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Synthesis and antihepatotoxic activity of some heterocyclic compounds containing the 1,4-dioxane ring system

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Silymarin isolated from *Silybum marianum* is a mixture of three isomers, silybin (**1**), silydianin (**2**) and silychristin (**3**). Silybin is the most active antihepatotoxic agent, and contains a 1,4-dioxane ring in addition to a flavonoid moiety. Based on the skeleton of silybin, we prepared some flavones and coumarins containing the 1,4-dioxane ring system and evaluated them for antihepatotoxic activity against carbon tetrachloride induced hepatotoxicity in albino rats. The degree of protection was determined by measuring biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALKP), total protein (TP) and total albumin (TA). The compounds namely 3′,4′(1″,4″-dioxino) flavone (**4f**), and 3′,4′(2-hydroxy methyl, 1″,4″-dioxino) flavone (**4g**) were found to exhibit a significant activity comparable to standard drug silymarin (silybon-70). Other compounds also exhibited good activity. The structure activity relationship (SAR) was also studied, and where the flavonoid analogues containing a hydroxy methyl group at position-2″ in the dioxane ring exhibited superior antihepatotoxic activity in comparison to coumarin derivatives.

1. Introduction

The liver is continuously exposed to a variety of xenobiotics, therapeutic agents and environmental pollutants leading to various disorders such as acute viral hepatitis, chronic viral hepatitis, liver cirrhosis and drug induced liver damage. A large number of medicinal plants have been chemically and pharmacologically investigated, as a result of which natural chemical components possessing antihepatotoxic activities have been obtained [1, 2]. Amongst them silymarin isolated from seeds of *Silybum marianum* commonly known as milk thistle has been found to be as most potent antihepatotoxic agent against a variety of toxicants [3], and is used as a safe drug (silybon-70) for the treatment of a variety of liver diseases. Silymarin is a mixture of three isomers namely, silybin (**1**), silydianin (**2**) and silychristin (**3**) [4]. Silybin is the most active component containing a 1,4-dioxane ring system, whereas the other isomers, silychristin and silydianin, do not possess a 1,4-dioxane ring [5], and thus do not display significant antihepatotoxic activity. We therefore thought that the 1,4-dioxane unit plays an important role in causing antihepatotoxic activity, and consequently prepared some heterocyclic compounds, namely coumarins and flavones, possessing the 1,4-dioxane ring system. The compounds synthesized are simple, of low molecular weight and can be easily synthesized in the laboratory in comparison to silybin. The compounds were screened for their antihepatotoxic activity in albino rats using CCl_4 as toxicant. The antihepatotoxic activity was monitored by estimating the liver enzymes, proteins and alkaline phosphatase. Some of the compounds namely 3′,4′(1″,4″-dioxino) flavone (**4f**), 3′, and 4′(2-hydroxy methyl, 1″,4″-dioxino) flavone (**4g**) showed a potent antihepatotoxic activity, whereas other exhibited only a moderate activity with respect to the standard drug silybon-70.

Scheme 1

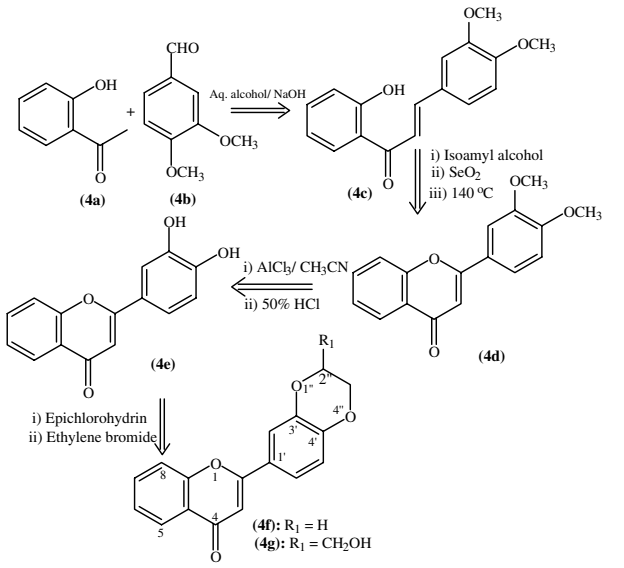


Table: Effect of the synthesized compounds on serum enzymes, alkaline phosphatase, total proteins and albumin in CCl_4 induced liver damage in rats[a]

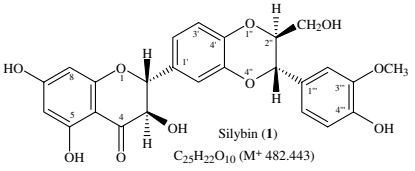
Groups n = 5	Treatment	Substituent	Dose mg/kg/b.w	SGOT ± S.E units/ml	SGPT ± S.E units/ml	ALKP ± S.E units/ml	TP ± S.E g/dl	TA ± S.E g/dl
I	Normal	–	–	36.28 ± 1.19	30.3 ± 0.196	15.8 ± 0.37	5.32 ± 0.156	3.32 ± 0.168
II	Toxic	–	1 ml/kg	71.52 ± 1.36	59.4 ± 0.354	46.34 ± 1.043	4.306 ± 0.48	4.30 ± 0.075
III	Silybon-70	–	10 mg	53.84 ± 0.65***	45.8 ± 0.62***	28.88 ± 0.23***	6.219 ± 0.186**	3.76 ± 0.13**
IV	Compd. **4f**	–	10 mg	57.04 ± 1.02***	47.7 ± 0.649***	34.56 ± 0.607***	5.18 ± 0.023*	3.71 ± 0.029***
V	Compd. **4g**	2″-CH_2OH	10 mg	53.88 ± 0.373***	47.06 ± 0.65***	30.76 ± 0.236***	5.72 ± 0.128**	3.59 ± 0.089***
VI	Compd. **5e**	–	10 mg	61.54 ± 0.727*	49.5 ± 0.348*	36.06 ± 0.266***	4.696 ± 0.094	3.818 ± 0.034***
VII	Compd. **5f**	4-Me	10 mg	59.66 ± 0.278***	47.68 ± 0.517***	34.9 ± 0.345***	4.96 ± 0.045	3.802 ± 0.053***
VIII	Compd. **5g**	4-Me, 2″-CH_2OH	10 mg	67.80 ± 0.48***	54.24 ± 1.65***	39.02 ± 0.252***	4.756 ± 0.077	3.856 ± 0.047***
IX	Compd. **5h**	2″-CH_2OH	10 mg	60.34 ± 0.525*	48.62 ± 0.232*	35.143 ± 0.256***	4.567 ± 0.083	3.816 ± 0.029***

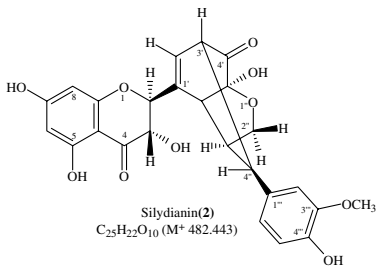
[a] SGOT = Serum glutamic oxaloacetic transaminase; SGPT = Serum glutamic pyruvic transaminase; ALKP = Alkaline Phosphatase; TP = Total Protein; TA = Total Albumin.
* $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$ Vs intoxicated control using Student's t-test.
Values are mean ± S.E. of five rats.

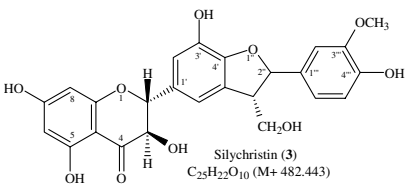
2.1. Investigations, results and discussion

Details of the compounds synthesized and their pharmacological screening have been summarized in the Table. The compounds were obtained in good yield and were characterized by their spectral and chemical data. The levels of enzymes such as SGOT, SGPT, ALKP were elevated on administration of CCl_4 to 71.52, 59.4 and 46.34 units/ml in comparison to normal values of 36.28, 30.3 and 15.8 units/ml respectively. The administration of the compounds under investigation decreased the enzyme levels to values in the range of 53.88–67.8 units/ml for SGOT, 47.06–54.24 units/ml for SGPT, and 30.76–39.02 units/ml for ALKP, which were found to be comparable to the reduced enzyme levels with the standard drug silybon-70 (53.84, 45.8 and 28.88 units/ml respectively). The most potent compounds that exhibited an antihepatotoxic activity almost comparable with that of the standard drug silybon-70 were found to be dioxino flavone **4f** (57.04, 47.7 and 34.56 units/ml respectively), and hydroxy methyl dioxino flavone **4g** (53.88, 47.06 and 30.76 units/ml respectively). The other compounds also exhibited a moderate antihepatotoxic activity (60.34–67.8 units/ml, 47.68–54.24 units/ml and 30.9–39.06 units/ml respectively). The results show that the flavone derivatives exhibited a more potent activity in comparison to the coumarin derivatives, which indicates that in addition to the 1,4-dioxane ring system, the flavone moiety also plays an important role in antihepatotoxic activity.

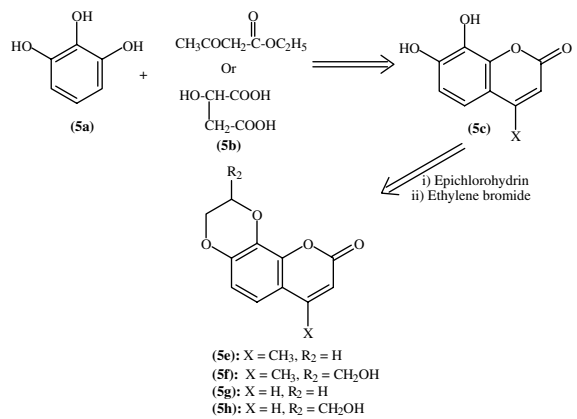
The toxicant CCl_4 also reduced the level of total protein (4.306 g/dl) and increased the level of total albumin (4.30 g/dl) in comparison to normal values (5.32 g/dl, 3.32 g/dl respectively). The administration of the test compounds elevated the reduced level of total protein to values in the range of 4.567–5.72 g/dl and decreased the elevated values of total albumin in the range of 3.59–







Scheme 2



3.856 g/dl in comparison to the standard drug silybon-70 (6.219 and 3.76 g/dl respectively). The most potent compounds namely dioxino flavone **4f** (5.18, 3.71 g/dl) and hydroxy methyl dioxino flavone **4g** (5.72, 3.59 g/dl) displayed values comparable to the standard drug silybon-70 (6.219, 3.76 g/dl), and normal values (5.32, 3.32 g/dl respectively) (Table). In contrast, the coumarin derivatives showed inferior results exhibiting total protein levels in the range of 4.567–4.96 g/dl, and total albumin levels between 3.802–3.856 g/dl. This again indicated that the flavone moiety as well as the 1,4-dioxane ring system are necessary for exhibiting a potent antihepatotoxic activity. This is also in accordance with the silybin moiety, which contains both a flavone unit and a 1,4-dioxane ring system.

It was also observed that the introduction of a hydroxy methyl group ($-CH_2OH$) at position-2″ in the dioxane ring of the flavonoid derivative, e.g. compound **4g**, increased the antihepatotoxic activity. The levels of liver enzymes were comparatively lower for compound **4g** (SGOT = 53.88; SGPT = 47.06; ALKP = 30.76 units/ml) than for compound **4f** (SGOT = 57.04; SGPT = 47.7; ALKP = 34.56 units/ml). In the same way the total protein was increased (5.72 g/dl), while total albumin was decreased (3.59 g/dl) in compound **4g** as compared to compound **4f** (TP = 5.18; TA = 3.71g/dl), giving a further indication that the presence of a hydroxy methyl group at position-2″ in the dioxane ring plays a significant role in poducing antihepatotoxic activity. This is also in accordance with the above view as silybin, too, possesses the same group at the same position. On the other hand the introduction of a hydroxy methyl group at the same position and a methyl group at position-4 in coumarin analogues had no significant role in producing the antihepatotoxic activity. It has, however, been observed that the introduction of a hydroxy methyl group at position-2″ in the dioxane ring had negative effects in the case of coumarin derivatives, e.g. compound **5h**, and resulted in a decrease in activity. The introduction of a methyl group at position-4 in the coumarin series, e.g. compound **5f** however, slightly enhanced the antihepatotoxic activity (Table). The coumarin derivatives containing both a hydroxy methyl group at position-2′ in the dioxane ring, and a methyl group at position-4 in the aromatic ring, e.g. compound **5g**, displayed least activity, consequently indicating a combined negative effect on antihepatotoxic activity.

The studies have shown that compounds which contain both a flavone moiety and a 1,4-dioxane ring together with a 2-hydroxy methyl group in the molecule could exhibit potent antihepatotoxic activity, and in turn, could be used for the treatment of various ailments of the liver. The compounds synthesized are simple, and of low molecular weight and thus 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 silymarin, being simple and of low molecular weight. Moreover, additional compounds could also be synthesized to find comparatively superior molecules possessing better antihepatotoxic activity.

3. Experimental

3.1. Synthesis of the compounds

Melting points were determined in capillary tubes and are uncorrected. IR (KBr) spectra in cm^{-1} were recorded on a Hitachi IR-270–300 spectrophotometer. ^1H NMR (300 MHz) spectra were recorded on a Bruker model DRX-300 NMR spectrometer in $CDCl_3$ and DMSO-d_6 using TMS as an internal reference, chemical shift in d ppm, and J values in Hz. The MS were obtained on a Jeol-JMS DX-303 spectrometer. Purity of the compounds was checked on silica gel G plates using iodine vapour as the visualizing agent.

3.1.1. Synthesis of 2-hydroxy 3′,4′-dimethoxy chalcone (**4c**) [6]

Ortho-hydroxy acetophenone (1.36 g, 10 mmol) dissolved in oxygen free ethanol (20 ml) was added to verateraldehye (1.66 g, 10 mmol) in a solution of sodium hydroxide (40%, 8 ml) in oxygen free water drop wise with constant stirring at 0 °C. The solution was stirred for 2 h on a magnetic stirrer and then acidified with dilute hydrochloric acid. A yellow colored solid so separated was filtered, washed with sodium bicarbonate solution (2%) and then with ice-cold water and dried. It was then recrystallized from methanol. m.p. 96–98 °C; yield: 87%; R_f: 0.73 (benzene : methanol, 9 : 1); IR (KBr): ν_{max} 3429 (OH), 2985 (CH_3), 1719 (C=O), 1520 (C=C), 1426, 1344, 1223, 1108, 1040 (CO), 927, 773, 653 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.98 (6 H, s, $2 \times OCH_3$), 6.935 (1 H, q, J = 1.8, 8.5 Hz, H-6), 6.976 (1 H, q, J = 1.8, 8.5 Hz, H-4), 7.003 (1 H, q, J = 1.8, 8.5 Hz, H-5), 7.07 (1 H, q, J = 1.8, 8.5 Hz, H-3), 7.202 (1 H, d, J = 1.8 Hz, H-2′), 7.288 (1 H, q, J = 2.1, 8.5 Hz, H-6′), 7.496 (1 H, d, J = 8.5 Hz, H-5′), 7.525 (1 H, d, J = 15.6 Hz, H-α), 7.948 (1 H, d, J = 15.6 Hz, H-β).

3.1.2. Synthesis of 3′,4′-dimethoxy flavone (**4d**) [6]

A mixture of chalcone **4c** (2.82 g, 10 mmol) and selenium dioxide (2.0 g) was dissolved in dry isoamyl alcohol (30 ml). It was refluxed at 140–150 °C for 12–14 h under anhydrous conditions. The precipitated selenium was filtered off, and the filtrate was steam distilled to remove isoamyl alcohol. The compound was then recrystallized from methanol, m.p. 112–113 °C; yield: 73%; R_f: 0.62 (benzene : methanol, 9 : 1); IR (KBr): ν_{max} 2835 (CH_3), 1654 (C=O), 1518 (C=C), 1466, 1371, 1267, 1029 (CO), 941, 773 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.994 (6 H, s, $2 \times OCH_3$), 6.79 (1 H, s, H-3), 7.0 (1 H, d, J = 8.7 Hz, H-5′), 7.41 (2 H, q, J = 1.8, 8.5 Hz, H-7, 8), 7.58 (1 H, d, J = 1.5 Hz, H-2′), 7.61 (1 H, q, J = 1.8, 8.5 Hz, H-5), 7.69 (1 H, q, J = 1.8, 8.5 Hz, H-6), 8.42 (1 H, q, J = 1.5, 8.7 Hz, H-6′); MS (70 eV): m/z 282 (M^+ $C_{17}H_{14}O_4$), 267, 254, 251, 220, 162, 121, 91.

3.1.3. Synthesis of demethylated flavone (**4e**)

The flavone **4d** (2.83 g, 10 mmol) was added to a solution of anhydrous aluminum chloride (5.0 g) in dry acetonitrile (200 ml) and the mixture was refluxed for 3 h. The solvent was distilled off and the aluminium complex was broken with ice cold hydrochloric acid (1 : 1, 100 ml). The aqueous solution was extracted with ethyl acetate (50 ml × 3) and then the combined extract was dried over anhydrous sodium sulphate. The solvent was evaporated to obtain a yellow mass, which was crystallized from dilute alcohol, m.p. 185–186 °C; yield: 41%; R_f: 0.68 (benzene : methanol, 9 : 1), IR (KBr): ν_{max} 3248 (OH), 1657 (C=O), 1502 (C=C), 1432, 1366, 1289, 1128, 1092 (CO), 932, 704 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.75 (1 H, s, H-3), 7.1 (1 H, d, J = 8.6 Hz, H-5′), 7.45 (2 H, q, J = 1.8, 8.5 Hz, H-7, 8), 7.60 (1 H, d, J = 1.5 Hz, H-2′), 7.63 (1 H, q, J = 1.8, 8.5 Hz, H-5), 7.68 (1 H, q, J = 1.8, 8.5 Hz, H-6), 8.44 (1 H, q, J = 1.5, 8.6 Hz, H-6′).

3.1.4. Synthesis of 3′,4′(1″,4″-dioxino) flavone (**4f**)

A solution of potassium hydroxide (1.84 g, 33.0 mmol) in water (15.0 ml) was added to a mixture of demethylated flavone **4e** (2.54 g, 10.0 mmol) and 1, 2-dibromoethane (4.23 g, 22.5 mmol) in water (10 ml) with stirring. After 20 h at reflux, the solvent and excess 1,2-dibromoethane were removed *in vacuo*. The residue was taken up in chloroform, the insoluble material was filtered off, and the organic layer was dried over anhydrous sodium sulphate and evaporated to give a yellowish solid, which was recrystallized from methanol; m.p. 225–226 °C; yield: 51.5%; R_f: 0.38 (benzene : methanol, 4 : 1); IR (KBr): ν_{max} 2875 (CH_2), 1647 (C=O), 1510 (C=C), 1461, 1376, 1289, 1265, 1065 (CO), 892, 751 cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.326 (4 H, dddd, J = 1.5, 6.3, 1.2, 6.8 Hz, $2 \times CH_2$), 6.740 (1 H, s, H-3), 6.976 (1 H, q, J = 2.0, 8.5 Hz, H-5), 7.39 (1 H, q, J = 2.0, 8.5 Hz, H-8), 7.434 (1 H, q, J = 2.0, 8.5 Hz, H-7), 7.475 (1 H, q, J = 9.0 Hz, H-5′), 7.562 (1 H, d, J = 2.1 Hz, H-2′), 7.667 (1 H, q, J = 2.0, 8.5 Hz, H-6), 8.213 (1 H, q, J = 2.1, 9.0 Hz, H-6′); MS (70 eV): m/z 281 (M^+ $C_{17}H_{13}O_4$), 253, 221, 160, 121, 100, 93.

3.1.5. Synthesis of 3′,4′(2-hydroxy methyl, 1″,4″-dioxino) flavone (**4g**)

The demethylated flavone **4e** (2.54 g, 10 mmol) was dissolved in aqueous ethanol (30 ml of 95% alcohol in 17.1 ml of water) containing sodium hydroxide (0.5 g). To this epichlorohydrin (8.0 ml, 9.0 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 which settled at the bottom of the flask was separated from the aqueous layer and concentrated to get a solid crude product, which was recrystal-

lized from aqueous alcohol; m.p. 108–110 °C; yield: 54%; R_f: 0.41 (benzene : methanol, 4 : 1); IR (KBr): ν_{max} 3425 (OH), 2885 (CH_2), 1685 (C=O), 1509 (C=C), 1461, 1277, 1117, 1031 (C–O), 812, 759 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.49 (2 H, brm, CH_2OH), 3.68 (1 H, brm, C*H*), 4.032 (2 H, brm, CH_2), 6.74 (1 H, s, H-3), 6.976 (1 H, q, J = 2.0, 8.5 Hz, H-5), 7.39 (1 H, q, J = 2.0, 8.5 Hz, H-8), 7.485 (1 H, q, J = 2.1, 9.0 Hz, H-5′), 7.582 (1 H, d, J = 2.1 Hz, H-2′), 7.679 (1 H, q, J = 2.0, 8.5 Hz, H-6), 8.213 (1 H, q, J = 2.1, 9.0 Hz, H-6′); MS (70 eV): m/z 311 (M^+ $C_{18}H_{15}O_5$), 309, 283, 280, 221, 190, 161, 143, 121, 111, 93.

3.1.6. Synthesis of 4-methyl 7,8-dihydroxy coumarin (**5c**)

100 ml conc. H_2SO_4 was placed in a 500 ml flask fitted with a thermometer. The flask was immersed in an ice bath, and when the temperature fell below 10 °C, a solution of pyragallol (12.0 g, 91.0 mmol) in ethyl acetoacetate (13.5 ml, 103.0 mmol) was added drop wise with stirring. During addition the temperature was maintained below 10 °C by means of an ice salt bath. The reaction mixture was kept at room temperature for 18 h and then placed over crushed ice with vigorous stirring. The solid obtained was recrystallized from aqueous ethanol; m.p. 228–229 °C; yield: 69%; R_f: 0.92 (toluene : ethyl acetate : formic acid, 5 : 4 : 1); IR (KBr): ν_{max} 3255 (OH), 1651 (C=O), 1513 (C=C), 1410, 1141, 1007 (CO), 964, 875, 804, 769, 630 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.81 (3 H, s, CH_3), 6.12 (1 H, s, H-3), 6.80 (1 H, d, J = 8.7 Hz, H-5), 7.078 (1 H, d, J = 8.7 Hz, H-6), 9.01 (1 H, brs, O*H*), 10.75 (1 H, brs, O*H*); MS (70 eV): m/z 192 (M^+ $C_{10}H_8O_4$), 164, 151, 135, 118, 94.

3.1.7. Synthesis of 7,8-dihydroxy coumarin (**5d**)

Pyragallol (12.0 g, 91 mmol) was dissolved in conc. sulphuric acid (100 ml) and heated to 120–130 °C. DL-malic acid (1.34 g, 110 mmol) was then added portion wise over a period of 1 h until the evolution of CO_2 ceased. The final dark viscous liquid was poured on to crushed ice, whereupon a brown precipitate separated, which was recrystallized from aqueous ethanol; m.p. 235–236 °C; yield: 61%; R_f: 0.42 (benzene : ethyl acetate, 3 : 2); IR (KBr): ν_{max} 3422 (OH), 1707 (C=O), 1578 (C=C), 1318 1152, 1040 (CO), 989, 898, 781; ^1H NMR (DMSO-d_6): δ 5.036 (1 H, dd, J = 2.1, 9.3 Hz H-4), 6.143 (1 H, d, J = 9.3 Hz, H-3), 6.843 (1 H, d, J = 8.5 Hz, H-6), 7.864 (1 H, dd, J = 8.5, Hz, H-5), 9.61 (2 H, brs, 2 × O*H*).

3.1.8. Synthesis of 7,8-(1′,4′-dioxino) coumarin (**5e**)

This was prepared by the usual method starting from 7,8-dihydroxy coumarin (**5d**) and 1,2-dibromoethane; m.p. 210–211 °C; yield: 47%; R_f: 0.57 (benzene : methanol, 4 : 1); ^1H NMR (DMSO-d_6): δ 4.349 (4 H, m, 2 × CH_2), 5.04 (1 H, dd, J = 2.1, 9.0 Hz, H-4), 6.214 (1 H, d, J = 9.0 Hz, H-3), 6.873 (1 H, d, J = 8.5 Hz, H-5), 7.20 (2 H, d, J = 8.5 Hz, H-6); MS (70 eV): m/z 204 (M^+ $C_{11}H_8O_4$), 176, 163, 160, 144, 132, 117, 88.

3.1.9. Synthesis of 7,8-(1′,4′-dioxino)-4-methyl coumarin (**5f**)

This was prepared by the usual method starting from 4-methyl-7,8-dihydroxy coumarin (**5c**) and 1,2-dibromoethane; m.p. 142–143 °C; yield: 47%; R_f: 0.61 (benzene : methanol, 4 : 1); IR (KBr): ν_{max} 2879 (CH_2), 1728 (C=O), 1577 (C=C), 1173, 1107, 1026 (C–O), 942, 896, 798, 759; ^1H NMR (DMSO-d_6): δ 3.85 (3 H, s, CH_3), 3.325 (2 H, d, J = 2.0 Hz, CH_2), 4.34 (2 H, d, J = 2.0 Hz, CH_2), 6.212 (1 H, s, H-3), 6.86 (1 H, d, J = 8.5 Hz, H-5), 7.193 (1 H, d, J = 8.5 Hz, H-6); MS (70 eV): m/z 218 (M^+ $C_{12}H_{10}O_4$), 192, 175, 159, 134, 106, 89.

3.1.10. Synthesis of 7,8-(2′-hydroxy methyl, 1′,4′-dioxino)-4-methyl coumarin (**5g**)

This was also synthesized by the usual method starting from 4-methyl-7,8-dihydroxy coumarin (**5c**) and epichlorohydrin; m.p. 228–229 °C; yield: 56%; R_f: 0.68 (benzene : ethyl acetate, 9 : 1); IR (KBr): ν_{max} 3376 (OH), 2798 (CH_2), 1724 (C=O), 1591 (C=C), 1281, 1077, 1008 (CO), 981, 916, 848, 742; ^1H NMR (DMSO-d_6): δ 2.362 (3 H, s, CH_3), 4.06 (2 H, dd, J = 7.6, 7.5 Hz, CH_2OH), 4.27 (1 H, brm, $W_{1/2}$ = 5.1 Hz, C*H*), 4.448 (2 H, dd, J = 11.7, 1.8 Hz, CH_2), 5.15 (1 H, s, O*H*), 6.21 (1 H, s, H-3), 6.886 (1 H, J = 8.5 Hz, H-5), 7.21 (1 H, J = 8.5 Hz, H-6); MS (70 eV): m/z 248 (M^+ $C_{13}H_{12}O_5$), 220, 217, 203, 189, 187, 175, 159, 134, 106, 91.

3.1.11. Synthesis of 7,8-(2′-hydroxy methyl, 1′,4′-dioxino) coumarin (**5h**)

This was also prepared by the usual method starting from 7,8 dihydroxy coumarin (**5d**) and epichlorohydrin; m.p. 243–244 °C; yield: 41%; R_f: 0.87 (benzene: ethyl acetate, 9 : 1); ^1H NMR (DMSO-d_6): δ 3.524 (2 H, dd, J = 7.2, 8.1 Hz, CH_2OH), 3.914 (1 H, brm, $W_{1/2}$ = 4.8 Hz, C*H*), 4.358 (2 H, dd, J = 5.7, 2.0 Hz, CH_2), 6.269 (1 H, d, J = 7.5 Hz, H-3), 7.867 (1 H, dd, J = 8.5, 2.0 Hz, H-5), 7.135 (1 H, d, J = 8.5, 2.0 Hz, H-6), 7.91 (1 H, dd, J = 7.5, 2.0 Hz, H-4); MS (70 eV): m/z 234 (M^+ $C_{12}H_{10}O_5$), 206, 204, 193, 175, 174, 147, 119, 91.

3.2. Pharmacological screening

3.2.1. Experimental animals

The antihepatotoxic studies of compounds **4(f–g)** and **5(e–h)** were carried out on Wistar albino rats (150–200 g) of either sex as reported in the literature [7]. The rats were bred in a colony in the Central Animal House of Jamia Hamdard. They were fed with a standard pellet diet (Gold Mohar, Lipton India Ltd., Calcutta) and water *ad libitum*. Before and during the experiment, the rats were kept in standard environmental conditions (temp. 25–28 °C and 12 h light/dark cycle). They were divided into nine groups of five animals each in all sets of experiments. Carbon tetrachloride mixed with liquid paraffin (1 : 1) was used as the hepatotoxic agent. The drugs were administered for seven days after CCl_4 administration, in the form of an aqueous suspension made from carboxymethyl cellulose. On the last day, four rats from each group were taken for biochemical evaluation.

3.2.2. Treatment schedule

Group I (Normal control) was neither given CCl_4 nor any drug. Group II (Toxic control) was treated with CCl_4 (1.0 ml/kg) on the first day of the study to produce toxicity in the liver. Group III (Silymarin treated) was given a single dose of CCl_4 (1.0 ml/kg) on the first day and then silymarin (silybon-70, 10 mg/kg, daily) was given for seven days. Groups IV, V, VI, VII, VIII and IX received a single dose of CCl_4 (1.0 ml/kg) on the first day followed by oral treatment with a daily dose (10.0 mg/kg) of compounds **4f**, **4g**, **5e**, **5f**, **5g** and **5h** for seven days.

3.2.3. Assessment of the liver function

The biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT) [8], serum glutamic pyruvate transaminase (SGPT) [8] and alkaline phosphatase (ALKP) [9], were estimated by reported methods. The total protein and total albumin were also measured according to the reported methods [10, 11].

4.3.4. Statistical analysis

The results of the biochemical estimations are reported as mean ± S.E. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA). Student's test and Dennett's test were used for determining the significance [12, 13].

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