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Research Article

ANTI HYPERLIPIDEMIC ACTIVITY OF BISINDOLE SUBSTITUTED COUMARIN DERIVATIVES

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ABSTRACT

Some Bis indole substituted coumarin derivatives prepared in 3 steps. The final compound III is react with different substituted indole compounds and aceto nitrile at room temperature for 30 minutes the compounds S₁-S₆ is obtained. The present investigation deals with the synthesized compounds having anti hyperlipidemic activity. Table 1 and 2 show the levels of Cholesterol, Triglycerides, HDL, LDL and VLDL of control and experiment rats respectively. Serum of hyperlipidemic rats showed significantly increased levels of Cholesterol, Triglycerides, LDL - C and low HDL - C, when compared with normal rats. In rats treated with various synthetic drugs and Atorvastatin there was significant decrease in the content cholesterol, TGs, LDL - C, and VLDL and increases HDL - C, when compared with cholesterol control rats.

Keywords: Bisindole derivatives, Anti hyperlipidemic activity.

INTRODUCTION

Coumarins and its derivatives have been proved as useful precursors for the synthesis of variety of medicinal agents¹. The heterocycles derived from these have also been tested for their anti-HIV, anti-inflammatory, anti-convulsant, antioxidant, antibacterial, antifungal, anti-carcinogenic and anti-histaminic activities^{2,3}. On the basis of our observation the present research work was carried out to synthesis some coumarin derivatives and further evaluate antioxidant and anti coagulant activity. The bis indolecoumarin derivatives have anti microbial^{4,6}, antioxidant, analgesic and anti inflammatory⁷, anti cancer⁸, Hepatoprotective⁹, anti histamine¹⁰ activities. Dyslipidemia further raises risk of atherosclerosis in people with diabetes. Dyslipidemia affects people with type 2 diabetes more often than those with type 1 diabetes. The most common Dyslipidemia in diabetes is the combination of high triglycerides and low HDL levels. People with diabetes may also have elevated LDL cholesterol. Among the drugs available to treat Dyslipidemia, statins are often the first choice for lowering total and LDL cholesterol levels, other drugs that lowers cholesterol include cholesterol-adsorption blockers, bile acids, sequestrants and nicotinic acids. These may be used in combination, if a single drug is not effective in reaching target levels. Fibrates and extended release niacin may be used to lower triglycerides (or) raise HDL cholesterol levels^{11,12}. Hyperglycemia and dyslipidemia are significant and independent risk factors for the vascular complications and suggested to cause cardiovascular pathological changes in diabetic states through the following molecular mechanism, formation and accumulation of advanced glycation products, increased oxidative stress, activation of protein kinase C pathway, increased activity of

hexosamine pathway and vascular inflammation and the impairment of insulin action in the vascular tissues¹³.

Synthetic Procedure

Step I

14.4 g of α -naphthol [I] (0.1M) was weighed and taken in 500 ml RB flask. To this 14 g of hexamine (0.1M) and 80 ml of trifluoro acetic acid were added. Then this RB was fixed under reflux condenser. Then it was refluxed on boiling water bath for 3 hours at 100° C. After 3 hours of reflux, 10 % sulphuric acid was to the reaction mixture. Then this was again refluxed under bath for 2 hours at 100°C the reddish brown naphtholdehyde was formed in the reaction mixture. This was separated by extracting the mixture with ether. Then the ether was evaporated to obtain reddish brown color naphtholdehyde compound [II]. The yield of this compound was 12 g.

Step II

About 10 g of (0.05M) of naphtholdehyde [II] obtained in step I was weighed and taken in a 500 ml RB flask. To this 8 ml of diethyl malonate (0.05M), 5 ml ethanol and 5 ml of piperidine were added and shaken well. This reaction mixture was refluxed under water bath using reflux condenser for 30-40 minutes. Now the yellowish orange coumarinic adduct namely ethyl 6-formyl-2-oxo-2H-benzo[h]chromene-3-carboxaldehyde [III] was formed. This was then separated carefully by filtration using suction funnel. The product of this was re crystallized by using methanol. Yield was about 10 g.

Step III

The coumarinic compound obtained in step III was decided to use for the synthesis six various Bisindole substituted coumarin derivatives. By treating the coumarinic adduct with different substituted indoles in the presence of acetonitrile and iodine. This mixture was shaken for 30 minutes to get product.

Compounds and their codes

- Compound 1 [code-compound S₁]
- Compound 2 [code-compound S₂]
- Compound 3 [code-compound S₃]
- Compound 4 [code-compound S₄]
- Compound 5 [code-compound S₅]
- Compound 6 [code-compound S₆]

Procedure for Individual Synthesis**Synthesis of Compound S₁**

About 1.4 g (0.005M) of coumarinic adducts [III] which was obtained in step III weighed and taken in RB flask. To this 1.45 g of (0.01M) Indole-2-carboxaldehyde was added. Then 0.63 g of iodine and 20 ml of acetonitrile were added. This mixture was then shaken well for 30 minutes at room temperature. The reddish brown crystals of compound S₁ was separated by filtration. Re crystallization was done by using ethanol. The yield was about 1.2 g.

Synthesis of Compound S₂

1.4 g (0.005M) of coumarinic adducts [III] was weighed and taken in RB flask. To this 1.45 g of (0.01M) Indole-4-carboxaldehyde was added. Then 0.63 g of iodine and 20 ml of acetonitrile were added. This mixture was then shaken well for 30 minutes at room temperature. The reddish powders of compound S₂ was separated by filtration. Re crystallization was done by using ethanol. The yield was about 0.86 g.

Synthesis of Compound S₃

About 1.4 g (0.005M) compound [III] which was obtained in step III weighed and taken in RB flask. To this 1.61 g of (0.01M) Indole-2-carboxylic acid was added. Then 0.63 g of iodine and 20 ml of acetonitrile were added. This mixture was then shaken well for 30 minutes at room temperature. The orange color compound S₃ was separated by filtration. Re crystallization was done by using chloroform. The yield was about 0.98 g.

Synthesis of Compound S₄

About 1.4 g (0.005M) of coumarinic adducts [III] which was obtained in step III weighed and taken in RB flask. To this 1.61 g of (0.01M) Indole-4-carboxylic acid was added. Then 0.63 g of iodine and 20 ml of acetonitrile were added. This mixture was then shaken well for 30 minutes at room temperature. The brown crystals of compound S₄ was separated by filtration. Re crystallization was done by using chloroform. The yield was about 0.89 g.

Synthesis of Compound S₅

About 1.4 g (0.005M) of coumarinic compound [III] was weighed and taken in RB flask. To this 1 g of (0.01M) Indole-5-carbonitrile was added. Then 0.63 g of iodine and 20 ml of acetonitrile were added. This mixture was then shaken well for 30 minutes at room temperature. The yellowish white crystals of compound S₅ was separated by

filtration. Re crystallization was done by using methanol. The yield was about 0.74 g.

Synthesis of Compound S₆

About 1.4 g (0.005M) of coumarinic adducts [III] which was obtained in step III weighed and taken in RB flask. To this 1.45 g of (0.01M) Indole-5-carboxamide was added. Then 0.63 g iodine and 20 ml of acetonitrile were added. This mixture was then shaken well for 30 minutes at room temperature. The pale yellow powders of compound S₆ was separated by filtration. Re crystallization was done by using ethyl acetate. The yield was about 1.3 g.

Experimental**Materials and Methods**

Melting points of synthesized compounds were determined in open capillary tubes and are therefore uncorrected. The structures of compounds were established on the basis of elemental analysis and spectral data. The IR spectra were recorded in range of 4000-450 cm⁻¹ using KBr pellets on a Perkin Elmer spectrophotometer. H NMR spectra were recorded on a Bruker DRX300 Mhz spectrophotometer using CDCl₃/DMSO-d₆ as solvent. Purity of synthesized compounds was checked by silica gel G plates of 2 mm thickness using hexane, ethylacetate, water as solvent system and iodine chamber as developer¹⁴⁻¹⁹.

Compound S₁**IR Spectral Data**

3747.89 (NH Stretching), 2929 (CH Stretching), 2366 (CH Stretching), 1637 (CO Stretching), 1297 (CO Stretching), 998 (CH Bending), 834 (NH Bending)

NMR Spectral Data

3.304 (Singlet, CH proton adjacent to OCOAr), 3.375-5.18 (Singlet, CH proton adjacent to OCOAr), 6.7659-7.1884 (Singlet, aromatic protons), 7.404-7.9 (Singlet, aromatic protons), 8.48-8.5 (Singlet, NH protons)

Compound S₂**IR Spectral Data**

3293 (NH Stretching), 2923 (CH Stretching), 2850 (CH Stretching), 2365 (CH Stretching), 1464 (CH Bending), 1123 (CO Stretching), 1064 (CO Stretching)

NMR Spectral Data

3.3842 (Singlet, CH proton adjacent to OCOAr), 6.7959-7.4241 (Singlet, aromatic protons), 7.85-7.94 (Singlet, aromatic protons), 8.4-8.5 (singlet NH Protons)

Compound S₃**IR Spectral Data**

3757 (NH Stretching), 3431 (NH Stretching), 2956 (NH Stretching), 2870 (NH Stretching), 1635 (C-O Stretching), 1592 (C=C Stretching), 1431 (CH Bending), 955 (CH Bending), 848 (NH Bending)

NMR Spectral Data

1.3015 (Singlet CH₃ proton), 1.5602 (Singlet CH₃ proton), 3.3029-3.64 (Singlet CH proton adjacent to OCOAr), 4.8 (Singlet CH proton adjacent to OCOAr), 7.05-7.3 (Multiplet, aromatic proton), 7.5-7.543 (Multiplet, aromatic proton)

Compound S₄**IR Spectral Data**

3754 (NH Stretching), 2923 (CH Stretching), 2854 (CH Stretching), 1627 (CO Stretching), 1440 (CH Bending), 748 (NH Bending)

NMR Spectral Data

1.31-1.7 (Singlet, CH₃ proton), 3.3-3.6 (Singlet, CH₃ proton), 7.03-7.52 (Singlet, aromatic proton), 8.48-8.5 (Singlet, NH proton)

Compound S₅**IR Spectral Data**

3757 (NH Stretching), 3431 (NH Stretching), 2956 (NH Stretching), 2870 (NH Stretching), 1635 (C-O Stretching), 1592 (C=C Stretching), 1431 (CH Bending), 955 (CH Bending), 848 (NH Bending)

NMR Spectral Data

1.38 (Singlet, CH₃ proton), 2.2-2.3 (Singlet, CH₂ proton), 3.2-4.8 (Singlet CH proton adjacent to OCOAr), 7.05-7.5 (Singlet Aromatic proton)

Compound S₆**IR Spectral Data**

3747.89 (NH Stretching), 2929 (CH Stretching), 2366 (CH Stretching), 1637 (CO Stretching), 1297 (CO Stretching), 998 (CH Bending), 834 (NH Bending)

NMR Spectral Data

1.39-2.3 (Singlet, CH₃ proton), 3.3-3.339 (Singlet CH proton adjacent to OCOAr), 7.26-7.5 (Singlet Aromatic proton)

Thin Layer Chromatography

Evaluation of various mobile phases as solvents alone or in combination was done for each compound. The R_f value of synthesis compounds have been found out using appropriate solvents system. Adsorbent or stationary phase is Silica gel G. The developing Solvent or Mobile phase is Ethanol and Chloroform. The detecting agent is Iodine vapor. Color of the spots Yellow [for S₁ S₅ S₆] and Brown [for S₂ S₃ S₄], Table 2²⁰

Development of Chromatogram

Removed about 2 mm of the adsorbent from each edge of the plate to give a sharply defined edge, Applied about 2-5 µl volumes of a 10 mg⁻¹ solution of the mixture and of reference substances in an organic solvent to the plate. Allowed the solvent to evaporate and transferred the plate to a previously prepared developing tank, this preparation was done about 30 minutes before insertion of the plate. Allowed the solvent to rise a distance of about 10 to 15 cm, removed the plate and allowed to dry.

Detection**Coloring agent**

Coloring agent used for the detection of the spot was done by using Iodine vapor. 1 g of iodine crystals were placed on a chamber and was kept closed using a lid for saturation of the chamber with iodine vapor. The dried plates were kept over the chamber and were observed for spot in each plate. Color of the spot was yellow.

Melting Point

The melting point is one of the physical data to evaluate the purity of the synthesized compounds. This was done by open capillary method. The melting range of the compounds defines its purity. Table 1

MATERIALS AND METHODS**Animals**

Male albino strains of wistar rats were obtained from central animal house, K. M. College of pharmacy, Madurai, India. The animals were given standard rodent diet and water *ad libitum* throughout the study. The rats used in the present study were maintained in accordance with guidelines of the national institute of nutrition, Indian council for medical research, Hyderabad, India and study approved by Institutional animal ethical committee.

Materials

- Various synthetic drugs such as S1 to S6
- Cholesterol extra pure for feeding purpose was obtained from S.D fine-chem. limited, Mumbai, India. Coconut oil was used as a vehicle for cholesterol feeding.
- Atorvastatin was obtained from Micro labs, Bangalore, India.

Experimental Procedure

All the animals were weighed and divided into nine groups each of six animals. Group I is served as Normal control. Group II is Cholesterol control. Fed cholesterol at a dose of 400 mg/kg body weight for 30 days; Group III is fed cholesterol as in group II and Atorvastatin 1 mg/kg body weight from days 15 to day 30. [3] Group IV is fed cholesterol as in group II and synthetic compound S1 at a dose of 50 mg/kg body weight from days 15 to day 30. Group V is fed cholesterol as in group II and synthetic compound S2 at a dose of 150 mg/kg body weight from days 15 to day 30. Group VI is fed cholesterol as in group II and synthetic compound S3 at a dose of 50 mg/kg body weight from days 15 to day 30. Group VII is fed cholesterol as in group II and synthetic compound S4 at a dose of 50 mg/kg body weight from days 15 to day 30. Group VIII is fed cholesterol as in group II and synthetic compound S5 at a dose of 50 mg/kg body weight from days 15 to day 30. Group IX is fed cholesterol as in group II and synthetic compound S6 at a dose of 50 mg/kg body weight from days 15 to day 30. At the end of 30 days all the rats were sacrificed, blood was collected, allowed to clot and serum was obtained by centrifugation. The serum samples were used for various biochemical procedures Table 3, 4²¹⁻²⁵.

Biochemical Analysis

The serum was analyzed for total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), by using standard protocol methods. (Auto Analyzer)²⁶⁻²⁹

Atherogenic index (AI) and LDL-C/HDL-C ratio

- The AI was calculated by the following formula
- $AI = (total\ cholesterol - HDL-C)/HDL-C$
- LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C Levels.

Statistical Analysis

All the values were expressed as mean $\pm$ SEM. Data was analyzed by one way analysis of variance (ANOVA) followed by Newman keul's multiple range tests. The P values < 0.05 were considered as statistically significant^{30,31}.

RESULTS

Table 3 and 4 show the levels of Cholesterol, Triglycerides, HDL, LDL and VLDL of control and experiment rats respectively. Serum of hyperlipidemic rats showed significantly increased levels of Cholesterol, Triglycerides, LDL - C and low HDL - C, when compared with normal rats. In rats treated with various synthetic drugs and Atorvastatin there was significant decrease in the content cholesterol, TGs, LDL - C, and VLDL and increases HDL - C, when compared with cholesterol control rats.

Atherogenic index (AI) and LDL - C / HDL - C ratio

Table 3 and 4 show the changes of Atherogenic Index and LDL - C / HDL - C ratio in control and treated rats. It appears clear from these results that the cholesterol induction significantly affects the cardio vascular risk markers. Indeed, AI was statistically increased in cholesterol control group 90 % compared with the values found in their normal control group. Besides there were significant further increase of LDL - C / HDL - C ratio in cholesterol control group compared to normal control group. Promising results in lowering of AI by various synthetic drugs that is bisindole substituted coumarin derivatives was found in Table 3 and 4. The various synthetic drugs showed an improvement of the cardio vascular risk level by decrease of AI in the treated groups when compared to the cholesterol control group. The ratio of LDL - C to HDL - C is also a protective indicator of cardio vascular disease incidence. The cholesterol induction produced a significant increase of this marker. In contrast, elevated ratio in treated group and Atorvastatin group returned to basal value when the data were compared in the same period to the data found for cholesterol rats.

Scheme of Work

General scheme

Scheme for Individual Synthesis
Synthesis of Compound S₁

Synthesis of Compound S₂

Synthesis of Compound S₃

Synthesis of Compound S₄

Synthesis of Compound S₅

Compound S₅

Synthesis of Compound S₆

Compound S₆

Table 1: Effect of various synthetic drugson Lipid Profile

Groups	Total cholesterol (Mg/dl)	Tri glycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	AI	LDL/HDL
Normal Control	52.60 ± 2.75	57.10 ± 2.89	26.40 ± 1.16	14.30 ± 0.70	31.88 ± 1.58	0.99 ± 1.37	0.54
Cholesterol Control	115.40 ± 4.50 ^{**a)}	160.4 ± 5.68 ^{**a)}	11.90 ± 0.65 ^{**a)}	31.95 ± 1.86 ^{**a)}	13.10 ± 0.70 ^{**a)}	8.69 ± 5.92 ^{**a)}	2.68
Standard Control	73.65 ± 3.52 ^{**b)}	80.8 ± 3.78 ^{**b)}	21.8 ± 1.06 ^{**b)}	21.01 ± 1.04 ^{**b)}	26.90 ± 0.92 ^{**b)}	2.37 ± 2.32 ^{**b)}	0.96
Treatment control (S1)	90.80 ± 3.75 ^{**b)}	118.42 ± 4.90 ^{**b)}	18.2 ± 0.90 ^{**b)}	25.23 ± 1.58 ^{**b)}	19.90 ± 0.45 ^{**b)}	3.98 ± 3.16 ^{**b)}	1.38
Treatment control (S2)	88.90 ± 2.94 ^{**b)}	94.3 ± 3.77 ^{**b)}	20.22 ± 1.20 ^{**b)}	22.08 ± 1.12 ^{**b)}	21.20 ± 0.88 ^{**b)}	3.39 ± 1.45 ^{**b)}	1.09

Table 2: Effect of various synthetic drugson Lipid Profile

Groups	Total cholesterol (Mg/dl)	Tri glycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	AI	LDL/HDL
Treatment control (S3)	85.83 ± 2.82 ^{**b)}	96.4 ± 3.80 ^{**b)}	19.32 ± 1.30 ^{**b)}	20.15 ± 1.72 ^{**b)}	23.30 ± 0.96 ^{**b)}	3.44 ± 1.16 ^{**b)}	1.04
Treatment control (S4)	83.80 ± 2.65 ^{**b)}	98.6 ± 3.92 ^{**b)}	21.30 ± 1.42 ^{**b)}	20.35 ± 1.62 ^{**b)}	22.35 ± 0.86 ^{**b)}	3.13 ± 2.93 ^{**b)}	0.95
Treatment control (S5)	85.90 ± 2.90 ^{**b)}	92.0 ± 3.07 ^{**b)}	20.32 ± 1.08 ^{**b)}	21.42 ± 1.82 ^{**b)}	23.32 ± 0.98 ^{**b)}	3.22 ± 1.68 ^{**b)}	1.05
Treatment control (S6)	87.80 ± 2.98 ^{**b)}	94.8 ± 3.12 ^{**b)}	21.55 ± 1.35 ^{**b)}	22.42 ± 1.72 ^{**b)}	22.86 ± 0.95 ^{**b)}	3.07 ± 1.20 ^{**b)}	1.04

Values are expressed as Mean ± SEM, Values were find out by using ONE WAY ANOVA followed by Newman Keul's multiple range tests, ^{**a)} values were significantly different from normal control at P < 0.01, ^{**b)} Values were significantly different from hyperlipidemic control at P < 0.01

Table 3: Data showing R_f values of compounds

S. No	Compound	Adsorbent	Mobile phase and ratio	R _f value
1	S ₁	Silica gel G	Ethanol : chloroform 6 : 4	0.68
2	S ₂		Ethanol : chloroform 8 : 2	0.73
3	S ₃		Chloroform : benzene 7 : 3	0.62
4	S ₄		Chloroform : benzene 6 : 4	0.79
5	S ₅		Ethanol : hexane 5 : 5	0.70
6	S ₆		Ethanol : hexane 3 : 7	0.59

CONCLUSION

From the physical characteristics and spectral data of compound S₁ – S₆ showed that the synthesized compounds were our expected substituted compounds. So these were selected to study their anti-hyperlipidemic potential. So it was concluded that synthetic reactions were completed and yielded our final compounds. Hyperlipidemia is considered to be major risk factor for the premature atherosclerosis and essentially the cholesterol in atherosclerotic plaque is derived from that of circulatory cholesterol. The anti hyperlipidemic effect of various synthetic drugs of bisindole substituted coumarin could be considered as a possible therapeutic value.

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