Hypolipidemic Activity in Rodents of Phenobarbital and Related Derivatives

I.H. Hall, M.A. Patrick, and J.H. Maguire

Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7360, USA

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A series of 5-alkyl-5-phenylbarbituric acid analogues were shown to be potent hypolipidemic agents in rats and mice at 20 mg/kg/day. This dose is lower than that required for hypolipidemic activity for clofibrate and nicotinic acid derivatives in rodents and man. These new derivatives reduced both serum cholesterol and triglyceride levels in rodents by either the oral or intraperitoneal route of administration. Previous studies have demonstrated that similar heterocyclic compounds, *i.e.* cyclic imides, glutarimides and hydantoins are potent hypolipidemic agents in rodents. The barbituric acid derivatives probably interfered with *de novo* synthesis of cholesterol and fatty acids in the early steps since the agents inhibit the activities of ATP-dependent citrate lyase and acetyl-CoA synthetase. Triglyceride synthesis may be blocked since the agents inhibited the rate limiting enzyme, sn-glycerol-3-phosphate-acyl-transferase. Rat tissue lipids especially cholesterol and triglycerides were reduced after 14 days treatment. Fecal lipids were increased in cholesterol and phospholipid content by selected compounds. The rat serum lipoprotein after 14 days drug administration showed reduced VLDL-cholesterol and chylomicron-cholesterol content with elevated HDL-cholesterol and HDL-triglyceride contents. The modulation of the lipid content of the serum lipoproteins by the barbituric acids suggest that these agents may be helpful in treating clinical hyperlipidemic disease states.

Lipidsenkende Wirkung von Phenobarbital und verwandten Verbindungen bei Nagetieren

Verschiedene 5-Alkyl-5-phenylbarbitursäuren erwiesen sich bei Ratten und Mäusen in einer Dosierung von 20 mg/kg/Tag als wirksame Lipidsenker. Diese Dosis liegt unter der, die bei Clofibrat und Nikotinsäurederivaten beim Menschen und bei Nagetieren eingesetzt werden muß. Diese neuen Barbitursäurederivate erniedrigten nach peroraler oder i.p. Gabe sowohl den Serumcholesterin- als auch den Triglycerid-Spiegel. Nach früheren Untersuchungen sind ähnliche Heterocyclen - cyclische Imide, Glutarimide und Hydantoine - bei Nagetieren wirksame Lipidsenker. Diese Barbitursäuren greifen möglicherweise in die ersten Stufen der *de novo*-Synthese des Cholesterols und der Fettsäuren ein, da diese Wirkstoffe die ATP-abhängige Citrat-Lyase und die Acetyl-CoA-Synthetase hemmen. Auch die Triglycerid-Synthese könnte blockert werden, da diese Wirkstoffe die sn-Glycerol-3-phosphat-acyl-Transferase, das die Geschwindigkeit bestimmende Enzym, hemmen. Nach 14-tägiger Behandlung waren in Rattengeweben insbesondere Cholesterol und Triglyceride vermindert. Einige Verbindungen erhöhten den Gehalt an Cholesterol und Phospholipiden in den Fäzes. In den Lipoproteinen des Rattenserums waren nach 14-tägiger Applikation VLDL-Cholesterol und das Chylomikronen-Cholesterol vermindert, HDL-Cholesterol und HDL-Triglyceride vermehrt. Die Beeinflussung des Lipidgehaltes der Serumlipoproteine durch diese Barbitursäuren deutet darauf hin, daß sie bei der Behandlung von Hyperlipidaemien hilfreich sein könnten.

The ability to significantly lower serum cholesterol and triglyceride levels in rodents has been demonstrated by a series of cyclic imides, e.g., phthalimide[1], saccharin[2], diphenimides[3] and reduced diphenimides[4], 1,8-naphthalimide[5], 2,3-dihydrophthalazine-1,4-diones[6], 3- and 4-phenylpiperidine-2,6-diones[7], and 5-ethyl-5-phenylhydantoins[8]. Clinically, phenytoin increases the cholesterol content of the high density lipoprotein fraction (HDL) in man[9,10]. Phenobarbital appears also to affect lipid metabolism in man probably by a mechanism which is consistent with its ability to induce liver enzyme since lecithin-cholesterol acyl transferase activity is stimulated by phenobarbital[11] treatment. Additional studies have shown that the combination of phenytoin with phenobarbital and/or carbamazepine led to elevated high-density lipoprotein concentrations, a high-density lipoprotein cholesterol/total cholesterol ratio and a reduced low/high density lipoprotein cholesterol level[12]. In man, *Durrington* has demonstrated that phenobarbital at 180 mg/day elevated HDL-cholesterol as well as total plasma and LDL apoprotein B content after three weeks[13]. Phenobarbital treatment induces rat liver apolipoprotein A-1 mRNA synthesis which may correlate with the low incidence of coronary heart disease in epileptics[14]. Alcohol use and exposure to pesticides elevates HDL-cholesterol content but cigarette smoking markedly reduces HDL-cholesterol content in man[15]. Cholesterol in the diet [2%] has been shown to modulate cytochrome P-450 monooxygenase activities in the liver, small intestine and lung[16]. Thus, a study was undertaken to examine a series of 5-alkyl-5-phenylbarbituric acids for their effects on lipid metabolism and serum lipoproteins in rodents, to ascertain their potential as hypolipidemic agents.

Experimental Part

NMR: Jeol-FT FX-60 NMR or Varian 80 MHz FT-NMR ($CDCl_3$/TMS), unless otherwise specified, A Bruker 250 MHz, Varian 400 MHz FT-NMR Chem. shifts in δ (ppm). - IR: Perkin-Elmer 1320. - Melting points: Thomas-Hoover "Unimelt" apparatus, uncorrected. - Elemental analysis (C, H, N, ± 0.40 % theoretical): Desert Analytics of Tucson, Arizona.

Source of Compounds

Diethyl-2-alkyl-2-phenylmalonates **1-5**

Diethyl 2-alkyl-2-phenyl-malonates were synthesized by modification of the method of *Beres* et al.[17]. The appropriate alkyl iodide (methyl, n-propyl, iso-propyl, n-butyl, iso-butyl) (2 equiv.) was added and the reaction was stirred at room temp. for 20-24 h.

5-Alkyl-5-phenylbarbituric Acids **6-10**

Barbituric acids analogs were synthesized from compounds **1-5** by the method of *Cook* and *Tallent*[18]. The mixtures were stirred at room temp. for 3-12 h. The more bulky diethyl-2-alkyl-2-phenylmalonates required prolonged reaction and additional NaH.

Phenobarbital **(11)**

11 was obtained from Sigma Chemical Co. and was recrystallized from water/ethanol; its purity was determined by elemental analysis.

  0365-6233/90/0909-0579 $ 3.50 + .25/0

Ethyl 4-hydroxyphenylacetate (**12**)

Compound **12** was synthesized by the method of *Furstoss* et al.[19].

Ethyl-4-Benzyloxyphenylacetate (**13**)

Compound **13** was synthesized from **12** by the method of *Baggaley* et al.[20]. NMR: 1.2 (t, J = 7 Hz, 3H), 3.50 (s, 2H), 4.10 (q, J = 7 Hz, 2H), 4.98 (s, 2H), 7.04 (m, 4H), 7.34 (s, 5H).

Diethyl 2-(4'-benzyloxyphenyl)malonate (**14**)

Compound **14** was prepared by a modification of the method of *Krapcho* et al.[21]. Dry toluene (120 mL) was added to washed NaH (13 g, 0.32 mol, 60% mineral oil dispersion). Freshly distilled diethyl carbonate (53 mL, 0.44 mol) was added, and the solution was heated under reflux. **13** (30 g, 0.11 mol) was added dropwise over 1 h, and the mixture was heated under reflux for 19 h. The solution was cooled to 0°C, and 2 mL of glacial acetic acid was added slowly. The white mass that formed dissolved upon addition of cold water (50 mL). The solution was extracted with hexane (4 x 75 mL), and the org. extract was washed with cold water, dried ($MgSO_4$), filtered and evaporated. The orange oil (36.1 g, 95% yield) solidified to a waxy material at room temp. The NMR spectra was in agreement with the structure, and the product was used without further purification. ^{1}H-NMR: 1.25 (t, 6H), 4.20 (q, 4H), 4.59 (s, 1H), 5.05 (s, 2H), 7.2 (m, 4H), 7.4 (s, 5H).

Diethyl 2-isopropyl-2-(4'-benzyloxyphenyl)malonate (**15**)

Compound **15** was prepared from **14** by adaptation of the method of *Beres* et al.[17]. The product distilled at 160-162°C (0.02 mm) in 77% yield. ^{1}H-NMR: 0.88 (d, 6H), 1.2 (t, 6H), 2.5-3.0 (m, 1H), 4.2 (q, 4H), 5.05 (s, 2H), 7.12 (m, 4H), 7.38 (s, 5H).

5-Isopropyl-5-(4'-benzyloxyphenyl)barbituric acid (**16**)

Compound **16** was prepared from **15** by a modification of the procedure of *Cook* and *Tallent*[18]. The reaction mixture containing urea and washed (hexane) NaH was cooled to 0°C before the substituted malonate **15** in 5-10 mL DMSO was added slowly. The product was crystallized from ethyl acetate - hexane and recystallized from water - ethanol 58%, mp. 214-215. - ^{1}H-NMR: 1.06 (d, 6H), 2.8-3.3 (m, 1H), 5.08 (s, 2H), 7.15 (m, 4H), 7.42 (s, 5H), 8.2 (broad, 2H).

Ethyl 3-hydroxyphenylacetate (**17**)

Compound **17** was prepared by adaptation of the method described for the synthesis of **12**. The product distilled at 121-123°C (0.4 mm) in 94% yield. - ^{1}H-NMR: 1.2 (t, 3H), 3.52 (s, 2H), 4.12 (q, 2H), 6.5-7.3 (m, 4H).

Ethyl 3-benzyloxyphenylacetate (**18**)

Compound **18** was prepared from **17** by adaptation of method described for the synthesis of ethyl 4-benzyloxyphenylacetate (**13**). The *meta*-compound distilled at 170-172°C (0.8 mm), 58% yield. - ^{1}H-NMR: 1.22 (t, 3H). 3.58 (s, 2H), 4.15 (q, 2H), 5.03 (s, 2H), 6.7-7.5 (m, 4H), 7.38 (s, 5H).

Diethyl 2-(3'-benzyloxyphenyl)malonate (**19**)

Compound **19** was prepared from **18** by adaptation of the method described for the synthesis of **14**. The product was distilled at 174-178°C (0.05 mm) and was obtained in 72% yield. - ^{1}H-NMR: 1.23 (t, 6H), 4.2 (q, 4H), 4.6 (s, 1H), 5.05 (s, 2H), 6.8-7.6 (m, 4H), 7.38 (s, 5H).

Diethyl 2-isopropyl-2-(3'-benzyloxyphenyl)malonate (**20**)

Compound **20** was synthesized from **19** by adaptation of the procedure described for compound **15**. The product (78%) was distilled at 164-7°C (0.01 mm). - ^{1}H-NMR: 0.9 (d, 6H), 1.22 (t, 6H), 2.6-3.1 (m, 1H), 4.22 (q, 4H), 5.07 (s, 2H), 6.7-7.5 (m, 4H), 7.35 (s, 5H).

5-Isopropyl-5-(3'-benzyloxyphenyl)barbituric acid (**21**)

Compound **21** was prepared from **20** by adaptation of the method used for compound **16**. The product was dissolved in $CHCl_3$, applied to a silica gel column, and eluted with 95:5 $CHCl_3$/MeOH. The solvent was evaporated, and the product was crystallized twice from hexane-ethyl acetate. mp. 198-199°C, 47% yield. - ^{1}H-NMR: 1.05 (d, 6H), 2.8-3.4 (m, 1H), 5.05 (s, 2H), 6.7-7.6 (m, 4H), 7.39 (s, 5H), 8.17 (s, 2H).

2-Benzyloxyethanol (**22**)

The procedure of *Bennett* and *Hock*[22] was adapted to synthesize compound **22** from ethylene glycol. The product distilled at 99-100°C (1.5 mm) to yield 33.1 g of oil **22** (73%). - IR (neat): 3100-3600 (O-H); 3000-3100 (arom. C-H) cm^{-1}. - ^{1}H-NMR: 3.5-3.9 (m, 4H), 4.55 (s, 2H), 7.35 (s, 5H).

2-Benzyloxyethylchloride (**23**)

Compound **23** was synthesized from **22** according to *Ludeman* et al.[23].

Diethyl 2-(2'-benzyloxyethyl)-2-phenylmalonate (**24**)

Compound **24** was synthesized from **23** according to the modified procedure for the synthesis of **15**. The product was distilled at 170-173°C (0.05 mm) as a colorless oil (73%). - ^{1}H-NMR: 1.18 (t, 6H), 2.67 (t, 2H), 3.48 (t, 2H), 4.12 (q, 4H), 4.4 (s, 2H), 7.1-7.6 (m, 10H).

5-(2'-Benzyloxyethyl)-5-phenylbarbituric acid (**25**)

The method of *Dickey* and *Gray*[24] was adapted to synthesize compound **24**. The product (mp. 198-199°C) was crystallized twice from hexane-ethyl acetate (18%). - ^{1}H-NMR (400 MHz): 2.83 (t, 2H), 3.65 (t, 2H), 7.2-7.45 (m, 10H), 7.8 (s, 2H).

Hypolipidemic Screens in Normal Rodents

Test compounds **6-11, 13, 16, 21,** and **25** were suspended in an aqueous 1% carboxymethyl-cellulose solution, homogenized, and administered to CF_1 male mice (~25 g) intraperitoneally for 16 days. On days 9 and 16, blood was obtained by tail vein bleeding and the serum separated by centrifugation for 3 min. *Sprague Dawley* male rats (~350 g) were administered compounds **6, 9,** or **11** orally by intubation needle at 20 mg/kg/day. Blood was collected on days 9 and 14. The serum cholesterol levels were determined with a modification of the *Liebermann-Burchard* reaction[26]. Serum was also analyzed for triglyceride content as determined by a commercial kit [BioDynamics/bmc single vial Triglycerides colorimetric method 348201]. Food and water were available *ad libitum* for animals in experiments. Animals were maintained at 22°C with 12 h light and 12 h dark.

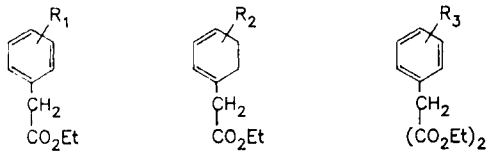
Animal Weights and Food Intake

Food consumption was determined daily as g food/rat/day for control rats and for those treated orally with compounds **6, 9,** and **11** at 20 mg/kg/day. Body weights were obtained during the experiments and expressed as a percentage of the animal's weight on day 0. After dosing for 14 days with compounds, selected organs were excised, trimmed of fat and weighed.

Enzymatic Studies

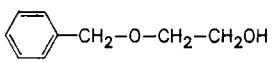
In vitro enzymatic studies were determined using 10% homogenates of CF_1 male mouse liver with 50-200 μM concentrations of compounds **6, 9,**

Scheme



Compd	R_1	Compd	R_2	Compd	R_3
12	4-OH	13	4-OBzl	14	4-OBzl
17	3-OH	18	3-OBzl	19	3-OBzl

Compd	R_4	R_5	Compd	R_6	R_7
1	H	methyl	6	CH_3	C_6H_5
2	H	*n*-propyl	7	*n*-propyl	C_6H_5
3	H	*iso*-propyl	8	*iso*-propyl	C_6H_5
4	H	*n*-butyl	9	*n*-butyl	C_6H_5
5	H	*iso*-butyl	10	*iso*-butyl	C_6H_5
15	4-OBzl	*iso*-propyl	11	C_2H_5	C_6H_5
20	3-OBzl	*iso*-propyl	16	*iso*-propyl	4-C_6H_4OBzl
24	H	$(CH_2)_2$OBzl	21	*iso*-propyl	3-C_6H_4OBzl
			25	C_6H_5	$(CH_2)_2$OBzl



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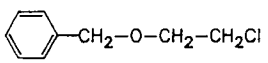

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Table 1. Structure Activity Relationships for Hypolipidemic Activity in C. F.-1 Mice at 20 mg/kg/day I.P.

[N-6] Compound	Percent of Control (X ± S.D.) Serum Cholesterol Levels Day 9	Serum Cholesterol Levels Day 16	Serum Triglyceride Levels Day 16
6	58+4*	57+5*	81+6
7	80+7*	72+4*	55+4*
8	50+4*	45+4*	66+5*
9	50+4*	59+6*	70+6*
10	51+3*	54+3*	69+5*
11	68+6*	52+4*	50+4*
13	65+5*	64+6*	64+5*
16	62+3*	59+5*	63+7*
21	54+5*	61+5*	65+5*
25	77+7*	61+6*	66+5*
Clofibrate 150 mg/kg/day	88+7	87+5	75+7*
1% CMC	100+6[a]	100+5[b]	100+6[c]

a=75 mg%, b=78 mg%, c=137 mg%

* $P < 0.001$ Student's Test

Table 2. The Effects of Phenobarbital Derivatives on Sprague Dawley Male Rats Serum Lipids After Oral Administration at 20 mg/kg/day

		Percent of control [mg%]			
	Food Consumption	Serum Cholesterol		Serum Triglyceride	
[N=6]	µg/day/rat	Day 7	Day 14	Day 7	Day 14
Control	19.24	100±5[a]	100±6[b]	100±5[c]	100±7[d]
Cpd. 6	21.55	81±5*	45±4*	79±6*	67±5*
Cpd. 11	23.50	74±4*	51±5*	86±5	53±4*
Cpd. 9	24.08	67±5*	66±4*	76±7*	60±5*
Lovastatin[e]	-	85±6	82±5*	89±6	86±8

[a]75 m%, [b]76 mg%, [c]137 mg%, [d]136 mg%, [e]8 mg/kg/day orally

* $P < 0.001$

Table 3. The Effect of 5-Alkyl-5-Phenylbarbituric Acid on Sprague Dawley Rat Tissue Lipids and Serum Lipoproteins After 14 Day Oral Administration at 20 mg/kg/day.

		Percent of Control				
[N=6]	mg of lipid extracted	Cholesterol	Triglyceride	Neutral Lipids	Phospholipids	Protein
Liver						
Control	100±6[a]	100±5[b]	100±7[c]	100±7[d]	100±6[e]	100±5[f]
Cpd. 6	53±4*	71±6*	72±6*	109±8	88±7	154±6
Cpd. 11	74±5*	68±6*	73±7*	109±7	74±5*	175±7
Cpd. 9	64±4*	62±5*	60±6*	155±6*	19±8*	170±6
Small Intestine						
Control	100±7[g]	100±6[h]	100±7[i]	100±8[j]	100±7[k]	100±6[l]
Cpd. 6	83±5	43±4*	78±7*	92±7	55±6*	114±7
Cpd. 11	112±7	54±5*	86±6*	91±6	300±3*	168±5
Cpd. 9	131±8*	48±4*	77±5*	111±6	360±5*	186±8
Aorta						
Control	100±6[m]	100±7[n]	100±6[o]	100±7[p]	100±4[q]	100±5[r]
Cpd. 6	89±5	58±5*	89±7	98±6	209±5*	98±6
Cpd. 11	90±4	43±4*	97±5*	96±7	200±6*	96±7
Cpd.9	88±6	33±5*	61±4*	124±6*	218±5*	102±4
Feces						
Control	100+7[s]	100±6[t]	100±5[u]	100±6[v]	100±8[w]	100±6[x]
Cpd.6	76+6*	109±4	78±6*	100±8	122±6*	93±5
Cpd.11	87+7	147±6*	100±5	82±7	118±5	107±6
Cpd. 9	105+8	87±6	56±6*	95±7	73±6*	93±6

[a]50.0 mg lipid/g wet tissue
[b]9.18 mg cholesterol/g wet tissue
[c]6.37 mg triglyceride/g wet tissue
[d]15.70 mg neutral lipid/g wet tissue
[e]27.19 mg phospholipid/g wet tissue
[f]4.5 mg protein/g wet tissue
[g]68.20 mg lipid/g wet tissue
[h]12.02 mg cholesterol/g wet tissue
[i]11.20 mg triglyceride/g wet tissue
[j]16.98 mg neutral lipid/g wet tissue
[k]20.06 mg phospholipid/g wet tissue
[l]42.0 mg protein/g wet tissue
[m]67.5 mg lipid/g wet tissue
[n]5.77 mg/cholesterol/g wet tissue
[o]9.85 mg triglyceride/g wet tissue
[p]15.28 mg neutral lipid/g wet tissue
[q]28.8 mg phospholipid/g wet tissue
[r]11.71 mg protein/g wet tissue
[s]11.58 mg of lipid/g wet tissue
[t]2.84 mg cholesterol/g wet tissue
[u]1.86 mg triglyceride/g wet tissue
[v]3.39 mg neutral lipid/g wet tissue
[w]5.70 mg phospholipid/g wet tissue
[x]6.99 mg protein/g wet tissue

and **11**. The enzyme activities were determined by following literature procedures; acetyl coenzyme A synthetase[27]; adenosine triphosphate dependent citrate lyase[28]; mitochondrial citrate exchange[29,30]; cholesterol-7α-hydroxylase[31]; 3-hydroxy-3-methylglutaryl coenzyme A reductase[32,33]; acyl CoA cholesterol acyl transferase[34]; neutral cholesterol ester hydrolase[35]; acetyl coenzyme A carboxylase activity[36]; sn-glycerol-3-phosphate acyl transferase[37]; phosphotidylate phosphohydrolase activity[38]; and heparin activated hepatic lipoprotein lipase[39]. Protein was determined for the cell extract appropriate for each enzyme assay by the *Lowry* et al.[40] technique.

Liver, Small Intestine, and Fecal Lipid Extraction

In *Sprague Dawley* rats that had been administered compounds **6**, **9**, or **11** for 14 days, the liver, small intestine, and fecal materials (24 h collection) were removed, extracted[41,42] and analyzed for cholesterol levels[26], triglyceride levels (BioDynamics/bmc Triglyceride Kit), neutral lipid content[43], phospholipid content[44] and protein levels[40].

Serum Lipoprotein Fractions

Sprague Dawley male rats (~300 g) were administered compounds **6**, **9**, and **11** at 20 mg/kg/day orally. Blood was collected from the abdominal

Table 3 cont'd

[N-6]	Cholesterol	Triglyceride	Neutral Lipids	Phosopholipids	Protein
Chylomicron					
Control	100±6[a]	100±6[b]	100±7[c]	100±7[d]	100±5[e]
Cpd. 6	84±5	124±7*	115±6	78±5*	83±7
Cpd. 11	69±4*	120±5*	108±6	129±6*	97±7
Cpd. 9	62±5*	97±6	123±7*	84±7	98±5
VLDL					
Control	100±6[f]	100±7[g]	100±7[h]	100±8[i]	100±6[j]
Cpd. 6	44±5*	90±6	88±5	94±5	79±6
Cpd. 11	34±4*	116±7	88±6	106±6	81±5
Cpd. 9	22±4*	71±6*	85±4	96±5	93±6
LDL					
Control	100±7[k]	100±6[l]	100±6[m]	100±6[n]	100±6[o]
Cpd. 6	89±6	141±5*	95±5	64±5	189±6
Cpd. 11	94±5	124±5*	123±8	103±6*	112±7
Cpd. 9	74±6*	134±5*	91±7	138±6	61±5*
HDL					
Control	100±6[p]	100±7[q]	100±6r	100±7[s]	100±4[t]
Cpd. 6	176±7*	122±6*	87±5	111±6	108±5
Cpd. 11	200±7*	133±7*	47±5*	89±5	108±6
Cpd. 9	167±5*	124±5*	81±6	97±6	105±4

[a]337 µg cholesterol/ml serum
[b]420 µg triglyceride/ml serum
[c]67 µg neutral lipid/ml serum
[d]149 µg phospholipids/ml serum
[e]184 µg protein/ml serum
[f]190 µg cholesterol/ml serum
[g]22 µg triglyceride/ml serum
[h]98 µg neutral lipid/ml serum
[i]26 µg phospholipids/ml serum
[j]50 µg protein/ml serum
[k]210 µg cholesterol/ml serum
[l]45 µg triglyceride/ml serum
[m]10 µg neutral lipids/ml serum
[n]41 µg phospholipids/ml serum
[o]122 µg protein/ml serum
[p]544 µg cholesterol/ml serum
[q]27 µg triglyceride/ml serum
[r]620 µg neutral lipid/ml serum
[s]153 µg phospholipids/ml serum
[t]657 µg protein/ml serum

Table 4. Effects of 5-Alkyl-Phenylbarbituric Acids on CF_1 Mouse Hepatic *In Vitro* Enzyme Activities Involving Lipid Metabolism

	Percent of Control								
		Compound 6 µM			Compound 11 µM			Compound 9 µM	
Enzyme Assay (N-6)	Control	25	50	100	25	50	100	25	50	100
Mitochondrial Citrate Exchange	100[a]	101	98	95	91	89	89	112	111	92
ATP Dependent Citrate Lyase	100[b]	63	50	39	72	56	40	84	80	74
Acetyl CoA Synthetase	100[c]	103	95	72	113	106	81	113	111	102
HMG CoA Reductase	100[d]	89	92	99	104	105	112	105	107	107
Cholesterol-7α-Hydroxylase	100[e]	104	106	142	104	113	134	92	99	106
Acyl CoA Cholesterol Acyl Transferase	100[f]	49	44	42	45	32	32	42	36	28
Cholesterol Ester Hydrolase	100[g]	94	99	110	101	108	114	116	117	126
Acetyl CoA Carboxylase	100[h]	101	99	96	104	97	96	100	97	95
sn-glycerol-3-phosphate-acyl Transferase	100[i]	126	78	53	119	80	67	96	66	65
Phosphatidylate Phosphohydrolase	100[j]	153	153	191	211	186	99	120	99	93

Standard deviations were all within 6% for all values

[a]30.8 % exchange of mitochondria citrate
[b]30.5 mg citrate hydrolyzed/g wet tissue/20 min
[c]28.5 mg acetyl CoA formed/g wet tissue/20 min
[d]384,900 dpm cholesterol formed/g wet tissue/60 min
[e]4808 dpm/mg microsomal protein/20 min
[f]224,000 dpm/mg microsomal protein/30 min
[g]56,436 dpm/g wet tissue/h
[h]32010 dpm/g wet tissue/30 min
[i]537,800 dpm/wet tissue/20 min
[j]16.7 µg/iP/g wet tissue/15 min

aorta vein and lipoprotein fractions were obtained by the method of *Hatch* and *Lees*[45] and *Havel* et al.[46] as modified for the rat[47]. Each of the fractions was analyzed for cholesterol[26], triglyceride, neutral lipids, phospholipids[44] and protein levels[40].

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Results

The 5-alkyl-5-phenylbarbituric acid derivatives proved to be potent hypolipidemic agents in rodents at 20 mg/kg/day. Compound **6** demonstrated potent activity in the SAR mouse screen at 20 mg/kg/day I.P., lowering serum cholesterol levels 42% on day 9 and 43% on day 16 of the screen [Table 1]. The reduction of the serum triglycerides was marginal at 19% on day 16 for compound **6**. Compound **7** resulted in only 28% reduction of serum cholesterol levels and 45% reduction of serum triglycerides on day 16. Compound **8**, the isopropyl derivative resulted in 55% reduction in serum cholesterol level and 34% reduction of serum triglycerides. Compound **9**, the *n*-butyl derivative afforded 41% reduction of serum cholesterol levels in mice with a 30% reduction of serum triglycerides. The isobutyl derivative, compound **10**, resulted in 46% reduction of serum cholesterol and 31% reduction of serum triglycerides after 16 days dosing in mice. Phenobarbital (**11**), at the same dose, 20 mg/kg/day, resulted in the best activity in both screens lowering serum cholesterol levels 48% and serum triglycerides 50% after 16 days. Compounds **13, 16, 21**, and **25** afforded significant activity but no significant improvement over the previous derivatives with 41 to 36% reduction of serum cholesterol and 37 to 34% reduction of serum triglycerides levels after 16 days at 20 mg/kg/day. Consequently, compounds **6, 9**, and **11** were selected for further examination of hypolipidemic activity in rats.

When these three compounds were tested *in vivo* in rats [Table 2] compound **6** afforded a 55% reduction, compound **9** a 34% reduction and compound **11** a 49% reduction of serum cholesterol on day 14. Serum triglycerides were reduced 33%, 40%, and 47%, respectively, by compounds **6, 9**, and **11**. The compounds did lower tissue lipids after 14 days dosing at 20 mg/kg/day. Cholesterol and triglyceride levels were reduced significantly in the liver, small intestinal mucosa and aorta tissue. Phospholipids were reduced in the liver and elevated in the aorta. Protein content of the aorta was not significantly altered by drug treatment for 14 days; however, it was elevated by drug therapy in the liver and small intestinal mucosa. The fecal lipids were also altered by drug therapy with compound **11** elevating fecal cholesterol content by 47% and compounds **6** and **11** elevating phospholipid content 18-22%. Serum lipoprotein fractions also demonstrated alterations in lipid content after treatment of rats with these three compounds for 14 days. The cholesterol content of the chylomicron and VLDL were significantly reduced by all three compounds, with elevations of 67 to 100% of the cholesterol content in the HDL fraction. Triglyceride content was elevated by all three compounds in the LDL and HDL fractions. Compound **9** lowered triglyceride content of the VLDL fraction. Whereas neutral lipids were lowered in VLDL and HDL fractions by all three agents, phospholipids were lowered by compounds **6** and **11** in the chylomicron fraction and by **6** in the LDL fraction. Compound **11** afforded reductions in the phospholipid content of the HDL fractions. Protein content was lowered by compound **6** in the chylomicron and VLDL fractions, and compound **11** afforded a reduction in protein content of the VLDL fraction.

Examination of the effects of these three compounds on *in vitro* hepatic enzyme activities demonstrated that all three significantly reduced ATP-dependent citrate lyase activity in a dose dependent manner with compound **6** affording 61%, compound **11** 60% and compound **9** 26% reduction at 100 μM. Acetyl-CoA synthetase activity was reduced 28% by compound **6** and 19% by compound **11** at 100 μM. HMG-CoA reductase, cholesterol-7α-hydroxylase, and neutral cholesterol ester hydrolase activities were not significantly affected by the presence of the compounds *in vitro*.

One exception was that compound **9** did elevate neutral cholesterol ester hydrolase activity in a dose dependent manner from 16 to 26%. Acyl-CoA cholesterol acyl transferase activity was reduced in a dose dependent manner by all three compounds with compound **9** affording 72%, compound **11** 68% and compound **6** 58% reduction at 100 μM concentration. In the triglyceride *de novo* synthetic pathway acetyl-CoA carboxylase and phosphatidylate phosphohydrolase activities were not inhibited by the three compounds. In fact the latter enzyme was actually increased in activity by all three compounds at 25 μM concentration. sn-Glycerol-3-phosphate acyl transferase activity was inhibited in a dose dependent manner by the three drugs with compound **6** resulting in 47%, compound **9** 35% and compound **11** 33% inhibition at 100 μM concentration (Table 4).

Discussion

These studies have demonstrated that barbituric acid derivatives possess potent hypolipidemic activity in rodents. This may be due to the fact that all of these derivatives are alkylphenyl, benzyloxyalkylphenyl, or alkyl benzyloxyphenyl substituted cyclic diimides. The presence of the phenyl group in position 5 indicated that this moiety was necessary for hypolipidemic activity. The ethyl substitution in position 5, i.e. phenobarbital, afforded the best lipid lowering effects in both lipid screens in mice. These compounds were also effective in rats when administered orally. The three compounds tested in rats lowered serum cholesterol levels more than clofibrate at 150 mg/kg/day which resulted in a 13% reduction and lovastatin at 8 mg/kg/day which caused a 18% reduction in serum cholesterol levels in rats. The barbituric acid derivatives did not cause displacement of lipids from the plasma compartment to other body tissues; in fact, the agents lowered liver and small intestinal muscosa lipids, significantly. This may have been due to the enzyme induction capability of phenobarbital so that more lipids were

being catabolized by the endoplasmic reticulium of these two tissues. Enzyme inducers raise the hepatic levels of protein and phospholipids of the endoplasmic reticulium which was evident from these studies also. Not only will enzyme induction raise the level of enzymes participating in lipid metabolism, but it has also been correlated with elevations in HDL-cholesterol, and apo A-1[11]. After treatment with anti-epileptic enzyme inducers the overall reductions in total serum cholesterol level were marginal, i.e. phenytoin has been observed to afford a small decrease in man[10] but its effects in lowering serum cholesterol levels in rats are more noted[9]. Cholesterol in the diet of rats was shown to be related to the ability of phenobarbital and methyl-cholanthrene to induce Cyto P-450 isoenzymes in the GI mucosa and liver[16]. The Cyto P-450 oxidative reactions not only play a role in cholesterol synthesis but they also participate in the oxidation of the side chain of cholesterol and conjugation of bile acids. Thus, it is somewhat difficult to determine the exact effects of enzyme inducers on the overall total serum cholesterol levels. The present studies also indicate that some lipids were being excreted in the bile to the feces in the presence of barbituric acids, consequently lowering the total serum lipid levels over 14 days. Additionally, the barbituric acid derivatives afforded a part of their lipid lowering effects by inhibiting the activities of regulatory enzymes involved in *de novo* synthesis of lipids in the liver. This effect appears to be early in the syntheses of cholesterol and fatty acids at the ATP-dependent citrate lyase and acetyl-CoA synthetase steps. Cholesterol ester synthesis was reduced because the compounds blocked the activity of acyl-CoA cholesterol acyltransferase activity, and triglyceride synthesis was blocked because the agents reduced the activity of rate limiting enzyme sn-glycerol-3-phosphate acyl transferase.

The effects of the agents on serum lipoprotein lipid levels were important. Since VLDL and LDL conduct lipids to peripherial tissue including plaque cells in the aorta, it is important that a potential hypolipidemic agent lowers the cholesterol and triglyceride content of these two fractions. High HDL-cholesterol content protects man from myocardial infarcations and other cardiovacular disease[48], since HDL conducts cholesterol from plaque cells to the liver for clearance from the body. The barbituric acid derivatives significantly elevate HDL-cholesterol in rats. Clofibrate affords only a 4 to 16% increase in HDL cholesterol content and nicotinic acid affords a 25% increase, whereas probucol, d-thyroxine and cholestyramine cause no increase in human HDL cholesterol content. In fact, reductions in human HDL cholesterol have been reported with the use of these agents. Lovastatin causes a 28% increase in rat HDL-cholesterol after 8 weeks to rats at 8 mg/kg/day (unpublished results).

This study indicates that barbituric acid lowers serum lipids and modulate serum lipoproteins in a favorable therapeutic direction to afford beneficial removal of cholesterol from peripheral tissue. Further studies of these derivatives are desirable to characterize their pharmacological usefulness as hypolipidemic agents for potential clinical use.

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