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Synthesis and antihepatotoxic activity of 5-(2,3-dihydro-1,4-benzodioxane-6-yl)-3-substituted-phenyl-4,5-dihydro-1*H*-pyrazole derivatives

Habibullah Khalilullah [a,b,*], Shamshir Khan [b], Mohamed Jawed Ahsan [a], Bahar Ahmed [b]

[a] Department of Pharmaceutical Chemistry, Alwar Pharmacy College, Alwar 301030, Rajasthan, India
[b] Antihepatotoxic Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, (Hamdard University), New Delhi 110062, India

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ABSTRACT

In continuance of our search for newer antihepatotoxic agents some novel pyrazoline derivatives containing 1,4-dioxane ring system were synthesized starting from 3-(2,3-dihydro-1,4-benzodioxane-6-yl)-1-substituted-phenylprop-2-en-1-one. Some of 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.

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. 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. Herbal-based therapeutics for liver disorders have 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 lack of standardization of the herbal drugs, lack of identification of active ingredients/principles, lack of randomized controlled clinical trials and 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 to be a potent antihepatotoxic agent against a variety of toxicants. Silymarin has been found to be a mixture of three isomers that is silybin, silychristin and silydianin. The main component silybin, which usually makes up 20–30% of the total flavonolignans contains 1,4-benzodioxane ring system and possess significant antihepatotoxic activity;[6,7] other constituents silychristin and silydianin do not possess 1,4-dioxan ring system and thus do not display significant antihepatotoxic activity. On the basis of the above hypothesis we taught that 1,4-dioxane ring system plays an important role in exhibiting antihepatotoxic activity, and thus we have prepared some new pyrazoline derivatives bearing 1,4-dioxane ring system and evaluated some of them for antihepatotoxic activity against CCl_4-induced hepatotoxicity in rats. Among them, compounds **3d** and **3j** have shown significant antihepatotoxic activity as comparable to stan-

* Corresponding author. Tel.: +91 0144 5121027; mobile: +91 8058790282.
E-mail address: shabib79@yahoo.co.in (H. Khalilullah).

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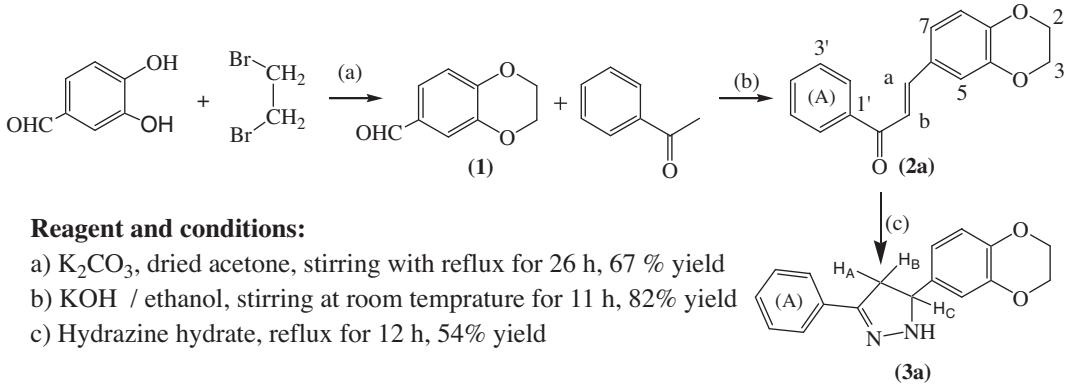


Reagent and conditions:

a) K_2CO_3, dried acetone, stirring with reflux for 26 h, 67 % yield

b) KOH / ethanol, stirring at room temprature for 11 h, 82% yield

c) Hydrazine hydrate, reflux for 12 h, 54% yield

Scheme 1. Synthetic route for the preparation of 5-(2,3-dihydro-1,4-benzodioxane-6-yl)-3-phenyl)-4,5-dihydro-1*H*-pyrazole.

dard drug silymarin. Other compounds of the series showed moderate antihepatotoxic activity.

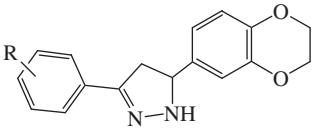
The synthetic route used to prepare starting materials and the title compounds is outlined in Scheme 1. The starting material 2,3-dihydro-1,4-benzodioxane-6-carbaldehyde was prepared[12] by reaction between 3,4-dihydroxybenzaldehyde and ethylene dibromide in dry acetone in the presence of anhydrous potassium carbonate, which on Claisen–Schmidt condensation with substituted acetophenone in absolute ethanol and 10% potassium hydroxide afforded corresponding 3-(2,3-dihydro-1,4-benzodioxane-6-yl)-1-substituted-phenylprop-2-en-1-one derivatives **2a–o**.[13] The chalcones are considered to be useful intermediates in several cyclization reactions to produce different types of heterocyclic compounds of diverse biological importance. The titled pyrazolines **3a–o** (Table 1) were synthesized[14] by cyclocondensation of chalcones **2a–o** with hydrazine hydrate in absolute ethanol and few drops of glacial acetic acid in presence of molecular sieve (reaction time varies from 10 to 15 h). The highest yield (63%) was observed for the compound **3d** having 2,4-dihydroxy substitution on the aromatic ring whereas the compounds **3l** and **3m** having methyl substitution in the aromatic ring have shown lowest yield (28% and 35%). It has also been observed that compounds substituted in the *meta* position of phenyl ring have shown greater yield than those substituted with the same functional group in the *para* position.

Both analytical and spectral data of all the synthesized compounds were in full agreement with the proposed structures. The ^{1}H NMR spectrum of **1** showed two broad singlets at δ 4.30 and δ 4.33 corresponding to the protons due to $-CH_2$ at position 2 and 3, respectively. A singlet at δ 9.79 could be assigned to the aldehydic protons at position-6. The formation of compounds **2a–o** is observed in ^{1}H NMR by the disappearance of the aldehydic proton signal and the appearance of a signal of the olefinic proton at about 7.54–7.95 ppm. The ^{1}H NMR spectrum of compounds **2a–o** also showed doublets at δ 4.20 due to methylenic protons of dioxane ring system. The IR spectra of the compounds **3a–o** revealed absorption bands in the regions 1523–1590 cm^{-1} corresponding to C=N stretching bands because of ring closure. In addition, the absorption bands at 1420–1480 cm^{-1} were attributed to the (N–N) stretch vibrations, which also confirm the formation of the desired pyrazoline ring in all the compounds. The ^{1}H NMR spectrum of **3a–o** showed peaks due to the protons at positions 2 and 3 of benzodioxane as broad singlets (unresolved doublet) at δ 4.12–4.38. The compounds also exhibited the presence of two magnetically nonequivalent protons of a methylene group (H_A/H_B) at δ 3.00–3.40 and δ 3.72–3.80, respectively, coupled with each other and in turn with the vicinal methine proton at δ 5.14–5.24. The phenyl protons were observed at the expected chemical shifts and integral values. The mass spectrum gave molecular ion peak corresponding to the molecular formula of the compounds.

The CCl_4-induced hepatotoxicity is mediated by primary and secondary bond formation of reactive species to 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 $CCl_3^{\cdot}$ 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 shown 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.

Some of the synthesized compounds were evaluated for antihepatotoxic activity against CCl_4-induced hepatic damage in rats. The

Table 1
Chemical structures, melting point and percentage yield of the synthesized pyrazoline derivatives



S. No	Compd	R	Mol. Formula	Mp (°C)	Yield (%)
1	**3a**	H	$C_{17}H_{16}N_2O_2$	115–117	54
2	**3b**	2-Hydroxy	$C_{17}H_{16}N_2O_3$	124–125	52
3	**3c**	4-Hydroxy	$C_{17}H_{16}N_2O_3$	119–122	49
4	**3d**	2,4-Dihydroxy	$C_{17}H_{15}N_2O_4$	140–42	63
5	**3e**	2-Chloro	$C_{17}H_{15}ClN_2O_2$	110–12	58
6	**3f**	4-Chloro	$C_{17}H_{15}ClN_2O_2$	165–67	43
7	**3g**	4-Fluoro	$C_{17}H_{15}FN_2O_2$	98–100	36
8	**3h**	4-Bromo	$C_{17}H_{15}BrN_2O_2$	102–04	33
9	**3i**	4-Methoxy	$C_{18}H_{18}N_2O_3$	134–36	37
10	**3j**	3,4-Dimethoxy	$C_{19}H_{20}N_2O_4$	154–56	45
11	**3k**	2-Methyl	$C_{18}H_{18}N_2O_2$	175–77	47
12	**3l**	3-Methyl	$C_{18}H_{18}N_2O_2$	97–99	28
13	**3m**	4-Methyl	$C_{18}H_{18}N_2O_2$	138–140	35
14	**3n**	4-Nitro	$C_{17}H_{15}N_3O_4$	149–151	45
15	**3p**	*N*,*N*-Dimethylamino	$C_{19}H_{21}N_3O_2$	180–182	52

Table 2
Effect of selected pyrazoline derivatives on serum enzymatic activity 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**	61.29 ± 1.78**	48.62 ± 3.385**	4.2 ± 0.84**
IV	Compd **3a**	10 mg/kg (p.o.)	62.85 ± 1.654**	65.00 ± 1.458**	53.14 ± 0.172*	4.04 ± 0.10**
V	Compd **3d**	10 mg/kg (p.o.)	58.57 ± 1.457**	62.09 ± 0.700*	58.22 ± 1.097	3.82 ± 0.438*
VI	Compd **3f**	10 mg/kg (p.o.)	63.42 ± 1.471**	65.81 ± 1.463**	46.06 ± 0.495**	3.91 ± 0.63**
VII	Compd **3j**	10 mg/kg (p.o.)	58.85 ± 1.948**	64.95 ± 1.105**	49.48 ± 0.254**	3.82 ± 0.15*
VIII	Compd **3l**	10 mg/kg (p.o.)	64.85 ± 1.726**	64.13 ± 0.828*	55.74 ± 0.595	3.98 ± 0.43**

SGOT, serum glutamate Oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase; ALP, alkaline phosphatase; TP, total protein; p.o., per oral.
***P <0.0001; **P <0.01; *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 changes in liver of Wistar rats

Groups $n = 5$	Treatment	Microscopic observations
I	Normal control	Normal liver architecture, no degeneration, necrosis, or inflammation
II	Toxic control	Prominent centrilobular necrosis enlarged central vein, significant periportal inflammation reflecting liver damage
III	Silymarin (standard drug)	Hepatocytes with uniformly staining cytoplasm, mild dilatation of sinusoidal spaces, central vein was clearly visible, good recovery with the absence of necrosis
IV	Compd **3a**	Moderate sinusoidal dilatation around the central vein, fine vascular change is also seen in scattered hepatocytes
V	Compd **3d**	Moderate dilatation of sinusoids, normal parenchyma
VI	Compd **3f**	Sinusoidal dilatation around the central vein, fine vascular changes in scattered hepatocytes
VII	Compd **3j**	Mild dilatation of sinusoids in centrizonal area, liver parenchyma is otherwise normal
VIII	Compd **3l**	Little dilatation of sinusoids and central vein appeared clear

degree of hepatoprotection of the synthesized compounds **3a, 3d, 3f, 3j** and **3l** (10 mg/kg, p.o., respectively) was determined by measuring the various biochemical parameters such as serum glutamate oxaloacetate transaminase (GOT),[15] glutamate pyruvate transaminase (GPT),[15] alkaline phosphatase (ALP),[16] and total protein.[17] The extent of CCl_4-induced liver injury and hepatoprotective effect of the synthesized compounds was also analyzed through histopathological observation.[18]

Details of antihepatotoxic activity of the synthesized pyrazole derivatives **3a, 3d, 3f, 3j** and **3l** have been summarized in Table 2. As shown in Table 2, the activities of the liver enzymes SGOT, SGPT, ALP were markedly increased and 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. Silymarin (10 mg/kg) had significantly decreased the level of SGOT, SGPT, and ALP and increased that 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 values in the range of 58.57–64.85 IU/L for SGOT, 62.09–65.81 IU/L for SGPT and 46.06–58.22 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 3.82–4.04 g/dl. 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. The central vein appeared clearly indicating a potent antihepatotoxic activity.

In summary, the study has shown that some of the synthesized compounds possess significant antihepatotoxic activity. They are simple, low molecular weight compounds and could be prepared easily. On the other hand, silybin is a complex molecule of high molecular weight and thus cannot be prepared easily. Furthermore the compounds are expected to be easily metabolizable, in comparison to silybin being simple and of low molecular weight.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.056.

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12. *Synthesis of 2,3-dihydro-1,4-benzodioxane-6-carbaldehyde (**1**)*: Anhydrous potassium carbonate (21 g) was added in portions to a stirred solution of 27.6 g of 3,4-dihydroxy benzaldehyde in 100 ml of dry acetone followed by the dropwise addition of 4.3 ml of ethylene dibromide. Another 21 g of potassium carbonate and 4.3 ml of ethylene dibromide were added similarly and this was repeated twice more using a total of 84 g of potassium carbonate and 17.2 g of ethylene dibromide. Stirring and refluxing was continued for another 25 h. The reaction mixture was then filtered by sintered glass funnel and the solid residue was washed several times with acetone. The combined filtrate was concentrated to about 25 ml and the residue was poured onto crushed ice, a solid was separated which was filtered, dried and crystallized with methanol to give a low melting solid; mp 35–37 °C; 67% yield; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.30 (2H, d, J = 8.7 Hz, CH_2-2), 4.33 (2H, d, J = 8.7, CH_2-3), 7.03 (1H, d, J = 8.4 Hz, ArH-8), 7.36 (1H, d, J = 2.5 Hz, ArH-5), 7.43 (1H, dd, J = 8.4, 2.5 Hz, ArH-7), 9.79 (1H, s, Ar-CHO).
13. *General method for the synthesis of 3-(2,3-dihydro-1,4-benzodioxane-6-yl)-1-substituted-phenylprop-2-en-1-one (**2a–o**)*: 25% solution of potassium hydroxide in aqueous ethanol (5 ml) was added dropwise to a mixture of substituted acetophenone (0.01 mol) and aldehyde **1** (0.01 mol) in 25 ml ethanol at 0–5 °C with stirring. The stirring was further continued for 10–12 h at room temperature. After completion of the reaction, the reaction mixture was poured onto crushed ice; the resulting solid was filtered, washed with water, dried and crystallized from ethanol. *Compound **2a***: mp 82–84 °C; 82% yield **:** ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.20 (4H, unresolved doublet , 2 $\times$ CH_2), 7.36–7.55 (5H, m, Ring A), 7.26 (1H, s, ArH-5), 7.10 (1H, d, ArH-7), 6.91 (1H, d, J = 9.0 Hz, ArH-8), 7.57 (1H, d, J = 18.9 Hz, H-a), 7.68 (1H, d, J = 15.6 Hz, H-b); FTIR (KBr) cm^{-1}: 3052 (=C-H, aromatic), 1647 (C=O), 1590 (C=C str.); HRMS (m/z): 266.2911; $[M]^+$ Anal. Calcd for C, 76.68; H, 5.30; O, 18.02. Found: C, 76.63; H, 5.25; O, 18.06.
14. *General method for the synthesis of 5-(2,3-dihydro-1,4-benzodioxane-6-yl)-3-phenyl-4,5-dihydro-1H-pyrazole (**3a–o**)*: To a solution of chalcone **(2a–o)** in absolute ethanol, hydrazine hydrate (99%) and a few drops of glacial acetic acid was added. The reaction mixture was heated under reflux for 10–15 h in presence of molecular sieves and then cooled and poured onto crushed ice. The solid thus obtained was filtered and recrystallized from ethanol. *Compound **3a***: ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.30 (4H, br s, 2 $\times$ OCH_2), 3.15 (1H, dd, J_{AB} = 17.2 Hz, J_{AC} = 2.5 Hz, CH_2-4_{HA}), 3.45 (1H, dd, J_{AB} = 17.2 Hz, J_{BC} = 10.8 Hz,CH_2-4_{HB}), 5.2 (1H, dd, J_{AB} = 17.2 , J_{AC} = 2.5 and J_{BC} = 10.8 Hz, CH-4_{HC}), 5.7 (1H, s, NH-pyrazoline), 6.92 -7.18 (m, 5H, Ar-ring A), 7.31 (1H, d, J = 2.5 Hz, ArH-5), 7.22 (1H, dd, J = 8.4, 2.5 Hz, ArH-7), 7.14 (1H , s Ar H-8); FTIR (KBr) cm^{-1}: 3052 (=C–H, aromatic), 3300 (pyrazoline NH), 1653 (C=C), 1590 (ring C=N), 1480 (ring N-N); HRMS (m/z): 280.3210 $[M]^+$; Anal. Calcd for: $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99; O, 11.42. Found: C, 72.89; H, 5.78; N, 10.02; O, 11.41.
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