Communication

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Design and development of novel thiazole-sulfonamide derivatives as a protective agent against diabetic cataract in Wistar rats via inhibition of aldose reductase

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Abstract: In recent years, ALR2 (aldose reductase) inhibitors have attracted attention for their effective ability to reduce the progression of diabetes-associated cataracts. Therefore, in the present article, we intended to develop novel thiazole-sulfonamide hybrids as a potent inhibitor of ALR2. These molecules significantly inhibited the ALR2 level in the rat lenses homogenate, where the most potent compound **7b** showed activity comparable to sorbinil as standard. In Wistar rats, compound **7b** improved the insulin level and body weight of the experimental animal together with a reduction in the glucose output. Compound **7b** showed a significant reduction in the expression of ALR2 in rat lenses in western blot analysis.

Keywords: ALR2, compound synthesis, hybrid, diabetes, cataract

1 Introduction

Diabetes mellitus (DM) is a lifelong disease that currently affects 285 million people across the world. According to the International Diabetes Federation (IDF), by 2030, DM will affect approximately 439 million people across the world [1,2]. In diabetic patients, cataract is the major cause of visual impairment and poses a serious threat to clinical management of DM patients. Various clinical and epidemiological studies suggest that the risk for cataracts has been increasing proportionately with the progress of DM [3,4]. Cataract surgery is the most frequently used clinical option. However, this procedure has been associated with a higher incidence of surgery-related complications due to the presence of DM [5]. Thus, it is worthwhile to discover an alternative to surgery that can effectively control or minimize cataracts.

ALR2 (aldose reductase) inhibitors have recently gained attention for their effective ability to reduce the progression of diabetes-associated cataracts. ALR2 inhibitors target the ALR2 enzyme (ALR2; EC 1.1.1.21), which plays a vital role in the polyol pathway and is responsible for catalyzing the transformation of glucose into sorbitol [6]. It also promotes the transformation of NADPH to $NADP^+$. Upon activation, the polyol pathway increases the concentration of glucose via ALR2, which is later converted to sorbitol and deposited in lens tissues. This deposition alters the osmotic balance and induces the formation of cataracts due to increased sodium concentration and reduced potassium and glutathione levels [7–9]. Consequently, ALR2 inhibitors stop the activation of the polyol pathway and significantly reduce the burden of diabetic cataracts. Numerous ALR2 inhibitors are in different stages of drug development, and Epalrestat (ONO-2235) is the only ARI currently available in the market [10,11].

Thiazole is a well-known heterocyclic molecule endowed with diverse pharmacological properties, such as antibacterial [12], antimalarial [13], anticancer [14,15], antifungal, etc. Many of the clinically relevant drugs that contain thiazole as a core scaffold are, e.g., antimicrobial agents (acinitrazole and sulfathiazole), antibiotic (penicillin), antidepressant (pramipexole), antineoplastic agents (Bleomycin and Tiazofurin), anti-HIV drug (Ritonavir), anti-asthmatic drug (cinalukast) and antiulcer agent (Nizatidine) [16,17]. On the other hand, sulfonamides are another important pharmacophore responsible for diverse biological activities and are found as a core fragment in many clinically used drugs, such as sulfonamide antibiotics

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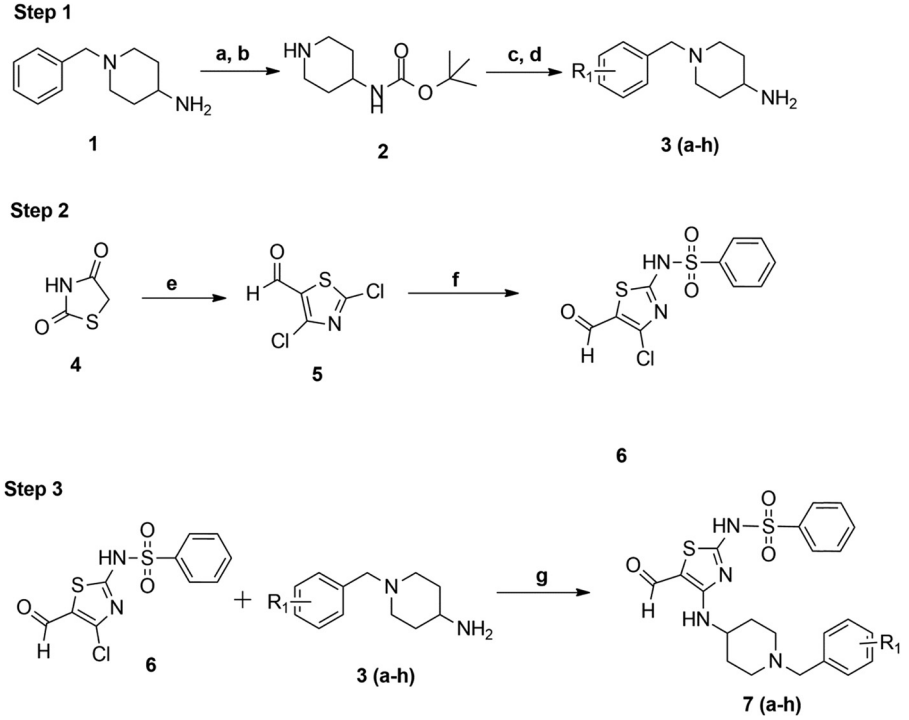
(sulfamethoxazole-trimethoprim and erythromycin-sulfisoxazole), antidiabetic (glyburide), anti-migraine (sumatriptan), anti-inflammatory (Celecoxib) and diuretics (hydrochlorothiazide and furosemide) [18–20]. Both thiazole and sulfonamide compounds have shown selective and potent ALR2 inhibitory properties. Thus, in the present article, we wish to combine these two pharmacophores into a single skeleton in a search for a novel class of ALR2 inhibitors.

2 Results and discussion

The synthesis of target molecules has been achieved in three steps as shown in Scheme 1. Step 1 corresponds to the synthesis of 4-amino-1-benzylpiperidine **3(a–h)**. The synthesis was started with the BOC protection of primary amine of 4-aminopiperdine using *di*-tert-butyl dicarbonate in THF. The resulting compound was then debenzylated in methanol and subsequently alkylated with the substituted benzyl bromides/chlorides in THF. Compound **3(a–h)** was obtained after BOC deprotection using 10% HCl. Step 2 started with the synthesis of compound **5**(2,4-dichlorothiazole-5-carbaldehyde) using 2,4-thiazolidinedione and phosphoryl chloride in DMF. Compound **5** was further reacted with benzenesulfonamide to yield *N*-(2-chloro-5-formylthiazol-4-yl)benzenesulfonamide (compound **6**) in the presence of a base. The last step gave the title hybrid derivatives **7**(**a–h**), which were obtained after heating to reflux compound **6** and corresponding **3**(**a–h**).

3 Aldose reductase inhibitory (ALR2) activity

The designed compounds were then evaluated for their ALR2 inhibitory activity using rat lenses. Various studies have suggested a strong correlation between human and rat ALR2 in terms of the active site for drug action. Thus, it serves as an excellent model for *in vitro* drug testing. The assay was based on the principle of NADPH oxidation, which was then identified by the spectrophotometric method. The comparative inhibitory activities of the compounds are tabulated in Table 1. As shown in Table 1, compound **7a** with no substitution showed the lowest activity among the tested series with IC_{50} of



Scheme 1: Synthesis of various thiazole sulfonamide derivatives, where (a) Boc_2O, TEA, THF, rt, 12 h; (b) 10% Pd/C, H_2, methanol, 24 h (93%); (c) substituted benzyl bromide or chloride, THF, reflux, 24 h; (d) 10% HCl, 24 h (85–92%); (e) $POCl_3$, DMF (87%); (f) K_2CO_3, reflux (90%); and (g) $NaHCO_3$, reflux (73–88%).

Table 1: Inhibitory activities of compounds **7(a–h)** against ALR2

S. No.	Compound	Substituent	% Inhibition[a] (±SD)[b]	IC_{50} (±SD)[b] (μM)
1	**7a**	H	18.1 ± 1.4	67.4 ± 6.4
2	**7b**	4-Cl	72.3 ± 6.7	4.5 ± 1.6
3	**7c**	3,4-Cl	65.4 ± 6.0	10.2 ± 2.4
4	**7d**	4-Br	42.2 ± 4.3	32.1 ± 4.4
5	**7e**	4-F	59.2 ± 5.1	19.4 ± 3.5
6	**7f**	4-NO_2	51.5 ± 4.6	27.3 ± 4.1
7	**7g**	4-OCH_3	39.6 ± 3.7	41.4 ± 5.3
8	**7h**	4-CH_3	31.4 ± 2.8	49.6 ± 5.5
9	Sorbinil		—	3.42[c]

[a] At 100 μM; [b] $n = 3$; [c] Reported IC_{50} [21].

67.4 μM and 18.1% inhibition of rat ALR2. On the other hand, the introduction of substitution seems favorable for inhibitory activity. For instance, compound **7b** with chloro substitution showed the highest inhibitory activity among the tested molecules with IC_{50} of 4.5 μM and 72.3% inhibition of rat ALR2 at 100 μM. The introduction of another chloro atom in the phenyl ring at the *meta* position along with *para* (**7c**) showed a moderate reduction in the activity. The inhibitory activity was further reduced drastically in the case of compound **7d**. On replacing bromo with a fluoro group (**7e**), a significant increase in the inhibitory activity was reported. A subsequent reduction in the activity was reported by remaining compounds containing, *para*-nitro, *para*-methoxy, and *para*-methyl substitutions, compounds **7f**, **7g,** and **7h**, respectively. The structure–activity relationship studies suggest that the inhibitory activity was influenced by the substitution on the phenyl ring, whereas the activity was found to be reduced in the case of non-substituted derivatives. On closely inspecting the inhibitory profile of the substituted derivatives, it was observed that the compounds containing electron-withdrawing substitution were found to be more active than their electron-donating substituted counterparts. Moreover, the presence of the di halogen group on the phenyl ring showed reduced activity (**7c**) as compared to the mono-substituted derivative (**7b**). Thus, it could be inferred that the presence and the nature of substitution have a strong influence on the inhibitory activity against the rat ALR2 enzyme. It could be suggested that substituted analogs might occupy the binding site of ALR2 with higher affinity than their non-substituted counterpart. Moreover, the electron density of the respective substituent might be the reason for their diverse inhibitory profile.

4 Pharmacological activity

Prompted by the potent inhibitory activity of compound **7b**, we next aimed to investigate its protective effect against experimentally induced diabetic cataracts in Wistar rats. Initially, the effect of compound **7b** was determined on the body weight, blood glucose, and insulin; the results are presented in Figure 1. It has been found that compound **7b**-treated rats showed improvement in body weight along with an increase in the level of insulin in the blood (Figure 1a and c). Moreover, the level of blood glucose was found drastically reduced in a dose-dependent manner in the **7b**-treated group (Figure 1b).

To further verify the activity of compound **7b**, its inhibitory activity was tested on the expression of ALR2 in the lens tissue homogenate of the experimental subjects. As shown in Figure 2, compound **7b** causes a dose-dependent reduction of ALR2 expression.

5 Conclusion

In the present study, we have successfully developed a series of thiazole-sulfonamide as a novel class of potent ALR2 inhibitors for the potential benefit against diabetic cataracts.

6 Experimental

6.1 Chemistry

The chemicals used in the present study were obtained from Sigma Aldrich (USA). ^{1}H NMR spectra were recorded in DMSO-d6 on a Bruker Avance-400 NMR spectrometer with TMS as the internal reference. 13C NMR spectra were recorded on a Bruker Avance-100 NMR spectrometer in DMSO-d6 on the same spectrometers with TMS as the internal reference. The multiplicity of a signal is indicated as s – singlet, d – doublet, t – triplet, q – quartet, m – multiplet, br – broad, dd – doublet of doublets, etc. Coupling constants (J) are quoted in Hz and reported to the nearest 0.1 Hz. Infrared spectra were recorded as a neat thin film on a Perkin-Elmer Spectrum One FT-IR spectrometer using Universal ATR sampling accessories. Melting points were obtained using MEL-TEMP (model 1001D). MS spectra were recorded on an Agilent 1100 LC/MS. Elemental analysis was performed with Vario

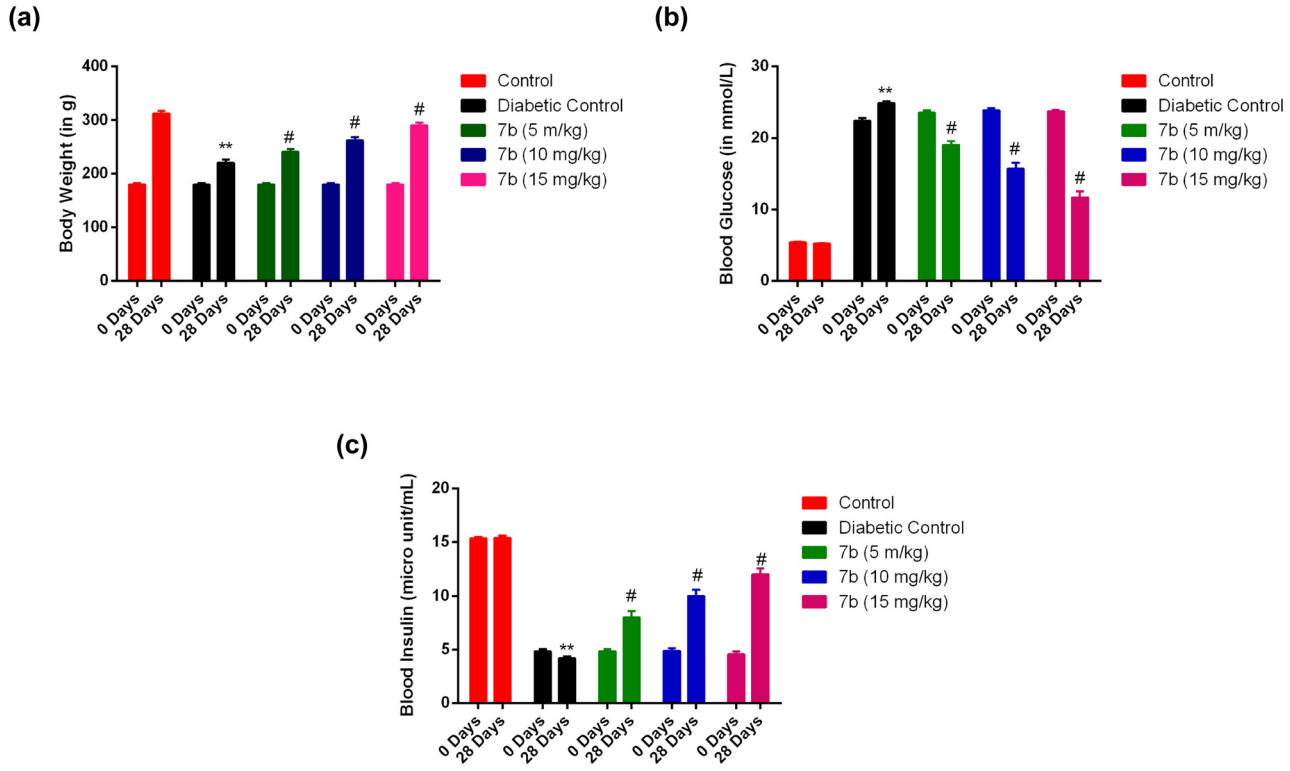


Figure 1: Effect of compound **7b** on the (a) body weight, (b) blood glucose, and (c) insulin. $^{**}P < 0.5$ (diabetic control vs normal group) and $^{\#}P < 0.05$ (treated groups vs diabetic control). Values represent the mean ± SEM and are representative of three independent experiments.

elemental analyser. Compounds **3**(**a–h**) were synthesized according to the previously reported procedure and obtained in excellent yields (85–92%). The authenticity of the samples was analyzed via melting point and elemental analysis of compounds and they were found to be within acceptable limits [22]. Compounds **5** and **6** were synthesized according to the previously published procedure obtained in yields of 87 and 90% [23]. The authenticity of compounds was established using elemental analysis, which was within acceptable limits (±0.4%).

6.2 General procedure for the synthesis of substituted (thiazol-2-yl) benzenesulfonamide derivatives 7(a–h)

A mixture of *N*-(4-chloro-5-formylthiazol-2-yl)benzenesulfonamide (**6**) (0.02 mol), substituted 1-benzylpiperidin-4-amine (**3a–h**) (0.02 mol), and sodium bicarbonate (0.02 mol) in 20 mL of 50% ethanol was refluxed for 12 h. The completion of the reaction was ascertained using ethyl acetate/*N*-hexane/methanol as the solvent system by TLC. The crude product was crystallized from ethanol to obtain target compounds **7**(**a–h**) in excellent yields.

6.2.1 *N*-(4-((1-Benzylpiperidin-4-yl)amino)-5-formylthiazol-2-yl)benzenesulfonamide (7a)

White crystalline powder; yield: 82%; m.p.: 218–219°C; MW: 456.58; R_f: 0.72; FTIR (ν_{max}; cm^{-1} KBr): 3,254 (N–H stretching, NH), 3,142 (C–H stretching, aromatic), 2,942 (CH_2 stretching, aliphatic), 1,723 (C=O stretching), 1,653 (C=N stretching), 1,641 (C=C), 1,376 (CH_2 bending), 1,149 (SO_2 stretching), 1,078 (C–N stretching), 648 (C–S stretching); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ ppm: δ 10.02 (s, 1H, CHO), 7.73 (d, 2H, J = 1.48 Hz, Ar-H), 7.64 (t, 1H, J = 1.46 Hz, Ar-H), 7.54 (d, 2H, J = 1.63 Hz, Ar-H), 7.31 (t, 1H, J = 7.74 Hz, Ar-H), 7.29 (d, 2H, J = 1.36 Hz, Ar-H), 7.28 (d, 2H, J = 1.84 Hz, Ar-H), 3.97 (s, 2H, NH × 2), 3.67 (t, 1H, J = 3.25 Hz, piperidine-H) 3.62 (s, 2H, aliphatic CH2), 2.56 (d, 4H, J = 2.93 Hz, CH_2 × 2, piperidine-H), 1.79 (d, 4H, J = 3.26 Hz, CH_2 × 2, piperidine-H); ^{13}C NMR (100 MHz, DMSO-d6) δ ppm: 188.7, 166.2, 142.2, 139.9, 138.5, 137.4, 131.8, 129.3, 128.9, 128.2, 127.3, 127.1, 64.8, 56.2, 51.9, 30.5;

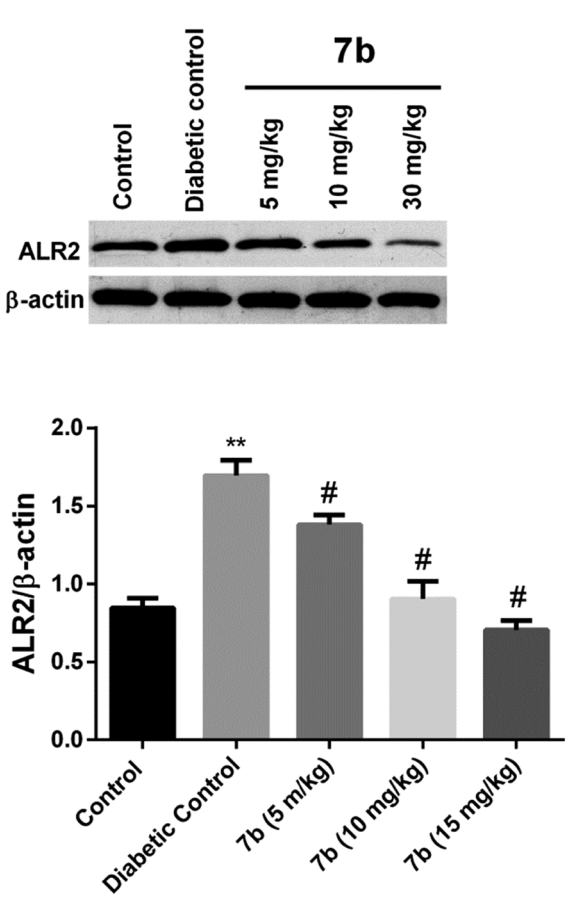


Figure 2: Effect of compound **7b** on protein expression of aldose reductase in the lens tissue homogenate. (a) Representative western blot of aldose reductase, (b) densitometry analysis of aldose reductase by western blot. $^{**}P < 0.5$ (diabetic control vs normal group) and $^{\#}P < 0.05$ (treated groups vs Diabetic control). Values represent the mean ± SEM and are representative of three independent experiments.

mass: 457.59 $(M + H)^+$; elemental analysis for $C_{22}H_{24}N_4O_3S_2$: calculated: C, 57.87; H, 5.30; N, 12.27. Found: C, 57.92; H, 5.28; N, 12.29.

6.2.2 *N*-(4-((1-(4-Chlorobenzyl)piperidin-4-yl)amino)-5-formylthiazol-2-yl)benzenesulfonamide (7b)

Off-white crystalline powder; yield: 78%; m.p.: 231–232°C; MW: 491.02; R_f: 0.79; FTIR (ν_{max}; cm^{-1} KBr): 3,258 (N–H stretching, NH), 3,141 (C–H stretching, aromatic), 2,946 (CH_2 stretching, aliphatic), 1,721 (C=O stretching), 1,657 (C=N stretching), 1,643 (C=C), 1,372 (CH_2 bending), 1,152 (SO_2 stretching), 1,074 (C–N stretching), 796 (C–Cl stretching) 645 (C–S stretching); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ ppm: δ 10.03 (s, 1H, CHO), 7.76 (d, 2H, J = 1.43 Hz, Ar-H), 7.62 (t, 1H, J = 1.46 Hz, Ar-H), 7.52 (d, 2H, J = 1.59 Hz, Ar-H), 7.44 (d, 2H, J = 1.34 Hz, Ar-H), 7.37 (d, 2H, J = 1.34 Hz, Ar-H), 3.99 (s, 2H, NH × 2), 3.65 (t, 1H, J = 3.28 Hz, piperidine-H) 3.61 (s, 2H, aliphatic CH_2), 2.58 (d, 4H, J = 2.95 Hz, CH_2 × 2, piperidine-H), 1.78 (d, 4H, J = 3.24 Hz, CH_2 × 2, piperidine-H); ^{13}C NMR (100 MHz, DMSO-d6) δ ppm: 188.9, 166.3, 142.2, 139.8, 137.4, 136.9, 132.5, 131.7, 131.3, 129.2, 128.6, 127.4, 64.8, 56.2, 51.9, 30.4; mass: 492.01 $(M + H)^+$; elemental analysis for $C_{22}H_{23}ClN_4O_3S_2$: calculated: C, 53.81; H, 4.72; N, 11.41. Found: C, 53.85; H, 4.70; N, 11.39.

6.2.3 *N*-(4-((1-(3,4-Dichlorobenzyl)piperidin-4-yl)amino)-5-formylthiazol-2-yl)benzenesulfonamide (7c)

Buff-white amorphous solid; yield: 73%; m.p.: 254–255°C; MW: 525.46; R_f: 0.84; FTIR (ν_{max}; cm^{-1} KBr): 3,263 (N–H stretching, NH), 3,145 (C–H stretching, aromatic), 2,949 (CH_2 stretching, aliphatic), 1,718 (C=O stretching), 1,653 (C=N stretching), 1,649 (C=C), 1,378 (CH_2 bending), 1,151 (SO_2 stretching), 1,079 (C–N stretching), 799 (C–Cl stretching) 648 (C–S stretching); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ ppm: δ 10.02 (s, 1H, CHO), 7.75 (d, 2H, J = 1.47 Hz, Ar–H), 7.64 (t, 1H, J = 1.42 Hz, Ar-H), 7.54 (d, 2H, J = 1.57 Hz, Ar-H), 7.48 (d, 1H, J = 1.31 Hz, Ar-H), 7.45 (d, 1H, J = 1.32 Hz, Ar-H), 7.23 (d, 1H, J = 1.30 Hz, Ar-H), 3.95 (s, 2H, NH × 2), 3.67 (t, 1H, J = 3.25 Hz, piperidine-H) 3.62 (s, 2H, aliphatic CH_2), 2.56 (d, 4H, J = 2.93 Hz, CH_2 × 2, piperidine-H), 1.76 (d, 4H, J = 3.21 Hz, CH_2 × 2, piperidine-H); ^{13}C NMR (100 MHz, DMSO-d6) δ ppm: 188.4, 166.2, 142.1, 139.9, 137.4, 135.1, 132.2, 131.9, 130.1, 129.9, 129.2, 129.0, 128.9, 127.4, 64.2, 56.2, 51.9, 30.4; mass: 526.48 $(M + H)^+$; elemental analysis for $C_{22}H_{22}Cl_2N_4O_3S_2$: calculated: C, 50.29; H, 4.22; N, 10.66. Found: C, 50.31; H, 4.21; N, 10.65.

6.2.4 *N*-(4-((1-(4-Bromobenzyl)piperidin-4-yl)amino)-5-formylthiazol-2-yl)benzenesulfonamide (7d)

Yellowish crystalline powder; yield: 79%; m.p.: 245–247°C; MW: 535.48; R_f: 0.76; FTIR (ν_{max}; cm^{-1} KBr): 3,269 (N–H stretching, NH), 3,147 (C–H stretching, aromatic), 2,942 (CH_2 stretching, aliphatic), 1,724 (C=O stretching), 1,657 (C=N stretching), 1,646 (C=C), 1,498 (C–Br stretching), 1,372 (CH_2 bending), 1,153 (SO_2 stretching), 1,074 (C–N stretching), 647 (C–S stretching); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ ppm: δ 10.04 (s, 1H, CHO), 7.76 (d, 2H, J = 1.51 Hz, Ar–H), 7.62 (t, 1H, J = 1.45 Hz, Ar-H), 7.55 (d, 2H, J = 1.52 Hz, Ar-H), 7.45 (d, 2H, J = 1.38 Hz, Ar-H), 7.31 (d, 1H, J = 1.18 Hz, Ar-H), 3.98 (s, 2H, NH × 2), 3.66 (t, 1H, J =

3.23 Hz, piperidine-H) 3.61 (s, 2H, aliphatic CH_2), 2.58 (d, 4H, J = 2.91 Hz, CH_2 × 2, piperidine-H), 1.79 (d, 4H, J = 3.27 Hz, CH_2 × 2, piperidine-H); ^{13}C NMR (100 MHz, DMSO-d6) δ ppm: 188.9, 166.2, 142.3, 139.9, 137.8, 137.5, 131.8, 131.2, 131.0, 129.0, 127.4, 121.8, 64.3, 56.2, 51.8, 30.4; mass: 536.48 $(M + H)^+$; elemental analysis for $C_{22}H_{23}BrN_4O_3S_2$: calculated: C, 49.35; H, 4.33; N, 10.46. Found: C, 49.37; H, 4.35; N, 10.43.

6.2.5 *N*-(4-((1-(4-Fluorobenzyl)piperidin-4-yl)amino)-5-formylthiazol-2-yl)benzenesulfonamide (7e)

White crystalline powder; yield: 87%; m.p.: 229–230°C; MW: 474.57; R_f: 0.87; FTIR (ν_{max}; cm^{-1} KBr): 3,273 (N–H stretching, NH), 3,142 (C–H stretching, aromatic), 2,948 (CH_2 stretching, aliphatic), 1,721 (C═O stretching), 1,655 (C═N stretching), 1,648 (C═C), 1,379 (CH_2 bending), 1,156 (C–F stretching), 1,149 (SO_2 stretching), 1,076 (C–N stretching), 648 (C–S stretching); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ ppm: δ 10.03 (s, 1H, CHO), 7.75 (d, 2H, J = 1.58 Hz, Ar-H), 7.64 (t, 1H, J = 1.49 Hz, Ar-H), 7.56 (d, 2H, J = 1.54 Hz, Ar-H), 7.27 (d, 2H, J = 1.06 Hz, Ar–H), 6.94 (d, 2H, J = 1.24 Hz, Ar-H), 3.99 (s, 2H, NH × 2), 3.67 (t, 1H, J = 3.26 Hz, piperidine-H) 3.62 (s, 2H, aliphatic CH_2), 2.56 (d, 4H, J = 2.93 Hz, CH_2 × 2, piperidine-H), 1.77 (d, 4H, J = 3.23 Hz, CH_2 × 2, piperidine-H); ^{13}C NMR (100 MHz, DMSO-d6) δ ppm: 188.5, 166.2, 161.7, 142.3, 139.9, 137.5, 134.1, 131.8, 130.5, 129.1, 127.4, 115.4, 64.9, 56.2, 51.8, 30.5; mass: 475.58 $(M + H)^+$; elemental analysis for $C_{22}H_{23}FN_4O_3S_2$: calculated: C, 55.68; H, 4.89; N, 11.81. Found: C, 55.71; H, 4.90; N, 11.79.

6.2.6 *N*-(5-Formyl-4-((1-(4-nitrobenzyl)piperidin-4-yl)amino)thiazol-2-yl)benzenesulfonamide (7f)

White crystalline powder; yield: 80%; m.p.: 257–258°C; MW: 501.58; R_f: 0.82; FTIR (ν_{max}; cm^{-1} KBr): 3,275 (N–H stretching, NH), 3,148 (C–H stretching, aromatic), 2,941 (CH_2 stretching, aliphatic), 1,724 (C═O stretching), 1,657 (C═N stretching), 1,643 (C═C), 1,534 (NO_2 stretching), 1,382 (CH_2 bending), 1,152 (SO_2 stretching), 1,075 (C–N stretching), 646 (C–S stretching); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ ppm: δ 10.05 (s, 1H, CHO), 7.72 (d, 2H, J = 1.55 Hz, Ar-H), 7.62 (t, 1H, J = 1.42 Hz, Ar-H), 7.54 (d, 2H, J = 1.59 Hz, Ar-H), 7.67 (d, 2H, J = 1.68 Hz, Ar-H), 8.04 (d, 2H, J = 1.92 Hz, Ar-H), 3.95 (s, 2H, NH × 2), 3.64 (t, 1H, J = 3.23 Hz, piperidine-H) 3.66 (s, 2H, aliphatic CH_2), 2.59 (d, 4H, J = 2.91 Hz, CH_2 × 2, piperidine-H), 1.76 (d, 4H, J = 3.27 Hz, CH_2 × 2, piperidine-H); ^{13}C NMR (100 MHz, DMSO-d6) δ ppm: 188.4, 166.3, 146.5, 144.8, 142.1, 139.7, 137.2, 131.8, 129.9, 129.1, 127.5, 123.8, 64.8, 56.3, 51.8, 30.5; mass: 502.59 $(M + H)^+$; elemental analysis for $C_{22}H_{23}N_5O_5S_2$: calculated: C, 52.68; H, 4.62; N, 13.96. Found: C, 52.73; H, 4.61; N, 13.98.

6.2.7 *N*-(5-Formyl-4-((1-(4-methoxybenzyl)piperidin-4-yl)amino)thiazol-2-yl)benzenesulfonamide (7g)

White crystalline powder; yield: 86%; m.p.: 234–235°C; MW: 486.61; R_f: 0.74; FTIR (ν_{max}; cm^{-1} KBr): 3,279 (N–H stretching, NH), 3,142 (C–H stretching, aromatic), 2,946 (CH_2 stretching, aliphatic), 2,821 (OCH_3 stretching), 1,719 (C═O stretching), 1,659 (C═N stretching), 1,641 (C═C), 1,387 (CH_2 bending), 1,151 (SO_2 stretching), 1,079 (C–N stretching), 648 (C–S stretching); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ ppm: δ 10.02 (s, 1H, CHO), 7.75 (d, 2H, J = 1.57 Hz, Ar-H), 7.64 (t, 1H, J = 1.49 Hz, Ar-H), 7.52 (d, 2H, J = 1.52 Hz, Ar-H), 7.21 (d, 2H, J = 1.12 Hz, Ar-H), 6.82 (d, 2H, J = 1.78 Hz, Ar-H), 3.97 (s, 2H, NH × 2), 3.73 (s, 3H, OCH_3), 3.67 (t, 1H, J = 3.21 Hz, piperidine-H) 3.64 (s, 2H, aliphatic CH_2), 2.57 (d, 4H, J = 2.65 Hz, CH_2 × 2, piperidine-H), 1.79 (d, 4H, J = 3.21 Hz, CH_2 × 2, piperidine-H); ^{13}C NMR (100 MHz, DMSO-d6) δ ppm: 188.3, 166.2, 159.3, 142.4, 139.5, 137.4, 132.5, 131.8, 130.7, 129.1, 127.6, 114.6, 64.9, 56.3, 55.9, 51.8, 30.6; mass: 487.62 $(M + H)^+$; elemental analysis for $C_{23}H_{26}N_4O_4S_2$: calculated: C, 56.77; H, 5.39; N, 11.51. Found: C, 56.79; H, 5.38; N, 11.50.

6.2.8 *N*-(5-Formyl-4-((1-(4-methylbenzyl)piperidin-4-yl)amino)thiazol-2-yl)benzenesulfonamide (7h)

White crystalline powder; yield: 88%; m.p.: 261–262°C; MW: 470.61; R_f: 0.63; FTIR (ν_{max}; cm^{-1} KBr): 3,283 (N–H stretching, NH), 3,149 (C–H stretching, aromatic), 2,941 (CH_2 stretching, aliphatic), 2,863 (CH_3 stretching), 1,716 (C═O stretching), 1,654 (C═N stretching), 1,647 (C═C), 1,381 (CH_2 bending), 1,155 (SO_2 stretching), 1,075 (C–N stretching), 645 (C–S stretching); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ ppm: δ 10.04 (s, 1H, CHO), 7.78 (d, 2H, J = 1.61 Hz, Ar-H), 7.61 (t, 1H, J = 1.53 Hz, Ar-H), 7.57 (d, 2H, J = 1.46 Hz, Ar-H), 7.04 (d, 2H, J = 1.27 Hz, Ar-H), 6.98 (d, 2H, J = 1.08 Hz, Ar-H), 3.95 (s, 2H, NH × 2), 3.64 (t, 1H, J = 3.27 Hz, piperidine-H) 3.68 (s, 2H, aliphatic CH_2), 2.53 (d, 4H, J = 2.69 Hz, CH_2 × 2, piperidine-H), 2.23 (s, 3H, CH_3),1.75 (d, 4H, J = 3.18 Hz, CH_2 × 2, piperidine-H); ^{13}C NMR (100 MHz, DMSO-d6) δ ppm: 188.9, 166.5, 142.6, 139.4, 137.5, 136.8, 135.4,131.6,130.2, 129.3, 128.9, 127.7, 64.4, 56.3, 51.8, 30.5, 21.5; mass: 471.59 $(M + H)^+$; elemental analysis for $C_{23}H_{26}N_4O_3S_2$: calculated: C, 58.70; H, 5.57; N, 11.91. Found: C, 58.74; H, 5.56; N, 11.89.

7 Biological assays

7.1 *In vitro* enzyme inhibition studies

7.1.1 Extraction and purification of aldose reductase (ALR2)

The rat lenses (200–250 g) were added to the triple volume of cold water and homogenized for 20 min. The homogenate was then centrifuged at 10,000 rpm for 15 min at 4°C, and the supernatant was saturated with 70% ammonium sulfate. This was centrifuged at 10,000 rpm at 4°C for 15 min, and the supernatant was dialyzed overnight. The protein content was estimated via the Bradford method and the crude aldose reductase was stored at −80°C.

7.1.2 ALR2 enzyme inhibition assay

The assay mixture was composed of 20 µL of buffer (100 mM potassium dihydrogen phosphate, pH 6.2), 10 µL of test compound (1 mM), and 70 µL of enzyme; it was incubated for 10 min at 37°C followed by the addition of 40 µL of the substrate (D,L-glyceraldehyde 50 mM for ALR2) and 50 µL of NADPH (0.5 mM) (nicotinamide adenine dinucleotide phosphate) as a co-factor. After 30 min of incubation, the optical density was measured at 340 nm ELISA (Bio-Tek EL×800™ Instrument, Inc. USA) based spectrophotometric analysis in 96 well plates. Sorbinil was used as a positive control for ALR2.

Ethical approval: The research related to animal use has been complied with all the relevant national regulations and institutional policies for the care and use of animals and has been approved by the Animal Ethical Committee for Biomedical Research of General Hospital of the Tianjin Medical University.

7.1.3 Experimental animals

Male Wistar rats (170–200 g) were obtained from an Institutional animal house and had free access to food and water. The rats were kept in polypropylene cages with an alternate day/night cycle of 12 h per day under strict hygienic conditions.

7.1.4 Experimental diabetes induction and treatment

Experimental diabetes was induced by the intraperitoneal administration of streptozotocin (60 mg/kg of body weight, 0.1 M citrate buffer, pH 4.5), the test compound (**7b**) suspended in 2% carboxymethylcellulose (CMC), and administered to rats by oral gavage. The experimental groups were categorized as follows.

Group 1: Normal control (only vehicle).

Group 2: Diabetic control (diabetes induction with no treatment)

Treatment Groups

Group 3: Diabetes induction + compound **7b** (5 mg/kg)

Group 4: Diabetes induction + compound **7b** (10 mg/kg)

Group 5: Diabetes induction + compound **7b** (15 mg/kg)

7.1.5 Preparation of the lens tissue homogenate

At the end of the experiment, the rats were killed by cervical dislocation, and eyeballs were excised immediately for the collection of the lens. The lens after removal was washed and centrifuged in PBS (10%) at 10,000*g* for 5 min. The resulting supernatant was taken for further biochemical estimation.

7.1.5.1 Western blot analysis

The lens tissues were lysed with RIPA buffer, PMSF, and phosphorylase inhibitor cocktail (MCE, New Jersey, USA) mixed according to 100:1:1. The BCA protein quantitation kit (Boster, California, USA) was used to determine the protein concentration. Then, the loading buffer was added to the protein sample and boiled for 10 min at 100°C to denature the proteins. The protein extract (30 µg) was resolved by 12% SDS-PAGE electrophoresis and electrotransferred onto a membrane of nitrocellulose. The membrane was blocked up using TrisPBS (TPBS) with Tween 20 and 5% skimmed milk and probed with primary antibodies. Next, the secondary antibody conjugated to HRP (1:10,000; Abcam/Cell Signaling Technology) in TPBS was added and incubated at 37°C for 1 h. Bands were visualized using an enhanced chemiluminescence image analyzer, and protein levels were quantified using Image-Pro Plus software (Media Cybernetics, Inc., MD, USA).

7.1.5.2 Statistical analysis

All the data were expressed as mean ± standard error (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc multiple comparison test using statistical software

GraphPad Prism 5.0 (California, USA). The P-value < 0.05 was considered statistically significant.

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Conflict of interest: The authors state no conflicts of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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