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Synthesis and antihepatotoxic activity of some new xanthones containing 1, 4-dioxane ring system

Shah Alam Khan[1] and Bahar Ahmed*[2]

[1] Jeddah Community College, King Abdul Aziz University, Jeddah, Kingdom of Sausi Arabia.
[2] Antihepatotoxic Research Laboratory Department of Pharmaceutical Chemistry, Faculty of Pharmacy Jamia Hamdard, New Delhi-110062, India.

Abstract

A few xanthone derivatives containing 1,4 dioxane ring system were newly synthesized and evaluated for antihepatotoxic activity against carbon tetrachloride induced hepatotoxicity in albino rats. Compound 4b (3,4 (2' hydroxy 1',4' dioxano) xanthone showed a potent antihepatotoxic activity comparable to standard drug silybon-70 (silymarin), whereas other compounds exhibited moderate activity.

Key words: Silymarin, antihepatotoxic activity, xanthone, 1,4 dioxane.

Introduction

Silymarin isolated from seeds of *Silybum marianum* commonly known as *Milk thistle* (Morazzoni and Bombardelli 1995) has been used as a potent antihepatotoxic agent against a variety of toxicants (Flora et al. 1998). Silymarin is a mixture of three isomers namely, silybin, silydianin and silychristin (Khan et al. 2003) . Silybin is the most active component containing 1, 4 dioxane ring system, whereas other isomers i.e. silychristin and silydianin do not possess 1, 4 –dioxane ring, and thus do not display significant activity (Ahmed et al. 2003). We, therefore, thought that 1, 4 dioxane unit plays an important role in exhibiting antihepatotoxic activity and thus have prepared some new xanthone derivatives containing 1, 4 -dioxane ring system. These compounds were screened for antihepatotoxic activity in albino rats using Carbon tetrachloride as toxicant and by conducting the estimation of liver enzymes such as SGOT, SGPT, total proteins, total albumin and alkaline phosphatase. One of the compound 4b namely 3, 4-(2′-hydroxy methyl 1′, 4′-dioxano) xanthone showed a potent antihepatotoxic activity, whereas other compounds exhibited moderate activity with respect to standard drug silybon-70.

Materials and Methods

All melting points were determined in open capillaries and are uncorrected. 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 $CDCl_3$ and DMSO-d_6 using TMS as an internal reference (chemical shift in δ ppm) and mass spectra on Jeol JMS DX-303 spectrometer. Purity of the compounds was checked on silica gel G plates using iodine vapors as visualizing agent.

Synthesis of 3, 4 -dihydroxy xanthone (3) (Ahmed et al. 2000)

$POCl_3$ (30 ml) and fused $ZnCl_2$ (13 g) were added to salicylic acid (**1**) (4.14 g, 30 mmol) and pyragallol (2) (6.3 g, 50 mmol).

***Corresponding author:** drbahmed@rediffmail.com

It was then heated over sand bath for 2.5 h at 70 ^{0}. 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 **3**; R_f: 0.81 (petroleum ether : ethyl acetate, 3:2); yield: 66% ; m.p. 141- 142 ^{0}; IR (KBr): ν_{max} 3380 (OH), 1670 (C=O), 1491 (C=C), 1154, 1062 (C-O), 983, 759 and 628; ^{1}H-NMR ($CDCl_3$): δ 6.557 (d,1H, J=8.7 Hz, H-1), 6.68 (d, 1H, J=8.1 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 (70ev): m/z 228(M^+, $C_{13}H_8O_4$) (7.4), 200 (2.3), 192 (5.5), 150 (5.7), 121 (100), 92 (16.2).

Synthesis of 5, 7 dinitro 3, 4 -dihydroxy xanthone (3a)

It was prepared by the above procedure using 3, 5 dinitro salicylic acid (1a) (2.01, 10 mmol); R_f: 0.87 (benzene:ethyl acetate, 8:2); yield: 81% ; m.p. 116-118 ^{0}; IR (KBr): ν_{max} 3300 (OH), 3040 (Ar-CH), 1740 (C=O), 1590, 1560, 1470, 1400 (NO_2), 1270, 1170, 860, 750, 640; ^{1}H-NMR ($CDCl_3$): δ 6.35(dd, 2H, J=3.3, 8.4Hz, H-1, 2), 6.89 (d, 1H, J=2.5 Hz, H-6), 7.01 (d, 1H, J=2.5 Hz, H-8), 9.60 (brs,1H, OH), 10.43 (brs,1H, OH).

Synthesis of 5, 7 dibromo 3,4 -dihydroxy xanthone (3b)

It was prepared by the above procedure using 3,5 dibromo salicylic acid (3.16, 10 mmol); R_f: 0.71 (benzene:ethyl acetate, 8:2); yield: 76% ; m.p. 185-187 ^{0}; IR (KBr): ν_{max} 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 (Br); ^{1}H-NMR (DMSO-d_6): δ 6.37(d, 1H, J= 8.4Hz, H-1), 6.91 (d, 1H, J=8.7 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 a mixture of 3 (2.28 g, 10mmol) and 1,2 dibromo ethane (4.23 g, 22.5mmol) 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 taken up in chloroform and the insoluble material was filtered off, the organic layer was dried over anhydrous Na_2SO_4 and evaporated to get the yellowish solid, which was recrystallized from methanol; R_f: 0.47 (Benzene: Methanol, 4:1); yield: 33%; m.p. 197-198 ^{0}; IRν_{max} (KBr): 2497 (CH_2), 1654 (C=O), 1560, 1508 (C=C), 1260, 1190, 1082 (C-O), 898, 789, 621; ^{1}H-NMR (DMSO- d_6): δ 4.19 (m, 4H, 2 x CH_2), 6.56 (d, 1H, J= 8.7Hz, H-1), 6.69 (d, 1H, J= 8.0 Hz, H-2), 7.02 (brm, 2H, H-7, 8), 7.57 (brm, 2H, H-5, 6); MS (70 eV): m/z 254 (M^+ $C_{15}H_{10}O_4$) (100), 226(4.5), 194 (9.6), 175 (18.1), 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 **3** was dissolved in 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 ^{0} 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 settled down at the bottom of the flask was separated from the aqueous layer and concentrated to get semi-solid crude product; R_f: 0.63 (Benzene: Ethyl acetate, 9:1) ; yield: 47 %; ^{1}H-NMR (DMSO- d_6): δ 3.73 (ddd, 1H, J=2.1, 7.8, 2.4 Hz, H_a-3''), 3.76 (ddd, 1H, , J=2.4, 7.9, 2.1 Hz, H_b-3''), 3.85 (brm, 1H, 1/2w=5.2, -C<u>H</u>-CH_2OH), 4.09 (ddd, 1H, J= 2.3, 7.4, 2.1 H_a-C<u>H_2</u>OH), 4.175 (ddd, 1H, J= 2.3, 9.0, 2.4 H_b-C<u>H_2</u>OH),. 6.50 (d, 1H, J= 8.2Hz, H-1), 6.61 (d, 1H, J= 8.0 Hz, H-2), 7.02 (brm, 2H, H-7.8), 7.28 (brm, 2H, H-5,6); MS (70 eV): m/z 284 (M^+ $C_{16}H_{12}O_5$) (22.3), 256 (7.0), 254 (3.9), 226 (5.8), 205 (4.9), 121 (58.6), 93 (10.3).

Synthesis of 5, 7-dinitro 3, 4-(2' hydroxy methyl-1', 4'-dioxano) Xanthone (4c)

This compound was synthesized by the usual method; R_f: 0.71(benzene: ethyl acetate, 9:1) ; yield: 43.6 %; IR (KBr): ν_{max} 3305 (OH), 1673 (C=O), 1508 (C=C), 1461 (NO_2), 1340, 1243, 1109, 966, 850, 789, 69; ^{1}H-NMR (DMSO- d_6): δ 4.07 (dd, 2H, J=1.8, 7.2 Hz, 3'-CH_2), 4.51 (dd, 2H, J=2.1, 8.4 Hz, C<u>H_2</u>OH), 4.76 (brm, 1H, 2'-CH),. 8.37 (d, 2H, J= 8.5Hz, H-6, 8), 8.51 (d, 2H, J= 8.5 Hz, H-1,2).

Synthesis of 5, 7 dibromo 3, 4-(1', 4'- dioxano) xanthone (4d)

This compound was synthesized by the usual method; R_f: 0.72 (benzene: ethyl acetate, 9:1) ; yield: 39 %;m.p.111-112 ^{0}; ^{1}H-NMR (DMSO- d_6): δ 3.68 (dddd, 2H, J=3.0, 9.0,3.7, 9.2 Hz, 2'-CH_2), 4.18 (dddd, 2H, J=3.2,9.0,3.0,7.2 Hz, 3'-CH_2), 6.37 (d, 1H, J= 8.4Hz, H-1),. 6.91 (d, 1H, J= 8.7Hz, H-2), 7.75 (d, 1H, J= 2.7 Hz, H-6), 7.89(d, 1H, J= 2.7Hz, H-8); MS (70 eV): m/z 412 (M^+ $C_{15}H_8O_4Br_2$) (3.6), 384 (4.1), 352 (6.3), 279 (5.8), 175(14.1), 93 (15.3).

Synthesis of 5, 7 dibromo 3, 4-(2' hydroxy methyl, 1', 4'- dioxano) xanthone (4e)

This compound was also synthesized by the usual method; R_f: 0.59 (benzene: ethyl acetate, 9:1) ; yield: 46 %; IR (KBr): ν_{max} 3245 (OH), 1674 (C=O), 1571, 1510 (C=C), 1349, 1240, 1204, 1002 (C-O), 808, 761 (Br), 649; ^{1}H-NMR (DMSO- d_6): δ 3.64 (d, 1H, $W_{1/2}$=3.6Hz, 2'-C*H*), 3.95 (ddd, 2H, J=5.1,8.4, 4.5 Hz, -CH_2OH), 4.22 (ddd, 2H, J= 7.3, 3.2, 7.0Hz, 3'-CH_2), 6.50 (d, 1H, J= 8.4Hz, H-1), 6.93 (d, 1H, J= 8.7 Hz, H-2), 7.48(d, 1H, J= 2.7Hz, H-6), 7.92(d, 1H, *J*= 2.7Hz, H-8).

Antihepatotoxic activity

The antihepatotoxic studies of compounds 4a-4e were carried out on Wistar albino rats (150-200 g). They were divided into eight groups of five animals each in all sets of experiments. CCl_4 mixed with liquid paraffin (1:1) was used as hepatotoxic agent. The drugs were administered for seven days after CCl_4 administration, in the form of aqueous suspension made from carboxymethyl cellulose. On the last day, four rats from each group were taken for biochemical evaluation.

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) for the first day of 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 to VIII were administered with a single dose of CCl_4 (1.0 ml/kg) on the first day followed by oral treatment with a daily dose (10 mg/kg) of xanthones 4a to 4e for seven days.

The biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT) (Rietman and Frankel 1957), serum glutamic pyruvate transaminase (SGPT) (Rietman and Frankel 1957 and alkaline phosphatase (Kind and King 1954) (ALKP), total protein and total albumin were analyzed according to the reported methods (Wooton 1954).

The results of the biochemical estimations are reported as mean ± S.E.M. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA). Student's't' test was used for determining the significance.

Results and Discussion

The results of the synthesized compounds 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. The levels of enzymes like SGOT, SGPT, ALKP were enhanced on administration of CCl_4 by 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 have decreased the enzyme levels in the range of 58.9 - 66.8 units/ml in case of SGOT, 47.24-54.20 units/ml in case of SGPT and 35.92 - 39.7 units/ml in case of ALKP, which were found to be comparable to the enzyme levels reduced by standard drug silybon-70 (53.84, 45.8 and 28.88 units/ml, respectively). The most potent compound, which exhibited almost similar antihepatotoxic activity as that of standard drug silybon-70 was found to be compound (58.9, 47.2 and 35.92 units/ml, respectively). Other compounds also exhibited a moderate activity (61.04 - 66.8 units/ml, 50.68 - 54.2 units/ml and 37.4 - 39.7 units/ml, respectively).

The toxicant CCl_4 also reduced the level of total protein (4.306 g/dl) and increased the level of total albumin (4.3 gm/dl) in comparison to normal values (5.32 g/dl, 3.32 gm/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- 4.0 m/dl in comparison to standard drug silybon-70 (6.219 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.

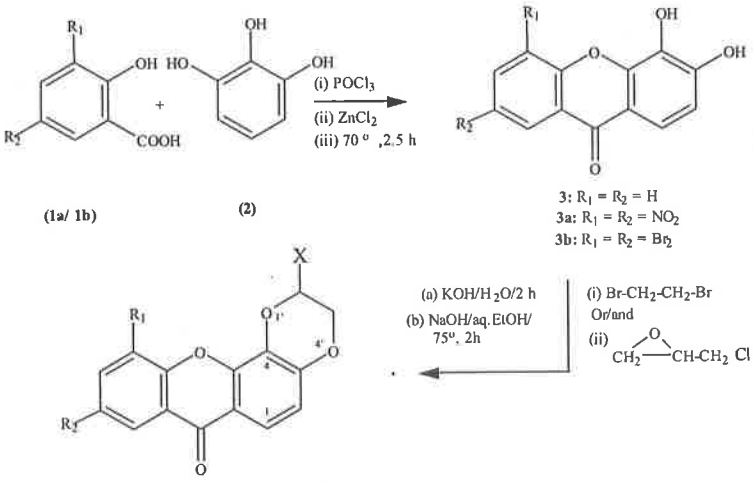
Table 1. Estimation of biochemical parameters of synthesized compounds in CCl_4 induced hepatotoxicity in albino rats.

Groups	Treatment	Dose mg/kg/b.w	SGOT units/ml	SGPT units/ml	ALKP units/ml	TP g/dl	TA 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	53.84±0.65*	45.8±0.62*	28.88±0.23*	6.219±0.186*	3.76±0.13*
IV	Xanthone-4a	10	61.51±0.321	51.4±0.55	38.56±0.29*	4.93±0.045*	3.95±0.079
V	Xanthone-4b	10	58.9±0.345*	47.24±0.47*	35.92±0.91*	5.15±0.045*	3.75±0.025*
VI	Xanthone-4c	10	65.26±1.01*	50.68±0.33*	38.38±0.29*	4.95±0.08*	3.89±0.026*
VII	Xanthone-4d	10	66.8±0. 80*	54.2±0.464	39.7±0.21*	4.75±0.077*	4.0±0. 072*
VIII	Xanthone-4e	10	61.04±0.366*	50.9±0.394*	37.4±0.18*	4.49±0.074	3.94±0.046*

Values are Mean±S.E.M. (n= 5 animal per group); SGOT- Serum glutamic oxaloacetic acid transaminase; SGPT-Serum gluatamic pyruvic transaminase; ALKP- Alkaline Phosphatase; TP- Total Protein; TA- Total Albumin; *P<0.05 vs CCl_4; Student's *t* test

It was also observed that compound 4b possess 2-hydroxy methyl group at position 2 of the dioxane ring of xanthone derivative, which has also indicated 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.

The substitutions in the aromatic ring of xanthones have no significant role in exhibiting antihepatotoxic activity. However, it was observed that the unsubstituted xanthone derivatives have better activity in comparision to nitro- and bromo- substituted xanthones.



S.No.	R_1	R_2	X
4a	H	H	H
4b	H	H	CH_2OH
4c	NO_2	NO_2	CH_2OH
4d	Br	Br	H
4e	Br	Br	CH_2OH

Figure 1. Synthetic route.

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