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Effect of C7 Modifications on Benzothiadiazine-1,1-dioxide Derivatives on Their Inhibitory Activity and Selectivity toward Aldose Reductase

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The development and progression of chronic complications in diabetic patients, such as retinopathy, nephropathy, neuropathy, cataracts, and stroke, are related to the activation and/or overexpression of aldose reductase (ALR2), which is a member of the aldo–keto reductase superfamily. A structure–activity relationship study focused on the C7 position of 1,2,4-benzothiadiazine-1,1-dioxide derivatives was pursued in an attempt to discover ALR2 inhibitors with enhanced potency and selectivity. These studies led to a series of new C7-substituted compounds, which were evaluated for their inhibitory activity against ALR2; they exhibited IC_{50} values in the range of 2.80–45.13 nM. Two compounds with a C7-dimethylcarbamoyl and a C7-diethylcarbamoyl substituent, respectively, were found to be the most active and presented excellent selectivity for ALR2 over aldehyde reductase (ALR1). The structure–activity relationship analyses and molecular modeling studies presented herein highlight the importance of hydrophobic and bulky groups at the C7 position for inhibitory activity and selectivity toward ALR2.

Introduction

Diabetes mellitus as a complex metabolic disorder remains a life-threatening disease that is spreading around the world. Diabetic patients have a high risk for onset of chronic complications including retinopathy, nephropathy, neuropathy, cataracts, and stroke. The development and progression of these complications are related to the activation and/or overexpression of the enzyme aldose reductase (ALR2, EC 1.1.1.21), which is a member of the aldo–keto reductase superfamily.[1] During periods of normal blood sugar levels, glucose is preferentially metabolized through the glycolytic pathway, while during hyperglycemia conditions, the polyol metabolic pathway is activated and leads to the accumulation of sorbitol. ALR2, which catalyzes the reduction of excess glucose to sorbitol in the presence of NADPH as a cofactor, is the first and rate-determining enzyme in the polyol pathway (Figure 1). Thus, inhibition of ALR2 has received considerable attention for the prevention and treatment of diabetic complications.

A large number of ALR2 inhibitors (ARIs) have been developed in the past few decades, and some typical ARIs are shown in Figure 2.[2] However, only epalrestat has reached the market, in Japan in 1992 and recently in China and India, for the treatment of diabetic neuropathy.[3] Many ARIs that appeared to be promising have yet to be clinically successful because of undesirable effects. The side effects are believed to result from a lack of selectivity relative to aldehyde reductase (ALR1, EC 1.1.1.2) which plays a detoxification role in specifically metabolizing toxic aldehydes such as hydroxynonenal (HNE), 3-deoxyglucosone, and methylglyoxal.[4] ALR2 and ALR1 share a high degree of homology in their primary and secondary structures, substrate specificities, and kinetic mechanisms.[5] Our effort has focused on the development of more selective and effective ARIs. We have recently designed a number of potent ARIs based on the scaffolds of 1,2,4-benzothiadiazine-1,1-dioxide, pyrido[2,3-*e*][1,2,4]thiadiazine-1,1-dioxide, 1,2-benzothiazine-1,1-dioxide and quinoxalinone, and we found that substitutions at the C7 position of the benzothiadiazine scaffold greatly increase the activity.[6] Herein, we report further modifications at the C7 position and discuss structure–activity relationships (SAR).

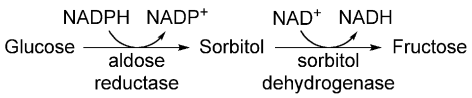

Figure 1. The polyol pathway of glucose metabolism.

Results and Discussion

Chemistry

2-Benzyl-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid derivatives with diverse substitutions at the C7 position (**8a–d** and **16a–l**) were prepared following the synthetic pathway depicted in Scheme 1.[6a] Reaction of commercially available 4-substituted anilines **1a–b** with chlorosulfonyl isocyanate formed ureas, which were then sulfonylated intramolecularly in

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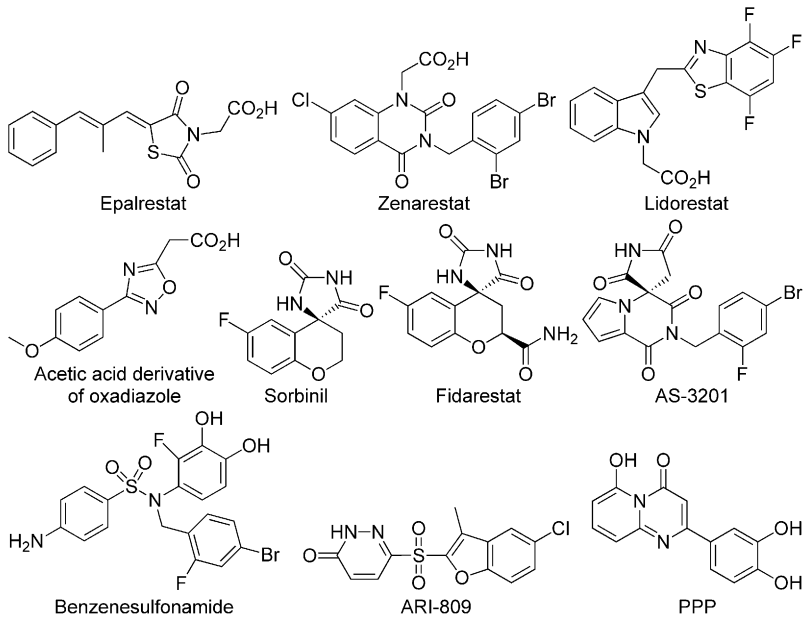


Figure 2. Structures of aldose reductase inhibitors.

the presence of anhydrous aluminum chloride to produce 2*H*-benzo[*e*][1,2,4]thiadiazin-3(4*H*)-one-1,1-dioxides (**2a**–**b**). Aminobenzenesulfonamides **3a**–**b** were obtained by the decarboxylation of **2a**–**b** using sulfuric acid. They were then reacted with triethyl orthoformate to give **4a**–**b**, which were alkylated with methyl bromoacetate to form methyl esters **5a**–**b**. Reduction of the double bond at the 2,3 positions of **5a**–**b** with sodium borohydride gave **6a**–**b**. A benzyl group was introduced at the N2 position with benzylbromide, and the products (**7a**–**d**) were then treated with aqueous sodium hydroxide to provide compounds **8a**–**d**. Alternatively, **2a** was used in the preparation of compounds **16a**–**l** and was oxidized by means of potassium permanganate to form carboxylic acid **9**. Preparation of **11** from **9** was completed by using the same method as for preparation of **4a**–**b** by **2a**–**b**. Acid **11** was converted into ester **12a** by treatment with methanol and sulfuric acid (step k). Conversely, **11** was treated with thionyl chloride (step l) and then with secondary amines in the presence of triethylamine (step m) to form amides **12b**–**f**. Alkylation at the N2 and N4 positions of **12a**–**f** led to **15a**–**l** by using the same method as for the modification of **4a**–**b** to produce **7a**–**d**. The ester groups in the N4 side chain of **15a**–**l** and in the C7 side chain of **15a** and **15g** were hydrolyzed with sodium hydroxide to yield **16a**–**l**.

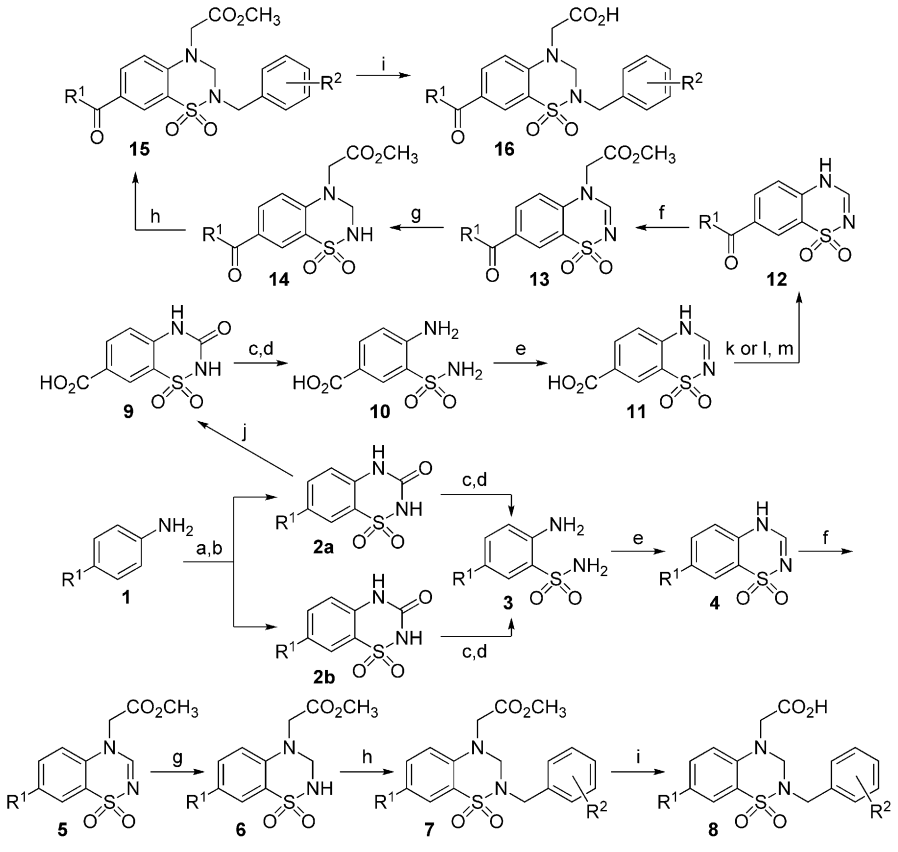
Biological activity and SAR studies

With regard to the design of effective ARIs based on benzothiadiazine-1,1-dioxide and pyridothiadiazine-1,1-dioxide core structures, an N4-acetate group and a substituted N2-benzyl group have proven to be essential side chains.[6a,b] 2-Fluoro-4-bromobenzyl and 2,4,5-trifluorobenzyl turned out to be two of the most potent structures among the substituted N2-benzyl groups. The present study then focused on derivatives resulting from modifications to the C7 position of the 1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-ylacetic acid framework containing an N2 side chain of 2-fluoro-4-bromobenzyl or 2,4,5-trifluorobenzyl. This synthetic work led to two series of compounds, including derivatives of 2-(2-fluoro-4-bromobenzyl)-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-ylacetic acid (**8a**–**b** and **16a**–**f**) and 2-(2,4,5-trifluorobenzyl)-1,1-dioxido-2*H*-benzo[*e*]-[1,2,4]thiadiazin-4(3*H*)-ylacetic acid (**8c**–**d** and **16g**–**l**) as shown in Table 1. All of these compounds were tested for their potential inhibitory activity toward ALR2 isolated from rat lenses and ALR1 isolated from rat kidneys. IC_{50} values were determined by linear regression analysis of the log of the concentration–response curve.[6c] In order to study the SAR, compounds **17a**–**f** (reported previously[6a]) were retested along with the newly synthesized compounds in the present study (Table 1).

ALR2 inhibition

Among all of the compounds synthesized in the present study and previously, as shown in Table 1, **16l** and **16k** (with IC_{50} values of 2.80 and 3.20 nM, respectively) appeared to have the most potent inhibitory activity against ALR2. Further SAR analysis found that C7-carbamoyl derivatives **16f** (IC_{50} = 4.57 nM) and **16e** (IC_{50} = 6.43 nM) were the most active compounds in the N2-2-fluoro-4-bromobenzyl series (**8a**–**b**, **16a**–**f**, and **17a**–**c**). The C7-chloro derivative **17b**, C7-*N*,*N*-methyl-(phenyl)carbamoyl derivative **16d**, and C7-methyl derivative **8a** also exhibited considerable ALR2 inhibitory activity. The ranking order of the compounds displaying the highest levels of ALR2 inhibitory activity in this series is **16f** > **16e** > **17b** > **16d** > **8a** > **17c** > **8b**, therefore giving the relative preference for increased ALR2 inhibition with a diethylcarbamoyl C7 substituent, followed by dimethylcarbamoyl, chloro, *N*,*N*-methyl(phenyl)carbamoyl, methyl, bromo, and methoxy groups.

Highly similar results to the compounds in the N2-2-fluoro-4-bromobenzyl series were also obtained for compounds in the N2-2,4,5-trifluorobenzyl series (**8c**–**d**, **16g**–**l**, and **17d**–**f**). The C7-carbamoyl derivatives **16l** (IC_{50} = 2.80 nM) and **16k** (IC_{50} = 3.20 nM) were the most active compounds. In addition, C7-chloro derivative **17e**, C7-methyl derivative **8c**, and C7-*N*,*N*-methyl(phenyl)carbamoyl derivative **16j** showed excellent ALR2 inhibitory activity. The ranking for the inhibitory activity of the most potent compounds in this series is **16l** > **16k** > **17e** > **8c** > **16j** > **17f** > **8d**, indicating the preferred order



1a–6a: $R^1 = CH_3$
1b–6b: R^1 = OMe
7a, 8a: $R^1 = CH_3$; R^2 = 2-F, 4-Br
7b, 8b: R^1 = OMe; R^2 = 2-F, 4-Br
7c, 8c: $R^1 = CH_3$; R^2 = 2,4,5-F_3
7d, 8d: R^1 = OMe; R^2 = 2,4,5-F_3
12a–14a: R^1 = OMe
12b–14b: R^1 = piperidin-1-yl
12c–14c: R^1 = morpholino
12d–14d: R^1 = methyl(phenyl)amino
12e–14e: R^1 = dimethylamino
12f–14f: R^1 = diethylamino
15a: R^1 = OMe; R^2 = 2-F, 4-Br; **16a:** R^1 = OH; R^2 = 2-F, 4-Br
15b, 16b: R^1 = piperidin-1-yl; R^2 = 2-F, 4-Br
15c, 16c: R^1 = morpholino; R^2 = 2-F, 4-Br
15d, 16d: R^1 = methyl(phenyl)amino; R^2 = 2-F, 4-Br
15e, 16e: R^1 = dimethylamino; R^2 = 2-F, 4-Br
15f, 16f: R^1 = diethylamino; R^2 = 2-F, 4-Br
15g: R^1 = OMe; R^2 = 2,4,5-F_3; **16g:** R^1 = OH; R^2 = 2,4,5-F_3
15h, 16h: R^1 = piperidin-1-yl; R^2 = 2,4,5-F_3
15i, 16i: R^1 = morpholino; R^2 = 2,4,5-F_3
15j, 16j: R^1 = methyl(phenyl)amino; R^2 = 2,4,5-F_3
15k, 16k: R^1 = dimethylamino; R^2 = 2,4,5-F_3
15l, 16l: R^1 = diethylamino; R^2 = 2,4,5-F_3

Scheme 1. *Reagents and conditions*: a) $ClSO_2NCO$, CH_3NO_2, −40 °C, 0.5 h; b) $AlCl_3$, 110 °C, 1 h, 64–70%; c) 50% H_2SO_4, 140 °C, 6 h; d) $NaOH_{(aq)}$, 0 °C, 0.5 h, 46–56%; e) $HC(OEt)_3$, reflux, 2 h, 86–92%; f) $BrCH_2COOCH_3$, K_2CO_3, CH_3CN, 75 °C, 2 h, 68–84%; g) $NaBH_4$, CH_3OH, 0 °C, 5 min, 56–75%; h) Bn-Br, K_2CO_3, CH_3CN, 70 °C, 2 h, 69–86%; i) 1,4-dioxane, NaOH, RT, 2 h, 69–88%; j) NaOH, $KMnO_4$, 80 °C, 7 h, 68%; k) CH_3OH, H_2SO_4, reflux, 5 h, 41%; l) $SOCl_2$, 75 °C, overnight; m) Et_3N, secondary amines, CH_3CN, RT, 0.5 h, 67–78%.

of effect of the C7 substituents as diethylcarbamoyl > dimethylcarbamoyl > chloro > methyl > *N*,*N*-methyl(phenyl)carbamoyl > bromo > methoxy.

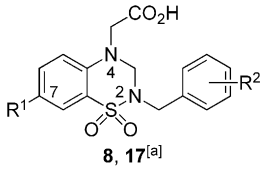
In addition, both of the C7-carboxy derivatives (**16a** and **16g**) had the lowest ALR2 inhibitory activity in each series. C7-piperidine-1-carbonyl derivatives **16b** and **16h** and C7-morpholine-4-carbonyl derivatives **16c** and **16i** also showed relatively low ALR2 inhibitory activity. Therefore, it is likely that this decrease in ALR2 inhibitory activity was caused by the hydrophilicity of the C7 substituents. Furthermore, it was found that the C7-piperidine-1-carbonyl and C7-morpholine-4-carbonyl substituents, compared with the C7-*N*,*N*-methyl(phenyl)carbamoyl substituent in both series, yielded quite different effects on ALR2 inhibition despite their similarity in size. For example, **16b** and **16c** exhibited significantly lower activity than **16d**, and **16h** and **16i** showed lower activity than **16j**. The decreased inhibitory effect for the C7-piperidine-1-carbonyl and C7-morpholine-4-carbonyl substituents might result from that fact that their structures are more constrained than that of the C7-*N*,*N*-methyl(phenyl)carbamoyl substituent. This is also evidenced by the fact that the most potent compounds, including **16f**, **16e**, **16l**, and **16k**, have the most flexible C7-carbamoyl substituents in each series compared with the other C7-carbamoyl derivatives. Moreover, the chloro, methyl, and *N*,*N*-methyl(phenyl)carbamoyl C7 substituents are very different in size but showed similar activity. These results suggest that the effect of C7 substituents depends more on the hydrophobicity and flexibility of the substituents than on the size.

Furthermore, all compounds in the N2-2,4,5-trifluorobenzyl series were more potent than their counterparts in the N2-2-fluoro-4-bromobenzyl series, demonstrating that the N2-2,4,5-trifluorobenzyl group is favorable for the design of ALR2 inhibitors based on a benzothiadiazine-1,1-dioxide core structure. This is consistent with recently reported study results.[6a,b]

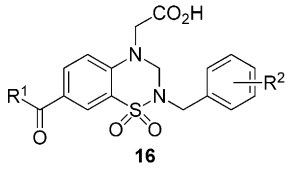
ALR1 inhibition

All of the synthetic compounds were evaluated for ALR1 inhibition at a concentration of 5 μM, as shown in Table 1. Notably, C7-carbamoyl derivatives (**16b**–**f** and **16h**–**l**) showed low inhibitory potency within both of the two series, whereas the C7-halogen derivatives (**17a**–**c** and **17d**–**f**) displayed higher inhibitory activities. The inhibitory activities of C7-carboxy derivatives were similar to those shown by the C7-carbamoyl derivatives. The activity of C7-methyl and C7-methoxy derivatives was moderate. Among the C7-carbamoyl derivatives, C7-piperidine-1-carbonyl and C7-morpholine-4-carbonyl compounds (**16b**–**c** and **16h**–**i**) were the least active with regard to ALR1 inhibition in each of the two series, respectively, although they

Table 1. Biological data for the synthesized compounds.



8, **17**[a]



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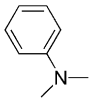
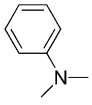
Compd	R^1	R^2	ALR2 IC_{50} [nM][b]	ALR1 Inhib. [%][c]
8a	CH_3	2-F-4-Br	8.23 (6.96–9.51)	23.4
8b	OMe	2-F-4-Br	20.17 (17.02–23.31)	22.3
16a	OH	2-F-4-Br	45.13 (41.02–49.25)	18.4
16b		2-F-4-Br	25.97 (21.83–30.10)	14.9
16c		2-F-4-Br	35.07 (27.19–42.95)	12.2
16d		2-F-4-Br	8.20 (4.45–11.95)	17.8
16e		2-F-4-Br	6.43 (5.02–7.85)	18.3
16f		2-F-4-Br	4.57 (3.94–5.19)	15.6
17a	F	2-F-4-Br	25.83 (18.62–33.04)	25.6
17b	Cl	2-F-4-Br	7.30 (5.81–8.79)	30.2
17c	Br	2-F-4-Br	9.50 (7.71–11.29)	28.3
8c	CH_3	2,4,5-F_3	4.36 (3.09–5.64)	46.1
8d	OMe	2,4,5-F_3	8.43 (6.50–10.36)	38.3
16g	OH	2,4,5-F_3	15.97 (12.72–19.21)	25.1
16h		2,4,5-F_3	11.10 (8.00–14.20)	14
16i		2,4,5-F_3	13.20 (7.75–18.65)	22
16j		2,4,5-F_3	5.50 (3.76–7.24)	35.8
16k		2,4,5-F_3	3.20 (2.12–4.28)	25
16l		2,4,5-F_3	2.80 (2.14–3.46)	22.8
17d	F	2,4,5-F_3	13.77 (9.29–18.24)	57.8
17e	Cl	2,4,5-F_3	3.87 (2.83–4.90)	52.9
17f	Br	2,4,5-F_3	7.70 (4.65–10.75)	50.8
	Epalrestat		14.32 (12.26–16.08)	79.2

[a] Compounds prepared previously.[6a] [b] IC_{50} (95% CL) values represent the concentration of the tested compounds required to decrease enzymatic activity by 50%. [c] Inhibitory effects were evaluated at a concentration of 5 μM.

did not exhibit the highest ALR2 inhibition, as described above. The C7-diethylcarbamoyl and C7-dimethylcarbamoyl compounds (**16e–f** and **16k–l**) that were the most potent inhibitors of ALR2 exhibited low inhibitory activity against ALR1. Accordingly, it is likely that bulky and rigid substituents at the C7 position greatly decrease the ALR1 inhibitory activity of the compounds. These results, together with the data from the compounds in the ALR2 inhibition assay, suggest that the C7-carbamoyl derivatives are highly selective inhibitors for ALR2 over ALR1. In particular, the C7-diethylcarbamoyl derivatives (**16f** and **16l**) proved to be potent and selective ALR2 inhibitors.

Molecular docking

Docking studies were performed to examine the manner by which newly prepared compounds interact with ALR2, which may explain the above-described SARs and mechanistic details of the biological activity. The most active compound, **16l**, was docked into the conformation of human ALR2 from the complex with $NADP^+$/IDD594 (PDB code: 1US0) as shown in Figure 3.

Docking results revealed that inhibitor **16l** fit tightly within the active sites of ALR2. The carboxy group was bound to an anion-binding pocket through three tight hydrogen bonds with the hydroxy group of Tyr48 (3.30 Å), the Nε2 atom of His110 (2.70 Å), and the Nε1 of Trp111 (2.82 Å), which are three key residues in binding and catalysis.[7] The carboxy head also formed a stabilizing electrostatic interaction with the positively charged nicotinamide moiety of the cofactor $NADP^+$ (N–O = 4.15 Å). The substituted benzyl ring fit well in the specificity pocket formed by Leu300, Cys303, Cys298, Thr113, Phe122, and Trp111, among which Trp111 π-stacked perfectly against the benzyl ring. In addition, the benzothiadiazine-1,1-dioxide core was trapped in the hydrophobic cage formed by the side chains of Trp20, Phe122, Trp79, and Trp219, which in turn led to the placement of the relatively bulky 7-diethylcarbamoyl substituent in the deep cavity of the hydrophobic cage formed by Phe121, Phe122, Gln49, and Val47. The ability of the core, together with the bulky 7-substituent, to be accommodated by the hydrophobic cage is likely due to the relatively open space of the pocket,[2c] suggesting that this pocket may also play an important role in ligand binding. This binding behavior is different from that of compound **17e** (reported previously[6a]) in which the phenyl ring of the benzothiadiazine-1,1-dioxide core penetrates the specificity pocket, while the benzyl ring occupies the hydrophobic cage, which consequently may explain the higher ALR2 inhibitory activity of **16l** as compared with **17e**.

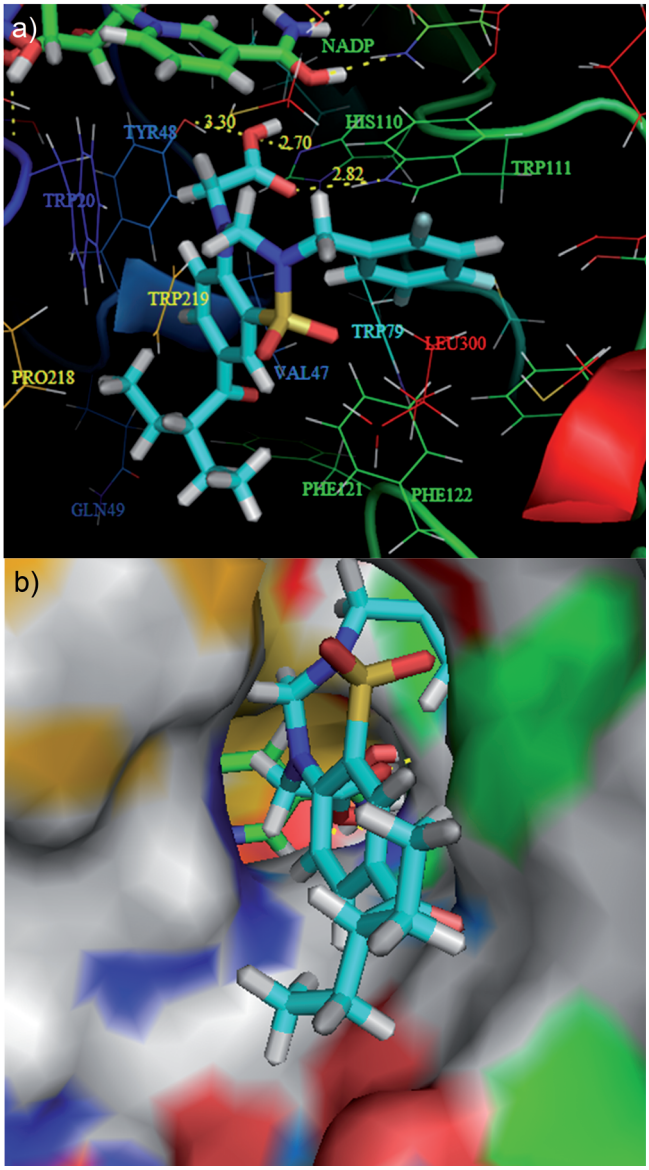


Figure 3. Docking of inhibitor **16l** into the active site of ALR2. Ligand **16l** and NADP are shown as stick models, while protein structures are shown as a) ribbon diagrams and b) in surface representation. The docked pose of **16l** is shown in cyan (C), red (O), blue (N), yellow (S), and grey (F); hydrogen bonds are shown as yellow dashed lines.

Conclusions

A group of new derivatives of benzothiadiazine-1,1-dioxide were synthesized with modifications at the C7 position and showed ALR2 inhibitory activity, with IC_{50} values ranging from 2.80 nM to 45.13 nM. This allowed us to investigate the effect of various C7 substituents of the benzothiadiazine-1,1-dioxide core on inhibitory activity against aldose reductase and selectivity. SAR analysis, combined with docking studies, demonstrated that bulky hydrophobic groups were favorable for C7 substitution in the design of ARIs. In particular, we found that a flexible amide group at the C7 position not only increased ALR2 inhibition activity but also greatly decreased the inhibition against ALR1, demonstrating a pronounced effect of the C7 substituent both on activity and selectivity. Consequently, the present study determined the important role of the side chain at the C7 position of benzothiadiazine-1,1-dioxide. This side chain, together with the other two essential side chains of the N4-carboxylic acid and the N2-benzyl determined by our recent studies,[6a] provides a structural basis for effective ARIs, as well as insight into the pharmacophore requirements for ALR2 inhibition.

Experimental Section

Chemistry

General: Melting points were recorded on an X-4 microscopic melting point apparatus and were uncorrected. All reactions were routinely checked by TLC on Merck 60F254 silica gel. ^{1}H NMR spectra were recorded at 400 MHz, while ^{13}C NMR spectra were recorded at 100 MHz in [D_6]DMSO (unless otherwise stated). Data for ^{1}H NMR are presented as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant J (Hz), and integration. Data for ^{13}C NMR are reported in chemical shifts (δ, ppm). A Hitachi D-2000 Elite HPLC system was used to determine the purity of the final acetic acid derivatives with the following method: Inertsil ODS-2 250 mm × 10 mm, 5 μm column; mobile phase: CH_3CN (0.2% TFA)/CH_3OH = 1:1 for 8 min; room temperature; flow rate: 1 mL min^{-1}; detection at λ 254 nm.

The *p*-toluidine (**1a**), 4-methoxyaniline (**1b**), methyl bromoacetate, 4-bromo-2-fluorobenzyl bromide, 2,4,5-trifluorobenzyl bromide, piperidine, morpholine, dimethylamine, diethylamine and *N*-methylaniline used to obtain the target inhibitors were from Alfa Aesar. D,L-Glyceraldehyde, sodium D-glucuronate, and NADPH were from Sigma–Aldrich. All other chemicals were of reagent grade. Wistar rats (200–250 g) were supplied by Vital River, Beijing, China.

General procedure for the synthesis of 2a–b: A three-necked flask (500 mL) was charged with chlorosulfonyl isocyanate (13 mL, 150 mmol) and $MeNO_2$ (150 mL). The flask was cooled in a dry ice/CH_3CN bath, and a solution of the corresponding aniline **1** (135 mmol) in $MeNO_2$ (100 mL) was then added dropwise to the flask with vigorous stirring. After the addition was complete, the reaction mixture was stirred for an additional 30 min. Anhydrous aluminum chloride (20 g, 150 mmol) was then added to the resulting suspension, and the mixture was stirred for 1 h at 110 °C. The resulting mixture was then poured into ice, and the precipitate was collected by filtration and washed with cold H_2O, then dry Et_2O, to yield the final product.

7-Methyl-2*H*-benzo[*e*][1,2,4]thiadiazin-3(4*H*)-one-1,1-dioxide (2a): White solid (yield: 18.3 g, 64%): mp: >300 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 11.10 (s, 1H), 7.58 (s, 1H), 7.51 (d, J = 8 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 2.34 ppm (s, 3H).

7-Methoxy-2*H*-benzo[*e*][1,2,4]thiadiazin-3(4*H*)-one-1,1-dioxide (2b): Brown solid (yield: 21.5 g, 70%): mp: >300 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 11.08 (s, 1H), 7.24 (m, 3H), 3.81 ppm (s, 3H).

7-Carboxy-2*H*-benzo[*e*][1,2,4]thiadiazin-3(4*H*)-one-1,1-dioxide (9): Compound **2a** (10 g, 47 mmol) was first suspended in H_2O (600 mL) and then dissolved during the addition of saturated aqueous NaOH (10 mL). $KMnO_4$ (24.5 g, 155 mmol) was added portionwise at 0 °C, and the reaction mixture was stirred at 80 °C for 7 h. The insoluble material was removed by filtration, and the filtrate

was acidified to pH 1 with concentrated HCl. The precipitate was collected by filtration, washed with H_2O, and dried to yield the product as a white solid (yield: 7.7 g, 68%): mp: >300 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.77 (s, 1H), 9.83 (s, 1H), 8.02 (s, 1H), 7.84 (dd, J_1 = 2 Hz, J_2 = 8.4 Hz, 1H), 7.01 ppm (d, J = 8.4 Hz, 1H).

General procedure for the synthesis of 3a–b, 10: Compound **2** or **9** (28 mmol) was added to 50% aqueous H_2SO_4 (120 mL) and stirred at 140 °C for 6 h. The resulting mixture was cooled to room temperature and then poured over ice. Saturated aqueous NaOH (60 mL) was added to neutralize the mixture. The precipitate was collected by suction filtration, washed with cold H_2O, and dried in vacuo to give desired product **3** or **10**.

2-Amino-5-methylbenzenesulfonamide (3a): White solid (yield: 2.9 g, 56%): mp: 107–108 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.35 (s, 1H), 7.14 (s, 2H), 7.05 (d, J = 8 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 5.63 (s, 2H), 2.17 ppm (s, 3H).

2-Amino-5-methoxybenzenesulfonamide (3b): Brown solid (yield: 2.9 g, 52%); mp: 118–119 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.22 (s, 2H), 7.10 (s, 1H), 6.93 (m, 1H), 6.76 (d, J = 8.8 Hz, 1H), 5.43 (s, 2H), 3.68 ppm (s, 3H).

4-Amino-3-sulfamoylbenzoic acid (10): White solid (yield: 2.8 g, 46%); mp: 240–241 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.18 (d, J = 2 Hz, 1H), 7.75 (m, 1H), 7.38 (s, 2H), 6.81 (d, J = 8.8 Hz, 1H), 6.54 ppm (s, 2H).

General procedure for the synthesis of 4a–b, 11: The appropriate 2-aminophenylsulfonamide **3** or **10** (10 mmol) was suspended in triethylorthoformate (30 mL) and stirred at reflux for 2 h. The mixture was then cooled to room temperature, and the precipitate was collected by filtration, washed with Et_2O, and dried in vacuo to yield the product.

7-Methyl-4*H*-benzo[*e*][1,2,4]thiadiazine-1,1-dioxide (4a): White solid (yield: 1.8 g, 90%): mp: 278–279 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.23 (s, 1H), 7.96 (s, 1H), 7.63 (m, 1H), 7.50 (m, 1H), 7.23 (d, J = 8.4 Hz, 1H), 2.38 ppm (s, 3H).

7-Methoxy-4*H*-benzo[*e*][1,2,4]thiadiazine-1,1-dioxide (4b): White solid (yield: 2 g, 92%): mp: 244–246 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.22 (s, 1H), 7.92 (s, 1H), 7.29 (m, 1H), 7.28 (m, 1H), 7.23 (m, 1H), 3.83 ppm (s, 3H).

4*H*-Benzo[*e*][1,2,4]thiadiazine-7-carboxylic acid-1,1-dioxide (11): White solid (yield: 1.9 g, 86%): mp: >300 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.59 (s, 1H), 8.25 (s, 1H), 8.18 (d, J = 8.4 Hz, 1H), 8.07 (s, 1H), 7.43 (d, J = 8.8 Hz, 1H), 3.4 ppm (s, 1H).

4*H*-Benzo[*e*][1,2,4]thiadiazine-7-carboxylate-1,1-dioxide (12a): Compound **11** (10 mmol, 2.3 g) was suspended in a solution of H_2SO_4 (1.3 mL) and CH_3OH (20 mL), and the mixture was stirred at reflux for 5 h. The solvent was removed in vacuo, and the residue was suspended in H_2O and adjusted to pH 8 with aqueous NaOH (10% *w*/*v*). The precipitate was collected by filtration, washed with H_2O, and dried to yield the product (yield: 984 mg, 41%): mp: 235–236 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.63 (s, 1H), 8.27 (s, 1H), 8.20 (m, 1H), 8.08 (s, 1H), 3.89 ppm (s, 3H).

General procedure for the synthesis of 12b–f: Compound **11** (10 mmol, 2.3 g) was suspended in $SOCl_2$ (10 mL) and stirred at reflux overnight. The remaining $SOCl_2$ was removed by distillation in vacuo. The residue was then dissolved in CH_3CN (20 mL), to which a solution of the corresponding secondary amine (12 mmol), Et_3N (20 mmol), and CH_3CN (5 mL) was added dropwise. The resulting mixture was stirred at room temperature for 30 min, then the solvent was evaporated in vacuo, and the residue was washed with H_2O and dried to yield the product.

(1,1-Dioxido-4*H*-benzo[*e*][1,2,4]thiadiazin-7-yl)(piperidin-1-yl)methanone (12b): Light-brown solid (yield: 2.3 g, 78%): mp: >300 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.45 (s, 1H), 8.03 (s, 1H), 7.72 (m, 2H), 7.37 (d, J = 8.4 Hz, 1H), 3.57 (s, 2H), 3.33 (s, 2H), 1.56 ppm (d, 6H).

(1,1-Dioxido-4*H*-benzo[*e*][1,2,4]thiadiazin-7-yl)(morpholino)methanone (12c): Light-brown solid (yield: 2.2 g, 76%): mp: 298–299 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.45 (s, 1H), 8.03 (s, 1H), 7.83 (s, 1H), 7.72 (d, J = 7.2 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 3.4 ppm (d, 8H).

***N*-Methyl-*N*-phenyl-4*H*-benzo[*e*][1,2,4]thiadiazine-7-carboxamide-1,1-dioxide (12d)**: Light-brown solid (yield: 2.1 g, 67%): mp: 289–291 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.35 (s, 1H), 7.94 (s, 1H), 7.65 (s, 1H), 7.50 (m, 1H), 7.24 (m, 5H), 7.13 (d, J = 8.4 Hz, 1H), 3.38 ppm (s, 3H).

***N*,*N*-Dimethyl-4*H*-benzo[*e*][1,2,4]thiadiazine-7-carboxamide-1,1-dioxide (12e)**: Light-brown solid (yield: 1.9 g, 76%): mp: 252–253 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.46 (s, 1H), 8.03 (s, 1H), 7.74 (m, 2H), 7.37 (d, J = 8.4 Hz, 1H), 2.96 ppm (d, 6H).

***N*,*N*-Diethyl-4*H*-benzo[*e*][1,2,4]thiadiazine-7-carboxamide-1,1-dioxide (12f)**: Light-brown solid (yield: 2.1 g, 74%): mp: 210–211 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.47 (s, 1H), 8.04 (s, 1H), 7.73 (m, 1H), 7.68 (m, 1H), 7.66 (m, 1H), 3.42 (s, 2H), 3.18 (s, 2H), 1.13 ppm (d, 6H).

General procedure for the synthesis of 5a–b, 13a–f: The corresponding compound **4** or **12** (20 mmol) was dissolved in CH_3CN (60 mL). K_2CO_3 (3 g, 21.7 mmol) and methyl bromoacetate (3.25 g, 20 mmol) were successively added to this solution, and the mixture was stirred at 75 °C for 2 h. The solvent was evaporated in vacuo and the residue was washed with H_2O, dried, and then recrystallized from EtOAc to give pure product.

Methyl 2-(7-methyl-1,1-dioxido-4*H*-benzo[*e*][1,2,4]thiadiazin-4-yl)acetate (5a): White solid (yield: 4.5 g, 84%): mp: 178–179 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.04 (s, 1H), 7.71 (s, 1H), 7.54 (dd, J_1 = 1.6 Hz, J_2 = 8.8 Hz, 1H), 7.23 (d, J = 8.8 Hz, 1H), 5.06 (s, 2H), 3.72 (s, 3H), 2.40 ppm (s, 3H).

Methyl 2-(7-methoxy-1,1-dioxido-4*H*-benzo[*e*][1,2,4]thiadiazin-4-yl)acetate (5b): White solid (yield: 4.1 g, 72%): mp: 163–164 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.02 (s, 1H), 7.32 (m, 3H), 5.08 (s, 2H), 3.86 (s, 3H), 3.70 ppm (s, 3H).

Methyl 4-(2-methoxy-2-oxoethyl)-4*H*-benzo[*e*][1,2,4]thiadiazine-7-carboxylate-1,1-dioxide (13a): White solid (yield: 4.9 g, 78%): mp: 205–206 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.33 (s, 1H), 8.21 (m, 2H), 7.54 (d, J = 8.8 Hz, 1H), 5.15 (s, 2H), 3.90 (s, 3H), 3.73 ppm (s, 3H).

Methyl 2-(1,1-dioxido-7-(piperidine-1-carbonyl)-4*H*-benzo[*e*]-[1,2,4]thiadiazin-4-yl)acetate (13b): White solid (yield: 5.7 g, 78%): mp: 243–244 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.13 (s, 1H), 7.86 (s, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 5.12 (s, 2H), 3.74 (s, 3H), 3.60 (s, 2H), 3.32 (s, 2H), 1.55 ppm (d, 6H).

Methyl 2-(7-(morpholine-4-carbonyl)-1,1-dioxido-4*H*-benzo[*e*]-[1,2,4]thiadiazin-4-yl)acetate (13c): White solid (yield: 5 g, 68%): mp: 142–144 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.12 (s, 1H), 7.93 (s, 1H), 7.77 (s, 1H), 7.44 (d, J = 8.4 Hz, 1H), 5.13 (s, 2H), 3.74 (s, 3H), 3.60 ppm (s, 8H).

Methyl 2-(7-(methyl(phenyl)carbamoyl)-1,1-dioxido-4*H*-benzo[*e*]-[1,2,4]thiadiazin-4-yl)acetate (13 d): White solid (yield: 5.4 g, 70%): mp: 190–191 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.04 (s, 1H), 7.72 (s, 1H), 7.56 (m, 1H), 7.27 (m, 5H), 7.21 (d, J = 8.8 Hz, 1H), 5.01 (s, 2H), 3.69 ppm (s, 3H).

Methyl 2-(7-(dimethylcarbamoyl)-1,1-dioxido-4*H*-benzo[*e*]-[1,2,4]thiadiazin-4-yl)acetate (13 e): White solid (yield: 5.1 g, 78%): mp: 179–180 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.13 (s, 1H), 7.90 (d, J = 1.6 Hz, 1H), 7.79 (m, 1H), 7.43 (d, J = 8.8 Hz, 1H), 5.13 (s, 2H), 3.74 (s, 3H), 2.96 ppm (d, 6H).

Methyl 2-(7-(diethylcarbamoyl)-1,1-dioxido-4*H*-benzo[*e*]-[1,2,4]thiadiazin-4-yl)acetate (13 f): White solid (yield: 5.2 g, 74%): mp: 205–206 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.13 (s, 1H), 7.83 (d, J = 1.6 Hz, 1H), 7.73 (dd, J_1 = 1.6 Hz, J_2 = 8.6 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 5.13 (s, 2H), 3.74 (s, 3H), 3.44 (s, 2H), 3.16 (s, 2H), 1.11 ppm (d, 6H).

General procedure for the synthesis of 6 a–b and 14 a–f: $NaBH_4$ (1.5 g, 40 mmol) was added to a solution of the appropriate acetate **5** or **13** (10 mmol) in CH_3OH. The reaction mixture was stirred at room temperature for 5 min, then the residue was poured into ice, and the alkaline suspension was acidified with 0.1 N HCl. The resulting precipitate was collected by filtration, washed with H_2O, dried, and recrystallized from EtOAc to give the desired product.

Methyl 2-(7-methyl-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (6 a): White solid (yield: 2.0 g, 75%): mp: 181–182 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.05 (m, 1H), 7.22 (m, 1H), 7.18 (m, 1H), 6.64 (d, J = 8.8 Hz, 1H), 4.72 (d, J = 8 Hz, 2H), 4.30 (s, 2H), 3.65 (s, 3H), 2.22 ppm (s, 3H).

Methyl 2-(7-methoxy-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (6 b): White solid (yield: 2.1 g, 72%): mp: 127–128 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.10 (t, J = 8 Hz, 1H), 7.04 (m, 2H), 6.73 (d, J = 8.8 Hz, 1H), 4.70 (d, J = 8 Hz, 2H), 4.30 (s, 2H), 3.72 (s, 3H), 3.65 ppm (s, 3H).

Methyl 4-(2-methoxy-2-oxoethyl)-3,4-dihydro-2*H*-benzo[*e*]-[1,2,4]thiadiazine-7-carboxylate-1,1-dioxide (14 a): White solid (yield: 2.4 g, 75%): mp: 133–134 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.41 (t, J = 7.6 Hz, 1H), 8.07 (s, 1H), 7.85 (d, J = 9.2 Hz, 1H), 6.87 (d, J = 9.2 Hz, 1H), 4.84 (d, J = 7.6 Hz, 2H), 4.45 (s, 2H), 3.82 (s, 3H), 3.68 ppm (s, 3H).

Methyl 2-(1,1-dioxido-7-(piperidine-1-carbonyl)-2*H*-benzo[*e*]-[1,2,4]thiadiazin-4(3*H*)-yl)acetate (14 b): White solid (yield: 2.5 g, 68%): mp: 206–207 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.26 (s, 1H), 7.55 (s, 1H), 7.41 (m, 1H), 6.78 (d, J = 8 Hz, 1H), 4.80 (s, 2H), 4.40 (s, 2H), 3.68 (s, 3H), 3.60 (s, 2H), 3.44 (s, 2H), 1.55 ppm (d, 6H).

Methyl 2-(7-(morpholine-4-carbonyl)-1,1-dioxido-2*H*-benzo[*e*]-[1,2,4]thiadiazin-4(3*H*)-yl)acetate (14 c): White solid (yield: 2.6 g, 70%): mp: 180–181 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.29 (t, J = 8 Hz, 1H), 7.59 (dd, J_1 = 2 Hz, J_2 = 12 Hz, 1H), 7.45 (dd, J_1 = 2 Hz, J_2 = 8.8 Hz, 1H), 6.79 (d, J = 8.8 Hz, 1H), 4.80 (d, J = 8.4 Hz, 2H), 4.41 (s, 2H), 3.68 (s,3H), 3.59 (s, 4H), 3.49 ppm (s, 4H).

Methyl 2-(7-(methyl(phenyl)carbamoyl)-1,1-dioxido-2*H*-benzo[*e*]-[1,2,4]thiadiazin-4(3*H*)-yl)acetate (14 d): White solid (yield: 2.2 g, 56%): mp: 192–193 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.19 (m, 1H), 7.48 (m, 1H), 7.32 (m, 2H), 7.20 (m, 4H), 6.54 (d, J = 9.2 Hz, 1H), 4.72 (d, J = 8, 2H), 4.30 (s, 2H), 3.63 (s, 3H), 3.35 ppm (s, 3H).

Methyl 2-(7-(dimethylcarbamoyl)-1,1-dioxido-2*H*-benzo[*e*]-[1,2,4]thiadiazin-4(3*H*)-yl)acetate (14 e): White solid (yield: 2.2 g, 68%): mp: 176–178 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.27 (m, 1H), 7.60 (m, 1H), 7.46 (m, 1H), 6.78 (d, J = 8.8 Hz, 1H), 4.81 (s, 2H), 4.40 (s, 2H), 3.68 (s, 3H), 2.96 ppm (d, 6H).

Methyl 2-(7-(diethylcarbamoyl)-1,1-dioxido-2*H*-benzo[*e*]-[1,2,4]thiadiazin-4(3*H*)-yl)acetate (14 f): White solid (yield: 2.1 g, 60%): mp: 167–168 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.74 (s, 1H), 7.44 (d, J = 8.8 Hz, 1H), 6.63 (d, J = 8.8 Hz, 1H), 5.55 (s, 1H), 4.87 (d, J = 7.6 Hz, 2H), 4.16 (s, 2H), 3.70 (s, 3H), 3.41 (m, 4H), 1.19 ppm (m, 6H).

General procedure for the synthesis of 7 a–b and 15 a–l: A mixture of corresponding acetate **6** or **14** (5 mmol), K_2CO_3 (1.38 g, 10 mmol), and 2-fluoro-4-bromobenzyl bromide (1.3 g, 5 mmol) in CH_3CN (20 mL) was stirred at 70 °C for 2 h. After evaporation of the solvent in vacuo, the residue was washed with H_2O, dried, and recrystallized from EtOAc to give the product.

Methyl 2-(2-(4-bromo-2-fluorobenzyl)-7-methyl-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (7 a): The mixture was purified using a petroleum ether (PE)/EtOAc (1:1) eluent to obtain **7 a** as a white solid (yield: 1.9 g, 86%): mp: 145–146 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.58 (m, 1H), 7.44 (m, 3H), 7.26 (m, 1H), 6.69 (d, J = 8.8 Hz, 1H), 4.86 (s, 2H), 4.32 (s, 2H), 4.25 (s, 2H), 3.66 (s, 3H), 2.25 ppm (s, 3H).

Methyl 2-(2-(4-bromo-2-fluorobenzyl)-7-methoxy-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (7 b): The mixture was purified using a PE/EtOAc (1:1) eluent to obtain **7 b** as a white solid (yield: 1.8 g, 78%): mp: 163–164 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.59 (d, J = 10 Hz, 1H), 7.45 (m, 2H), 7.11 (m, 2H), 6.77 (d, J = 10 Hz, 1H), 4.84 (s, 2H), 4.32 (s, 2H), 4.26 (s, 2H), 3.75 (s, 3H), 3.66 ppm (s, 3H).

Methyl 2-(7-methyl-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (7 c): The mixture was purified using a PE/EtOAc (1:1) eluent to obtain **7 c** as a white solid (yield: 1.7 g, 83%): mp: 144–145 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.53 (m, 2H), 7.47 (m, 1H), 7.28 (m, 1H), 6.67 (d, J = 9.6 Hz, 1H), 4.89 (s, 2H), 4.33 (s, 2H), 4.25 (s, 2H), 3.67 (s, 3H), 2.25 ppm (s, 3H).

Methyl 2-(7-methoxy-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (7 d): The mixture was purified using a PE/EtOAc (1:1) eluent to obtain **7 d** as a white solid (yield: 1.7 g, 80%): mp: 157–158 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.37 (m, 1H), 7.03 (m, 1H), 6.95 (m, 1H), 6.53 (d, J = 8.8 Hz, 1H), 4.84 (s, 2H), 4.37 (s, 2H), 4.00 (s, 2H), 3.80 (s, 3H), 3.75 ppm (s, 3H).

Methyl 2-(4-bromo-2-fluorobenzyl)-4-(2-methoxy-2-oxoethyl)-3,4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine-7-carboxylate-1,1-dioxide (15 a): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15 a** as a white solid (yield: 1.9 g, 75%): mp: 177–178 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.14 (m, 1H), 7.95 (m, 1H), 7.59 (d, J = 9.6, 1H), 7.43 (m, 2H), 6.94 (d, J = 9.2, 1H), 4.99 (s, 2H), 4.45 (s, 2H), 4.26 (s, 2H), 3.83 (s, 3H), 3.69 ppm (s, 3H).

Methyl 2-(2-(4-bromo-2-fluorobenzyl)-1,1-dioxido-7-(piperidine-1-carbonyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (15 b): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15 b** as a white solid (yield: 2.2 g, 81%): mp: 89–90 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.60 (m, 2H), 7.17 (m, 3H), 6.83 (d, J = 8.8 Hz, 1H), 4.94 (s, 2H), 4.40 (s, 2H), 4.28 (s, 2H), 3.69 (s, 3H), 3.45 (s, 4H), 1.57 ppm (d, 6H).

Methyl 2-(2-(4-bromo-2-fluorobenzyl)-7-(morpholine-4-carbonyl)-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (15 c): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15 c** as a white solid (yield: 1.94 g, 70%): mp: 152–

153 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.68 (s, 1H), 7.58 (m, 1H), 7.52 (m, 1H), 7.46 (m, 2H), 6.84 (d, J = 8.8 Hz, 1H), 4.94 (s, 2H), 4.40 (s, 2H), 4.28 (s, 2H), 3.69 (s, 3H), 3.60 (s, 4H), 3.51 ppm (s, 4H).

Methyl 2-(2-(4-bromo-2-fluorobenzyl)-7-(methyl(phenyl)carbamoyl)-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (15d): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15d** as a white solid (yield: 2.1 g, 72%): mp: 199–200 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.56 (d, J = 8 Hz, 1H), 7.49 (d, J = 1.2 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.33 (m, 4H), 7.21 (m, 3H), 6.61 (d, J = 8.8 Hz, 1H), 4.86 (s, 2H), 4.30 (s, 2H), 4.02 (s, 2H), 3.64 (s, 3H), 3.36 ppm (s, 3H).

Methyl 2-(2-(4-bromo-2-fluorobenzyl)-7-(dimethylcarbamoyl)-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (15e): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15e** as a white solid (yield: 2.0 g, 79%): mp: 157–158 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.68 (m, 1H), 7.55 (m, 2H), 7.44 (m, 2H), 6.83 (d, J = 8.8 Hz, 1H), 4.95 (s, 2H), 4.40 (s, 2H), 4.28 (s, 2H), 3.69 (s, 3H), 2.97 ppm (s, 6H).

Methyl 2-(2-(4-bromo-2-fluorobenzyl)-7-(diethylcarbamoyl)-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (15f): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15f** as a white solid (yield: 1.9 g, 70%): mp: 114–116 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.58 (m, 2H), 7.43 (m, 3H), 6.83 (d, J = 8.8 Hz, 1H), 4.95 (s, 2H), 4.40 (s, 2H), 4.29 (s, 2H), 3.69 (s, 3H), 3.40 (s, 4H), 1.16 ppm (m, 6H).

Methyl 4-(2-methoxy-2-oxoethyl)-2-(2,4,5-trifluorobenzyl)-3,4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine-7-carboxylate-1,1-dioxide (15g): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15g** as a white solid (yield: 1.7 g, 76%): mp: 155–157 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): 8.47 (s, 1H), 8.06 (d, J = 8.8 Hz, 1H), 7.36 (m, 1H), 6.95 (m, 1H), 6.59 (d, J = 8.8 Hz, 1H), 4.93 (s, 2H), 4.34 (s, 2H), 4.08 (s, 2H), 3.90 (s, 3H), 3.79 ppm (s, 3H).

Methyl 2-(1,1-dioxido-7-(piperidine-1-carbonyl)-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (15h): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15h** as a white solid (yield: 2.0 g, 77%): mp: 159–160 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.82 (s, 1H), 7.53 (d, J = 8.8 Hz, 1H), 7.34 (m, 1H), 6.94 (m, 1H), 6.58 (d, J = 8.8 Hz, 1H), 4.91 (s, 2H), 4.34 (s, 2H), 4.05 (s, 2H), 3.78 (s, 3H), 3.55 (s, 4H), 1.64 ppm (d, 6H).

Methyl 2-(7-(morpholine-4-carbonyl)-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (15i): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15i** as a white solid (yield: 1.8 g, 69%): mp: 141–143 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.84 (s, 1H), 7.55 (d, J = 8.8 Hz, 1H), 7.35 (m, 1H), 6.96 (m, 1H), 6.60 (d, J = 8.4 Hz, 1H), 4.92 (s, 2H), 4.34 (s, 2H), 4.06 (s, 2H), 3.79 (s, 3H), 3.71 (s, 4H), 3.66 ppm (s, 4H).

Methyl 2-(7-(methyl(phenyl)carbamoyl)-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (15j): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15j** as a white solid (yield: 1.9 g, 71%): mp: 130–131 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.67 (s, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.30 (m, 1H), 7.24 (m, 3H), 7.08 (m, 2H), 6.92 (m, 1H), 6.34 (d, J = 8.8 Hz, 1H), 4.83 (s, 2H), 4.21 (s, 2H), 3.96 (s, 2H), 3.74 (s, 3H), 3.49 ppm (s, 3H).

Methyl 2-(7-(dimethylcarbamoyl)-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (15k): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15k** as a white solid (yield: 1.8 g, 78%): mp: 110–111 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.86 (s, 1H), 7.57 (m, 1H), 7.34 (m, 1H), 6.95 (m, 1H), 6.59 (d, J = 9.2 Hz, 1H), 4.91(s, 2H), 4.32 (s, 2H), 4.05 (s, 2H), 3.19 (s, 3H), 3.08 ppm (s, 6H).

Methyl 2-(7-(diethylcarbamoyl)-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetate (15l): The mixture was purified using a CH_2Cl_2/CH_3OH (60:1) eluent to obtain **15l** as a white solid (yield: 1.8 g, 74%): mp: 90–91 °C; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.81 (s, 1H), 7.49 (m, 1H), 7.35 (m, 1H), 6.95 (m, 1H), 6.58 (d, J = 8.8 Hz, 1H), 4.91 (s, 2H), 4.34 (s, 2H), 4.05 (s, 2H), 3.78 (s, 3H), 3.43 (s, 4H), 1.23 ppm (s, 6H).

General procedure for the synthesis of 8a–8b and 16a–16f: A mixture of the appropriate acetate **7** or **15** (1 mmol), 1,4-dioxane (5 mL), and saturated aqueous NaOH (1 mL) was stirred at room temperature for 2 h. The alkaline suspension was poured into ice and acidified with 0.1 N HCl (0.6 mL). The resulting precipitate was collected by filtration, washed with H_2O, dried, and recrystallized from CH_3OH to give the pure product.

2-(2-(4-Bromo-2-fluorobenzyl)-7-methyl-1,1-dioxido-2*H*-benzo[*e*]-[1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (8a): White solid (yield: 390 mg, 88%, purity: 99.90%): mp: 141–142 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.92 (s, 1H), 7.57 (m, 1H), 7.42 (m, 3H), 7.28 (m, 1H), 6.68 (d, J = 8.4 Hz, 1H), 4.86 (s, 2H), 4.26 (s, 2H), 4.19 (s, 2H), 2.25 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 171.14, 160.45 (d, J = 250 Hz), 140.28, 134.76, 132.59 (d, J = 4.6 Hz), 127.70 (d, J = 3.4 Hz), 126.71, 125.06, 122.98 (d, J = 14.1 Hz), 121.36 (d, J = 9.5 Hz), 119.694, 118.89 (d, J = 24.4), 113.62, 65.48, 49.87, 43.65, 19.57 ppm; HRMS (ESI+) m/z calcd for $[M+H]^+$ 443.0076, found 443.0069.

2-(2-(4-Bromo-2-fluorobenzyl)-7-methoxy-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (8b): White solid (yield: 376 mg, 82%, purity: 98.86%): mp: 133–134 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.94 (s, 1H), 7.59 (m, 1H), 7.45 (m, 2H), 7.14 (m, 2H), 6.76 (d, J = 8.8, 1H), 4.82 (s, 2H), 4.27 (s, 2H), 4.09 (s, 2H), 3.73 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 171.32, 160.48 (d, J = 249.9 Hz), 150.97, 136.81, 132.63 (d, J = 4.2 Hz), 127.71 (d, J = 3.0 Hz), 122.94 (d, J = 14.1 Hz), 121.94, 121.40 (d, J = 9.5 Hz), 119.97, 118.90 (d, J = 24.4 Hz), 115.34, 108.41, 65.49, 55.76, 50.08, 43.63 ppm; HRMS (ESI+) m/z calcd for $[M+H]^+$ 459.0026, found 459.0017.

2-(7-Methyl-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*]-[1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (8c): White solid (yield: 340 mg, 85%, purity: 99.90%): mp: 112–113 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.55 (m, 2H), 7.43 (s, 1H), 7.28 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 8.8 Hz, 1H), 4.88 (s, 2H), 4.26 (s, 2H), 4.12 (s, 2H), 2.25 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 171.27, 140.30, 134.81, 126.74, 125.07, 119.66, 119.06, 118.86, 113.62, 106.46, 106.24, 106.18, 105.97, 65.43, 49.87, 43.53, 40.15, 19.57 ppm; HRMS (ESI+) m/z calcd for $[M+H]^+$ 401.0783, found 401.0773.

2-(7-Methoxy-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*]-[1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (8d): White solid (yield: 324 mg, 78%, purity: 98.24%): mp: 146–147 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.55 (m, 2H), 7.13 (m, 2H), 7.76 (d, J = 7.2 Hz, 1H), 4.87 (s, 2H), 4.21 (s, 2H), 4.19 (s, 2H), 3.74 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 171.39, 151.00, 136.79, 121.99, 119.93, 119.07, 118.88, 115.32, 108.42, 106.45, 106.24, 106.18, 105.96, 65.40, 55.76, 49.97, 43.47 ppm; HRMS (ESI+) m/z calcd for $[M+H]^+$ 417.0732, found 417.0721.

2-(4-Bromo-2-fluorobenzyl)-4-(carboxymethyl)-3,4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine-7-carboxylic acid-1,1-dioxide (16a): White solid (yield: 331 mg, 70%, purity: 99.90%): mp: 173–174 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 13.05 (s, 2H), 8.11 (d, J = 2 Hz,

1H), 7.95 (m, 1H), 7.59 (m, 1H), 7.44 (m, 2H), 6.89 (d, $J=9.2$ Hz, 1H), 4.97 (s, 2H), 4.31 (s, 2H), 4.28 ppm (s, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.46$, 166.00, 160.47 (d, $J=250.4$ Hz), 145.55, 134.41, 132.63 (d, $J=4.2$ Hz), 127.71 (d, $J=3.4$ Hz), 127.12, 122.67 (d, $J=14.5$ Hz), 121.55 (d, $J=9.9$ Hz), 119.15 (d, $J=19.5$ Hz), 118.88, 118.81, 113.77, 65.71, 50.05, 44.19 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 472.9818, found 472.9820.

2-(2-(4-Bromo-2-fluorobenzyl)-1,1-dioxido-7-(piperidine-1-carbonyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (16b): White solid (yield: 470 mg, 87%, purity: 98.95%): mp: 136–137 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=13.12$ (s, 1H), 7.59 (m, 2H), 7.44 (m, 3H), 6.81 (d, $J=9.2$ Hz, 1H), 4.94 (s, 2H), 4.29 (s, 2H), 4.28 (s, 2H), 3.52 (s, 2H), 3.46 (s, 2H), 1.56 ppm (d, 6H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.77$, 167.46, 160.48 (d, $J=250.3$ Hz), 143.20, 132.69, 127.67, 124.73, 122.67 (d, $J=15.2$ Hz), 121.54, 119.08, 119.00, 118.76, 113.34, 109.43, 65.57, 50.04, 44.01, 29.48, 25.63, 24.05 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 540.0604, found 540.0602.

2-(2-(4-Bromo-2-fluorobenzyl)-7-(morpholine-4-carbonyl)-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (16c): White solid (yield: 428 mg, 79%, purity: 98.23%): mp: 137–138 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=7.68$ (s, 1H), 7.54 (m, 2H), 7.42 (m, 2H), 6.81 (d, $J=9.2$ Hz, 1H), 4.94 (s, 2H), 4.29 (s, 2H), 4.27 (s, 2H), 3.70 (s, 4H), 3.45 ppm (s, 4H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.73$, 167.70, 160.52 (d, $J=249.9$ Hz), 143.44, 132.88 (d, $J=35.4$ Hz), 127.74, 125.30, 123.92, 122.71 (d, $J=13.8$ Hz), 121.55 (d, $J=9.5$ Hz), 119.14 (d, $J=16.4$ Hz), 118.81, 113.37, 109.50, 66.41 (2C), 66.10 (2C), 65.63, 50.00, 44.10 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 542.0397, found 542.0397.

2-(2-(4-Bromo-2-fluorobenzyl)-7-(methyl(phenyl)carbamoyl)-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (16d): White solid (yield: 483 mg, 86%, purity: 99.62%): mp: 152–153 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=7.54$ (m, 1H), 7.49 (m, 1H), 7.42 (m, 3H), 7.35 (m, 2H), 7.31 (m, 3H), 6.58 (d, $J=9.2$ Hz, 1H), 4.85 (s, 2H), 4.16 (s, 2H), 4.15 (s, 2H), 3.36 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.68$, 167.44, 160.42 (d, $J=249.9$ Hz), 144.80, 143.19, 134.12, 132.54 (d, $J=4.6$ Hz), 129.39 (2C), 127.67 (d, $J=3.0$ Hz), 126.91 (2C), 126.71 (d, $J=13.8$ Hz), 124.06, 122.72 (d, $J=13.7$ Hz), 121.46 (d, $J=9.5$ Hz), 119.01, 118.76, 118.31, 112.65, 65.53, 49.93, 43.99, 38.35 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 562.0448, found 562.0446.

2-(2-(4-Bromo-2-fluorobenzyl)-7-(dimethylcarbamoyl)-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (16e): White solid (yield: 410 mg, 82%, purity: 98.53%): mp: 149–150 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=13.08$ (s, 1H), 7.67 (m, 1H), 7.58 (m, 2H), 7.43 (m, 2H), 6.81 (d, $J=8.8$ Hz, 1H), 4.94 (s, 2H), 4.29 (s, 2H), 4.28 (s, 2H), 2.98 ppm (s, 6H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.73$, 168.53, 160.48 (d, $J=219.9$ Hz), 143.20, 133.03, 132.65 (d, $J=4.2$ Hz), 127.70 (d, $J=3.4$ Hz), 125.07, 124.81, 122.72 (d, $J=14.5$ Hz), 121.50 (d, $J=9.6$ Hz), 118.91 (d, $J=23.7$ Hz), 113.20, 109.46, 65.58, 49.95, 44.04 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 500.0291, found 500.0280.

2-(2-(4-Bromo-2-fluorobenzyl)-7-(diethylcarbamoyl)-1,1-dioxido-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (16f): White solid (yield: 449 mg, 85%, purity: 99.78%): mp: 247–248 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=13.10$ (s, 1H), 7.58 (m, 2H), 7.45 (m, 3H), 6.80 (d, $J=9.6$ Hz, 1H), 4.94 (s, 2H), 4.29 (s, 2H), 4.26 (s, 2H), 3.33 (s, 2H), 3.16 (s, 2H), 1.23 (s, 3H), 1.13 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.76$, 168.42, 160.48 (d, $J=249.9$ Hz), 142.96, 132.64 (d, $J=4.6$ Hz), 132.11, 127.67 (d, $J=3.4$ Hz), 125.72, 124.06, 122.69 (d, $J=14.4$ Hz), 121.49 (d, $J=9.9$ Hz), 119.06 (d, $J=10.3$ Hz), 118.77, 113.42, 65.57, 49.92, 44.03, 13.41; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 528.0604, found 528.0596.

4-(Carboxymethyl)-2-(2,4,5-trifluorobenzyl)-3,4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine-7-carboxylic acid-1,1-dioxide (16g): White solid (yield: 297 mg, 69%, purity: 98.84%): mp: 244–245 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.96$ (s, 1H), 8.12 (s, 1H), 7.95 (d, $J=8.8$ Hz, 1H), 7.57 (m, 2H), 6.89 (d, $J=8.8$ Hz, 1H), 4.99 (s, 2H), 4.32 (s, 2H), 4.27 ppm (s, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.56$, 165.97, 145.54, 134.42, 127.11, 120.17, 119.23, 118.99, 118.81, 113.73, 106.49, 106.28, 106.21, 106.00, 65.53, 49.93, 43.96 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 431.0525, found 431.0512.

2-(1,1-Dioxido-7-(piperidine-1-carbonyl)-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (16h): White solid (yield: 383 mg, 77%, purity: 99.98%): mp: 218–219 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=7.56$ (m, 4H), 6.80 (d, $J=8.8$ Hz, 1H), 4.95 (s, 2H), 4.28 (s, 2H), 4.26 (s, 2H), 3.56 (s, 4H), 1.61 ppm (d, 6H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.85$, 167.48, 160.09, 157.07, 143.17, 132.76, 124.72, 120.21, 119.17, 118.98, 113.33, 106.45, 106.16, 105.96, 65.45, 49.94, 43.85, 25.56, 24.00 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 498.1311, found 498.1296.

2-(7-(Morpholine-4-carbonyl)-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (16i): White solid (yield: 364 mg, 73%, purity: 98.34%): mp: 138–139 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=7.68$ (s, 1H), 7.55 (m, 3H), 6.82 (d, $J=8.8$ Hz, 1H), 4.95 (s, 2H), 4.28 (s, 2H), 4.27 (s, 2H), 3.58 ppm (d, 8H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.82$, 167.61, 143.39, 133.04, 125.23, 123.89, 120.09, 119.08, 118.98, 113.35, 106.49, 106.21, 106.00, 66.03, 65.43, 49.95, 43.82 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 500.1103, found 500.1086.

2-(7-(Methyl(phenyl)carbamoyl)-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (16j): White solid (yield: 410 mg, 79%, purity: 99.99%): mp: 240–241 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=7.54$ (m, 3H), 7.31 (m, 6H), 6.59 (d, $J=8.0$ Hz, 1H), 4.87 (s, 2H), 4.16 (s, 4H), 3.36 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.78$, 167.39, 144.78, 143.17, 134.14, 129.37, 126.88, 126.77, 126.61, 124.02, 118.00 (d, $J=20.9$ Hz), 118.21, 112.61, 106.20 (dd, $J_1=21.3$ Hz, $J_2=28.2$ Hz), 65.38, 49.92, 43.75, 38.33 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 520.1154, found 520.1143.

2-(7-(Dimethylcarbamoyl)-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (16k): White solid (yield: 334 mg, 73%, purity: 99.49%): mp: 224–225 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=7.67$ (s, 1H), 7.55 (m, 3H), 6.81 (d, $J=8.4$ Hz, 1H), 4.95 (s, 2H), 4.21 (s, 4H), 2.97 ppm (s, 6H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.87$, 168.51, 143.19, 133.07, 125.07, 124.85, 118.97, 113.22, 65.45, 49.91, 43.85 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 458.0998, found 458.0992.

2-(7-(Diethylcarbamoyl)-1,1-dioxido-2-(2,4,5-trifluorobenzyl)-2*H*-benzo[*e*][1,2,4]thiadiazin-4(3*H*)-yl)acetic acid (16l): White solid (yield: 388 mg, 80%, purity: 99.91%): mp: 223–224 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=7.52$ (m, 4H), 6.81 (d, $J=8.0$ Hz, 1H), 4.96 (s, 2H), 4.29 (s, 2H), 4.27 (s, 2H), 3.33 (s, 4H), 1.12 ppm (s, 6H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=170.92$, 168.44, 142.99, 132.17, 125.72, 124.07, 120.14, 119.21, 119.16, 119.04, 113.43, 106.20 (dd, $J_1=20.9$ Hz, $J_2=27.8$ Hz), 65.47, 49.97, 43.85 ppm; HRMS (ESI+) *m/z* calcd for $[M+H]^+$ 486.1311, found 486.1299.

Biology

General: ALR2 was prepared according to the method of Hayman and Kinoshita[8] and La Motta et al.[2i] Lenses freshly acquired from rats were homogenized (Glas-Potter) in three volumes of cold deionized H_2O in an ice bath. The homogenate was then centrifuged at 12000 rpm (rotor type: 12154-H) at 0–4 °C for 30 min. The supernatant was precipitated with $(NH_4)_2SO_4$, first at 40% and then at 50% saturation. The combined supernatant from the first two precipitations was then precipitated with $(NH_4)_2SO_4$ at 75%. The final precipitate obtained from the 75% saturated fraction, which was determined to possess ALR2 activity, was re-dissolved in 0.05 M NaCl and then dialyzed overnight in 0.05 M NaCl. The dialyzed material was used in the enzymatic assay.

ALR1 was prepared in accordance with a previously reported method.[2i] Kidneys were removed from rats and homogenized (Glas-Potter) in three volumes of 10 mM sodium phosphate buffer pH 7.2 containing 0.25 M sucrose, 2.0 mM EDTA di-potassium salt, and 2.5 mM β-mercaptoethanol. The homogenate was centrifuged at 12000 rpm (rotor type: 12154-H) at 0–4 °C for 30 min, and the supernatant was subjected to a 40–75% $(NH_4)_2SO_4$ fractionation, following the same procedure previously described for ALR2. The precipitate obtained from the 75% $(NH_4)_2SO_4$ saturation, possessing ALR1 activity, was re-dissolved in 50 volumes of 10 mM sodium phosphate buffer pH 7.2 containing 2.0 mM EDTA di-potassium salt and 2.0 mM β-mercaptoethanol and was dialyzed overnight using the same buffer. The dialyzed material was used in the enzymatic assay.

Enzyme activity was assayed spectrophotometrically on a Shimadzu UV-1800 UV spectrophotometer by measuring the decrease in absorption of NADPH at λ 340 nm, which accompanies the oxidation of NADPH catalyzed by ALR2 and ALR1.

Enzymatic assays: Measurement of ALR2 activity was carried out at 30 °C in a reaction mixture containing 0.10 mM NADPH (0.25 mL), 0.1 M sodium phosphate buffer (pH 6.2, 0.25 mL), enzyme extract (0.1 mL), deionized H_2O (0.15 mL), and 10 mM D,L-glyceraldehyde (0.25 mL) as substrate in a final volume of 1 mL. With the exception of D,L-glyceraldehyde, the reaction mixture was incubated at 30 °C for 10 min. The substrate was then added to initiate the reaction, which was monitored for 4 min. The ALR1 activity assay was performed at 37 °C in a reaction mixture containing 0.12 mM NADPH (0.25 mL), enzyme extract (0.1 mL), 0.1 M sodium phosphate buffer (pH 7.2, 0.25 mL), deionized H_2O (0.15 mL) and 20 mM sodium D-glucuronate (0.25 mL) as substrate in a final volume of 1 mL. With the exception of the sodium D-glucuronate, the reaction mixture was incubated at 37 °C for 10 min. The substrate was then added to initiate the reaction, which was monitored for 4 min.

The inhibitory activity of the newly synthesized compounds against ALR2 and ALR1 was assayed by adding 5 μL of the inhibitor solution to the reaction mixture described above. All compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted with deionized H_2O. To correct for the non-enzymatic oxidation of NADPH, the rate of NADPH oxidation in the presence of all of the reaction mixture components except the substrate was subtracted from each experimental rate. The inhibitory effect of the synthetic compounds was routinely estimated at a concentration of 10^{-4} M (the concentration refers to that of the compound in the reaction mixture). The compounds found to be active were tested at additional concentrations between $10^{-4}$and 10^{-8} M. Each dose–response curve was generated using at least four concentrations of compound with inhibitory activity between 20% and 80%, with three replicates at each concentration. The 95% confidence limits (95% CL) were calculated from t values for $n-2$, where n is the total number of determinations.

Docking studies

Docking studies were performed using Molegro Virtual Docker, version 5.0. The crystal structure of human aldose reductase with bound inhibitor IDD594 (PDB code: 1US0) retrieved from the RCSB Protein Data Bank was used for docking. All solvent molecules within the protein structure were removed for the docking procedure. All structural parameters of ligands such as bond orders, hybridization, explicit hydrogen atoms, and charges were assigned when necessary in the Molegro Virtual Docker software. To obtain better potential binding sites in the protein, detection of possible binding cavities was implemented, and five cavities were obtained. The cavity around the anion binding site (volume of ~124 Å^3) was chosen for docking calculations. All docking calculations were carried out using the grid-based MolDock score (GRID) function with a grid resolution of 0.30 Å. The best ligand poses were chosen on the basis of the MolDock score and ReRank score. Docking calculations were performed with a dual processor Windows 7-based computer with 4 GB RAM, and each docking process lasted 4–6 min.

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