: 417.983695 found: 417.98112.

3-((4-methoxybenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (20d). The white compound was obtained from **13d** by following the experimental conditions described for Method **A** (69%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.73 (s, 3H), 4.39 (2H, d, *J*=5.8 Hz), 6.93 (1H, d, *J*=8.7 Hz), 7.18 (1H, d, *J*=8.2 Hz), 7.37 (m, 3H), 7.49 (br, 1H), 7.55 (2H, t, *J*=8.3 Hz), 7.66 (1H, dd, *J*=7.8, 2 Hz), 10.68 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.75, 55.53, 114.27, 116.98, 123.10, 123.30, 124.18, 129.16, 130.85, 132.84, 136.15, 151.53, 158.91; HRMS (ESI) m/z calcd for $C_{15}H_{15}N_3O_3S$ $[M+Na]^+$: 340.073184 found: 340.0722 .

3-(benzylamino)-7-fluoro-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (21a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (61%): ^{1}H NMR (400 MHz, DMSO-d6):): δ 4.48 (2H, d, *J*=5.8 Hz), 7.26 (m, 2H), 7.34 (m, 4H), 7.51 (m, 2H) 7.65 (br, 1H), 10.70 (br, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 44.35, 109.25, 109.49, 119.45, 120.54, 123.87, 127.84, 129.07, 132.94, 138.97, 151.86, 156.83, 159.25; HRMS (ESI) m/z calcd for $C_{14}H_{12}FN_3O_2S$ $[M+Na]^+$: 328.0531 found: 328.0523.

3-(benzylamino)-7-bromo-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (21c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (70%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.48 (2H, d, *J*=5.8 Hz), 7.21 (1H, d, *J*=8.7 Hz), 7.27 (m, 1H), 7.34 (m, 4H), 7.72 (2H, dd, *J*=8.7, 2.2 Hz), 7.78 (1H, d, *J*=2.2 Hz), 10.87 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 44.33, 115.08, 119.63, 124.57, 125.39, 127.59, 127.67, 128.87, 135.50, 135.64, 138.84, 151.57; HRMS (ESI) m/z calcd for $C_{14}H_{12}BrN_3O_2S$ $[M+Na]^+$: 387.97313 found: 387.97103.

3-(benzylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (21d). The white compound was obtained from **13d** by following the experimental conditions described for Method **A** (67%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.49 (2H, d, *J*=5.8 Hz), 7.22 (1H, d, *J*=8.2 Hz), 7.26 (m, 2H), 7.34 (4H, d, *J*=4.5 Hz), 7.55 (2H, t, *J*=8.3 Hz), 7.70 (1H, d, *J*=7.8 Hz), 10.74 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 44.26, 117.03, 123.10, 123.31, 124.22, 127.56, 127.67, 128.87, 136.16, 139.03, 151.65; HRMS (ESI) m/z calcd for $C_{14}H_{13}N_3O_2S$ $[M+Na]^+$: 310.0626 found: 310.0621.

7-fluoro-3-(methylsulfinyl)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (13aa). The 7-fluoro-3-methylsulfanyl-4H-1,2,4-benzothiadiazine1,1-dioxide (**13a**, 0.5g, 2.03 mmol) was suspended in an aqueous solution of sodium carbonate (2.2 g/25 ml) and the aqueous solution 2 N NaOH was added until the mixture was completely dissolved. At room temperature, bromine (0.2 mL, 2.03 mmol) was added under vigorous stirring for 30 min, the resulting suspension was adjusted to pH 2-3 by adding 6 N HCl. The insoluble compound was collected by filtration, washed twice with water, and suspended under stirring in methanol (10 mL). The resultant white precipitate was collected by filtration, washed with water and methanol, and air dried (0.443 g, 83%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.45 (s, 3H), 7.66 (m, 1H), 7.76-7-81 (m, 2H); HRMS (ESI) m/z calcd for $C_8H_7FN_2O_3S_2$ $[M+Na]^+$: 284.9780, found: 284.9831.

6-chloro-3-(methylsulfinyl)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (13ba). The white compound was obtained from **13b** (1 g, 3.81 mmol) by following the experimental conditions described for **13aa** (0.965 g, 91%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.44 (s, 3H), 7.66 (m, 1H), 7.74-7-80 (m,2H) (s, 1H); HRMS (ESI) m/z calcd for $C_8H_7ClN_2O_3S_2$ $[M+Na]^+$: 300.9484, found: 300.9526.

7-bromo-3-(methylsulfinyl)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (13ca). The white compound was obtained from **13c** (1 g, 3.26 mmol) by following the experimental conditions described for **13aa** (0.725 g, 75%): ^{1}H NMR (400 MHz, DMSO-d): δ 3.45 (s, 3H), 7.66 (m, 1H), 7.76-7-81 (m,1H); HRMS (ESI) m/z calcd for $C_8H_7BrN_2O_2S_2$ $[M+Na]^+$: 328.9030, found: 328.9018.

7-fluoro-3-(cyclopentylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (22a). A mixture of 7-fluoro-3-methylsulfinyl4H-1,2,4-benzothiadiazine 1,1-dioxide (**13aa**) (0.25 g, 0.953 mmol) and cyclopentylamine (0.3 mL,2.89 mmol) was dissolved in 1,4-Dioxane (5 mL) and heated in a sealed vessel overnight at 160 °C. The

solvent and excess amine was removed in vacuo, and the residue was dissolved in a hydromethanolic (1:1) 2% w/v solution of NaOH (10mL). The alkaline solution was treated with charcoal and was filtered, and the filtrate was adjusted to pH 4-5 with 6N HCl. The precipitate was collected by filtration, washed with water, and air dried. The dried compound was suspended in an aqueous solution of sodium bicarbonate $NaHCO_3$ (1 g/40 mL). The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 4-5 with 6 N HCl. The white precipitate was collected by filtration, washed with water, and air dried. The white compound was recrystallized from methanol/water (0.185 g, 68%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.46 -1.66 (m, 6H), 1.90 (m, 2H), 4.07 (m,1H), 7.27 (s, 2H), 7.45 (m, 2H), 10.35 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 23.72, 32.71, 52.82, 109.42, 119.46, 120.66, 132.74, 151.31, 156.78, 159.20; HRMS (ESI) m/z calcd for $C_{12}H_{14}FN_3O_2S$ $[M+Na]^+$: 306.0688, found: 306.0675.

6-chloro-3-(cyclopentylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (22b). The white compound was obtained from **13ba** (0.25 g, 0.897 mmol) by following the experimental conditions described for **22a** (0.166 g, 61%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.48 -1.67 (m, 6H), 1.91 (m, 2H), 4.06 (m,1H), 7.29 (2H, dd, *J*=8.5, 2 Hz), 7.39 (br, 1H),7.69(1H, d, *J*=8.7 Hz), 10.32 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 22.65, 32.66, 52.85, 116.63, 121.98, 124.23, 125.38, 136.91, 137.44, 150.99; HRMS (ESI) m/z calcd for $C_{12}H_{14}ClN_3O_2S$ $[M+Na]^+$: 322.0392, found: 322.0407.

7-bromo-3- (cyclopentylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (22c). The white compound was obtained from **13ca** (0.25 g,0.77 mmol) by following the experimental conditions described for **22a** (0.132 g, 49%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.48 -1.67 (m, 6H), 1.91 (m, 2H), 4.06 (m,1H), 7.19 (br, 2H), 7.28 (br, 1H), 7.76 (m, 1H), 10.40 (s, 1H);); ^{13}C NMR (100 MHz, DMSO-d6): δ 23.64, 32.69, 52.84, 114,99, 119.63, 124.65, 125.34, 135.40, 135.54, 151.05; HRMS (ESI) m/z calcd for $C_{12}H_{14}BrN_3O_2S$ $[M+Na]^+$: 365.98878, found: 365.98796.

7-fluoro-3-((3-phenylpropyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (23a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (76%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.83 (m, 2H), 2.63 (2H, t, *J*=7.8 Hz), 3.24 (q, 2H) 7.16- 7.30 (m, 7H), 7.42- 7.51 (m, 2H) 10.68 (br, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 31.01, 32.78, 109.20, 109.45, 119.40, 120.47, 120.70, 123.90,

126.28, 128.79, 132.87, 141.86, 151.75, 156.78, 159.19; HRMS (ESI) m/z calcd for $C_{16}H_{16}FN_3O_2S$ $[M+Na]^+$: 356.084497 found: 356.0811.

7-bromo-3-((3-phenylpropyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (23c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (74%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.83 (m, 2H), 2.63 (2H, t, *J*=7.8 Hz), 3.26 (q, 2H) 7.16- 7.31 (m, 7H), 7.71 (1H, dd, *J*=8.7, 2.2 Hz), 7.76 (1H, d, *J*=2.2 Hz), 10.73 (br, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 30.95, 32.78, 114.97, 119.56, 124.61, 125.36, 126.28, 128.74, 128.79, 135.50, 135.55, 141.85, 151.49; HRMS (ESI) m/z calcd for $C_{16}H_{16}BrN_3O_2S$ $[M+Na]^+$: 416.00443 found: 416.00367.

3-((3-phenylpropyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (23d). The white compound was obtained from **13d** by following the experimental conditions described for Method **A** (61%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.83 (m, 2H), 2.64 (2H, t, *J*=7.5 Hz), 3.24 (q, 2H) 7.17- 7.31 (m, 8H), 7.54 (1H, t, *J*=8.3 Hz), 7.65 (1H, d, *J*=7.8 Hz), 10.59 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 31.04, 32.80, 116.94, 123.10, 123.27, 124.09, 126.28, 128.75, 128.80, 132.78, 136.19, 141.89, 151.61; HRMS (ESI) m/z calcd for $C_{16}H_{17}N_3O_2S$ $[M+Na]^+$: 338.093919 found: 338.0938.

7-fluoro-3-((1-phenylethyl)amino)-4H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (24a). The white compound was obtained from **13a** by following the experimental conditions described for Method **B** (76%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.48 (3H, d, *J*=7 Hz), 5.02 (m, 1H), 7.27 (m, 2H), 7.39 (m, 4H), 7.48 (m, 2H), 7.70 (br, 1H), 10.52 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 32.91, 50.47, 109.48, 119.67, 120.54, 122.89, 126.41, 127.55, 128.91, 132.66, 143.84, 150.95, 156.85, 159.27; HRMS (ESI) m/z calcd for $C_{15}H_{14}FN_3O_2S$ $[M+Na]^+$: 342.068847 found: 342.0676.

6-chloro-3-((1-phenylethyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (24b). The white compound was obtained from **13b** by following the experimental conditions described for Method **B** (59%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.48 (3H, d, *J*=7 Hz), 5.02 (m, 1H), 7.28 (m, 3H), 7.38 (m, 4H), 7.66 (1H, d, *J*=8.3 Hz), 7.84 (s, 1H), 10.58 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 22.87, 50.48, 116.64, 120.80, 121.88, 124.34, 125.47, 126.43, 127.57, 128.92, 137.00, 137.63, 143.74, 150.68; HRMS (ESI) m/z calcd for $C_{15}H_{14}ClN_3O_2S$ $[M+Na]^+$: 358.039297 found: 358.0385.

7-bromo-3-((1-phenylethyl)amino)-4H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (24c). The white compound was obtained from **13c** by following the experimental conditions described for Method **B** (46%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.49 (3H, d, *J*=7 Hz), 5.02 (m, 1H), 7.19 (1H, d, *J*=8.7 Hz), 7.26 (m, 1H), 7.38 (m, 4H), 7.71 (2H, dd, *J*=8.7, 2.2 Hz), 7.76 (1H, d, *J*=2.2 Hz), 10.57 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 22.84, 50.50, 115.12, 119.69, 124.58, 125.38, 126.43, 127.14, 127.57, 128.91, 129.23, 135.31, 135.61, 143.73, 150.71; HRMS (ESI) m/z calcd for $C_{15}H_{14}BrN_3O_2S$ $[M+Na]^+$: 401.98878 found: 401.98855.

3-((1-phenylethyl)amino)-4H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (24d). The white compound was obtained from **13d** by following the experimental conditions described for Method **B** (52%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.47 (3H, d, *J*=6.9 Hz), 5.03 (m, 1H), 7.19 (1H, d, *J*=8.2 Hz), 7.25 (m, 2H), 7.39 (m, 4H), 7.54 (2H, t, *J*=8.3 Hz), 7.58 (br, 1H), 7.67 (1H, d, *J*=7.6 Hz), 10.42 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 22.96, 50.36, 117.06, 123.08, 123.28, 124.26, 126.41, 127.54, 128.92, 132.86, 135.98, 143.91, 150.82; HRMS (ESI) m/z calcd for $C_{15}H_{15}N_3O_2S$ $[M+Na]^+$: 324.078269 found: 324.0782.

7-fluoro-3-((4-(trifluoromethyl)benzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (25a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (53%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.58 (2H, d, *J*=5.9 Hz), 7.30 (m, 1H), 7.45- 7.56 (m, 4H), 7.71 (2H, d, *J*=8.2 Hz), 7.76 (br, 1H), 11.00 (br, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 43.94, 109.27, 119.52, 120.62, 123.41, 123.82, 125.69, 126.11, 128.18, 132.81, 144.05, 151.82, 156.88, 159.30; HRMS (ESI) m/z calcd for $C_{15}H_{11}F_4N_3O_2S$ $[M+Na]^+$: 396.040581 found: 396.0388.

6-chloro-3-((4-(trifluoromethyl)benzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (25b). The white compound was obtained from **13b** by following the experimental conditions described for Method **A** (48%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.50 (2H, d, *J*=5.8 Hz), 7.30 (s, 1H), 7.32 (1H, d, *J*=2 Hz), 7.37 (2H, d, *J*=8.5 Hz), 7.45 (2H, d, *J*=8.7 Hz), 7.70 (1H, d, *J*=8.7 Hz), 7.86 (s, 1H), 10.90 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 43.66, 116.65, 119.26, 121.50, 121.81, 121.87, 124.35, 125.45, 129.55, 137.01, 137.53, 138.48, 147.83, 151.48; HRMS (ESI) m/z calcd for $C_{15}H_{11}ClF_3N_3O_2S$ $[M+Na]^+$: 412.011031 found: 412.1504.

7-bromo-3-((4-(trifluoromethyl)benzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (25c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (39%): ^{1}H

NMR (400 MHz, DMSO-d6): δ 4.58 (2H, d, *J*=5.2 Hz), 7.22 (1H, d, *J*=8.7 Hz), 7.55 (2H, d, *J*=8.1 Hz), 7.73 (m, 3H), 7.78 (1H, d, *J*=2.2 Hz), 7.83 (br, 1H), 11.04 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 43.94, 115.16, 119.28, 123.41, 124.50, 125.41, 125.71, 126.11, 127.99, 128.21, 128.30, 135.51, 135.69, 143.97, 151.61; HRMS (ESI) m/z calcd for $C_{15}H_{11}BrF_3N_3O_2S$ $[M+Na]^+$: 455.960514 found: 455.95914.

7-fluoro-3-((2-methoxybenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (26a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (71%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.48 (s, 3H), 4.43 (2H, d, *J*=5.7 Hz), 6.93 (1H, t, *J*=7.3 Hz), 7.02 (1H, d, *J*=8.1 Hz), 7.28 (m, 3H), 7.46 (2H, t, *J*=8.7 Hz), 7.52 (1H, dd, *J*=7.5, 2.8 Hz), 10.78 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 55.83, 109.51, 111.03, 119.47, 120.68, 123.79, 126.14, 128.38, 129.05, 132.78, 151.84, 156.83, 157.21, 159.25; HRMS (ESI) m/z calcd for $C_{15}H_{14}FN_3O_3S$ $[M+Na]^+$: 358.063762 found: 358.0608.

6-chloro-3-((2-methoxybenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (26b). The white compound was obtained from **13b** by following the experimental conditions described for Method **A** (67%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.48 (s, 3H), 4.43 (2H, d, *J*=5.7 Hz), 6.93 (1H, t, *J*=7.3 Hz), 7.03 (1H, d, *J*=8.2 Hz), 7.28 (m, 4H), 7.66 (br, 1H), 7.68 (1H, d, *J*=8.3 Hz), 10.47 (br, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 55.86, 111.08, 116.57, 120.68, 121.84, 124.25, 125.43, 126.04, 128.44, 129.08, 136.99, 137.53, 151.55, 157.23; HRMS (ESI) m/z calcd for $C_{15}H_{14}ClN_3O_3S$ $[M+Na]^+$: 374.034212 found: 374.0336.

7-bromo-3-((2-methoxybenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (26c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (56%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.84 (s, 3H), 4.42 (2H, d, *J*=5.3 Hz), 6.93 (1H, t, *J*=7.3 Hz), 7.03 (1H, d, *J*=8.2 Hz), 7.18 (1H, d, *J*=8.5 Hz), 7.22 (1H, d, *J*=7.2 Hz), 7.28 (1H, t, *J*=7.7 Hz) 7.46 (br, 1H), 7.72 (1H, dd, *J*=8.7, 2 Hz), 7.77 (1H, d, *J*=2 Hz), 10.69 (br, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 55.86, 111.08, 115.00, 119.60, 120.69, 124.51, 125.40, 126.06, 128.42, 129.08, 135.61, 151.61, 157.22; HRMS (ESI) m/z calcd for $C_{15}H_{14}BrN_3O_3S$ $[M+Na]^+$: 417.983695 found: 417.98163.

7-fluoro-3-((4-methylbenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (27a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (74%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.28 (s, 3H), 4.44 (2H, d, *J*=5.7 Hz), 7.16 (2H, d, *J*=7.8 Hz), 7.22 (2H, d, *J*=7.8 Hz), 7.28 (m,

1H), 7.46 (m, 1H), 7.52 (1H, dd, *J*=7.5, 2.8 Hz), 7.64 (br, 1H), 10.81 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 21.13, 44.10, 109.49, 119.54, 120.53, 120.77, 123.89, 127.66, 129.40, 132.84, 135.86, 136.68, 151.75, 156.83, 157.21, 159.25; HRMS (ESI) m/z calcd for $C_{15}H_{14}FN_3O_2S$ $[M+Na]^+$: 342.068847 found: 342.0672.

7-bromo-3-((4-methylbenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (27c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (61%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.28 (s, 3H), 4.43 (2H, d, *J*=4.6 Hz), 7.15- 7.23 (m, 5H), 7.67 (br, 1H), 7.72 (1H, dd, *J*=8.7, 2.2 Hz), 7.77 (1H, d, *J*=2.2 Hz), 10.83 (s, 1H) ; ^{13}C NMR (100 MHz, DMSO-d6): δ 21.48, 44.10, 116.67, 121.90, 124.23, 125.42, 127.70, 129.40, 135.77, 136.70, 136.95, 137.65, 151.48; HRMS (ESI) m/z calcd for $C_{15}H_{14}BrN_3O_2S$ $[M+Na]^+$: 401.98878 found: 401.98857.

7-bromo-3-((3-methoxybenzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (28c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (74%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.74 (s, 3H), 4.44 (s, 2H), 6.85 (1H, d, *J*=7.9 Hz), 6.92 (2H, d, *J*=10.5 Hz), 7.20 (1H, d, *J*=8.6 Hz), 7.26 (1H, t, *J*=7.8 Hz), 7.72 (2H, dd, *J*=8.7, 2 Hz), 7.77 (1H, d, *J*=2 Hz), 10.79 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 44.28, 55.47, 113.01, 113.36, 115.05,119.68, 119.79, 124.57, 125.39, 129.95, 135.62, 140.45, 151.59, 159.81; HRMS (ESI) m/z calcd for $C_{15}H_{14}BrN_3O_3S$ $[M+Na]^+$: 417.983695 found: 417.98193.

7-Fluoro-3-((4-(trifluoromethoxy)benzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (30a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (59%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.51 (2H, d, *J*=5.7 Hz), 7.29 (q, 1H), 7.37 (2H, d, *J*=8.2 Hz), 7.44- 7.52 (m, 4H), 7.73 (br, 1H), 10.91 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.64, 109.51, 119.26, 119.59, 120.60, 121.51, 123.85, 129.50, 132.80, 138.63, 147.80, 151.77, 156.87, 159.29; HRMS (ESI) m/z calcd for $C_{15}H_{11}F_4N_3O_3S$ $[M+Na]^+$: 412.035496 found: 412.0304.

7-bromo-3-((4-(trifluoromethoxy)benzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (30c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (68%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.50 (2H, d, *J*=5 Hz), 7.21 (1H, d, *J*=8.7 Hz), 7.36 (2H, d, *J*=8.2 Hz), 7.45 (2H, d, *J*=8.5 Hz), 7.74 (1H, d, *J*=8.6, 2 Hz), 7.78 (2H, d, *J*=2 Hz), 10.84 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ

43.65, 115.10, 116.72, 119.26, 119.68, 121.49, 121.81, 124.54, 125.40, 129.53, 135.55, 135.64, 138,52, 147.82, 151.58; HRMS (ESI) m/z calcd for $C_{15}H_{11}BrF_3N_3O_3S$ $[M+Na]^+$: 471.955429 found: 471.95405.

3-((4-(trifluoromethoxy)benzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (30d). The white compound was obtained from **13d** by following the experimental conditions described for Method **A** (72%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.52 (2H, d, *J*=5.8 Hz), 7.22 (1H, d, *J*=8.2 Hz), 7.26 (1H, t, *J*=7.6 Hz), 7.37 (2H, d, *J*=8.2 Hz), 7.46 (2H, d, *J*=8.6 Hz), 7.56 (1H, t, *J*=8.2 Hz), 7.61 (br, 1H), 7.67 (1H, d, *J*=7.2 Hz), 10.83 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.55, 117.04, 119.27, 121.50, 121.81, 123.05, 123.30, 124.27, 129.50, 132.89, 136.12, 138.71, 147.79, 151.62; HRMS (ESI) m/z calcd for $C_{15}H_{12}F_3N_3O_3S$ $[M+Na]^+$: 394.044918 found: 394.0439.

3-((4-aminobenzyl)amino)-7-bromo-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (31c). The white compound was obtained from **13c** by following the experimental conditions described for Method **B** (48%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.26 (s, 2H), 5.03 (s, 2H) 6.52 (s, 2H), 7.00 (s, 2H), 7.16 (s, 1H), 7.46 (s, 1H), 7.76 (d, 2H), 10.15 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 44.28, 114.02, 114.17, 114.73, 119.76, 124.63, 125.35, 128.93, 130.36, 135.49, 135.89, 148.39, 151.56; HRMS (ESI) m/z calcd for $C_{14}H_{13}BrN_4O_2S$ $[M+Na]^+$: 402.984029 found: 402.98192.

3-(allylamino)-7-fluoro-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (32a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (81%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.88 (m, 3H), 5.12- 5.23 (m, 2H), 5.88 (m, 1H), 7.28 (q, 1H), 7.36 (br, 1H), 7.43- 7.57 (m, 2H), 10.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.15, 109.47, 116.23, 119.52, 120.75, 123.86, 132.80, 134.92, 151.65, 156.82, 159.24; HRMS (ESI) m/z calcd for $C_{10}H_{10}FN_3O_2S$ $[M+Na]^+$: 278.037547 found: 278.0351.

3-(allylamino)-6-chloro-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (32b). The white compound was obtained from **13b** by following the experimental conditions described for Method **A** (76%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.88 (m, 3H), 5.15 (m, 1H), 5.29 (m, 1H), 5.88 (m, 1H), 7.29 (s, 1H), 7.31 (1H, d, *J*=8.7 Hz), 7.50 (br, 1H), 7.68 (d, 1H), 10.74 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.16, 116.32, 116.61, 121.85, 124.28, 125.42, 134.81, 136.69, 137.53, 151.33; HRMS (ESI) m/z calcd for $C_{10}H_{10}ClN_3O_2S$ $[M+Na]^+$: 294.007997 found: 294.0068.

3-(allylamino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (32d). The white compound was obtained from **13d** by following the experimental conditions described for Method **A** (88%): ^{1}H NMR (400 MHz, DMSO-d6): δ 3.88 (m, 3H), 5.12- 5.23 (m, 2H), 5.88 (q, 1H), 7.21 (1H, d, *J*=8.2 Hz), 7.25 (2H, t, *J*=8.3 Hz), 7.55 (1H, t, *J*=8.3 Hz), 7.65 (1H, dd, *J*=7.8, 2 Hz), 10.64 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.05, 116.19, 116.98, 123.05, 123.28, 124.19, 132.82, 135.01, 136.13, 151.49; HRMS (ESI) m/z calcd for $C_{10}H_{11}N_3O_2S$ $[M+Na]^+$: 260.046969 found: 260.0338.

7-bromo-3-((4-fluorophenethyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (33c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (73%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.84 (2H, t, *J*=7.2 Hz), 3.47 (q, 2H), 7.14 (m, 4H), 7.29 (m, 2H), 7.73 (1H, dd, *J*=8.7, 2.2 Hz), 7.77 (1H, d, *J*=2.2 Hz), 10.78 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 34.15, 42.41, 115.01, 115.46, 115.66, 119.51, 124.53, 125.39, 130.91, 130.99, 135.44, 135.58, 151.46, 160.19, 162.59; HRMS (ESI) m/z calcd for $C_{15}H_{13}BrFN_3O_2S$ $[M+Na]^+$: 419.979358 found: 419.97927.

7-bromo-3-((4-methoxyphenethyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (34c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (67%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.78 (2H, t, *J*=7.2 Hz), 3.43 (q, 2H), 3.72 (s, 3H), 6.89 (2H, d, *J*=8.6 Hz), 7.14 (br, 1H), 7.16 (3H, d, *J*=8.6 Hz), 7.71 (1H, dd, *J*=8.7, 2.2 Hz), 7.77 (1H, d, *J*=2.2 Hz), 10.75 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 34.13, 42.66, 55.45, 114.42, 115.00, 119.52, 124.53, 125.38, 130.12, 130.06, 135.44, 135.58, 151.42, 158.27; HRMS (ESI) m/z calcd for $C_{16}H_{16}BrN_3O_3S$ $[M+Na]^+$: 431.999345 found: 431.99962.

7-bromo-3-((2-(trifluoromethyl)benzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (35c). The white compound was obtained from **13c** by following the experimental conditions described for Method **A** (42%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.67 (2H, d, *J*=5.4 Hz), 7.22 (1H, d, *J*=8.7 Hz), 7.54 (m, 1H), 7.67- 7.79 (m, 5H), 11.09 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 41.12, 114.22, 119.71, 123.53, 124.44, 125.45, 126.37, 128.15, 128.85, 133.72, 135.45, 135.73, 137.07, 151.61; HRMS (ESI) m/z calcd for $C_{15}H_{112}BrF_3N_3O_2S$ $[M+Na]^+$: 455.960514 found: 455.95883.

3-((2-(1H-indol-3-yl)ethyl)amino)-7-fluoro-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (36a). A mixture of 7-fluoro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**13a**) (0.25 g, 1.02 mmol) and tryptamine (0.19 g

,1.21 mmol) was dissolved in 1,4-dioxane (10 mL) and refluxed for 72 h. The reaction was allowed to cool and the solvent and excess amine removed in vacuo and the resulting residue dissolved in an aqueous 2% w/v solution of sodium hydroxide (7 mL). This solution was treated with charcoal and was filtered. The filtrate was adjusted to pH 3-4 using 6N HCl. The precipitated compound was collected by filtration, washed with water and air dried. The dried compound was suspended in an aqueous solution of sodium bicarbonate $NaHCO_3$ (1 g/40 mL). The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 4-5 with 6N HCl. The white precipitate was collected by filtration, washed twice with water, and air dried. (0.197 g, 53%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.97 (2H, t, *J*=7.2 Hz), 3.56 (q, 2H), 6.99 (1H, t, *J*=7.7 Hz), 7.09 (2H, t, *J*=7.8 Hz), 7.22 (m, 2H), 7.35 (1H, d, *J*=8.1 Hz), 7.45 (1H, t, *J*=8.7 Hz), 7.53 (1H, dd, *J*=7.5, 2.8 Hz), 7.59 (1H, d, *J*=7.8 Hz), 10.72 (br, 1H), 10.90 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 25.21, 41.70, 109.50, 111.53, 111.87, 118.78, 119.37, 120.73, 121.49, 123.37, 123.84, 127.59, 132.85, 136.74, 151.73, 156.77, 159.18; HRMS (ESI) m/z calcd for $C_{17}H_{15}FN_4O_2S$ $[M+Na]^+$: 381.079746 found: 381.0749.

3-((2-(1H-indol-3-yl)ethyl)amino)-6-chloro-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (36b). The white compound was obtained from **13b** by following the experimental conditions described for **36a** (62%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.97 (2H, t, *J*=7.2 Hz), 3.55 (q, 2H), 6.99 (1H, t, *J*=7.9 Hz), 7.08 (1H, t, *J*=7.9 Hz), 7.22 (1H, d, *J*=2.5 Hz),7.23 (br, 1H), 7.30 (2H, dd, *J*=8.4, 2 Hz), 7.34 (1H, d, *J*=8.1 Hz), 7.61 (1H, d, *J*=7.8 Hz), 7.68 (1H, d, *J*=8.4 Hz), 10.71 (br, 1H), 10.90 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 25.15, 41.71, 111.51, 111.87, 116.47, 118.78, 121.49, 123.38, 124.18, 125.44, 126.53, 127.59, 136.74, 136.93, 137.55, 151.43, 161.92; HRMS (ESI) m/z calcd for $C_{17}H_{15}ClN_4O_2S$ $[M+Na]^+$: 397.050196 found: 397.0498.

3-((4-chlorophenethyl)amino)-7-fluoro-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (37a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (81%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.85 (2H, t, *J*=7.1 Hz), 3.47 (q, 2H), 7.14 (br, 1H), 7.24 (m, 1H), 7.30 (1H, d, *J*=8.3 Hz), 7.36 (2H, d, *J*=8.3 Hz), 7.45 (1H, t, *J*=8.7 Hz), 7.51 (1H, dd, *J*=7.5, 2.8 Hz), 10.70 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 34.32, 42.18, 109.48, 119.45, 120.74, 128.79, 131.08, 131.39, 131.60, 132.78, 138.36, 151.67, 159.22, 16,91; HRMS (ESI) m/z calcd for $C_{15}H_{13}ClFN_3O_2S$ $[M+Na]^+$: 376.029875 found: 376.0270.

7-fluoro-3-((4-((trifluoromethyl)thio)benzyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (38a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (72%): ^{1}H NMR (400 MHz, DMSO-d6): δ 4.57 (2H, d, *J*=5.9 Hz), 7.30 (m, 1H), 7.44- 7.53 (m, 4H), 7.7 (2H, d, *J*=8.1 Hz), 7.77 (br, 1H), 11.05 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 43.87, 109.51, 119.50, 120.84, 121.77, 123.83, 128.54, 129.00, 131.60, 132.82, 136.79, 143.08, 151.86, 156.88, 159.30; HRMS (ESI) m/z calcd for $C_{15}H_{11}F_4N_3O_2S_2$ $[M+Na]^+$: 428.012653 found: 428.0131 .

7-fluoro-3-((4-methoxyphenethyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (39a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (63%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.79 (2H, t, *J*=7.2 Hz), 3.44 (q, 2H), 3.72 (s, 3H), 6.88 (2H, d, *J*=8.6 Hz), 7.10 (br, 1H), 7.16 (2H, d, *J*=8.5 Hz), 7.24 (br, 1H), 7.44 (1H, t, *J*=8.7 Hz), 7.52 (1H, dd, *J*=7.5, 2.8 Hz) 10.73 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 34.20, 42.66, 55.42, 109.47, 114.30, 119.40, 120.47, 120.71, 123.75, 130.12, 131.09, 132.82, 151.70, 156.78, 158.26, 159.20; HRMS (ESI) m/z calcd for $C_{16}H_{16}FN_3O_3S$ $[M+Na]^+$: 372.0794 found: 372.0720 .

7-fluoro-3-((3-(trifluoromethyl)phenethyl)amino)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (41a). The white compound was obtained from **13a** by following the experimental conditions described for Method **A** (75%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.97 (2H, t, *J*=7 Hz), 3.52 (q, 2H), 7.25 (br, 2H), 7.45 (1H, t, *J*=8.7 Hz), 7.50 (1H, dd, *J*=7.5, 2.8 Hz), 7.57 (q, 3H), 7.63 (s, 1H), 10.71 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 34.69, 42.06, 109.45, 119.45, 120.48, 120.72, 123.51, 123.83, 125.72, 129.84, 132.78, 133.40, 140.81, 151.74, 156.87, 159.24; HRMS (ESI) m/z calcd for $C_{16}H_{13}F_4N_3O_2S$ $[M+Na]^+$: 410.0562 found: 410.0497.

7-fluoro-4-methyl-3-(methylthio)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (42a). To a solution of 7-fluoro-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine1,1-dioxide (**13a**) (1 g, 3.8 mmol) in acetonitrile/DMF, 4:1 (15 mL) at room temperature was added K_2CO_3 (0.48 g, 3.45 mmol) and methyl iodide (1 mL, 6.9 mmol). The mixture was stirred for 10 h and the solvent was removed in vacuo. The solid residue was taken up in water (20 mL). The resulting aqueous suspension was adjusted to pH 2 by means of formic acid, and the precipitate was collected by filtration and washed with water. The crude compound was recrystallized in methanol/water to provide the title compound as a white powder (0.93 g, 89%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.55 (s, 3H), 3.71 (s, 3H),

7.68 (m, 2H), 7.73 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d6): δ 16.09, 36.41, 110.29, 120.66, 121.48, 125.01, 135.26, 158.17, 160.63, 166.35; HRMS (ESI) m/z calcd for $C_9H_9FN_2O_2S_2$ $[M+Na]^+$: 282.99872 found: 282.9954.

4-methyl-3-(methylthio)-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (42d). The white compound was obtained from **13d** by following the experimental conditions described for **42a** (76%): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.54 (s, 3H), 3.70 (s, 3H), 7.60 (m, 2H), 7,79 (1H, t, *J*=8.6 Hz), 7.86 (1H, dd, *J*=7.8, 2 Hz); ^{13}C NMR (100 MHz, DMSO-d6): δ 15.98, 36.13, 117.45, 123.96, 126.74, 133.82, 138.43, 166.09 HRMS (ESI) m/z calcd for $C_9H_{10}N_2O_2S_2$ $[M+Na]^+$: 265.008142 found: 264.992.

3-(isopropylamino)-4-methyl-4H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (43d). To a soluton of 4-methyl-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**42d**, 0.2 g, 0.79 mmol) in 1,4-dioxane (3 mL) in a sealed vessel was added isopropylamine (0.2 mL, 3.16 mmol) and the mixture heated for 24 h at 130 °C. The excess solvent and amine were removed by distillation under reduced pressure, and the residue was suspended in water (15 mL). The mixture was stirred for 1 h at room temperature, the resultant precipitate was collected by filtration, washed twice with water, and recrystallized from methanol/water to yield the title compound as a white powder (0.11 g, 55%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.21 (6H, d, *J*=6.5 Hz), 3.46 (s, 3H), 4.05 (q, 1H), 7.36 (1H, t, *J*=7.5 Hz), 7.45 (2H, d, *J*=8.3 Hz), 7.65 (1H, t, *J*=8.5 Hz), 7.70 (1H, dd, *J*=7.8, 2 Hz); ^{13}C NMR (100 MHz, DMSO-d6): δ 22.53, 35.09, 44.80, 66.80, 117.22, 122.76, 124.42, 126.45, 132.77, 139.26, 153.37; HRMS (ESI) m/z calcd for $C_{11}H_{15}N_3O_2S$ $[M+Na]^+$: 276.0552 found: 276.0782.

Complex II inhibition assay: Mitochondria were isolated either from mouse liver by differential centrifugation in sucrose-based buffer as previously described.[45] Complex II enzymatic activity was determined spectrophotometrically as the rate of succinate-driven, co-enzyme Q2-linked reduction of dichlorophenolindophenol (DCPIP).[51] Freeze/thawed mitochondria were incubated in phosphate buffer (pH 7.4) containing 40 μM DCPIP, 1 mM KCN, 10 μM rotenone, and 50 μM co-enzyme Q2. The rate of reduction of DCPIP to $DCPIPH_2$ was followed at 600 nM (ε = 21,000 M^{-1}). At the end of each run thenoyltrifluoroacetone (1 mM) was added and the residual TTFA-insensitive rate subtracted. Varying amounts of benzothiadiazine derivatives were used to determine an IC_{50} value.

Cell Culture: Cell lines (22Rv1 prostate cancer and MDA-MB-468 triple-negative breast cancer cells) were purchased from ATCC. The cells were cultured in RPMI-1640 Medium (ATCC® 30-2001™) for 22Rv1 cells and in Dulbecco's Modified Eagle Medium (DMEM) (ThermoFisher Scientific) for MDA-MB-468 cells with fetal bovine serum (ATCC 30-2020) to a final concentration of 10% and Corning™ Penicillin-Streptomycin Solution (Catalog No. MT30001CI) according to the supplier's recommended protocol.

Cytotoxicity Assays: To determine the cell growth inhibition ability of the synthesized compounds the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) assay used according to the manufacturer's recommended protocol. Stock solutions of the synthesized compounds were prepared in DMSO. Cells were seeded at a density of 1 x 10^5 cells in 96-well plates. After 24 hours, cells were treated at the indicated concentrations of test compounds, limiting the final DMSO concentration to less than 1%. After incubation at 37 °C in an environment of 5% CO_2 for 48-72 hours, 10 µL of MTS reagent (CellTiter 96® AQueous One Solution Reagent) was added to each well and incubated at the above mentioned conditions for 2-4 hr. Absorbance was recorded at 490 nm on a BioTek Synergy Mx multimode plate reader and the viability of cells were plotted as percentage of controls. MDA-MB-468 cells were seeded at a density of 1 x 10^5 cells/dish in 12-well plates. After incubation at 37 °C in an environment of 5% CO_2 for 48-72 hours, the numbers of viable cells were counted using a hemocytometer. The relative cell viability was calculated by normalizing the cell number with drug treatment to that with DMSO.

Statistical Analysis: Experiments were repeated at least thrice, and the statistical significance was calculated using the unpaired *t* test. A p value of <0.05 was considered statistically significant. IC_{50} values were calculated by GraphPad prism software.

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Conflict of interest

A provisional patent (US 63/027180, '*Halogenated Benzothiazines for the Treatment of Cancer'*) describing the compounds in this manuscript has been filed.

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