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Original Article

SYNTHESIS AND ASSESSMENT OF HEPATOPROTECITVE ACTIVITY OF SOME NEW 2-ARYL TETRAHYDROQUINOLINE AND SPIROOXYINDOLYL TETRAHYDROQUINOLINE DERIVATIVES

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ABSTRACT

Objective: To evaluate the hepatoprotecitve potential of some newly synthesized tetrahydroquinoline derivatives against carbon tetrachloride induced hepatic damage in wister rats.

Methods: A series of 2-aryl, 4-*N* vinyl pyrrolido/caprolacto and spirooxy indolyl tetrahydroquinolines were synthesized by imino Diels-Alder reaction using Antimony (III) sulfate as catalyst. The titled compounds were characterized by IR, ^{1}HNMR spectroscopy and screened for hepatoprotecitve activity. Hepatopotoxicity was induced in male Wister rats by intrperitoneal injection of CCl_4. Wister rats weighing 150-200g were randomly assigned in to various groups of six animals each. Group I – Normal control received only 1% Tween in distilled water, Group II – Served as negative control received CCl_4 in liquid paraffin 1 ml/kg p. o. at every 72 h for 10 days. Group III – X were intoxicated with CCl_4 1 ml/kg p. o. before the administration of Silymarin 100 mg/kg p. o. and suspension of synthetic derivatives in polyethylene glycol-400 at the dose of 25 mg/kg p. o. for 10 days. Different hepatic biochemical parameters viz. SGOT, SGPT, SALP, Total and direct bilirubin were evaluated before and after treatment to investigate the hepatoprotecitve activity.

Results: It was observed that in CCl_4 intoxicated group; total and direct bilirubin, SGOT, SGPT, SALP levels were significantly increased as compared to control group. Administration of synthesized tetrahydroquinoline derivatives at the dose of 25 mg/kg p. o. reduced these pathological damages caused by CCl_4 intoxication compared to normal and Silymarin treated groups.

Conclusion: The present study revealed that synthesized tetrahydroquinoline derivatives, showed hepatoprotecitve potential against CCl_4 induced hepatotoxicity in wister rats, thus offering a novel synthetic formulation as a hepatoprotecitve drug.

Keywords: Tetrahydroquinolines, Imino diels-Alder reaction, Antimony (III) sulfate, Hepatoprotecitve, Silymarin, Liver, Carbon tetrachloride.

INTRODUCTION

Liver plays a major role in metabolism, secretion, storage, detoxification and excretion of many endogenous and exogenous compounds. Liver cell injury caused by systemic drugs, foods, preservatives, agrochemicals, microbial agents and excessive alcohol consumption, leads to many disorders ranging from elevation of liver enzymes to liver failure [1]. Administration of CCl_4 causes liver and kidney damage through free radical mediated process. Also CCl_4 increases the serum level of marker enzymes SGOT, SGPT, SALP and bilirubin marking the induction of hepatotoxicity. Though the modern medical system as advanced phenomenally there are no potential drugs which can completely cure all liver disorders [2]. Silymarin has been proved to possess hepatoprotecitve potential by prevention of absorption of toxins in to hepatocytes by occupying binding sites as well as inhibiting many transport proteins at the membrane. These actions along with aniperoxidative property make Silymarin suitable for the treatment of toxic liver disease [3].

In an attempt to expand the spectrum of hepatoprotecitve agents, the present study was carried out to synthesize some new tetrahydroquinoline and spirooxyindolyl tetrahydroquinoline derivatives and evaluate their hepatoprotecitve potential against CCl_4 induced toxicity in wister rats. Biological importance of tetrahydroquinolines has been demonstrated by recent studies as hundreds of them bearing various simple or complex substituents have shown interesting biochemical and pharmaceutical activity [4-8]. Further the presence of spirooxy indole core in number of natural products has evolved significant interest in synthesis of spirooxy indole derivatives [9]. In the present work, a series of 2-aryl, 4-*N* vinyl pyrrolido/caprolacto and spirooxy indolyl tetrahydroquinolines were synthesized by imino Diels-Alder reaction in the presence of Antimony (III) sulfate as catalyst and were screened for hepatoprotecitve activity against CCl_4 induced hepatotoxicity in wister rats.

MATERIALS AND METHODS

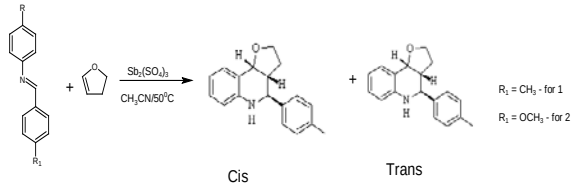
Synthesis and characterization of compounds (1-7)

All melting points were recorded in open capillaries and were uncorrected. The purity of the compounds was monitored by TLC and they were purified by column chromatography. ^{1}H NMR spectra were recorded on a Bruker-300 Hz spectrometer using TMS as an internal standard. IR spectra were obtained using a FTS-135 spectrometer. The identity of compounds (1-7) was established by means of IR, ^{1}HNMR, mass spectral study and elemental analysis.

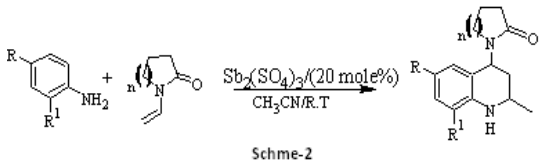
Procedure for synthesis of compounds

(1) and (2): Antimony (III) sulfate (0.2m. mol) was added to a mixture of 1.0 m. mol *N*-benzylidene and 2.3 dihydrofuran in 5 cm^3 acetonitrile. The reaction mixture was stirred at 50^{o}C for 4 hrs. After completion of the reaction (as indicated by TLC) the mixture was quenched with saturated $NaHCO_3$, extracted with ethyl acetate, dried over (anhydrous Na_2SO_4) and purified by column chromatography on SiO_2 with an ethyl acetate and petroleum ether as elutent (Scheme -1).

(3) and (4): A mixture of aromatic amine (1m. mol) and *N*-vinyl pyrrolidine and *N*-vinyl caprolactum and Antimony (III) sulfate (0.28 m. mol) in 10 cm^3 acetonitrile was stirred at room temperature. After completion of reaction (as indicated by TLC), the reaction mixture was quenched in water, extracted with ethylether, dried, concentrated, and separated by column chromatography (Scheme-2).

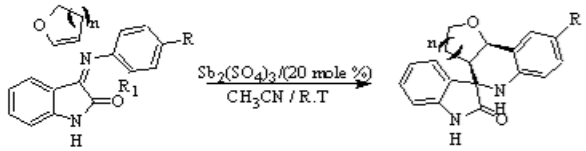


Scheme – 1



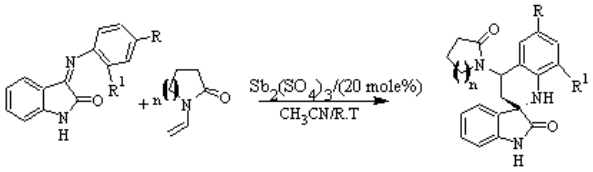
Schme-2

(5) and (6): Isatin amine was made to react with 2.3 dihydrofuran/3,4 dihydro 2H-pyran in presence of Antimony (III) sulfate (20 mol%) in acetonitrile, mixed for 2 hours at room temperature to give corresponding Spirooxy indolyl tetrahydro quinolines (Scheme-3).



Scheme - 3

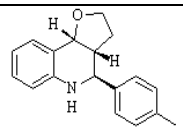
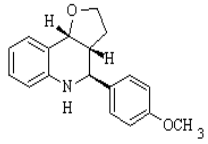
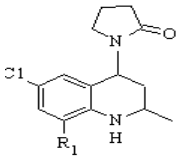
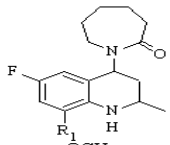
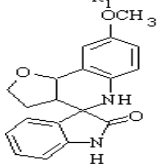
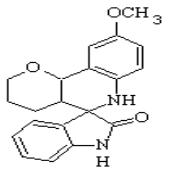
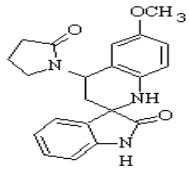
(7): Isatin Schiff base was made to react with N-vinyl pyrrolidine in presence of Antimony (III) sulfate catalyst (20 mol %) in acetonitrile, mixed for 1 hour at room temperature to get the target Spirooxy indolyl tetrahydroquinolines (Scheme-4).



Scheme-4

Physical and analytical data of newly synthesized compounds were reported in table 1.

Table 1: Physical and analytical data of synthesized components

Compound	Structure	MP(°C)	Yield	Molecular formula	Mol. Wt
1		92-94°C	40	$C_{18}H_{19}O$	265
2		94-96°C	40	$C_{18}H_{19}O_2$	281
3		105-152°C	93	$C_{14}H_{17}C_1N_2O$	265
4		138-140°C	93	$C_{16}H_{21}FN_2O$	277
5		150°C	90	$C_{19}H_{18}N_2O_3$	321
6		120°C	91	$C_{20}H_{18}N_2O_3$	333
7		290-294°C	91	$C21H_{20}N_3O_3$	364

Spectral data

(1): Colorless crystalline solid, IR (KBr): $\bar{\nu}$ = 3401 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 7.26-7.46 (6H, m), 6.99 (d, *J* = 7.4 Hz, 1H), 6.72 (t, *J* = 7.5 Hz, 1H), 4.55 (d, *J* = 4.9 Hz, 1H), 4.00-4.08 (m, 2H), 3.75-3.84 (m, 2H), 2.40-2.47 (m, 1H), 2.08 (s, 3H,), 1.88-1.95 (m, 1H), 1.62-1.69 (m, 1H) ppm.

(2): Colorless crystalline solid, IR (KBr): $\bar{\nu}$ = 3298 cm^{-1}; ^1H NMR ($CDCl_3$): δ = 7.39-7.46 (m, 5H), 6.99 (d, *J* = 2.8 Hz, 1H), 6.80 (dd, *J* = 8.1, 2.8 Hz, 1H), 6.61 (d, *J* = 8.1 Hz, 1H), 4.63 (d, *J* = 5.3 Hz, 1H,), 4.06 (m, 1H), 3.78 (s, 3H), 3.73-3.87 (2H, m), 2.49 (1H, br), 1.98-2.04 (m, 1H), 1.68-1.73 (m, 1H), 1.18-1.24 (1H, m) ppm.

(3): Colourless crystalline solid, IR (KBr): = 3395 cm^{-1}; 1H NMR (400 MHz, $CDCl_3$): δ = 1.24 (d, 3H, *J* = 6.2 Hz), 1.75 (ddd, 1H, *J* = 12.3, 5.5, 2.2 Hz), 1.95 (ddd, 1H, *J* = 11.6, 5.9, 2.4 Hz), 1.99-2.15 (m, 2H), 2.42-2.59 (m, 2H), 3.11-3.30 (m, 2H), 3.47-3.61 (m, 1H), 4.5 (brs, 1H, NH), 5.5 (dd, 1H, *J* = 11.9, 5.9 Hz), 6.48 (d, 1H, *J* = 8.5 Hz), 6.78 (s, 1H), 6.96 (dd, 1H, *J* = 8.6 Hz, 2.0 Hz) ppm.

(4): White crystalline solid; mp.138-140 ^{0}C; 1H NMR (400 MHz, DMSO-d_6): δ = 1.13 (d, *J* = 6.2 Hz, 3H), 1.23-1.29 (m, 1H), 1.40-1.51 (m, 3H), 1.77-1.82 (m, 4H), 2.37-2.43 (m, 1H), 2.72 (t, *J* = 12.4 Hz, 1H), 2.83-2.88 (m, 1H), 3.14-3.20 (m, 1H), 3.37-3.41 (m, 1H), 5.61 (s, 1H), 5.74 (dd, *J* = 11.6, 5.0 Hz, 1H), 6.47 (m, 2H), 6.72-6.77 (m, 1H) ppm.

(5 and 6): crystalline yellow solid; ^1H NMR (400 MHz DMSO-d_6): 10.4 (br, s), 7.2 (t, *J*=5.4 Hz, 1H), 6.7 (d, *J*=2.3 Hz, 1H), 6.6 (t, *J*= 3.7 Hz, 1H), 6.5 (d, *J*=13.1Hz, 1H), 5.01 (s, NH), 3.6 (t, *J*=4.5 Hz, 1H), 2.4 (t, *J*=6 Hz, 1H), 2.2 (d, *J*=4.2 Hz, 1H), 1.9 (t, *J*=9 Hz, 1H), 1.8 (d, *J*=5.8 Hz, 1H).

(7): Crystalline yellow solid; ^1H NMR (400 MHz DMSO-d_6): 10.2 (br, s, NH), 7.3 (d, *J*=5.49 Hz, 1H), 7.2 (t, *J*=7.64 Hz, 1H), 7.0 (t, *J*=7.44 Hz, 1H), 6.8 (d, *J*=7.72 Hz, 1H), 6.6 (d, *J*=2.36 Hz, 1H), 6.5 (d, *J*=8.64, 1H), 6.2 (s, 1H), 6.1 (s, 1H), 3.6 (s, 3H), 3.3 (t, *J*=7.16 Hz, 1H), 3.1 (d, *J*=5.6 Hz, 1H), 2.3 (m, 3H), 1.9 (q, *J*=4.68, 2H), 1.6 (s, 1H).

Hepatoprotecitve activity

Chemicals

All the solvents and chemicals used were of analytical grade. Standard kits for SGOT, SGPT and Bilirubin (Teco Diagnostic, USA), Standard drug Silymarin (Micro laboratory, India), were used in the present study.

Animals

Adult mice (25-30g) and wister rats (180-200g) were used in the present study. The animals were procured from disease free animal house, National Institute of Pharmacy, Shivamogga, Karnataka, India. All the animals were kept in quarantine for 10 days under standard husbandry conditions with temperature (25-27 ^{o}C), 12-h light/12-h dark cycle and relative air humidity 40-60% and were given standard food (Hindustan lever Ltd. Mumbai) and water *adlibitum*. The experimental protocol was approved by an Institutional Animal Ethics Committee (IAEC) and care of laboratory animals was taken as per CPCSEA guidelines.

Experimental procedure

Acute toxicity was conducted on Swiss albino mice weighing between 20-25g using stair case/up and down method. The newly synthesized derivatives were orally administered to different groups of mice at a dose of 10-50 mg/kg p. o. Convulsion, sedation, body temperature, mortality and behavioral changes if any were observed [10]. Synthetic derivatives did not produce any toxicity up to dose level of 50 mg/Kg. Hence 25 mg/kg p. o was selected to screen for hepatoprotecitve activity.

Assessment of Hepatoprotecitve activity

In the experiment wister rats weighing 150-200 g were used. The rats were divided into ten groups of six animals in each group. **Group I:** Served as normal control and received 1% Tween 80 in distilled water. **Group II:** Served as negative control and received CCl_4 in liquid paraffin (1:1), 1 ml/kg, i. p. Intraperitoneally on 3rd, 6th and 10th day. **Group III**: Treated with Standard drug Silymarin at the dose of 100 mg/kg p. o for ten days. The **Group IV, V, VI, VII, VIII, IX and X** received **s**uspensions of synthetic derivatives (1-7) in polyethylene glycol-400 at the dose of 25 mg/kg p. o. for 10 days [11]. Groups III – X were intoxicated with CCl_4 1h before the administration of Silymarin or synthetic compounds for ten days.

On the eleventh day after administration of last dose of synthetic derivatives, the rats were anesthetized by light ether anesthesia and blood was collected from the retro-orbital plexus. It was allowed to coagulate for 30 minutes and serum was separated by cold centrifugation at 3000 rpm. The centrifugate was used to estimate the serum glutamate pyruvate transminase (SGPT), Serum glutamate oxaloacetate (SGOT) [12]and serum alkaline phosphatase (SALP**)** [13]. Total and direct bilirubin levels [14] were also determined.

Statistical analysis

The data obtained from this study were expressed as mean value ± SEM (n=6) for each parameter. The data was analyzed using one-way ANOVA followed by Dunnet's multiple comparison tests. A probability level of less than 5% ($p<0.05$) was considered statistically significant [15].

RESULTS

Synthetic derivatives **(1-7)** did not show any toxicity and behavioral changes in mice up to dose level of 50mg/kg. Hence, the doses selected were 25 mg/kg. p. o.

Hepatoprotecitve activity

Rats treated with CCl_4 (1.0 ml/kg in liquid paraffin, 1:1, i. p.) suffered from hepatotoxicity. The serum levels of SGOT, SGPT, SALP, bilirubin (Total and direct) levels were significantly elevated. Newly synthesized compounds **(2-7)** (25 mg/kg/p. o.) exhibited significant hepatoprotecitve activity ($p<0.01$) by decreasing the elevated enzyme levels when compared with compound 1 against CCl_4 induced hepatotoxicity (Table-2 and Figs. 1-2). The higher activity of 2-7 may be due to presence of Methoxy, Chloro and Fluoro groups as substituents in the synthetic derivatives.

Table 2: Effect of some tetrahydroquinoline derivatives on CCl_4 induced hepatotoxicity

Groups	SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Normal	140.53 ± 2.33	65.16 ± 1.78	142.74 ± 6.21	1.01± 0.20	0.18 ± 0.01
$CC1_4$ (1m1/kg p. o)	435.46 ± 7.31[a]	296.27 ± 8.20[a]	442.61 ± 8.21[a]	3.66 ± 0.38[a]	1.60 ± 0.30[a]
Silymarin+$CC1_4$ (100mg/kg p. o)	159.22 ± 3.10 [c]	79.34 ±2.00 [c]	167.81 ± 4.28 [c]	0.96 ±0.05[c]	0.24 ± 0.02[c]
(1)+ $CC1_4$ (25mg/kg p. o)	309.46 ± 4.12[a]	238.28 ± 4.62[a]	402.32 ±5.42[a]	2.62 ± 0.29	1.28 ± 0.29
(2)+ $CC1_4$ (25 mg/ kg p. o)	256.34 ± 3.22[b]	228 ± 4.85[b]	355.54 ± 4.30[b]	0.88 ± 0.34[a]	0.98 ± 0.08[a]
(3)+$CC1_4$(25mg /kg p. o)	265.3 ± 3.42[b]	250.35 ± 4.6[b]	365.34 ± 5.45[b]	2.86 ± 0.58[a]	1.29 ± 0.47[b]

(4)+CC1$_4$(25mg/kg p. o)	259.46 ± 3.26^b	238.21 ± 4.89 ^{b}	379.28 ± 4.32^b	2.68 ± 0.26^b	1.58 ± 0.56^b
(5)+ CC1$_4$(25 mg /kg p. o)	265.38 ± 3.29^b	245.18 ± 4.92^b	370.31 ± 4.35^b	2.65 ± 0.25^b	1.56 ± 0.54^b
(6)+ CC1$_4$(25 mg /kg p. o)	260.12 ± 3.32^b	225.28 ± 4.6^b	362.41 ± 4.25^b	2.56 ± 0.32^b	1.26 ± 0.20^b
(7)+ CC1$_4$(25 mg /kg p. o)	265.42 ± 3.42^b	235.16 ± 4.58^b	365.45 ± 4.18^b	2.58 ± 0.32^b	1.25 ± 0.30^b

a p<0.05 as compared to normal group, b p<0.01 as compared to normal and CCl_4 group, c p<0.001 as compared to normal and CCl_4 group

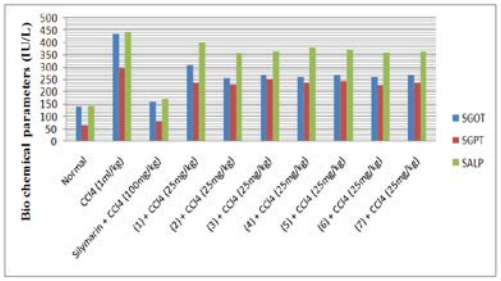


Fig. 1: Effect of synthetic derivatives on SGOT, SGPT and SALP in CCl_4 induced hepatotoxicity

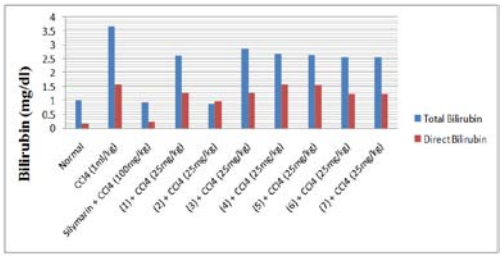


Fig. 2: Effect of synthetic derivatives on total and direct bilirubin in CCl_4 induced hepatotoxicity

DISCUSSION

Carbon tetrachloride has been used as a tool to induce hepatotoxicity in experimental rats. The hepatotoxic effects of CCl_4 is due to its hepatic conversion, catalyzed by cytochrome P-450 enzyme system, in to highly reactive trichloromethyl radical (CCl_3^o) which gives trichloromethyl peroxide radical (Cl_3COO^o) by reaction with oxygen. These activated radicals bond covalently with sulfohydroxyl group of several membrane molecules like glutathione, which is considered as the initial step in the chain of events leading to lipid peroxidation and hepatic tissue destruction [16-19]. The degree of hepatotoxicity developed by CCl_4, can be observed by elevated levels of biochemical parameters SGOT, SGPT, SALP and bilirubin which are attributed to the generation of trichloromethyl free radical during metabolism by hepatic microsomes which in turn cause peroxidation of lipids of cellular membrane [20]. Hepatocellular necrosis lead to the very high level of SGOT, SGPT released from liver in the blood. Among the two, SGPT is a better index of liver injury, as liver SGPT activity represents 90% of total enzyme present n the body [21]. SALP activities on the other hand are related to the functioning of the hepatocytes. Increase in SALP level is due to increased synthesis in presence of increased biliary pressure [22]. Reduction in levels of SGOT and SGPT towards the respective normal value in synthetic compounds treated groups of rats was an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by carbon tetra chloride. The serum levels of transminase return to normal with healing of hepatic parenchyma and regeneration of hepatocytes. Suppression of increased SALP activity with concurrent depletion of raised bilirubin level suggests the stability of the biliary dysfunction in rat liver during chronic hepatic injury with CCl_4 [23].

CONCLUSION

The results suggest that synthetic compounds (2-7) treated groups exhibited significant hepatoprotecitve activity when compared with the compound (1) treated groups. The possible hepatoprotecitve mechanism of synthetic tetrahydroquinolines may be through inhibition of the cytochrome P-450 activity which prevents the process of lipid peroxidation leading to stabilization of hepatocelluar membrane. The higher liver protective effect of synthetic derivatives (2-7) may be due to the presence of methoxy, fluoro and Chloro groups as substituents. The present study leads to conclude that synthetic derivatives (2-7) may be employed in the management of hepatic disorders.

ACKNOWLEDGEMENT

Authors are thankful to the President and Principal, National College of Pharmacy, Shivamogga for providing necessary facilities to carry out the experimental work.

Ethical clearance

The research work was approved by Institutional Animal Ethics Committee (NCP/IAEC/CLEAR/25/02/2009-10, dated 09/03/2010)

CONFLICT OF INTERESTS

Declared None

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