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Discovery of Novel 1,4-Benzodioxane Containing Thiazolidinone Derivatives as Potential Antihepatotoxic Agent

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In continuance of our search for newer antihepatotoxic agents some novel thiazolidinone derivatives containing 1,4-benzodioxane ring system were synthesized starting from 2,3-dihydro-1,4-benzodioxane-2-carbohydrazide. The synthesized compounds were evaluated for antihepatotoxic activity against CCl_4-induced hepatotoxicity in rats. Among them some compounds have shown significant antihepatotoxic activity comparable to standard drug silymarin.

Key Words : 1,4-Benzodioxane, Thiazolidinone, Antihepatotoxic activity

Introduction

Liver pathologies affect hundreds of millions of patients worldwide. The most common cause of hepatopathy are chronic hepatitis C and alcoholism, nonalcoholic fatty liver diseases, autoimmune and drug induced hepatic disorders. Many of these conditions can be prevented or treated, but if not, they can lead to progressive liver injury, liver fibrosis and ultimately cirrhosis, portal hypertension, liver failure, and in some instances cancer.

Liver is considered to be one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it is also involved in the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them. Herbal-based therapeutics for liver disorders has been in use in India for a long time. Despite the significant popularity of several herbal medicines in general, and for liver diseases in particular, they are still unacceptable treatment modalities for liver diseases. The limiting factors that contribute to this eventuality are (i) lack of standardization of the herbal drugs; (ii) lack of identification of active ingredients/principles; (iii) lack of randomized controlled clinical trials and (iv) lack of toxicological evaluation.[1] Traditional drugs used in the treatment of liver diseases are sometimes inadequate to cater the need of large population. In spite of tremendous strides in the modern medicine, there are not much drugs available for the treatment of liver disorders. Many natural products of herbal origin are in use for the treatment of liver ailments.[2-5] The drug available in the modern systems of medicine are mainly corticosteroids and immunosuppressive agents, which brings about only symptomatic relief and in most of the cases have no influence on the disease process. Further their use is associated with the risk of relapses and danger of side effects.

Benzodioxane represents a series of synthetic and natural compounds of considerable medicinal importance. Compounds containing dioxane ring systems exhibited different biological activities like antihepatotoxic,[6-8] α-adrenergic blocking agent,[9] *anti*-inflammatory,[10] and D_2 antagonist/5-HT_{1A} partial agonist activity.[11]

Silymarin isolated from seeds of *Silybum marianum* commonly known as 'milk thistle' has been found most potent antihepatotoxic agent against a variety of toxicants. Silymarin has been found to be a mixture of three isomers of flavonolignan i.e. silybin, silychristin and silydianin. Among them silybin is found to be the most potent component containing 1,4-benzodioxane ring system. Other isomer silychristin and silydianin do not possess 1,4-benzodioxane ring system and thus do not display significant antihepatotoxic activity. It has also been previously reported that that 1,4-dioxane ring system plays an important role in exhibiting antihepatotoxic activity.[6-8] Thiazolidinone derivatives are also reported to possess hepatoprotective and curative antihepatotoxic effects and especially in the case of a prolonged treatment, do not exhibit any undesired choleretic and other side effects.[12] We have recently reported the antihepatotoxic activity of 1,3,4-oxadiazole[8] and pyrazoline[13] derivatives based on 1,4-benzodioxane moiety. Keeping in view of the above facts, we have undertaken the current work and synthesized some novel 1,4-benzodioxane derivatives containing thiazolidinone ring system and evaluated some of them for antihepatotoxic activity against CCl_4-induced hepatotoxicity in rats. Among them compounds **4b**, **4d**, **4e** and **4j** have shown significant antihepatotoxic activity as comparable to standard drug silymarin. Other compounds of the series showed moderate antihepatotoxic activity.

Experimental

The IR spectra were recorded on Brucker. The mass spectra were recorded on a Bruker daltronics high resolution

mass spectrometer, ^{1}H-NMR (300 MHz) was recorded on Bruker DPX 300 spectrometer in CD_3OD and DMSO-d_6 using TMS as internal standard reference and chemical shifts are in δ (ppm). Elemental analyses were performed on Elementar Vario EL III, Carlo Erba 1108. The melting points were determined by capillary method.

Synthesis of Ethyl-l,4-benzodioxane-2-carboxylate (1). Anhydrous potassium carbonate (50 g) was added in portions to a stirred solution of 55 g of catechol in 200 mL of dry acetone followed by the dropwise addition of 34.5 g of ethyl-2,3-dibromopropionate. Another 50 g of potassium carbonate and 34.5 g of the dibromoester were added similarly and this was repeated two times more using a total of 200 g of potassium carbonate and 137.5 g of ester. Stirring and refluxing was continued for another 15 hrs. The reaction mixture was then filtered and the solid was washed several times with acetone. The filtrate was concentrated to about 75 mL and the residue was diluted with 50 mL of cold water. The oily layer was separated from the aqueous layer; the latter was extracted repeatedly with ether. The combined oily layer and ether extracts were washed with water, dried over magnesium sulfate, and evaporated. The dark residue was distilled at 96-97 °C (0.1 mm) to yield 38 g of ester **1** as colorless liquid. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.23 (3H, t, J = 7.1 Hz, CH_3-12), 4.20 (2H, q, J = 7.1, 5.7 Hz, CH_2-15), 4.30 (2H, d, J = 2.7, CH_2-3), 4.77 (1H, t, J = 2.7, CH-2), 6.84 (4H, m, Ar-H); FTIR cm^{-1}: 3052 (=C-H, aromatic), 1772 (C=O), 1653 (C=C), 1292 (C-O, ester)

Synthesis of 2,3-Dihydro-1,4-benzodioxane-2-carbohydrazide (2). To a solution of ethyl-1,4-benzodioxane-2-carboxylate (0.01 mol) in ethanol (20 mL), hydrazine hydrate (0.01 mol) was added and the reaction mixture was refluxed. The progress of the reaction was monitored by TLC. After the completion of the reaction (usually 16 hrs), the excess solvent was removed under reduced pressure. The reaction mixture was poured over crushed ice. The solid thus separated was filtered, dried and crystallized with methanol to give a white powder; m.p: 110-112 °C; Yield: 80%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.91 (2H, br-s, NH_2-13), 4.24 (1H, dd, J = 6.0, 11.4 Hz, H_a-3), 4.46 (1H, dd, J = 6.0, 11.4 Hz, H_b-3), 4.78 (1H, d, J = 6.0, CH_2), 6.91 (4H, m, Ar-H), 7.78 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3052 (=C-H, aromatic), 1772 (C=O), 1673 (C=C), 1259 (-NH_2), 1195 (-NH), 758 (C=C); Anal. Calcd. for $C_9H_{10}N_2O_3$: C, 55.67; H, 5.19; N, 14.43; O, 24.72. Found: C: 55.37; H, 5.02; N, 14.67; O, 24.73.

General Method for the Synthesis of *N′*-[substituted-phenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3a-o). Equimolar quantities of 2,3-dihydro-1,4-benzodioxane-2-carbohydrazide and substituted benzaldehyde were taken in absolute ethanol. To this solution few drops of glacial acetic acid was added and the mixture was refluxed for 8-9 hrs in molecular sieves. The excess solvent was removed under reduced pressure and the residue was poured onto the crushed ice to obtain solid product which was filtered, dried and crystallized from ethanol.

***N′*-Benzylidene-2,3-dihydro-1,4-benzodioxine-2-carbohydrazide (3a):** mp 140-142 °C; Yield: 78%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.35 (2H, d, J = 12 Hz, CH_2-3), 4.95 (1H, brs (unresolved doublet), CH_2-2), 6.85-7.03 (4H, m, ArH-ring A), 7.42 (5H, m, ArH-ring B), 8.02 (1H, s, N=CH), 11.59 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3208-2875 (NH), 3056 (=C-H, aromatic), 1656 (C=C), 1645 (C=O); Anal. Calcd. for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92; O, 17.00; Found: C, 68.03; H, 5.06; N, 9.85; O, 17.05.

***N′*-[2-Hydroxyphenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3b):** mp 199-201 °C; Yield: 89%; ^{1}H-NMR (300 MHz, DMSO- d_6): δ 4.36 (2H, d, J = 9 Hz, CH_2-3), 4.99 (1H, brs (unresolved doublet), CH_2-2), 6.88-7.20 (4H, m, ArH-ring A), 7.50-7.68 (4H, m, ring B), 8.32 (1H, s, N=CH), 10.97 (1H, s, Ar-OH), 11.62 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3208-2680 (NH), 3064 (=C-H, aromatic), 1663 (C=C), 1655 (C=O); Anal. Calcd. for $C_{16}H_{14}N_2O_4$ C, 64.42; H, 4.73; N, 9.39; O, 21.45; Found: C, 64.35; H, 4.79; N, 9.41; O, 21.43.

***N′*-[3-Hydroxyphenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3c):** mp 180-182 °C; Yield: 85%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.42 (2H, d, J = 10 Hz, CH_2-3), 5.02 (1H, brs (unresolved doublet), CH_2-2), 6.75-7.32 (4H, m, ArH-ring A), 7.50-7.72 (4H, m, ring B), 8.41 (1H, s, N=CH), 10.85 (1H, s, Ar-OH), 11.58 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3210-2675 (NH), 3034 (=C-H, aromatic), 1653 (C=C), 1640 (C=O); Anal. Calcd. for $C_{16}H_{14}N_2O_4$ C, 64.42; H, 4.73; N, 9.39; O, 21.45; Found: C, 64.38; H, 4.78; N, 9.42; O, 21.40.

***N′*-[4-Hydroxyphenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3d):** mp 167-169 °C; Yield: 88%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.25 (2H, d, J = 10.5 Hz, CH_2-3), 5.12 (1H, brs (unresolved doublet), CH_2-2), 6.68-7.25 (4H, m, ArH-ring A), 7.35- 7.57 (4H, m, ring B), 8.46 (1H, s, N=CH), 10.73 (1H, s, Ar-OH), 11.35 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3180-2753(NH), 3045 (=C-H, aromatic), 1665 (C=C), 1652 (C=O); Anal. Calcd. for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 9.39; O, 21.45; Found: C, 64.35; H, 4.79; N, 9.35; O, 21.51.

***N′*-[3,4-Dihydroxyphenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3e):** mp 157-59 °C; Yield: 75%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.32 (2H, d, J = 10.5 Hz, CH_2-3), 5.15 (1H, brs (unresolved doublet), CH_2-2), 6.56-7.14 (4H, m, ArH-ring A), 7.25- 7.45 (3H, m, ring B), 8.32 (1H, s, N=CH), 10.65 (2H, s, Ar-OH), 11.43 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3085-2836 (NH), 3042 (=C-H, aromatic), 1670 (C=C), 1648 (C=O); Anal. Calcd. for $C_{16}H_{14}N_2O_5$: C, 61.14; H, 4.49; N, 8.91; O, 25.45; Found: C, 61.08; H, 4.55; N, 8.89; O, 25.43.

***N′*-[2-Chlorophenyl) methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3f):** mp 165-67 °C; Yield: 73%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.23 (2H, d, J = 11 Hz, CH_2-3), 5.05 (1H, brs (unresolved doublet), CH_2-2), 6.75-7.20 (4H, m, ArH-ring A), 7.26- 7.52 (4H, m, ring B), 8.25 (1H, s, N=CH), 11.45 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3075-2850(NH), 3054 (=C-H, aromatic), 1680 (C=C), 1652 (C=O), 745 (C-Cl); Anal. Calcd. for $C_{16}H_{13}ClN_2O_3$: C,

60.67; H, 4.14; N, 8.84; O, 15.15; Found: C, 60.75; H, 4.21; N, 8.88; O, 15.17.

***N′*-[4-Chlorophenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3g):** mp 175-77 °C; Yield: 90%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.34 (2H, d, J = 12 Hz, CH_2-3), 5.61 (1H, brs (unresolved doublet), CH_2-2), 6.83-7.00 (4H, m, ArH-ring A), 7.50-7.69 (4H, m, Ar-ring B), 8.07 (1H, s, N=CH), 11.69 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3210-2878 (NH), 3048 (=C-H, aromatic), 1658 (C=C), 1663 (C=O); Anal. Calcd. for $C_{16}H_{13}ClN_2O_3$: C, 60.67; H, 4.14; N, 8.84; O, 15.15; Found: C, 60.72; H, 4.18; N, 8.86; O, 15.13.

***N′*-[4-Flourophenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3h):** mp 136-38 °C; Yield: 65%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.29 (2H, d, J = 12 Hz, CH_2-3), 5.15 (1H, brs (unresolved doublet), CH_2-2), 6.42-7.13 (4H, m, ArH-ring A), 7.23-7.59 (4H, m, ring B), 8.35 (1H, s, N=CH), 11.56 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3042-2875 (NH), 3054 (=C-H, aromatic), 1675 (C=C), 1648 (C=O), 745 (C-F); Anal. Calcd. for $C_{16}H_{13}FN_2O_3$: C, 64.00; H, 4.36; N, 9.33; O, 15.98; Found: C, 63.96; H, 4.56; N, 9.36; O, 15.95.

***N′*-[4-Methoxyphenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3i):** mp 132-34 °C; Yield: 82%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.36 (3H, s, 3× OCH_3), 4.35 (2H, d, J = 12 Hz, CH_2-3), 5.62 (1H, brs (unresolved doublet), CH_2-2), 6.85-6.94 (4H, m, ArH-ring A), 7.63-7.65 (4H, m, ring B), 8.31 (1H, s, N=CH), 11.52 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3032-2845 (NH), 3032 (=C-H, aromatic), 1675 (C=C), 1645 (C=O); Anal. Calcd. for $C_{17}H_{16}N_2O_4$: C, 65.38; H, 5.16; N, 8.97; O, 20.49; Found: C, 65.35; H, 5.19; N, 8.95; O, 20.50.

***N′*-[3,4-Dimethoxyphenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3j):** mp 150-52 °C; Yield: 88%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.78 (6H, s, 2×OCH_3), 4.39 (2H, d, J = 12.5 Hz, CH_2-3), 4.93 (1H, brs (unresolved doublet), CH_2-2), 6.87-7.08 (4H, m, ArH-ring A), 7.18-7.28 (3H, m, Ar-ring B), 7.94 (1H, s, N=CH), 11.48 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3220-2879 (NH), 3056 (=C-H, aromatic), 1654 (C=C), 1672 (C=O); Anal. Calcd. for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30; N, 8.18; O, 23.37; Found: C, 63.17; H, 5.28; N, 8.20; O, 23.35.

***N′*-[3-Hydroxy-4-methoxyphenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3k):** mp 194-96 °C; Yield: 81%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.25 (3H, s, OCH_3), 4.42 (2H, d, J = 12 Hz, CH_2-3), 5.54 (1H, brs (unresolved doublet), CH_2-2), 6.67-7.12 (4H, m, ArH-ring A), 7.25-7.54 (3H, m, ring B), 8.31 (1H, s, N=CH), 10.25 (1H, s, Ar-OH), 11.40 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3043-2845 (NH), 3045 (=C-H, aromatic), 1667 (C=C), 1643 (C=O); Anal. Calcd. for $C_{17}H_{16}N_2O_5$:C, 62.19; H, 4.91; N, 8.53; O, 24.37; Found: C, 62.21; H, 4.88; N, 8.55; O, 24.34.

***N′*-[2-Methyl-phenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3l):** mp 125-27 °C; Yield: 65%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.52 (3H, s, CH_3), 4.53 (2H, d, J = 10.5 Hz, CH_2-3), 5.53 (1H, brs (unresolved doublet), CH_2-2), 6.52-7.02 (4H, m, ArH-ring A), 7.34-7.52 (4H, m, ArH-ring B), 8.12 (1H, s, N=CH), 11.53 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3230-2875 (NH), 3035 (=C-H, aromatic), 1654 (C=C), 1645 (C=O); Anal. Calcd. for $C_{17}H_{16}N_2O_3$:C, 68.91; H, 5.44; N, 9.45; O, 16.20; Found: Anal. Calcd. for C, 68.87; H, 5.43; N, 9.51; O, 16.18.

***N′*-[3-Methyl-phenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3m):** mp 119-21 °C; Yield: 63%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.48 (3H, s, CH_3), 4.40 (2H, d, J = 11.5 Hz, CH_2-3), 5.63 (1H, brs (unresolved doublet), CH_2-2), 6.82-6.99 (4H, m, ArH-ring A), 7.24-7.30 (4H, m, Ar-ring B), 8.08 (1H, s N=CH), 11.67 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3215-2872 (NH), 3028 (=C-H, aromatic), 1664 (C=C), 1665 (C=O); Anal. Calcd. for $C_{17}H_{16}N_2O_3$:C, 68.91; H, 5.44; N, 9.45; O, 16.20; Found: C, 68.85; H, 5.47; N, 9.47; O, 16.21.

***N′*-[2-Nitrophenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3n):** mp 145-47 °C; Yield: 78%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.35 (2H, d, J = 10.5 Hz, CH_2-3), 5.23 (1H, brs (unresolved doublet), CH_2-2), 6.75-7.24 (4H, m, ArH-ring A), 7.32-7.67 (4H, m, ArH-ring B), 7.98 (1H, s, N=CH), 11.43 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3125-2856 (NH), 3043 (=C-H, aromatic), 1690 (C=C), 1643 (C=O); Anal. Calcd. for $C_{16}H_{13}N_3O_5$: C, 58.72; H, 4.00; N, 12.84; O, 24.44; Found: C, 58.70; H, 4.03; N, 12.87; O, 24.40.

***N′*-[4-Nitrophenyl-methylidene]-2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (3o):** mp 185-87 °C; Yield: 88%; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.45 (2H, m (unresolved doublet), CH_2-3), 5.02 (1H, brs (unresolved doublet), CH_2-2), 6.87-7.02 (4H, m, ArH-ring A), 7.21-7.56 (4H, m, ArH-ring B), 7.97 (1H, s, N=CH), 11.96 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3215-2884 (NH), 3054 (=C-H, aromatic), 1663 (C=C), 1687 (C=O); HRMS (*m/z*): 328.1941 [M]$^+$ (Calcd for $C_{16}H_{11}BrN_2O_3$, 327.2915); Anal. Calcd. for $C_{16}H_{11}BrN_2O_3$: C, 58.72; H, 4.00; N, 12.84; O, 24.44: Found: C, 58.70; H, 3.98; N, 12.90; O, 24.39.

General Method for the Synthesis of *N*-(4-oxo-2-phenyl)-1,3-thiazolidin-3-yl)-2,3-dihydro-1,4-benzodioxane-2-carboxamide (4a-o). Different hydrazones **3a-o** (0.01 mol) were dissolved in dry toluene (75 mL). To this solution thioglycollic acid (0.01 mol) was added dropwise. The resulting solution was refluxed in Dean stark apparatus for 36 hrs thus removing the water formed during the reaction by means of azeotropic distillataion. The excess solvent was removed under reduced pressure and the residue was poured onto the crushed ice to obtain solid product which was filtered, dried and crystallized from ethanol.

***N*-(4-Oxo-2-phenyl)-1,3-thiazolidin-3-yl)-2,3-dihydro-1,4-benzodioxane-2-carboxamide (4a):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.46 (2H, dd (J = 16.2, 15.9 Hz), CH_2-15), 4.45 (2H, d, dd (J = 11.4, 5.1, 5.4 Hz), CH_2-3), 4.61 (1H, s, CH-17), 5.51 (1H, brs (unresolved doublet), CH-2), 7.07-7.18 (4H, m, Ar H-ring A), 6.55 (5H, m, ArH-ring B), 10.22 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3273 (NH), 3052 (=C-H, aromatic), 168, 1752 (2, C=O), 1264 (CS); HRMS (*m/z*): 356.3932 [M]$^+$; Anal. Calcd. for $C_{18}H_{16}N_2O_4S$: C, 60.66; H, 4.53; N, 7.86; O, 17.96; S, 9.03; Found: C, 60.65;

H, 4.50; N, 7.88; O, 17.96; S, 9.00.

***N*-[2-(2-Hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4b):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.42 (2H, dd (J = 16.3, 15.7 Hz), CH_2-15), 4.40 (2H, d, dd (J = 11.3, 5.2, 5.6 Hz), CH_2-3), 4.54 (1H, s, CH-17), 9.10 (1H, s, Ar-OH), 5.65 (1H, brs (unresolved doublet), CH-2), 7.25-7.34 (4H, m, ArH-ring A), 6.68 (4H, m, Ar-H-ring B), 10.34 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3265 (NH), 3028 (=C-H, aromatic), 1676 , 1768 (2, C=O), 1243 (CS): Anal. Calcd. for $C_{18}H_{16}N_2O_5S$: C, 58.05; H, 4.33; N, 7.52; O, 21.48; S, 8.61; Found: C, 58.02; H, 4.36; N, 7.57; O, 21.45; S, 8.58.

***N*-[2-(3-Hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxa mide (4c):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.34 (2H, dd (J = 15.6, 16.3 Hz), CH_2-15), 4.51 (2H, d, dd (J = 11.6, 5.2, 5.6 Hz), CH_2-3), 4.46 (1H, s, CH-17), 5.10 (1H, s, Ar-OH), 5.62 (1H, brs (unresolved doublet), CH-2), 7.15-7.21 (4H, m, ArH-ring A), 6.45 (4H, m, Ar-H-ring B), 10.31 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3260 (NH), 3049 (=C-H, aromatic), 1665, 1732 (2, C=O), 1252 (CS); Anal. Calcd. for $C_{18}H_{16}N_2O_5S$: C, 58.05; H, 4.33; N, 7.52; O, 21.48; S, 8.61; Found: C, 58.08; H, 4.29; N, 7.51; O, 21.46; S, 8.62.

***N*-[2-(4-Hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4d):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.60 (2H, dd (J = 16.7, 15.4 Hz), CH_2-15), 4.37 (2H, d, dd (J = 11.8, 5.3, 5.5 Hz), CH_2-3), 4.57 (1H, s, CH-17), 9.14 (1H, s, Ar-OH), 5.29 (1H, brs (unresolved doublet), CH-2), 7.27-7.31 (4H, m, ArH-ring A), 6.55 (4H, m, Ar-H-ring B), 9.89 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3167 (NH), 3028 (=C-H, aromatic), 1694, 1738 (2, C=O), 1253 (CS); Anal. Calcd. for $C_{18}H_{16}N_2O_5S$: C, 58.05; H, 4.33; N, 7.52; O, 21.48; S, 8.61; Found: C, 58.04; H, 4.35; N, 7.51; O, 21.44; S, 8.59.

***N*-[2-(3,4-Dihydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4e):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.46 (2H, dd (J = 16.5, 15.4 Hz), CH_2-15), 4.24 (2H, d, dd (J = 11.3, 5.7, 5.9 Hz), CH_2-3), 4.72 (1H, s, CH-17), 9.23 (2H, s, Ar-OH), 5.49 (1H, brs (unresolved doublet), CH-2), 7.13-7.17 (4H, m, ArH-ring A), 6.82 (3H, m, Ar-H-ring B), 10.12 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3265 (NH), 2987 (=C-H, aromatic), 1692, 1743 (2, C=O), 1242 (CS); Anal. Calcd. for $C_{18}H_{16}N_2O_6S$: C, 55.66; H, 4.15; N, 7.21; O, 24.72; S, 8.26; Found: C, 55.64; H, 4.18; N, 7.20; O, 24.73; S, 8.24.

***N*-[2-(2-Chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4f):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.24 (2H, dd (J = 15.8, 16.3 Hz), CH_2-15), 4.36 (2H, d, dd (J = 10.9, 6.2, 5.9 Hz), CH_2-3), 4.45 (1H, s, CH-17), 5.25 (1H, brs (unresolved doublet), CH-2), 7.27-7.38 (4H, m, ArH-ring A), 6.79 (4H, m, ArH-ring B), 10.34 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3143 (NH), 2980 (=C-H, aromatic), 1705, 1767 (2, C=O), 1246 (CS), 746 (C-Cl); Anal. Calcd. for $C_{18}H_{15}ClN_2O_4S$: C, 55.31; H, 3.87; Cl, 9.07; N, 7.17; O, 16.37; S, 8.20; Found: C, 55.28; H, 3.90; N, 7.15; O, 16.39; S, 8.19.

***N*-[2-(4-Chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4g):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.46 (2H, dd (J = 16.2, 15.9 Hz), CH_2-15), 4.01 (2H, d, dd (J = 11.4, 5.1, 5.4 Hz), CH_2-3), 4.61 (1H, s, CH-17), 5.51 (1H, brs (unresolved doublet), CH-2), 7.07-7.18 (4H, m, ArH-ring A), 6.55 (4H, m, ArH-ring B), 10.22 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3260 (NH), 3134 (=C-H, aromatic), 1667, 1745 (2, C=O), 1256 (CS), 742 (C-Cl). HRMS (*m/z*): 391.2709 $[M+H]^+$; Anal. Calcd. for $C_{18}H_{15}ClN_2O_4S$: C, 55.31; H, 3.87; Cl, 9.07; N, 7.17; O, 16.37; S, 8.20; Found: C, 55.25; H, 3.90; N, 7.20; O, 16.35; S, 8.22.

***N*-[2-(4-Fluorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4h):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.45 (2H, dd (J = 15.8, 15.2 Hz), CH_2-15), 4.27 (2H, d, dd (J = 11.3, 5.3, 5.8 Hz), CH_2-3), 4.54 (1H, s, CH-17), 5.23 (1H, brs (unresolved doublet), CH-2), 7.12-7.35 (4H, m, ArH-ring A), 6.54 (4H, m, ArH-ring B), 10.12 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3243 (NH), 3120 (=C-H, aromatic), 1675, 1733 (2, C=O), 1226 (CS), 756 (C-F); Anal. Calcd. for $C_{18}H_{15}FN_2O_4S$: C, 57.75; H, 4.04; F, 5.07; N, 7.48; O, 17.09; S, 8.56; Found: C, 57.78; H, 4.07; N, 7.43; O, 17.10; S, 8.53.

***N*-[2-(4-Methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4i):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.23 (2H, dd (J = 16.6, 15.8 Hz), CH_2-15), 3.53 (3H, S, Ar-OCH_3), 4.27 (2H, d, dd (J = 11.6, 5.6, 5.2 Hz), CH_2-3), 4.56 (1H, s, CH-17), 5.45 (1H, brs (unresolved doublet), CH-2), 7.24-7.34 (4H, m, ArH-ring A), 6.55 (4H, m, ArH-ring B), 10.22 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3263 (NH), 2985 (=C-H, aromatic), 1692, 1734 (2, C=O), 1254 (CS); Anal. Calcd. for $C_{19}H_{18}N_2O_5S$: C, 59.06; H, 4.70; N, 7.25; O, 20.70; S, 8.30. Found: C, 59.03; H, 4.72; N, 7.22; O, 20.68; S, 8.31.

***N*-[2-(3,4-Dimethoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4j):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.48 (2H, dd (J = 16.3, 15.7 Hz), CH_2-15), 3.10 (6H, s, Ar-OCH_3), 4.35 (2H, d, dd (J = 11.5, 5.0, 5.6 Hz), CH_2-3), 4.76 (1H, s , CH-17), 5.21 (1H, s, Ar-OH), 5.65 (1H, brs (unresolved doublet), CH-2), 7.15-7.23 (4H, m, ArH-ring A), 6.73 (4H, m, ArH-ring B), 10.32 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3235 (NH), 3071 (=C-H, aromatic), 1697, 1720 (2, C=O), 1234 (CS); Anal. Calcd. for $C_{20}H_{20}N_2O_6S$: C, 57.68; H, 4.84; N, 6.73; O, 23.05; S, 7.70; Found: C, 57.69; H, 4.86; N, 6.71; O, 23.03; S, 7.72.

***N*-[2-(3-Hydroxy-4methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4k):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.87 (2H, dd (J = 16.8, 15.3 Hz), CH_2-15), 3.15 (3H, s, Ar-OCH_3), 4.58 (2H, d, dd (J = 11.4, 5.1, 5.4 Hz), CH_2-3), 4.81 (1H, s, CH-17), 9.12 (1H, s, Ar-OH), 5.65 (1H, brs (unresolved doublet), CH-2), 7.15-7.23 (4H, m, ArH-ring A), 6.73 (4H, m, Ar-H-ring B), 10.32 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3273 (NH), 3052 (=C-H, aromatic), 1686, 1752 (2, C=O), 1264 (CS); Anal. Calcd. for $C_{19}H_{18}N_2O_6S$: C, 56.71; H, 4.51; N, 6.96; O, 23.85; S, 7.97; Found: C, 56.68; H, 4.50; N, 6.98; O, 23.86; S, 7.95.

***N*-[2-(2-Methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-**

dihydro-1,4-benzodioxine-2-carboxamide (4l): ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.25 (1H, s, Ar-CH_3), 3.48 (2H, dd (J = 16.8, 15.7 Hz), CH_2-15), 4.34 (2H, d, dd (J = 11.6, 5.8, 5.5 Hz), CH_2-3), 4.96 (1H, s, CH-17), 5.28 (1H, brs (unresolved doublet), CH-2), 6.97-7.18 (4H, m, ArH-ring A), 6.63 (4H, m, ArH-ring B), 10.22 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3257 (NH), 3043 (=C-H, aromatic), 1685, 1745 (2, C=O), 1226 (CS); Anal. Calcd. for $C_{19}H_{18}N_2O_4S$: C, 61.61; H, 4.90; N, 7.56; O, 17.28; S, 8.66; Found: C, 61.59; H, 4.88; N, 7.59; O, 17.27; S, 8.68.

***N*-[2-(3-Methylphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4m):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.30 (1H, s, Ar-CH_3), 3.56 (2H, dd (J = 15.6, 15.2 Hz), CH_2-15), 4.63 (2H, d, dd (J = 12.5.0, 6.4, 6.2 Hz), CH_2-3), 5.12 (1H, s, CH-17), 5.65 (1H, brs (unresolved doublet), CH-2), 7.29-7.34 (4H, m, ArH-ring A), 6.9-7.2 (4H, m, ArH-ring B), 10.12 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3134 (NH), 3050 (=C-H, aromatic), 1661, 1705 (2, C=O), 1206 (CS); Anal. Calcd. for $C_{19}H_{18}N_2O_4S$: C, 61.61; H, 4.90; N, 7.56; O, 17.28; S, 8.66; Found: C, 61.65; H, 4.88; N, 7.53; O, 17.29; S, 8.68.

***N*-[2-(2-Nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4n):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.32 (2H, dd (J = 16.5, 15.7 Hz), CH_2-15), 4.40 (2H, d, dd (J = 12.2, 5.6, 5.3 Hz), CH_2-3), 4.65 (1H, s, CH-17), 5.55 (1H, brs (unresolved doublet), CH-2), 7.12-7.15 (4H, m, ArH-ring A), 6.76 (4H, m, ArH-ring B), 10.15 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3265 (NH), 3064 (=C-H, aromatic), 1695, 1746 (2, C=O), 1245 (CS); Anal Calc. for $C_{18}H_{15}N_3O_6S$: C, 53.86; H, 3.77; N, 10.47; O, 23.92; S, 7.99; Found: C, 53.83; H, 3.74; N, 10.50; O, 23.94; S, 7.98.

***N*-[2-(4-Nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2,3-dihydro-1,4-benzodioxine-2-carboxamide (4o):** ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.57 (2H, dd (J = 16.7, 15.5 Hz), CH_2-15), 4.52 (2H, d, dd (J = 11.6, 5.5, 5.3 Hz), CH_2-3), 4.72 (1H, s, CH-17), 5.53 (1H, brs (unresolved doublet), CH-2), 7.12-7.15 (4H, m, ArH-ring A), 6.98 (4H, m, ArH-ring B), 10.25 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3267 (NH), 3023 (=C-H, aromatic), 1692, 1735 (2, C=O), 1256 (CS); Anal. Calcd. for $C_{18}H_{15}N_3O_6S$: C, 53.86; H, 3.77; N, 10.47; O, 23.92; S, 7.99; Found: C, 53.86; H, 3.77; N, 10.47; O, 23.92; S, 7.99.

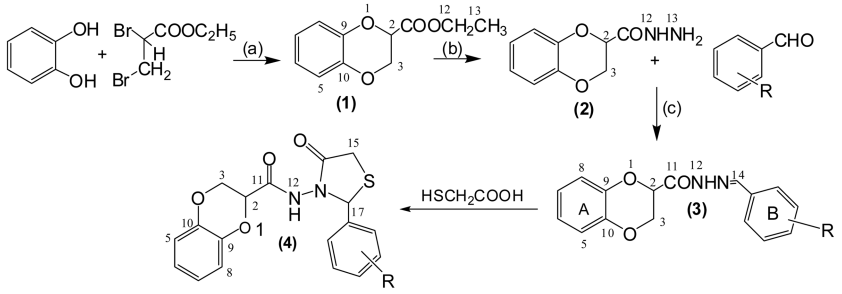
Antihepatotoxic Activity of Synthesized Compounds. Wistar Albino rats of either sex weighing 150-200 g were used for the study. The animals were housed in clean metabolic cages. They were fed with a standard pellet diet and water *ad libitum*. The animals were maintained at 25 °C to 28 °C with 40-70% relative humidity and 12 hrs light/dark cycles and were fastened for 12 hrs prior to the experiment. The protocol was approved by Institutional Animal Ethical Committee constituted by Jamia Hamdard for such purpose.

Wistar Albino rats were divided into thirteen groups consisting of five animals in each group. Group I received liquid paraffin only (1.5 mL/kg, orally) and served as normal control. Group II received suspension of carbon tetrachloride (CCl_4) in liquid paraffin (1:1, v/v, 1.5 mL of CCl_4/kg, per oral.) to induce hepatic damage and served as toxic control. Group III received CCl_4 suspension, in addition to silymarin (10 mg/kg, p.o.) daily. Groups IV-XIII received suspension of carbon tetrachloride in liquid paraffin (1:1, v/v, 1.5 mL/kg, per oral.) on first day followed by treatment with synthesized compounds **4a**, **4b**, **4d**, **4e**, **4g**, **4i**, **4j**, **4k**, **4l** and **4m** (10 mg/kg, p.o., respectively) for seven days. Blood was withdrawn through retro-orbital plexus of rats on 8th day. Serum was separated from blood of each rat by centrifugation for estimation of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT),[13] alkaline phosphatase (ALP),[14] and total protein.[15] The rats were sacrificed and liver rapidly exercised immediately after sacrifice. Liver was fixed in formalin (10%), serially sectioned and microscopically examined after staining with hematoxylin and eosin.

Statistical Analysis. The data obtained were analyzed by one-way ANOVA followed by Dunnett's test. The level of significance was set at $P < 0.05$.

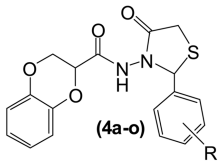
Results and Discussion

The synthetic route used to prepare starting materials and the title compounds is outlined in Scheme 1. The starting material ethyl-l,4-benzodioxane-2-carboxylate (**1**) was prepared by reaction between catechol and ethyl-2,3-dibromopropionate in dry acetone in the presence of anhydrous potassium carbonate. The IR spectrum of compound **1** showed an intense peak at 1772 cm^{-1} for carbonyl C=O; 1653 cm^{-1} for C=C; 3052 cm^{-1} for =C-H; and 1292 cm^{-1} for C-O ester groups. The ^{1}H-NMR spectrum of **1** showed a triplet at δ 1.23 (J = 7.1 Hz) and a quartet at δ 4.20 (J = 7.1, 5.7 Hz) due to -CH_3 at position 13 and -CH_2- at position 12



Scheme 1. Reagents and conditions: (a) K_2CO_3, acetone, reflux with stirring (b) NH_2-NH_2. H_2O, reflux (c) abs. ethanol, reflux (d) dry toluene, reflux in Dean stark apparatus.

Table 1. Physical constants of the synthesized compounds



Compd.	R	mp (°C)	Yield (%)	Compd.	R	mp (°C)	Yield (%)
4a	H	218-220	35	**4i**	4-OCH_3	186-188	58
4b	2-OH	212-214	40	**4j**	3,4-di-OCH_3	190-192	48
4c	3-OH	216-218	45	**4k**	3-OH,4-OCH_3	160-162	45
4d	4-OH	208-210	52	**4l**	2-CH_3	175-177	30
4e	3,4-di-OH	199-201	48	**4m**	3-CH_3	170-172	25
4f	2-Cl	202-204	62	**4n**	2-NO_2	228-230	55
4g	4-Cl	196-198	65	**4o**	4-NO_2	230-232	54
4h	4-F	180-182	31	-	-	-	-

respectively. Cyclization of the product was revealed by appearance of signals in ^{1}H-NMR at δ 4.77 as a triplet and δ 4.3 as a doublet due to the protons of CH_2- at position 2 and 3.

Treatment of **1** with hydrazine hydrate afforded corresponding hydrazide **2**. The IR spectrum of compound **2** showed an intense peak at 1725 cm^{-1} for carbonyl C=O; 1642 cm^{-1} for C=C; 3045 cm^{-1} for =C-H aromatic ring. The ^{1}H-NMR spectrum of **2** showed two double doublets at δ 4.24 and 4.46 (J = 6.0) corresponding to C-H_a and C-H_b of position 3, a doublet at δ 4.78 was assigned to the protons of CH_2 at position 2, two protons of NH_2- at position 13 were appeared as a broad singlet at δ 3.91, and a proton as a singlet at δ 7.78 (J = 7.5) was assigned to the protons of NH- at position-12. Aromatic protons were appeared as multiplets at δ 6.91.

The reaction of hydrazide (**2**) with substituted aromatic aldehyde in absolute ethanol in presence of molecular sieves and catalytic amount of glacial acetic acid afforded the corresponding hydrazone derivatives **3a-o** in moderate to good yields. Their structures were confirmed on the basis of elemental and spectral data. The IR spectra of the compounds revealed disappearance of NH_2 bands between 3425 and 3439 cm^{-1}. The ^{1}H-NMR spectrum of the compound **3a-o** displayed single signals corresponding to resonance of azomethine protons at δ 7.94-8.46. The hydrazine hydrazone NH protons were observed at δ 10.97-11.59. Aromatic protons were located at d 6.85-7.42.

Moreover, refluxing **3a-o** with thioglycollic acid afforded titled thiazolidinone derivatives **4a-o**. The formation of thiazolidinone proceeds by the attack of mercaptoacetic acid upon CH=N- group with the SCH_2COOH adding to the carbon atom followed by the capture of a proton by nitrogen and subsequent cyclization. The ^{1}H-NMR spectrum of the thiazolidinone derivatives **4a-o** exhibited single NH (amide CONH) resonance at δ 9.89-10.34. In the ^{1}H-NMR spectra methylene protons of 4-thiazolidinone ring displayed signals appearing as double doublet at δ 3.24-3.87. The methine protons of thiazolidinone **4a-o** showed resonance at δ 4.45-5.12. Chemical structures, melting point and percentage yield of the synthesized compounds are reported in Table 1.

The CCl_4-induced hepatotoxicity is mediated by primary and secondary bond formation of reactive species with critical cellular molecules such as DNA, lipid, proteins or carbohydrates. It is well established that hepatotoxicity by CCl_4 is due to enzymatic activation to release CCl_3 radical in free state, which in turn disrupts the structure and function of lipid and protein macromolecules in the membrane of the cell organelles. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane due to toxicity produced by CCl_4. Significant rise in serum enzymatic concentration *viz.* SGOT and SGPT could be taken as an index of liver damage. It generally induces deposition of fat in liver and plays a significant role in inducing triacyl glycerol accumulation, depletion of GSH, increase lipid oxidation, membrane damage, and depression of protein synthesis and loss of enzyme activity. Being cytoplasmic in location, the damage marker enzymes SGOT, SGPT are released in serum. It has been reported that protective agents exert their action against CCl_4 mediated lipid peroxidation, either through decreased production of free radical derivatives or due to the *anti*-oxidant activity of the protective agent itself.

As shown in Table 2, the activities of the liver enzymes serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate oxaloacetate transaminase (SGPT), alkaline phosphatase (ALP) were markedly increased and total proteins (TP) were decreased in CCl_4-treated rats in comparison with normal values. Administration of silymarin (standard drug) and synthesized compounds at the dose levels 10 mg/kg body weight, prevented CCl_4-induced elevation of SGOT, SGPT, ALP and also prevented decrease in total protein. The activities of the liver enzymes such as SGOT and SGPT were elevated on administration of CCl_4 to 74.52 and 85.27 IU/L in comparison to normal values of 34.82 and 45.60 IU/L respectively. The level of alkaline phosphatase was also elevated on administration of CCl_4 to 66.15 KA units as compared to normal values of 42.08 KA units. The administration of compound under investigation significantly reduced the elevated enzyme level to the values in the range of 55.28-61.85 IU/L for SGOT, 61.76-70.54 IU/L for

Table 2. Effect of the synthesized compounds and Silymarin on serum biochemical parameters in CCl_4 induced liver damage in rats

Groups n=5	Treatment	Dose	SGOT (IU/L)	SGPT (IU/L)	ALP KA units	Total protein (g/dl)
I	Normal control	-	34.82 ± 0.697	45.60 ± 1.18	42.08 ± 3.57	6.46 ± 0.53
II	Toxic control	1.5 mL/kg (p.o.)	74.52 ± 0.695	85.27 ± 2.05	66.157 ± 2.886	3.54 ± 0.67
III	Silymarin (standard drug)	10 mg/kg (p.o.)	56.19 ± 0.808^b	61.29 ± 1.78^b	48.62 ± 3.385^b	4.2 ± 0.84^b
IV	Compd. **4a**	10 mg/kg (p.o.)	61.57 ± 1.229^c	64.63 ± 0.620^b	56.72 ± 1.110	3.92 ± 0.41^b
V	Compd. **4b**	10 mg/kg (p.o.)	59.13 ± 1.020^b	65.41 ± 0.426^b	52.80 ± 0.272	3.95 ± 0.62^a
VI	Compd. **4d**	10 mg/kg (p.o.)	58.28 ± 1.725^a	63.76 ± 0.401^b	51.46 ± 0.152	3.98 ± 0.321^a
VII	Compd. **4e**	10 mg/kg (p.o.)	55.28 ± 1.325^a	61.76 ± 0.603^a	55.16 ± 0.069^a	3.77 ± 0.678
VIII	Compd. **4g**	10 mg/kg (p.o.)	61.85 ± 2.515^b	62.93 ± 0.526^b	57.97 ± 0.713	3.82 ± 0.13^a
IX	Compd. **4i**	10 mg/kg (p.o.)	61.12 ± 1.134^a	65.27 ± 0.423^b	53.53 ± 0.342	3.78 ± 0.34^a
X	Compd. **4j**	10 mg/kg (p.o.)	59.57 ± 2.657^a	67.06 ± 0.699^a	48.72 ± 0.326^b	3.85 ± 0.61^a
XI	Compd. **4k**	10 mg/kg (p.o.)	61.32 ± 2.516^a	65.34 ± 0.425^b	56.66 ± 0.243	3.85 ± 0.321^a
XII	Compd. **4l**	10 mg/kg (p.o.)	62.12 ± 1.234^b	66.21 ± 0.236^a	57.38 ± 0.423	3.81 ± 0.24^b
XIII	Compd. **4m**	10 mg/kg (p.o.)	61.85 ± 1.429^b	70.54 ± 0.392^b	53.11 ± 0.770^a	3.92 ± 0.12^a

SGOT, *Serum glutamate Oxaloacetate transaminase*; SGPT, *Serum glutamate pyruvate transaminase*; ALP, *alkaline phosphatase*: TP, total protein; p.o., per oral. ^{c}P < 0.0001. ^{b}P < 0.01. ^{a}P < 0.05 *vs* CCl4; P > 0.05 ns. Values are mean ± SEM (n = 5). ANOVA followed by Dunnett test was performed.

Table 3. Histopathological studies of the synthesized compounds and Silymarin on CCl_4 induced liver damage in rats

Groups n=5	Treatment	Microscopic observations
I	Normal control	Normal architecture of liver without any degeneration, necrosis, or inflammation.
II	Toxic control	Prominent centrilobular necrosis with prominent and enlarged central vein, significant periportal inflammation.
III	Silymarin (standard drug)	Hepatocytes with uniformly staining cytoplasm and mild dilatation of sinusoidal spaces, clearly visible central vein, absence of necrosis.
IV	Compd. **4a**	Significant recovery of hepatocytes, absence of necrosis.
V	Compd. **4b**	Partial decrease in portal inflammation and centrizonal sinusoidal dilatation.
VI	Compd. **4d**	Portal track inflammation, no sinusoidal dilatation.
VII	Compd. **4e**	Significant reduction in periportal inflammation and sinusoidal dilatation, absence of necrosis, the central vein and the portal vein clearly visible.
VIII	Compd. **4g**	Collection of inflammatory cells within the portal triad, vacuolated hepatocytes in the periportal zone.
IX	Compd. **4i**	Disappearance of fatty deposits but not completely.
X	Compd. **4j**	Few scattered lymphocytes around the portal triad, mild dilatation of sinusoids in the centrizonal area.
XI	Compd. **4k**	Inflammatory cells within the portal triad, little sinusoidal dilatation.
XII	Compd. **4l**	Scattered lymphocytes around the portal triad, normal portal triad.
XIII	Compd. **4m**	Mild dilatation of sinusoids in the centrizonal area, normal portal triad.

SGPT and 48.72-57.38 KA units for ALKP which were found to be comparable to the reduced enzyme level with the standard drug silymarin (56.19 and 61.29 IU/L for SGOT and SGPT respectively). The toxicant CCl_4 reduced the level of total protein (3.54 g/dl) in comparison to normal values (6.46 g/dl). The administration of the test compound elevated the reduced level of total protein to values in the range of 3.77-3.98 g/dl. Silymarin (10 mg/kg) had significantly decreased the level of SGOT, SGPT, and ALP and increased that in total protein.

The histopathological studies also showed significant recovery of hepatocytes of the liver in the standard drug and compound-treated animals which has again correlated with the biochemical parameters. The results of the liver histopathological studies have been presented in Table 3, which showed hepatocytes swelling and necrosis in CCl_4-treated rats in comparison with normal control rats. Administration of synthesized compounds exhibited a significant protection of hepatocytes injury and showed normalization of the tissues as neither fatty accumulation nor necrosis was observed as shown in Table 3. The central vein appeared clearly indicating a potent antihepatotoxic activity.

Conclusion

The study has shown that the 1,4-benzodioxane ring containing thiazolidinone derivatives possess significant hepatoprotective activity. The synthesized compounds are simple and of low molecular weight and can be easily synthesized as compared to silybin. Furthermore the compounds are expected to be easily metabolizable, in comparison to silybin.

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Conflict of Interest. The authors report no conflict of interest.

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