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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5347–5350

Bis[3-(4′-substituted phenyl)prop-2-ene]disulfides as a new class of antihyperlipidemic compounds

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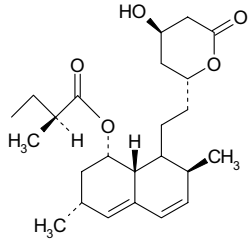
Received 1 June 2004; revised 5 August 2004; accepted 7 August 2004
Available online 8 September 2004

Abstract—A series of bis[3-(4′-substituted phenyl)prop-2-ene]disulfides were prepared and their hypolipidemic activities were examined in hypercholesterolemic Wistar rats. Introduction of an electron withdrawing group to the phenyl ring in the parent compound led to the identification of compound **8** as a potent and efficacious hypolipidemic agent.

1. Introduction

At present, the statins (**1**, Fig. 1) remain the drug of choice for lowering cholesterol levels.[1] However the statins have been known to be associated with undesirable side effects including severe myopathy and statin associated memory loss.[2] Consequently there emerges a need for new therapeutic agents. The lipid-soluble compounds diallyldisulfide (DADS) (**2**, Fig. 1), diallyltrisulfide (DATS) and dipropyldisulfide (DPDS), found in garlic, have been shown to depress cholesterol synthesis by 10–25% at low concentration (<0.5 mmol/L). It has been shown that incubation of rat hepatocyte culture with diallydisulfide [a metabolite of allicin (**3**, Fig. 1)] at a concentration of about 10 μM blocked the formation of 7-dehydrocholesterol and reduced the production of cholesterol.[3]



1 Lovastatin

$CH_2{=}CH{-}CH_2{-}S{-}S{-}CH_2{-}CH{=}CH_2$

2 Diallydisulphide

$CH_2{=}CH{-}CH_2{-}S(O){-}S{-}CH_2{-}CH{=}CH_2$

3 Diallythiosulphinate (Allicin)

Figure 1.

The use of diallyldisulfide is restricted due to its extreme volatility.[4] Our objective was to synthesize substituted derivatives of diallyldisulfide with greater stability and greater hypolipidemic activity as compared to diallyldisulfide.

2. Chemistry

The compounds reported in Table 3 were synthesized by oxidation of 2-mercaptoethanol (**4**) with Br_2 in the presence of potassium bicarbonate as shown in Scheme 1 to yield bis-(2-hydroxy ethyl)disulfide[5] (**5**). The compound **5** was heated with 48% HBr along with concd H_2SO_4 in a steam bath to yield bis-(2-bromoethyl)disulfide[6] (**6**). Compound **6** was refluxed with triphenylphosphine in dry DMF[7] to yield the phosphonium salt (**7**). The phosphonium salt (**7**) was refluxed with the appropriate aldehydes in the presence of lithium ethoxide to afford the target compounds[8,9] (**8**–**12**). Structural determination of all synthesized compounds was carried out by spectroscopic analysis (^{1}H NMR, IR and Mass) and CHN analysis.[9]

Keywords: Diallyldisulphide; Hypercholesterolemia; HMG-CoA reductase.
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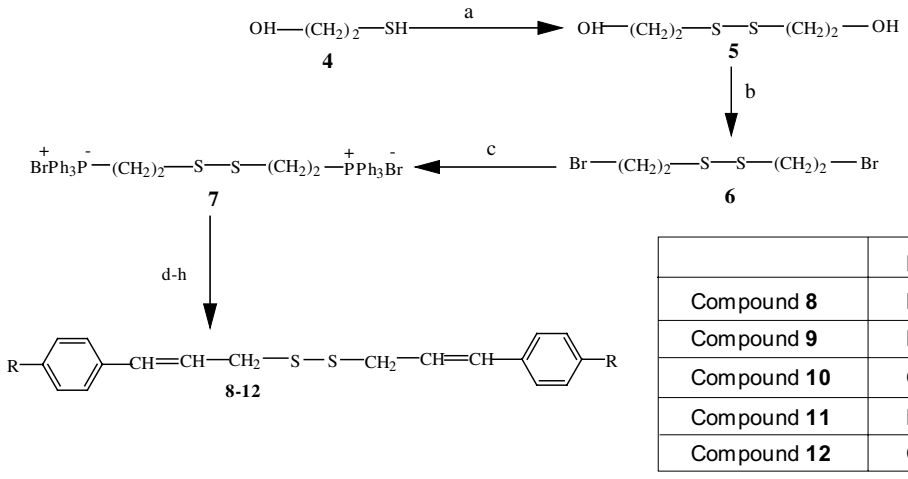
doi:10.1016/j.bmcl.2004.08.015



	R
Compound **8**	NO_2
Compound **9**	NH_2
Compound **10**	OCH_3
Compound **11**	H
Compound **12**	COOH

Scheme 1. Reagents and conditions: (a) Br_2, $KHCO_3$, DCM (33.8% from **4**): (b) HBr, H_2SO_4 (55% from **5**); (c) PPh_3, DMF, reflux (71.7% from **6**); (d) lithium ethoxide, *p*-nitro benzaldehyde (60.6% from **7**); (e) lithium ethoxide, *p*-amino benzaldehyde (67.0% from **7**); (f) lithium ethoxide, *p*-methoxy benzaldehyde (77.8% from **7**); (g) lithium ethoxide, benzaldehyde (69.3% from **7**); (h) lithium ethoxide, *p*-carboxy benzaldehyde (49.2% from **7**).

3. Animal treatment

Adult male rats of Wistar strain weighing 150–200 g were used in the investigation. The rats were selected at random from the stock colony maintained in the animal house facility, Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi. Animals were maintained in an air-conditioned room. The room was maintained at 25 ± 2 °C with natural daytime and no light after 1900 h, until morning. Lovastatin was procured from Ranbaxy Labs India.

The rats were divided into nine groups of six animals each. The control group was treated with saline with a normal diet. Gold Mohur rat feed was used supplied by Brooke Bond Lipton India Ltd. Control hypercholesterolemic group animals were fed with 5% cholesterol in their diet for one week (group 2) (Table 1). The thiosulfinate fraction of garlic and lovastatin (20 mg/kgbwt) were taken as two positive control groups and supplemented with 5% cholesterol in their diet (groups 3, 4). The animals in groups 5, 6, 7, 8 and 9 were fed 5% cholesterol containing diet supplemented with synthesized compounds (**8**–**12**) (20 mg/kgbwt). The experiment was repeated three times. Lovastatin and test compounds were orally administered (suspension in water) to Wistar rats.

The food intake of the animals and weight gain taking place in the animals is elaborated in Table 1.

Table 1. Food intake and body weight gain in rats fed the respective diets for 7 days (values are mean ± SE of six rats in each group)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Food intake (g)							
Group 1	10.25 ± 1.25	10.50 ± 0.55	10.00 ± 1.0	10.30 ± 0.74	11.05 ± 1.0	10.75 ± 0.5	10.05 ± 1.0
Group 2	11.00 ± 0.95	10.05 ± 1.0	11.50 ± 2.0	12.10 ± 0.45	11.50 ± 2.0	11.20 ± 3.0	9.85 ± 1.5
Group 3	11.05 ± 0.75	11.95 ± 0.35	12.50 ± 2.5	12.00 ± 1.5	11.20 ± 2.0	11.25 ± 1.0	11.06 ± 3.0
Group 4	10.95 ± 1.24	10.45 ± 1.0	10.40 ± 1.5	12.90 ± 2.0	13.05 ± 1.0	13.00 ± 2.0	13.75 ± 2.0
Group 5	12.85 ± 1.34	12.00 ± 1.25	12.75 ± 1.0	12.00 ± 1.5	10.05 ± 2.0	9.95 ± 1.5	10.94 ± 2.0
Group 6	13.05 ± 0.5	12.95 ± 0.25	12.35 ± 0.75	12.30 ± 2.0	9.85 ± 0.85	10.65 ± 3.0	10.55 ± 0.5
Group 7	10.50 ± 0.5	10.85 ± 0.5	10.00 ± 0.85	11.05 ± 0.95	11.25 ± 2.0	9.75 ± 1.5	9.25 ± 0.5
Group 8	11.94 ± 1.5	13.56 ± 2.0	12.45 ± 0.53	12.80 ± 2.56	10.35 ± 1.5	12.90 ± 2.0	12.40 ± 1.0
Group 9	11.00 ± 1.0	10.55 ± 0.95	10.35 ± 0.80	11.00 ± 0.95	12.05 ± 3.0	12.45 ± 1.5	9.55 ± 2.0
Body weight (g)							
Group 1	175 ± 5.25	175 ± 3.5	177 ± 2.5	177 ± 5.5	178 ± 5.5	178 ± 5.5	179 ± 5.5
Group 2	170 ± 4.85	170 ± 5.5	172 ± 4.5	172 ± 8.5	173 ± 8.5	174 ± 4.5	176 ± 4.5
Group 3	160 ± 10.05	160 ± 10.05	161 ± 5.05	162 ± 4.05	162 ± 5.05	163 ± 5.05	163 ± 9.05
Group 4	165 ± 5.5	166 ± 4.5	166 ± 7.5	167 ± 5.5	167 ± 5.5	168 ± 5.5	168 ± 7.5
Group 5	180 ± 4.5	180 ± 4.6	182 ± 2.6	182 ± 6.5	183 ± 5.5	185 ± 5.5	185 ± 3.5
Group 6	175 ± 5.65	176 ± 3.65	177 ± 1.5	177 ± 4.5	177 ± 4.5	178 ± 4.5	178 ± 2.5
Group 7	185 ± 4.5	186 ± 4.4	187 ± 3.5	187 ± 9.5	187 ± 9.5	188 ± 4.5	188 ± 1.5
Group 8	180 ± 8.5	180 ± 8.6	182 ± 5.6	183 ± 6.5	183 ± 6.5	184 ± 5.5	185 ± 5.5
Group 9	190 ± 6.5	191 ± 5.5	191 ± 2.5	192 ± 2.5	192 ± 2.5	193 ± 2.5	194 ± 2.5

All the test compounds were found to be soluble in organic solvents and insoluble in water. The compounds were stable under ordinary conditions of use and storage.

4. Biochemical assays

The animals were anaesthetized at the end of the experimental period, after overnight starvation. Blood was drawn from retroorbital sinus using capillary tubes, into dried test tubes. The serum was separated for cholesterol estimation. The animals were immediately dissected to remove their tissues, which were washed in ice-cold saline (0.85% NaCl). The extraneous material was removed.[10,11] Approximately 1 g of tissue was kept for estimation of lipids and the remaining was used for other biochemical assays.[12]

Hepatic and serum cholesterol was determined using the method of Zlatkis et al.[13] Hepatic triglyceride method was determined by the method of Van Handel and Zilversmit.[14] HMG-CoA reductase activity was determined by the method of Venugopala Rao et al.[15]

Hepatotoxicity was evaluated by measuring alanine transferase,[16] aspartate transferase[17] and alkaline phosphatase[18] levels in serum. Nephrotoxicity was evaluated by measuring blood urea nitrogen levels[19] and creatinine[20] in serum (Tables 3 and 4).

5. Results and discussion

In the present communication we describe the synthesis of several derivatives of diallyldisulfide and an evaluation of their antilipidemic activity[12–14] in male Wistar rats. The rats were fed 5% cholesterol in their diet for one week (Table 1). The test compounds were simultaneously administered to the rats for 1 week at a dose of 20 mg/kgbwt. No significant difference was found in body weight gain and diet consumption in all the treated groups as compared to control (Table 1). During the course of this search it was found that, bis[3-(4′-nitrophenyl)prop-2-ene]disulfide showed significant antihyperlipidemic activity.

The derivatives with electron withdrawing substituents (**8**, **12**) on the phenyl ring were found to lower hepatic cholesterol levels by 37% and 13%, respectively, and inhibit the activity of HMG-CoA reductase by 56% and 24%, respectively (Table 2). The derivatives with electron donating substituents (**9** and **10**) on the phenyl ring showed weaker activity as evidenced by their reduced ability to lower hepatic cholesterol levels and inhibit HMG-CoA reductase. The derivative with no substituents on the phenyl ring showed low antihyperlipidemic activity. A comparison of these percentages with those evoked on treatment with lovastatin demonstrated a potential use of bis-(4-nitrophenyl)disulfide in lowering lipid levels.

Table 2. The hypolipidemic activities of derivatives of diallyldisulfide (**8**–**12**, allicin and lovastatin)

	Cholesterol				Triglyceride			
	Liver mg/g wet tissue (mean ± SE)	% Dec.*	Serum mg/dL (mean ± SE)	% Dec.*	Liver mg/g wet tissue (mean ± SE)	% Dec.*	Ratio of HMG-CoA/mevalonate	% Dec. in the HMG-CoA reductase activity*
Compound **8**	3.15 ± 0.03[b]	36.5	44.15 ± 4.90[b]	30.2	14.85 ± 0.029	9.21	2.53 ± 0.02[a]	55.69
Compound **9**	4.81 ± 0.05	3.13	59.76 ± 3.01	5.5	14.58 ± 0.148	6.71	1.747 ± 0.13	7.50
Compound **10**	4.9 ± 0.03	1.2	61.95 ± 4.45	2.05	14.85 ± 0.029	4.91	1.685 ± 0.24	3.69
Compound **11**	4.86 ± 0.07	2.0	60.95 ± 4.25	3.63	14.19 ± 0.029	4.9	1.721 ± 0.31	5.90
Compound **12**	4.30 ± 0.05[b]	13.3	58.82 ± 3.25	7.01	14.33 ± 0.12	8.28	2.02 ± 0.23[b]	24.18
Lovastatin	2.84 ± 0.03[a]	42.3	43.5 ± 1.0[b]	31.2	9.86 ± 0.163[a]	36.91	2.20 ± 0.21[a]	35.38
Allicin	3.59 ± 0.16[b]	27.6	50.25 ± 1.25[b]	20.5	9.44 ± 0.193[a]	39.6	2.075 ± 0.19[b]	27.6
Control hypercholesterolemic	4.96 ± 0.03		63.25 ± 2.25		15.63 ± 0.163		1.625 ± 0.20	

*Percentages change from the initial value in saline treated rats as mean from at least two experiments.
[a] $p < 0.01$ versus control.
[b] $p < 0.05$ versus control.

Table 3. Effect of single and five doses of synthesized compounds on liver marker enzymes

	Single dose			Five doses		
	Alanine transferase (GPT) IU	Aspartate transferase (GOT) IU	Alklaline phosphatase KA Units	Alanine transferase (GPT) IU	Aspartate transferase (GOT) IU	Alklaline phosphatase KA Units
Normal range	18–30	46–81	14–32	18–30	46–81	14–32
Control	22 ± 3	41 ± 6	30.2 ± 18.2	22 ± 4	48.5 ± 2.5	45.59 ± 5.8
Compound **8**	21 ± 3.5	51 ± 1	29.5 ± 12.5	15.5 ± 0.50	49 ± 1	48 ± 8
Compound **9**	26.5 ± 1.5	51.5 ± 2.50	40.6 ± 5.2	21 ± 2	37 ± 7	46.9 ± 6.7
Compound **10**	26.5 ± 0.5	49 ± 4	45.6 ± 9.0	23 ± 2	29.5 ± 4.5	25.2 ± 9.4
Compound **11**	17.5 ± 0.5	51 ± 6	34.0 ± 0.5	27 ± 1	39 ± 3	33.6 ± 12.5
Compound **12**	25 ± 1.0	53 ± 3.5	31.0 ± 0.3	28 ± 2.5	56 ± 4	32.8 ± 2.5

Table 4. Effect of single and five doses of synthesized compounds on kidney marker enzymes

	Single dose		Five doses	
	Blood urea nitrogen mg/dL	Creatinine mg/dL	BUN mg/dL	Creatinine mg/dL
Normal range	5–29	0.20–0.80	5–29	0.20–0.80
Control	10.45 ± 1.41	0.715 ± 0.075	10.8 ± 1.89	0.79 ± 0.04
Compound **8**	7.65 ± 0.15	0.4 ± 0.06	6.48 ± 0.015	0.8 ± 0.04
Compound **9**	12.96 ± 0.07	0.55 ± 0.28	14.64 ± 1.23	0.84 ± 0.175
Compound **10**	7.43 ± 0.65	0.595 ± 0.145	8.48 ± 0.59	0.495 ± 0.06
Compound **11**	12.39 ± 1.05	0.58 ± 0.06	7.37 ± 0.31	0.57 ± 0.05
Compound **12**	20.23 ± 2	0.61 ± 0.15	17.4 ± 0.45	0.70 ± 0.03

6. Conclusion

Results shows that certain novel derivatives of diallyldisulfide such as bis[3(4-nitrophenyl)prop-2-ene]disulfide are effective in lowering cholesterol levels and would be potentially beneficial in treating hypercholesterolemia.

Acknowledgements

The authors wish to acknowledge the financial support received from the Department of Science and Technology, India and the facilities provided by the Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110007.

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