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Design, Synthesis of Rhodanine Derivatives and *In-vitro* Evaluation as Aldose Reductase Inhibitors

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Abstract: - Aldose reductase enzyme plays a significant role in conversion of excess amount of glucose into sorbitol in diabetic condition, inhibitors of which decreases the secondary complication of Diabetes Mellitus. To understand the structural interaction of inhibitors with aldose reductase (ALR) enzyme and develop more effective aldose reductase inhibitors, a series of substituted 5-phenylbenzoate containing *N*-substituted rhodanine derivatives were synthesized and evaluated for their *in vitro* ALR inhibitory activity. Docking studies of these compounds were carried out which revealed that the 5-phenylbenzoate moiety deeply influenced the key π-π stacking while 4-oxo-2-thioxothiazolidines contributed in hydrogen bond interactions. The phenyl ring of benzylidene system occupied in specific pocket constituted from Phe115, Phe122, Leu300 and Cys303 while the rhodanine ring forms a tight net of hydrogen bond with Val47 at anionic binding site of the enzyme. The structural insights obtained from the docking study gave better understanding of rhodanine and

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macromolecular interaction and will help us in further designing and improving of aldose reductase inhibitory activity of rhodanine analogs.

Keywords: Rhodanine, Aldose Reductase Inhibitors, Docking, Diabetes Mellitus.

Introduction

Diabetes mellitus (DM) is a common chronic disease in constant growth that is taking on epidemic proportions especially in developing countries. More than 220 million people worldwide suffer from DM and this figure is expected to increase to 400 million cases by 2030[1]. Diabetic complications, such as neuropathy, retinopathy, nephropathy or cataract, are serious and disabling pathologies associated with DM[2]. Hyperglycemia is a typical condition of DM and plays a crucial role in the development and advancement of these complications which arise from acute and reversible changes in cellular metabolism as well as from irreversible long-term damage in biological macromolecules.

Prospective studies have highlighted that good control of blood glucose levels delays the onset or slows the progression of diabetic complications[3]. Despite the wide availability of effective oral antidiabetic drugs, it is difficult to keep glycemia under tight control and thus the onset of long-term damage is unavoidable. Considerable efforts have been made to identify effective agents that are able to counteract the biological mechanisms responsible for the development of diabetic complications but so far very few drugs have been marketed[4]. Numerous mechanisms like non-enzymatic glycation of proteins, glucose auto-oxidation, the activation of protein kinase C (PKC) isoforms and polyol pathway triggered by the chronic exposure to high levels of glucose[5]. Moreover these biochemical pathways sustain in various tissue types and increases in hyperglycemic induced oxidative stress which is observed in both clinical and experimental DM and leads to the deleterious effects on cellular functions and signaling underlying the appearance of both macro and microangiopathy[5-8]. In hyperglycemic conditions, the auto-oxidation reactions of glucose and other α-hydroxyaldehydes produce reactive oxygen species (ROS) which can damage biomolecules, cellular membranes and tissues by promoting intermolecular cross-linking and fragmentation reactions as well as lipid oxidation[5, 6]. Moreover the highly reactive dicarbonyl compounds produced in the course of glucose auto-oxidation reactions lead to the glycation of macromolecules which culminates in the synthesis of advanced glycation end-products (AGEs)[7, 8]. The significant increase of glucose flux through the polyol pathway increases

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both cellular oxidative and osmotic stress, thereby significantly contributing to tissue injury in which the glucose up-take is insulin independent linked to DM[4,5,7].

Aldose reductase (E.C.1.1.1.21), a ubiquitous enzyme which has been identified in brain, kidney, liver, lens and skeletal muscle tissue, is an aldo-keto reductase that catalyzes the NADPH-dependent reduction of glucose into sorbitol in the first step of the polyol pathway which further dehydrogenase through an NAD^+ dependent reaction into fructose. Although not determinant, the osmotic stress generated by sorbitol accumulation has been proposed as contributing to the development of tissue damage above all in the lens[8,9]. Moreover, the increase in the NADPH/$NADP^+$ ratio promotes the activation of PKC isoforms which are found to be crucial mediators of biochemical and functional alterations triggered by hyperglycemia. Thus, the oxidative stress triggered by the glucose-oxidation process and upheld by increased aldose reductase activity is rightly considered to be a major and unifying mechanism responsible for the onset of diabetic complications[5]. Over the last three decades, numerous ALR inhibitors (ARIs) have been identified; most of them belong to either carboxylic acid (such as epalrestat; **Figure 1**) or hydantoin (such as sorbinil, fidarestat; **Figure 1**) classes of compounds[10-14]. However, many of the clinically tested ARIs proved to be inadequate as drug candidates because of adverse pharmacokinetics, toxic side effects or low efficacy. At present, epalrestat is the only ARI available on the market[10,11,15].

Literature reveals that in last few years, numerous 5-arylidene-2, 4-thiazolidinediones (**Figure 1**) derivatives produced appreciable ALR inhibition similar to that identified[16,17] but their effectiveness generally decreases *in-vivo*, probably due to their poor penetrability to key target tissues, in particular peripheral nerves[18-20]. Thus, to develop new ARIs, with pK_a values higher than those of carboxylic acids, the 2-oxo group of 2,4-thiazolidinediones was bioisosterically replaced to 4-oxo-2-thioxothiazolidines which may alter the physicochemical properties and enhanced the potential with better bioavailability for the successful development of new clinical agents[21-22].

In the present study new active analogues of 5-arylidene-4-oxo-2-thioxothiazolidines were identified, designed and synthesized as potent *in-vitro* ALR inhibitors. In particular, 5-benzylidene moieties bearing hydroxy, methoxy, nitro, chloro and/or dichloro substituted benzoates were prepared which is potentially able to enhance stability of enzyme inhibitor complex.

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Method and Material

Docking

The detailed interaction study of synthesized rhodanine analogs and aldose reductase enzyme was carried out through *Glide* version *5.5* module of *Schrodinger* suite[23]. Grid based ligand docking with energetics (*Glide*) was used for ligand docking in XP (extra precision) mode. This docking method is fast and accurate and consists of mainly two steps.

A) *Ligand preparation:* The initial structure of ligands were drawn on *ChemDraw ultra* 11.0 and converted to 3D by using *Chem3D ultra* 11.0 and then minimized by using MM2 force field and truncated newton minimizer corresponding to RMS gradient 0.100 and saved as mole file. Subsequently these mole files were imported in to the *Maestro* workspace and combined to one molecule file as sdf format. This file was used for ligands preparation with the help of *LigPrep* module of the *Schrodinger* suite keeping state generation pH condition 7±2 value and output file used for docking study.

B) *Protein preparation:* The X-ray crystal structure of the aldose reductase enzyme (2PDG) was retrieved from protein data bank[24]. All the water molecules were removed from the enzyme and subsequently protein was prepared in *Maestro 9.0* using protein preparation wizard. Co-factor (NAP) and ligand (47D) of enzyme were prepared using "Generate Het States" module of protein preparation wizard and default Het States of NAP & 47D were used for docking. Hydrogen atoms were added to the protein. The H-bonds were optimized using sample water orientations. Finally, the protein structure (hydrogen only) was minimized to the default Root Mean Square Deviation (RMSD) value of 0.30Å. Receptor grid was generated using centroid of work space ligand. The XYZ coordinates of centroid are 16.93, -6.27, & 14.46 respectively. The active site was defined as an enclosing box and bounding box around the assigned center (16.93, -6.27, & 14.46) of lengths 10 Å & 30 Å respectively. The grid was used for the next step of docking.

Docking protocol was validated by comparing the RMSD of crystal structure conformation of ligand with docked conformation of the ligand obtain through docking methodology. The RMSD value of these two conformers was found to be 1.91Å which is under acceptable limit. The minimized structures of ligand's and protein were subsequently used in docking simulation. The conformation flexibility of ligands were considered by exhaustive conformational search within glide augmented by heuristic screen that removed

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conformations which were not suitable for aldose reductase binding or had long range hydrogen bonds, whereas aldose reductase conformation remained fixed. An exhaustive systemic search of the conformational space and a series of hierarchical filters to locate the possible position of ligands in the docking simulation were done. The shape and properties were represented on grid by different fields, which provided more accurate position and orientation of ligands (termed pose) in the Aldose reductase. Ten poses were calculated per ligand molecule, which were docked to Aldose reductase. The resulting dock conformations were analyzed using *Glide* pose viewer tool. The conformation/poses that made the maximum number of interaction were considered to further analysis. *G* score method was considered for selection of the compounds.

Biological Evaluation

***In- vitro* aldose reductase inhibitory activity**

A purified goat lens extract was prepared in accordance with the method of Hayman and Kinoshita with slight modifications[25]. Lenses were quickly removed from goat eye following euthanasia and homogenized (Glass-Potter) in 5 volume of cold deionized water. The homogenate was centrifuged at 10,400 rpm at 0-4°C for 30min. Saturated ammonium sulfate solution was added to the supernatant fraction to form a 40% solution, which was stirred for 30min at 0-4ºC and subsequently centrifuged at 10,400 rpm for 20 min. Recovered supernatant was subsequently treated with saturated ammonium sulfate solution to make a 50% solution and follow the above mentioned stirring and centrifugation condition. The obtained supernatant was adjusted to 75% salt saturation by saturated ammonium sulfate solution and repeated the stirring and centrifugation procedure. The precipitate recovered from the 75% saturated fraction, possessing ALR enzyme. The precipitate was dissolved in 0.05M NaCl and dialyzed overnight in 0.05M NaCl. The dialyzed material was used for the enzymatic assay.

Chemistry

The purification of intermediates and final compounds was carried out through recrystallization and column chromatography technique. Melting points were recorded by open capillary method on melting points apparatus and are uncorrected. TLC controls were carried out on precoated silica gel plates. ^{1}H NMR spectra were recorded on Bruker Avance II 400 magnetic resonance spectrometer from Central Drug Research Institute, Lucknow.

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Chemical shifts are given in δ units (ppm) relative to internal standard TMS and refer to DMSO-D_6 solutions. The IR spectra of the intermediates and synthesized compounds were recorded on FTLA 2000 ABB spectrophotometer at SCOPE, Indore. MS spectral data were recorded from SAIF Punjab University, Chandigarh using THERMO Finnigan LCQ Advantage max ion trap MS for the entire synthesized compound. Unless stated otherwise, all materials were obtained from commercial suppliers and used without further purification.

Results

Docking Analysis

The docking results for all the compounds are summarized in Table 1. Visual inspection of the top-ranked poses for every ligand–protein combination showed that, in contrast to the cross-docking of the crystal structure ligands, the compounds often had similar poses in 2PDG protein. *Glide* score was calculated based on number of parameters such as hydrogen bonds (H bond), hydrophobic (Lipo), van der Waals (vdW), columbic (coul), polar interaction in the binding-site (site), metal-binding term (metal) and penalty for buried polar group (Bury P), and freezing rotable bonds (Rot B). Among these compounds, compound C-2 having lowest *Glide* score when docked into 2PDG, suggesting that the π-π stacking and hydrogen bonds interaction with the amino acid. 2D representation of docking interaction of proto type design compound & 3D docked poses view using *PyMOL*[26] interface are shown in **Figure 2** & **3**. The phenyl ring of benzylidene system takes the position in specific pocket constituted from Phe115, Phe122, Leu300 and Cys303 and interacts with Trp111 through π-π stacking. At the same time rhodanine occupied position in the anionic binding site and nitrogen of rhodanine forms a hydrogen bond with O of Val47.

Aldose Reductase Inhibition

All the synthesized rhodanine derivatives were evaluated for their ability to inhibit the *in vitro* reduction of D,L-glyceraldehydes by partially purified ALR from goat lenses; sorbinil was used as a reference drug (**Table 1**). Among the synthesized compounds, (4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-2-chlorobenzoate (C-2) showed IC_{50} value; 1.82 μM (Table 1) which was very similar to that of sorbinil. In contrast, analogues C-3 and C-9, bearing an 4-Chloro and 2,4-dichloro benzoate substituent in the 5-arylidene moiety produced no or only moderate ALR inhibition. In particular, 4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl) phenyl 4-methoxybenzoate (C-6) (2.57 μM) proving to be more or same active than the corresponding 4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl) phenyl 3,4-

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dimethoxybenzoate (C-4) (2.68 μM) whereas its 2-methoxy derivative (C-6) was shown to be 5 times less effective at the same dose.

The displacement of methyl group of C-5 from *para* to *ortho* position in C-7 of the 5-benzylidene ring markedly increased inhibitory effectiveness, while replacement of phenyl substitution with benzyl moiety which are totally inefficacious as an ALR inhibitors.

Conclusion

In conclusion, rhodanine derivatives were synthesized and evaluated as aldose reductase inhibitors. Preliminary structure activity relationship revealed that rhodanine derivatives are having improved aldose reductase inhibitory activity as compare to *N*-acetic acid rhodanine derivatives. Electron withdrawing group are favourable at 2^{nd} position of terminal phenyl ring whereas electron withdrawing group at 4^{th} position of terminal phenyl ring decreases activity. Similarly di-substituted electronegative derivatives are having less activity while electropositive and bulky group at 4^{th} position showed comparable activity. Methylene bridge between benzylidene and terminal phenyl group decreases activity. *N*-acetic acid rhodanine derivatives showed that electron withdrawing group at terminal phenyl ring increases activity, while methoxy substitution showed comparable activity. Docking study reveal that synthesized compounds are having the same orientation like ligand protein complex (2PDG). Phenyl ring of benzylidene system takes the position in specific pocket constituted from Phe115, Phe122, Leu300 and Cys303 and interacts with Trp111 through π-π stacking which is similar to that of 1,3-benzothiazolyl moiety of ligand co-crystal structure. Among the synthesized rhodanine derivatives, C-2 and C-6 exhibited ALR inhibiting affinity at micro molar concentration which will be used as starting points for a future drug discovery program.

General Method of Synthesis

Synthesis of substituted benzoyl chloride (A1-12):

Different substituted benzoic acid derivatives (0.01mol) were refluxed with thionyl chloride for 3-4 hr and monitored the reaction through TLC. After completion of reaction, evaporate excess of thionyl chloride under reduced pressure, the crude solid was used as such in next step. **(Scheme 1)**

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Synthesis of substituted 4-formylphenyl benzoate (B1-12):

A cooled solution of benzoyl chloride analogs was mixed slowly with triethylamine (0.03mol) with constant stirring, followed by adding p-hydroxy benzaldehyde (0.01mol). Stirred the reaction mixture at 0°C for 2 hr. and continued stirring at RT for overnight. After evaporation of the solvent under reduced pressure, the crude solid was washed with saturated solution of sodium bicarbonate, brine solution and water. Organic phase was separated and pass through anhydrous Na_2SO_4 and re-crystallized using ethanol. **(Scheme 1)**

Synthesis of substituted 4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl benzoate (C1–12):

All the rhodanine derivatives (C1–C12) were synthesized by Knoevenagel condensation[27,28] by reacting rhodanine with prepared 4–formyl phenyl benzoate derivatives. A mixture of derivatives of 4–formyl phenyl benzoate (0.0025mol) and rhodanine (0.0025mol) in glacial acetic acid containing catalytic amount of sodium acetate (0.080 gm.) was stirred and heat at 100-105°C for 10-12 hr. After completion of reaction, cooled to RT and filtered the crystalline product, washed with cold acetic acid and purified by column chromatography. **(Scheme 1)**

Synthesis of substituted 2-(5-(4-(benzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid derivatives (C13-C24):

Equimolar concentration of derivative of 4–formyl phenyl benzoate (0.025mol) and rhodanine-*N*-acetic acid (0.025mol) were taken in round-bottomed flask containing glacial acetic acid. To this a catalytic amount of sodium acetate (0.080 gm.) was added. The reaction mixture was stirred and heat at 100-105°C for 10-12 h. Progress of the reaction mixture was checked through TLC. After completion of reaction, mixture was kept aside for overnight at RT. Crystalline product was filtered, washed with cold acetic acid and purified by column chromatography. **(Scheme 1)**

All the compounds were analyzed and assigned structures were confirmed by spectroscopic data as follows.

1. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl) benzoate** (**C-1**): Yield 64%; MP 218–222 °C; IR (ATR) 3132 (C-H), 1686 (C=C), 1165 (C-O), 1731 (C=O), 3132 (N-H); ^{1}H NMR (DMSO) δ 9.7 (s, 1H, N-H), 8.16- 8.18 (t, 2H, arom. CH), 7.55– 7.80 (m, 5H, arom. CH), 7.35-7.50 (m, 2H, arom. CH), 7.25 (s, 1H, ethylene CH); MS (ESI^+): 342

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$[M+H]^+$

2. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-2-chlorobenzoate (C-2):** Yield 52%; MP 210–214 °C; IR (ATR) 3033 (C-H), 1685 (C=C), 1159 (C-O), 1748 (C=O), 3648 (N-H), 736 (C-Cl); ^{1}H NMR (DMSO) δ 9.65 (s, 1H, N-H), 7.9-8.1 (d, 1H, arom. CH), 7.55-7.75 (m, 5H, arom. CH), 7.45-7.55 (m, 2H, arom. CH), 7.38 (s, 1H, ethylene CH); MS (ESI^+): 375 $[M]^+$
3. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-4-chlorobenzoate (C-3):** Yield 69%; MP 215–220 °C; IR (ATR) 2984 (C-H), 1676 (C=C), 1267 (C-O), 1732 (C=O), 1590 (N-H), 759 (C-Cl). ^{1}H NMR (DMSO) δ 9.85 (s, 1H, N-H), 7.95-8.25 (m, 2H, arom. CH), 7.6-7.75 (m, 4H, arom. CH), 7.3-7.5 (m, 2H, arom. CH), 7.15 (s, 1H, ethylene CH); MS (ESI^+): 374.1 $[M]^+$
4. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-3,4-dimethoxybenzoate (C-4):** Yield 45%; MP 248–250 °C; IR (ATR) 3061 (C-H), 1697 (C=C), 1138 (C-O), 1734 (C=O), 3181 (N-H), 1212 (-OCH_3). ^{1}H NMR (DMSO) δ 9.95 (s, 1H, N-H), 7.8-8.0 (m, 2H, arom. CH), 7.61-7.75 (m, 2H, arom. CH), 7.35-7.50 (m, 2H, arom. CH), 7.1 (s, 1H, ethylene CH), 6.9-7.2 (m, 1H, arom. CH), 3.9-4.25 (d, 6H, methyl CH). MS (ESI^+): 402$[M+H]^+$
5. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-4-methylbenzoate (C-5):** Yield 36%; MP 198–200 °C; IR (ATR) 3055 (C-H), 1683 (C=C), 1220 (C-O), 1732 (C=O), 3647 (N-H). ^{1}H NMR (DMSO) δ 9.75 (s, 1H, N-H), 8.0 (t, 2H, arom. CH), 7.5-7.8 (m, 2H, arom. CH), 7.3-7.5 (m, 4H, arom. CH), 7.3 (s, 1H, ethylene CH), 2.5 (s, 3H, methyl CH). MS (ESI^+): 356.6$[M+H]^+$
6. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-4-methoxybenzoate (C-6):** Yield 39%; MP 202–208 °C; IR (ATR) 3077 (C-H), 1698 (C=C), 1165 (C-O), 1748 (C=O), 3647 (N-H), 1204 (-OCH_3). ^{1}H NMR (DMSO) δ 10.05 (s, 1H, N-H), 7.7-8.1 (m, 2H, arom. CH), 7.5-7.7 (m, 2H, arom. CH), 7.3-7.5 (m, 2H, arom. CH), 7.03 (s, 1H, ethylene CH), 6.9-7.2 (m, 2H, arom. CH), 3.9 (s, 3H, methyl CH). MS (ESI^+): 372 $[M+H]^+$
7. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-2-methylbenzoate (C-7):** Yield 47%; MP 212–215 °C; IR (ATR) 2998(C-H), 1683 (C=C), 1166 (C-O), 1733 (C=O), 3647 (N-H). ^{1}H NMR (DMSO) δ 9.90 (s, 1H, N-H), 8.1 (d, 1H, arom. CH), 7.6-7.8 (m, 3H, arom. CH), 7.4-7.55 (m, 4H, arom. CH), 7.35 (s, 1H, ethylene CH), 2.5-2.6 (m, 3H, methyl CH). MS (ESI^+): 356.2 $[M+H]^+$
8. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl) phenyl)-2-methoxybenzoate (C-8):**

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Yield 71%; MP 228–230 °C; IR (ATR) 3037 (C-H), 1658 (C=C), 1125 (C-O), 1708 (C=O), 3587 (N-H), 1244 (-OCH_3).^{1}H NMR (DMSO) δ 9.80 (s, 1H, N-H), 7.85-8.15 (d, 1H, arom. CH), 7.5-7.70 (m, 3H, arom. CH), 7.3-7.45 (m, 2H, arom. CH), 7.2 (s, 1H, ethylene CH), 6.8-7.1 (m, 2H, arom. CH), 3.8 (s, 3H, methyl CH). MS (ESI^+): 372 $[M+H]^+$

9. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-2,4-dichlorobenzoate (C-9):** Yield 57%; MP 231–235 °C; IR (ATR) 3084 (C-H), 1546 (C=C), 1252 (C-O), 1712 (C=O), 1610 (N-H), 785 (C-Cl).^{1}H NMR (DMSO) δ 10.0 (s, 1H, N-H), 7.8-8.1 (d, 1H, arom. CH), 7.5-7.7 (m, 4H, arom. CH), 7.35-7.45 (m, 2H, arom. CH), 7.3 (s, 1H, ethylene CH). MS (ESI^+): 410 $[M+H]^+$
10. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-4-nitrobenzoate (C-10):** Yield 38%; MP 238–241 °C; IR (ATR) 3025 (C-H), 1653 (C=C), 1260 (C-O), 1710 (C=O), 3627 (N-H). ^{1}H NMR (DMSO) δ 10.15 (s, 1H, N-H), 8.0-8.3 (m, 4H, arom. CH), 7.65-7.85 (m, 2H, arom. CH), 7.25-7.55 (m, 2H, arom. CH), 7.1 (s, 1H, ethylene CH). MS (ESI^+): 386.8$[M]^+$
11. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-3,5-dinitrobenzoate (C-11):** Yield 31%; MP 210–213 °C; IR (ATR) 2945 (C-H), 1663 (C=C), 1242 (C-O), 1718 (C=O), 3627 (N-H). ^{1}H NMR (DMSO) δ 9.90 (s, 1H, N-H), 8.9-9.3 (d, 2H, arom. CH), 8.6 (s, 1H, arom. CH), 7.69-7.85 (m, 2H, arom. CH), 7.5-7.65 (m, 2H, arom. CH), 7.2 (s, 1H, ethylene CH). MS (ESI^+): 431 $[M]^+$
12. **(4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl)-2-phenylacetate (C-12):** Yield 30%; MP 224–230 °C; IR (ATR) 3032 (C-H), 1661 (C=C), 1175 (C-O), 1721 (C=O), 3172 (N-H). ^{1}H NMR (DMSO) δ 10.05 (s, 1H, N-H), 7.7- 7.95 (t, 2H, arom. CH), 7.3– 7.6 (m, 7H, arom. CH), 7.25 (s, 1H, ethylene CH), 3.7 (s, 2H, methylene CH). MS (ESI^+): 356.8 $[M+H]^+$
13. **2-(5-(4-(benzoyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid (C-13):** Yield 71%; MP 109-111°C; IR (cm^{-1}) 3142 (C-H), 1696 (C=C), 1175 (C-O), 1721 (C=O), 3142 (N-H); ^{1}H NMR (DMSO) (δ) 11.2 (s, 1H, O-H), 8.16- 8.18 (t, 2H, arom. CH), 7.55– 7.8 (m, 5H, arom. CH), 7.38-7.50 (m, 2H, arom. CH), 7.33-7.35 (d, 1H, ethylene CH), 3.68 (s, 2H, methylene); MS (ESI^+): 401.7 $[M+H]^+$
14. **2-(5-(4-(2-chlorobenzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid (C-14):** Yield 56%; MP 98-101°C; IR (cm^{-1}) 3043 (C-H), 1695 (C=C), 1169 (C-O), 1758 (C=O), 3658 (N-H), 746 (C-Cl); ^{1}H NMR (DMSO) (δ) 11.05 (s, 1H, O-H), 7.8-8.1 (d, 1H, arom. CH), 7.65-7.75 (m, 5H, arom. CH), 7.45-7.55 (m, 2H, arom. CH), 7.38-

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7.45 (d, 1H, ethylene CH), 3.34 (s, 2H, methylene); MS (ESI^+): 433.1 $[M]^+$

15. **2-(5-(4-(4-chlorobenzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid** (**C-15):** Yield 65%; MP 102-104°C; IR (cm^{-1}) 2994 (C-H), 1686 (C=C), 1277 (C-O), 1722 (C=O), 1600 (N-H), 769 (C-Cl); ^1H NMR (DMSO) (δ) 10.87 (s, 1H, O-H), 7.95-8.25 (m, 2H, arom. CH), 7.6-7.75 (m, 4H, arom. CH), 7.3-7.55 (m, 2H, arom. CH), 7.15-7.25(d, 1H, ethylene CH), 3.58 (s, 2H, methylene); MS (ESI^+): 433.2 $[M+H]^+$
16. **2-(5-(4-(3,4-dimethoxybenzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid** (**C-16):** Yield 70%; MP 100-102°C; IR (cm^{-1}) 3071 (C-H), 1687 (C=C), 1148 (C-O), 1724 (C=O), 3191 (N-H), 1222 ($-OCH_3$); ^1H NMR (DMSO) (δ) 11.04 (s, 1H, O-H), 7.8-8.0 (m, 2H, arom. CH), 7.61-7.75 (m, 2H, arom. CH), 7.35-7.50 (m, 2H, arom. CH), 7.1-7.2 (d, 1H, ethylene CH), 6.9-7.2 (m, 1H, arom. CH), 3.9-4.25 (d, 6H, methyl CH), 3.67 (s, 2H, methylene); MS (ESI^+): 460 $[M+H]^+$
17. **2-(5-(4-(4-methylbenzoyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid** (**C-17):** Yield 50%; MP 99-105°C; IR (cm^{-1}) 3065 (C-H), 1693 (C=C), 1230 (C-O), 1726 (C=O), 3657 (N-H); ^1H NMR ($CDCl_3$) (δ) 11.09 (s, 1H, O-H), 8.0-8.2 (t, 2H, arom. CH), 7.58-7.8 (m, 2H, arom. CH), 7.3-7.5 (m, 4H, arom. CH), 7.2-7.3 (d, 1H, ethylene CH), 2.5 (s, 3H, methyl CH), 3.36 (s, 2H, methylene); MS (ESI^+): 411.5 $[M-H]^+$
18. **2-(5-(4-(4-methoxybenzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid** (**C-18):** Yield 63%; MP 113-115°C; IR (cm^{-1}) 3047 (C-H), 1668 (C=C), 1135 (C-O), 1718 (C=O), 3597 (N-H), 1254 ($-OCH_3$); ^1H NMR (DMSO) (δ) 11.07 (s, 1H, O-H), 8.1-8.34 (m, 2H, arom. CH), 7.6-8.0 (m, 2H, arom. CH), 7.3-7.5 (m, 2H, arom. CH), 7.16-7.3 (d, 1H, ethylene CH), 6.71-7.0 (m, 2H, arom. CH), 3.8 (s, 3H, methyl CH), 3.54 (s, 2H, methylene); MS (ESI^+): 430.2 $[M+H]^+$
19. **2-(5-(4-(2-methylbenzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid** (**C-19):** Yield 64%; MP 94-97°C; IR (cm^{-1}) 2988(C-H), 1673 (C=C), 1156 (C-O), 1723 (C=O), 3637 (N-H); ^1H NMR ($CDCl_3$) (δ) 10.99 (s, 1H, O-H), 8.1 (d, 1H, arom. CH), 7.6-7.8 (m, 3H, arom. CH), 7.2-7.3 (m, 4H, arom. CH), 6.8-7.1 (d, 1H, ethylene CH), 2.5-2.6 (m, 3H, methyl CH), 3.64 (s, 2H, methylene); MS (ESI^+): 412.8 $[M]^+$
20. **2-(5-(4-(2-methoxybenzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid** (**C-20):** Yield 69 %; MP 92-94°C; IR (cm^{-1}) 3067 (C-H), 1688 (C=C), 1155 (C-O), 1728 (C=O), 3657 (N-H), 1214 ($-OCH_3$); ^1H NMR ($CDCl_3$) (δ) 10.91 (s, 1H, O-H), 7.7-8.15 (d, 1H, arom. CH), 7.5-7.60 (m, 3H, arom. CH), 7.3-7.5 (m, 2H, arom. CH), 7.01-7.03 (d, 1H, ethylene CH), 6.78-6.91 (m, 2H, arom. CH), 3.9 (s, 3H, methyl CH), 3.40 (s, 2H, methylene); MS (ESI^+): 430 $[M+H]^+$

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21. **2-(5-(4-(2,4-dichlorobenzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid** (**C-21):** Yield 55%; MP 88-91°C; IR (cm^{-1}) 3094 (C-H), 1556 (C=C), 1262 (C-O), 1718 (C=O), 1620 (N-H), 795 (C-Cl); ^1H NMR (DMSO) (δ) 10.87 (s, 1H, O-H), 8.3-8.4 (d, 1H, arom. CH), 7.5-8.1 (m, 4H, arom. CH), 7.48-7.55 (m, 2H, arom. CH), 7.4-7.5 (d, 1H, ethylene CH), 3.62 (s, 2H, methylene); MS (ESI^+): 465.6 $[M-H]^+$
22. **2-(5-(4-(4-nitrobenzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid** (**C-22):** Yield 58%; MP 111-114°C; IR (cm^{-1}) 3035 (C-H), 1663 (C=C), 1270 (C-O), 1720 (C=O), 3637 (N-H); ^1H NMR ($CDCl_3$) (δ) 11.14 (s, 1H, O-H), 7.7-8.1 (m, 4H, arom. CH), 7.41-7.65 (m, 2H, arom. CH), 7.25-7.4 (m, 2H, arom. CH), 7.1-7.2 (d, 1H, ethylene CH), 3.55 (s, 2H, methylene); MS (ESI^+): 445.3 $[M+H]^+$
23. **2-(5-(4-(3,5-dinitrobenzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid** (**C-23):** Yield 70%; MP 95-97°C;IR (cm^{-1}) 2955 (C-H), 16673 (C=C), 1252 (C-O), 1721 (C=O), 3637 (N-H); ^1H NMR (DMSO) (δ) 10.92 (s, 1H, O-H), 7.9-8.3 (d, 2H, arom. CH), 8.5 (s, 1H, arom. CH), 7.69-7.85 (m, 2H, arom. CH), 7.5-7.65 (m, 2H, arom. CH), 7.2-7.35 (d, 1H, ethylene CH), 3.67 (s, 2H, methylene); MS (ESI^+): 489.5$[M]^+$
24. **2-(5-(4-(2-phenylacetoxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid** (**C-24):** Yield 72 %; MP 107-110°C; IR (cm^{-1}) 3042 (C-H), 1671 (C=C), 1185 (C-O), 1711 (C=O), 3182 (N-H); ^1H NMR ($CDCl_3$) (δ) 10.95 (s, 1H, O-H), 8.22- 8.38 (t, 2H, arom. CH), 7.8– 8.19 (m, 7H, arom. CH), 7.5-7.8 (d, 1H, ethylene CH), 3.4 (s, 2H, methylene CH), 3.8 (s, 2H, methylene); MS (ESI^+): 412.2 $[M-H]^+$

In-vitro **Enzymatic Assay**

ALR activity was assayed at 30ºC in a reaction mixture containing 0.75mL of 10mM D,L-glyceraldehyde, 0.5mL of 0.104mM NADPH, 0.75mL of 0.1M sodium phosphate buffer (pH = 6.2), 0.3mL of enzyme extract and 0.7mL of deionized water in a total volume of 3mL. All the above reagents, except D,L-glyceraldehyde, were incubated at 30ºC for 10 min; the substrate was then added to start the reaction, which was monitored for 5min. Enzyme activity was calibrated by diluting the enzymatic solution in order to obtain an average reaction rate of 0.011 ± 0.0010 absorbance units/min for the sample.

The sensitivity of aldose reductase to different compounds was tested in the above assay conditions in the presence of inhibitors dissolved at proper concentration in DMSO (1 % v/v). IC_{50} values were determined by non linear regression analysis. Each log dose-inhibition curve was generated using at least seven concentrations of inhibitor causing an inhibition between

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10 and 90. The 95% confidence limits (95% CL) were calculated using GraphPad Prism software. The inhibitory activity of the compounds is shown in **Table 1**.

Conflict of interest

The authors report no conflict of interest.

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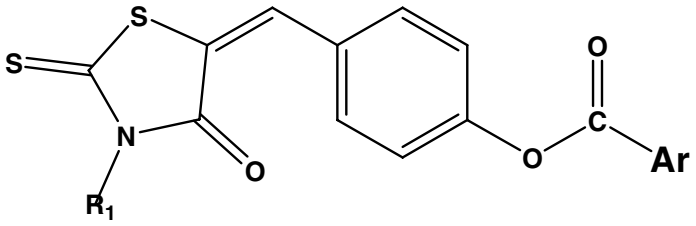
Table 1: Glide Score and Aldose Reductase Inhibitory activity of Synthesized Compounds



Compound No.	R_1	Ar	Glide Score	[a]Aldose Reductase Inhibitory activity (IC_{50} in µM)
C-1	H	C_6H_5-	-10.34	2.47 (2.28-2.91)
C-2	H	2-Cl-C_6H_4-	-11.87	1.82 (1.13-1.49)
C-3	H	4-Cl-C_6H_4-	-10.64	5.89 (5.63-6.29)
C-4	H	3,4-$(OCH_3)_2$-C_6H_3-	-9.19	2.68 (2.43-2.87)
C-5	H	4-CH_3-C_6H_4-	-11.09	5.82 (5.32-6.09)

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C-6	H	4-OCH_3-C_6H_4-	-10.34	2.57 (2.37-2.71)
C-7	H	2-CH_3-C_6H_4-	-11.84	3.65 (3.32-3.94)
C-8	H	2-OCH_3-C_6H_4-	-09.93	4.50 (4.36-4.89)
C-9	H	2-$(Cl)_2$-C_6H_3-	-9.14	12.5 (11.03-13.09)
C-10	H	4-NO_2-C_6H_4-	-10.58	15.54 (14.43-16.07)
C-11	H	3,5-$(NO_2)_2$-C6H3-	-11.08	9.73 (9.14-10.26)
C-12	H	C_6H_5-CH_2-	-9.73	[b]14% (12.5 µM)
C-13	-CH_2COOH	C_6H_5-	-11.34	16.9 (15.75-17.62)
C-14	-CH_2COOH	2-Cl-C_6H_4-	-11.73	3.17 (2.88-3.41)
C-15	-CH_2COOH	4-Cl-C_6H_4-	-12.15	7.02 (6.13-7.49)
C-16	-CH_2COOH	3,4-$(OCH_3)_2$-C_6H_3-	-11.11	[b]51 % (12.5 µM)
C-17	-CH_2COOH	4-CH_3-C_6H_4-	-10.85	10.18 (9.43-10.87)
C-18	-CH_2COOH	4-OCH_3-C_6H_4-	-12.13	15.02 (14 -15.09)
C-19	-CH_2COOH	2-CH_3-C_6H_4-	-8.61	4 .57 (4.37-4.71)
C-20	-CH_2COOH	2-OCH_3-C_6H_4-	-12.34	[b]34% (12.5 µM)
C-21	-CH_2COOH	2,4-$(Cl)_2$-C_6H_3-	-7.94	12.01 (11.36-12.49)
C-22	-CH_2COOH	4-NO_2-C_6H_4-	-5.15	11.5 (11.03-12.09)
C-23	-CH_2COOH	3,5-(NO_2)-C_6H_3-	-5.03	5.40 (4.97-6.03)
C-24	-CH_2COOH	C_6H_5-CH_2-	-3.82	6.13 (6.1-6.26)
Standard		Sorbinil		1.32 (1.12-1.65)

[a] Fifty percent inhibitory concentration in µM with 95% confidence limit; [b] Percentage inhibition at the concentration given in the parenthesis

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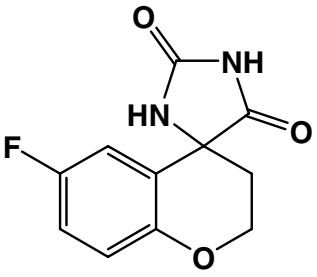
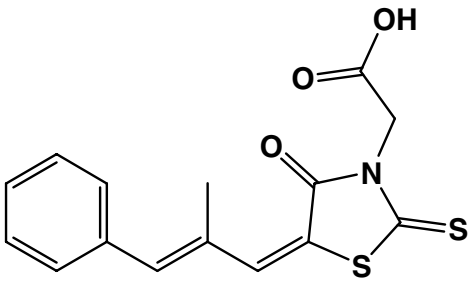


Sorbinil



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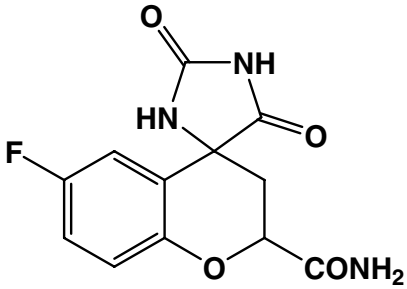

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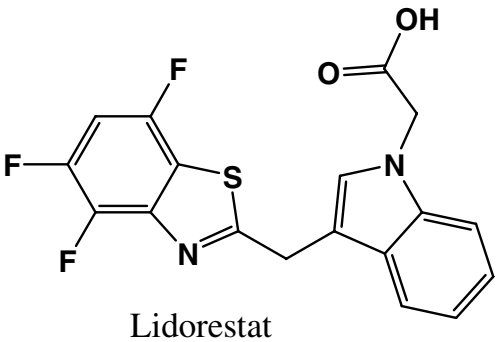

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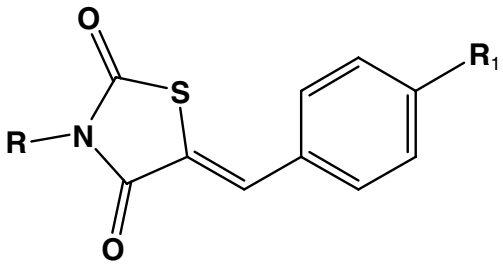

5-arylidene-2,4-thiazolidinediones

1 R= H; R_1= H
1a R= H; R_1= OH
2 R= CH_2COOCH_3; R_1= H
3 R= CH_2COOH; R_1= H

Figure 1: Structure of aldose reductase inhibitors

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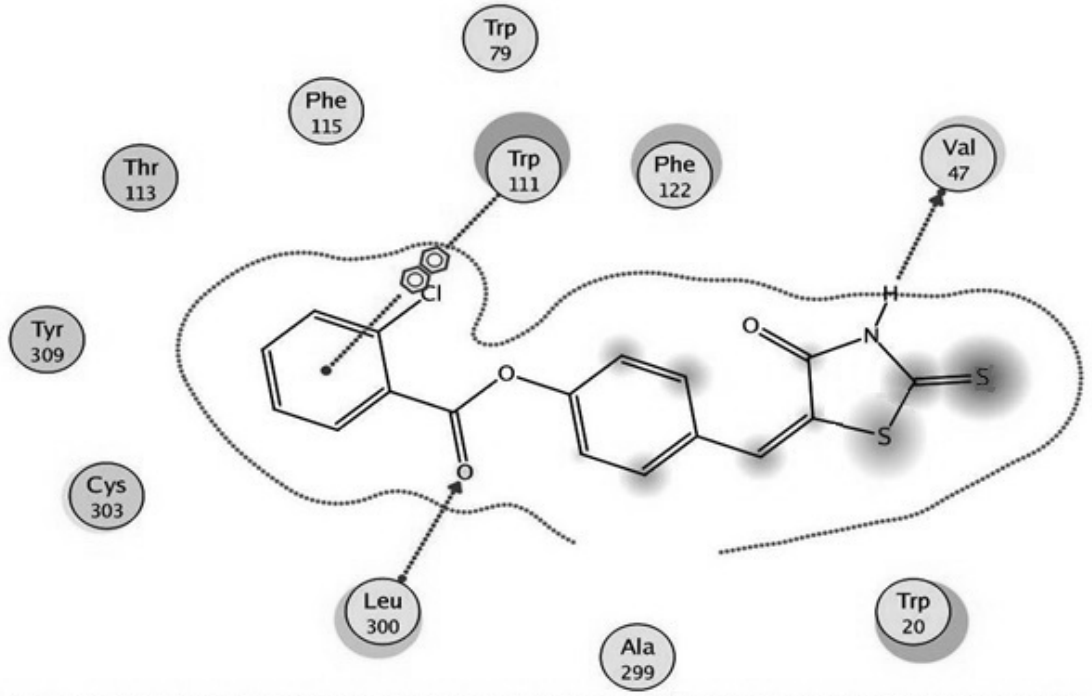

Figure 3: 2D Representation of docking interaction of the 4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl 2-chlorobenzoate (C-2)

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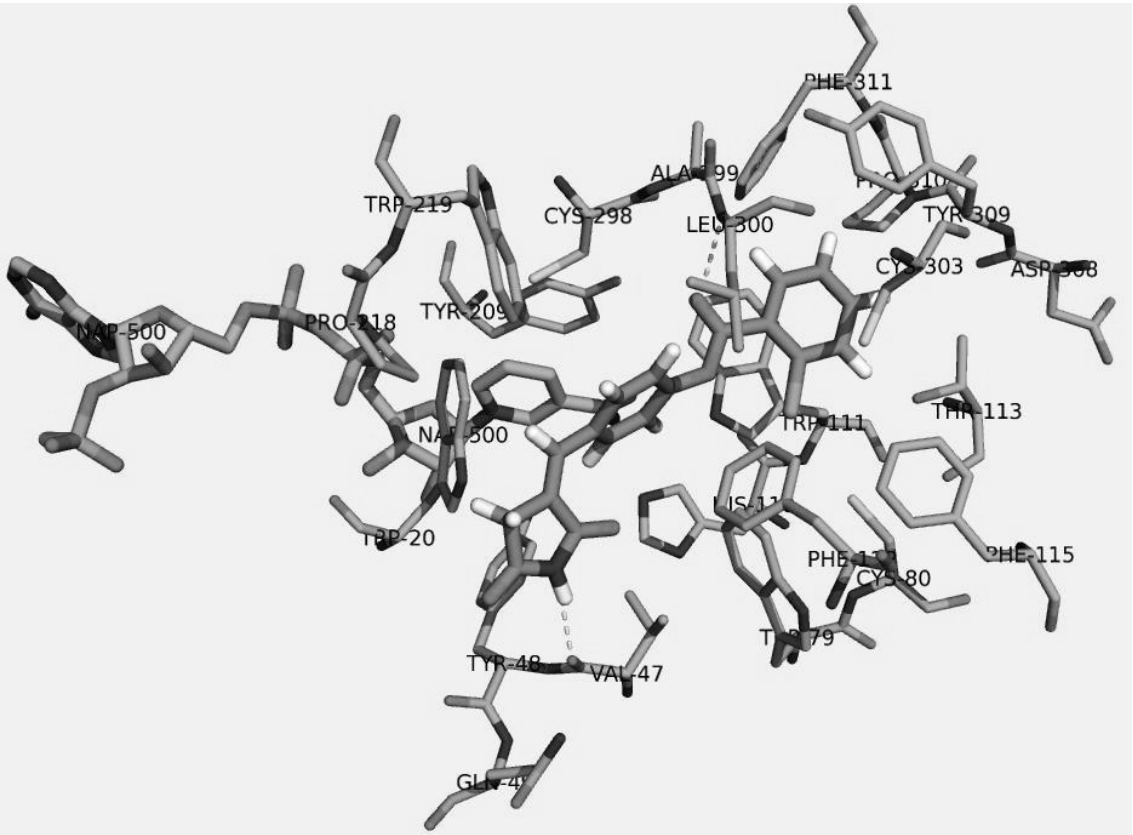

Figure 4: Docking pose and Interaction of 4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl 2-chlorobenzoate(C-2) with 2PDG.

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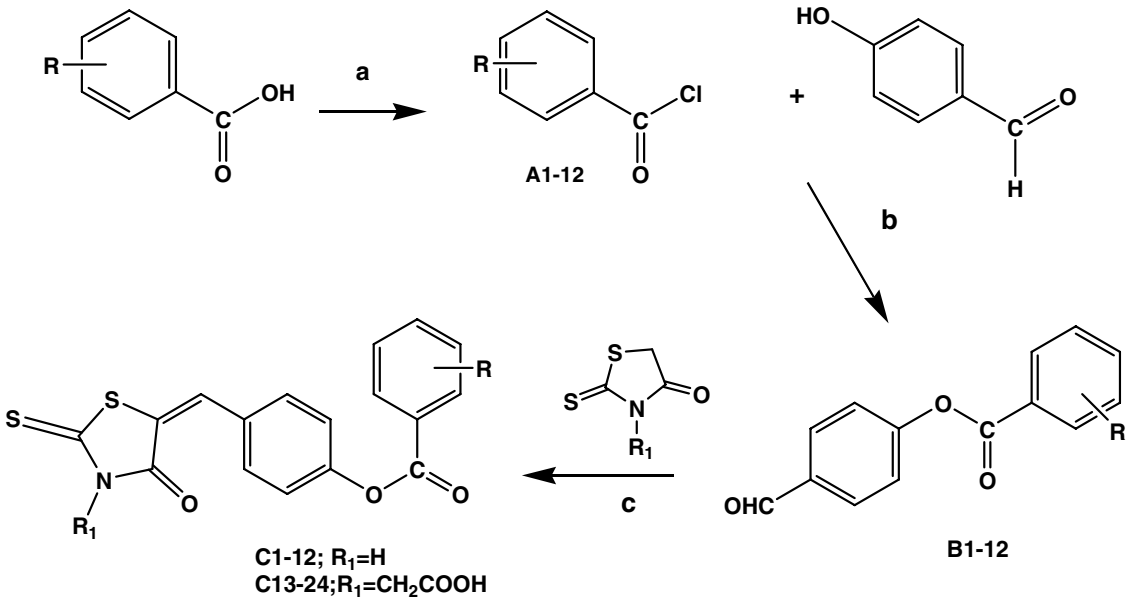

Reagents and conditions: (a) $SOCl_2$, Reflux; (b) $(C_2H_5)N$, CH_2Cl_2, RT;(c) CH_3COONa, CH_3COOH, Reflux.

Scheme 1: General synthesis scheme of rhodanine derivatives

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