

Anti-hyperlipidemic Activity of Thieno – [2, 3-d] - Pyrimidin-4-(3H)-ones

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Abstract

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Key words: Thieno – [2, 3-D] - pyrimidin-4-(3H)-ones; Hyperlipidemic; Hypotriglyceridaemia; Atherogenic index.

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Aim: The present study was aimed to evaluate some newly synthesized thieno – [2, 3-d] - pyrimidin-4-(3H)-ones namely SR-C1 to SR-C12 for their anti-hyperlipidemic activity.

Materials and Methods: Anti-hyperlipidemic activity was evaluated using Triton 1339-induced hyperlipidemia in rats as an experimental model. Plasma triglycerides, total cholesterol, HDL-C, LDL-C, atherogenic index (AI) and LDL-C/HDL-C ratio were determined to assess the anti-hyperlipidemic activity.

Results: SR-C1, SR-C4, SR-C5, SR-C6, SR-C7, SR-C11 and SR-C12 have shown significant anti-hyperlipidemic activity by decreasing the total cholesterol and triglyceride, LDL-cholesterol, atherogenic index and LDL/HDL ratio ($p < 0.001$) and by increasing the HDL-Cholesterol ($p < 0.001$)

Conclusion: The findings of the present study clearly demonstrates that methyl, methoxy, chloro, dimethylamino, dimethoxy and trimethoxy functional groups possess cholesterol-suppressive capacities and has an ability to attenuate the accelerated development of atherosclerosis in hypercholesterolemic models. However, hydroxyl and nitro derivatives did not show any hypolipidemic activity.

Introduction

Various studies have shown that the plasma hypercholesterolemic state could contribute to the development of atherosclerosis and related cardiovascular system diseases (CVD) which are the most common causes of death in both western and eastern societies [1]. Indeed, clinical trials have demonstrated that the increase in plasma low density lipoprotein cholesterol (LDL-C) levels is implicated in the early development and progression of atherosclerosis. However, high density lipoprotein cholesterol (HDL-C) is an anti-atherogenic fraction [2]; Triglycerides (TGs) may

also be a risk factor, especially in individuals with diabetes [3]. A logical strategy to prevent or to treat atherosclerosis and to reduce the incidence of cardiovascular disease events is to target hyperlipidemia by drugs and/or dietary intervention [4]. With this aim, efforts to develop effective and better hypolipidemic drugs have led to discovery of many moieties with lipid-lowering property.

Triton WR-1339, a non-ionic detergent (oxyethylated tertiary octyl phenol formaldehyde polymer), has been widely used to produce acute hyperlipidemia in animal models in order to screen natural or chemical drugs [5] and to study cholesterol

and triacylglycerol metabolism [6]. The accumulation of plasma lipids by this detergent appears to be especially due to the inhibition of lipoprotein lipase activity [7]. Pyrimidin is a unique molecule. The applications of pyrimidin and its derivatives in chemotherapy are of versatile character. Being an active principle, it is found in numerous compounds like purines, nucleosides, nucleotides, anticancer drugs, antiviral drugs, antibiotics, vitamins, anti malarials, sulpha drugs and lot more [8, 9].

The close association of pyrimidin nucleus with biologically important compounds has drawn attention of number of workers. Although much work has been done on condensed pyrimidin with several other heterocycles, only a handful of reports are available concerning to thieno- (2,3-d) pyrimidin-4- ones. Thieno[2,3-d]pyrimidines a derivative of pyrimidine, are a large group of heterocycles with diverse and interesting biological activities. These compounds are reported to possess significant analgesic [10, 11], fungicidal [8], antiviral [9] and anti-inflammatory [12,13] activities. Also, some thieno[2,3-d]pyrimidines show CNS depressing activity [14] and are useful as muscle relaxants [15], sedatives [15], diuretics [16], pesticides and herbicides [17]. Various methods have already been proposed for the synthesis of these compounds and the most general ones involves cyclocondensation of suitably functionalized thiophenes with different electrophiles such as chloroformamide [18], a-substituted acetonitriles [19], formic acid [19], phosgen [20], ethyl chloroformate [21] and guanidine [22].

The present investigation makes use of the elegant method described by Gewald et al for thieno (2, 3-d) pyrimidine synthesis. The appropriate thieophene 2-amino-3-carbethoxy-4,5,6,7-tetra hydro benzo (b)thieophene was prepared by Gewald reaction [23] starting from cyclo hexanone and active methylene compound that is ethyl cyano acetate. Reaction of 2-amino-3-carbethoxy-4,5,6,7-tetra hydro benzo (b) thieophene with acetic anhydride followed by treatment with hydrazine hydrate yielded the starting material 2-methyl-3- N-amino -5,6,7,8-tetrahydro benzothieno[2,3-d]- pyrimidin -4-ones (SR-C)

Materials and Methods

Drugs and Chemicals

Ethylcyanoacetate (Sisco Research Laboratories Pvt. Ltd., India.), Cyclohexanone (Sisco Research Laboratories Pvt. Ltd., India.), Sulphur (Helix

Lab Tech, Bangalore, India.), Hydrazine Hydrate (Helix Lab Tech, Bangalore, India), Triton WR-1339 (Tyloxapol, Sigma-Aldrich, USA), the solvents and other chemical used for the study were of analytical grade and purchased from local firms.

Animals

Wistar Rats weighing 150–200 g were procured from In vivo Biosciences, Kachohalli, Bangalore for experimental purpose. Then all the animals were acclimatized at least under standard husbandry conditions, i.e.; room temperature of $24 \pm 1^\circ \text{C}$; relative humidity 45–55% and a 12 : 12 h light/dark cycle. The animals had free access to standard rat pellet (Pranav Agro Industry, Bangalore), with water supplied *ad libitum* under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed anywhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any non- specific stress. The approval of the Institutional Animal Ethical Committee (IAEC/2007/) of P.E.S. College of Pharmacy Bangalore (Karnataka) was taken prior to the start of experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines by committee for the purpose of control and Supervision of Experimental on Animals (CPCSEA, India).

Experimental design

Experimental design is been given in Figure 1.

Procedure

The appropriate thieophene 2-amino-3-carbethoxy-4,5,6,7-tetra hydro benzo (b) thieophene was prepared by Gewald reaction starting from cyclo hexanone and active methylene compound that is ethyl cyano acetate. Reaction of 2-amino-3-carbethoxy-4,5,6,7-tetra hydro benzo (b) thieophene with acetic anhydride followed by treatment with hydrazine hydrate yielded the starting material 2-methyl-3- N-amino -5,6,7,8-tetrahydro benzothieno[2,3-d]- pyrimidin -4-one.

Step 1- Synthesis of 2-Amino-3- carbethoxy-4,5,6,7-tetrahydro benzo(b) thiophene (SR-A). A mixture of cyclohexanone (3.92 g ; 0.04 mol), ethyl cyanoacetate(4.52g; 0.04 mol) and sulphur powder(1.28 g ; 0.04 mol) in ethanol (40 ml) was added diethylamine (4.0 ml) drop wise with stirring . The mixture was stirred further for 1hr at 45-50°C, chilled overnight and the solid

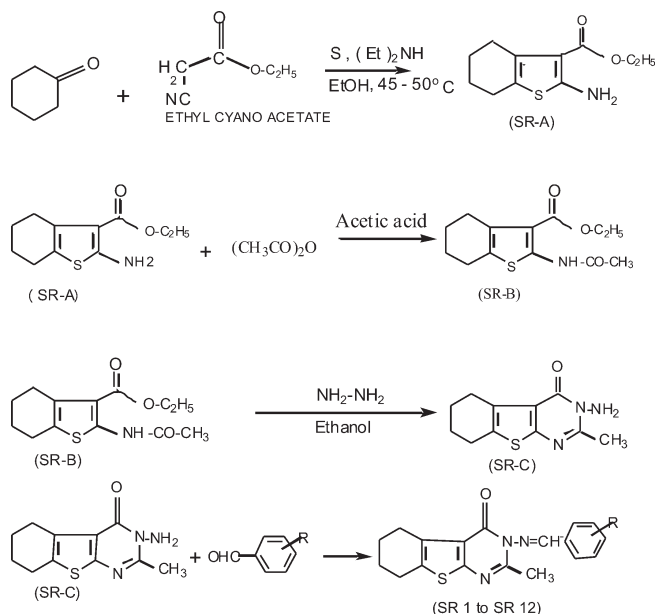


Figure 1: Experimental design of the thieno – [2, 3-d] - Pyrimidin-4-(3H)-ones.

obtained was filtered, washed and recrystallised from ethanol .

Step 2- Synthesis of 2-Aceto-3- carbethoxy-4,5,6,7-tetrahydro benzo(b) thiophene (SR-B). A mixture of 2-Amino-3- Carbethoxy-4,5,6,7-Tetrahydro benzo(b) Thiophene (SRA) (2.25 g, 0.01 mol), acetic anhydride (6 ml) and zinc dust (0.25 g) was stirred and irradiated with microwave heating involving kenstar microwave oven (2450 MHz, 900 w) for 15 seconds, when the solid dissolved the reaction mixture was cooled and the resulting white solid was crystallized from methanol, yield = 75%, M.P=122°C.

Step 3- Synthesis of 2-methyl-3- N-amino -5,6,7,8-tetrahydro benzothieno [2,3-d]- pyrimidin –4-one (SR-C). A mixture of SR-B (2.35 g, 0.01 mol), hydrazine hydrate (15 ml) and ethanol (20 ml) was irradiated for 20 seconds until the solid dissolved, the irradiation was continued until solid separates out from the reaction mixture. Then the reaction mixture was cooled to room temperature, a white crystalline product was obtained which was crystallised from aqueous acetone (1:2).

Yield = 50%, MP-86°C.

Step 4- Synthesis of 3-N-[(substituted aryl)-methylene]-imino-2-methyl –5,6,7,8-tetra hydrobenzo thieno[2,3-d]-pyrimidin-4(3H)-ones (SR 1-SR 12). A mixture of SR-C (2.35 g, 0.01 mol) and the appropriate

aryl aldehydes (0.01 mol) in propanol containing catalytic amount of glacial acetic acid (2 ml) was irradiated for 20 seconds then the mixture was cooled to get the corresponding title compounds (SR1-SR12) and are crystallised from propanol or ethyl acetate to obtain the pure compounds.

Acute Toxicity Test

Acute toxicity studies were performed according to Organization for Economic Co-Operation and Development (OECD) guidelines 425. The acute toxicity of some Thieno – [2, 3-D] - Pyrimidin-4-(3H)-ones. was determined by using female swiss mice (18-25 g) those maintained under standard husbandry conditions. The animals were fasted 3 hrs prior to the experiment. Animals were administered with single dose of test compounds and observed for their mortality during 48 hours study period (short term) toxicity. Based on short-term profile of drug, the dose of the drug was fixed.

Triton model of hyperlipideamia

Triton WR-1339 (Tyloxapol, Sigma–Aldrich, USA) was dissolved in normal saline (pH 7.4) and administered intraperitoneally to the rat (200 mg/kg B.W) in order to develop an acute hyperlipidemia in them.

Experimental design

Overnight fasted rats were randomly divided into groups of six animals each. The first group, serving as a normal control (NCG), received an intraperitoneal administration of normal saline and water by gavage; the second, hyperlipidemic control group (HCG) was treated with Triton and gavaged by distilled water; the third, hyperlipidemic plus DMSO (4% v/v in distilled water) control group (HDCG) received an intraperitoneal injection of Triton and was gavaged with DMSO 4% v/v (in distilled water). Fourth group received the standard drug Atorvastatin 10 mg/kg suspended orally followed by Triton. The remaining groups (SR) received all the derivatives at 10 mg/kg and 30 mg/kg BW of drug dissolved in DMSO-distilled water mixture followed by intraperitoneal injection of Triton.

After treatments (7 h and 24 h), animals were anaesthetized briefly with diethyl ether and blood was taken from retro orbital puncture using a heparinised capillary. The blood samples were immediately centrifuged (2500 rpm/10 min) and the plasma was used for lipid analysis.

Analytical procedures

Triglycerides in plasma were quantified by an enzymatic method using Span Diagnostic kits. Briefly, after enzymatic hydrolysis with lipases, the formation of quinoneimine from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic effect of peroxidase, was followed spectrophotometrically at 505 nm. Total cholesterol levels were determined by the cholesterol oxidase enzymatic method, using Span Diagnostic Kits cholesterol was hydrolyzed and, in the presence of phenol, the quinoneimine as indicator was formed from hydrogen peroxide and 4-aminoantipyrine via peroxidase catalysis and spectrophotometrically measured at 505 nm. HDL-cholesterol concentrations were quantified by the same method as used to determine total cholesterol after removal of other lipoproteins by precipitation with phosphotungstic acid (PTA) and $MgCl_2$ (Span Diagnostic kit, Inc, India). The LDL-cholesterol was calculated by the Friedwald formula [24]:

$$\text{LDL-Cholesterol} = \text{totalcholesterol} - [\text{HDL-Cholesterol} + \text{triglycerides}] / 5$$

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

The AI was calculated by the following formula: $AI = (\text{total cholesterol} - \text{HDL-C})/\text{HDL-C}$ and the LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C levels.

Statistical analysis

Data are basically expressed as the mean value (mean) and standard deviation (S.D.). Statistical analysis was performed with the Dunnett's test (JMP, SAS Institute). Asterisks are used to denote a significant difference at the level of $p < 0.05$.

Results

Acute toxicity studies

There was no adverse effects or mortality detected in the mice up to 1000 mg/kg, p.o., during the 48 h observation period. LD_{50} was found to be 310 mg/

Table 1: Effect of Some Novel Thieno – [2, 3-d] - Pyrimidin-4-(3H)-ones on plasma lipid levels in Triton WR-1339-induced hyperlipemic rats after 7 h.

GROUPS	Dose	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal control (NCG)	---	180.2 ± 3.135 ^c	165.2 ± 23.57 ^c	126.2 ± 1.259	121.8 ± 2.12 ^c
Hyperlipidemic Control (HCG)	Triton 200 mg/kg p.o.	430.1 ± 3.135 ^c	256.2 ± 23.57 ^c	123.2 ± 1.259	353.4 ± 2.12 ^c
Vehicle control (HDCG)	DMSO 4% v/v	452.4 ± 4.632 ^c	268 ± 35.43 ^c	132.56 ± 1.13	372.5 ± 12.78 ^c
Standard (Atorvastatin)	10 mg/kg p.o.	284.43 ± 65.11 ^z	196.2 ± 34.16 ^z	155.04 ± 5.6 ^z	213.8 ± 2.14 ^z
SR-C 1	10 mg/kg p.o.	290.32 ± 22.43 ^z	202.3 ± 2.425 ^z	162.2 ± 6.431	217.0 ± 2.15 ^y
SR-C 1A	30 mg/kg p.o.	245.5 ± 2.24 ^z	186.2 ± 3.02 ^z	155.42 ± 2.76	176.8 ± 2.83 ^c
SR-C 2	10 mg/kg p.o.	436.2 ± 22.24	245.2 ± 05.24	165.2 ± 4.357	354.3 ± 53.26
SR-C 2A	30 mg/kg p.o.	455.72 ± 22.68	262.3 ± 35.13	154.44 ± 6.23	371.8 ± 31.25
SR-C 3	10 mg/kg p.o.	510.2 ± 20.47	285.3 ± 14.62	172.35 ± 2.175	418.6 ± 21.19
SR-C 3A	30 mg/kg p.o.	545 ± 2.11	292.4 ± 45.22	181.22 ± 2.57	450.7 ± 28.62
SR-C 4	10 mg/kg p.o.	210.3 ± 8.372 ^z	171.9 ± 8.09 ^x	164.56 ± 1.49	141.6 ± 8.64 ^y
SR-C 4A	30 mg/kg p.o.	195.3 ± 7.390 ^z	165.6 ± 11.8 ^x	158.2 ± 2.428	130.6 ± 6.512
SR-C 5	10 mg/kg p.o.	226.0 ± 3.803 ^z	198.6 ± 11.29 ^x	152.57 ± 1.76	156.4 ± 4.27 ^a
SR-C 5A	30 mg/kg p.o.	235.5 ± 7.751 ^z	210.3 ± 13.23 ^x	160.8 ± 19.01	161.5 ± 22.3 ^a
SR-C 6	10 mg/kg p.o.	206.3 ± 18.85	181.1 ± 10.97	166.9 ± 3.672	136.4 ± 21.67
SR-C 6A	30 mg/kg p.o.	215.4 ± 6.168	162.0 ± 10.58	154.16 ± 2.799	151.6 ± 6.126
SR-C 7	10 mg/kg p.o.	191.7 ± 9.85 ^x	167.7 ± 9.114 ^x	150.06 ± 3.126	128.1 ± 11.69 ^y
SR-C 7A	30 mg/kg p.o.	185.5 ± 7.225 ^z	154.6 ± 7.55 ^x	157.59 ± 2.237	123.0 ± 7.729 ^y
SR-C 8	10 mg/kg p.o.	424.8 ± 9.761	260.2 ± 10.89	119.90 ± 1.734	348.9 ± 11.20
SR-C 8A	30 mg/kg p.o.	429.8 ± 11.70	265.3 ± 9.861	122.8 ± 2.601	352.0 ± 12.89
SR-C 9	10 mg/kg p.o.	520.4 ± 6.168	289.7 ± 8.244	174.7 ± 2.159	426.7 ± 7.287
SR-C 9A	30 mg/kg p.o.	528.4 ± 3.163	281.7 ± 6.244	167.9 ± 5.449	437.8 ± 5.261
SR-C 10	10 mg/kg p.o.	459.8 ± 9.480	270.7 ± 7.845	127.84 ± 2.688	380.0 ± 8.716
SR-C 10A	30 mg/kg p.o.	468.4 ± 5.866	276.3 ± 8.985	135.1 ± 2.422	386.2 ± 5.165
SR-C 11	10 mg/kg p.o.	280.4 ± 18.50 ^z	211.9 ± 4.586 ^x	170.8 ± 1.424	203.4 ± 18.95 ^y
SR-C 11A	30 mg/kg p.o.	264.9 ± 8.663 ^z	196.6 ± 8.147 ^y	162.64 ± 2.484	193.2 ± 10.80 ^a
SR-C 12	10 mg/kg p.o.	301.9 ± 9.952 ^z	210.2 ± 16.42 ^y	201.9 ± 1.781	218.0 ± 11.47 ^x
SR-C 12A	30 mg/kg p.o.	309.8 ± 8.651 ^z	201.5 ± 16.21 ^z	190.23 ± 1.34	230.8 ± 8.51 ^z

Values are means ± SEM from six animals in each group. NCG, normal control group; HCG, hyperlipidemic control group; HDCG, hyperlipidemic + 4% DMSO control group; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. HCG and HDCG are compared with NCG. The derivatives are compared with HCG. NS, not significant. a, x, $P < 0.05$; b, y, $P < 0.001$; c, z, $P < 0.0001$.

Table 2: Effect of Some Novel Thieno – [2, 3-d] - Pyrimidin-4-(3H)-ones on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 24 h.

GROUPS	Dose	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal control	---	184.03 ± 2.860	172.13 ± 4.7	135.30 ± 0.0	122.6 ± 2.330
Hyperlipidemic Control	Triton 200 mg/kg p.o.	454.8 ± 9.475 ^c	263.8 ± 46.57 ^c	150.45 ± 1.349 ^c	271.55 ± 15.69 ^c
Vehicle control	DMSO 4% v/v	464.4 ± 3.212 ^c	275 ± 12.22 ^c	156.1 ± 1.243 ^c	378.0 ± 18.41 ^c
Standard (Atorvastatin)	10 mg/kg p.o.	260.3 ± 10.96 ^y	189.9 ± 20.56 ^z	162.1 ± 2.013 ^z	188.8 ± 14.92 ^z
SR-C1	10 mg/kg p.o.	251.7 ± 9.851 ^z	198.1 ± 5.978 ^z	164.73 ± 2.72 ^z	178.9 ± 9.362 ^y
SR-C 1A	30 mg/kg p.o.	236.3 ± 6.283 ^z	194.5 ± 16.92 ^z	171.8 ± 5.532	162.8 ± 7.89 ^z
SR-C 2	10 mg/kg p.o.	456.2 ± 14.46	252.1 ± 16.38	164.13 ± 2.883	373.1 ± 15.85
SR-C 2A	30 mg/kg p.o.	461.2 ± 14.46	268.1 ± 16.38	155.13 ± 2.883	376.1 ± 15.85
SR-C 3	10 mg/kg p.o.	525.4 ± 18.12	304.7 ± 28.12	182.22 ± 1.22	426.6 ± 31.44
SR-C 3A	30 mg/kg p.o.	550.3 ± 41.19	312.6 ± 13.12	186.23 ± 1.307	450.0 ± 41.97
SR-C 4	10 mg/kg p.o.	215.3 ± 8.372 ^z	164.9 ± 8.093 ^z	169.56 ± 1.493 ^y	134.9 ± 8.642 ^z
SR-C 4A	30 mg/kg p.o.	202.3 ± 7.390 ^y	160.6 ± 11.8 ^z	175.26 ± 2.428 ^z	135.0 ± 6.512 ^z
SR-C 5	10 mg/kg p.o.	230.3 ± 7.390 ^y	179.6 ± 11.8 ^z	160.26 ± 2.428 ^z	162.0 ± 6.512 ^z
SR-C 5A	30 mg/kg p.o.	205.0 ± 3.803 ^z	171.6 ± 11.29 ^z	163.57 ± 1.768 ^z	138.3 ± 4.273 ^z
SR-C 6	10 mg/kg p.o.	198.3 ± 18.85 ^z	185.1 ± 10.97 ^z	164.9 ± 3.672 ^z	128.1 ± 21.67 ^a
SR-C 6A	30 mg/kg p.o.	190.4 ± 6.168 ^z	178.0 ± 10.58 ^z	168.16 ± 2.799 ^z	120.5 ± 6.126 ^z
SR-C 7	10 mg/kg p.o.	194.7 ± 9.851 ^z	169.7 ± 9.114 ^z	152.06 ± 3.126 ^z	131 ± 11.69 ^z
SR-C 7A	30 mg/kg p.o.	189.5 ± 7.225 ^z	161.6 ± 7.537 ^z	159.6 ± 2.237 ^z	126.2 ± 7.729 ^z
SR-C 8	10 mg/kg p.o.	431.8 ± 9.761	266.2 ± 10.89	109.90 ± 1.734	356.8 ± 11.20
SR-C 8A	30 mg/kg p.o.	438.4 ± 7.521	271.7 ± 4.401	115.03 ± 1.247	360.6 ± 7.842
SR-C 9	10 mg/kg p.o.	524.8 ± 11.70	298.3 ± 9.861	162.8 ± 2.601	432.5 ± 12.89
SR-C 9A	30 mg/kg p.o.	532.4 ± 6.168	301.7 ± 8.244	164.7 ± 2.459	438.5 ± 7.287
SR-C 10	10 mg/kg p.o.	464.8 ± 9.480	284.7 ± 7.845	121.84 ± 2.688	383.66 ± 8.716
SR-C 10A	30 mg/kg p.o.	472.4 ± 5.866	281.3 ± 8.985	130.1 ± 2.422	389.6 ± 5.165
SR-C 11	10 mg/kg p.o.	197.4 ± 18.50 ^z	222.9 ± 4.586 ^z	145.6 ± 1.424 ^x	113.4 ± 18.95 ^z
SR-C 11A	30 mg/kg p.o.	189.9 ± 8.663 ^z	200.6 ± 8.147 ^z	153.64 ± 2.484 ^y	119.0 ± 10.80 ^z
SR-C 12	10 mg/kg p.o.	198.9 ± 9.952 ^z	154.2 ± 16.42 ^z	145.9 ± 1.781 ^x	139.0 ± 11.47 ^z
SR-C 12A	30 mg/kg p.o.	194.8 ± 8.651 ^z	159.8 ± 5.506 ^z	151.9 ± 1.417 ^y	132.6 ± 8.518 ^z

Values are means ± SEM from six animals in each group. NCG, normal control group; HCG, hyperlipidemic control group; HDCG, hyperlipidemic + 4% DMSO control group; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. HCG and HDCG are compared with NCG. The derivatives are compared with HCG. NS, not significant. a, x, P < 0.05; b, y, P < 0.001; c, z, P < 0.0001.

kg. Based on the results obtained from this study, the dose for anti-hyperlipidemic activity was fixed at 10 mg and 30 mg/kg.

Induction of hyperlipidemia by Triton WR-1339

The plasma total cholesterol and triglyceride levels of all groups 7 h and 24 h after treatments are shown in Tables 1 and 2. In comparison with the normal control group (NCG), Triton WR-1339 caused a marked increase of plasma total cholesterol and triglyceride levels of the hyperlipidemic control group (HCG) and HCG+DMSO 4% (HDCG), at both 7 h and 24 h after injection.

In fact, 7 h after Triton administration the increase of plasma total cholesterol concentration were 139% in HCG and 151% in HDCG with respect to the NCG. Triglyceride levels were also elevated by 55% and 62% in HCG and HDCG, respectively. Again, 24 h after treatment, the elevated plasma lipid profile was maintained, either in the hyperlipidemic group rats (HCG) or in those receiving Triton, followed by 4% DMSO (HDCG). In the HCG, the increase of total cholesterol

was 147% and that of triglycerides was 53%. In the HDCG, a similar significant pattern of change was observed for blood total cholesterol (+152%) and triglyceride levels (+59%) when compared to the NCG. HDL and LDL-cholesterol concentrations are shown in Tables 1 and 2. There was no significant change in HDL cholesterol, however both at 7h & 24 h in HCG and HDCG with respect to control group (NCG), while a significant increase on LDL-cholesterol levels occurred at 7 h and was maintained until 24 h from Triton injection. LDL-cholesterol concentrations in HCG and HDCG were respectively, 191% and 200% higher than those in normal control grouped animals after 7 h. Also, the increase of this parameter was maintained at +122% and +200% in HCG and HDCG, respectively, 24 h after the beginning of the experiment.

Table 3 shows the changes of atherogenic index (AI) and LDL-C/HDL-C ratio in control and treated rats. It appears clear from these results that the Triton administration significantly affects the cardiovascular risk markers. Indeed, the AI was statistically increased in both HCG (+250%) and HDCG (251%) when compared with values found in their relative normolipidemic control

Table 3: Effect of Some Novel Thieno – [2, 3-d] - Pyrimidin-4-(3H)-ones on atherogenic index (AI) and LDL/HDL-C ratio in Triton WR-1339-induced hyperlipemic rats after 7 h and 24 h on plasma.

Groups	AI (7 hr)	AI (24 hr)	LDL/HDL (6 hr)	LDL/HDL (24 hr)
Normal (NCG)	0.42 ± 0.01	0.36 ± 0.043	0.8 ± 0.16	0.36 ± 0.03
Control (HCG)	2.44 ± 0.10	2.01 ± 0.25 ^c	2.83 ± 0.18 ^b	1.81 ± 0.38 ^c
Vehicle (HDCG)	2.42 ± 0.15 ^b	1.97 ± 0.23 ^b	2.81 ± 0.53 ^c	2.42 ± 0.21 ^c
Std	0.83 ± 0.08 ^y	2.18 ± 0.21 ^z	1.37 ± 0.11 ^y	2.08 ± 0.26 ^x
1	0.79 ± 0.08 ^z	0.47 ± 0.17 ^z	1.34 ± 0.11 ^z	0.98 ± 0.14 ^z
1A	0.58 ± 0.23 ^z	0.25 ± 0.34 ^z	1.14 ± 0.16 ^z	1.65 ± 0.2 ^y
2	1.64 ± 0.09	4.01 ± 0.28	2.14 ± 0.10	2.98 ± 0.31
2A	1.95 ± 0.14	4.45 ± 0.49	2.41 ± 0.13	2.98 ± 0.50
3	1.96 ± 0.65	4.46 ± 0.50	2.43 ± 0.12	4.13 ± 0.48
3A	2.01 ± 0.07	5.64 ± 1.25	2.48 ± 0.07	5.18 ± 1.16
4	0.27 ± 0.14 ^z	0.57 ± 0.33 ^z	0.86 ± 0.10 ^z	4.07 ± 0.3
4A	0.23 ± 0.19 ^z	1.24 ± 0.27 ^z	0.82 ± 0.12 ^z	3.95 ± 0.22
5	0.48 ± 0.15 ^z	2.60 ± 0.18 ^x	1.02 ± 0.14 ^y	2.66 ± 0.15 ^y
5A	0.46 ± 0.12 ^z	0.25 ± 0.75 ^z	1.00 ± 0.08 ^y	2.71 ± 0.57 ^z
6	0.33 ± 0.33 ^z	0.36 ± 0.59 ^z	0.81 ± 0.10 ^z	0.94 ± 0.54 ^z
6A	0.39 ± 0.11 ^z	2.03 ± 0.23 ^z	0.98 ± 0.0 ^z	1.78 ± 0.16 ^z
7	0.28 ± 0.22 ^z	2.02 ± 0.43 ^c	0.85 ± 0.18 ^y	1.10 ± 0.40 ^y
7A	0.17 ± 0.12 ^z	1.08 ± 0.25 ^c	0.77 ± 0.11 ^y	1.08 ± 0.19 ^y
8	2.54 ± 0.18	2.92 ± 0.47	2.90 ± 0.13	3.24 ± 0.39
8A	1.45 ± 0.12	2.80 ± 0.29	2.86 ± 0.10	3.13 ± 0.28
9	1.97 ± 0.26	2.18 ± 0.43	2.44 ± 0.18	2.67 ± 0.35
9A	2.14 ± 0.11	2.22 ± 0.29	2.60 ± 0.12	2.65 ± 0.25
10	2.59 ± 0.14	2.81 ± 0.28	2.85 ± 0.11	2.99 ± 0.24
10A	2.46 ± 0.20	2.63 ± 0.33	0.04 ± 0.17 ^z	4.40 ± 0.23
11	0.63 ± 0.11 ^z	0.35 ± 0.40 ^z	1.18 ± 0.08 ^y	0.77 ± 0.40 ^z
11A	0.61 ± 0.12 ^z	0.23 ± 0.33 ^z	1.17 ± 0.09 ^y	0.77 ± 0.28 ^z
12	0.42 ± 0.16 ^z	0.36 ± 0.38 ^z	1.08 ± 0.15 ^z	0.95 ± 0.34 ^z
12A	0.63 ± 0.14 ^z	0.28 ± 0.18 ^z	1.21 ± 0.14 ^y	0.87 ± 0.185 ^z

Values are means ± SEM from six animals in each group. NCG, normal control group; HCG, hyperlipidemic control group; HDCG, hyperlipidemic + 4% DMSO control group; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. HCG and HDCG are compared with NCG. The derivatives are compared with HCG. NS, not significant. a, x, P < 0.05; b, y, P < 0.001; c, z, P < 0.0001.

at 7 h. Similar results were noticed at 24 h. The AI of HCG was +450% higher than that of NCG and +447% in HDCG with respect to NCG.

Besides, there was significant further increase of LDL-C/HDL-C ratios in Triton-injected animals (HCG and HDCG). In contrast to normolipidemic rats, 7 h after Triton treatment produced an elevated ratio either in hyperlipidemic group animals (493%) or in HDCG (487%). This changing pattern was maintained until 24 h when the ratios were increased by 400% and 570% in HCG and HDCG, respectively, compared to NCG.

Effect of Some Novel Thieno – [2, 3-d] - Pyrimidin-4-(3H)-ones on plasma profile

Total cholesterol and triglycerides

The plasma total cholesterol and triglyceride levels of the Novel Thieno – [2, 3-d] - Pyrimidin-4-(3H)-ones are shown in Tables 1 and 2. Importantly, the elevated total cholesterol concentrations produced by Triton administration after 7 h were significantly (P values < 0.001) suppressed by more than 50% in animals gavaged with SR-C1, SR-C4, SR-C5, SR-C6, SR-C7, SR-C11, and SR-C12. However, there was no reduction of total cholesterol by the remaining derivatives compared

to its relative hyperlipidemic control group (HDCG). Plasma TG levels of SR-C 1, 11 and 12 were significantly less when compared to HDCG (P < 0.001). While the plasma TG levels of the rats treated with SR-C4, 5 & 7 were comparatively less (P < 0.05) with respect to the levels found in animals of HDCG. Again, from 7 h, the similar lipid lowering effect of various extracts was maintained until 24 h in SR-C1, 11 & 12, while in SR-C5 & 7 the plasma TG levels were not significantly reduced.

HDL and LDL-cholesterol

Neither at 7 h nor at 24 h there was a significant differences in blood HDL-C between any treated groups were observed. However the LDL-cholesterol was significantly reduced in SR-C1, 4, 5, 7, 11 and 12 treated groups. Similarly the other derivatives did not show any significant ameliorative action on plasma elevated LDL-cholesterol caused by TritonWR-1339.

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

Promising results in lowering of the AI by the SR-C1, 4, 5, 6, 7, 11 and 12 in Triton-induced hyperlipidemic rats were found. This cardiovascular predictive marker in other derivatives not significantly different from the hyperlipidemic control group rats treated with 4% DMSO (HDCG). These compounds are substituted with methyl, methoxy, chloro, di and trimethoxy showed an improvement of the cardiovascular risk level by the decrease of AI by more than 147% (P values are less than 0.0001) at 7 h and 100% at 24 h (P values are less than 0.001) when compared to their corresponding hyperlipidemic control (HCG).

The ratio of LDL-C to HDL-C is also a predictive indicator of cardiovascular disease incidence. The Triton injection produced a significant increase of this marker and as with similar other biomarkers the SR-C1, 4, 5, 6, 7, 11 and 12 there was a significant reduction (p < 0.001 to < 0.00001) both at 7 h or at 24 h after treatment. In contrast, other derivatives did not show any significant activity in any of the lipid profile biomarkers.

Discussion

Triton WR-1339 has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidaemia in several animals [25]. This model is widely used for a number of different aims [26] and, in particular, in rats it has been used for screening natural or chemical hypolipidaemic drugs because it is convenient in terms of length of treatment period and handling. A

parenteral administration of a dose of Triton WR-1339 to adult rats induced hyperlipidaemia [27]. The maximum plasma triglycerides and total cholesterol were reached at 20 h, followed by a decline to normal values. Similar results were observed when investigating with the same model, the hypolipidaemic effect of *Mucuna pruriens* [28] and *Achyranthusaspera* [29], respectively. In our study too, the same model gave similar pattern of lipid profile changes either at 24 h after Triton injection (Table 1) and demonstrates the feasibility of using it of acute hyperlipidaemia, to assess the hypolipemic activity.

In our study this model gave similar plasma lipid profile changes, both at 7 h and at 24 h after Triton WR-1339 injection in rats, these results demonstrates the feasibility of using Triton induced hyperlipidemic rats as an experimental model to investigate the hypolipidemic effect of Novel Thieno – [2, 3-d] - Pyrimidin-4-(3H)-ones. It is clear from our results that the compounds SR-C1, 4, 5, 6, 11 and 12 decreased plasma total cholesterol in a marked manner, both at 7 h and 24 h after Triton treatment. The reduction of plasma total cholesterol was associated with a decrease in LDL fraction which is a major, potentially modifiable risk factor of cardiovascular diseases and the target of many hypocholesterolemic therapies. These findings suggest that the cholesterol-lowering activity of these derivatives appears to be due to the enhancement of LDL-C catabolism through hepatic receptors [29].

In addition, these derivatives showed protective action by the increase of HDL-cholesterol levels, which is reported to have a preventive function against atherogenesis since an independent inverse relationship between blood HDL-C levels and cardiovascular risk incidence has been documented and reported beyond any doubt [30]. This lipoprotein called “good cholesterol” facilitates the mobilization of triglycerides and cholesterol from plasma to liver where it is catabolised and eliminated in the form of bile acids. The possible mechanism of this activity may result from the enhancement of lecithin cholesteryl acyl transferase (LCAT) and inhibition of hepatic triglyceride lipase (HTL) on HDL which may lead to a rapid catabolism of blood lipids through extrahepatic tissues [31]. It is also recently reported that triglycerides play a key role in the regulation of lipoprotein interactions to maintain normal lipid metabolism. Indeed, the elevated plasma TGs levels were associated with an increased incidence of coronary artery disease [32]. Moreover, these higher plasma TG levels have been attributed mainly to an increased population of small, dense LDL deposits which are very atherogenic [33] and enhanced

cholesteryl ester mass transfer from apolipoprotein B-containing lipoproteins (VLDL and LDL) [34]. TGs have also been proposed to be a major determinant of cholesterol esterification, its transfer and HDL remodelling in human plasma [35]. These derivatives suppressed the elevated blood concentrations of TGs. This result suggests that the derivatives are able to restore, almost to normal level, the catabolism of triglycerides. The underlying mechanism of this activity is not elucidated by the present study. However, as hypothesised by many works [36] the restoration of catabolic metabolism of triglycerides could be due to an increased stimulation of the lipolytic activity of plasma lipoprotein lipase (LPL).

The derivatives also provide a beneficial action on rat lipid metabolism in regard to the reduction of AI. In fact, the AI was decreased in SR-C1, 4, 5, 6, 7, 11 and 12 treated groups. This ameliorative action was due to the plasma lipid-lowering activity of different derivatives. It is also desirable to have higher plasma HDL and lower LDL-cholesterol to prevent atherogenesis, since there is a positive correlation between an increased LDL-C/HDL-C ratio and the development of atherosclerosis. Again, the derivatives SR-C1, 4, 5, 6, 7, 11 and 12 significantly suppressed the higher values of LDL-C/HDL-C ratio showing the beneficial effect in preventing atherosclerosis incidence.

Conclusion

The results clearly demonstrate that the functional groups methyl, methoxy, chloro, dimethylamino, dimethoxy and trimethoxy groups possess cholesterol-suppressive capacities and has an ability to attenuate the accelerated development of atherosclerosis in hypercholesterolemic models. However hydroxyl and nitro derivatives did not show any hypolipidemic activity.

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