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Acetic Acid Derivatives of 3,4-Dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide as a Novel Class of Potent Aldose Reductase Inhibitors

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A series of novel benzothiadiazine 1,1-dioxide derivatives were synthesized and tested for their inhibitory activity against aldose reductase. Of these derivatives, 17 compounds, having a substituted N2-benzyl group and a N4-acetic acid group on the benzothiadiazine, were found to be potent and selective aldose reductase inhibitors in vitro with IC_{50} values ranging from 0.032 to 0.975 μM. **9m** proved to be the most active in vitro. The eight top-scoring compounds coming from the in vitro test for ALR2 inhibition activity were then tested in vivo, whereby three derivatives, **9i**, **9j**, and **9m**, demonstrated a significantly preventive effect on sorbitol accumulation in the sciatic nerve in the 5-day streptozotocin-induced diabetic rats in vivo. Structure−activity relationship and molecular docking studies highlighted the importance of substitution features of N4-acetic acid group and halogen-substituted N2-benzyl group in the benzothiadiazine scaffold and indicated that substitution with hallogen at C-7 had a remarkably strong effect on ALR2 inhibition potency.

Introduction

Diabetes mellitus is a chronic disease that requires long-term medical attention to limit the development of its devastating complications and to manage them when these are active. This disease is a major threat to global public health that is rapidly getting worse, as it afflicted over 171 million people worldwide in 2000 and the incidence is expected to grow steadily to 366 million by 2030.[1] China is among the top 3 countries and may bear a higher diabetes-related burden than any other country. A very recent report showed that the prevalence of diabetes and prediabetes was 9.7% and 15.5%, respectively, accounting for 92.4 million adults with diabetes and 148.2 million adults with prediabetes.[2] These estimates showed approximately 2 times higher factor of than those reported in 2003. It is a disproportionately expensive disease; in 2002, the per capita cost of health care was $13,243 for people with diabetes while it was $2560 for those without diabetes.[1]

The complications that are specific to diabetes include retinopathy, nephropathy, and neuropathy.[3] Also, a substantial increase in atherosclerotic disease of large vessels, including cardiac, cerebral, and peripheral vascular disease, is seen in the complications.[3,4] Patients with all forms of diabetes of sufficient duration, including insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus, are vulnerable to these complications, which cause serious morbidity. Increasing evidence suggests that the enzyme aldose reductase (ALR2,[a] EC 1.1.1.21) may provide a common biochemical link in the pathogenesis of many of diabetic complications[5−7] and that hyperactivity of the polyol metabolic pathway catalyzed by the enzyme in individuals with high blood glucose levels contributes to the progression of diabetic complications.[8−10]

Aldose reductase, a member of the aldo−keto reductase (AKR) superfamily of enzymes,[11] together with sorbitol dehydrogenase forms the polyol pathway. In this pathway aldose reductase initially catalyzes the NADPH-dependent reduction of the aldehyde form of glucose to form sorbitol. Sorbitol dehydrogenase in turn, utilizing NAD^+, oxidizes this intermediate polyol to fructose (Figure 1).[8] Therefore, aldose reductase have been a potential target for drug design and aldose reductase inhibitors (ARIs) have received attention as possible therapeutic drugs for the prevention and treatment of diabetic complications.[4,11] Since tetramethyleneglutaric acid was identified as the first ARI in the 1960s,[12] a number of structurally different ARIs including at least two chemical classes of structures have been developed (Figure 2). The first chemical class is carboxylic acids such as alrestatin,[13] tolrestat,[14] epalrestat,[15] zopolrestat,[16] zenarestat,[17] ponalrestat,[18] and recently developed lidorestat,[19] naphtho[1,2-*d*]isothiazole derivatives,[20] and acetic acid derivatives of oxadiazole.[21] Epalrestat is the only ARI as a drug commercially available in Japan and recently in China, and lidorestat and derivatives of oxadiazole have been identified as a highly potent and

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[a]Abbreviations: ALR2, aldose reductase; ALR1, aldehyde reductase; ARI, aldose reductase inhibitor; AKR, aldo−keto reductase; AGEs, advanced glycation end products; AMPA, 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate; DMSO, dimethylsulfoxide; HNE, hydroxynonenal; NADPH, β-nicotinamide adenine dinucleotide phosphate reduced form; $NADP^+$, β-nicotinamide adenine dinucleotide phosphate; NADH, β-nicotinamide adenine dinucleotide reduced form; NAD^+, β-nicotinamide adenine dinucleotide; STZ, streptozotocin; SAR, structure−activity relationship.

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selective ARIs with a favorable pharmacokinetic profile,[19,21] although poor tissue penetration has been observed as the major shortcoming in some of this class.[22,23] The second chemical class is spirohydantoins involving sorbinil[24] and fidarestat.[25] Fidarestat and the spirohydantoin-like spiroimide ARI ranirestat (AS-3201)[26] have been identified as powerful ARIs with remarkable efficacy and safety,[25,27,28] although the hypersensitivity has been observed for some of the spirohydantoins.[22,23] Very recently, new classes of ARIs that are SO_2 linker-bearing pyridazinone based series including most potent 6-(5-chloro-3-methylbenzofuran-2-sulfonyl)-2*H*-pyridazin-3-one[29] and flavinoid bioisoster pyridopyrimidinones (PPP)[30] have been reported for their excellent selectivity and potent inhibitory activity both in vitro and in vivo. Despite these excellent efforts, to date, none of the ARIs have been approved for clinical use worldwide. Many ARIs that had promising activity against ALR2 during in vitro and in vivo studies with animal models failed to be advanced through late stage of clinical trials because of their side effects or poor efficacy. The side effects are widely believed to be mainly due to a lack of an ARI selectivity, which is often determined by measuring its activity against aldehyde reductase (EC 1.1.1.2, ALR1), an enzyme that is closely related to ALR2. Both ALR1 and ALR2 are members of the aldo−keto reductase (AKR) superfamily and present in a number of tissues. They share a high degree of sequence (~65%) and structural homology[31] with the majority of the differences present at the C-terminal end of the enzyme proteins,[32] which is the region containing the least conserved residues and lining the hydrophobic pocket of the active site called the "specificity pocket".[33] The "specificity pocket" is responsible for substrate and inhibitor specificity in the AKRs.[34] ALR1 plays a detoxification role, as it specifically metabolizes toxic aldehydes such as hydroxynonenal (HNE), 3-deoxyglucosone, and methylglyoxal, which arise in large quantities from pathological conditions connected with oxidative stress, as in hyperglycemia, and are intermediates for the formation of the advanced glycation end products (AGEs).[35−37] Thus, ALR1 inhibition may account for some of the undesirable side effects associated with the present ARIs. It is believable that the development of more structurally diverse ARIs and in turn the identification of molecularly targeted candidates that specifically block ALR2 are important approaches in the search of new drugs.

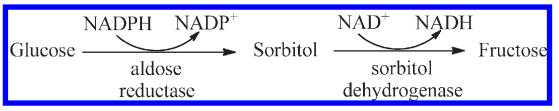


Figure 1. Polyol pathway of glucose metabolism.

In the present study, we have developed a new class of potent ARIs having a benzothiadiazine 1,1-dioxide scaffold and N4-acetic acid and substituted N2-benzyl residues. Since the 1950s, various pharmacological investigations of newly synthesized benzothiadiazines demonstrated interesting pharmacological activities and showed great potential for development of new medications for treating diseases. They were studied extensively for their diuretic, hypoglycemic, antihypertensive, and potassium channels opener activity.[38,39] Apart from the above-mentioned activities, benzothiadiazine derivatives also showed some interesting efficacy as highly potent HIV-1 non-nucleoside reverse transcriptase inhibitors[40] and potentiators of AMPA receptors (Figure 3).[41−46] Therefore, various benzothiadiazine compounds are of considerable interest for their diverse pharmaceutical uses. The above considerations prompted us to design and synthesize a new series of substituted benzothiadiazine 1,1-dioxide derivatives and to test their ALR2 inhibition activity both in vitro and in vivo. The present work describes the synthesis of the benzothiadiazine 1,1-dioxide derivatives as well as SAR and molecular docking studies on their ALR2 inhibition activity.

Chemistry

All compounds including **5a**, **6**−**10**, and **13**−**15** (Figure 4) described in this study have been obtained by synthesis either

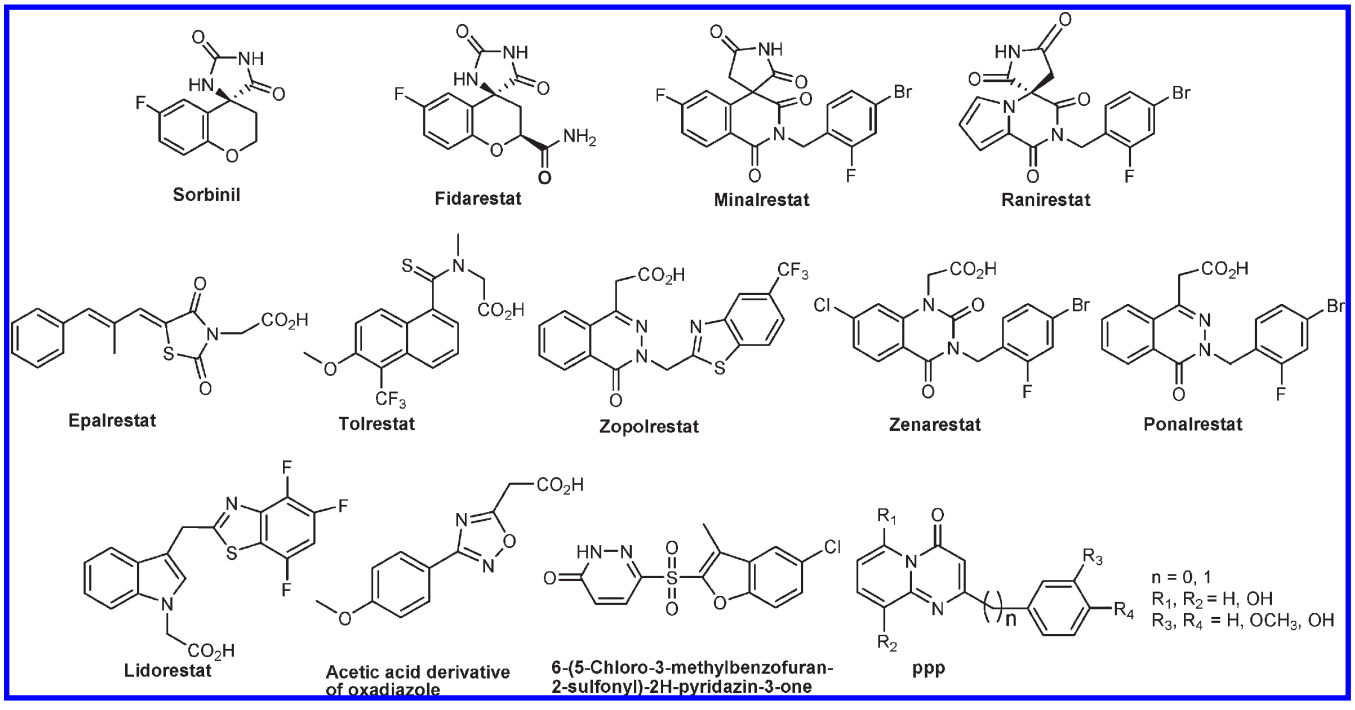


Figure 2. Chemical structures of aldose reductase inhibitors.

starting from aniline or substituted aniline (**1a**−**d**), as shown in Schemes 1 and 2.

N4-Acetic Acid Derivatives of 1,2,4-Benzothiadiazine 1,1-Dioxide. Compounds **2a**−**d** were synthsized in three steps from aniline and 4-substituted aniline (**1a**−**d**) as shown in Scheme 1.[47] N-Acylation of anilines **1a**−**d** with chlorosulfonyl isocyanate in nitromethane formed ureas, which were then sulfonylated intramolecularly in the presence of aluminum chloride to produce 3-oxo-1,2,4-benzothiadiazine 1,1-dioxides by ring closure. Removal of the carbonyl group of these products by treatment with sulfuric acid and then sodium hydroxide yielded the corresponding aminobenzenesulfonamides **2a**−**d**.

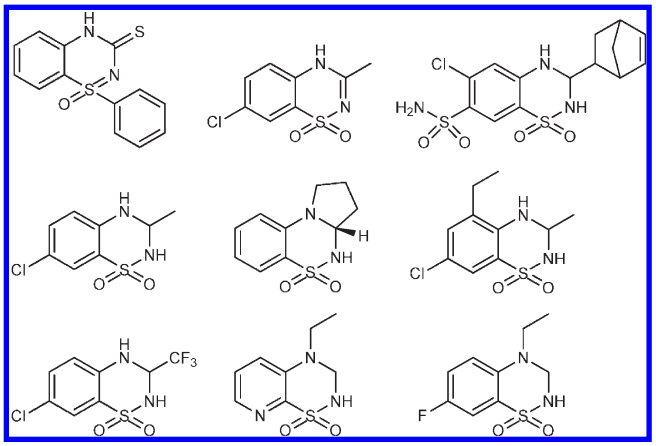


Figure 3. Chemical structures of 1,2,4-benzothiadiazine 1,1-dioxide derivatives according to Francotte and co-workers.[46]

Compounds **5a**−**d** served as intermediates for the synthesis of novel N4-carboxylic acid derivatives of benzothiadiazine 1,1-dioxide **6**−**10**. The preparation of the intermediates **5a**−**d** from the aminobenzenesulfonamides **2a**−**d** was carried out using the synthetic procedure described by Francotte and co-workers.[46] Compounds **2a**−**d** reacted with triethyl orthoformate to give corresponding **3a**−**d** by ring closure. To introduce an alkylcarboxylic acid residue as a side chain at the N4 position, compounds **3a**−**d** were alkylated with the methyl bromoacetate in the presence of potassium carbonate in acetonitrile, yielding compounds **4a**−**d**. Saturation of the double bond in the 2,3-positions of **4a**−**d** was achieved in 2-propanol by means of sodium borohydride to give the methyl esters **5a**−**d**. The C-7 unsubstituted ester **5a** was hydrolyzed with aqueous sodium hydroxide or aminolyzed

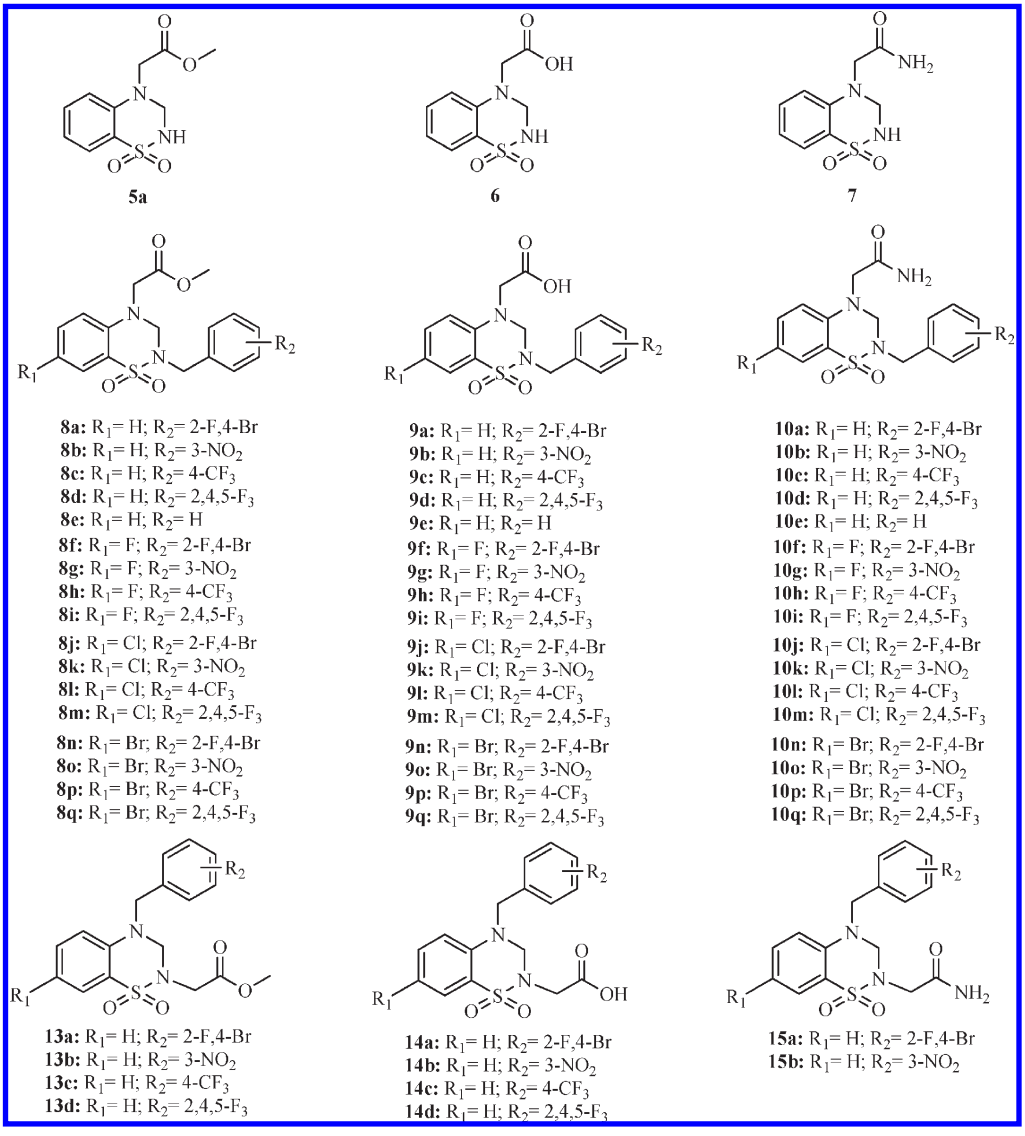
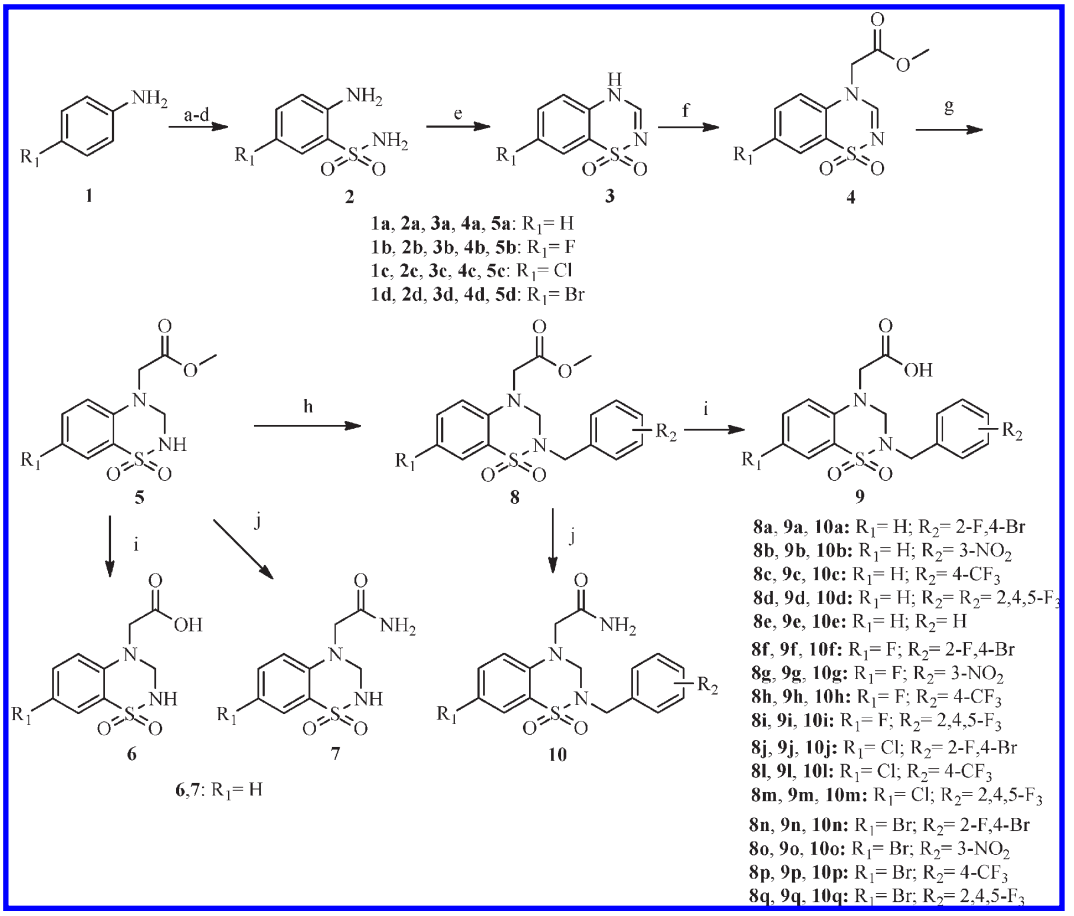


Figure 4. Substituted 1,2,4-benzothiadiazine 1,1-dioxide derivatives (**5a**, **6**−**10**, and **13**−**15**).

Scheme 1[a]

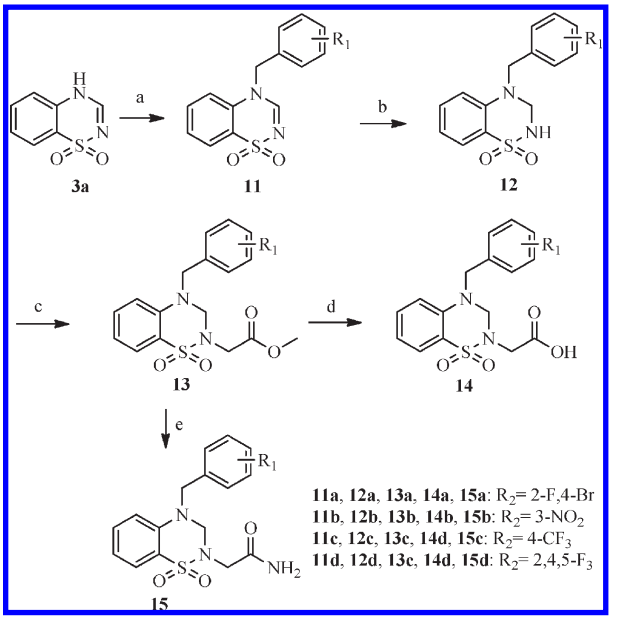


[a] Reagents and conditions: (a) $ClSO_2NCO$, CH_3NO_2, −40 °C; (b) $AlCl_3$, 110 °C; (c) 50% H_2SO_4, 140 °C; (d) aq NaOH; (e) $HC(OEt)_3$, reflux; (f) $BrCH_2COOCH_3$, K_2CO_3, CH_3CN, 70 °C; (g) $NaBH_4$, 2-propanol; (h) Bn-Br, K_2CO_3, CH_3CN, 70 °C; (i) 1,4-dioxane, NaOH; (j) NH_3, CH_3OH.

with ammonia to generate acid product **6** and amide **7**, respectively. In the synthesis of the other end products, introduction of a benzyl group as another side chain at the N2 position of esters **5a**−**d** was achieved by the alkylation reaction with the appropriate benzyl bromide to afford the corresponding methyl esters **8a**−**q**, which were then converted to the carboxylic acids **9a**−**q** and amides **10a**−**q**, respectively, by using the same procedures as those for the formation of the carboxylic acid **6** and the amide **7**.

N2-Carboxylic Acid Derivatives of 1,2,4-Benzothiadiazine 1,1-Dioxide. The same synthetic methods as described above were applied for the preparation of the expected compounds **13**−**15** starting from compound **3a** (Scheme 2). Alkylation of compound **3a** at the N4 position with the appropriate benzyl bromide produced **11a**−**d**, which were then hydrogenated with sodium borohydride to give compounds **12a**−**d**. A second alkylation at the N2 position by the reaction of **12a**−**d** with the methyl bromoacetate afforded the methyl esters **13a**−**d**. Saponification of the esters with aqueous sodium hydroxide led to carboxylic acids **14a**−**d**, and aminolysis of the esters **13a** and **13b** with ammonia provided amides **15a** and **15b**, respectively.

Scheme 2[a]

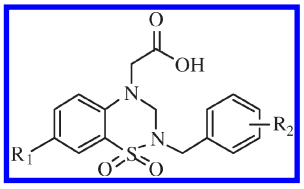


[a] Reagents and conditions: (a) Bn-Br, K_2CO_3, CH_3CN, 70 °C; (b) $NaBH_4$, 2-propanol; (c) $BrCH_2COOCH_3$, K_2CO_3, CH_3CN, 70 °C; (d) 1,4-dioxane, NaOH; (e) NH_3, CH_3OH.

Results and Discussion

Inhibition of Enzymes in Vitro. To ascertain which structural features are required for ALR2 inhibition activity and selectivity of substituted benzothiadiazine 1,1-dioxide derivatives, the structurally diverse compounds described above (Figure 4) were designed and synthesized for testing (Tables 1 and 2). The all synthesized compounds including **5a**, **6**−**10**,

Table 1. In Vitro Data for N2-Benzyl-N4-carboxyl Derivatives of Benzothiadiazine 1,1-Dioxide



	substituent		IC_{50} (μM) [a]	
compd	R_1	R_2	ALR2	ALR1
9a	H	2-F,4-Br	0.125 (0.093−0.157)	70.3 (51.5−89.2)
9b	H	3-NO_2	0.219 (0.153−0.286)	55.8 (34.7−76.8)
9c	H	4-CF_3	0.975 (0.760−1.189)	42% [c]
9d	H	2,4,5-F_3	0.111(0.095−0.127)	*b*
9e	H	H	101.5 (85.9−117.2)	*b*
9f	F	2-F,4-Br	0.090 (0.064−0.116)	121.9 (108.1−135.8)
9g	F	3-NO_2	0.108 (0.088−0.129)	61.9 (51.9−71.9)
9h	F	4-CF_3	0.931 (0.721−1.141)	40% [c]
9i	F	2,4,5-F_3	0.066 (0.054−0.077)	80.9 (62.5−99.2)
9j	Cl	2-F,4-Br	0.041 (0.034−0.048)	58.9 (47.2−70.6)
9k	Cl	3-NO_2	0.094 (0.068−0.120)	82.5 (69.8−95.2)
9l	Cl	4-CF_3	0.595 (0.465−0.725)	45% [c]
9m	Cl	2,4,5-F_3	0.032 (0.024−0.040)	50.6 (36.0−65.2)
9n	Br	2-F,4-Br	0.071 (0.052−0.090)	69.4 (57.7−81.1)
9o	Br	3-NO_2	0.116 (0.089−0.143)	102.9 (86.5−119.3)
9p	Br	4-CF_3	0.674 (0.525−0.824)	109.6 (87.5−131.8)
9q	Br	2,4,5-F_3	0.052 (0.038−0.067)	54.1 (36.6−71.6)
fidarestat			0.029 (0.020−0.037)	*b*

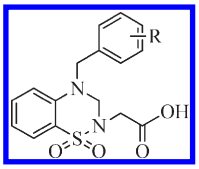
[a] IC_{50} (95% CL) values represent the concentration required to produce 50% enzyme inhibition. [b] Not determined. [c] The inhibitory effect was estimated at 10^{-4} M.

and **13**−**15** were tested for their potential inhibitory effect on ALR2 isolated from rat lenses. Only significantly active compounds coming from in vitro test for ALR2 inhibition activity were then subjected to test for their inhibition activity against aldehyde reductase (ALR1), isolated from rat kidneys, in order to evaluate their selectivity for ALR2 inhibition. The results are expressed as IC_{50} (μM) or the percentage of enzyme inhibition (%) summarized in Tables 1, 2, and S3−S5.

For the building of target ARIs based on the benzothiadiazine 1,1-dioxide scaffold, initially, carboxylic acid derivatives (ester **5a**, acid **6**, and amide **7**) were prepared by introducing an acetic acid group as only a side chain to the N4 position because carboxylic acid residue such as acetic acid group in well-known ARIs (Figure 2)[13−19] is believed to hold an important role in the interaction with ALR2 enzyme, especially in physiological conditions.[48] Recently successful examples of the carboxylic acid ARIs have been reported by Van Zandt, La Motta, and their co-workers.[19,21]

Of the three benzothiadiazine 1,1-dioxide derivatives, the acid **6** showed a little inhibition activity against ALR2 with IC_{50} value of 93.9 μM, whereas both the ester **5a** and the amide **7** were inactive (Table S3), indicating that simply retaining the carboxylic acid residue as in **6** is not sufficient for ALR2 inhibition activity. Therefore, we assumed that an additional substituent at the N2 position of the acid **6** as a second side chain could improve ALR2 inhibition potency. This consideration led to the modification, the acid derivative **9a**, by attaching a 2-fluoro-4-bromobenzyl residue to the N2 position of **6** because this substituent as a side chain had already been employed to design potent ARIs such as

Table 2. In Vitro Data for N2-Carboxyl-N4-benzyl Derivatives of Benzothiadiazine 1,1-Dioxide



compd	R	ALR2 IC_{50} (μM)[a]
14a	2-F,4-Br	58.4 (46.3−70.4)
14b	3-NO_2	74.5 (58.9−90.1)
14c	4-CF_3	41.3% [b]
14d	2,4,5-F_3	14.0 (9.25−18.7)

[a] IC_{50} (95% CL) values represent the concentration required to produce 50% enzyme inhibition. [b] The inhibitory effect was estimated at 10^{-4} M.

ranirestat, minalrestat, zenarestat, and ponalrestat (Figure 2). This modification was supported by docking studies. It was found that **9a** showed a significantly enhanced ALR2 inhibition effect compared with the parent compound **6**, and the activity (IC_{50} = 0.125 μM) was approximately 3 orders of magnitude greater than that of **6** (IC_{50} = 0.125 versus IC_{50} = 93.9 μM). By taking into account the key structural features required for ALR2 inhibition activity, we sought to design and synthesize variants that display a greater activity. Therefore, further substitutions at the N2-benzyl ring combined with substitutions at the 7-position resulted in the preparation of a series of acetic acid derivatives **9b**−**q**. We also obtained the corresponding ester derivatives **8a**−**q** and amide derivatives **10a**−**q** (Figure 4) in order to investigate structure−activity relationships.

As expected above and shown (Table 1), all of the N2-benzyl and N4-acetic acid substituted derivatives (**9a**−**q**) except **9e** showed significantly inhibitory activity against ALR2. Of these acid derivatives, 2-(2,4,5-trifluorobenzyl)-4-carboxymethyl-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **9m** was the most active compound having an IC_{50} value of 0.032 μM. Four compounds **9i**, **9j**, **9n**, and **9q** also demonstrated potent ALR2 inhibition activity with IC_{50} values of 0.066, 0.041, 0.071, and 0.052 μM, respectively. However, only compound **9e** exhibited an almost negligible ALR2 inhibition activity (IC_{50} = 101.5 μM), and the remaining 11 compounds including **9c**, **9h**, **9p**, **9l**, **9b**, **9a**, **9o**, **9d**, **9g**, **9k**, and **9f** showed varying levels of efficiency with IC_{50} values ranging from 0.975 to 0.090 μM.

We will now discuss SAR relative to substituents at the N2-benzyl ring in the N4-acetic acid substituted derivatives **9a**−**q**, which could be arranged according to 7-substituent in series: **9a**−**e** without 7-substitution, **9f**−**i** with the 7-fluoro substituent, **9j**−**m** with the 7-chloro substituent, and **9n**−**q** with the 7-bromo substituent (Table 1). By comparison of the compounds in series **9a**−**e**, compound **9e** without substitution at the N2-benzyl ring greatly lost ALR2 inhibition activity (IC_{50} = 101.5 μM) while compounds with substitutions of 2-F with 4-Br (**9a**), 3-NO_2 (**9b**), 4-CF_3 (**9c**), and 2,4,5-F_3 (**9d**) at the benzyl ring all retained potent activities (IC_{50} = 0.125, 0.219, 0.975, 0.111 μM). The 2,4,5-F_3-benzyl derivative **9d** was found to be the most active within this series. Similarly, comparing compounds in each of the remaining series **9f**−**i**, **9j**−**m**, and **9n**−**q** revealed that compounds **9i**, **9m**, and **9q** all having 2,4,5-trifluoro substituents

at the benzyl ring were the most active within each subseries, respectively. Moreover, it was found that effects of substitution groups at the benzyl ring on ALR2 inhibition activity were in the rank order of 2,4,5-F_3 > 2-F,4-Br > 3-NO_2 > 4-CF_3 as deduced from the comparison of compounds in 7-unsubstituted derivative series (**9d** > **9a** > **9b** > **9c**), 7-fluoro substituted derivative series (**9i** > **9f** > **9g** > **9h**), 7-chloro derivative series (**9m** > **9j** > **9k** > **9l**), and 7-bromo derivative series (**9q** > **9n** > **9o** > **9p**). These results suggest that apart from the acetic acid residue at the N4 position, the N2 benzyl residue with halogen substituents at the benzyl ring is essential for the activity of ARIs based on the benzothiadiazine 1,1-dioxide scaffold.

Further SAR studies on substituents at the 7-position in the derivatives **9a**−**q** except for **9e** indicated that ALR2 inhibition activity of the derivatives obtained from the introduction of the N4-acetic acid group combined with substitutions at the N2-benzyl ring could be further increased by substitutions with halogen atoms at the 7-position. It was evidenced by comparing biological activities of compounds in the 2-F,4-Br-benzyl derivative series including **9f** with 7-F group, **9j** with 7-Cl, **9n** with 7-Br, and **9a** with 7-unsubstitution, in which **9j** together with **9n** and **9f** proved to be more potent than **9a** (IC_{50} = 0.041, 0.071, 0.090 μM vs IC_{50} = 0.125 μM). Identically, comparing the activities of compounds in the 3-NO_2-benzyl derivative series including **9g** with 7-F, **9k** with 7-Cl, **9o** with 7-Br, and **9b** with 7-unsubstitution revealed that **9k**, **9o**, and **9g** were more active than **9b** (IC_{50} = 0.094, 0.116, and 0.108 μM vs IC_{50} = 0.219 μM). Again, comparison of the activities of compounds in the 4-CF_3-benzyl derivative series including **9h** with 7-F, **9l** with 7-Cl, **9p** with 7-Br, and **9c** with 7-unsubstitution revealed that **9l**, **9p**, and **9h** were more active than **9c** (IC_{50} = 0.595, 0.674, and 0.931 μM vs 0.975 μM). Regarding the 2,4,5-F_3-benzyl derivative series, there were four individuals including substitution at C-7 with F **9i**, Cl **9m**, Br **9q**, and unsubstituted **9d**. Within this series, **9m** was the most active (IC_{50} = 0.032 μM) while **9d** was the least active (IC_{50} = 0.111 μM). Consequently, effects of substitutions at C-7 on ALR2 inhibition activity showed the rank order to be Cl > Br > F > H as deduced from the above comparisons (**9j** > **9n** > **9f** > **9a**, **9k** > **9o** ~ **9g** > **9b**, **9l** > **9p** > **9h** > **9c**, and **9m** > **9q** > **9i** > **9d**) and also showed that C-7 chloro substituted derivatives **9j**, **9k**, **9l**, and **9m** were the most active in each series, respectively, while unsubstituted **9a**, **9b**, **9c**, and **9d** showed the lowest activity. However, unlike the substitutions at the N4 position and at the benzyl ring, the 7-halogen was not essential for the activity.

In contrast to the compounds in the N4-acetic acid derivative series **9a**−**q**, except for **9e**, which presented potent ALR2 inhibition activities as described above, all of compounds in the corresponding N4-acetic acid ester derivative series **8a**−**q** and the N4-amide derivative series **10a**−**q** revealed little or no inhibitory activity against ALR2 (Table S4). In this case, substitutions neither at the N2-benzyl ring nor at the 7-position could obviously influence biological activity. It is possible to conclude from the results given above that esterification and amidation of the N4-acetic acid group result in a considerable loss of ALR2 inhibition activity, and subsequently the carboxylic acid anion may be the key structural feature required for ALR2 inhibition activity of benzothiadiazine 1,1-dioxide derivatives.

In order to investigate the influence of positions of carboxyl and benzyl groups in the benzothiadiazine 1,1-dioxide on ALR2 inhibition activity, compounds **13**−**15** (Figure 4) were prepared by repositioning of the carboxyl and the benzyl residues on the benzothiadiazine 1,1-dioxide scaffold of the corresponding compounds **8**−**10**. Of these compounds, however, only the N2-acetic acid derivatives **14a**, **14c**, and **14d** showed a somewhat inhibitory activity against ALR2 (IC_{50} = 58.4 μM, 74.5 μM, and 14.0 μM), while the remaining N2-esters **13a**−**d** and the N2-amides **15a** and **15d** exhibited negligible activity as shown in Tables 2 and S5, indicating that the N2-acetic acid-N4-benzyl substitutions were not desirable. These results suggest that positions of carboxylic acid and benzyl residues as side chains on the benzothiadiazine 1,1-dioxide scaffold greatly influence biological activity in which substitution of the carboxylic acid residue at the N4 position combined with substitution of the benzyl residue at the N2 position drastically increases the ALR2 inhibition activity other than substitutions of the two residues at reversed positions. Comparable results were found in the cases of compounds **9a**−**d** versus their corresponding compounds **14a**−**d** (**9a** with N2-(2-F,4-Br-benzyl), IC_{50} = 0.125 μM vs **14a** with N4-(2-F,4-Br-benzyl), IC_{50} = 58.4 μM; **9b** with N2-(3-NO_2-benzyl), IC_{50} = 0.219 μM vs **14b** with N4-(3-NO_2-benzyl), IC_{50} = 74.5 μM; **9c** with N2-(4-CF_3-benzyl), IC_{50} = 0.975 μM vs **14c** with N4-(4-CF_3-benzyl), inhibition of 43% at 10^{-4} μM; **9d** with N2-(2,4,5-F_3-benzyl), IC_{50} = 0.111 μM vs **14d** with N4-(2,4,5-F_3-benzyl), IC_{50} = 14.0 μM).

As shown in Table 1, the N2-benzy-N4-carboxylic acid derivatives **9a**−**q** except for **9e** with significantly potent ALR2 inhibitory activity were tested for their inhibition ability against ALR1. All of the tested compounds were found to be slightly active to inactive (IC_{50} > 50 μM), demonstrating their selectivity for ALR2.

Biological Activity in Vivo. Of the 16 significantly potent ARIs identified in the in vitro test as described above, the eight top ranked compounds were then subjected to test for oral in vivo activity in streptozotocin diabetic rat model as measured by the ability of the inhibitors to normalize sorbitol that had become elevated in diabetic nerve. In particular, we focused our attention on the comparison of 2,4,5-F_3-benzyl derivative series containing **9i**, **9m**, and **9q**. As shown in Table 3, three compounds **9i**, **9j**, and **9m** showed significant ALR2 inhibition activity in vivo, as they normalized elevated sorbitol levels of the sciatic nerve of diabetic rats by 68%, 61%, and 62%, respectively. Although no statistically significant data from in vivo investigation were available for the other test compounds, a trend toward the normalization of sorbitol levels was observed. Particular interest is that all three in vivo active compounds were most readily distinguished by their trifluoro substituents on the N2-benzyl ring. The finding is consistent with previous results in which 2-fluoro substituted derivatives were found to have the best oral activities among tolrestat analogues containing 2-halo and other substituents.[49] It seems therefore likely that the significant in vivo potency of the compounds **9i** and **9m** may be greatly attributed to the presence of the trifluoro substituents. This can be explained by assuming that multifluoro substituted compounds are more favorable for tissue penetration than less fluoro substituted compounds in the present study and subsequently show more potent activity in vivo. The assumption is strengthened by comparing in vivo activities of 2,4,5-F_3-benzyl derivative series including **9i**, **9m**, and **9q**. Within these three compounds, **9i** showed the most potent activity in vivo (68%)

Table 3. Biochemical and Nerve Sorbitol Assessment with **9f**, **9g**, **9i**, **9j**, **9k**, **9m**, **9n**, and **9q** in 5-Day STZ-Induced Diabetic Rat Screening Model[a]

group	blood glucose (mmol/L)	nerve sorbitol(μmol/g)	% improvement[b]
nondiabetic control	6.6 ± 0.43	0.1062 ± 0.0102	
diabetic control	22.2 ± 2.72	0.2055 ± 0.0542	
9f	17.9 ± 1.18	0.1585 ± 0.0211	47.3
9g	19.8 ± 2.99	0.1622 ± 0.0301	43.6
9i	18.4 ± 0.98	0.1378 ± 0.0154*	68.2
9j	18.2 ± 0.91	0.1455 ± 0.0114*	60.7
9k	19.7 ± 1.09	0.1610 ± 0.0099	45.0
9m	17.9 ± 0.84	0.1444 ± 0.0240*	61.5
9n	18.4 ± 1.39	0.1634 ± 0.0170	42.4
9q	20.1 ± 2.25	0.1546 ± 0.0184	51.2
fidarestat	18.5 ± 3.25	0.1039 ± 0.0105**	100

[a] Five days of diabetes followed by 5 days of drug treatment at 100 (mg/kg)/day: (*) $p < 0.05$, (**) $p < 0.01$ compared to untreated diabetic control group. [b] Percent improvement of drug-treated group relative to the difference between nondiabetic and diabetic control groups.

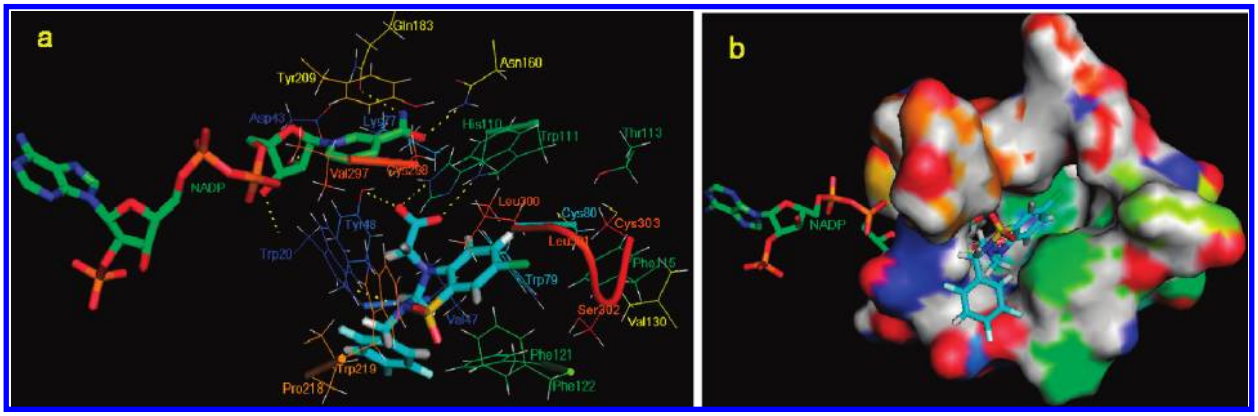


Figure 5. Docking of the inhibitor **9m** into the active site of ALR2. (a) Protein structure is shown as a cartoon diagram with selected and labeled residues shown in line representation, and ligand **9m** and NADP are shown as stick models. Docked pose of **9m** is shown in cyan (C), red (O), blue (N), yellow (S), green (Cl), and gray (F). The hydrogen bonds are shown as yellow dashed lines. (b) Protein residues are in surface representation.

while **9m** showed the decreased activity in vivo (62%), although it was more potent in vitro than **9i** (IC_{50} = 0.032 μM vs 0.066 μM). **9q** was inactive in vivo probably because of its greater molecular weight than those of **9i** and **9m**, although it also contained trifluoro substituents on the N2-benzyl ring.

Given that the N4-acid derivative **9i** appeared to have the most in vivo potency, we evaluated in vivo activity of its bioisosteric N4-amide counterpart **10i**. However, no activity in vivo was found for the amide **10i**. Also, we investigated the in vivo activity of the N4-ester **8m**, which might be considered to be a prodrug of its acid counterpart **9m** having the most potent in vitro activity, but did not find improvement in the in vivo activity over **9m** (data not shown). However, we have noted that ester and amide derivatives in some cases could function as prodrugs of corresponding acid ARIs reported respectively by La Motta et al.[21] and Wrobel et al.[49] In the present study, the possibility of the N4-amide and N4-ester compounds of benzothiadiazine 1,1-dioxide as prodrugs of the N4-acid derivatives may be excluded.

Docking Studies. To better understand the inhibitory mechanism of the newly synthesized ARIs and to design new compounds, docking experiments were performed. Comparison of different aldose reductase-inhibitor X-ray complexes by Sotriffer and co-workers showed that there are three main kinds of binding conformations of ALR2,[50] best represented by the complexes with ligands sorbinil (PDB entry code 1AH0[51]), tolrestat (PDB entry code 2FDZ[52]), and IDD594 (PDB entry code 1US0[53]). The potent derivatives identified in the present study had more similarity with the ligand tolrestat compared to the other ligands, and therefore, the tolrestat bound conformation (2FDZ)[52] was used for the docking. Compound **9m**, the most active in vitro as described above, was docked using the FlexX docking into the conformation 2FDZ of ALR2 as shown in Figure 5. Docking results reveal that the inhibitor **9m** is tightly bound in the active site of ALR2. The carboxylate group is well inserted in the anion binding site, hydrogen-bonding with Tyr48, His110, and Trp111 side chains and engaging a stabilizing electrostatic interaction with the positively charged nicotinamide moiety of the cofactor $NADP^+$ (N−O = 4.71 Å, Figure 5). Further, N2-nitrogen is hydrogen-bonded to the Trp20 side chain. Moreover, the 7-chloro substituted phenyl ring of benzothiadiazine 1,1-dioxide core penetrates the crevice formed between the Leu300 and Phe122 residues of the specificity pocket, while the 7-chloro atom at the phenyl ring is well placed in the deep hole of the pocket formed by the side chains of Phe115, Trp79, and Val130 (Figure 5). In addition, these interactions together with the presence of the benzothiadiazine ring well orient the substituted benzyl ring in a hydrophobic pocket mainly formed by the side chains of the following ALR2 residues: Phe121, Phe122, Trp20, Val47, and Tyr48.

Conclusions

In this paper we described the synthesis, biological activity, and structure−activity relationships of a series of benzothiadiazine 1,1-dioxide derivatives bearing different substituent

groups at the N2, N4, and 7 positions of the benzothiadiazine scaffold. Most of the derivatives (**9a**−**d** and **9f**−**q**) bearing a N4-acetic acid group and a N2-benzyl group with different substituents at the benzyl ring showed strong and selective in vitro inhibition effect on ALR2 with IC_{50} values in the range of 0.032−0.975 μM, whereby three of them (**9i**, **9j**, and **9m**) showed more prominent ALR2 inhibitory activity both in vitro and in vivo, indicating high ALR2 binding affinity as well as sufficient intracellular availability. On the other hand, the SAR analysis seemed to corroborate the importance of the structural requirements of the N4-acetic acid and N2-benzyl substituents in the benzothiadiazine 1,1-dioxide structure in order to achieve good activity. Either conversion of the acid group into the corresponding ester and amide groups or removal of substituents at the benzyl ring diminished in vitro activity. The ester and amide compounds were not yet active in vivo more than their acid counterparts, making them unavailable as prodrugs. Furthermore, the positions of the acetic acid group and the substituted benzyl group on benzothiadiazine scaffold played a role in their ALR2 inhibitory activity, whereby N4-acetic acid group combined with N2-benzyl group retained the potent activity while exchange of their positions led to drastic loss of the activity. Consequently, it is suggested that an acid group at the N4 position and a substituted benzyl group at the N2 position are minimum requirements for ALR2 inhibitory activity of benzothiadiazine 1,1-dioxide derivatives. In addition, substitutions at the 7-position are not essential but greatly increase the activity. These results were also supported by molecular docking studies in which the inhibitor is tightly anchored in the active site with its acid residue trapped in the anion-binding pocket, with the halogen-substituted phenyl ring of the benzothiadiazine core fit in the specificity pocket, and with its benzyl group inserted in a hydrophobic pocket. The results from the present study have encouraged us to continue our investigations into the design of more potent and selective analogues by conducting appropriate modifications based on the benzothiadiazine scaffold.

Experimental Section

Chemistry. Melting points were recorded on a X-4 microscopic melting point apparatus and were uncorrected. All reactions were routinely checked by TLC on silica gel Merck 60F254. The ^{1}H NMR spectra were recorded on a Bruker Advance 400 spectrometer (400 MHz, Bruker (Beijing) Technologies and Services Co., Ltd.) or Bruker Advance 500 spectrometer (500 MHz, Beijing Normal University, China). Chemical shifts are given in δ units (ppm) relative to internal standard TMS and refer to $CDCl_3$ or DMSO-d_6 solutions. Elemental analyses (C, H, N) were performed at the analytical instrumentation center of Peking University (China) and realized on an Elementar Vario MICRO CUBE elemental analyzer.

The following HPLC methods were used to determine the purity of acetic acid derivatives using a Shimadze LC-20AT liquid chromatograph (Shimadze Corporation, Japan). All acetic acid derivatives tested in biological assays were ≥95% pure. Conditions were as follows. Method A: column, C18, 5 μm, 250 mm × 4.6 mm; mobile phase MeCN (0.1% TFA)/MeOH = 6:4, for 6 min; room temperature; flow rate of 1 mL/min; detection at 254 nm. Method B: column, C18, 5 μm, 250 mm × 4.6 mm; mobile phase H_2O (0.1% TFA)/MeOH = 1:9, for 8 min; room temperature; flow rate of 1 mL/min; detection at 254 nm.

The 4-fluoroaniline, **1b**, 4-chloroaniline, **1c**, 4-bromoaniline, **1d**, and 2-aminobenzenesulfonamide, **2a**, methyl bromoacetate, 4-bromo-2-fluorobenzyl bromide, 3-nitrobenzyl bromide, 4-(trifluoromethyl)benzyl bromide, 2,4,5-trifluorobenzyl bromide, used to obtain the target inhibitors, were from Alfa Aesar.

General Synthetic Procedure for 2b−d. A solution of chlorosulfonyl isocyanate (26 mL, 300 mmol) in 200 mL of nitroethane was cooled to −40 °C, and a solution of the appropriate aniline **1** (270 mmol) in 100 mL of nitroethane was then added dropwise from a dropper funnel with stirring. After the addition was completed, the reaction mixture was stirred for an additional 30 min and aluminum chloride (39 g, 300 mmol) was added. The mixture was then heated to 110 °C with stirring for 1 h. The crude material was then poured into ice, and the precipitate was collected by suction filtration, washed with cold water and dry ether. The residue was added to 320 mL of 50% aqueous sulfuric acid and heated to 140 °C for 8 h. The solution was then poured over ice and neutralized at 0 °C with saturated aqueous sodium hydroxide. The precipitate was collected by suction filtration, washed with cold water, and dried in vacuo to give the desired product.

2-Amino-5-fluorobenzenesulfonamide (2b). Yield 51%; mp 149−152 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.71 (s, 2H), 6.80 (m, 1H), 7.14 (m, 1H), 7.28 (m, 1H), 7.35 (s, 2H).

2-Amino-5-chlorobenzenesulfonamide (2c). Yield 62%; mp 155−157 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.96 (s, 2H), 6.80 (d, J = 8.8 Hz, 1H), 7.24 (m, 1H), 7.36 (s, 2H), 7.48 (d, J = 2.4 Hz, 1H).

2-Amino-5-bromobenzenesulfonamide (2d). Yield 45%; mp 179−181 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.02 (s, 2H), 6.77 (d, J = 8.5 Hz, 1H), 7.38 (m, 1H), 7.40 (s, 2H), 7.61 (d, J = 2.4 Hz, 1H).

General Synthetic Procedure for 3a−d. The appropriate 2-aminobenzenesulfonamide **2** (100 mmol) was heated in triethyl orthoformate (150 mL) to reflux during 2 h. After the mixture was cooled to room temperature, the desired compound was collected by filtration, washed with diethyl ether, and dried in vacuo.

4*H*-1,2,4-Benzothiadiazine 1,1-Dioxide (3a). Yield 89%; mp 226−228 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28 (m, 1H), 7.43 (m, 1H), 7.65 (m, 1H), 7.78 (m, 1H), 7.96 (s, 1H), 12.28 (s, 1H).

7-Fluoro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (3b). Yield 92%; mp 261−264 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 (m, 1H), 7.58 (m, 1H), 7.69 (m, 1H), 7.98 (s, 1H), 12.39 (s, 1H).

7-Cholro-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (3c). Yield 95%; mp 244−247 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.34 (d, J = 8.8 Hz, 1H), 7.72 (m, 1H), 7.85 (d, J = 2.4 Hz, 1H), 8.01 (s, 1H), 12.42 (d, J = 4.4 Hz,1H).

7-Bromo-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (3d). Yield 88%; mp 280−281 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27 (d, J = 8.8 Hz, 1H), 7.86 (m, 1H), 7.95 (d, J = 2.4 Hz, 1H), 8.02 (s, 1H), 12.43 (s, 1H).

General Synthetic Procedure for 4a−d. A mixture of the appropriate 4*H*-1,2,4-benzothiadiazine 1,1-dioxide **3** (80 mmol), potassium carbonate (12 g), and methyl bromoacetate (13.4 g, 88 mmol) in acetonitrile (180 mL) was heated at 70 °C for 2 h. After evaporation of the solvent under reduced pressure, the crude solid was washed with water, dried, and then recrystallized in ethyl acetate to give pure product.

(4*H*-1,2,4-Benzothiadiazine 1,1-dioxide-4-yl)acetic Acid Methyl Ester (4a). Colorless crystal, yield 83%; mp 154−156 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.74 (s, 3H), 5.10 (s, 2H), 7.34 (d, J = 8.5 Hz, 1H), 7.55 (m, 1H), 7.74 (m, 1H), 7.91 (d, J = 9.0 Hz, 1H), 8.10 (s, 1H).

(7-Fluoro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl)acetic Acid Methyl Ester (4b). Colorless crystal, yield 85%; mp 197−200 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.73 (s, 3H), 5.12 (s, 2H), 7.49 (m, 1H), 7.67 (m, 1H), 7.84 (m, 1H), 8.10 (s, 1H).

(7-Cholro-4*H*-1,2,4-benzothiadiazine 1,1-dioxid-4-yl)acetic Acid Methyl Ester (4c). Colorless crystal, yield 79%; mp 248−250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.72 (s, 3H),

5.09 (s, 2H), 7.42 (d, J = 9.2 Hz, 1H), 7.79 (m, 1H), 7.95 (d, J = 2.4 Hz, 1H), 8.10 (s, 1H).

(7-Bromo-4*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl)acetic Acid Methyl Ester (4d). Colorless crystal, yield 82%; mp 214−216 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.73 (s, 3H), 5.11 (s, 2H), 7.38 (d, J = 9.5 Hz, 1H), 7.94 (m, 1H), 8.08 (d, J = 2.0 Hz, 1H), 8.13(s, 1H).

General Synthetic Procedure for 5a−d. To a solution of the appropriate acetate **4** (50 mmol) in 2-propanol−acetonitrile (1:1, 80 mL) with stirring was added $NaBH_4$ (8 g). Then the mixture was stirred at room temperature for 5 min. The solvent was then removed by distillation under reduced pressure, and the residue was suspended in water (25 mL). The alkaline suspension was treated with 0.1 N HCl until the pH value indicated that the mixture was acidic. The resulting insoluble material was collected by filtration, washed with water, dried, and then recrystallized in ethyl acetate to give pure desired product.

(3,4-Dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl)acetic Acid Methyl Ester (5a). Colorless crystal, yield 84%; mp 146−148 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75 (s, 3H), 4.16 (s, 2H), 4.85 (d, J = 7.8 Hz, 2 H), 5.26 (m, 1 H), 6.66 (d, J = 6.4 Hz,1H), 6.94 (m, 1H), 7.37 (m, 1H), 7.74 (m, 1H).

(7-Fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl)acetic Acid Methyl Ester (5b). Colorless crystal, yield 67%; mp 175−176 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.66 (s, 3H), 4.36 (s, 2H), 4.74 (d, J = 8.0 Hz, 2 H), 6.81 (m, 1 H), 7.31 (m, 1H), 7.44 (m, 1H), 8.27 (m, 1H).

(7-Cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxid-4-yl)acetic Acid Methyl Ester (5c). Colorless crystal, yield 71%; mp 186−187 °C, ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.67 (s, 3H), 4.38 (s, 2H), 4.76 (d, J = 8.0 Hz, 2 H), 6.81 (d, J = 9.0 Hz, 1 H), 7.44 (m, 1H), 7.57 (d, J = 2.5 Hz, 1H), 8.34 (m, 1H).

(7-Bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl)acetic Acid Methyl Ester (5d). Colorless crystal, yield 77%; mp 178−182 °C; (400 MHz, DMSO-d_6) δ 3.65 (s, 3H), 4.35 (s, 2H), 4.75 (d, J = 8.0 Hz, 2 H), 6.79 (d, J = 9.2 Hz, 1 H), 7.41 (m, 1H), 7.54 (d, J = 2.4 Hz, 1H), 8.27 (m, 1H).

(3,4-Dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl)-acetic Acid (6). A mixture of **5a** (0.51 g, 2 mmol), 1,4-dioxane (8 mL), and saturated aqueous sodium hydroxide (8 mL) was stirred at room temperature for 2 h. The alkaline suspension was adjusted to be acidic with 0.1 N HCl and extracted 3-fold with ethyl acetate (3 × 20 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure and the residue of the desired compound was recrystallized in methanol to give pure product as a colorless crystal (0.40 g, 83%): mp 171−173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.20 (s, 2H), 4.75 (d, J = 8.0 Hz, 2H), 6.70 (m, 1H), 6.82 (m, 1H), 7.37 (m, 1H), 7.54 (m, 1H), 8.06 (m, 1H), 12.8 (s, 1H). Anal. ($C_9H_{10}N_2O_4S$) C, H, N.

(3,4-Dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl)acetamide (7). A solution of **5a** (0.51 g, 2 mmol) in 25 mL of methanol with stirring was treaded with NH_3 (gas) for 30 min. Then the mixture was stirred at room temperature for 18 h. After evaporation of the solvent under reduced pressure, the crude solid was recrystallized in ethyl acetate−dichloromethane (1:1) to give pure product as a colorless crystal (0.36 g, 75%): mp 225−227 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.95 (s, 2H), 4.80 (s, 2H), 6.60 (d, J = 8.5 Hz, 1H), 6.83 (m, 1H), 7.28 (s, 1H), 7.40 (m, 1H), 7.55 (m, 1H), 7.57 (m, 1H), 7.93 (s, 1H). Anal. ($C_9H_{11}N_3O_3S$) C, H, N.

General Synthetic Procedure for 8a−q. A mixture of appropriate acetate **5** (10 mmol), potassium carbonate (3 g), and appropriate benzyl bromide (11 mmol) in acetonitrile (30 mL) was heated at 70 °C for 2 h. After evaporation of the solvent under reduced pressure, the crude solid was washed with water, dried, and then recrystallized in ethyl acetate.

[2-(4-Bromo-2-fluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8a). Colorless crystal, yield 79%; mp 128−131 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75(s, 3H), 4.01 (s, 2H), 4.36 (s, 2H), 4.86 (s, 2H), 6.55 (d, J = 8.4 Hz, 1H), 6.92 (m, 1H), 7.27 (m, 2H), 7.40 (m, 2H), 7.75 (m, 1H). Anal. ($C_{17}H_{16}BrFN_2O_4S$) C, H, N.

[2-(3-Nitrobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8b). Colorless crystal, yield 80%; mp 163−165 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (s, 3H), 3.97 (s, 2H), 4.47 (s, 2H), 4.82 (s, 2H), 6.56 (d, J = 8.8 Hz, 1H), 6.95 (m, 1H), 7.45 (m, 1H), 7.57 (m, 1H), 7.80 (m, 2H), 8.18 (m, 1H), 8.26 (s, 1H). Anal. ($C_{17}H_{17}N_3O_6S$) C, H, N.

[2-(4-(Ttrifluoromethyl)benzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8c). Colorless crystal, yield 86%; mp 120−121 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.76 (s, 3H), 3.94 (s, 2H), 4.41 (s, 2H), 4.79 (s, 2H), 6.55 (d, J = 8.4 Hz, 1H), 6.93 (m, 1H), 7.42 (m, 1H), 7.54 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 8.0 Hz, 2H), 7.78 (m, J = 8.0 Hz, 1H). Anal. ($C_{18}H_{17}F_3N_2O_4S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8d). Colorless crystal, yield 73%; mp 110−112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (s, 3H), 4.01 (s, 2H), 4.11 (s, 2H), 4.89 (s, 2H), 6.56 (d, J = 8.4 Hz, 1H), 6.95 (m, 2H), 7.41 (m, 2H), 7.77 (m, 1H).

(2-Benzyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl)acetic Acid Methyl Ester (8e). Colorless crystal, yield 75%; mp 80−82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73 (s, 3H), 3.91 (s, 2H), 4.30 (s, 2H), 4.75 (s, 2H), 6.54 (d, J = 8.4 Hz, 1H), 6.91 (m, 1H), 7.35 (m, 6H), 7.76 (m, 1H). Anal. ($C_{17}H_{18}N_2O_4S$) C, H, N.

[2-(4-Bromo-2-fluorobenzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8f). Colorless crystal, yield 89%; mp 108−110 °C (500 MHz, DMSO-d_6) δ 3.68 (s, 3H), 4.27 (s, 2H), 4.37 (s, 2H), 4.89 (s, 2H), 6.87 (m, 1H), 7.40 (m, 2H), 7.47 (d, J = 8.0 Hz, 1H), 7.55 (m, 1H), 7.59 (d, J = 10.0 Hz, 1H). Anal. ($C_{17}H_{15}BrF_2N_2O_4S$) C, H, N.

[2-(3-Nitrobenzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8g). Colorless crystal, yield 81%; mp 149−150 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.68(s, 3H), 4.34(s, 2H), 4.42 (s, 2H), 4.94 (s, 2H), 6.87 (m, 1H), 7.40 (m, 1H), 7.56 (m, 1H), 7.69 (m, 1H), 7.83 (d, J= 7.5 Hz, 1H), 8.20 (m, 2H). Anal. ($C_{17}H_{16}FN_3O_6S$) C, H, N.

[2-(4-(Trifluoromethyl)benzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8h). Colorless crystal, yield 77%; mp 102−104 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.68 (s, 3H), 4.35 (s, 2H), 4.37 (s, 2H), 4.91 (s, 2H), 6.87 (m, 1H), 7.41 (m, 1H), 7.55 (m, 1H), 7.60 (d, J = 8.0 Hz, 2H), 7.76 (d, J = 8.0 Hz, 2H). Anal. ($C_{18}H_{16}F_4N_2O_4S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8i). Colorless crystal, yield 84%; mp 147−149 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.68 (s, 3H), 4.27 (s, 2H), 4.38 (s, 2H), 4.90 (s, 2H), 6.87 (m, 1H), 7.40 (m, 1H), 7.54 (m, 2H), 7.61 (m, 1H). Anal. ($C_{17}H_{14}F_4N_2O_4S$) C, H, N.

[2-(4-Bromo-2-fluorobenzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8j). Colorless crystal, yield 75%; mp 155−157 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.68 (s, 3H), 4.27 (s, 2H), 4.38 (s, 2H), 4.90 (s, 2H), 6.86 (d, J = 9.5 Hz, 1H), 7.40 (m, 1H), 7.47 (d, J = 11.5 Hz, 1H), 7.50 (m, 1H), 7.59 (d, J = 9.0 Hz, 1H), 7.67 (d, J = 1.0 Hz, 1H). Anal. ($C_{17}H_{15}BrClFN_2O_4S$) C, H, N.

[2-(3-Nitrobenzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8k). Colorless crystal, yield, 80%; mp 169−170 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.69 (s, 3H), 4.36 (s, 2H), 4.41 (s, 2H), 4.96 (s, 2H), 6.86 (d, J = 9.0 Hz, 1H), 7.50 (m, 1H), 7.68 (m, 2H), 7.83 (d, J = 7.5 Hz, 1H), 8.20 (m, 2H). Anal. ($C_{17}H_{16}ClN_3O_6S$) C, H, N.

[2-(4-(Trifluoromethyl)benzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8l). Colorless crystal, yield 83%; mp 114−116 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.68 (s, 3H), 4.36 (s, 4H), 4.93 (s, 2H), 6.86 (d, J = 9.0 Hz, 1H), 7.50 (d, J = 9.0 Hz, 1H), 7.58

(d, J = 8.0 Hz, 2H), 7.68 (s, 1H), 7.57 (d, J = 8.0 Hz, 2H). Anal. ($C_{18}H_{16}ClF_3N_2O_4S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic acid Methyl Ester (8m). Colorless crystal, yield 80%; mp 191−193 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.68 (s, 3H), 4.26 (s, 2H), 4.39 (s, 2H), 4.93 (s, 2H), 6.87 (d, J = 9.0 Hz, 1H), 7.52 (m, 2H), 7.62 (m, 1H), 7.67 (s, 1H). Anal. ($C_{17}H_{14}ClF_3N_2O_4S$) C, H, N.

[2-(4-Bromo-2-fluorobenzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazin 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8n). Colorless crystal, yield 88%; mp 150−152 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.68 (s, 3H), 4.26 (s, 2H), 4.37 (s, 2H), 4.90 (s, 2H), 6.80 (d, J = 9.5 Hz, 1H), 7.40 (m, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.61 (m, 2H), 7.75 (d, J = 1.5 Hz, 1H). Anal. ($C_{17}H_{15}Br_2FN_2O_4S$) C, H, N.

[2-(3-Nitrobenzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8o). Colorless crystal, yield 87%; mp 174−176 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.69 (s, 3H), 4.33 (d, J = 9.5 Hz, 2H), 4.41 (s, 2H), 4.96 (s, 2H), 6.80 (d, J = 9.0 Hz, 1H), 7.60(m, 1H), 7.68 (m, 1H), 7.76 (d, J = 1.5 Hz, 1H), 7.82 (d, J = 7.5 Hz, 1H), 8.20 (m, 2H). Anal. ($C_{17}H_{16}BrN_3O_6S$) C, H, N.

[2-(4-(Trifluoromethyl)benzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8p). Colorless crystal, yield 92%; mp 117−118 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.68 (s, 3H), 4.35 (s, 4H), 4.93 (s, 2H), 6.80 (d, J = 9.5 Hz, 1H), 7.60 (m, 3H), 7.56 (m, 3H). Anal. ($C_{18}H_{16}BrF_3N_2O_4S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid Methyl Ester (8q). Colorless crystal, yield 79%; mp 124−126 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.68 (s, 3H), 4.26 (s, 2H), 4.38 (s, 2H), 4.92 (s, 2H), 6.81 (d, J = 9.0 Hz, 1H), 7.52 (m, 1H), 7.61 (m, 2H), 7.75 (d, J = 1.5 Hz, 1H). Anal. ($C_{17}H_{14}BrF_3N_2O_4S$) C, H, N.

General Synthetic Procedure for 9a−q. A mixture of the appropriate acetate **8** (1 mmol), 1,4-dioxane (5 mL), and saturated aqueous sodium hydroxide (5 mL) was stirred at room temperature for 2 h. The alkaline suspension was adjusted to be acidic with 0.1 N HCl and extracted 3-fold with ethyl acetate (3 × 20 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure, and the residue of the desired compound was recrystallized in methanol to give pure product.

[2-(4-Bromo-2-fluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9a). Colorless crystal, yield 79%; mp 200−203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.21 (s, 2H), 4.27 (s, 2H), 4.89 (s, 2H), 6.76 (d, J = 8.8 Hz, 1H), 6.87 (m, 1H), 7.44 (m, 3H), 7.59 (m, 2H), 12.96 (s, 1H). Anal. ($C_{16}H_{14}BrFN_2O_4S$) C, H, N.

[2-(3-Nitrobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9b). Colorless crystal, yield 85%; mp 166−169 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.20 (s, 2H), 4.42 (s, 2H), 4.95 (s, 2H), 6.76 (d, J = 8.4 Hz, 1H), 6.89 (m, 1H), 7.46 (m, 1H), 7.65 (m, 2H), 8.17 (m, 1H), 8.20 (m, 2H), 12.98 (s, 1H). Anal. ($C_{16}H_{15}N_3O_6S$) C, H, N.

[2-(4-(Ttrifluoromethyl)benzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9c). Colorless crystal, yield 83%; mp 152−155 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (s, 2H), 4.39 (s, 2H), 4.79 (s, 2H), 6.58 (d, 1H), 6.96 (m, 1H), 7.43 (m, 1H), 7.51 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 8.0 Hz, 2H), 7.78 (m, 1H). Anal. ($C_{17}H_{15}F_3N_2O_4S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9d). Colorless crystal, yield 85%; mp 172−174 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.21 (s, 2H), 4.25 (s, 2H), 4.90 (s, 2H), 6.75 (d, J = 8.4 Hz, 1H), 6.88 (m, 1H), 7.45 (m, 1H), 7.50 (m, 1H), 7.56 (m, 1H), 7.62 (m, 1H). Anal. ($C_{16}H_{13}F_3N_2O_4S$) C, H, N.

(2-Benzyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl)acetic Acid (9e). Colorless crystal, yield 90%; mp 173−175 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83 (s, 2H), 4.18 (s, 2H), 4.62 (s, 2H), 6.47 (d, J = 8.4 Hz, 1H), 6.80 (m, 1H), 7.23 (m, 5H), 7.64 (d, J = 8.0 Hz, 1H), 8.69 (s, 1H). Anal. ($C_{16}H_{16}N_2O_4S$) C, H, N.

[2-(4-Bromo-2-fluorobenzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9f). Colorless crystal, yield 78%; mp 167−169 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.24 (s, 2H), 4.28 (s, 2H), 4.88 (s, 2H), 6.83 (m, 1H), 7.40 (m, 2H), 7.45 (d, 1H), 7.52 (m, 1H), 7.60 (m, 1H). Anal. ($C_{16}H_{13}BrF_2N_2O_4S$) C, H, N.

[2-(3-Nitrobenzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9g). Colorless crystal, yield 86%; mp 178−180 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.21 (s, 2H), 4.43 (s, 2H), 4.94 (s, 2H), 6.83 (m, 1H), 7.39 (m, 1H), 7.54 (m, 1H), 7.68 (m, 1H), 7.82 (d, J = 7.5 Hz, 1H), 8.18 (d, J = 8.5 Hz, 1H), 8.21 (s, 1H). Anal. ($C_{16}H_{14}FN_3O_6S$) C, H, N.

[2-(4-(Trifluoromethyl)benzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9h). Colorless crystal, yield 79%; mp 191−193 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.22 (s, 2H), 4.37 (s, 2H), 4.90 (s, 2H), 6.83 (m, 1H), 7.40 (m, 1H), 7.53 (m, 1H), 7.58 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H). Anal. ($C_{17}H_{14}F_4N_2O_4S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9i). Colorless crystal, yield 92%; mp 146−149 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.24 (s, 2H), 4.27 (s, 2H), 4.89 (s, 2H), 6.84 (m, 1H), 7.40 (m, 1H), 7.52 (m, 2H), 7.60 (m, 1H). Anal. ($C_{16}H_{12}F_4N_2O_4S$) C, H, N.

[2-(4-Bromo-2-fluorobenzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9j). Colorless crystal, yield 86%; mp 179−181 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.25 (s, 2H), 4.27 (s, 2H), 4.90 (s, 2H), 6.85 (m, 1H), 7.42 (m, 1H), 7.52 (m, 1H), 7.58 (m, 1H), 7.65 (d, J = 2.5 Hz, 1H), 7.69 (s, 1H). Anal. ($C_{16}H_{13}BrClFN_2O_4S$) C, H, N.

[2-(3-Nitrobenzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9k). Colorless crystal, yield 78%; mp 201−204 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.15 (s, 2H), 4.42 (s, 2H), 4.96 (s, 2H), 6.80 (d, J = 9.5 Hz, 1H), 7.48 (m, 1H), 7.64 (d, J = 2.5 Hz, 1H), 7.67 (m, 1H), 7.82 (d, J = 7.5 Hz, 1H), 8.17 (d, J = 8.5 Hz, 1H), 8.21 (s, 1H). Anal. ($C_{16}H_{14}ClN_3O_6S$) C, H, N.

[2-(4-(Trifluoromethyl)benzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9l). Colorless crystal, yield 85%; mp 197−199 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.21 (s, 2H), 4.37 (s, 2H), 4.93 (s, 2H), 6.83 (d, J = 9.0 Hz, 1H), 7.52 (m, 1H), 7.58 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 2.0 Hz, 1H), 7.74 (d, J= 8.0 Hz, 2H). Anal. ($C_{17}H_{14}ClF_3N_2O_4S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9m). Colorless crystal, yield 76%; mp 140−142 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.23 (s, 2H), 4.25 (s, 2H), 4.90 (s, 2H), 6.83 (d, J = 8.8 Hz, 1H), 7.49 (m, 1H), 7.55 (m, 1H), 7.62 (m, 1H), 7.64 (s, 1H), 13.0 (s, 1H). Anal.($C_{16}H_{12}ClF_3N_2O_4S$) C, H, N.

[2-(4-Bromo-2-fluorobenzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9n). Colorless crystal, yield 83%; mp 176−179 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.19 (s, 2H), 4.28 (s, 2H), 4.89 (s, 2H), 6.76 (d, J = 9.0 Hz, 1H), 7.43 (m, 2H), 7.59 (m, 2H), 7.72 (d, J = 1.0 Hz, 1H). Anal. ($C_{16}H_{13}Br_2FN_2O_4S$) C, H, N.

[2-(3-Nitrobenzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9o). Colorless crystal, yield 90%; mp 212−215 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.22 (s, 2H), 4.42 (s, 2H), 4.96 (s, 2H), 6.77 (d, 1H), 7.61 (m, 1H), 7.68 (m, 1H), 7.75 (d, J = 2.5 Hz, 1H), 7.82 (d, J = 7.5 Hz, 1H), 8.18 (d, J = 7.5 Hz, 1H), 8.21 (s, 1H), 13.10 (s, 1H). Anal. ($C_{16}H_{14}BrN_3O_6S$) C, H, N.

[2-(4-(Trifluoromethyl)benzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9p). Colorless crystal, yield 80%; mp 192−193 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.16 (s, 2H), 4.37 (s, 2H), 4.91 (s, 2H), 6.76 (d, J = 9.5 Hz, 1H), 7.58 (s, 1H), 7.61 (m, 2H), 7.73 (m, 2H), 7.75 (s, 1H). Anal. ($C_{17}H_{14}BrF_3N_2O_4S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetic Acid (9q). Colorless crystal, yield 89%; mp 197−201 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.25 (s, 2H), 4.26 (s, 2H), 4.91 (s, 2H), 6.78 (d, J = 9 Hz, 1H), 7.52 (m, 1H), 7.60 (m, 2H), 7.74 (m, 1H). Anal. ($C_{16}H_{12}BrF_3N_2O_4S$) C, H, N.

General Synthetic Procedure for 10a−q. Appropriate acetate **8** (1 mmol) was dissolved in 25 mL of methanol and was treaded with NH_3 (gas) for 30 min with stirring. The mixture was then stirred at room temperature for 18 h. After evaporation of the solvent under reduced pressure, the crude solid was recrystallized in ethyl acetate−dichloromethane (1:1) to give pure product.

[2-(4-Bromo-2-fluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10a). Colorless crystal, yield 89%; mp 166−168 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (s, 2H), 4.33 (s, 2H), 4.86 (s, 2H), 5.55 (s, 1H), 5.92 (s, 1H), 6.63 (d, J = 8.4 Hz, 1H), 6.97 (m, 1H), 7.29 (m, 2H), 7.33 (m, 2H), 7.43 (d, J = 6.0 Hz, 1H). Anal. ($C_{16}H_{15}BrFN_3O_3S$) C, H, N.

[2-(3-Nitrobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10b). Yellow crystal, yield 90%; mp 172−175 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (s, 2H), 4.45 (s, 2H), 4.82 (s, 2H), 5.55 (s, 1H), 5.75 (s, 1H), 6.64 (d, J = 8.4 Hz, 1H), 6.97 (m, 1H), 7.44 (m, 1H), 7.57 (m, 1H), 7.79 (s, 1H), 7.80 (s, 1H), 8.19 (s, 1H), 8.27 (s, 1H). Anal. ($C_{16}H_{16}N_4O_5S$) C, H, N.

[2-(4-(Ttrifluoromethyl)benzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10c). Colorless crystal, yield 73%; mp 171−172 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.00 (s, 2H), 4.40 (s, 2H), 4.92 (s, 2H), 6.68 (d, J = 8.4 Hz, 1H), 6.87 (m, 1H), 7.22 (s, 1H), 7.46 (m, 1H), 7.59 (d, J = 8.4 Hz, 2H), 7.60(m, 1H), 7.62 (m, 1H), 7.73 (d, J = 8.0 Hz, 2H). Anal. ($C_{17}H_{16}F_3N_3O_3S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10d). Colorless crystal, yield 77%; mp 208−211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.02 (s, 2H), 4.29 (s, 2H), 4.91 (s, 2H), 6.66 (d, J = 8.4 Hz, 1H), 6.86 (m, 1H), 7.25 (s, 1H), 7.45 (m, 1H), 7.54 (m, 1H), 7.60 (m, 2H), 7.64 (s, 1H).

(2-Benzyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl)acetamide (10e). Colorless crystal, yield 72%; mp 144−146 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.96 (s, 2H), 4.26 (s, 2H), 4.83 (s, 2H), 6.66 (d, J = 8.4 Hz, 1H), 6.85 (m, 1H), 7.29 (m, 1H), 7.31 (m, 1H), 7.34 (m, 2H), 7.36 (m, 1H), 7.43 (m, 2H). Anal. ($C_{16}H_{17}N_3O_3S$) C, H, N.

[2-(4-Bromo-2-fluorobenzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10f). Colorless crystal, yield 74%; mp 123−125 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.03 (s, 2H), 4.31 (s, 2H), 4.90 (s, 2H), 6.72 (m, 1H), 7.28 (s, 1H), 7.40 (m, 1H), 7.45 (m, 2H), 7.51 (m, 1H), 7.58 (d, J = 9.0 Hz, 1H), 7.65 (s, 1H). Anal. ($C_{16}H_{14}BrF_2N_3O_3S$) C, H, N.

[2-(3-Nitrobenzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10g). Yellow crystal, yield 89%; mp 180−183 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.00 (s, 2H), 4.46 (s, 2H), 4.96 (s, 2H), 6.70 (m, 1H), 7.29 (s, 1H), 7.41 (m, 1H), 7.53 (m, 1H), 7.63 (s, 1H), 7.68 (m, 1H), 7.83 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 1.5 Hz, 1H), 8.21 (s, 1H). Anal. ($C_{16}H_{15}FN_4O_5S$) C, H, N.

[2-(4-(Trifluoromethyl)benzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10h). Colorless crystal, yield 94%; mp 103−105 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.01 (s, 2H), 4.41 (s, 2H), 4.92 (s, 2H), 6.72 (m, 1H), 7.27 (s, 1H), 7.42 (m, 1H), 7.52 (m, 1H), 7.61 (d, J = 8.0 Hz, 2H), 7.63 (m, 1H), 7.76 (d, J = 8.0 Hz, 2H). Anal. ($C_{17}H_{15}F_4N_3O_3S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-7-fluoro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10i). Colorless crystal, yield 80%; mp 175−177 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.04 (s, 2H), 4.30 (s, 2H), 4.91 (s, 2H), 6.70 (m, 1H), 7.31 (s, 1H), 7.42 (m, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.61 (m, 2H), 7.68 (s, 1H). Anal. ($C_{16}H_{13}F_4N_3O_3S$) C, H, N.

[2-(4-Bromo-2-fluorobenzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10j). Colorless crystal, yield 93%; mp 188−191 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.05 (s, 2H), 4.31 (s, 2H), 4.91 (s, 2H), 6.72 (d, J = 9.0 Hz, 1H), 7.30 (s, 1H), 7.44 (m, 2H), 7.53 (d, J = 9.0 Hz, 1H), 7.58 (d, J = 9.5 Hz, 1H), 7.64 (s, 1H), 7.66 (s, 1H). Anal. ($C_{16}H_{14}BrClFN_3O_3S$) C, H, N.

[2-(3-Nitrobenzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10k). Yellow crystal, yield 80%; mp 186−188 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.01 (s, 2H), 4.46 (s, 2H), 4.98 (s, 2H), 6.70 (d, J = 9.5 Hz, 1H), 7.31 (s, 1H), 7.53 (d, J = 9.0 Hz, 1H), 7.65 (s, 2H), 7.67 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 7.5 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.23 (s, 1H). Anal. ($C_{16}H_{15}ClN_4O_5S$) C, H, N.

[2-(4-(Trifluoromethyl)benzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10l). Colorless crystal, yield 89%; mp 176−178 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.02 (s, 2H), 4.40 (s, 2H), 4.93 (s, 2H), 6.72 (d, J = 9.5 Hz, 1H), 7.29 (s, 1H), 7.53 (m, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.64 (d, J = 2.0 Hz, 2H), 7.74 (s, J = 8.5 Hz, 2H). Anal. ($C_{17}H_{15}ClF_3N_3O_3S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-7-cholro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10m). Colorless crystal, yield 94%; mp 157−159 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.05 (s, 2H), 4.30 (s, 2H), 4.92 (s, 2H), 6.71 (d, J = 9.5 Hz, 1H), 7.34 (s, 1H), 7.52 (m, 1H), 7.58 (m, 2H), 7.60 (d, J = 4.0 Hz, 1H), 7.63 (s, 1H). Anal. ($C_{16}H_{13}ClF_3N_3O_3S$) C, H, N.

[2-(4-Bromo-2-fluorobenzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10n). Colorless crystal, yield 91%; mp 203−205 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.04 (s, 2H), 4.31 (s, 2H), 4.91 (s, 2H), 6.66 (d, J = 9.5 Hz, 1H), 7.30 (s, 1H), 7.43 (s, 1H), 7.44 (s, 1H), 7.59 (d, J = 10.5 Hz, 1H), 7.64 (m, 2H), 7.72 (d, J = 2.5 Hz, 1H). Anal. ($C_{16}H_{14}Br_2FN_3O_3S$) C, H, N.

[2-(3-Nitrobenzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10o). Yellow crystal, yield 75%; mp 166−168 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.01 (s, 2H), 4.46 (s, 2H), 4.97 (s, 2H), 6.65 (d, J = 9.0 Hz, 1H), 7.31 (s, 1H), 7.62 (m, 2H), 7.67 (m, 1H), 7.73 (d, J = 2.5 Hz, 1H), 7.83 (d, J = 7.5 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.23 (s, 1H). Anal. ($C_{16}H_{15}BrN_4O_5S$) C, H, N.

[2-(4-(Trifluoromethyl)benzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10p). Colorless crystal, yield 92%; mp 188−192 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.02 (s, 2H), 4.40 (s, 2H), 4.93 (s, 2H), 6.66 (d, J = 9.5 Hz, 1H), 7.29 (s, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.61 (s, 1H), 7.64 (m, 1H), 7.73 (d, J = 2.5 Hz, 1H), 7.73 (d, J = 8.0 Hz, 2H). Anal. ($C_{17}H_{15}BrF_3N_3O_4S$) C, H, N.

[2-(2,4,5-Trifluorobenzyl)-7-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-4-yl]acetamide (10q). Colorless crystal, yield 81%; mp 153−155 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.05 (s, 2H), 4.30 (s, 2H), 4.92 (s, 2H), 6.66 (d, d, J = 9.0 Hz, 1H), 7.34 (s, 1H), 7.63 (m, 2H), 7.65 (m, 1H), 7.68 (s, 1H), 7.72 (d, d, J = 2.0 Hz, 1H). Anal. ($C_{16}H_{13}BrF_3N_3O_3S$) C, H, N.

General Synthetic Procedure for 11a−d. A mixture of **3a** (20 mmol), potassium carbonate (3.0 g), and appropriate benzyl bromide (22 mmol) in acetonitrile (50 mL) was heated at 70 °C for 2 h. After evaporation of the solvent under reduced pressure, the crude solid was washed with water, dried, and then recrystallized in ethyl acetate.

4-(4-Bromo-2-fluorobenzyl)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (11a). Colorless crystal, yield 69%; mp 207−208 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (s, 2H), 7.01 (m, 2H), 7.29 (m, 1H), 7.47 (m, 1H), 7.56 (m, 1H), 7.78 (s, 1H), 8.04 (m, 1H).

4-(3-Nitrobenzyl)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (11b). Colorless crystal, yield 84%; mp 223−225 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.55 (s, 2H), 7.41 (d, J = 8.4 Hz, 1H), 7.51 (m, 1H), 7.69 (m, 3H), 7.91 (m, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.27 (s, 1H), 8.41 (s, 1H).

4-[4-(Trifluoromethyl)benzyl]-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (11c). Colorless crystal, yield 59%; mp 216−219 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24 (s, 2H), 6.95 (m, 1H), 7.36

(d, J = 8.0 Hz, 2H), 7.47 (m, 2H), 7.66 (d, J = 8.4 Hz, 2H), 7.79 (s, 1H), 8.05 (m, 1H).

4-(2,4,5-Trifluorobenzyl)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (11d). Colorless crystal, yield 76%; mp 203−206 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.40 (s, 2H), 7.43 (d, J = 8.4 Hz, 1H), 7.51 (m, 1H), 7.61 (m, 2H), 7.71 (m, 1H), 7.89 (m, 1H), 8.30 (d, J = 1.6 Hz, 1H).

General Synthetic Procedure for 12a−d. To a solution of the appropriate **11** (14 mmol) in 2-propanol−acetonitrile (1:1, 20 mL) with stirring was added $NaBH_4$ (2.0 g). Then the mixture was stirred at room temperature for 5 min. The solvent was removed by distillation under reduced pressure, and the residue was suspended in Cl_2H_2 (10 mL). The alkaline suspension was treated with 0.1 N HCl until the pH value indicated that the mixture was acidic. The resulting insoluble material was collected by filtration, washed with water, dried, and then recrystallized in ethyl acetate.

4-(4-Bromo-2-fluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (12a). Colorless crystal, yield 70%; mp 165−168 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.62 (s, 2H), 4.84 (d, J = 8.4 Hz, 2H), 6.66 (d, J = 8.8 Hz, 1H), 6.79 (m, 1H), 7.30 (m, 2H), 7.38 (m, 1H), 7.57 (m, 2H), 8.18 (m, 1H).

4-(3-Nitrobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (12b). Colorless crystal, yield 66%; mp 154−156 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.74 (s, 2H), 4.87 (d, J = 8.0 Hz, 2H), 6.65 (d, J = 8.4 Hz, 1H), 6.78 (m, 1H), 7.29 (m, 1H), 7.55 (m, 1H), 7.63 (m, 1H), 7.78 (d, J = 8.0 Hz, 1H), 8.11 (m, 1H), 8.19 (s, 1H), 8.24 (s, 1H).

4-[4-(Trifluoromethyl)benzyl]-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (12c). Colorless crystal, yield 70%; mp 187−188 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.64 (s, 2H), 4.90 (s, 2H), 6.62 (d, J = 8.8 Hz, 1H), 6.89 (m, 1H), 7.31 (m, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.74 (m, 1H).

4-(2,4,5-Trifluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (12d). Colorless crystal, yield 83%; mp 158−160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.54 (s, 2H), 4.90 (s, 2H), 6.64 (d, J = 8.8 Hz, 1H), 6.90 (m, 1H), 7.02 (m, 2H), 7.35 (m, 1H), 7.73 (m, 1H).

General Synthetic Procedure for 13a−d. A mixture of appropriate **12** (10 mmol), potassium carbonate (3 g), and methyl bromoacetate (1.68 g, 11 mmol) in acetonitrile (30 mL) was heated at 70 °C for 2 h. After evaporation of the solvent under reduced pressure, the crude solid was washed with water, dried, and then recrystallized in ethyl acetate to give pure product.

[4-(4-Bromo-2-fluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-2-yl]acetic Acid Methyl Ester (13a). Colorless crystal, yield 86%; mp 193−195 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73 (s, 3H), 3.89 (s, 2H), 4.53 (s, 2H), 5.16 (s, 2H), 6.70 (d, J = 8.8 Hz, 1H), 6.89 (m, 1H), 7.05 (m, 1H), 7.32 (m, 2H), 7.35 (m, 1H), 7.71 (m, 1H). Anal. ($C_{17}H_{16}BrFN_2O_4S$) C, H, N.

[4-(3-Nitrobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-2-yl]acetic Acid Methyl Ester (13b). Colorless crystal, yield 67%; mp 120−122 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73 (s, 3H), 3.95 (s, 2H), 4.66 (s, 2H), 5.22 (s, 2H), 6.65 (d, J = 8.4 Hz, 1H), 6.92 (m, 1H), 7.35 (m, 1H), 7.55 (m, 2H), 7.74 (m, 1H), 8.11 (s, 1H), 8.17 (m, 1H). Anal. ($C_{17}H_{17}N_3O_6S$) C, H, N.

[4-(4-(Ttrifluoromethyl)benzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-2-yl]acetic Acid Methyl Ester (13c). Colorless crystal, yield 78%; mp 177−179 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 (s, 3H), 3.92 (s, 2H), 4.63 (s, 2H), 5.18 (s, 2H), 6.67 (d, J = 8.8 Hz, 1H), 6.90 (m, 1H), 7.33 (d, J = 7.6 Hz, 2H), 7.36 (m, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.73 (m, 1H).

[4-(2,4,5-Trifluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-2-yl]acetic Acid Methyl Ester (13d). Colorless crystal, yield 72%; mp 128−130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (s, 3H), 3.91 (s, 2H), 4.53 (s, 2H), 5.18 (s, 2H), 6.69 (d, J = 8.4 Hz, 1H), 6.91 (m, 1H), 7.02 (m, 2H), 7.38 (m, 1H), 7.73 (m, 1H).

General Synthetic Procedure for 14a−d. A mixture of the appropriate acetate **13** (1 mmol), 1,4-dioxane (8 mL), and saturated aqueous sodium hydroxide (8 mL) was stirred at room temperature for 2 h. The alkaline suspension was adjusted to be acidic with 0.1 N HCl and extracted 3-fold with ethyl acetate (3 × 20 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure, and the residue of the desired compound was recrystallized in methanol.

[4-(4-Bromo-2-fluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-2-yl]acetic Acid (14a). Colorless crystal, yield 69%; mp 170−173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.77 (s, 2H), 4.69 (s, 2H), 5.10 (s, 2H), 6.85 (m, 2H), 7.20 (m, 1H), 7.40 (m, 2H), 7.59 (m, 2H), 12.90 (s, 1H). Anal. ($C_{16}H_{14}BrFN_2O_4S$) C, H, N.

[4-(3-Nitrobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-2-yl]acetic Acid (14b). Colorless crystal, yield 77%; mp 160−163 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.78 (s, 2H), 4.83 (s, 2H), 5.15 (s, 2H), 6.87 (m, 2H), 7.40 (m, 1H), 7.62 (m, 2H), 7.70 (m, 1H), 8.13 (m, 2H), 12.79 (s, 1H). Anal. ($C_{16}H_{15}N_3O_6S$) C, H, N.

[4-(4-(Ttrifluoromethyl)benzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-2-yl]acetic Acid (14c). Colorless crystal, yield 88%; mp 88−91 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.79 (s, 2H), 4.78 (s, 2H), 5.14 (s, 2H), 6.76 (d, J = 8.4 Hz, 1H), 6.85 (m, 1H), 7.37 (m, 1H), 7.46 (d, J = 8.4 Hz, 2H), 7.59 (m, 1H), 7.71 (d, J = 8.4 Hz, 2H). Anal. ($C_{17}H_{15}F_3N_2O_4S$) C, H, N.

[4-(2,4,5-Trifluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-2-yl]acetic Acid (14d). Colorless crystal, yield 91%; mp 159−161 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.75 (s, 2H), 4.67 (s, 2H), 5.09 (s, 2H), 6.87 (m, 2H), 7.41 (m, 2H), 7.58 (m, 2H). Anal. ($C_{16}H_{13}F_3N_2O_4S$) C, H, N.

General Synthetic Procedure for 15a, 15b. A solution of appropriate acetate **13** (1 mmol) in 25 mL of methanol with stirring was treated with NH_3 (gas) for 30 min and then stirred at room temperature for 18 h. After evaporation of the solvent under reduced pressure, the crude solid was recrystallized in ethyl acetate−dichloromethane (1:1) to give pure product.

[4-(4-Bromo-2-fluorobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-2-yl]acetamide (15a). Colorless crystal, yield 63%; mp 159−161 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.79 (s, 2H), 4.58 (s, 2H), 5.09 (s, 2H), 5.64 (s, 1H), 6.55 (s, 1H), 6.70 (d, J = 8.8 Hz, 1H), 6.92 (m, 1H), 7.05 (m, 1H), 7.29 (m, 2H), 7.38 (m, 1H), 7.71 (m, 1H). Anal. ($C_{16}H_{15}BrFN_3O_3S$) C, H, N.

[4-(3-Nitrobenzyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide-2-yl]acetamide (15b). Yellow crystal, yield 53%; mp 154−157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83 (s, 2H), 4.71 (s, 2H), 5.29 (s, 2H), 5.52 (s, 1H), 6.47 (s, 1H), 6.65 (d, J = 8.4 Hz, 1H), 6.95 (m, 1H), 7.40 (m, 1H), 7.57 (m, 2H), 7.77 (d, J = 1.2 Hz, 1H), 8.10 (s, 1H), 8.18 (m, 1H). Anal. ($C_{16}H_{16}N_4O_5S$) C, H, N.

Biology, Materials, and Methods. ALR2 and ALR1 were obtained from Wistar rats, 200−250 g, b.w., supplied by Vital River, Beijing, China. STZ was from Amresco. Sorbitol dehydrogenase was from Wako, Japan. D,L-Glyceraldehyde, sodium D-glucuronate, and NADPH were from Sigma-Aldrich. All other chemicals were of reagent grade.

ALR1 and ALR2 were prepared in accordance with the method of Kinoshita and Concettina La Motta.[12,30] Enzyme activity was assayed spectrophotometrically on an Unico 4802S UV/vis double beam spectrophotometer by measuring the decrease in absorption of NADPH at 340 nm, which accompanies the oxidation of NADPH catalyzed by ALR2 and ALR1.

Enzymatic Assays. ALR2 activity was performed at 32 °C in a reaction mixture containing 0.25 mL of 0.10 mM NADPH, 0.25 mL of 0.1 M sodium phosphate buffer (pH 6.2), 0.1 mL of enzyme extract, 0.15 mL of deionized water, and 0.25 mL of 10 mM D,L-glyceraldehyde as substrate in a final volume of 1 mL. The reaction mixture except for D,L-glyceraldehyde was incubated at 32 °C for 10 min. The substrate was then added to start the reaction, which was monitored for 4 min.

ALR1 activity was performed at 37 °C in a reaction mixture containing 0.25 mL of 0.12 mM NADPH, 0.1 mL of enzyme extract, 0.25 mL of 0.1 M sodium phosphate buffer (pH 7.2),

0.15 mL of deionized water, and 0.25 mL of 20 mM sodium D-glucuronate as substrate in a final volume of 1 mL. The reaction mixture except for sodium D-glucuronate was incubated at 37 °C for 10 min. The substrate was then added to start 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 dimethylsulfoxide (DMSO) and diluted with deionized water. To correct for the nonenzymatic 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 10^{-4} M (the concentration is referenced to that of the compound in the reaction mixture). Those compounds found to be active were tested at additional concentrations between 10^{-4} and 10^{-8} M. Each dose−effect curve was generated using at least four concentrations of inhibitor, causing an inhibition 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.

STZ-Induced Diabetic Rat Model (General). Male Wistar rats (200−250 g) were purchased from the Experimental Animal Center of Peking University (Certificate SCXK-2006-0008). The animals were allowed to acclimate for a week and then were rendered diabetic by an intraperitoneal injection of STZ (55 mg/kg), which had been freshly dissolved in 0.03 M citrate buffer, pH 4.5. Four days later, tail blood glucose was measured using a One Touch II blood glucose meter. Diabetic rats whose blood glucose levels were ≥16 mmol/L were randomized according to blood glucose levels into diabetic-control and diabetic-treatment groups (n = 6−8).

Five-Day STZ-Induced Diabetic Rat Screening Model. Beginning on day 4 after induction of diabetes with STZ, test compounds suspended in a vehicle of 0.5% sodium carboxymethylcellulose were administered po consecutively for 5 days. Nondiabetic and diabetic controls received vehicle during this period. Six hours after the final dose was administered, the rats were sacrificed and sciatic nerves were removed, weighed, and stored at −80 °C for sorbitol analysis.

Tissue Sorbitol Analysis. The sciatic nerve sample (30−60 mg) was placed into water (1.0 mL/40 mg of tissue), heated in a boiling bath for 2 min, and then homogenized with a Polytron instrument in 6% perchloricacid. The homogenate was centrifuged at 1050g for 15 min at 4 °C The supernatant was neutralized with 2 M K_2CO_3 and used as tissue extract for the assaying of sorbitol. Sorbitol was assayed by an enzymatic method in which sorbitol dehydrogenase catalyzes the stoichiometric conversion of NAD^+ by sorbitol to a fluorogenic product, NADH.[54] The reaction mixture contained 30 mM glycine buffer (pH 9.4), 1.3 mM NAD^+, 1.3 U/mL sorbitol dehydrogenase, and 1.0 mL of the tissue extract in a total volume of 3 mL. After the mixture was allowed to stand for 60 min at 37 °C, the fuorescence intensity was measured at 365 nm excitation wavelength and 430 nm emission wavelength using a fluorospectrophotometer. The sorbitol concentration was quantitated by comparison with standards of sorbitol. The sorbitol content in the sciatic nerve of each animal was expressed as nmol/wet weight. The activity of test compounds was expressed as the percent inhibition of sorbitol accumulation at a given dose, which was calculated according to the following equation:[55]

$$\% \text{ inhibition} = (S - T)/(S - N) \times 100$$

where S is the sorbitol content in the sciatic nerve of untreated diabetic control rats, T is the sorbitol content in the sciatic nerve of diabetic rats given test compounds, and N is the sorbitol content in the sciatic nerve of age-matched nondiabetic control rats.

FlexX Docking. Docking was performed using the SYBYL 7.1. The structure of the ligand was set up as follows: molecular model of compound was constructed using standard bond lengths and bond angles of the SYBYL fragment library, Gasteiger−Huckel charges were assigned, and the minimization was realized with the maximum number of iterations set to 1000. The X-ray crystal structure of human aldose reductase holoenzyme in complex with the inhibitor tolrestat (PDB entry code 2FDZ)[52] was retrieved from the Protein Data Bank (PDB). All the amino acid residues within 6.5 Å of the ligand tolrestat are defined as the active site. The docking and subsequent scoring were performed, and a maximum of 30 poses for the specified ligand was produced. The Total Score, Match Score, Lipo Score, Ambig Score, Clash Score, Rot Score, Match, Avg Volume, Max Volume, Frag No., Pdb Id, D_SCORE, PMF_SCORE, G_SCORE, CHEMSCORE, and CSCORE for each conformation were all exported.

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Supporting Information Available: Elemental analysis data, analytical HPLC purity data, and additional biochemical data. This material is available free of charge via the Internet at http://pubs.acs.org..

References

(1) World Health Organization. Disease Action Now Booklet. http://www.who/diabetes/pubs/pdf/ndfs.pdf (accessed Feb 6, 2010).
(2) Yang, W.; Lu, J.; Weng, J.; Jia, W.; Ji, L.; Xiao, J.; Shan, Z.; Liu, J.; Tian, H.; Ji, Q.; Zhu, D.; Ge, J.; Lin, L.; Chen, L.; Guo, X..; Zhao, Z.; Li, Q.; Zhou, Z.; Shan, G.; He, J. Prevalence of diabetes among men and women in China. *N. Engl. J. Med.* **2010**, *362*, 1090–1101.
(3) Nathan, D. M. Long-term complications of diabetes mellitus. *N. Engl. J. Med.* **1988**, *318*, 1315–1321.
(4) Alexiou, P.; Pegklidou, K.; Chatzopoulou, M.; Nicolaou, I.; Demopoulos, V. J. Aldose reductase enzyme and its implication to major health problems of the 21st century. *Curr. Med. Chem.* **2009**, *16*, 734–752.
(5) Hers, H. G. The mechanism of the transformation of glucose in fructose in the seminal vesicles. *Biochim. Biophys. Acta* **1956**, *22*, 202–203.
(6) van Heyningen, R. Formation of polyols by the lens of the rat with "sugar" cataract. *Nature* (*London*) **1959**, *184*, 194–195.
(7) Gabbay, K. H.; Merola, L. O.; Field, R. A. Sorbitol pathway: presence in nerve and cord with substrate accumulation in diabetes. *Science* **1966**, *151*, 209–210.
(8) Kinoshita, J. H.; Nishimura, C. The involvement of aldose reductase in diabetic complications. *Diabetes Metab. Rev.* **1988**, *4*, 323–337.
(9) Yabe-Nishimura, C. Aldose reductase in glucose toxicity: a potential target for the prevention of diabetic complications. *Pharmacol. Rev.* **1998**, *50*, 21–33.
(10) Chung, S. S.; Chung, S. K. Genetic analysis of aldose reductase in diabetic complications. *Curr. Med. Chem.* **2003**, *10*, 1375–1387.
(11) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. Aldose reductase inhibitors: a potential new class of agents for the pharmacological control of certain diabetic complications. *J. Med. Chem.* **1985**, *28*, 841–849.
(12) Hayman, S.; Kinoshita, J. H. Isolation and properties of lens aldose reductase. *J. Biol. Chem.* **1965**, *240*, 877–882.
(13) Dvornik, D.; Simard-Duquesne, N.; Keami, M.; Sestanj, K. Polyol accumulation in galactosemic and diabetic rats: control by an aldose reductase inhaibitor. *Science* **1973**, *182*, 1146–1147.
(14) Sestanj, K.; Bellini, F.; Fung, S.; Abraham, N.; Treasurywala, A.; Humber, L.; Simard-Duquesne, N.; Dvornik, D. *N*-[5-(Trifluoromethyl)-6-methoxy-1-naphthalenyl]thioxomethyl]-*N*-methylglycine (Tolrestat), a potent, orally active aldose reductase inhibitor. *J. Med. Chem.* **1984**, *27*, 255–256.
(15) Kikkawa, R.; Hatanaka, I.; Yasuda, H.; Kobayashi, N.; Shigeta, Y.; Terashima, H.; Morimura, T.; Tsuboshima, M. Effect of a new aldose reductase inhibitor, (*E*)-3-carboxymethyl-5-[(2*E*)-methyl-3-phenylpropenylidene]rhodanine (ONO-2235) on peripheral nerve disorders in streptozotocin-diabetic rats. *Diabetologia* **1983**, *24*, 290–292.

(16) Mylari, B. L.; Larson, E. R.; Beyer, T. A.; Zembrowski, W. J.; Aldinger, C. E.; Dee, M. F.; Siegel, T. W.; Singleton, D. H. Novel, potent aldose reductase inhibitors: 3,4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetic acid (zopolrestat) and congeners. *J. Med. Chem.* **1991**, *34*, 108–122.
(17) Ao, S.; Shingu, Y.; Kikuchi, C.; Takano, Y.; Nomura, K.; Fujiwara, T.; Ohkubo, Y.; Notsu, Y.; Yamaguchi, I. Characterization of a novel aldose reductase inhibitor, FR74366, and its effects on diabetic cataract and neuropathy in the rat. *Metabolism* **1991**, *40*, 77–87.
(18) Stribling, D.; Mirrlees, D. J.; Harrison, H. E.; Earl, D. C. Properties of ICI 128,436, a novel aldose reductase inhibitor, and its effects on diabetic complications in the rat. *Metabolism* **1985**, *34*, 336–344.
(19) Van Zandt, M. C.; Jones, M. L.; Gunn, D. E.; Geraci, L. S.; Jones, J. H.; Sawicki, D. R.; Sredy, J.; Jacot, J. L.; DiCioccio, A. T.; Petrova, T.; Mitschler, A.; Podjarny, A. D. Discovery of 3-[(4,5,7-trifluorobenzothiazol-2-yl)methyl]indole-*N*-acetic acid (lidorestat) and congeners as highly potent and selective inhibitors of aldose reductase for treatment of chronic diabetic complications. *J. Med. Chem.* **2005**, *48*, 3141–3152.
(20) Da Settimo, F.; Primofiore, G.; La Motta, C.; Sartini, S.; Taliani, S.; Simorini, F.; Marini, A. M.; Lavecchia, A.; Novellino, E.; Boldrini, E. Naphtho[1,2-*d*]isothiazole acetic acid derivatives as a novel class of selective aldose reductase inhibitors. *J. Med. Chem.* **2005**, *48*, 6897–6907.
(21) La Motta, C.; Sartini, S.; Salerno, S.; Simorini, F.; Taliani, S.; Marini, A. M.; Settimo, F. D.; Marinelli, L.; Limongelli, V.; Novellino, E. Acetic acid aldose reductase inhibitors bearing a five-membered heterocyclic core with potent topical activity in a visual impairment rat model. *J. Med. Chem.* **2008**, *57*, 3182–3193.
(22) Hamada, Y.; Nakamura, J. Clinical potential of aldose reductase inhibitors in diabetic neuropathy. *Treat. Endocrinol.* **2004**, *3*, 245–255.
(23) Costantino, L.; Rastelli, G.; Vianello, P.; Cignarella, G.; Barlocco, D. Diabetes complications and their potential prevention: aldose reductase inhibition and other approaches. *Med. Res. Rev.* **1999**, *19*, 3–23.
(24) Fukushi, S.; Merola, L. O.; Kinoshita, J. H. Altering the course of cataracts in diabetic rats. *Invest. Ophthalmol. Visual Sci.* **1980**, *19*, 313–315.
(25) Asano, T.; Saito, Y.; Kawakami, M.; Yamada, N. Fidarestat (SNK-860), a potent aldose reductase inhibitor, normalizes the elevated sorbitol accumulation in erythrocytes of diabetic patients. *J. Diabetes Complications* **2002**, *16*, 133–138.
(26) Negoro, T.; Murata, M.; Ueda, S.; Fujitani, B.; Kuromiya, M.; Suzuki, K.; Matsumoto, J. Novel, highly potent aldose reductase inhibitors: *R*-(−)-2-(4-bromo-2-fluorobenzyl)-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine-4-spiro-31-pyrrolidine-1,21,3,51-tetrone (AS-3201) and its congeners. *J. Med. Chem.* **1998**, *41*, 4118–4129.
(27) Kurono, M.; Fujii, A.; Murata, M.; Fujitani, B.; Negoro, T. Stereospecific recognition of a spirosuccinimide type aldose reductase inhibitor (AS-3201) by plasma proteins: a significant role of specific binding by serum albumin in the improved potency and stability. *Biochem. Pharmacol.* **2006**, *71*, 338–353.
(28) Giannoukakis, N. Drug evaluation: ranirestat, an aldose reductase inhibitor for the potential treatment of diabetic complications. *Curr. Opin. Invest. Drugs.* **2006**, *7*, 916–923.
(29) Mylari, B. L.; Armento, S. J.; Beebe, D. A.; Conn, E. L.; Coutcher, J. B.; Dina, M. S.; O'Gorman, M. T.; Linhares, M. C.; Martin, W. H.; Oates, P. J.; Tess, D. A.; Withbroe, G. J.; Zembrowski, W. J. A novel series of non-carboxylic acid, non-hydantoin inhibitors of aldose reductase with potent oral activity in diabetic rat models: 6-(5-chloro-3-methylbenzofuran-2-sulfonyl)-2*H*-pyridazin-3-one and congeners. *J. Med. Chem.* **2005**, *48*, 6326–6339.
(30) La Motta, C.; Sartini, S.; Mugnaini, L.; Simorini, F.; Taliani, S.; Salerno, S.; Marini, A. M.; Settimo, F. D.; Lavecchia, A.; Novellino, E.; Cantore, M.; Failli, P.; Ciuffi, M. Pyrido[1,2-*a*]pyrimidin-4-one derivatives as a novel class of selective aldose reductase inhibitors exhibiting antioxidant activity. *J. Med. Chem.* **2007**, *50*, 4917–4927.
(31) El-Kabbani, O.; Wilson, D. K.; Petrash, M.; Quiocho, F. A. Structural features of the aldose reductase and aldehyde reductase inhibitor-binding sites. *Mol. Vision* **1998**, *4*, 19–25.
(32) Bohren, K. M.; Grimshaw, C. E.; Gabbay, K. H. Catalytic effectiveness of human aldose reductase. Critical role of C terminal domain. *J. Biol. Chem.* **1992**, *267*, 20965–20970.
(33) Urzhumtsev, A.; Tete-Favier, F.; Mitscler, A.; Barbanton, J.; Barth, P.; Urzhumgtseva, L.; Biellmann, J. F.; Podjarny, A. D.; Moras, D. A "specificity" pocket inferred from the crystal structures of the complexes of aldose reductase with the pharmaceutically important inhibitors tolrestat and sorbinil. *Structure* **1997**, *5*, 601–612.
(34) Barski, O. A.; Gabbay, K. H.; Bohren, K. M. The C-terminal loop of aldehyde reductase determines the substrate and inhibitor specificity. *Biochemistry* **1996**, *35*, 14276–14280.
(35) Carper, D. A.; Wistow, G.; Nishimura, C.; Graham, C.; Watanabe, K.; Fujii, Y.; Hayashi, H.; Hayaishi, O. A superfamily of NADPH-dependent reductases in eukaryotes and prokaryotes. *Exp. Eye Res.* **1989**, *49*, 377–388.
(36) Feather, M. S.; Flynn, T. G.; Munro, K. A.; Kubiseski, T. J.; Walton, D. J. Catalysis of reduction of carbohydrate 2-oxoaldehydes (osones) by mammalian aldose reductase and aldehyde reductase. *Biochim. Biophys. Acta* **1995**, *1244*, 10–16.
(37) Ratliff, D. M.; Van der Jagt, D. J.; Eaton, R. P.; Van der Jagt, D. L. Increased levels of methylglyoxal-metabolizing enzymes in mononuclear and polymorphonuclear cells from insulindependent diabetic patients with diabetic complications: aldose reductase, glyoxalase I, and glyoxalase II-a clinical research center study. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 488–492.
(38) Timmemans, P. B. M.; Smith, R. D. In *Burger's Medicinal Chemistry and Drug Discovery*, 5th Ed.; Wolf, M. E., Ed.; John Wiley & Sons: New York, 1996; Vol. 2, pp 265−321.
(39) Longman, S. D.; Hamilton, T. C. Potassium channel activator drugs: mechanism of action, pharmacological properties, and therapeutic potential. *Med. Res. Rev.* **1992**, *12*, 73–148.
(40) Buckheit, R. W., Jr.; Fliakas-Boltz, V.; Decker, W. D.; Roberson, J. L.; Pyle, C. A.; White, E. L.; Bowdon, B. J.; McMahon, J. B.; Boyd, M. R.; Bader, J. P.; Nickell, D. G.; Barth, H.; Antonucci, T. K. Biological and biochemical anti-HIV activity of the benzothiadiazine class of nonnucleoside reverse transcriptase inhibitors. *Antiviral Res.* **1994**, *25*, 43–56.
(41) Yamada, K. A.; Tang, C. M. Benzothiadiazides inhibit rapid glutamate receptor desensitization and enhance glutamatergic synaptic currents. *J. Neurosci.* **1993**, *13*, 3904–3915.
(42) Lestage, P.; Danober, L.; Lockhart, B.; Roger, A.; Lebrun, C.; Robin, J.-L.; Desos, P.; Cordi, A. S18986, positive allosteric modulator of AMPA receptors as a novel cognition enhancer in rodents. *Res. Pract. Alzheimer's Dis.* **2002**, *6*, 253–259.
(43) Phillips, D.; Sonnenberg, J.; Arai, A. C.; Vaswani, R.; Krutzik, P. O.; Kleisli, T.; Kessler, M.; Granger, R.; Lynch, G.; Chamberlin, A. R. 5′-Alkyl-benzothiadiazides: a new subgroup of AMPA receptor modulators with improved affinity. *Bioorg. Med. Chem.* **2002**, *10*, 1229–1248.
(44) Braghiroli, D.; Puia, G.; Cannazza, G.; Tait, A.; Parenti, C.; Losi, G.; Baraldi, M. Synthesis of 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide derivatives as potential allosteric modulators of AMPA/kainate receptors. *J. Med. Chem.* **2002**, *45*, 2355–2357.
(45) Pirotte, B.; Podona, T.; Diouf, O.; de Tullio, P.; Lebrun, P.; Dupont, L.; Somers, F.; Delarge, J.; Morain, P.; Lestage, P.; Lepagnol, J.; Spedding, M. 4*H*-1,2,4-Pyridothiadiazine 1,1-dioxides and 2,3-dihydro-4*H*-1,2,4-pyridothiadiazine 1,1-dioxides chemically related to diazoxide and cyclothiazide as powerful positive allosteric modulators of (*R*/*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid receptors: design, synthesis, pharmacology, and structure−activity relationships. *J. Med. Chem.* **1998**, *41*, 2946–2959.
(46) Francotte, P.; de Tullio, P.; Goffin, E.; Dintilhac, G.; Graindorge, E.; Fraikin, P.; Lestage, P.; Danober, L.; Thomas, J.-Y.; Caignard, D.-H.; Pirotte, B. Design, synthesis, and pharmacology of novel 7-substituted3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides as positive allosteric modulators of AMPA receptors. *J. Med. Chem.* **2007**, *50*, 3153–3157.
(47) Pyridazinone Compounds. World Patent WO2006066079, June 22, 2006.
(48) Suzen, S.; Buyukbingol, E. Recent studies of aldose reductase enzyme inhibition for diabetic complications. *Curr. Med. Chem.* **2003**, *10*, 1329–1352.
(49) Wrobel, J.; Millen, J.; Sredy, J.; Dietrich, A.; Gorham, B. J.; Malamas, M.; Kelly, J. M.; Bauman, J. G.; Harrison, M. C.; Jones, L. R.; Guinosso, C.; Sestanj, K. Syntheses of tolrestat analogs containing additional substituents in the ring and their evaluation as aldose reductase inhibitors. Identification of potent, orally active 2- fluoro derivatives. *J. Med. Chem.* **1991**, *34*, 2504–2520.
(50) Sotriffer, C. A.; Krämer, O.; Klebe, G. Probing flexibility and "induced-fit" phenomena in aldose reductase by comparative crystal structure analysis and molecular dynamics simulations. *Proteins* **2004**, *56*, 52–66.
(51) Urzhumtsev, A.; Tete-Favier, F.; Mitschler, A.; Barbanton, J.; Barth, P.; Urzhumtseva, L.; Biellmann, J. F.; Podjarny, A.; Moras,

D. A "specificity" pocket inferred from the crystal structures of the complexes of aldose reductase with the pharmaceutically important inhibitors tolrestat and sorbinil. *Structure* **1997**, *5*, 601–612.
(52) Steuber, H.; Zentgraf, M.; Gerlach, C.; Sotriffer, C. A.; Heine, A.; Klebe, G. Expect the unexpected or caveat for drug designers: multiple structure determinations using aldose reductase crystals treated under varying soaking and co-crystallisation conditions. *J. Mol. Biol.* **2006**, *363*, 174–187.
(53) Howard, E. I.; Sanishvili, R.; Cachau, R. E.; Mitschler, A.; Chevrier, B.; Barth, P.; Lamour, V.; Van Zandt, M.; Sibley, E.; Bon, C.; Moras, D.; Schneider, T. R.; Joachimiak, A.; Podjarny, A. Ultrahigh resolution drug design i: details of interactions in human aldose reductase-inhibitor complex at 0.66 Å. *Proteins* **2004**, *55*, 792–804.
(54) Bergmeyer, H. V.; Gruber, W.; Gutman, I. In *Methods of Engymatic Analysis*; Bergmeyer, H. V., Ed.; Academic Press: New York, 1974; Vol. 3, pp 1323−1330.
(55) Murata, M.; Fujitani, B.; Mizuta, H. Synthesis and aldose reductase inhibitory activity of a new series of 5-[[2-(-carboxyalkoxy)aryl]methylene]-4-oxo-2-thioxothiazolidine derivatives. *Eur. J. Med. Chem.* **1999**, *34*, 1061–1070.