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# Hypolipidemic effects of α, β, and γ-alkylaminophenone analogs in rodents

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**Summary** — A number of *N*-substituted β-alkylaminophenone derivatives including two α- and two γ-alkylaminophenone analogs were synthesized and investigated for hypolipidemic activity in mice at 8 mg/kg/day ip. Most of these analogs were found to be significantly more active than lovastatin and clofibrate. *N*-Phenylpiperazinopropiophenone **16** was one of the best derivatives, lowering serum cholesterol levels 41% and serum triglyceride levels 48% after 16 days of drug administration in $CF_1$ mice. In Sprague–Dawley rats, *N*-phenylpiperazinopropiophenone at 8 mg/kg/day orally also demonstrated more potent hypolipidemic activity than clofibrate, gemfibrozil, and lovastatin at their therapeutic dosage. It significantly reduced tissue cholesterol and triglyceride levels in the aorta wall tissue and lowered the cholesterol and triglyceride levels in chylomicron, very low density lipid (VLDL) and low density lipid (LDL) fractions, while it significantly elevated the cholesterol levels in high density lipid (HDL) fraction. This compound also proved to be active in lowering both cholesterol and triglyceride levels in hyperlipidemic mice and rats induced with atherogenic diet. In vitro liver acetyl coenzyme A (CoA) synthetase, 3-hydroxy-3-methyl glutaryl (HMG) CoA reductase, acyl CoA cholesterol acyl transferase (ACAT), *sn*-glycerol-3-phosphate acyltransferase, phosphatidylate phosphohydrolase, and hepatic lipoprotein lipase activities were significantly inhibited by *N*-phenylpiperazinopropiophenone from 25 to 100 μM.

**hypolipidemic activity / alkylaminophenone analog / cholesterol lowering / elevated HDL**

## Introduction

Atherosclerosis continues to be the major cause of death in developed countries [1, 2]. Over the past several decades, tremendous efforts have been underway to develop effective therapeutic hypolipidemic agents, which can reduce both serum cholesterol and triglyceride levels without significant side effects. This has led to the discovery of hundreds of compounds that showed significant serum cholesterol and triglyceride lowering effects, such as bile acid sequestrants [3], nicotinic acid [4], fibric acids [5], 3-hydroxy-3-methyl glutaryl (HMG) coenzyme A (CoA) reductase inhibitors [6], probucol [7], and many others [8–12]. Based on the studies of phthalimide derivatives and other cyclic imides, pyrazolidinediones, triazolidinediones and isoxazolidinediones [12, 13], we proposed that alkylaminophenone derivatives might possess potent hypolipidemic activity. Thus we conducted an investigation on β-alkylaminophenone analogs as potential hypolipidemic agents and report the results herein.

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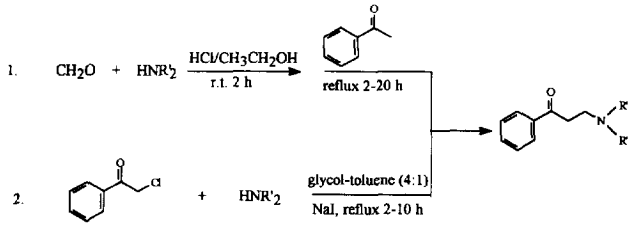
## Chemistry

β-Dimethylaminopropiophenone **1** was prepared according to the procedure described by Maxwell [14]. Derivatives **2–13**, **15**, and **17–19** were obtained by a modified method of Blike and Burckhalter [15]. Thus, an appropriately substituted amine was reacted with three equivalents of paraformaldehyde in absolute ethanol with addition of equivalent of concentrated HCl to form an intermediate iminium salt which was then reacted with the substituted phenone to afford the desired product (scheme 1-1). The analogs **14**, **16**, and **20–23** were synthesized by treating the




**Scheme 1.** The chemical synthesis of α, β, and γ-alkylaminophenone analogs.

**Table I.** Physical and structural characteristics of *N*-substituted α, β, and γ-alkylaminophenones[a].

| *Compound* | *Yield (%)* | *Mp (°C)* | *$^1$H NMR (DMSO-$d_6$) ppm* | *IR (KBr) ($cm^{-1}$)* | *Formula (element analysis)* |
|---|---|---|---|---|---|
| **1** | 67 | 156–157 (155–156)[b] | 8.03–7.58 (m, 5H, aromatic)<br>3.60–3.42 (m, α, β-CH2)<br>2.83 (s, 6H, $2CH_3$) | 2700–2400 (N-H)<br>1670 (C=O)<br>1240 (C-N) | $C_{11}H_{15}NO$·HCl<br>(C, H, N) |
| **2** | 76 | 108–110 (108–110)[c] | 8.14–7.57 (m, 5H, aromatic)<br>3.58–3.46 (m, α, β-$CH_2$)<br>3.23, 1.24 (ethyl) | 2700–2300 (N-H)<br>1670 (C=O)<br>1215 (C-N) | $C_{13}H_{19}NO$·HCl<br>(C, H, N) |
| **3** | 22 | 177–179 | 7.99–7.58 (m, 5H, aromatic)<br>3.51–3.28 (m, α, β-$CH_2$) | 2820–2400 (N-H)<br>1680 (C=O) | $C_{15}H_{21}NO$·HCl<br>(C, H, N) |
| **4** | 10 | 155–157 | 9.35 (s, OH)<br>8.01–7.58 (m, 5H, aromatic)<br>7.06–6.73 (d, 4H, aromatic) | 3100 (OH)<br>2800–2400 (N-H)<br>1690 (C=O) | $C_{17}H_{19}NO_2$·HCl<br>(C, H, N) |
| **5** | 30 | 165–167 | 8.02–7.58 (m, 5H, aromatic)<br>3.59–3.47 (m, α, β-$CH_2$) | 2700–2450 (N-H)<br>1670 (C=O) | $C_{13}H_{17}NO$·HCl<br>(C, H, N) |
| **6** | 88 | 193–195 | 8.02–7.58 (m, 5H, aromatic)<br>3.63–3.48 (m, α, β-$CH_2$) | 2720–2430 (N-H)<br>1680 (C=O) | $C_{14}H_{19}NO$·HCl<br>(C, H, N) |
| **7** | 20 | 173–175 | 8.00–7.58 (m, 5H, aromatic)<br>3.49–3.42 (m, α, β-$CH_2$) | 2730–2400 (N-H)<br>1680 (C=O) | $C_{15}H_{21}NO$·HCl<br>(C, H, N) |
| **8** | 58 | 182 (decomp) | 8.53–7.99 (m, 5H, aromatic)<br>4.35–4.00 (m, α, β-$CH_2$)<br>4.53 (m, morpholino) | 2700–2360 (N-H)<br>1680 (C=O)<br>1125 (C-O-C) | $C_{13}H_{17}NO_2$·HCl<br>(C, H, N) |
| **9** | 60 | 176–178 | 8.03–7.58 (m, 5H, aromatic)<br>3.64–3.49 (m, α, β-$CH_2$)<br>0.90 (d, methyl) | 2750–2300 (N-H)<br>1680 (C=O)<br>1220 (C-N) | $C_{15}H_{21}NO$·HCl<br>(C, H, N) |
| **10** | 41 | 173–175 | 8.02-7.58 (m, 5H, aromatic)<br>3.63–3.51 (m, α, β-$CH_2$)<br>0.93 (d, methyl) | 2720–2300 (N-H)<br>1680 (C=O)<br>1220 (C-N) | $C_{15}H_{21}NO$·HCl<br>(C, H, N) |
| **11** | 18 | 194–196 | 8.10–7.66 (m, 5H, aromatic)<br>3.75–3.50 (m, α, β-$CH_2$)<br>0.98 (d, $2CH_3$) | 2720–2400 (N-H)<br>1685 (C=O)<br>1220 (C-N) | $C_{16}H_{23}NO$·HCl<br>(C, H, N) |
| **12** | 42 | 159–161 | 8.03–7.58 (m, 5H, aromatic)<br>7.09 (b, $NH_2$) | 2760–2400 (N-H)<br>1630 (C=O) | $C_{15}H_{20}N_2O_2$·HCl<br>(C, H, N) |
| **13** | 23 | 197 (decomp) | 8.01–7.59 (m, 5H aromatic)<br>3.69–3.55 (m, α, β-$CH_2$)<br>2.84 (s methyl) | 2720–2430 (N-H)<br>1675 (c=O)<br>1220 (C-N) | $C_{14}H_{21}N_2O$·HCl<br>(C H, N) |
| **14** | 11 | 164–166 | 11.57 (CHO)<br>8.02–7.58 (m, 5H aromatic)<br>3.75–3.45 (m, α, β-$CH_2$) | 2700–2350(N-H)<br>1680 (C=O)<br>1220 (C-N) | $C_{14}H_{18}N_2O_2$·HCl<br>(C, H, N) |
| **15** | 10 | 197 (decomp) | 8.02–7.58 (m, 5H aromatic)<br>4.18 ($NCH_2C$=O) | 2720–2430 (N-H)<br>1670 (C=O) | $C_{19}H_{27}N_3O_3$·HCl<br>(C, H, N) |
| **16** | 87 | 186 (decomp) | 8.04–7.59 (m, 5H aromatic)<br>7.28–6.88 (m, 5H aromatic) | 2700–2450 (N-H)<br>1680 (C=O) | $C_{19}H_{22}N_2O$·HCl<br>(C, H, N) |
| **17** | 69 | 182–184 | 8.11–7.66 (m, 5H aromatic)<br>3.72–3.48 (m, α, β-$CH_2$) | 2740–2430 (N-H)<br>1680 (C=O) | $C_{16}H_{23}NO$·HCl<br>(C, H, N) |
| **18** | 15 | 167–169 | 7.75–7.47 (m, 4H, aromatic)<br>3.61–3.44 (m, α, β-$CH_2$)<br>3.18 (d, bridge $CH_2$) | 2750–2450 (N-H)<br>1720 (C=O)<br>1220 (C-N) | $C_{15}H_{19}NO$·HCl<br>(C, H, N) |
| **19** | 12 | 165–167 | 7.75–7.47 (m, 4H, aromatic)<br>3.60–3.47 (m, α, β-$CH_2$)<br>3.17 (d, bridge $CH_2$) | 2750–2450 (N-H)<br>1705 (C=O)<br>1220 (C-N) | $C_{14}H_{17}NO$·HCl<br>(C, H, N) |
| **20** | 75 | 233 (decomp) | 8.01–7.63 (m, 5H aromatic)<br>5.04 (d, O=$CCH_2N$) | 2720–2400 (N-H)<br>1700 (C=O) | $C_{13}H_{17}NO$·HCl<br>(C, H, N) |
| **21** | 20 | 224 (decomp) | 8.01–7.62 (m, 5H aromatic)<br>5.07 (d, O=$CCH_2N$) | 2720–2400 (N-H)<br>1695 (C=O) | $C_{14}H_{19}NO$·HCl<br>(C, H, N) |
| **22** | 14 | 180–182 | 7.98–7.55 (m, 5H aromatic)<br>3.19 (t, α-$CH_2$)<br>3.07 (m, γ-$CH_2$) | 2720–2400 (N-H)<br>1680 (C=O) | $C_{15}H_{21}NO$·HCl<br>(C, H, N) |
| **23** | 41 | 195–197 | 7.98–7.55 (m, 5H aromatic)<br>3.39 (t α-$CH_2$)<br>3.18 (m, γ-$CH_2$) | 2720–2400 (N-H)<br>1680 (C=O) | $C_{16}H_{23}NO$·HCl<br>(C, H, N) |

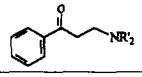
[a]See table II for structures; [b] reference [14]; [c] reference [15].

amine with potassium iodide and alkyl halide in glycol/toluene (4:1) under nitrogen (scheme 1). All prepared derivatives were obtained as the hydrochloric salt and purified by recrystallization from ethanol/water (95:5) three to five times, or by silica-gel column with chloroform/ethanol (9:1) as eluant. Elemental analyses (C, H, and N) were performed for all compounds and were found to be within 0.3% of the calculated values. The chemical and physical characteristics of these analogs are listed in table I for the known and new derivatives.

## Results

The structure–activity relationship study in mice at 8 mg/kg/day ip showed that β-alkylaminophenone analogs **1–17** were more active than lovastatin at 8 mg/kg/day and clofibrate at 150 mg/kg/day ip in lowering serum cholesterol levels (table II, III). Compounds **2–4**, **8**, **9**, **13**, **14**, **16** and **17** afforded >40% reduction of serum cholesterol and compounds **6**, **9**, **12**, **18** and **23** resulted in at least 60% decrease in serum triglyceride levels after 16 days of treatment at

**Table II.** In vivo hypolipidemic activity of β-alkylaminophenone analogs in $CF_1$ male mice at 8 mg/kg/day ip.



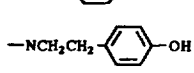
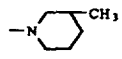
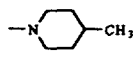
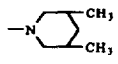
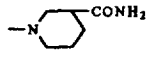
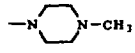
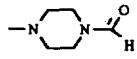
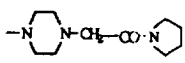
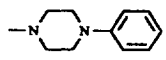
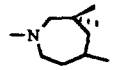
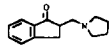
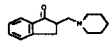
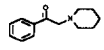
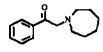
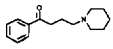
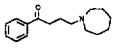
| *Analog* | *—NR'₂* | *Percent of control ($\bar{X} \pm SD$)* | | |
|---|---|---|---|---|
| | | *Serum cholesterol* | | *Serum triglyceride* |
| *(N = 6)* | | *Day 9* | *Day 16* | *Day 16* |
| **1** | —$N(CH_3)_2$ | 91 ± 7 | 66 ± 5* | 70 ± 6* |
| **2** | —$N(CH_2CH_3)_2$ | 79 ± 5 | 58 ± 4* | 64 ± 5* |
| **3** |  | 72 ± 4* | 53 ± 5* | 75 ± 5 |
| **4** |  | 72 ± 3* | 56 ± 4* | 67 ± 4* |
| **5** |  | 74 ± 6* | 68 ± 5* | 69 ± 7* |
| **6** |  | 55 ± 5* | 61 ± 5* | 60 ± 5* |
| **7** |  | 100 ± 7 | 83 ± 5 | 69 ± 6* |
| **8** |  | 70 ± 6* | 55 ± 7* | 75 ± 6* |
| **9** |  | 68 ± 4* | 56 ± 5* | 54 ± 5* |
| **10** |  | 68 ± 5* | 68 ± 4* | 67 ± 6* |
| **11** |  | 66 ± 5* | 64 ± 5* | 65 ± 4* |
| **12** |  | 83 ± 7 | 70 ± 5* | 60 ± 5* |
| **13** |  | 69 ± 5* | 55 ± 4* | 74 ± 5* |
| **14** |  | 68 ± 4* | 49 ± 4* | 77 ± 5* |
| **15** |  | 78 ± 6 | 60 ± 5* | 63 ± 5* |
| **16** | | 69 ± 4* | 59 ± 5* | 52 ± 4* |
| **17** |  | 76 ± 6 | 50 ± 4* | 73 ± 4* |

**Table III.** In vivo hypolipidemic activity of α-, β- or γ-alkylaminophenone analogs in $CF_1$ mice at 8 mg/kg/day ip.

| *Analog (N = 6)* | *Structure* | *Percent of control ($\bar{X} \pm SD$)* | | |
|---|---|---|---|---|
| | | *Serum cholesterol* | | *Serum triglyceride* |
| | | *Day 9* | *Day 16* | *Day 16* |
| **18** |  | 71 ± 4* | 67 ± 5* | 54 ± 5* |
| **19** |  | 85 ± 5 | 87 ± 5 | 60 ± 6* |
| **20** |  | 75 ± 6 | 71 ± 5* | 49 ± 5* |
| **21** |  | 76 ± 4 | 61 ± 4* | 46 ± 5* |
| **22** |  | 71 ± 5* | 61 ± 5* | 46 ± 4* |
| **23** |  | 77 ± 5 | 60 ± 6* | 51 ± 5* |
| Control | 1% CMC | 100 ± 5[a] | 100 ± 7[b] | 100 ± 6[c] |
| Lovastatin | (8 mg/kg/day) | 85 ± 4 | 82 ± 5 | 86 ± 7 |
| Clofibrate | (150 mg/kg/day) | 87 ± 6 | 78 ± 6 | 75 ± 6 |

*$P \leq 0.001$; [a] 125 mg/dL total serum cholesterol; [b] 128 mg/dL total serum cholesterol; [c] 137 mg/dL total serum triglycerides.

**Table IV.** In vivo hypolipidemic activity of β-alkylaminophenone analog **16** in Sprague–Dawley rats at 8 mg/kg/day after 7 and 14 days of oral administration.

| *N=6* | *Percent of control ($\bar{X} \pm SD$)* | | | |
|---|---|---|---|---|
| | *Serum cholesterol level* | | *Serum triglyceride level* | |
| | *Day 7* | *Day 14* | *Day 7* | *Day 14* |
| Control (1% CMC) | 100 ± 6[a] | 100 ± 6[b] | 100 ± 7[c] | 100 ± 5[d] |
| **16** | 65 ± 6* | 72 ± 5* | 76 ± 5* | 52 ± 5* |
| Clofibrate[e] | 89 ± 7 | 86 ± 5 | 83 ± 6 | 74 ± 7* |
| Gemfibrozil[f] | 91 ± 5 | 82 ± 7 | 78 ± 6* | 62 ± 5* |
| Lovastatin[g] | 85 ± 4 | 78 ± 5* | 91 ± 5 | 86 ± 7 |

*$P \leq 0.001$; [a] 73 mg/dL total serum cholesterol; [b] 75 mg/dL total serum cholesterol; [c] 111 mg/dL total serum triglyceride; [d] 112 mg/dL total serum triglyceride; [e] 150 mg/kg/day; [f] 90 mg/kg/day; [g] 8 mg/kg/day.

8 mg/kg/day ip. Compounds **9** and **16** afforded the best overall activity when considering the effects on both serum lipid levels.

Shortening the alkyl chain of the β-alkylaminophenones as in compounds **20** and **21**, or extending the alkyl chain by one carbon unit as in **22** and **23**, caused an improvement in the ability to lower serum triglyceride levels. The indanone derivatives **18** and **19** did not cause substantial improvement in hypolipidemic activity in mice. The morpholine and piperazine derivatives **8** and **13–16** demonstrated greatly improved hypocholesterolemic activity compared to most of the piperidine derivatives, but not their hypotriglyceridemic activity.

Since compound **16** demonstrated the best hypolipidemic activity, further investigations in rats were undertaken. At 8 mg/kg/day orally, it showed more hypolipidemic activity than gemfibrozil, clofibrate, and lovastatin at their effective doses by lowering both serum cholesterol (38%) and triglyceride (48%) levels after 14 days of treatment (table IV). Rat tissue lipid levels were also significantly altered by compound **16** after 14 days of treatment (table V). The liver cholesterol levels were lowered by 17% and the liver phospholipid levels were elevated significantly. The small intestine mucosa showed decreased cholesterol and neutral lipid levels with moderately elevated phospholipid levels. While in the aorta wall, cholesterol,

**Table V.** Effects of β-alkylaminophenone analog **16** on tissue and fecal lipid levels after 14 days of oral administration in Sprague–Dawley rats at 8 mg/kg/day.

| *Tissue* | | *Percent of control ($\bar{X} \pm SD$)* | | | | | |
|---|---|---|---|---|---|---|---|
| | *N=6* | *Lipid extracted* | *Cholesterol* | *Triglyceride* | *Neutral lipid*[y] | *Phospholipid* | *Protein* |
| Liver | Control | 100 ± 6[a] | 100 ± 7[b] | 100 ± 6[c] | 100 ± 8[d] | 100 ± 7[e] | 100 ± 5[f] |
| | **16** | 120 ± 7 | 83 ± 6 | 101 ± 5 | 90 ± 6 | 204 ± 6* | 98 ± 4 |
| Small intestine | Control | 100 ± 8[g] | 100 ± 7[h] | 100 ± 7[i] | 100 ± 8[j] | 100 ± g[k] | 100 ± 9[l] |
| | **16** | 93 ± 5 | 88 ± 6 | 93 ± 7 | 69 ± 7* | 140 ± 9* | 89 ± 4 |
| Aorta | Control | 100 ± 5[m] | 100 ± 6[n] | 100 ± 5[o] | 100 ± 6[p] | 100 ± 7[q] | 100 ± 5[r] |
| | **16** | 88 ± 5 | 50 ± 3* | 50 ± 4* | 69 ± 5* | 117 ± 5 | 118 ± 9 |
| Feces | Control | 100 ± 7[s] | 100 ± 6[t] | 100 ± 7[u] | 100 ± 8[v] | 100 ± 8[w] | 100 ± 5[x] |
| | **16** | 115 ± 6 | 112 ± 4 | 120 ± 6 | 106 ± 5 | 124 ± 5* | 82 ± 3 |

*$P \leq 0.001$; [a] 50.5 mg total lipid/g wet tissue; [b] 9.18 mg cholesterol/g wet tissue; [c] 6.37 mg triglyceride/g wet tissue; [d] 15.7 mg neutral lipid/g wet tissue; [e] 27.19 mg phospholipid/g wet tissue; [f] 12.02 mg protein/g wet tissue; [g] 68.2 mg total 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 total 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 total 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; [y] = mono- and diglycerols after subtracting the triglycerides from the total neutral lipids.

triglyceride, and neutral lipid levels were all significantly reduced after 14 days of treatment. The fecal lipid levels were slightly increased, but the difference from the control was not statistically significant. Compound **16** had no effect on the total body weight, individual organ weights or daily food consumption compared to the control values for 14 days.

The lipid levels of the rat serum lipoproteins were also examined after 14 days of treatment (table VI). Cholesterol, triglyceride, and neutral lipid levels were all reduced in chylomicrons, very low density lipid (VLDL), and low density lipid (LDL), whereas high density lipid (HDL) cholesterol triglyceride, and neutral lipid levels were elevated. The phospholipid levels were increased in the VLDL and HDL fractions. The protein content was reduced in LDL but increased in the HDL fraction.

The agent was also active in hyperlipidemic mice and rats with approximately the same degree of activity as in normalipidemic animals in reducing the serum cholesterol levels. In hyperlipidemic mice, the serum cholesterol levels were reduced to approximately half of the baseline control levels, while the serum triglyceride levels were reduced close to the baseline control levels after 16 days of treatment (table VII). In hyperlipidemic rats, serum cholesterol and triglyceride levels were reduced but not as significantly as in hyperlipidemic mice after 14 days of treatment (table VIII). Tissue lipid levels of hyperlipidemic rats showed that liver cholesterol, triglyceride, and neutral lipid levels were reduced but were still above the baseline control levels (table IX). Small intestine lipid levels were more significantly reduced, approximately to the baseline control levels. The aorta wall lipid levels were reduced significantly, but the cholesterol levels were still far above the baseline control levels. Fecal excretion was reduced but was not statistically significant. The serum lipoprotein cholesterol and triglyceride levels were reduced in VLDL, and LDL fractions, but elevated in HDL fraction (table X). Phospholipid levels in VLDL were increased significantly, and protein levels in VLDL and HDL were elevated.

In vitro mouse hepatic acetyl CoA synthetase activity was reduced significantly at 50 and 100 μM of compound **16** in 60 min (table XI). HMG CoA reductase and acyl coenzyme A cholesterol acyl transferase (ACAT) activities were reduced significantly at all concentrations employed. Cholesterol 7α-hydrolase activity was reduced at 50 and 100 μM. *sn*-Glycerol-3-phosphate acyltransferase activity was moderately reduced by compound **16**. Whereas the phosphatidylate phosphohydrolase activity was markedly reduced even at 25 μM. Hepatic lipase activity was also reduced significantly at all concentrations of compound **16**.

The mean survival dose of compound **16** in $CF_1$ mice was 250 mg/kg ip as a single administration after 7 days of observation.

**Table VI.** Effects of β-alkylaminophenone analog **16** on Sprague–Dawley rat lipoprotein lipid levels after 14 days of oral administration at 8 mg/kg/day.

| *Tissue* | *N=6* | *Percent of control ($\bar{X} \pm SD$)* | | | | |
|---|---|---|---|---|---|---|
| | | *Cholesterol* | *Triglyceride* | *Neutral lipid* | *Phospholipid* | *Protein* |
| Chylomicrons | Control | 100 ± 6[a] | 100 ± 5[b] | 100 ± 7[c] | 100 ± 6[d] | 100 ± 6[e] |
| | **16** | 71 ± 3* | 84 ± 4 | 86 ± 6 | 103 ± 5 | 95 ± 4 |
| VLDL | Control | 100 ± 7[f] | 100 ± 6[g] | 100 ± 7[h] | 100 ± 6[i] | 100 ± 6[j] |
| | **16** | 34 ± 4* | 79 ± 6* | 87 ± 4 | 204 ± 7* | 80 ± 4* |
| LDL | Control | 100 ± 6[k] | 100 ± 6[l] | 100 ± 7[m] | 100 ± 8[n] | 100 ± 6[o] |
| | **16** | 44 ± 3* | 78 ± 5* | 75 ± 4* | 114 ± 5 | 27 ± 3* |
| HDL | Control | 100 ± 7[p] | 100 ± 6[q] | 100 ± 6[r] | 100 ± 8[s] | 100 ± 7[t] |
| | **16** | 721 ± 6* | 127 ± 5* | 303 ± 8* | 183 ± 8* | 131 ± 5* |

* $P \leq 0.001$; [a] 337 μg cholesterol/mL serum; [b] 420 μg triglyceride/mL serum; [c] 67 μg neutral lipid/mL serum; [d] 149 μg phospholipid/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 phospholipid/mL serum; [j] 50 μg protein/mL serum; [k] 210 μg cholesterol/mL serum; [l] 45 μg triglyceride/mL serum; [m] 10 μg neutral lipid/mL serum; [n] 41 μg phospholipid/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 phospholipid/mL serum; [t] 657 mg protein/mL serum.

**Table VII.** In vivo hypolipidemic activity of β-alkylaminophenone analog **16** in hyperlipidemic $CF_1$ mice after 9 and 16 days of ip administration at 8 mg/kg/day.

| *N=6* | *Percent of control ($\bar{X} \pm SD$)* | | | |
|---|---|---|---|---|
| | *Serum cholesterol level* | | *Serum triglyceride level* | |
| | *Day 9* | *Day 16* | *Day 9* | *Day 16* |
| Diet control | 100 ± 4[a] | 100 ± 6[b] | 100 ± 5[c] | 100 ± 5[d] |
| **16** | 55 ± 6* | 50 ± 3* | 67 ± 6* | 50 ± 5* |
| Baseline control | 29 ± 4* | 28 ± 4* | 59 ± 5* | 57 ± 5* |

* $P \leq 0.001$; [a] 387 mg/dL total serum cholesterol; [b] 398 mg/dL total serum cholesterol; [c] 236 mg/dL total serum triglycerides; [d] 249 mg/dL total serum triglycerides.

**Table VIII.** In vivo hypolipidemic activity of β-alkylaminophenone analog **16** in hyperlipidemic Sprague–Dawley rats after 2 and 4 weeks of oral administration at 8 mg/kg/day.

| *N=6* | *Percent of control ($\bar{X} \pm SD$)* | | | |
|---|---|---|---|---|
| | *Serum cholesterol levels* | | *Serum triglyceride levels* | |
| | *Week 2* | *Week 4* | *Week 2* | *Week 4* |
| Diet control | 100 ± 5[a] | 100 ± 6[b] | 100 ± 5[c] | 100 ± 5[d] |
| **16** | 116 ± 7 | 60 ± 5* | 73 ± 5* | 80 ± 4* |
| Baselin control** | 21 ± 4* | 17 ± 3* | 40 ± 5* | 43 ± 3* |

* $P \leq 0.001$; [a] 460 mg/dL; [b] 520 mg/dL; [c] 408 mg/dL; [d] 512 mg/dL; ** Sprague–Dawley rats with normal lipid levels administered 1% CMC and fed with normal food during the entire period of the experiment.

**Table IX.** Effects of β-alkylaminophenone analog **16** on hyperlipidemic Sprague–Dawley rat tissue and fecal lipid levels after 4 weeks of oral administration at 8 mg/kd/day.

| *Tissue* | *N=6* | *Percent of control ($\bar{X} \pm SD$)* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Lipid extracted* | *Cholesterol* | *Triglyceride* | *Neutral lipid* | *Phospholipid* | *Protein* |
| Liver | Diet control | 100 ± 5[a] | 100 ± 5[b] | 100 ± 6[c] | 100 ± 7[d] | 100 ± 9[e] | 100 ± 7[f] |
| | **16** | 78 ± 5 | 79 ± 6 | 78 ± 5* | 85 ± 5 | 78 ± 6 | 85 ± 6 |
| | Base control | 8 ± 2* | 6 ± 2* | 43 ± 4* | 32 ± 3* | 31 ± 3* | 73 ± 5* |
| Small intestine | Diet control | 100 ± 4[g] | 100 ± 6[h] | 100 ± 6[i] | 100 ± 8[j] | 100 ± 8[k] | 100 ± 6[l] |
| | **16** | 81 ± 5 | 74 ± 5* | 57 ± 5* | 67 ± 6* | 69 ± 7* | 104 ± 5 |
| | Base control | 91 ± 5 | 65 ± 5* | 71 ± 6* | 62 ± 5* | 87 ± 7 | 127 ± 7 |
| Aorta | Diet control | 100 ± 4[m] | 100 ± 5[n] | 100 ± 6[o] | 100 ± 7[p] | 100 ± 8[q] | 100 ± 7[r] |
| | **16** | 142 ± 4* | 68 ± 5* | 34 ± 4* | 76 ± 6* | 120 ± 9 | 73 ± 6* |
| | Base control | 176 ± 6* | 7 ± 2* | 55 ± 6* | 72 ± 5* | 71 ± 6* | 78 ± 6 |
| Feces | Diet control | 100 ± 6[s] | 100 ± 6[t] | 100 ± 6[u] | 100 ± 7[v] | 100 ± 7[w] | 100 ± 6[x] |
| | **16** | 89 ± 5 | 84 ± 5 | 97 ± 7 | 76 ± 6* | 101 ± 6 | 74 ± 5* |
| | Base control | 37 ± 1* | 4 ± 2* | 26 ± 4* | 79 ± 6 | 92 ± 5 | 156 ± 8* |

* $P \leq 0.001$; [a] 312 mg total lipid/g wet tissue; [b] 155 mg cholesterol/g wet tissue; [c] 14.77 mg triglyceride/g wet tissue; [d] 49.3 mg neutral lipid/g wet tissue; [e] 87.33 mg phospholipid/g wet tissue; [f] 16.42 mg protein/g wet tissue; [g] 85.3 mg total lipid/g wet tissue; [h] 18.54 mg cholesterol/g wet tissue; [i] 15.89 mg triglyceride/g wet tissue; [j] 27.3 mg neutral lipid/g wet tissue; [k] 22.97 mg phospholipid/g wet tissue; [l] 32.9 mg protein/g wet tissue; [m] 119.18 mg total lipid/g wet tissue; [n] 8.19 mg cholesterol/g wet tissue; [o] 18.05 mg triglyceride/g wet tissue; [p] 13.63 mg neutral lipid/g wet tissue; [q] 41.11 mg phospholipid/g wet tissue; [r] 15.06 mg protein/g wet tissue; [s] 31.21 mg total lipid/g wet tissue; [t] 7.49 mg cholesterol/g wet tissue; [u] 7.13 mg triglyceride/g wet tissue; [v] 4.29 mg neutral lipid/g wet tissue; [w] 6.16 mg phospholipid/g wet tissue; [x] 4.47 mg protein/g wet tissue.

**Table X.** Effects of β-alkylaminophenone analog **16** on hyperlipidemic Sprague–Dawley rat lipoprotein lipid levels after 4 weeks of oral administration at 8 mg/kg/day.

| *Tissue* | *N=6* | *Percent of control ($\bar{X} \pm SD$)* | | | | |
|---|---|---|---|---|---|---|
| | | *Cholesterol* | *Triglyceride* | *Neutral lipid* | *Phospholipid* | *Protein* |
| Chylomicrons | Diet control | 100 ± 6[a] | 100 ± 6[b] | 100 ± 7[c] | 100 ± 6[d] | 100 ± 6[e] |
| | **16** | 93 ± 5 | 98 ± 7 | 96 ± 6 | 67 ± 7* | 95 ± 6 |
| | Base control | 33 ± 3* | 47 ± 5* | 58 ± 6* | 42 ± 4* | 91 ± 5 |
| VLDL | Diet control | 100 ± 5[f] | 100 ± 6[g] | 100 ± 7[h] | 100 ± 8[i] | 100 ± 6[j] |
| | **16** | 69 ± 4* | 83 ± 6 | 99 ± 6 | 190 ± 8* | 201 ± 7* |
| | Base control | 50 ± 5* | 68 ± 5* | 108 ± 7 | 76 ± 5* | 44 ± 4* |
| LDL | Diet control | 100 ± 6[k] | 100 ± 5[l] | 100 ± 6[m] | 100 ± 8[n] | 100 ± 7[o] |
| | **16** | 52 ± 4* | 73 ± 6* | 63 ± 5* | 97 ± 7 | 40 ± 4* |
| | Base control | 65 ± 6* | 66 ± 5* | 50 ± 4* | 98 ± 7 | 26 ± 3* |
| HDL | Diet control | 100 ± 7[p] | 100 ± 7[q] | 100 ± 6[r] | 100 ± 5[s] | 100 ± 7[t] |
| | **16** | 361 ± 6* | 189 ± 6* | 133 ± 5* | 110 ± 5 | 829 ± 11* |
| | Base control | 142 ± 6* | 117 ± 4 | 108 ± 5 | 57 ± 5* | 288 ± 7* |

* $P \leq 0.001$; [a] 371 μg cholesterol/mL serum; [b] 554 μg triglyceride/mL serum; [c] 79 μg neutral lipid/mL serum; [d] 231 μg phospholipid/mL serum; [e] 196 μg protein/mL serum; [f] 773 μg cholesterol/mL serum; [g] 142 μg triglyceride/mL serum; [h] 294 μg neutral lipid/mL serum; [i] 78 μg phospholipid/mL serum; [j] 15 μg protein/mL serum; [k] 836 μg cholesterol/mL serum; [l] 62 μg triglyceride/mL serum; [m] 54 μg neutral lipid/mL serum; [n] 231 μg phospholipid/mL serum; [o] 73 μg protein/mL serum; [p] 689 μg cholesterol/mL serum; [q] 16 μg triglyceride/mL serum; [r] 397 μg neutral lipid/mL serum; [s] 1184 μg phospholipid/mL serum; [t] 315 mg protein/mL serum.

**Table XI.** In vitro effects of β-alkylaminone analog **16** on the activities of $CF_1$ mouse liver enzymes.

| *Enzyme* (*N* = 6) | *Percent of control* ($\bar{X} \pm SD$) | | | |
|---|---|---|---|---|
| | *Control* | *25 μM* | *50 μM* | *100 μM* |
| Acetyl CoA synthetase | 100 ± 5[a] | 102 ± 4 | 68 ± 5* | 38 ± 4* |
| ATP dependent citrate lyase | 100 ± 6[b] | 121 ± 7 | 96 ± 6 | 88 ± 5 |
| HMG CoA reductase | 100 ± 6[c] | 54 ± 6* | 52 ± 5* | 51 ± 5* |
| ACAT | 100 ± 5[d] | 69 ± 6* | 61 ± 6* | 27 ± 3* |
| Cholesterol ester hydrolase | 100 ± 4[e] | 62 ± 7* | 77 ± 8* | 104 ± 6 |
| Cholesterol 7α-hydrolase | 100 ± 7[f] | 98 ± 6 | 69 ± 6* | 65 ± 5* |
| Acetyl CoA carboxylase | 100 ± 4[g] | 99 ± 5 | 95 ± 4 | 94 ± 4 |
| sn-Glycerol-3-phosphate acyltransferase | 100 ± 5[h] | 79 ± 6* | 76 ± 5* | 61 ± 5* |
| Phosphatidylate phosphohydrolase | 100 ± 6[i] | 32 ± 3* | 15 ± 2* | 6 ± 2* |
| Hepatic lipoprotein lipase | 100 ± 7[j] | 62 ± 6* | 61 ± 5* | 45 ± 4* |

* $P \leq 0.001$; [a] 28.5 mg acetyl CoA formed/g wet tissue; [b] 30.5 mg citrate hydrolyzed/g wet tissue; [c] 384 900 dpm cholesterol formed/g wet tissue; [d] 224 000 dpm/mg microsomal protein; [e] 56 436 dpm/mg wet tissue; [f] 4808 dpm/mg microsomal protein; [g] 537 800 dpm/mg wet tissue; [h] 302 010 dpm/mg wet tissue; [i] 16.7 μg pi formed/g wet tissue; [j] 278 538 dpm/g wet tissue.

## Discussion

β-Alkylaminophenone analogs proved to be potent hypolipidemic agents at the low dose of 8 mg/kg/day ip and orally. Both serum cholesterol and triglyceride levels were significantly reduced compared to the control levels after 14–16 days of treatment. The tissue cholesterol, triglyceride, and neutral lipid levels were also reduced in most cases, whereas the lipoprotein lipid levels were significantly altered by lowering VLDL and LDL cholesterol and triglyceride levels and increasing the HDL cholesterol levels.

Altering the lipoprotein lipids in this manner by drug therapy is desirable for hyperlipidemic patients, since VLDL- and LDL-cholesterol levels are elevated and HDL-cholesterol levels are lowered in the diseased state. VLDL and LDL conduct cholesterol to peripheral tissue including the aorta wall, causing the growth of plaques. HDL conducts free cholesterol released from tissues back to the liver where it is released from the lipoprotein to be secreted into the bile for elimination from the body. As can be seen, the β-alkylaminophenones alter the lipoprotein ratio in favor of clearance of cholesterol from the body.

The reduction of mouse acetyl CoA synthetase activity by the agent shoud reduce the available cytoplasmic acetyl CoA for fatty acid and cholesterol de novo synthesis. The reduction of HMG CoA reductase activity by the agent should lower cholesterol synthesis additively. Reduction of ACAT activity would lower the levels of cholesterol esters, the storage form of cholesterol in tissue. If a similar process occurs in the aorta wall, lowering of the cholesterol esters should reduce the size of the plaques which lead to atherosclerosis since it is dependent directly on the deposition of cholesterol esters.

Reduction of hepatic *sn*-glycerol-3-phosphate acyl transferase and phosphatidylate phosphohydolase activities by the agent would explain the reduction of triglyceride levels in the serum and tissues. Furthermore, reduction of hepatic lipoprotein lipase activity by the compound would reduce the uptake of triglycerides by the heptocytes as they were released from the lipoproteins.

In the hyperlipidemic induced animals, the agent achieved reductions in serum and tissue lipids which are therapeutically desirable and reverse the LDL-cholesterol and HDL-cholesterol ratio in a favorable direction to reduce the hyperlipidemic state. The 4-week dosing period may need to be extended to obtain baseline serum lipid levels in rats. However, the mice had serum lipid levels after 2 weeks administration which were closer to the baseline values. The tissue lipids in hyperlipidemic rats were responding to treatment with compound **16**, but again had not achieved baseline tissue lipid levels. The serum lipoproteins after 4 weeks administration in hyperlipidemic rats demonstrated lowered VLDL- and LDL-cholesterol levels and higher HDL-cholesterol levels, indicating the effectiveness of compound **16** to reverse the ratio in a favorable direction.

## Experimental protocols

*Materials and apparatus*

All chemicals used for organic synthesis were purchased from Aldrich Chemical Company (Milwaukee, WI). All liquid reagents, as well as solvents, were distilled prior to use. Solid chemicals were used as received from the manufacturer. Melting points were determined on a Thomas-Hoover apparatus (capillary method), and were uncorrected. Infrared (IR)

spectra were recorded on a Perkin Elmer 1320 IR spectrophotometer. Thin layer chromatography (TLC) was performed using silica-gel 60F 254 plates (silica gel on aluminum, Aldrich Chemical Company). Column chromatography was performed using silica gel 200-400 mesh, 60 Å from Aldrich Chemical Company. Elemental analyses were performed by M-H-W laboratories (Phoenix, AZ) and by the School of Pharmacy, University of North Carolina at Chapel Hill. $^1$H NMR spectra were recorded on a 300 MHz NMR spectrometer (Varian) using tetramethylsilane as a external standard and DMSO-$d_6$ as the solvent.

All chemicals used in biological studies were purchased from Sigma Company (Saint Louis, MO). All isotopes were obtained from New England Nuclear (Boston, MA). $^3$H and $^{14}$C were counted in Scintiverse II (Fisher Scientific) scintillation fluid using a Packard Beta counter and were corrected for quenching using a standard curve. Ultraviolet (UV) and visible spectrophotometric measurements were made using a Bausch and Lomb Spectronic 1001. Assay blanks were run for all experiments and their values were subtracted from those of the test samples. Centrifugations were performed on an ICE centrifuge (small blood samples in microcapillary tubes) or a Beckman Ultracentrifuge (Model L5-65).

*Chemistry*

*Synthesis of β-alkylaminophenone derivatives* ***3–13***, ***15***, *and* ***17–19***

A mixture of 0.02 mol alkylamine, 2.0 g (0.066 mol) paraformaldehyde, 2.4 mL concentrated HCl, and 20 mL absolute ethanol in a 100 mL three-necked flask reaction bottle was refluxed for 4 h. Then 2.4 g (0.02 mol) acetophenone in 15 mL ethanolbenzene (8:2) were added slowly to the reaction mixture. The reaction mixture was refluxed for another 10 h. The reaction was poured into 100 mL ice-cold water. The aqueous solution was extracted with ether three times (3 x 20 mL). The organic phase was discarded. The water phase was neutralized with 1 N NaOH adjusting to pH 10, and extracted with ethyl acetate three times (3 x 20 mL). The fractions of the ethyl acetate were combined and washed with NaCl-saturated water three times (3 x 20 mL). The ethyl acetate phase was dried with $Na_2SO_4$ for 12 h. The $Na_2SO_4$ was removed by filtration. The organic solvent was evaporated under reduced pressure to a small volume. A silica-gel column was used to purify the expected product with $CHCl_3$/$CH_3CH_2OH$ (9:1). The desired fractions were combined and evaporated to a small volume. Ice-cold concentrated HCl (2.3 mL) in 35 mL ethanol was added slowly. The solution was evaporated under reduced pressure to dryness. The product was recrystallized from 90% ethanol to give the desired product.

*β-Alkylaminophenone analogs* ***14***, ***16*** *and* ***20–23***

A solution of 0.03 mol alkylamine and 4.0 g KI in glycol/toluene (4:1) was heated to 100 °C under $N_2$ protection. A solution of 0.03 mol β-chloropropiophenone in toluene was added dropwise to the above solution. The reaction was carried while stirring and under $N_2$ protection for 12 h. The resultant reaction mixture was evaporated under reduced pressure to a small volume and then poured into ice-cold 2 N HCl (100 mL). The organic phase was extracted with ether (3 x 25 mL). The aqueous phase was neutralized with 1 N NaOH to pH 10 and then extracted with ether (3 x 25 mL). The ether phases were combined and washed with NaCl saturated water (3 x 30 mL), and dried with anhydrous $Na_2SO_4$ over 12 h. The $Na_2SO_4$ was removed by filtration, and the solution evaporated to a small volume. An equimolar quantity of 2 N HCl in EtOH was added to the small residue which was then evaporated under reduced pressure to a solid residue. The solid was then recrystallized from EtOH/$H_2O$ (9–7:1) three times to give the proper product.

*Pharmacology*

*Hypolipidemic screen in normal CF$_1$ mice*

All synthesized analogs were homogenized with 1% carboxymethylcellulose/$H_2O$ and administered intraperitoneally to $CF_1$ male mice (25–30 g) at 8 mg/kg/day for 16 days. On days 9 and 16, blood was obtained by tail-vein bleeding using microcapillary tubes. The serum was separated by centrifugation (3500 *g* x 3 min) using an IEC MB microcentrifuge. The serum cholesterol levels were determined by modification of the Liebermann–Burchard reaction [16, 17]. The triglyceride levels were determined by a commercial kit (Sigma/Triglyceride GPO-Trinder) [18].

*Hypolipidemic assay in normal Sprague–Dawley rats*

The active analog, obtained from the preliminary hypolipidemic screen in $CF_1$ mice, was suspended and homogenized with 1% carboxymethylcellulose/$H_2O$, and administered orally to Sprague–Dawley male rats (200–250 g) at 8 mg/kg/day for 14 days. Animal weights were determined periodically during the course of the experiment. The average daily food intake in grams per animal was also determined over the period of study. Blood was obtained by tail-vein bleeding on days 7 and 14 and centrifuged. Serum cholesterol [16, 17] and triglyceride levels (Sigmal/Triglyceride GPO-Trinder) [18] were determined.

Liver, small intestine, aorta wall, and fecal materials (24 h collection) were obtained from Sprague–Dawley male rats after 14 days of treatment with test drugs at 8 mg/kg/day orally. A 10% homogenate was prepared in a solution of 0.25 M sucrose and 0.001 M EDTA. An aliquot (2 mL) of the homogenates was extracted using $CHCl_3$/$CH_3OH$ (2:1) by the methods of Folch et al [19] and Bligh and Dyer [20], and the amount of the total lipid was determined gravimetrically. The lipid was then taken up in ethyl acetate and the cholesterol levels [16, 17], triglyceride levels (Sigma/Triglyceride GPO-Trinder) [18], neutral lipid content [21, 27], phospholipid content [22, 27], and homogenate protein content [23] were determined.

The blood was collected from the abdominal vein of Sprague–Dawley rats after 14 days of treatment with drugs and centrifuged at 3500 rpm to obtain the serum. Aliquots (10 mL) of the separated serum were subjected to density gradient ultracentrifugation according to the methods of Hatch and Lees [24], and Havel et al [25] to give chylomicrons, VLDL, LDL, and HDL as modified for rats [26, 27]. Each of the fractions was analyzed for cholesterol [26], triglycerides [18], neutral lipids [21, 27], phospholipids [22, 27], and protein levels [23].

*Hypolipidemic assay in hyperlipidemic CF$_1$ male mice*

$CF_1$ male mice (25–30 g) were fed with commercial diet (US Biochemical Corporation Basal Atherogenic Diet, which for 1000 g was composed of 400 g butterfat, 60 g cellulose, 53 g cholesterol, 4 g choline dihydrogen citrate, 40 g salt/oil mixture, 20 g sodium cholate, 223 g sucrose, 200 g vitamin-free casein and 0.2 g vitamin supplement) mixed with regular ground rodent chow (Agway/Prolab Animal Diet, formula 3000) (1:1) with 5% fructose for 14 days. After the plasma cholesterol and triglyceride levels were assayed and observed to be elevated significantly above the normal control (more than three times), the mice were administered the test drugs intraperitoneally at 8 mg/kg/day for an additional 16 days while continuing the diet. On days 9 and 16 of the administration of drugs, the serum cholesterol and triglyceride levels were determined by the same methods described above.

*Hypolipidemic study in hyperlipidemic rats*

Sprague–Dawley male rats (200–250 g) were placed on the Basal Atherogenic Diet (US Biochemical Corporation) mixed with regular ground rodent chow (Agway/Prolab Animal Diet, formula 3000) (1:1) and 5% fructose for 4 weeks. After the lipid levels were observed to be significantly elevated above the normal control (more than three times), the rats were orally administered the test drugs for an additional 4 weeks while being maintained on the Basal Atherogenic Diet throughout the experiment. All animal weights were recorded periodically during the course of study. The average daily food intake per animal was also recorded each day over the experiment period. After 2 weeks of drug administration, blood was collected from the tail vein, and cholesterol and triglyceride levels were determined as described previously. After 4 weeks of drug administration, rats were anesthetized with ether. Blood was collected from the abdominal vein, and different organ tissues as well as feces (24 h collection) were collected. Serum cholesterol and triglyceride levels, tissue lipid levels, and lipoprotein lipid levels were determined using the same methods described above.

*Enzymatic assays*

In vitro enzymatic studies were performed using 10% homogenates of $CF_1$ male mouse liver in 0.25 M sucrose and 0.001 M EDTA incubated with 25, 50 and 100 μM of test drugs. Acetyl coenzyme A (CoA) synthetase [28] and ATP-dependent citrate lyase [28] activities were determined spectrophotometrically at 540 nm as the hydroxamate of acetyl CoA formed after 60 min at 37 °C. Cholesterol-7α-hydroxylase activity was determined using $^{14}$C-cholesterol and the microsomal fraction [30]. Neutral cholesterol ester hydrolase activity was determined using $^{14}$C-cholesterol oleate [27]. Acyl CoA cholesterol acyl transferases (ACAT) activity was determined using the microsomal preparation and $^{14}$C-oleic acid (56.7 mCi/mmol) by the method of Balasubramaniam et al [31]. HMG CoA reductase was measured using [1-$^{14}$C]-acetyl CoA (56 mCi/mmol) in a post-mitochondrial supernatant (9000 *g*, 20 min) for 60 min at 37 °C (32). The digitonide derivative of cholesterol was isolated and counted [33]. Fatty acids and acetyl CoA carboxylase activity were measured by the method of Greenspan and Lowenstein [34] using sodium $^{14}$C-bicarbonate (41.0 mCi/mmol). *sn*-Glycerol-3-phosphate acyl transferase was determined with $^{14}$C-glycerol-3-phosphate [35]. Phosphatidylate phosphohydrolase activity was determined as inorganic phosphate released after 15 min by the method of Mavis et al [36]. Hepared-induced lipoprotein lipase and hepatic lipase activities were determined using the method of Chait et al [37] using glycerol tri[1-$^{14}$C]palmitate (60 mCi/mmol). The protein content of the fractions was determined by the method of Lowry et al [23].

The safety of the compound **16** was obtained as a single dose IP in male $CF_1$ mice (~ 28 g) dosing from 100 to 500 mg/kg and using the probit method for determining the mean survival value [38].

Statistical analysis of data from these experiments included calculations of the mean ($\bar{X}$) and standard deviation (SD), and the *P*-values were determined using the Student's *t*-test and ANOVA.

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