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A scaffold replacement approach towards new sirtuin 2 inhibitors

Tina Seifert[a,*], Marcus Malo[a], Tarja Kokkola[b], E. Johanna L. Stéen[a], Kristian Meinander[c], Erik A.A. Wallén[c], Elina M. Jarho[b], Kristina Luthman[a]

[a] Department of Chemistry and Molecular Biology, Medicinal Chemistry, University of Gothenburg, SE-412 96 Göteborg, Sweden
[b] School of Pharmacy, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland
[c] Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, FI- 00014 Helsinki, Finland

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ABSTRACT

Sirtuins (SIRT1–SIRT7) are an evolutionary conserved family of NAD$^+$-dependent protein deacylases regulating the acylation state of ε-*N*-lysine residues of proteins thereby controlling key biological processes. Numerous studies have found association of the aberrant enzymatic activity of SIRTs with various diseases like diabetes, cancer and neurodegenerative disorders. Previously, we have shown that substituted 2-alkyl-chroman-4-one/chromone derivatives can serve as selective inhibitors of SIRT2 possessing an antiproliferative effect in two human cancer cell lines. In this study, we have explored the bioisosteric replacement of the chroman-4-one/chromone core structure with different less lipophilic bicyclic scaffolds to overcome problems associated to poor physiochemical properties due to a highly lipophilic substitution pattern required for achieve a good inhibitory effect. Various new derivatives based on the quinolin-4(1*H*)-one scaffold, bicyclic secondary sulfonamides or saccharins were synthesized and evaluated for their SIRT inhibitory effect. Among the evaluated scaffolds, the benzothiadiazine-1,1-dioxide-based compounds showed the highest SIRT2 inhibitory activity. Molecular modeling studies gave insight into the binding mode of the new scaffold-replacement analogues.

1. Introduction

Bioisosterism is a common strategy for optimization of lead compounds in medicinal chemistry which involves structural modifications of bioactive compounds by replacement of functional groups or scaffolds. We have used the chromone and chroman-4-one ring systems as scaffolds for the development of potent and selective sirtuin 2 (SIRT2) enzyme inhibitors (Fig. 1).[1,2] Sirtuins (SIRTs) catalyze the deacylation of lysine residues on numerous protein substrates requiring NAD$^+$ as a co-substrate.[3,4] The SIRT enzymes are considered to be important in various pathologies such as cancer, neurodegeneration, diabetes, inflammation and cardiovascular diseases and therefore, the development of SIRT inhibitor is of vast interest.[5–10] Despite the synthetic efforts to develop potent inhibitors the majority of the reported inhibitors show IC_{50} values in the submicromolar to micromolar range with only a handful of examples exibiting nanolar inhibitory activity.[9,11]

A drawback of the previously described chromone and chroman-4-one based SIRT2 inhibitors (e.g. **1**, Fig. 1)[1] was their high lipophilicity which prevented their use in more advanced biological assays as they precipitated at relevant test concentrations. Efforts to increase the hydrophilicity afforded compounds containing heterofunctional groups such as pyridyl or oxadiazole moieties (**3** and **5**, respectively, Fig. 1) that allowed them to be tested for their SIRT2-mediated antiproliferative effects in cancer cells.[2]

To further improve the physicochemical properties of potential SIRT2 inhibitors we decided to investigate the effect of replacing the chroman-4-one scaffold with other heterocyclic frameworks (Scheme 1).

In the present study, a set of compounds based on three types of bicyclic scaffolds has been synthesized and evaluated for their activity as sirtuin inhibitors.

Quinolin-4(1*H*)-ones (**A**) and bicyclic secondary sulfonamides such as benzothiazine-1,1-dioxides (**B**) and benzothiadiazine-1,1-dioxides (**C–D**), or saccharins (**E**) are versatile scaffolds found in bioactive compounds. The quinolin-4(1*H*)-one is structurally similar to the chromone and is a common scaffold in antibacterial agents such as

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Abbreviations: CDI, 1,1′-carbonyldiimidazole; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; HBA, hydrogen bond acceptor; HBD, hydrogen bond donator; HRMS, high resolution mass spectrometry; MMFF, Merck molecular force field; MW, microwave; NAD, nicotinamide adenine dinucleotide; NBS, *N*-bromosuccinimide; NMR, nuclear magnetic resonance; OPLS, optimized potentials for liquid simulations; PSA, polar surface area; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; TSA, toluenesulfonic acid

[*] Corresponding author.

E-mail address: tina.seifert@chem.gu.se (T. Seifert).

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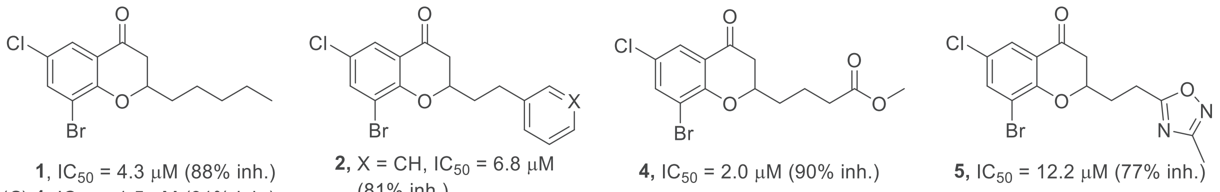


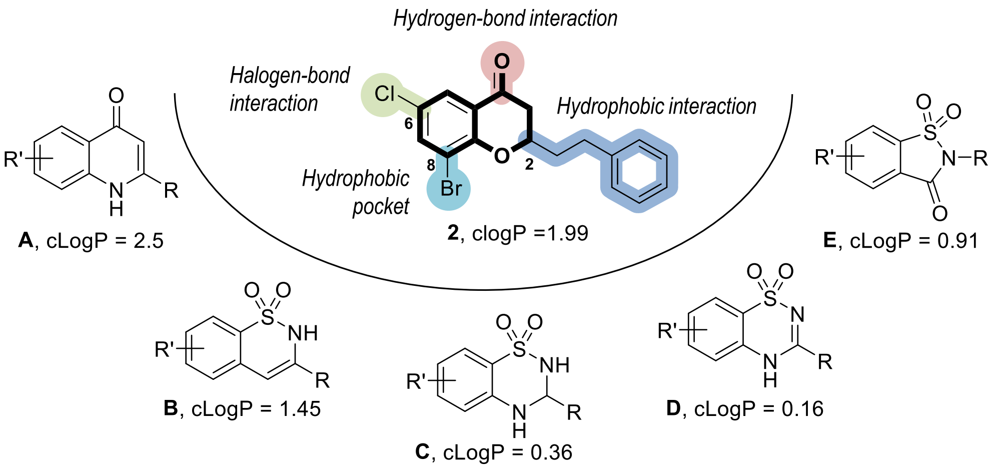
1, IC_{50} = 4.3 μM (88% inh.)
(*S*)-**1**, IC_{50} = 1.5 μM (91% inh.)
(*R*)-**1**, IC_{50} = 4.5 μM (70% inh.)

2, X = CH, IC_{50} = 6.8 μM (81% inh.)
3, X = N, IC_{50} = 3.7 μM (86% inh.)

4, IC_{50} = 2.0 μM (90% inh.)

5, IC_{50} = 12.2 μM (77% inh.)

Fig. 1. SIRT2 selective chroman-4-one based inhibitors. (%-Inhibition at 200 μM inhibitor conc.)



A, cLogP = 2.5

2, clogP =1.99

E, cLogP = 0.91

B, cLogP = 1.45

C, cLogP = 0.36

D, cLogP = 0.16

Scheme 1. Overview over the scaffolds envisioned to replace the chroman-4-one framework in the SIRT2 inhibitors. Apart from Scaffold **A**, the new bicyclic ring systems show lower lipophilicity than the chroman-4-one scaffold as reflected by the calculated logP values (logP values are calculated of the unsubstituted scaffolds (R = R′ = H)). The key-binding interactions of the chroman-4-one based inhibitors with SIRT2 have been highlighted in **2.**[2]

fluoroquinolones.[12] Benzothiadiazine-1,1-dioxide derivatives have been used as diuretic drugs since the 1950s and this scaffold is a frequent motif also in other biologically relevant substances.[13,14] Saccharin, widely used as an artificial sweetener, has been successfully used as a core structure in e.g. inhibitors of carbonic anhydrases[15] and is also a key element of repinotan, a highly selective 5-HT_{1A}-receptor agonist.[16] The new scaffold analogues contained comparable substitution patterns to the previously studied chroman-4-ones/chromones. The observed structure-activity relationships (SAR) were in agreement with the findings from docking studies conducted with a SIRT2 3D model previously published.[2]

2. Results and discussion

2.1. Design

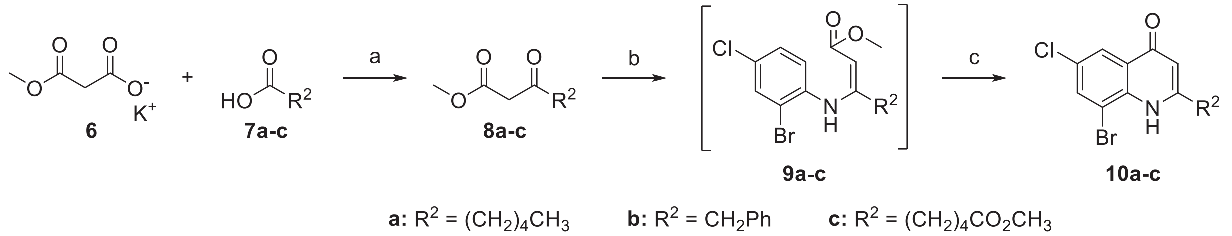
The design of new scaffold analogues was guided by SAR studies and the suggested binding mode achieved from molecular modelling trials performed around the parent chroman-4-one/chromone scaffold.[1,2] The main focus was the replacement of the core structure with less lipophilic ring systems in order to improve the physicochemical properties. To elucidate the influence of the scaffold replacement on the inhibitory properties, we aimed to retain a characteristic substitution pattern which has proven to be vital for inhibitory activity of derivatives based on the chroman-4-one/chromone framework. Hence, the scaffolds should contain a hydrogen bond acceptor (HBA) group mimicking the carbonyl group thereby retaining the hydrogen bond to a structural water molecule. In addition, the introduction of functional groups in positions mimicking the 2- and 6-position of the chroman-4-ones/chromones should be allowed (see Scheme 1 for numbering). The new compounds should preferably be brominated at the site corresponding to the 6-position of the parent scaffold since chroman-4-one derivatives holding a Br-substituent in this position showed higher inhibitory activity than other substituents due to a halogen bond interaction with a backbone carbonyl group of SIRT2. Introduction of an additional Br-group in the corresponding 8-position might be beneficial to increase the inhibitory activity even further due to hydrophobic interactions with the target protein. The substituent in the 2-position of the chroman-4-ones/chromones is located in a narrow hydrophobic tunnel pointing towards the surrounding water environment. Consequently, the group representing this substituent should be a linear aliphatic moiety which can be substituted with heterocycles and bulkier groups when separated from the core structure with at least an ethylene spacer.[1,2]

The heterocyclic frameworks shown in Scheme 1 emerged as promising alternatives as they are similar in size to the chroman-4-ones/chromones, readily accessible by synthesis and can be substituted with functional groups in equivalent positions as the parent scaffold to maintain the key binding properties of known, potent SIRT2-selective inhibitors (Fig. 1, **1–5**). The calculated logP values of scaffolds **B–E** are significantly lower than the one of the chroman-4-one scaffold, indicating a good starting point for addressing the issues of the less favourable physicochemical properties exhibited by the chroman-4-one series. In addition, the quinolin-4-(1*H*)-one framework (**A**) was also considered to be of interest due to its structural similarities to the chromone scaffold despite its higher clogP-value.

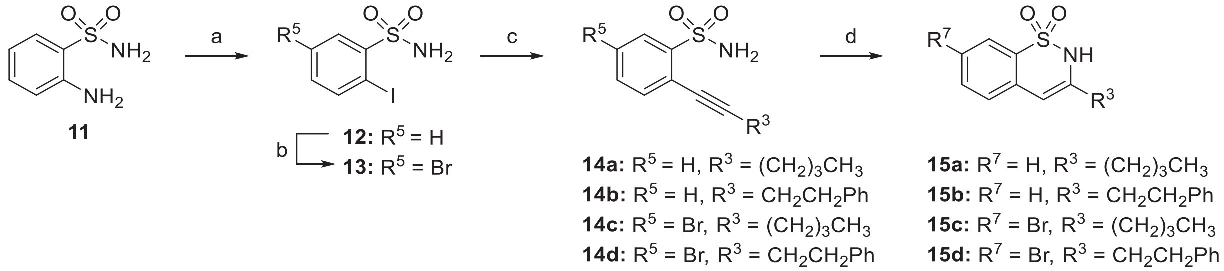
2.2. Chemistry

The synthesis towards the quinolin-4-(1*H*)-one-based analogues is shown in Scheme 2. The scaffold was synthesized from β-ketoesters and anilines employing the Conrad-Limpach reaction. The β-ketoesters **8a–c** were obtained in good yields by reacting monomethyl potassium malonate (**6**) with the CDI-activated carboxylic acids **7a–c** in anhydrous THF at room temperature.[17] 2-Bromo-4-chloroaniline was then reacted with **8a–c** under argon at 50 °C for 48 h to afford the intermediate enaminoesters **9a–c**. After removal of excess aniline and *p*-toluenesulfonic acid (*p*-TSA) the cyclization was achieved by heating the enaminoesters to 250 °C in diphenyl ether for 45 min using microwave heating. The quinolin-4-(1*H*)-ones **10a–c** were finally isolated by crystallization from hexane.

Four analogues with the benzothiazine-1,1-dioxide scaffold were



Scheme 2. Synthesis of quinolin-4-(1*H*)-one derivative **10a–c**. Reagents and conditions: (a) CDI, $MgCl_2$, THF, room temp, 16 h, 74–78%; (b) 2-bromo-4-chloroaniline, *p*-TSA, neat, 50 °C, 48 h; (c) i. cyclohexane, reflux, 30 min; ii. Ph_2O, 250 °C, 45 min, MW, 24–26% over two steps from **8a–c**.



Scheme 3. Synthesis of *2H*-benzo[*e*][1,2]thiazine-1,1-dioxide **15a–d.** Reagents and conditions: (a) i. $NaNO_2$, H_2O:HCl (6:4), 0 °C, 40 min; ii. KI, 90 °C, 5 h, 63%; (b) NBS, conc. H_2SO_4, 60 °C, 24 h; 87%; (c) **12** or **13**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, appropriate alkyne, DMF, room temp, overnight, 64–77%; (d) $Pd(PPh_3)_2(OAc)_2$, KOH, DMF, 60 °C, 4.5–5 h, 25–46%.

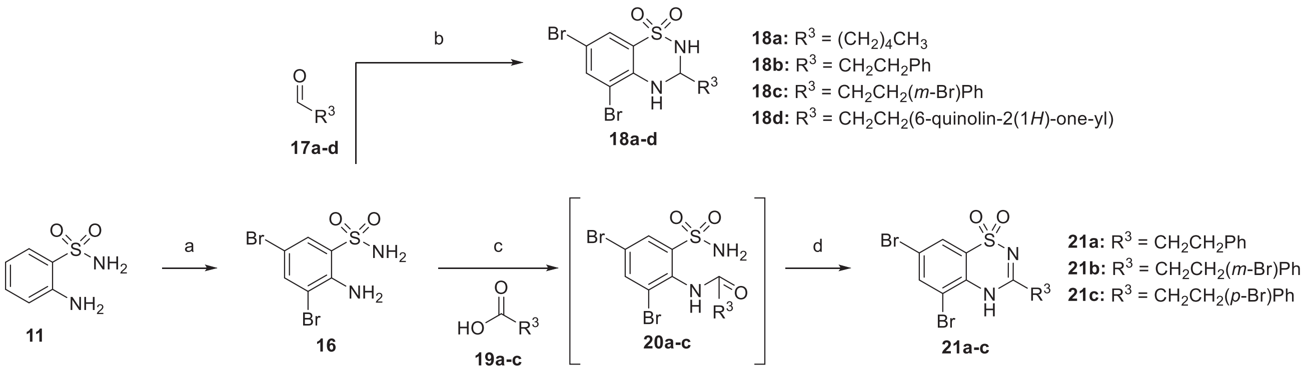
synthesized as outlined in Scheme 3. 2-Aminobenzenesulfonamide (**11**) was reacted in a Sandmeyer-type reaction with $NaNO_2$ at 0 °C followed by the addition of KI. Introduction of the bromide in the 5-position was pursued via the reaction of **12** with *N*-bromosuccinimide (NBS). Aryl iodides **12** and **13** were successfully used in a Sonogashira reaction yielding the precursor for the subsequent 6-*endo*-dig cyclization to afford the desired products **15a–d**.

The synthesis of derivatives based on the benzothiadiazine-1,1-dioxide scaffolds is shown in Scheme 4. 2-Amino-3,5-dibromobenzenesulfonamide **11** was obtained by reacting 2-aminobenzene-sulfonamide with Br_2 in DMF at room temperature. Sulfonamide **16** was then either reacted with various aldehydes or carboxylic acids which defined the substituent in the 3-position and the degree of saturation of the scaffold. Aldehydes **17a** and **17b** employed in the aforementioned reaction were commercially available. Whereas, compound **17c** was obtained via a reduction-oxidation protocol from 3-(3-bromophenyl)propionic acid (Supporting Information) and **17d**, used to introduce the quinolin-2(1*H*)-one moiety in the 3-position of **18d**, was synthesized in three steps as previously reported by our group.[2] The acid-catalyzed reaction of **16** with aldehydes **17a–d** was carried out under microwave heating at 120 °C in dioxane to afford **18a–d** in 34–53% yield.

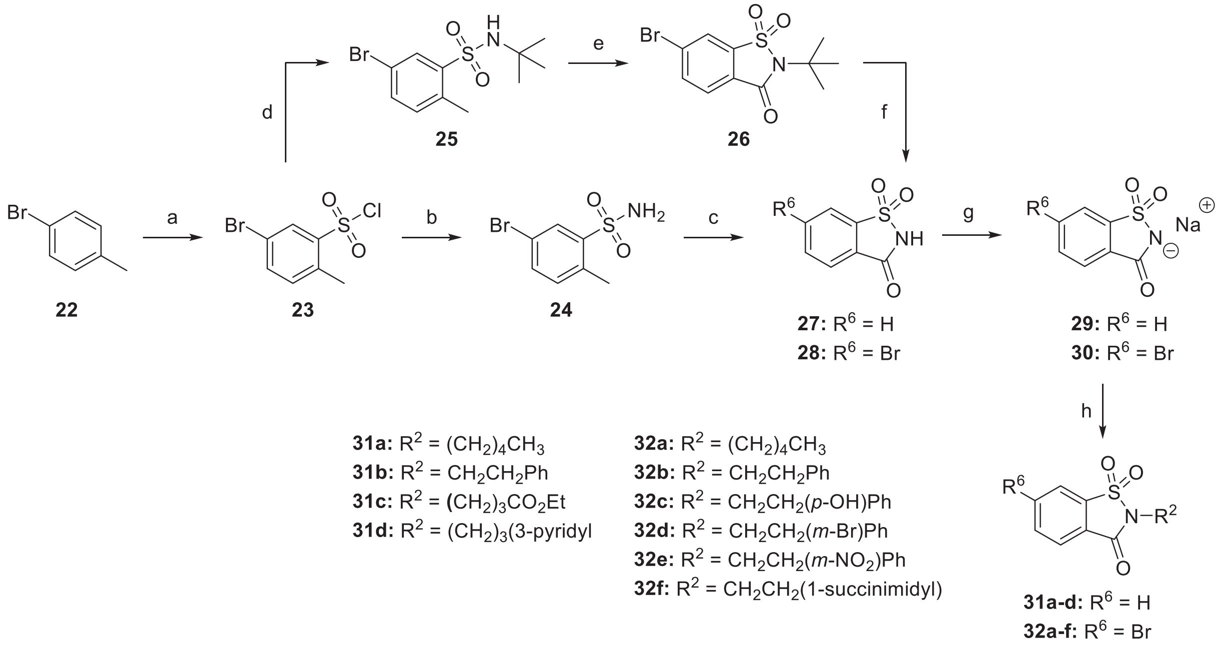
As outlined in Scheme 4, the unsaturated benzothiadiazine-1,1-dioxides **21a–c** were synthesized via a reaction of CDI-activated carboxylic acids with **16** leading to amide intermediates **20a–c** which were cyclized under microwave heating to afford **21a–c.**[18]

The synthesis of saccharin derivatives substituted in the 2-position is shown in Scheme 5. As earlier mentioned the SAR study of the chroman-4-ones revealed that halogen atoms in the 6- and 8-positions are essential for activity. Thus, dibrominated saccharins were considered promising derivatives. However, bromination of 2-methylbenzenesulfonamide by the previously used method (Br_2 in DMF) was unsuccessful. Attempts to obtain a dihalogenated precursor for saccharin synthesis via chlorosulfonation of 4-bromo-2-chlorotoluene yielded only the non-desired isomer, with the sulfonyl chloride moiety in the meta-position to the methyl group.

Instead, the mono-brominated derivative **23** was synthesized by chlorosulfonation of 4-bromotoluene (**22**). The desired isomer **23** was obtained together with small amounts of the meta-substituted regioisomer (14–21%). The isomeric mixture was used in the following reaction converting **23** to the corresponding sulfonamide **24** with aqueous ammonia. A subsequent oxidation reaction, as reported by Xu *et al.* using H_5IO_6 and sub-stoichiometric amounts of CrO_3 furnished 6-bromosaccharin (**28**) in 46% yield.[19] Alternatively, **28** was obtained in



Scheme 4. Synthesis of the saturated and unsaturated benzothiadiazine-1,1-dioxides **18a–d** and **21a–c**. Reagents and conditions: (a) Br_2, DMF, 10 °C → room temp, 22 h, 99% crude yield; (b) appropriate aldehyde, 4 M HCl in dioxane, 120 °C, MW, 1–2.5 h, 34–53%; (c) i. appropriate carboxylic acid, CDI, CH_2Cl_2, room temp, 1.5 h; ii. **16**, CH_2Cl_2, DMF, reflux, 21 h; (d) Cs_2CO_3, EtOH, 120 °C, 1.5 h, MW, 21–37% over two steps.



Scheme 5. Synthesis of saccharin derivatives. Reagents and conditions: (a) $ClSO_3H$, CH_2Cl_2, 0 °C → room temp, overnight, 81%; (b) NH_4OH, Et_2O, 0 °C → reflux → room temp, 48 h, 74%; (c) H_5IO_6, CrO_3, MeCN, reflux, 20 h, 38%; (d) *tert*-$BuNH_2$, Et_3N, Et_2O, 0 °C → room temp, 33 h, 81%; (e) H_5IO_6, CrO_3, Ac_2O, MeCN, 0 °C → room temp, 20 h; (f) TFA, reflux → room temp, 4 d, 51% over three steps (d–f); (g) NaOMe, MeOH, 6 h, room temp, > 99%; (h) Alkyl halide, DMF, MW, 145 °C, 15 min. or 1 h, 12–79%.

62% overall yield via oxidative cyclization under similar conditions of *N*-tert-butyl sulfonamide **27** followed by removal of the tert-butyl group in refluxing trifluoroacetic acid (TFA). However, the final deprotection step of **26** proceeds very slowly on larger scale and is therefore time consuming.

Finally, commercially available saccharin **27** and 6-bromosaccharin **28** were converted to their corresponding sodium salts using NaOMe in MeOH and were further subjected to an alkylation with a variety of alkyl halides containing different terminal heterofunctionalities and aryl groups (**31a–d**, **32a–f**).

2.3. Physicochemical evaluation

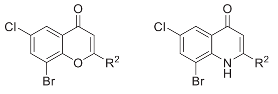
In this study we attained scaffold analogues with an improved physicochemical profile (Supporting Information, Table S1). The computed physicochemical properties (molecular weight, clogP, PSA, HBD, HBA) of the new derivatives conformed with Lipinski's rule of five. Even though we maintained the highly lipophilic substitution pattern required to assure a minor loss in inhibitory activity[1,2] a clear improvement in polarity can be seen in the increase in PSA and the number of HBD and HBA. Derivatives based on the bicyclic sulfonamides (**B–D**) and saccharins (**E**) showed at a least twofold increase in PSA and a decrease by one unit in clogP compared to active chroman-4-one based derivatives (e.g. **1–3**).

2.4. Biological evaluation

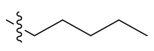
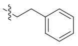
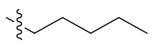
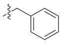
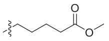
The synthesized compounds were evaluated in a fluorescence-based assay for their inhibitory activity against SIRT2 at a compound concentration of 200 μM.[2,20] The most active derivatives from each scaffold class were also tested for their inhibitory effect on SIRT1 and SIRT3 to investigate their selectivity towards the SIRT2-isoform. The results are summarized in Tables 1–3.

The quinolone-4-(1*H*)-one series (Table 1) resembles chromone in which the oxygen in the 1-position is replaced with a hydrogen bond donating NH-group. The evaluation of the inhibitory effect towards SIRT2 showed that similar biological effects cannot be achieved in this series despite its structural similarity to the chromone scaffold (Table 1).

Table 1
SIRT2 inhibitory activities of quinolin-4-(1*H*)-one derivatives **10a–c**. For selected analogues inhibition of SIRT1 and SIRT3 was investigated as well.[a,b,c].



33-34 10a-c

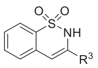
No.	R^2	Inhibition (%)[a,b,c]		
		SIRT1	SIRT2	SIRT3
33		9.8 ± 2.8	82 ± 0.4	4.5 ± 1.6
34		n.d.	75 ± 2.6	n.d.
10a		n.d.	26 ± 1.4	n.d.
10b		7.6 ± 2.2	58 ± 2.1	41 ± 3.0
10c		n.d.	53 ± 1.1	n.d.

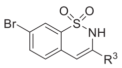
[a] SD, standard deviation (n = 3).
[b] Inhibition at 200 μM inhibitor concentration.
[c] n.d. = not determined.

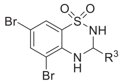
Further, as summarized in Table 2, we tested various analogues based on bicyclic sulfonamide scaffolds **B–D** substituted with aliphatic groups (butyl, pentyl, phenethyl) in the 3-position as well as the bulkier 2-quinolinone group (**18d**). The mono-brominated bicyclic sulfonamides **15c** and **15d** based on scaffold **B** (Table 2) displayed only poor inhibitory activity with the phenethyl derivative **15d** being the most active (35% inhibition at 200 μM concentration). Compared to non-brominated **15a** and **b**, introduction of the Br-group had only a marginal effect on the inhibitory activity as compared to the corresponding effect among the chroman-4-ones.[1]

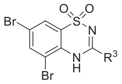
The di-brominated derivatives **18a–d** based on the saturated bicyclic sulfonamide core structure **C** showed increased activity

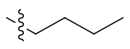
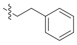
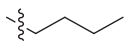
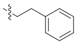
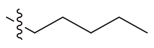
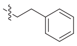
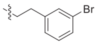
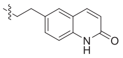
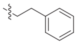
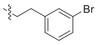
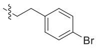
Table 2
SIRT2 inhibitory activities of benzothiazine-1,1-dioxide **15a–d** and benzothiadiazine-1,1-dioxides **18a–d** and **21a–c**. For selected analogues inhibition of SIRT1 and SIRT3 was also investigated.[a,b,c]


15a-b


15c-d

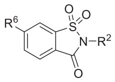

18a-d

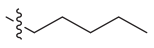
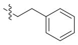
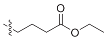
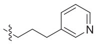
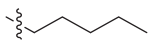
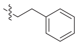
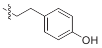
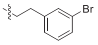
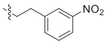
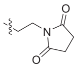

21a-c

No.	Scaffold	R^3	Inhibition (%)[a,b,c]		
			SIRT1	SIRT2	SIRT3
15a	B		n.d.	12 ± 2.3	n.d.
15b	B		n.d.	15 ± 6.5	n.d.
15c	B		n.d.	29 ± 4.1	n.d.
15d	B		n.d.	35 ± 1.7	n.d.
18a	C		4.4 ± 2.8	32 ± 2.2	5.8 ± 3.4
18b	C		4.6 ± 4.2	47 ± 1.9	54 ± 9.2
18c	C		9.7 ± 3.9	59 ± 0.4	9.9 ± 1.5
18d	C		25 ± 1.6	74 ± 1.3	28 ± 1.5
21a	D		n.d.	15 ± 3.1	n.d.
21b	D		0 ± 3.7	24 ± 0.8	3.2 ± 2.0
21c	D		n.d.	10 ± 1.5	n.d.

[a] SD, standard deviation (n = 3).
[b] Inhibition at 200 μM inhibitor concentration.
[c] n.d. = not determined.

Table 3
SIRT2 inhibitory activities of saccharin derivatives **31a–d** and **32a–f**. For selected analogues inhibition of SIRT1 and SIRT3 was also investigated.[a,b,c]


31a-d, 32a-f

No.	R^6	R^2	Inhibition (%)[a,b,c]		
			SIRT1	SIRT2	SIRT3
31a	H		n.d.	34 ± 2.5	n.d.
31b	H		14 ± 6.3	34 ± 2.8	14 ± 2.8
31c	H		8.7 ± 3.4	13 ± 5.0	7.2 ± 1.9
31d	H		17 ± 4.3	12 ± 4.9	14 ± 2.4
32a	Br		n.d.	6.9 ± 2.6	n.d.
32b	Br		3.8 ± 4.3	4.7 ± 3.5	1.6 ± 2.2
32c	Br		5.2 ± 4.0	12 ± 4.8	15 ± 5.6
32d	Br		n.d.	15 ± 1.9	n.d.
33e	Br		n.d.	6.2 ± 4.2	n.d.
32f	Br		n.d.	13 ± 4.4	n.d.

[a] SD, standard deviation (n = 3).
[b] Inhibition at 200 μM inhibitor concentration.
[c] n.d. = not determined.

compared to **15c–d.** Compound **18d** was the most potent compound of the benzothiadiazine-1,1-dioxides with 74% inhibition of SIRT2. The unsaturated analogues **21a–c** were considerably less potent (10–24% inhibition) with inhibitory activities in the range with the non-halogenated analogues based on scaffold **B**. Moreover, **21a–c** showed very low solubility in most solvents and were therefore not considered for future optimization.

The last scaffold evaluated in the study was the saccharin core, i.e. compounds **31a–d** and **32a–f**. As shown in Table 3, derivatives based on this core structure showed only a minimal inhibitory effect, **31a** and **31b** as most active showed 34% at 200 μM concentration. For this series, the introduction of a bromide in the aromatic ring (**32a–f**) did not improve the activity.

2.5. Structure-activity relationship and molecular modeling

In order to investigate and get a better understanding of the observed SAR among these new series of compounds molecular modeling and docking studies were performed. For the docking trials we used our 3D model of SIRT2 which was previously used for docking studies of the corresponding chromones and chroman-4-ones.[2] The docking was performed using Glide with the OPLS3 force field[21] as implemented in the Schrödinger software.[22] OPLS3 is superior in predicting protein–ligand binding energy to commonly used force fields (e.g. OPLS_2005 and MMFF)[23,24] and it is also parametrized to predict the geometry and energy contribution of aryl halogen bonds more accurately.[25] In the docking procedure the SIRT2 3D-structure was kept rigid while the ligands were flexible. A representative compound from each scaffold series was included together with a set of relevant previously published structurally analogous chroman-4-ones as reference ligands. Docking scores obtained from representative compounds of each compound class were compared (Supporting Information, Table S2). The rank of the docking scores was in good agreement with the decreasing inhibitory activity from the most potent chroman-4-one (**3**) to the saccharins comprising the least active series of the new analogues.

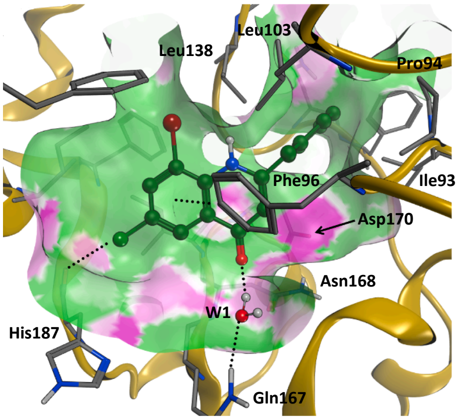


Fig. 2. Quinolone-4-(1*H*)-one analogue **10b** docked in the C-pocket of SIRT2. The key enzyme/inhibitor interactions are present, i.e. the halogen bond to His187, the hydrogen bond to W1, and the π-π interaction to Phe96. The NH moiety in **10b** is positioned in a hydrophobic region, close to two leucine residues (Leu103 and Leu138). The hydrophilic (purple) and hydrophobic (green) regions are highlighted on the surface of the binding site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

As illustrated in Fig. 2, docking studies of quinolone-4-(1*H*)-one **10b** revealed a similar binding mode as observed for the chroman-4-ones/chromones with the presence of the key enzyme/inhibitor interactions (see Fig. 1) such as the halogen bond and the hydrogen bond-interaction as well as the π-π interactions. However, in the suggested binding mode of this scaffold the polar NH-group of the quinolone-4-(1*H*)-ones is unfavourably positioned in a hydrophobic region close to Leu103 and 138 (Fig. 2). This in turn, might explain the decrease in inhibitory

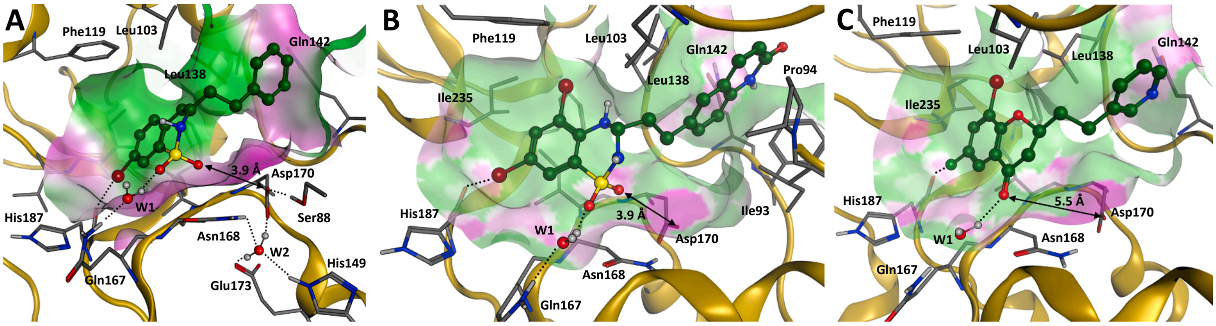


Fig. 3. (A) Binding pose of **15d** and the observed conserved hydrogen-bond network formed by Ser88, His149, Asp170, Asn168, Glu173 and a structural water molecule (W2). (B) Docking solution of **18d**. (C) The docking pose of the chroman-4-one analogue **2**. The carbonyl oxygen is interacting with the structural water molecule W1 and the distance to Asp170 is 5.5 Å. The hydrophilic (purple) and hydrophobic (green) regions are highlighted in on the molecular surface of the binding site.

activity.

The docking trial of the bicyclic sulfonamide scaffolds **B**–**D** revealed that they adopt similar binding poses as that observed for the parent scaffold. When looking at the binding pose of **15d** and **18d** as representative structures (Fig. 3A and B) in the SIRT2 homology model, the Br-group in the 7-position (corresponding to 6-position of the chroman-4-ones) forms the expected halogen-bond with a backbone carbonyl group of SIRT2. The other Br-group of the dibrominated derivatives (Fig. 3B) is buried in the hydrophobic pocket. The SO_2NH-group is placed in close proximity of a hydrogen bonding network comprising Ser88, His149, Thr166, Asn168, Asp170, Glu173 and a structural water molecule (W2) (see Fig. 3A), this network is highly conserved throughout the sirtuin family. The sulfonyl interacts with the protein via a hydrogen bond of one of the sulfonyl oxygens with W1, while the second oxygen is positioned in close proximity to Asp170 (3.9 Å) as shown in Fig. 3A and B. This results in an electrostatic repulsion as the side-chain conformation of the aspartate is rotationally restricted due to its participation in the conserved hydrogen bonding network. The distance between the sulfonyl oxygen atom and Asp170 (3.9 Å) is also considerably shorter as compared to that between the carbonyl oxygen of **2** and Asp170 (5.5 Å, Fig. 3C). The electrostatic repulsion could be the main contributor to the overall decreased inhibitory activity observed for scaffolds **B**–**D** as compared to the chroman-4-ones/chromones. In addition, a certain degree of flexibility in the scaffold might be required for reasonable binding to SIRT2 since derivatives **21a**–**c** that are based on the more rigid scaffold **D** only showed negligible activity.

In general, derivatives based on the saccharin scaffold compose a series of only weak SIRT2 inhibitors with 34% inhibitory activity as the best (**31a**–**b**). Docking studies of this compound class revealed that they can adopt two different binding poses with either the sulfonyl group (Fig. 4A) or the carbonyl moiety (Fig. 4B) interacting with the structural water (W1) thereby mimicking the carbonyl group of the chroman-4-ones. Depending on the nature of the hydrogen bond interacting group, the mono-brominated compounds can additionally, either form the halogen bond (Fig. 4A) or the π-π-interaction with Phe96 (Fig. 4B).

In this series we observed an opposite trend in the inhibitory effect where the lack of the halogen substituents slightly increased the activity. The non-halogenated saccharin derivatives can bind closer to His187 thereby increasing the distance between the sulfonyl oxygen to the acidic Asp170 (4.8 Å). At the same time the carbonyl group is positioned further away from the lipophilic leucine residues Leu103 and Leu138 which could result in a more favourable binding mode which could explain the slight increase in inhibitory activity.

3. Conclusion

We have shown that bicyclic sulfonamides can act as scaffolds for SIRT inhibitors. The new scaffold analogues comprised a set of promising SIRT2 inhibitors (50–75% inhibition at 200 μM) with **18d**, based on the sulfonamide scaffold **C** substituted with a 2-quinolinone containing alkyl group (Table 2), being the most potent inhibitor identified in our study. Further optimization guided by molecular modeling will be performed around the benzothiadiazine-1,1-dioxides, to identify compounds that can be expected to be more potent.

Interestingly, two compounds (**10b** and **18b**) show higher activity against SIRT3 than what we have previously seen for the chroman-4-ones. This could be a potential starting point for the development of either more general SIRT inhibitors, so called pan inhibitors, or of more SIRT3 selective compounds.

4. Experimental

4.1. General information

All reactions were carried out using magnetic stirring under ambient atmosphere if not otherwise stated. Room temperature corresponds to a temperature interval from 20 to 22 °C. All starting materials and reagents were obtained from commercial producers and were used without prior purification. Solvents were generally used as supplied by the manufacturer. Microwave reactions were carried out using a Biotage Initiator™ with fixed hold time modus in 0.5–2 mL, 2–5 mL or 10–20 mL capped microwave vials. All reactions were monitored by thin-layer chromatography (TLC) on silica plated aluminum sheets (Silica gel 60 F254, E. Merck). Spots were detected by UV light (254 or 365 nm). Purification by flash column chromatography was performed using an automatic Biotage SP4 Flash + ® instrument. Prefabricated columns of different cartridge sizes (surface area 500 m^2/g, porosity 60 Å, particle size 40–63 μm) were used. The NMR spectra were measured on a Varian 400-MR spectrometer or a 300 MHz instrument. ^1H and ^{13}C NMR spectra were measured at 400 MHz and 100 MHz, respectively. Chemical shifts are reported in ppm with the solvent residual peak as internal standard [$CHCl_3$ δ_H 7.26, $CDCl_3$ δ_C 77.16; CD_2HOD δ_H 3.31, CD_3OD δ_C 49.00; acetone-d_6 δ_H 2.05, δ_C 29.84; DMSO-d_6 δ_H 2.50, δ_C 39.52]. All NMR experiments were measured at ambient temperature. If not otherwise stated the NMR experiments were run in $CDCl_3$ at 400 MHz. Combustion analyses for CHN were measured on a Thermo Quest CE Instruments EA 1110 CHNS-O elemental analyzer or Perkin Elmer 2400 Series II CHNS/O system elemental analyzer. High-resolution mass spectrometry (HRMS) analysis was obtained from Recipharm OT Chemistry AB, Uppsala (Sweden).

4.2. General procedure for the synthesis of β-ketoesters **8a–c**

The carboxylic acid (1 equiv) and CDI (1.1 equiv) were dissolved in anhydrous THF (0.5 M) and stirred at room temperature for 1 h. Monomethyl potassium malonate **6** (1.2 equiv) and anhydrous $MgCl_2$

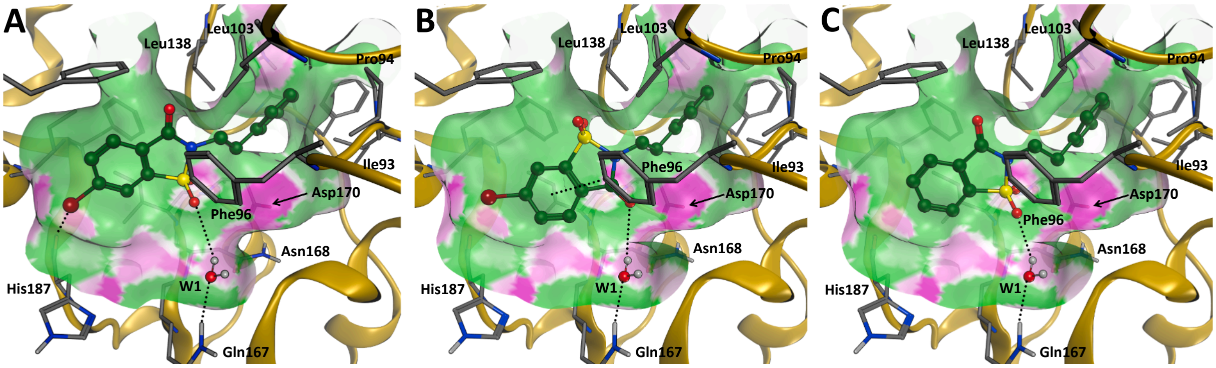


Fig. 4. (A). Binding pose of **32b** which is similar to that of the sulfonamides in where the sulfonyl group interacts with W1 and also forms a halogen bond with the backbone carbonyl moiety of His187. (B) The alternative pose of **32b** rotated 180° in which the hydrogen bond interaction with W1 is maintained via the carbonyl oxygen. However, this binding prevents any potential halogen bonding with His187. (C) Binding pose of the non-halogenated saccharin analogue **31b** which interacts with the structural water W1 and places the sulfonyl and carbonyl group further away from the acidic Asp170 (4.8 Å) and Leu103 and Leu138. However, the distance between the aromatic ring in the ligand and Phe96 is longer which weakens the π-π-interaction. The hydrophilic (purple) and hydrophobic (green) regions are highlighted in on the molecular surface of the binding site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(1.5 equiv) were added and the reaction was stirred at room temperature overnight. The reaction was treated with 1 M HCl (aq). The phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash column chromatography (2 → 22% EtOAc in pentane) afforded β-ketoesters **8a**–**c**. All ^{1}H NMR spectra also showed trace amounts of the enol tautomer of the product.

Methyl 3-oxooctanoate (8a). The compound was synthesized according to the general procedure from **7a** (1.39 g, 12.0 mmol) to afford **8a** (1.53 g, 74%) as a colourless oil. ^{1}H NMR (300 MHz) δ 3.73 (s, 3H), 3.45 (s, 2H), 2.53 (t, J = 7.4 Hz, 2H), 1.66–1.54 (m, 2H), 1.38–1.22 (m, 4H), 0.89 (t, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz) δ 202.8, 167.7, 52.3, 49.0, 43.0, 31.2, 23.2, 22.4, 13.9.

Methyl 3-oxo-4-phenylbutanoate (8b). The compound was synthesized according to the general procedure from **7b** (1.04 g, 7.63 mmol) to afford **8b** as a colourless oil (1.30 g, 88%). ^{1}H NMR (300 MHz) δ 7.37–7.13 (m, 5H), 3.80 (s, 2H), 3.68 (s, 3H), 3.44 (s, 2H). ^{13}C NMR (75 MHz) δ 200.3, 167.5, 133.3, 129.6, 128.9, 127.4, 52.3, 50.0, 48.0.

Methyl 7-methoxycarbonyl-3-oxooctanoate (8c). The compound was synthesized according to the general procedure from **7c** (1.46 g, 13.8 mmol) to afford **8c** as a colourless oil (1.51 g, 77%). ^{1}H NMR (300 MHz) δ 3.74 (s, 3H), 3.67 (s, 3H), 3.45 (s, 2H), 2.57 (t, J = 6.9 Hz, 2H), 2.33 (t, J = 7.1 Hz, 2H), 1.69–1.58 (m, 4H). ^{13}C NMR (75 MHz) δ 202.2, 173.8, 167.6, 52.4, 51.6, 49.0, 42.6, 33.8, 24.3, 22.9.

4.3. General procedure for the synthesis of 4-quinolones 10a–c

β-Ketoester (1 equiv), 2-bromo-4-chloroaniline (1.2 equiv) and p-TSA (0.1 equiv) were stirred in a sealed reaction vessel at 50 °C for approximately 48 h. The mixture was transferred to a flask and refluxed briefly in cyclohexane. The solid was filtered off and the solvent was evaporated. The residue was dissolved in diphenyl ether (5 mL) and the solution was heated in a microwave reactor at 250 °C for 45 min. Excess hexane was added and the product was collected the next day by filtration. The compounds were recrystallized from chloroform/hexane to remove traces of diphenyl ether.

8-Bromo-6-chloro-2-pentylquinolin-4(1H)-one (10a). The compound was synthesized according to the general procedure from **8a** (544 mg, 3.87 mmol) to afford **10a** (255 mg, 25%) as a brown solid. ^{1}H NMR (300 MHz) δ 8.28 (d, J = 2.3 Hz, 1H), 8.26 (br s, 1H), 7.78 (d, J = 2.3 Hz, 1H), 6.18 (s, 1H), 2.67 (t, J = 7.7 Hz, 2H), 1.85–1.66 (m, 2H), 1.46–1.32 (m, 4H), 0.99–0.83 (m, 3H). ^{13}C NMR (75 MHz) δ 177.2, 152.9, 135.8, 134.7, 129.5, 127.0, 125.7, 111.7, 109.7, 34.6, 31.2, 27.9, 22.5, 14.0. Anal. Calcd for $C_{14}H_{15}BrClNO$: C, 51.17; H, 4.60; N, 4.26. Found: C, 51.67; H, 4.80; N, 4.21.

2-Benzyl-8-bromo-6-chloroquinolin-4(1H)-one (10b). The compound was synthesized according to the general procedure from **8b** (322 mg, 1.68 mmol) to afford **10b** (143 mg, 25%) as a brown solid. ^{1}H NMR (300 MHz) δ 8.26 (d, J = 2.3 Hz, 1H), 8.20 (s, 1H), 7.72 (d, J = 2.3 Hz, 1H), 7.49–7.27 (m, 5H), 6.24 (s, 1H), 4.04 (s, 2H). ^{13}C NMR (75 MHz) δ 177.2, 150.8, 135.7, 134.7, 134.3, 129.72, 129.70, 129.5, 128.4, 126.8, 125.7, 111.9, 110.3, 40.4. Anal. ($C_{16}H_{11}BrClNO$) C, H, N.

Methyl 5-(8-bromo-6-chloro-quinoline-4(1H)-one-2-yl)pentanoate (10c). The compound was synthesized according to the general procedure from **8c** (248 mg, 1.15 mmol) to afford **10c** (129 mg, 30%) as a light brown solid. ^{1}H NMR (300 MHz) δ 8.41 (s, 1H), 8.27 (d, J = 2.3 Hz, 1H), 7.79 (d, J = 2.3 Hz, 1H), 6.18 (s, 1H), 3.69 (s, 3H), 2.70 (t, J = 7.2 Hz, 2H), 2.40 (t, J = 6.8 Hz, 2H), 1.90–1.68 (m, 4H). ^{13}C NMR (75 MHz) δ 177.2, 173.7, 152.4, 135.8, 134.8, 129.7, 127.0, 125.7, 111.8, 109.8, 51.8, 34.2, 33.6, 27.8, 24.1. Anal. ($C_{15}H_{15}BrClNO_3$) C, H, N.

4.4. 2-Iodobenzenesulfonamide (12)

To a suspension of 2-aminobenzenesulfonamide **11** (1.5 g, 8.71 mmol) in H_2O/HCl (16:11 mL) cooled to 0 °C was added a solution of $NaNO_2$ (0.91 g, 13.2 mmol) in H_2O (16 mL). The mixture was stirred until a clear yellow solution was formed. A solution of KI (4.34 g, 26.1 mmol) in H_2O (16 mL) was added and the red coloured mixture was heated to 90 °C for 4 h. The mixture was allowed to cool to room temperature and the formed solid was filtered off, washed with water and pentane and dried under vacuum to afford **12** (1.65 g, 63%) as a light brown solid. ^{1}H NMR (DMSO-d_6) δ 8.11 (dd, J = 7.8, 1.2 Hz, 1H), 8.01 (dd, J = 7.9, 1.5 Hz, 1H), 7.58 (ddd, J = 7.9, 7.4, 1.2 Hz, 1H), 7.49 (s, 2H), 7.27 (ddd, J = 7.4, 1.6 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 145.9, 142.1, 132.8, 128.3, 92.4.

4.5. 5-Bromo-2-iodobenzenesulfonamide (13)

2-Iodobenzenesulfonamide **12** (846 mg, 2.99 mmol) was suspended in conc. H_2SO_4 (20 mL) and warmed to 60 °C. *N*-Bromosuccinimde (638 mg, 3.59 mmol) was added in one portion and the mixture was stirred at 60 °C for 24 h. The mixture was poured on ice and the formed precipitate was filtered off, washed with water and pentane and dried *in vacuo* to afford **13** (941 mg, 87%) as off-white solid. ^{1}H NMR (DMSO-d_6) δ 8.08 (d, J = 2.3 Hz, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.67

(br s, 2H), 7.49 (dd, J = 8.3, 2.3 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 147.8, 144.0, 135.5, 130.6, 121.4, 91.3.

4.6. General procedure for synthesis of alkyne derivatives 14a–c

To a vial with the appropriate 2-iodobenzenesulfonamide (1 equiv), $Pd(PPh_3)_2Cl_2$ (0.1 equiv) and CuI (0.1 equiv) was added DMF (0.2 M) and Et_3N (10 equiv) and the mixture was purged with N_2 gas. The appropriate alkyne (1.5 equiv) was added and the reaction mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc and filtered through Celite™. The organic phase was washed with 0.1 M HCl, water (3 ×) and brine (2 ×), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by automated flash column chromatography (15%→20% EtOAc/pentane) to afford the desired products.

2-(Hex-1-yn-1-yl)benzenesulfonamide (14a) The compound was synthesized according to the general procedure from **12** (400 mg, 1.40 mmol), Purification by automated flash column chromatography (0 → 45% EtOAc/pentane) to afford **14a** (224 mg, 67%) as a white solid. ^{1}H NMR δ 8.07 (d, J = 2.0 Hz, 1H), 7.58 (dd, J = 8.2, 2.1 Hz, 1H), 7.40 (d, J = 8.2 Hz, 1H), 5.36 (s, 3H), 2.50 (t, J = 7.2 Hz, 2H), 1.69–1.56 (m, 2H), 1.54–1.40 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H). ^{13}C NMR δ 142.7, 134.3, 131.9, 127.6, 126.8, 121.1, 100.2, 77.4, 30.3, 22.0, 19.3, 13.5.

2-(4-Phenylbut-1-yn-1-yl)benzenesulfonamide (14b) The compound was synthesized according to the general procedure from **12** (400 mg, 1.40 mmol), Purification by automated flash column chromatography (0 → 45% EtOAc/pentane) to afford **14b** (309 mg, 77%) as a yellow solid. ^{1}H NMR δ 7.92 (dd, J = 7.9, 1.3 Hz, 1H), 7.51 (dd, J = 7.6, 1.4 Hz, 1H), 7.45 (td, J = 7.5, 1.4 Hz, 1H), 7.39–7.19 (m, 6H), 4.77 (br s, 1H), 2.96 (td, J = 7.0, 1.3 Hz, 2H), 2.86 (td, J = 6.8, 1.3 Hz, 2H). ^{13}C NMR δ 143.2, 140.1, 134.5, 132.1, 128.8, 128.7, 128.0, 127.2, 126.8, 121.0, 99.0, 78.4, 34.3, 21.7.

5-Bromo-2-(hex-1-yn-1-yl)benzenesulfonamide (14c) The compound was synthesized according to the general procedure from **13** (108 mg, 0.30 mmol), Purification by automated flash column chromatography (0 → 30% EtOAc/pentane) to afford **14c** (60 mg, 64%) as an off-white solid. ^{1}H NMR δ 8.11 (d, J = 2.0 Hz, 1H), 7.60 (dd, J = 8.2, 2.1 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 5.27 (s, 2H), 2.51 (t, J = 7.2 Hz, 2H), 1.69–1.58 (m, 2H), 1.54–1.41 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR δ 144.3, 135.7, 135.2, 130.2, 121.8, 120.2, 101.7, 76.9, 30.4, 22.2, 19.6, 13.7.

5-Bromo-2-(4-phenylbut-1-yn-1-yl)benzenesulfonamide (14d) The compound was synthesized according to the general procedure from **13** (105 mg, 0.29 mmol), Purification by automated flash column chromatography (0 → 40% EtOAc/pentane) to afford **14d** (68 mg, 65%) as an off-white solid. ^{1}H NMR δ 8.03 (d, J = 2.0 Hz, 1H), 7.55 (dd, J = 8.2, 2.1 Hz, 1H), 7.35 (d, J = 8.3 Hz, 1H), 7.34–7.19 (m, 5H), 4.79 (s, 3H), 2.95 (dd, J = 7.3, 5.8 Hz, 2H), 2.85 (td, J = 6.7, 1.2 Hz, 2H). ^{13}C NMR δ 144.4, 139.9, 135.7, 135.1, 130.1, 128.8, 128.6, 126.9, 121.9, 119.9, 100.2, 77.7, 34.1, 21.7.

4.7. General procedure for synthesis of benzothiazine-1,1-dioxides 15a–d

A solution of the alkyne (1 equiv) in DMF (0.1 M) was added to a oven-dried MW vial containing KOH (4 equiv) and $Pd(PPh_3)_2OAc_2$ (0.05 equiv). The mixture was heated to 60 °C for 4.5–5 h. The mixture was diluted with EtOAc and washed with 1 M HCl (aq.), water and brine, dried over $MgSO_4$, filtered and concentrated to afford the desire products after purification by automated flash column chromatography.

3-Butyl-2H-benzo[e][1,2]thiazine 1,1-dioxide (15a) The compound was synthesized according to the general procedure from **14a** (224 mg, 0.94 mmol). Purification by automated flash column chromatography (0 → 20% EtOAc/pentane) to afford **15a** (95 mg, 42%) as a colourless oil that crystalizes over time. ^{1}H NMR δ 7.93–7.79 (m, 1H), 7.54 (br s, 1H, NH), 7.53 (ddd, J = 7.9, 7.4, 1.3 Hz, 1H), 7.38 (td, J = 7.7, 1.2 Hz, 1H), 7.32–7.28 (m, 1H), 6.03 (s, 1H), 2.48–2.33 (m, 2H), 1.62 (ddt, J = 8.7, 7.5, 6.4 Hz, 2H), 1.53–1.28 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR δ 141.3, 133.8, 132.2, 130.4, 126.7, 126.6, 121.3, 104.8, 34.4, 29.2, 22.0, 13.9. Anal. ($C_{12}H_{15}NO_2S$) C, H, N.

3-Phenethyl-2H-benzo[e][1,2]thiazine 1,1-dioxide (15b) The compound was synthesized according to the general procedure from **14b** (309 mg, 1.08 mmol). Purification by automated flash column chromatography (0 → 20% EtOAc/pentane) to afford **15b** (110 mg, 36%) as a yellow oil. ^{1}H NMR δ 7.85 (dd, J = 8.0, 1.2 Hz, 1H), 7.80 (s, 1H), 7.50 (td, J = 7.6, 1.3 Hz, 1H), 7.36 (td, J = 7.7, 1.2 Hz, 1H), 7.32–7.15 (m, 6H), 5.97 (s, 1H), 2.95 (dd, J = 8.9, 6.7 Hz, 2H), 2.66 (dd, J = 8.8, 6.8 Hz, 2H). ^{13}C NMR δ 140.3, 140.2, 133.6, 132.2, 130.4, 128.6, 128.6 126.8, 126.7, 126.4, 121.2, 105.3, 36.7, 33.6. Anal. ($C_{16}H_{15}NO_2S{\cdot}0.6\ H_2O$) C, H, N.

7-Bromo-3-butyl-2H-benzo[e][1,2]thiazine 1,1-dioxide (15c) The compound was synthesized according to the general procedure from **14c** (60 mg, 0.19 mmol). Purification by automated flash column chromatography (0 → 20% EtOAc/pentane) to afford **15c** (15 mg, 25%) as a colourless oil that crystalizes over time. ^{1}H NMR δ 7.97 (d, J = 2.1 Hz, 1H), 7.64 (dd, J = 8.4, 2.0 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.13 (br s, 1H), 6.03 (s, 1H), 2.39 (t, J = 7.6 Hz, 2H), 1.63 (dddd, J = 8.6, 7.5, 7.0, 5.8 Hz, 2H), 1.47–1.33 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR δ 141.6, 135.3, 132.6, 131.8, 128.3, 124.2, 119.7, 104.8, 34.6, 29.2, 22.1, 13.9. HRMS (ESI) m/z calcd for $C_{12}H_{15}BrNO_2S$ $[M + H]^+$ 316.0007, found 316.0009.

7-Bromo-3-phenethyl-2H-benzo[e][1,2]thiazine 1,1-dioxide (15d) The compound was synthesized according to the general procedure from **14c** (68 mg, 0.19 mmol). Purification by automated flash column chromatography (0 → 20% EtOAc/pentane) to afford **15c** (31 mg, 46%) as a colourless oil that crystalizes over time. ^{1}H NMR δ 7.98 (d, J = 2.1 Hz, 1H), 7.64 (dd, J = 8.4, 2.0 Hz, 1H), 7.38–7.19 (m, 5H), 7.16 (d, J = 8.4 Hz, 1H), 6.79 (br s, 1H), 5.99 (s, 1H), 2.97 (t, J = 7.6 Hz, 1H), 2.69 (t, J = 7.6 Hz, 2H). ^{13}C NMR δ 140.5, 139.8, 135.4, 132.3, 132.0, 128.9, 128.6, 128.5, 126.8, 124.3, 120.1, 105.6, 36.9, 33.7. HRMS (ESI) m/z calcd for $C_{16}H_{15}BrNO_2S$ $[M+H]^+$ 364.0007, found 364.0011.

4.8. 2-Amino-3,5-dibromobenzenesulfonamide (16)

Br_2 (0.75 mL, 15 mmol) was added dropwise to a solution of 2-aminobenzenesulfonamide **15** (1.01 g, 5.85 mmol) in DMF (4 mL) at < 10 °C. The dark brown solution was stirred at 10–15 °C for 15 min and then continued to stir at room temperature for 23 h. Additional Br_2 (0.15 mL, 2.9 mmol) was added and the stirring was continued at room temperature for 22 h. The mixture was diluted with EtOAc and treated with 10% $Na_2S_2O_3$ (aq.). After 10 min of stirring water was added, the phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with sat. Na_2CO_3 (aq), water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford **16** (1.96 g, > 99%) as a light-brown solid, which was directly used in the next step. ^{1}H NMR (DMSO-d_6) δ 7.85 (d, J = 2.3 Hz, 1H), 7.71 (d, J = 2.4 Hz, 1H), 7.65 (s, 2H), 6.02 (s, 2H). ^{13}C NMR (DMSO-d_6) δ 141.5, 137.6, 129.7, 127.0, 110.6, 105.1.

4.9. General procedure for synthesis of saturated benzothiadiazine derivatives 18a–d

Compound **16** (1.1–1.2 equiv) and the appropriate aldehyde **17a**–**d** (1 equiv) were dissolved in 4 M HCl in dioxane (0.04–0.06 M). The mixture was heated in a microwave reactor at 120 °C for 1.5 h.

Work-up procedure A. The solvent was removed and the residue was diluted with EtOAc. The organic phase was washed with 2 M NaOH (aq), water, 0.1 M HCl (aq), water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure.

Work-up procedure B. The product was isolated by filtration and washed with EtOAc.

5,7-Dibromo-3-pentyl-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine-1,1-dioxide (18a). The compound was synthesized according to the general procedure from **16** (198 mg, 0.60 mmol), hexanal (**17a**, 0.05 mL, 0.50 mmol) and 4 M HCl in dioxane (10 mL). Work-up procedure A was used. Purification by flash column chromatography (10 → 20% EtOAc/pentane) and trituration with MeOH afforded **18a** (106 mg, 51%) as a white solid. ^{1}H NMR (acetone-d_6) δ 7.81 (d, J = 2.2 Hz, 1H), 7.69 (d, J = 2.2 Hz, 1H), 6.62 (d, J = 12.2 Hz, 1H), 5.79 (s, 1H), 5.02–4.88 (m, 1H), 2.16–2.06 (m, 1H), 2.02–1.89 (m, 1H), 1.70–1.48 (m, 2H), 1.43–1.31 (m, 4H), 0.97–0.79 (m, 3H). ^{13}C NMR (acetone-d_6) δ 141.0, 138.7, 126.9, 126.0, 111.1, 108.6, 67.5, 34.6, 32.0, 25.0, 23.1, 14.2. Anal. ($C_{12}H_{16}Br_2N_2O_2S$) C, H, N.

5,7-Dibromo-3-phenethyl-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine-1,1-dioxide (18b). The compound was synthesized according to the general procedure from **16** (200 mg, 0.61 mmol) and **17b** (0.08 mL, 0.50 mmol). Work-up procedure A was used. Purification by flash column chromatography (10 → 20% EtOAc/pentane) and trituration with MeOH afforded **18b** (117 mg, 53%) as an white solid. ^{1}H NMR (DMSO-d_6) δ 8.02 (d, J = 11.4 Hz, 1H), 7.90 (d, J = 2.3 Hz, 1H), 7.67 (dd, J = 2.2, 0.6 Hz, 1H), 7.35–7.13 (m, 5H), 6.38 (s, 1H), 4.76–4.62 (m, 1H), 2.86–2.62 (m, 2H), 2.44–2.28 (m, 1H), 2.12–1.96 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 140.8, 140.2, 138.0, 128.4, 128.4, 126.0, 125.6, 124.3, 110.3, 107.3, 65.6, 34.5, 30.2. Anal. ($C_{15}H_{14}Br_2N_2O_2S$) C, H, N.

5,7-Dibromo-3-(3-bromophenethyl)-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine-1,1-dioxide (18c). The compound was synthesized according to the general procedure from **16** (89 mg, 0.27 mmol) and **17c** (63 mg, 0.29 mmol) in a microwave reactor at 120 °C for 1.5 h. Work-up procedure A was used. Purification by flash column chromatography (20 → 100% EtOAc/pentane) and recrystallization from MeCN afforded **18c** (52 mg, 34%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 8.02 (d, J = 11.5 Hz, 1H), 7.91 (d, J = 2.2 Hz, 1H), 7.69 (dd, J = 2.3, 0.6 Hz, 1H), 7.51–7.46 (m, 1H), 7.43–7.36 (m, 1H), 7.31–7.23 (m, 2H), 6.40 (s, 1H), 4.77–4.64 (m, 1H), 2.90–2.60 (m, 2H), 2.45–2.27 (m, 1H), 2.13–1.95 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 143.7, 140.2, 138.0, 131.1, 130.5, 129.0, 127.6, 125.6, 124.3, 121.7, 110.3, 107.3, 65.5, 34.2, 29.7. Anal. ($C_{15}H_{13}Br_3N_2O_2S$) C, H, N.

5,7-Dibromo-3-(2-(quinolin-2(1H)-one-6-yl)ethyl)-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine-1,1-dioxide (18d). The compound was synthesized according to the general procedure from **16** (75 mg, 0.23 mmol) and **17d** (42 mg, 0.21 mmol). Work-up procedure B was used and **18d** (39 mg, 37%) was afforded as a light-brown solid. ^{1}H NMR (DMSO-d_6) δ 11.69 (s, 1H), 8.04 (d, J = 11.5 Hz, 1H), 7.91 (d, J = 2.2 Hz, 1H), 7.85 (d, J = 9.5 Hz, 1H), 7.69 (d, J = 2.2 Hz, 1H), 7.51 (d, J = 1.9 Hz, 1H), 7.40 (dd, J = 8.4, 1.9 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 6.48 (d, J = 9.5 Hz, 1H), 6.37 (s, 1H), 4.77–4.66 (m, 1H), 2.92–2.68 (m, 2H), 2.46–2.32 (m, 1H), 2.16–2.01 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 161.8, 140.2, 140.0, 137.9, 137.3, 134.1, 130.9, 127.0, 125.5, 124.3, 122.0, 119.1, 115.2, 110.3, 107.3, 65.5, 34.5, 29.5. HRMS (ESI) m/z calcd for $C_{18}H_{16}Br_2N_3O_3S$ $[M + H]^+$ 511.9279, found 511.9273.

*4.10. General procedure for synthesis of unsaturated benzothiadiazine derivatives **21a–c***

A solution of carboxylic acid (1.3 equiv) and CDI (1.2–1.8 equiv) in dry CH_2Cl_2 (0.4–0.7 M) was stirred at room temperature for 1.5–4 h. Sulfonamide **16** (1 equiv) dissolved in dry CH_2Cl_2 (0.4–0.5 M) and 15–50 vol% DMF was added and the solution was heated to reflux for 21 h. The solvent was removed and the residue was dissolved in a large quantity of EtOAc and washed with 0.1 M HCl (aq), sat. Na_2CO_3 (aq) and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was dissolved in EtOH, Cs_2CO_3 (2 equiv) was added and the mixture was heated in a microwave reactor at 120 °C for 1.5 h The solvent was removed and 1 M HCl (aq) was added. The formed precipitation was filtered off and washed with EtOAc.

5,7-Dibromo-3-phenethyl-4H-benzo[e][1,2,4]thiadiazine-1,1-dioxide (21a). The compound was synthesized according to the general procedure from **16** (373 mg, 1.13 mmol). Purification by recrystallization from MeCN afforded **21a** (120 mg, 21% over two steps) as an off-white solid.

^{1}H NMR (DMSO-d_6) δ 7.98 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 2.3 Hz, 1H), 7.31–7.20 (m, 4H), 7.14 (tt, J = 5.9, 3.0 Hz, 1H), 2.96 (m, 2H), 2.76 (t, J = 7.9 Hz, 2H). ^{13}C NMR (DMSO-d_6) δ 163.0, 162.8, 141.0, 137.4, 128.4, 128.3, 126.0, 124.8, 124.4, 115.4, 115.1, 39.5, 32.2. HRMS (ESI) m/z calcd for $C_{15}H_{13}Br_2N_2O_2S$ $[M + H]^+$ 442.9064, found 442.9053.

5,7-Dibromo-3-(3-bromophenethyl)-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine-1,1-dioxide (21b). The compound was synthesized according to the general procedure from **16** (251 mg, 0.76 mmol). Purification by recrystallization from MeCN afforded **21b** (97 mg, 24% over two steps) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 10.89 (s, 1H), 8.30 (d, J = 2.1 Hz, 1H), 8.01 (d, J = 2.1 Hz, 1H), 7.52 (dd, J = 2.2, 1.5 Hz, 1H), 7.40 (ddd, J = 7.5, 2.1, 1.5 Hz, 1H), 7.32–7.23 (m, 2H), 3.15–3.06 (m, 2H), 2.98–2.92 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 161.1, 142.9, 138.9, 132.7, 131.2, 130.5, 129.1, 127.6, 125.4, 123.7, 121.6, 117.7, 111.6, 36.3, 31.2. Anal. ($C_{15}H_{11}Br_3N_2O_2S$) C, H, N.

5,7-Dibromo-3-(4-bromophenethyl)-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine-1,1-dioxide (21c). The compound was synthesized according to the general procedure from **16** (253 mg, 0.77 mmol). Purification by recrystallization from MeCN afforded **21c** (146 mg, 37% over two steps) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 10.89 (s, 1H), 8.29 (d, J = 2.1 Hz, 1H), 8.01 (d, J = 2.1 Hz, 1H), 7.48 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 3.14–3.05 (m, 2H), 2.99–2.90 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 161.1, 139.5, 138.9, 132.7, 131.2, 130.7, 125.4, 123.8, 119.3, 117.7, 111.6, 36.3, 31.0. Anal. ($C_{15}H_{11}Br_3N_2O_2S$) C, H, N.

*4.11. 5-Bromo-2-methylbenzenesulfonyl chloride (**23**)*

A solution of $ClSO_3H$ (7.00 mL, 103 mmol) in CH_2Cl_2 (12 mL) was added dropwise to an ice-cold solution of 4-bromotoluene (2.61 g, 15 mmol) in CH_2Cl_2 (25 mL). The mixture was stirred in an ice-bath overnight, while the temperature increased to 10 °C. The solvent was removed and the residue was added dropwise to ice-water. The formed solid was filtered off and washed with water to afford a mixture (3.85 g, 94%, 86:14 ratio according to ^{1}H NMR) of **23** (major isomer, 81%) and 2-bromo-5-methylbenzenesulfonyl chloride as a colourless oil. ^{1}H NMR (**23**) δ 8.20 (d, J = 2.1 Hz, 1H), 7.72 (dd, J = 8.2, 2.0 Hz, 1H), 7.31 (dd, J = 8.2, 0.8 Hz, 1H), 2.74 (s, 3H). ^{13}C NMR (**23**) δ 144.1, 138.2, 137.0, 135.0, 131.4, 120.1, 20.0.

*4.12. 5-Bromo-2-methylbenzenesulfonamide (**24**)*

To an ice cold solution of **23** (5.83 g, 21.6 mmol) in Et_2O (100 mL) was added aqueous ammonia (25%, 20 mL). The mixture was refluxed for 2 h and finally stirred at room temperature for 48 h. The solvent was removed under reduced pressure and the formed solid was filtered off, extensively washed with water and dried *in vacuo* to afford a mixture (5.29 g, 98%, 83:17 ratio according to ^{1}H NMR signal of CH_3-group) of **24** (major isomer, 81%) and 2-bromo-5-methylbenzenesulfonamide (minor isomer, 17%) as a white solid. ^{1}H NMR (CD_3OD, **24**) δ 8.07 (d, J = 2.1 Hz, 1H), 7.61 (dd, J = 8.2, 2.2 Hz, 1H), 7.28 (d, J = 8.1 Hz, 1H), 2.61 (s, 3H). ^{13}C NMR (CD_3OD, **25**) δ 144.0, 135.3, 134.4, 134.4, 129.3, 118.4, 19.3.

*4.13. 6-Bromosaccharin (**28**) synthesized from **24***

To a mixture of H_5IO_6 (27.7 g, 122 mmol) and CrO_3 (304 mg, 3.0 mmol) in MeCN (150 mL) was added **24** (3.80 g, 15.2 mmol, isomer ratio 82:18) and the mixture was heated to reflux for 20 h. Isopropanol (15 mL) was added and the mixture was heated to reflux for additional

10 min. The mixture was allowed to cool to room temperature and the formed solid was filtered off, rinsed with acetone and the filtrate was concentrated under reduced pressure. The green coloured crude solid was triturated with 1 M H_2SO_4 (aq, 15 mL) and the solid material was filtered off, washed with water and pentane and dried *in vacuo* to afford **28** (1.60 g, 38% based on **24**) as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.51 (d, J = 1.7 Hz, 1H), 8.09 (dd, J = 8.2, 1.7 Hz, 1H), 7.87 (d, J = 8.1 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 160.9, 141.5, 137.4, 128.7, 127.5, 126.4, 124.1.

4.14. 6-Bromosaccharin (28) synthesized from 23

A solution of **23** (7.06 g, 26.2 mmol) in dry CH_2Cl_2 (60 mL) was dropwise added to a solution of *tert*-butylamine (2.88 mL, 27.5 mmol) and Et_3N (3.83 mL, 27.5 mmol) in CH_2Cl_2 (120 mL) at 0 °C. The mixture was stirred at 0 °C for 1.5 h, was then allowed to warm to room temperature and stirred at this temperature for 24 h. The mixture was washed with 0.1 M HCl (aq.) and sat. $NaHCO_3$ (aq.), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (17% EtOAc/pentane) to afford **25** (5.65 g, 70% yield, 23% of the other regioisomer). H_5IO_6 (15.5 g, 68.0 mmol) was vigorously stirred in MeCN (85 mL) for 1 h. CrO_3 (254 mg, 2.55 mmol) and acetic anhydride (6.4 mL, 68.0 mmol) were added and the suspension was stirred for 10 min at room temperature. The mixture was cooled in an ice-bath and **25** (2.60 g, 8.49 mmol, ratio 77:23 according to ^{1}H NMR spectra) was added in one portion. The mixture was stirred at 0 °C for 15 min and then allowed to warm to room temperature and stirred for 13 h. The solvent was removed and the remaining slurry was stirred in EtOAc (100 mL) for 10 min. The solid was filtered off, washed with EtOAc and acetone and the filtrate was concentrated under reduced pressure. Water was added and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with sat. $NaHCO_3$ (aq), sat. $Na_2S_2O_3$ (aq) and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was dissolved in TFA (20 mL) and heated to reflux for 48 h and thereafter stirred at room temperature for 4 d. The TFA was removed from the formed precipitate and concentrated under reduced pressure. The precipitate and the remaining solid after evaporation were treated with CH_2Cl_2, and dried *in vacuo* to afford **28** (1.14 g, 62% overall yield based on **25**). ^{1}H NMR (CD_3OD) δ 8.25 (d, J = 1.6 Hz, 1H), 8.07 (dd, J = 8.1, 1.6 Hz, 1H), 7.91 (d, J = 8.2 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 160.9, 141.5, 137.4, 128.7, 127.59, 126.4, 124.1.

4.15. General procedure for the synthesis of the sodium salt of saccharins (29 and 30)

To a 0.2 M solution of saccharin (1 equiv) in dry MeOH, NaOMe (1.01 equiv) was added and the mixture was stirred for 6 h at room temperature. The solvent was removed and the residue was dried *in vacuo*.

Sodium saccharin (29). The compound was synthesized according to the general procedure from saccharin **27** (8.0 g, 44 mmol) to afford **29** (8.8 g, 98%) as a white solid which was directly used in the next step.

Sodium 6-bromosaccharin (30). The compound was synthesized according to the general procedure from **28** (824 mg, 2.97 mmol) to afford **30** (896 mg, > 99%) as a white solid which was directly used in the next step.

4.16. General procedure for the alkyl substituted saccharins 31a–d and 32a–f

The sodium salts of saccharin **29** or **30** (1 equiv) was dissolved in DMF (1 M) and the appropriate halide (1.05 equiv) was added. The mixture was heated in a microwave reactor at 145 °C for 1 h if not otherwise stated. The mixture was poured on ice-water and the precipitate was filtered off, washed with water and pentane and dried *in vacuo*. If no precipitate was formed, the aqueous phase was extracted with EtOAc and the combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure.

2-Pentylbenzo[d]isothiazol-3(2H)-one-1,1-dioxide (31a). The compound was synthesized according to the general procedure from **29** (150 mg, 0.731 mmol). Purification by flash column chromatography (40% EtOAc/pentane) afforded **31a** (143 mg, 77%) as a colourless oil. ^{1}H NMR δ 8.08–8.02 (m, 1H), 7.93–7.89 (m, 1H), 7.89–7.78 (m, 2H), 3.76 (t, J = 7.6 Hz, 2H), 1.92–1.78 (m, 2H), 1.44–1.32 (m, 4H), 0.91 (t, J = 6.9 Hz, 3H). ^{13}C NMR δ 159.1, 137.9, 134.8, 134.4, 127.6, 125.2, 121.0, 39.6, 29.0, 28.2, 22.3, 14.0. Anal. ($C_{12}H_{15}NO_3S$) C, H, N.

2-Phenethylbenzo[d]isothiazol-3(2H)-one-1,1-dioxide (31b). The compound was synthesized according to the general procedure from **29** (200 mg, 0.97 mmol). Purification by flash column chromatography (30% EtOAc/pentane) afforded **31b** (197 mg, 67%) as a white solid. ^{1}H NMR DMSO-d_6) δ 8.30 (ddd, J = 7.6, 0.9, 0.9 Hz, 1H), 8.09–7.94 (m, 3H), 7.34–7.15 (m, 5H), 3.99–3.88 (m, 2H), 3.09–2.97 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 158.2, 137.6, 136.7, 135.8, 135.3, 128.7, 128.4, 126.6, 126.2, 125.0, 121.5, 39.8, 33.9. Anal. ($C_{15}H_{13}NO_3S$) C, H, N.

Ethyl 4-(1,1-dioxo-3-oxobenzo[d]isothiazol-2(3H)-yl)butanoate (31c). The compound was synthesized according to the general procedure from **29** (100 mg, 0.49 mmol) in a microwave reactor at 145 °C for 15 min. Purification by flash chromatography (25% EtOAc/pentane) afforded **31c** (111 mg, 73%) as a colourless oil. ^{1}H NMR δ 8.08–8.04 (m, 1H), 7.95–7.90 (m, 1H), 7.90–7.80 (m, 2H), 4.16 (q, J = 7.1 Hz, 2H), 3.86 (t, J = 7.0 Hz, 2H), 2.45 (t, J = 7.3 Hz, 2H), 2.18 (p, J = 7.2 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ 172.5, 159.2, 137.8, 134.9, 134.5, 127.5, 125.3, 121.1, 60.7, 38.7, 31.4, 23.8, 14.4. Anal. ($C_{13}H_{15}NO_5S$) C, H, N.

2-(3-(Pyridin-3-yl)propyl)benzo[d]isothiazol-3(2H)-one-1,1-dioxide (31d). The compound was synthesized according to the general procedure from **29** (88 mg, 0.43 mmol). Purification by flash column chromatography (30 → 80% EtOAc/pentane) afforded **31d** (15 mg, 12%) as a yellow oil.

^{1}H NMR δ 8.49 (d, J = 1.6 Hz, 1H), 8.45 (dd, J = 4.8, 1.6 Hz, 1H), 8.10–8.03 (m, 1H), 7.95–7.91 (m, 1H), 7.90–7.80 (m, 2H), 7.55 (ddd, J = 7.8, 2.3, 1.6 Hz, 1H), 7.22 (ddd, J = 7.8, 4.8, 0.9 Hz, 1H), 3.84 (t, J = 7.2 Hz, 2H), 2.83–2.68 (m, 2H), 2.24–2.13 (m, 2H). ^{13}C NMR δ 159.1, 150.1, 147.9, 137.8, 136.1, 135.9, 135.0, 134.5, 127.4, 125.3, 123.5, 121.1, 39.0, 30.3, 29.8. HRMS (ESI) *m/z* calcd for $C_{15}H_{15}N_2O_3S$ $[M + H]^+$ 303.0803, found 303.0776.

6-Bromo-2-pentylbenzo[d]isothiazol-3(2H)-one-1,1-dioxide (32a). The compound was synthesized according to the general procedure from **30** (100 mg, 0.33 mmol) in a microwave reactor at 145 °C for 30 min. to afford **32a** (67 mg, 58%) as an off-white solid. ^{1}H NMR δ 8.05 (dd, J = 1.6, 0.6 Hz, 1H), 7.95 (dd, J = 8.1, 1.6 Hz, 1H), 7.90 (dd, J = 8.2, 0.6 Hz, 1H), 3.79–3.71 (m, 2H), 1.84 (p, J = 7.5 Hz, 2H), 1.58–1.47 (m, 2H), 1.44–1.34 (m, 4H), 1.00–0.84 (m, 3H). ^{13}C NMR δ 158.3, 139.2, 137.7, 129.8, 126.5, 126.3, 124.3, 39.8, 29.0, 28.2, 22.3, 14.0. HRMS (ESI) *m/z* calcd for $C_{12}H_{14}BrNO_3S$ $[M]^+$ 330.9878, found 330.9877.

6-Bromo-2-phenethylbenzo[d]isothiazol-3(2H)-one-1,1-dioxide (32b). The compound was synthesized according to the general procedure from **30** (150 mg, 0.50 mmol). Purification by recrystallization from EtOAc/pentane afforded **32b** (148 mg, 77%) as a white solid. ^{1}H NMR δ 8.08–8.04 (m, 1H), 7.95–7.90 (m, 1H), 7.90–7.80 (m, 2H), 4.16 (q, J = 7.1 Hz, 2H), 3.86 (t, J = 7.0 Hz, 2H), 2.45 (t, J = 7.3 Hz, 2H), 2.18 (p, J = 7.2 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H). ^{13}C NMR δ 158.1, 139.2, 137.8, 137.4, 129.9, 129.0, 128.8, 127.1, 126.6, 126.2, 124.3, 40.8, 34.8. Anal ($C_{15}H_{12}BrNO_3S$) C, H, N.

6-Bromo-2-(4-hydroxyphenethyl)benzo[d]isothiazol-3(2H)-one-1,1-dioxide (32c). The compound was synthesized according to the general procedure from **30** (124 mg, 0.41 mmol). Purification by recrystallization from EtOAc/pentane afforded **32c** (108 mg, 66%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 9.22 (s, 1H), 8.74 (d, J = 1.6 Hz, 1H), 8.17 (dd, J = 8.2, 1.7 Hz, 1H), 7.96 (d, J = 8.2 Hz, 1H), 7.06 (d, J = 8.4 Hz, 2H), 6.67 (d, J = 8.4 Hz, 2H), 3.86 (dd, J = 8.4, 6.8 Hz, 2H), 2.91 (dd, J = 8.5, 6.7 Hz, 2H). ^{13}C NMR (DMSO-d_6) δ 157.6, 156.0, 138.3, 138.2, 129.7, 129.4, 127.6, 126.8, 125.4, 124.7, 115.3, 40.3, 33.1. Anal. ($C_{15}H_{12}BrNO_4S$) C, H, N.

6-Bromo-2-(3-bromophenethyl)benzo[d]isothiazol-3(2H)-one-1,1-dioxide (32d). The compound was synthesized according to the general procedure from **30** (114 mg, 0.38 mmol) to afford **32d** (133 mg, 76%) as a white solid. ^{1}H NMR δ 8.06 (dd, J = 1.6, 0.5 Hz, 1H), 7.96 (dd, J = 8.2, 1.6 Hz, 1H), 7.89 (dd, J = 8.2, 0.5 Hz, 1H), 7.47–7.43 (m, 1H), 7.39 (dt, J = 7.5, 1.7 Hz, 1H), 7.25–7.16 (m, 2H), 4.01–3.93 (m, 1H), 3.14–3.06 (m, 2H). ^{13}C NMR δ 158.1, 139.6, 139.1, 137.9, 132.1, 130.4, 130.3, 130.1, 127.7, 126.7, 126.1, 124.4, 122.8, 40.4, 34.4. Anal. ($C_{15}H_{11}Br_2NO_3S$) C, H, N.

6-Bromo-2-(3-nitrophenethyl)benzo[d]isothiazol-3(2H)-one-1,1-dioxide (32e). The compound was synthesized according to the general procedure from **30** (150 mg, 0.53 mmol) to afford **32e** (166 mg, 76%) as an off-white solid. ^{1}H NMR (DMSO-d_6) δ 8.74 (dd, J = 1.7, 0.5 Hz, 1H), 8.20–8.15 (m, 2H), 8.08 (ddd, J = 8.2, 2.4, 1.0 Hz, 1H), 7.95 (dd, J = 8.2, 0.5 Hz, 1H), 7.74 (ddd, J = 7.6, 1.7, 1.0 Hz, 1H), 7.58 (ddd, J = 8.1, 7.6, 0.4 Hz, 1H), 4.05 (t, J = 7.0 Hz, 1H), 3.19 (t, J = 6.9 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 157.8, 147.8, 140.1, 138.3, 138.1, 135.9, 129.9, 129.5, 126.8, 125.3, 124.8, 123.7, 121.7, 39.4[1], 33.2. Anal. ($C_{15}H_{11}BrN_2O_5S$) C, H, N.

6-Bromo-2-(2-(1-succinimidyl)ethyl)benzo[d]isothiazol-3(2H)-one-1,1-dioxide (32f). The compound was synthesized according to the general procedure from **30** (90 mg, 0.3 mmol). Purification by recrystallization from EtOAc/pentane afforded **32f** (71 mg, 59%) as a white solid.

^{1}H NMR (DMSO-d_6) δ 8.74 (dd, J = 1.7, 0.5 Hz, 1H), 8.20 (dd, J = 8.2, 1.7 Hz, 1H), 8.02 (dd, J = 8.2, 0.5 Hz, 1H), 3.91–3.80 (m, 2H), 3.80–3.70 (m, 2H), 2.55 (s, 4H). ^{13}C NMR (DMSO-d_6) δ 177.8, 157.9, 138.4, 137.9, 129.6, 126.8, 125.3, 125.0, 37.0, 35.6, 28.0. Anal. ($C_{13}H_{11}BrN_2O_5S$) C, H, N.

4.17. SIRT1–3 in vitro assay

The Fluor de Lys fluorescence assays were based on the method described in the BioMol product sheet (Enzo Life Sciences) using the BioMol KI177 substrate for SIRT1 and the KI179 substrate for SIRT2 and SIRT3. The determined K_m value of SIRT1 for KI177 was 58 µM, and the K_m of SIRT2 for KI179 was 198 µM.[26] The K_m of SIRT3 for KI179 was reported by Enzo Life Sciences to be 32 µM. The K_m values of SIRT1, SIRT2 and SIRT3 for NAD^+ were reported by BioMol to be 558 µM, 547 µM and 2 mM, respectively.

Briefly, assays were carried out using the Fluor de Lys acetylated peptide substrate at 0.7 K_m and NAD^+ (Sigma N6522 or BioMol KI282) at 0.9 K_m, recombinant GST-SIRT1/2-enzyme or recombinant His-SIRT3 and SIRT assay buffer (KI286). GST-SIRT1 and GST-SIRT2 were produced as described previously.[27,28] His-SIRT3 (BML-SE270) was purchased from Enzo Life Sciences. The buffer, Fluor de Lys acetylated peptide substrate, NAD^+ and DMSO/compounds in DMSO (2.5 µL in 50 µL total reaction volume; DMSO from Sigma, D2650) were preincubated for 5 min at room temperature. The reaction was started by adding the enzyme. The reaction mixture was incubated for one hour at 37 °C. After that, Fluor de Lys developer (KI176) and 2 mM nicotinamide (KI283) in SIRT assay buffer (total volume 50 µL) were added, and the incubation was continued for 45 min at 37 °C. Fluorescence readings were obtained using EnVision 2104 Multilabel Reader (PerkinElmer) with excitation wavelength 370 nm and emission 460 nm.

4.18. Molecular modeling

4.18.1. Docking

The previously published 3D-structure of SIRT2[2] was prepared using Protein Preparation Wizard[29] implemented in the Schrödinger Suite. Ensembles of conformations for each ligand to be docked were generated in order to prevent the starting conformation bias the final docking solutions. In case of a chiral compound, both enantiomers were docked. The docking was conducted using Glide XP (extra precision) into the proposed binding site for the chroman-4-one inhibitors.[2] In the docking trial the protein structure was held rigid while the ligands were flexible. The best scored docking solutions that fulfilled the inhibitor-enzyme key interactions were reported. Single point energies for each docked ligand conformation were calculated, to be able to determine their relative energies. All calculations have been performed using OPLS3 force field[21] in a water solvation model with tools implemented in Schrödinger Suite.[22]

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Electronic Supplementary Information (ESI) available. Synthetic procedures for compounds **17c, 17d** and 3-(3-bromopropyl)pyridine, ^{1}H NMR and ^{13}C NMR spectra of all compounds, elemental analysis, HRMS and purity data of all biologically tested compounds, as well as the calculated physicochemical properties.

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