Rhodanine-3-Acetamide Derivatives as Aldose and Aldehyde Reductase Inhibitors to Treat Diabetic Complications; Synthesis, Biological Evaluation and Molecular Docking Studies

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Research article

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Abstract

In diabetes, increased accumulation of sorbitol has been associated with diabetic complications through polyol pathway. Aldose reductase (AR) is one of the key factors involved in reduction of glucose to sorbitol, thereby its inhibition is considered to be important for the management of diabetic complications. In the present study, a series of seven 4-oxo-2-thioxo-1,3-thiazolidin-3-yl acetamide derivatives **3(a-g)** were synthesized by the reaction of 5-(4-hydroxy-3-methoxybenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl acetic acid (**2a**) and 5-(4-methoxybenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl acetic acid (**2b**) with different amines. The synthesized compounds **3(a-g)** were investigated for their *in vitro* aldehyde reductase (ALR1) and aldose reductase (ALR2) enzyme inhibitory potential. Compound **3c**, **3d**, **3e**, and **3f** showed ALR1 inhibition at lower micromolar concentration whereas all the compounds were more active than the standard inhibitor valproic acid. Most of the compounds were active against ALR2 but compound **3a** and **3f** showed higher inhibition than the standard drug sulindac. Overall the most potent compound against aldose reductase was **3f** with an inhibitory concentration of $0.12 \pm 0.01 \mu\text{M}$. *In vitro* results showed that vanillin derivatives exhibited better activity against both aldehyde reductase and aldose reductase. The molecular docking studies were carried out to investigate the binding affinities of synthesized derivatives with both ALR1 and ALR2.

Introduction

A number of long term complications such as nephropathy, retinopathy, cataract and neuropathy have been associated with chronic diabetes. Nevertheless, damage to the blood vessels may be considered as one of the major long-term complications. Ergo, the patients with diabetes have somewhat a higher risk of developing cardiovascular diseases that might result in increased mortality because of microvascular complications such as strokes and peripheral artery disease [1]. Numerous pathways are involved in the impediments of diabetes mellitus, one of them being the glucolytic pathway. During normal physiological balance, this pathway is responsible for the regulation of metabolic flux of glucose concentration [2]. Whereas, polyol pathway, which is associated with the NADPH dependent reduction of glucose into sorbitol via aldol reductase, is known to be responsible for secondary complications of diabetes [2]. In hyperglycemic conditions, the increase in glucose flux via polyol pathway was believed to cause the tissue damage. Various mechanisms for such response might be osmotic imbalance because of sorbitol accumulation [4], pyridine nucleotide redox state imbalance, advanced glycated end products increase and decrease in antioxidant cell capacity [5–8]. These cell-damaging processes may consequently cause the diabetic impediments like nephropathies, retinopathies, cataract and peripheral neuropathies. Therefore, in order to prevent the onset and to limit the progression of diabetic complications, aldol reductase (AR) has been considered as a drug target to develop AR inhibitor (ARIs). The advanced glycation toxic end products, such as methyl glyoxal and 3-deoxyglucosone, are reduced and metabolized by the enzyme aldehyde reductase (ALR1, EC 1.1.1.2) which is closely related to ALR2 (EC 1.1.1.21) [4]. Although ALR1 is preferably involved in the reduction of aromatic rather than aliphatic aldehydes [9], nonetheless, both of these homologous aldose reductases ALR1 and ALR2 are involved in the NADPH-

dependent reduction of aldehydes, xenobiotics aldehydes, ketones, trioses, triose phosphates [10]. It has been reported previously that most of the known ALR2 inhibitors (ARIs) also inhibit ALR1 demonstrating the fact that there are few common features in the active sites of both the enzymes by which they bind the inhibitor and substrate. A lot of ARIs have already been reported in the studies such as carboxylic acid derivatives and hydantoin with low IC_{50} values in micromolar and sub-micromolar ranges [11], yet the only known ALR2 inhibitor that is being marketed only in Japan for the treatment of diabetic neuropathy is epalrestat [12]. However, undesirable effects like hypersensitivity and Steven-Johnson syndrome are associated with these hydantoin type of inhibitors [13]. Recently Del-Corso A et al. reported the differential inhibition of AR using different molecules having both hydrophilic and lipophilic scaffolds present [14]. Furthermore, most of them consist of a chemical group of acetic acid on the core. However, a lower tissue penetration has been found to be the major shortcoming for some individual potent carboxylic acid ARIs [15, 16]. Therefore, it is proposed to check carboxylic acid derivatives including amides to be tested for AR inhibition. This approach can reduce the side effects associated with the use of AR inhibitors.

Rhodanine derivatives are known to possess various biological activities which include β -lactamase inhibitory potential [17], inhibitors of (JSP-1) JNK-stimulating phosphatase-1 [18], histidine decarboxylase inhibitors [19], anti-apoptotic action [20], antibacterial activity [21], fungicidal activity [22], HIV-1 integrase inhibitory activity and HIV-1 cell replication inhibition [23] and trypanocidal activity [15]. These compounds can stimulate the formation of parathyroid hormone, receptor-mediated cAMP and may be useful in the treatment of degenerative arthritis, osteoarthritis and rheumatoid arthritis both locally and systemically [16]. Previously, Rhodanine-3-acetic acid derivatives have been screened biologically against various targets including aldose reductase which resulted epalrestat being used in Japan for diabetic complications. Still there is a need to develop new AR inhibitors with better efficacy and safety profile which can overcome the complications in diabetic patients.

In view of the fact that rhodanine is essential moiety for AR inhibition, the present study was designed to synthesize new 5-benzylidene-(4-Oxo-2-thioxo-1,3-thiazolidin-3-yl) acetamide derivatives and explore their potential against aldehyde/aldose reductase enzymes. *In silico* studies were conducted to investigate the binding mode of synthesized compounds with target enzymes ALR1 and ALR2.

Results And Discussion

Chemistry

Synthesis of rhodanine-3-acetic acid (1) was accomplished in two steps by reported procedure. The resultant product was condensed with aromatic aldehydes (vanillin and 4-methoxy benzaldehyde) in the presence of few drops of glacial acetic acid to get the corresponding benzylidene derivatives 2(a-b). These benzylidene derivatives were treated with thionyl chloride and finally with respective amines in the presence of triethylamine to furnish the target carboxamide derivatives **3(a-g)**. All the synthesized acetamide derivatives were characterized by FTIR and 1H NMR data. IR data showed amide carbonyl stretchings in the range $1610-1650\text{ cm}^{-1}$. C = S stretching vibrations were observed at $1200-1300\text{ cm}^{-1}$.

In the ^1H NMR spectra of these compounds, methylene protons of acetamide group resonated at 4.42–5.47 ppm and signals at 7.71–7.84 ppm were assigned to vinylic protons of $\text{C}=\text{CH}$ confirming the formation of benzylidene derivatives. All the compounds exhibited singlet of methoxy protons above 3.4 ppm. Rest of the peaks were observed at expected position in the respective IR and NMR spectra.

ALR1 and ALR2 Enzyme Inhibition

The newly synthesized 5-benzylidene rhodanine-3-acetamide derivatives **3(a-g)** were evaluated for *in vitro* enzyme inhibitory potential on aldehyde and aldose reductase enzymes. Valproic acid for aldehyde reductase and sulindac for aldose reductase was used as reference drugs with sodium D-glucuronic acid and D, L-glyceraldehyde as substrate, respectively. $\text{IC}_{50} \pm \text{SEM}$ (μM) values were calculated for the compounds showing more than 50% inhibition against both the isozymes, (Table 1).

Table 1
Aldehyde and aldose reductase inhibition efficacy by 5-benzylidene rhodanine-3-acetamide derivatives **3(a-g)**

Codes	R1	R2	R'	ALR1	ALR2
				$\text{IC}_{50} \pm \text{SEM}$ (μM) /% inhibition	
3a	OH	OCH_3	$\text{C}_6\text{H}_5\text{NH}_2$	5.38 ± 0.07	0.25 ± 0.04
3b	OH	OCH_3	$\text{C}_4\text{H}_9\text{NO}$	6.07 ± 0.05	19.2 ± 0.08
3c	OH	OCH_3	$\text{C}_7\text{H}_7\text{NO}_3$	2.38 ± 0.02	6.38 ± 0.01
3d	OH	OCH_3	$\text{C}_7\text{H}_7\text{NO}_2$	3.76 ± 0.09	7.36 ± 0.02
3e	OCH_3	H	$\text{C}_6\text{H}_5\text{NH}_2$	2.87 ± 0.01	12.71 ± 0.09
3f	OCH_3	H	$\text{C}_4\text{H}_9\text{NO}$	2.18 ± 0.03	0.12 ± 0.01
3 g	OH	OCH_3	$\text{C}_4\text{H}_9\text{N}$	2.22 ± 0.04	2.42 ± 0.03
Valproic acid				57.4 ± 0.89	-
Sulindac				-	0.29 ± 0.08

All the synthesized compounds **3a-g** exhibited good inhibitory activity against both aldehyde reductase and aldose reductase. Especially the IC_{50} values of all derivatives are lower than the standard valproic acid for ALR1. The best inhibition was shown by compounds **3c** and **3f** with IC_{50} value of $2.38 \pm 0.02 \mu\text{M}$ and $2.18 \pm 0.03 \mu\text{M}$ respectively. Similarly, compounds **3a**, **3b** and **3e** have shown good inhibition towards aldehyde reductase. As far as ALR2 is concerned all the compounds exhibited moderate activity especially **3a** and **3f** were more active than the standard Sulindac with IC_{50} of 0.25 ± 0.04 and $0.12 \pm 0.03 \mu\text{M}$ respectively. If we look at the selectivity of our synthesized derivatives towards ALR1 and ALR2,

it was observed that compound **3a**, **3f** and **3g** are selective towards ALR2 while **3c**, **3d** and **3e** are more selective and potent inhibitors of ALR1.

Furthermore, it was observed that 4-methoxybenzylidene derivatives are less selective between two isozymes while other derivatives bearing vanillin moiety **3a-d** showed more selective behavior towards ALR1 and ALR2. If we look at the amide substitution of all compounds, it is evident that carboxylic group containing acetamides **3c** and **3d** are more active against ALR1 while the presence of morpholine and pyrrolidine moiety increases the inhibitory potential of compounds against both isozymes with lesser selectivity.

The pictorial representation of inhibition profile and the influence of various groups attached to synthetic compounds is presented in Fig. 1.

Docking analysis

For docking analysis, the protein structures were selected from protein databank. In case of aldose reductase, the crystal structure of human ALR2 was available (1US0) and downloaded. However, human aldehyde reductase crystal structure was not available and porcine aldehyde reductase structure (3FX4) was selected (as human and porcine show about 97% sequence homology). For the purpose of validation, before carrying out the docking studies, the cognate ligands of both the enzymes were extracted and docked inside the active site. After docking, root mean square deviation of co-crystallized ligands (FX4401 for 3FX4 and IDD594 for 1US0) was found less than 1.0 Å for the respective enzymes. After reproducing the cognate ligands and their binding poses inside the active pocket, the docking studies were performed. The compounds **3a**, **3f** and **3g** were docked inside aldose reductase, whereas, **3c**, **3e** and **3f** were docked in the active site of aldehyde reductase. After evaluation of all the docked poses by visual inspection, the poses were selected on the basis of interactions shown inside the active pockets of aldose reductase and aldehyde reductase. The docked compounds exhibited good affinity for the target enzymes. The results showed that all the docked inhibitors are involved in a network of hydrogen bonding interactions with the target enzymes. Compound **3a** has greater hydrogen bonding interactions against ALR2 while compound **3f** is forming more hydrogen bonds with ALR1. However, **3f** showed dual inhibition against both the isozymes, therefore, **3e** was docked inside ALR1 as selective inhibitor.

The experimental results suggested that compound **3a** showed greater inhibitory potential against aldose reductase (ALR2) as compared to ALR1, while compound **3e** was more active against ALR1 as compared to ALR2. To apprehend the molecular center of such variance in inhibitory activity, the molecular docking study for compound **3a** and **3e** were carried out against individual enzyme. Inside the binding site of ALR1, the oxygen atom of 4-oxo-2-thioxo-1,3-thiazolidine ring of compound **3e** (Fig. 1) make two hydrogen bonds with amino acid Ser215 and Ser211. Two arene-Hydrogen bonds were formed by the aromatic part of rhodanine ring with Tyr210 and Trp22. While amino acid Ile261 showed backbone donor interaction with the sulfur of rhodanine ring.

Moreover, compound **3a** (Fig. 2) showed eight hydrogen bonds and make stronger interaction with ALR2 binding pocket, two hydrogen bonds were formed by Lys77 and Asn160 with the sulfur at position two of rhodanine ring, the ring itself makes a arene-arene interaction with Tyr209, and the sulfur of thiazole ring makes hydrogen bond with Gln183. The oxygen of CH₂CO makes two more hydrogen bonds with Tyr48 and His110, the 4-oxo at rhodanine nucleus forms hydrogen bond with Cys296, which is also showing two more hydrogen binds with nitrogen and carbon adjacent to aniline, which could be the reason of high potency. The formation of strong hydrogen bond strengthens the potent inhibitor inside the active pocket and contribute towards the potent inhibition towards isozyme.

Conclusion

The 5-benzylidenerhodanione-3-acetamide derivatives were successfully synthesized and characterized in this study. The synthetic analogues were screened against aldose reductase and aldehyde reductase and compound **3f** was found dual inhibitor exhibiting an IC₅₀ values of 0.12 ± 0.01 and 2.18 ± 0.03 μ M, respectively. However, compound **3a** with an IC₅₀ value of 0.25 ± 0.04 μ M was selective inhibitor of aldose reductase and compound **3e** with an IC₅₀ value of 2.87 ± 0.01 μ M was selective inhibitor of aldehyde reductase. The *in silico* analysis with human aldose reductase (PDB ID: 1US0) and aldehyde reductase (PDB ID: 3FX4) were carried out with these compounds which further supported the results of *in vitro* study. Overall the computational studies of the synthesized compounds and *in vitro* enzyme inhibitory studies against ALR1 and ALR2 identified some potent compounds which can be used as lead molecules for further development to treat diabetic complications.

Experimental

Materials And Methods

All the reagents were purchased from Sigma Aldrich and Alfa Aesar and used without further purification. Melting points of the synthesized compounds were recorded using Gallenkamp melting point apparatus. Characterization of the synthesized compounds was done by FTIR and ¹HNMR and elemental analysis data. FTIR spectra were recorded on Thermoscientific NICOLET IS10 spectrophotometer, and ¹HNMR spectra were taken on Bruker AM300 MHz spectrophotometer, in which DMSO was used as solvent. The progress of reaction was monitored by TLC with pre-coated silica gel 60 F254 plates using ethyl acetate and petroleum ether as mobile phase.

General procedure for the synthesis of Rhodanine-3-acetic acid (1)

Glycine (2.4 g, 0.031 mol) was dissolved in 33% NH₄OH (20 mL), carbon disulfide (2.36 g, 0.031 mol) was added to the solution and stirred vigorously for 1 hour while color of the solution turned orange. Then aqueous solution of sodium chloroacetate (3.61 g, 0.031 mol) was added and refluxed for 3 hours, after completion, reaction mixture was acidified with dilute HCl to bring the pH to 1.0 and further refluxed for 1 h. Saturated NaHCO₃ solution was added to the reaction mixture to neutralize it and the resultant

solution was acidified again with dilute HCl. The solid separated was filtered and recrystallized with water to obtain rhodanine-3-acetic acid. Yield: 86.0%. M.p.: 145–148 °C [24].

General procedure for the synthesis of 5-benzylidenerhodanine-3-acetic acid 2(a-b)

Equimolar amounts of rhodanine-3-acetic acid, anhydrous sodium acetate and respective aldehyde were dissolved in glacial acetic acid (30 mL) and solution was put to reflux for 3–4 hours. After completion the reaction mixture was cooled and the solid separated was filtered, washed with water and recrystallized from ethanol [25].

Z-5-(4-hydroxy-3-methoxybenzylidene) rhodanine-3-acetic acid (2a)

Yield: 75.0%. M.p.: 144 °C [25].

(Z)-5-(4-methoxybenzylidene) rhodanine-3-acetic acid (2b)

Yield: 66.0%. M.p.: 249–250 °C

General procedure for the synthesis of 5-benzylidenerhodanine-3-acetamide derivatives 3(a-g)

The synthesized 5-benzylidenerhodanine-3-acetic acid 2(a-b) was stirred with excess of thionyl chloride in dichloromethane (20 ml) for 2 hours. After reaction completion solvent was evaporated and residue treated with equimolar amount of respective amine in the presence of triethylamine and dichloromethane as solvent. Progress of reaction was monitored by TLC, after completion product was isolated by evaporation and purified by column chromatography [26].

(Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(2-anilino-2-oxoethyl)-2-thioxothiazolidin-4-one (3a)

Yield 53.5%, Orange solid, m.p 201–202 °C, Rf 0.42 (ethyl acetate: Pet-ether 2:1), IR (KBR) cm^{-1} , 1635 (C = O), 1201 (C = S), 3440 (NH), 1020 (C-N), 3150 (OH), 1489 (-CH₃), ¹H-NMR (DMSO, 400 MHz δ ppm) 9.98 (s, 1H, OH), 7.51 (s, 1H, vinylic H) 7.15–7.46 (m, 7H, ArH), 7.01 (d, 1H, J = 8.7 Hz, ArH), 6.87 (d, 2H, J = 8.7 Hz, Ar-H) 5.52 (s, 2H, CH₂-CO) and 3.65 (s, 3H, OCH₃), ¹³CNMR (DMSO-d₆, 100 MHz, δ ppm): 149.7, 146.2, 134.3, 126.8, 116.7, 109.2, 138.4, 129.7, 129.7, 125.1, 119.9, 119.9 (Ar-C), 194.3, 167.2, 130.2 (thiazolidine-C), 162.6 (CONH), 133.7 (CH), 57.1 (OCH₃), 48.8 (CH₂), Anal. Calcd. For C₁₉H₁₆N₂O₄S₂: C, 56.93; H, 4.02; N, 6.99; S, 15.98; Found: C, 56.84; H, 3.99; N, 7.00; S, 15.99.

(Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(2-morpholino-2-oxoethyl)-2-thioxothiazolidin-4-one (3b)

Yield 61.5%, yellow solid, m.p 211–213 °C, Rf 0.41 (ethyl acetate: Pet-ether 2:1), IR (KBR) cm^{-1} , 1615 (C = O), 1300 (C = S), 1104 (C-N), 1192 (C-O), 3384 (OH), 1450 (-CH₃), ¹H-NMR (DMSO, 400 MHz δ ppm) 9.76 (s, 1H, OH), 7.80 (s, 1H, vinylic H), 7.37–7.43 (m, 1H, ArH), 7.14–7.22 (m, 1H, ArH), 7.02 (d, 1H, J = 8.4 Hz, J = 2.7 Hz ArH), 4.72 (s, 2H, CH₂-CO), 3.84 (s, 3H, OCH₃), 3.05 (t, 4H, J = 7.2 Hz Morpholine H), 1.19 (t, 4H, J = 7.2 Hz Morpholine H), ¹³CNMR (DMSO-d₆, 100 MHz, δ ppm): 150.1, 148.7, 136.8, 129.3, 117.1, 110.3 (Ar-C), 192.3, 166.1, 128.8 (thiazolidine-C), 70.1, 70.1, 45.3, 45.3 (Morpholine-C), 163.9 (CO), 132.1 (CH), 57.2 (OCH₃), 49.2 (CH₂), Anal. Calcd. For C₁₇H₁₈N₂O₅S₂: C, 51.71; H, 4.59; N, 7.09; S, 16.22; Found: C, 51.69; H, 4.61; N, 7.10; S, 16.20.

2-Hydroxy-4-({[(Z)-5-(4-hydroxy-3-methoxybenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]acetyl}amino)benzoic acid (3c)

Yield 59.2%, yellow solid, m.p 199–201 °C, Rf 0.48 (ethyl acetate: Pet-ether 2:1), IR (KBR) cm^{-1} , 1610 (C = O), 1300 (C = S), 1272 (C-N), 3130 (OH), 1370 ($-\text{CH}_3$), 1720 (COOH) $^1\text{H-NMR}$ (DMSO, 400 MHz δ ppm) 12.0 (s, 1H, COOH), 9.75 (s, 1H, OH), 9.35 (d, 1H, OH), 7.75 (s, 1H, vinylic H), 7.41 (m, 1H, ArH), 7.19 (m, 1H, ArH), 7.05 (d, 1H, J = 8.4 Hz, J = 2.7 Hz), 7.29–7.48 (m, 4H, ArH), 5.52 (s, 2H, $\text{CH}_2\text{-CO}$), 3.82 (s, 3H, OCH_3), $^{13}\text{CNMR}$ (DMSO- d_6 , 100 MHz, δ ppm): 150.2, 149.7, 136.2, 131.3, 118.2, 109.5, 167.1, 141.4, 133.7, 119.7, 101.2, 100.1 (Ar-C), 197.2, 171.6, 130.3 (thiazolidine-C), 174.6 (COOH) 166.2 (CONH), 133.1 (CH), 57.0 (OCH_3), 48.3 (CH_2), Anal. Calcd. For $\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_7\text{S}_2$: C, 52.12; H, 3.50; N, 6.08; S, 13.98; Found: C, 52.00; H, 3.55; N, 6.11; S, 14.01.

4-(((Z)-5-(4-hydroxy-3-methoxybenzylidene)-4-oxo-2-thioxo-1,3-thiazolidin-3-yl]acetyl)amino)benzoic acid (3d)

Yield 84.5%, orange solid, m.p 217–219 °C, Rf 0.45 (ethyl acetate: Pet-ether 2:1), IR (KBR) cm^{-1} , 1622 (C = O), 1299 (C = S), 1050 (C-N), 3330 (OH), 1368 ($-\text{CH}_3$), 1704 (COOH) $^1\text{H-NMR}$ (DMSO, 400 MHz δ ppm), 12.0 (s, 1H, CO-OH), 9.75 (s, 1H, OH) 7.71 (s, 1H, vinylic H), 7.41 (m, 1H, ArH), 7.19 (m, 1H, ArH), 7.05 (d, 1H, J = 8.4 Hz, J = 2.7 Hz, ArH), 7.29–7.48 (m, 5H, ArH), 4.75 (s, 2H, $\text{CH}_2\text{-CO}$), 3.83 (s, 3H, OCH_3), $^{13}\text{CNMR}$ (DMSO- d_6 , 100 MHz, δ ppm): 148.5, 147.3, 133.2, 128.6, 115.3, 108.8, 137.9, 130.4, 130.4, 123.2, 119.7, 119.7 (Ar-C), 193.3, 166.5, 129.4 (thiazolidine-C), 168.8 (COOH) 163.5 (CONH), 132.7 (CH), 56.4 (OCH_3), 49.6 (CH_2), Anal. Calcd. For $\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_6\text{S}_2$: C, 53.99; H, 3.62; N, 6.29; S, 14.39; Found: C, 54.02; H, 3.60; N, 6.31; S, 14.33.

(Z)-5-(4-methoxybenzylidene)-3-(2-anilino-2-oxoethyl)-2-thioxothiazolidin-4-one (3e)

Yield 60%, yellow solid m.p 190–192 °C Rf 0.44 (ethyl acetate: Pet-ether 2:1), IR (KBR) cm^{-1} , 1620 (C = O), 1216 (C = S), 3368 (NH), 1022 (C-N), 1375 ($-\text{CH}_3$), $^1\text{H-NMR}$ (DMSO, 400 MHz δ ppm) 7.56 (s, 1H, vinylic H) 7.29–7.48 (m, 5H, ArH), 7.10 (d, 2H, J = 8.7 Hz, ArH), 6.85 (d, 2H, J = 8.7 Hz, ArH), 5.63 (s, 2H, $\text{CH}_2\text{-CO}$) and 3.71 (s, 3H, OCH_3), $^{13}\text{CNMR}$ (DMSO- d_6 , 100 MHz, δ ppm): 160.0, 132.3, 132.3, 125.8, 114.5, 114.5, 137.6, 129.2, 129.2, 124.6, 120.9, 120.9 (Ar-C), 194.2, 167.7, 129.8 (thiazolidine-C), 164.2 (CONH), 133.3 (CH), 55.7 (OCH_3), 50.0 (CH_2), Anal. Calcd. For $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_3\text{S}_2$: C, 59.30; H, 4.19; N, 7.28; S, 16.64; Found: C, 59.28; H, 4.20; N, 7.29; S, 16.61.

(Z)-5-(4-methoxybenzylidene)-3-(2-morpholino-2-oxoethyl)-2-thioxothiazolidin-4-one (3f)

Yield 49.3%, yellow solid m.p 203–205 °C Rf 0.48 (ethyl acetate: Pet-ether 2:1), IR (KBR) cm^{-1} , 1650 (C = O), 1225 (C = S), 1101 (C-N), 1173 (C-O), 1375 ($-\text{CH}_3$), $^1\text{H-NMR}$ (DMSO, 400 MHz δ ppm) 7.85 (s, 1H, vinylic H), 7.64 (d, 2H, J = 8.7 Hz ArH), 7.13 (d, 2H, J = 8.7 Hz ArH), 4.73 (s, 2H, $\text{CH}_2\text{-CO}$), 3.84 (s, 3H, OCH_3), 3.79 (t, 4H, J = 7.5 Hz Morpholine H), 1.19 (t, 4H, J = 7.5 Hz Morpholine H), $^{13}\text{CNMR}$ (DMSO- d_6 , 100 MHz, δ ppm): 161.7, 133.3, 133.3, 127.2, 117.4, 117.4 (Ar-C), 190.8, 168.0, 128.3 (thiazolidine-C), 66.7, 66.7, 44.3 44.3 (Morpholine-C) 166.3 (CO), 134.9 (CH), 57.6 (OCH_3), 45.2 (CH_2), Anal. Calcd. For $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_4\text{S}_2$: C, 53.90; H, 4.79; N, 7.39; S, 16.91; Found: C, 53.87; H, 4.81; N, 7.37; S, 16.88.

(Z)-5-(4-hydroxy-3-methoxybenzylidene)-3-(2-pyrrolidino-2-oxoethyl)-2-thioxothiazolidin-4-one (3g)

Yield 39.2%, yellow solid, m.p 187–189 °C, Rf 0.31 (ethyl acetate: Pet-ether 2:1), IR (KBR) cm^{-1} , 1640 (C = O), 1215 (C = S), 1260 (C-N), 3201 (OH), 1451 ($-\text{CH}_3$), $^1\text{H-NMR}$ (DMSO, 400 MHz δ ppm) 9.76 (s, 1H, OH),

7.79 (s, 1H, vinylic H), 7.40 (m, 1H, ArH), 7.17 (m, 1H, ArH), 7.00 (d,d 1H, J = 8.4 Hz, J = 2.7 Hz, ArH), 4.72 (s, 2H, CH₂-CO), 3.83 (d, 3H, OCH₃), 3.04 (m, 4H, Pyrrolidine H), 1.19 (t, 4H, J = 7.5 Hz, Pyrrolidine H), ¹³CNMR (DMSO-d₆, 100 MHz, δ ppm): 159.8, 133.7, 133.7, 124.2, 115.1, 115.1 (Ar-C), 192.7, 165.3, 127.5 (thiazolidine-C), 48.5, 48.5, 23.7, 23.7 (pyrrolidine-C) 169.0 (CO), 134.7 (CH), 58.2 (OCH₃), 51.3 (CH₂), Anal. Calcd. For C₁₇H₁₈N₂O₃S₂: C, 56.28; H, 5.00; N, 7.72; S, 17.65; Found: C, 56.31; H, 4.99; N, 7.70; S, 17.67.

Enzyme inhibition studies

All required chemicals used in the enzyme extraction procedure were of high analytical grade. Enzyme inhibitory assay was performed on ELIZA microplate reader at 340 nm and 96 well-plates were used for the sample analysis. Micropipettes from Gilson were used for sample loading. Sodium-D-glucuronate and D,L-glyceraldehyde were used as substrates along with a cofactor i.e. NADPH (nicotinamide adenine dinucleotide phosphate) from Sigma Aldrich.

Extraction and purification of Aldehyde reductase (ALR1)

Aldehyde reductase enzyme was extracted from lamb kidney and the cortical part was separated carefully. The cortex was homogenized in triple volume of extraction buffer (2.0 mM EDTA, 0.25 M sucrose, 10 mM sodium phosphate and 2.5 mM β-mercaptoethanol at pH 7.2). The homogenate was centrifuged at 12,000 rpm at 4 °C for 30 min, the insoluble precipitates were discarded and the supernatant was saturated with 40%, 50% and 75% ammonium sulfate respectively and after each addition the solution was centrifuged at 12000 rpm at 4 °C for 30 min, each time the pellet was discarded and for the last saturation the supernatant was taken and dialyzed overnight in extraction buffer. Next day the protein content was calculated via Bradford method and the crude aldehyde reductase was stored at -80 °C [27].

Extraction and Purification of Aldose reductase (ALR2)

The enzyme aldose reductase was extracted from calf lenses. 200–300 g lenses were added to 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. The insoluble precipitates were discarded and the supernatant was saturated with 70% ammonium sulfate and after centrifugation at 10,000 rpm at 4 °C for 15 min the supernatant was dialyzed overnight and the protein contents was calculated via Bradford method and the crude aldose reductase was stored at -80 °C [28].

ALR1 Enzyme inhibition assay

The assay was performed on ELIZA (Bio-Tek ELx800TM Instrument, Inc. USA) based spectrophotometric analysis in 96 well plate. The assay mixture was composed of 20 μL buffer (100 mM potassium dihydrogen phosphate pH 6.2), 10 μL test compound (1 mM), 70 μL enzyme and incubated for 10 min at 37 °C followed by addition of 40 μL Glucuronate 50 mM (as a substrate) and 50 μL (0.5 mM) NADPH (nicotinamide adenine dinucleotide phosphate) as a co-factor. After 30 min incubation optical density was measured at 340 nm. Valproic acid was used as a positive control for ALR1 [29].

ALR2 Enzyme inhibition assay

The assay was performed on ELIZA (Bio-Tek ELx800™ Instrument, Inc. USA) based spectrophotometric analysis in 96 well plate. The assay mixture was composed of 20 µL buffer (100 mM potassium dihydrogen phosphate pH 6.2), 10 µL test compound (1 mM), 70 µL enzyme and incubated for 10 min at 37 °C followed by addition of 40 µL substrate (DL-glyceraldehyde 50 mM for ALR2) and 50 µL NADPH (0.5 mM) (nicotinamide adenine dinucleotide phosphate) as a co-factor. After 30 min incubation optical density was measured at 340 nm. Sulindac was used as positive control for ALR2 [30].

Results were analyzed by graph pad prism® software to calculate IC₅₀ and percentage inhibition was calculated by the following formula

$$\% \text{ Inhibition} = [100 - (\text{Absorbance test well} / \text{Absorbance control})] \times 100$$

Docking study

For docking studies, crystal structures of human aldose reductase (PDB ID: **1US0**) [30] and aldehyde reductase (PDB ID: **3FX4**) [31] were used. The proteins were prepared by minimizing energy after removal of water molecules and visualized by discovery studio [32], the inhibitors were prepared using chemsketch [33] and the energy was minimized by chemdraw 3D [34]. Molecular Operating Environment (MOE) [35] was used as a tool for docking, using MMFF94x force-field. The positioning of co-factor (NADPH) in both the enzymes was made sure for the activity of enzyme. The co-crystallized ligands were left in the receptor site for grid path. Fifty poses were generated by affinity dG force-field and placement of these poses was confirmed by London dG force-field. All the poses were visually evaluated on the base of interaction and best poses were selected as potent inhibitors of aldose reductase and aldehyde reductase inhibitors i.e. **3c**, **3e** and **3f** for aldehyde reductase and **3a**, **3e** and **3g** for aldose reductase.

Abbreviations

ALR1

aldehyde reductase; ALR2:aldose reductase; ARIs:AR inhibitors; NADPH:Nicotinamide Adenine Dinucleotide Phosphate; JNK:c-Jun N-terminal kinase; JSP-1:JNK-stimulating phosphatase-1; cAMP:Cyclic adenosine monophosphate; FTIR:Fourier Transform Infrared; ¹HNMR:Proton Nuclear Magnetic Resonance; Rf:Relative factor; mol.:Moles

Declarations

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Authors' contributions

Authors MMB and HN have synthesized and characterized the compounds. HN also designed and supervised the whole research. JI have co-supervised the research. SUR and MA have carried out computational evaluation. Biological Screening of synthesized compounds was carried out by SZ and AI. SS and MA prepared the manuscript. All authors read and approved the final manuscript.

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Availability of data and material

All the relevant data supporting the conclusions of this article is included in the article.

Competing interest:

The authors declare that they have no competing interests.

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Figures

Figure 1

3D binding interactions and docked poses of compound 3e (color green), 3f (color red) and 3c (color blue) inside the 3FX4

Figure 2

3D binding interactions and docked poses of compound 3a (color magenta), 3e (color red) and 3g (color cyan) inside the 1US0

Figure 3

Structures with potency of synthesized derivatives 3(a-g)

Supplementary Files

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