Abstract:

This study was aimed at the synthesis of few xanthone derivatives containing 1,4-dioxane ring system. The reaction of 3,4-dihydroxy xanthone derivatives (3a-c) with ethylene dibromide and epichlorohydrin under appropriate reaction conditions afforded xanthone derivatives containing 1,4-dioxane ring system (4a-e). The structures of the synthesized compounds were confirmed on the basis of their chemical and spectral data. This work was also extended to study the possible antihepatotoxic activity of the newly synthesized compounds against carbon tetrachloride (CCl₄)-induced hepatotoxicity in female Wistar albino rats by estimating the levels of biochemical parameters such as total proteins (TP), total albumin (TA), alkaline phosphatase (ALKP), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT). Compound 4b [3,4-(2′-hydroxy methyl-1′,4′-dioxano) xanthone] showed a potent antihepatotoxic activity comparable to the standard reference drug Silybon-70®, whereas other compounds exhibited moderate activity. It was also observed that the unsubstituted xanthone derivatives possessing 1,4-dioxane ring have better antihepatotoxic activity in comparison to nitro- and bromo-substituted xanthone derivatives.

Keywords: Antihepatotoxic activity; Xanthone; 1,4-Dioxane; Silymarin; Silybum marianum
Introduction

Liver disease is a leading cause of death in many countries which occurs due to alcohol consumption, malnutrition, continuous exposure to environmental pollutants, hepatotoxic drugs, chemicals toxins and infections [1]. It results in various disorders such as acute viral hepatitis, chronic viral hepatitis and liver cirrhosis. The different medical, surgical and therapeutic methods available at present for the treatment of liver diseases are inadequate with generally poor results. Therefore, it is essential to search for newer drugs for the treatment of liver diseases. It is well known that some naturally occurring medicinal plants are potent hepatoprotective drugs and are widely used in alternative system of medicine throughout the world to cure liver ailments [2]. Silymarin isolated from the seeds of *Silybum marianum*, commonly known as *Milk thistle* [3], has been used as a potent antihepatotoxic agent against a variety of toxicants in modern medicine [4]. Silymarin is a complex mixture of three flavolignan isomers namely, silybin, silydianin and silychristin [5]. Among the isomers, silybin is the major and the most active component and represents about 60-70%, followed by silychristin (20%) and silydianin (10%) [6]. Silybin contains 1, 4-dioxane ring system in its structure, whereas the other isomers i.e. silychristin and silydianin do not possess 1, 4-dioxane ring and they do not display significant activity [7]. We, therefore, proposed that 1, 4-dioxane unit plays an important part in exhibiting antihepatotoxic activity and thus incorporated 1, 4-dioxane ring system in chalcones and various heterocyclic compounds like coumarins, flavones and oxadiazoles [7,8] and studied the pharmacological role of 1, 4-dioxane on the liver. We got the favourable results which further encouraged us to prepare some new heterocyclic chemical compounds containing 1, 4-dioxane ring system in search of effective and safer antihepatotoxic agents.

Xanthones (9-H-xanthene-9-ones) are heterocyclic compounds with the dibenzo-γ-pyrone framework which does not occur in nature [9]. However, a number of xanthone derivatives are secondary plant metabolites and have been isolated from natural sources [10-12].

![Figure 1](image_url)

*Figure 1* Structures of a) silybin, b) silychristin and c) silydianin
These oxygenated heterocyclic derivatives bear a structural relationship to flavonoids and coumarins and possess diverse pharmacological properties such as antioxidant [13], anti-inflammatory [14], antimicrobial [15], antifungal [16], diuretic and monoamine oxidase (MAO) inhibitory activity [17]. Literature survey reveals that xanthone derivatives have not been paid much attention for their antihepatotoxic activity though they have marked antioxidant activity. Hence the present study was undertaken to synthesize few compounds having different nature and positions of substituents like-NO2, -Br on the xanthonic nucleus as well as -CH2OH side chain on position-2 of dioxane ring and to study their antihepatotoxic potential. In this paper, we report the synthesis of five new xanthone derivatives containing 1, 4-dioxane ring system (4a-e) and their antihepatotoxic activity in carbon tetrachloride-intoxicated rats. The aspect of structure activity relationship (SAR) of the synthesized compounds is also tentatively discussed.

Materials and Methods

All chemicals were purchased from E. Merck (Germany) and S. D. Fine Chemicals (India) and were of analytical reagent grade. All the kits were the products of Biosystems (Spain) and Sigma Chemical Company (USA). Silybon-70® tablets (a standardized extract of silymarin) manufactured by Micro Labs India, were purchased from the local Pharmacy. All melting points were determined in open capillaries and were uncorrected. Purity of the compounds was checked on thin layer chromatography (Silica gel G) plates using iodine vapours as visualizing agent. The IR (KBr) spectra were recorded on a Hitachi IR- 270-300 spectrometer (cm⁻¹). ¹H NMR spectra were recorded on 300 MHz (Bruker model DRX-300 NMR spectrometer) in CDCl₃ and DMSO-d₆ using TMS as an internal reference (chemical shift in δ ppm) and mass spectra on Jeol JMS DX-303 spectrometer and are presented as m/z.

Synthesis of 3, 4-dihydroxy xanthone (3a) [18]

A mixture of POCl₃ (30 mL) and fused ZnCl₂ (13 g) were added to salicylic acid (1a) (4.14 g, 30 mmol) and pyragallol (2) (6.3 g, 50 mmol). It was then heated over sand bath for 2.5 h at 70°C. After cooling it was poured in crushed ice where upon a brown solid appeared. It was purified by silica gel column chromatography using petroleum ether : ethyl acetate (4:1) as eluent to obtain the crystals of 3a; Rf: 0.81 (petroleum ether : ethyl acetate, 3:2); yield: 66%; m.p. 241-242°C (Ref [19] 238-240°C); IR (KBr): Vmax 3380 (OH), 1670 (C=O), 1491 (C=C), 1154, 1062 (C-O), 983, 759 and 628; ¹H-NMR (CDCl₃): δ 6.557 (d, 1H, J=8.4 Hz, H-1), 6.68 (d, 1H, J=8.3 Hz, H-2), 7.0 (brm, 2H, H-7,8), 7.474 (brm, 2H, H-5,6), 10.19 (brs, 1H, OH), 10.32 (brs, 1H, OH); MS (70 ev): m/z 228 (M⁺, C₁₃H₈O₄) (7.4), 200 (2.3), 192 (5.5), 150 (5.7), 121 (100), 92 (16.2).

Synthesis of 3, 4-dihydroxy xanthone (3b)

It was prepared by the above procedure using 3, 5 dinitro salicylic acid (1b) (2.01, 10 mmol); Rf: 0.87 (benzene:ethyl acetate, 8:2); yield: 81%; m.p. 116-118°C; IR (KBr): Vmax 3400 (OH), 3060 (Ar-CH), 1700 (C=O), 1630, 1590, 1560, 1470, 1400 (NO₂), 1270, 1170, 860, 750, 640; ¹H-NMR (CDCl₃): δ 6.37(d, 1H, J=8.4Hz, H-1), 6.91 (d, 1H, J=8.3 Hz, H-2), 7.75 (d, 1H, J=2.7 Hz, H-6), 7.89 (d, 1H, J=2.7 Hz, H-8), 8.72 (brs, 1H, OH), 9.60 (brs, 1H, OH).

Synthesis of 3, 4-dihydroxy xanthone (3c)

It was prepared by the above procedure using 3, 5 dibromo salicylic acid (1c) (3.16, 10 mmol); Rf: 0.71 (benzene:ethyl acetate, 8:2); yield: 76%; m.p. 185-187°C; IR (KBr): Vmax 3400 (OH), 3060 (Ar-CH), 1700 (C-O), 1630, 1590, 1560, 1500 (C=C), 1460, 1340, 1300, 1200, 1150, 1060, 940, 860, 760 (Br); 650; ¹H-NMR (DMSO-d₆): δ 6.37(d, 1H, J=8.4Hz, H-1), 6.91 (d, 1H, J=8.3 Hz, H-2), 7.75 (d, 1H, J=2.7 Hz, H-6), 7.89 (d, 1H, J=2.7 Hz, H-8), 8.72 (brs, 1H, OH), 9.60 (brs,1H, OH).

Synthesis of 3, 4-(1’,4’-dioxano) xanthone (4a)

A solution of KOH (1.84 g, 33 mmol) in water (15 mL) was added to the mixture of 3a (2.28 g, 10 mmol) and 1,2-dibromo ethane (4.23 g, 22.5 mmol) in water (10 mL) with stirring. After 20 h at reflux, the solvent and excess of 1,2-dibromo ethane were removed under...
vacuum. The residue was taken up in chloroform and the insoluble material was filtered off, the organic layer was dried over anhydrous Na₂SO₄ and evaporated to get the yellowish solid, which was recrystallized from methanol; Rₖ: 0.47 (benzene: methanol, 4:1); yield: 33%; m.p. 197–198°C; IR (KBr): Vmax 2497 (CH₂), 1654 (C=O), 1560, 1508 (C=C), 1260, 1190, 1082 (C-O), 898, 789, 621; ¹H-NMR (DMSO-d₆): δ 4.19 (m, 4H, 2 x CH₂), 6.56 (d, 1H, J=8.4Hz, H-1), 6.69 (d, 1H, J=8.3 Hz, H-2), 7.02 (brm, 2H, H-7, 8), 7.57 (brm, 2H, H-5, 6); MS (70 eV): m/z 254 (M+ C₁₅H₁₀O₄) (100), 226(4.5), 194 (9.6), 175 (19.1), 121 (84.2), 93 (20.1).

Synthesis of 3, 4-(2′-hydroxy methyl, 1′, 4′-dioxano) xanthone (4b)

2.28 g (10 mmol) of compound 3a was dissolved in an aqueous ethanol [30 mL of alcohol (95%) in 17.1 mL of water] containing sodium hydroxide (0.5 g). To this epichlorohydrin (8.0 mL, 9 mmol) was added and the resulting solution was heated under reflux at 75°C for about 2 h with stirring. The solution was then further stirred for 3 h at room temperature. Ice-cold water was added to the reaction mixture. The oily fraction that settled down at the bottom of the flask was separated from the aqueous layer and concentrated to get the semi-solid crude product; Rₖ: 0.63 (benzene: ethyl acetate, 9:1); yield: 47%; ¹H-NMR (DMSO-d₆): δ 3.73 (ddd, 1H, J=2.1, 7.2, 2.4 Hz, Ha-3″), 3.76 (ddd, 1H, J=2.4, 7.9, 2.1 Hz, Hb-3″), 3.85 (brm, 1H, W₂=5.2, -CH₂OH), 4.09 (ddd, 1H, J=2.3, 7.4, 2.1 H₂O), 4.175 (ddd, 1H, J=2.3, 9.0, 2.4 H₂O), 6.50 (d, 1H, J=8.4Hz, H-1), 6.61 (d, 1H, J=8.0 Hz, H-3), 7.02 (brm, 2H, H-7,8). 7.28 (brm, 2H, H-5,6); MS (70 eV); m/z 284 (M+ C₁₆H₁₂O₅) (22.3), 256 (7.0), 254 (3.9), 226 (5.8), 205 (4.9), 121 (58.6), 93 (10.3).}
tetrachloride (CCl₄) mixed with liquid paraffin (1:1) was used as a hepatotoxic agent. The drugs were administered for seven days after CCl₄ administration, in the form of an aqueous suspension made from carboxymethyl cellulose as per the following treatment schedule.

Group I (normal control) was given only distilled water. Group II (toxic control) was treated with CCl₄ (1.0 mL/kg) for the first day of study to produce toxicity in the liver. Group III (Silybon-70® treated) was given a single dose of CCl₄ (1.0 mL/kg) on the first day and then Silybon-70® 10 mg/kg, daily) was given for seven days. Groups IV to VIII were administered with a single dose of CCl₄ (1.0 mL/kg) on the first day followed by an oral treatment with a daily dose (10 mg/kg) of xanthones 4a-e respectively for seven days. On the last day, blood was collected directly from the retro plexus orbital of four rats from each group and serum was separated for biochemical analysis.

**Biochemical analysis**

The biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT) [20], serum glutamate pyruvate transaminase (SGPT) [20], alkaline phosphatase (ALKP) [21], total proteins (TP), total albumin (TA) were analyzed according to the reported methods [22].

**Statistical analysis**

The results of the biochemical estimations were reported as mean ± S.E.M. The statistical analysis was done by one-way analysis of variance (ANOVA) followed by Student’s ‘t’ test by using 13th version of SPSS software.

**Results and Discussion**

The results of the synthesized compounds (Scheme 1) and their pharmacological screening have been summarized in Table 1. The compounds were obtained in average yield and were characterized by their spectral and chemical data. Single dose of CCl₄ (1 mL/kg) significantly elevated the SGPT, SGOT and ALKP activities (71.52, 59.40 and 46.34 units/mL) when compared to the normal animals (36.28, 30.30 and 15.80 units/mL) respectively indicating hepatocellular damage. Treatment of the rats with the compounds under investigation have decreased the enzyme levels in the range of 58.90-66.80 units/mL for SGOT, 47.24-54.20 units/mL for SGPT and 35.92-39.70 units/mL for ALKP, which were found to be comparable to the enzyme levels (SGOT, SGPT, ALKP) reduced by standard drug Silybon-70 (53.84, 45.80 and 28.88 units/mL, respectively). Silybon-70, a standardized extract of silymarin, is a well established hepatoprotective drug capable of decreasing the elevated levels of liver enzymes in various drug induced hepatotoxicity [23]. The most potent compound which significantly reversed the hepatotoxicity and exhibited almost similar activity comparable to standard drug Silybon-70 was found to be the compound 4b (58.90, 47.20 and 35.92 units/mL, respectively). Other compounds also exhibited a moderate activity (61.04-66.80 units/mL, 50.68-54.20 units/mL and 37.40-39.70 units/mL respectively).

The toxicant CCl₄ also reduced the level of total protein (4.31 g/dL) and increased the level of total albumin (4.30 g/dL) in comparison to normal values (5.32 g/dL, 3.32 g/dL, respectively). The administration of test compounds enhanced the reduced level of total protein in the range of 4.49-5.15 g/dL, and decreased the elevated values of total albumin in the range of 3.75-4.00 g/dL in comparison to standard drug Silybon-70 (6.22 and 3.76 g/dL, respectively). The most potent compound 4b i.e., 3, 4 (2′-hydroxy methyl 1′, 4′-dioxano) xanthone (5.15 and 3.75 g/dL, respectively) displayed these values comparable to standard drug Silybon-70, whereas other derivatives showed inferior results.

It was also observed that compound 4b possess 2-hydroxy methyl group at position-2 of the dioxane ring of xanthone derivative, which also suggests that the presence of hydroxy methyl group at position-2 in dioxane ring might play a significant role in exhibiting the antihepatotoxic activity. This is in accordance with the view that silybin too possess the same group at the same position which might be responsible for its better absorption and bioavailability.

The substitution in the aromatic ring of xanthones has no significant role in exhibiting antihepatotoxic activity. However, it was observed that the unsubstituted xanthone derivatives have better activity in comparison to
nitro- and bromo-substituted xanthones. Thus it could be inferred that introduction of electron withdrawing groups like -NO₂ or -Br on ring B of xanthone nucleus decreases the antihapatotoxic activity.

Table 1 Biochemical parameters of synthesized compounds in CCl₄ induced hepatotoxicity in albino rats

<table>
<thead>
<tr>
<th>S.No.</th>
<th>R₁</th>
<th>R₂</th>
<th>X</th>
<th>TA (g/dL)</th>
<th>TP (g/dL)</th>
<th>ALKP (units/mL)</th>
<th>SGPT (units/mL)</th>
<th>SGOT (units/mL)</th>
<th>Dose (mg/kg/b.wt)</th>
<th>Treatment</th>
<th>Groups</th>
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<td>3.32 ± 0.17</td>
<td>4.32 ± 0.08</td>
<td>3.76 ± 0.13*</td>
<td>3.95 ± 0.08*</td>
<td>3.75 ± 0.03*</td>
<td>3.89 ± 0.03*</td>
<td>4.00 ± 0.07*</td>
<td>3.94 ± 0.05*</td>
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<tr>
<td>5.32 ± 0.16</td>
<td>4.31 ± 0.48</td>
<td>6.22 ± 0.19*</td>
<td>4.93 ± 0.05*</td>
<td>5.15 ± 0.05*</td>
<td>4.95 ± 0.08*</td>
<td>4.75 ± 0.08*</td>
<td>4.49 ± 0.07</td>
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<tr>
<td>15.80 ± 0.37</td>
<td>46.34 ± 1.04</td>
<td>28.88 ± 0.23*</td>
<td>38.56 ± 0.29*</td>
<td>35.92 ± 0.91*</td>
<td>38.38 ± 0.29*</td>
<td>39.70 ± 0.21*</td>
<td>37.40 ± 0.18*</td>
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<tr>
<td>30.30 ± 0.19</td>
<td>59.40 ± 0.36</td>
<td>45.80 ± 0.62*</td>
<td>51.40 ± 0.55*</td>
<td>47.24 ± 0.47*</td>
<td>50.68 ± 0.33*</td>
<td>54.20 ± 0.46*</td>
<td>50.90 ± 0.39*</td>
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<tr>
<td>36.28 ± 1.19</td>
<td>71.52 ± 1.36</td>
<td>53.84 ± 0.65*</td>
<td>61.51 ± 0.32*</td>
<td>58.90 ± 0.35*</td>
<td>65.26 ± 1.01*</td>
<td>66.80 ± 0.80*</td>
<td>61.04 ± 0.37*</td>
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</table>

Conclusion

In the present study, administration of xanthon derivatives containing 1, 4-dioxane ring system significantly protected against CCl₄ induced hepatotoxicity.

Scheme 1 Synthesis of xanthone derivatives
in rats. Compound 4b having a hydroxymethyl group on the 1,4-dioxane ring was found to be the most potent antihepatotoxic agent among the synthesized compounds. The exact mechanism by which it protects the liver is unknown however the effect could be due to the significant antioxidant activity of xanthones [13] and due to the presence of 1,4-dioxane ring. Further detailed studies are needed to ascertain their exact mechanism of action.

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