# Hypolipidemic Activity of Cyclic Imido Alkyl Ethers, Thioethers, Sulfoxides, and Sulfones

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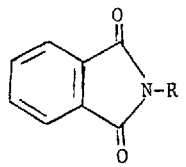
**Abstract** □ *N*-Substituted alkyl ethers, thioethers, sulfoxides, and sulfones of cyclic imides (e.g., phthalimide, saccharin, 1,8-naphthalimide, succinimide, and 2,3-dihydrophthalazine-1,4-dione) were shown to have potent hypolipidemic activity at doses of 10 and 20 mg/kg/d in rodents. These *N*-substitutions afforded no improvement over other known *N*-substitutions (e.g., butyl, 3-butanone, or the propionic acid derivatives of phthalimide, saccharin, and 2,3-dihydrophthalazine-1,4-dione) compared with the respective parent compounds. However, 2-(methoxyethyl)-1*H*-benz[de]isoquinoline-1,3-(2*H*)dione (**3a**), 2-[2-methylsulfinyl]ethyl-1*H*-benz[de]isoquinoline-1,3-(2*H*)dione (**3c**), 1-(2-methylsulfinyl)-2,5-pyrrolidenedione (**4c**), and 1-(2-methoxyethyl-2,5-pyrrolidenedione (**4a**) significantly improved activity compared with parent compounds, as well as previously reported *N*-substituted analogues, reducing serum cholesterol levels and serum triglyceride levels by 40%. The thioether of succinimide afforded a 54% reduction of serum cholesterol and a 41% reduction of serum triglyceride levels in mice after 16 d. The alkyl thioethers of 1,8-naphthalimide and succinimide significantly lowered cholesterol levels in serum VLDL and LDL, while the alkyl thioethers of succinimide elevated HDL cholesterol content. Tissue lipids were reduced in the liver and aorta by these selected derivatives. The activities of regulatory enzymes in de novo synthesis of hepatic cholesterol and triglyceride were inhibited by the selected 1,8-naphthalimide derivatives. In situ cholesterol and cholic acid reabsorption from intestines were suppressed by the presence of the agents.

A number of alkyl (e.g., butyl, pentyl, butanone, and propionic acid) *N*-substituted derivatives of cyclic imides have been examined for hypolipidemic activity in rodents.[1-7] Oral or intraperitoneal administration of these agents to rodents at a dose of 20 mg/kg/d significantly lowered both serum cholesterol and triglyceride levels by >35%. Unfortunately, these agents were generally not effective in elevating HDL cholesterol levels after 14 d of administration. An exception to this observation, however, is *o*-(*N*-phthalimido)acetophenone, which afforded a 43% increase in HDL cholesterol in rats after 8 weeks of drug administration.[8] This effect was not evident after 2 weeks of drug administration.[9] Clinical studies in humans indicate that if an agent is to be useful from the standpoint of reducing serum lipid levels and clearance of cholesterol esters from atherogenic plaques, then the agent should lower serum LDL cholesterol and elevate HDL cholesterol levels. The objective of the present research is to investigate alkyl ether, thioether, sulfoxide, and sulfone *N*-substitutions of cyclic imides to determine their hypolipidemic activity in rodents and to assess their ability to modulate the cholesterol content of LDL and HDL in a clinically favorable direction.

## Results

**Chemistry**—Alkylation of phthalimide, saccharin, 1,8-naphthalimide, and phthalhydrazide with either 2-chloro-

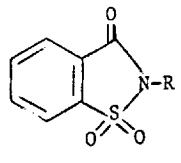


1a. R = $-CH_2CH_2OCH_3$

1b. R = $-CH_2CH_2SCH_3$

1c. R = $-CH_2CH_2SOCH_3$

1d. R = $-CH_2CH_2SO_2CH_3$

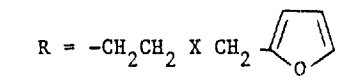


2a. R = $-CH_2CH_2OCH_3$

2b. R = $-CH_2CH_2SCH_3$

2c. R = $-CH_2CH_2SOCH_3$

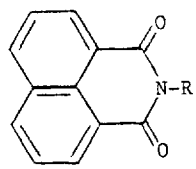
2d. R = $-CH_2CH_2SO_2CH_3$



1e. X = S

1f. X = SO

1g. X = $SO_2$

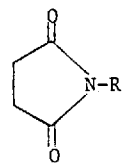


3a. R = $-CH_2CH_2OCH_3$

3b. R = $-CH_2CH_2SCH_3$

3c. R = $-CH_2-CH_2SOCH_3$

3d. R = $-CH_2CH_2SO_2CH_3$

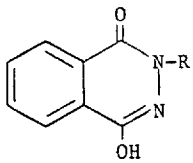


4a. R = $-CH_2CH_2OCH_3$

4b. R = $-CH_2CH_2SCH_3$

4c. R = $-CH_2CH_2SOCH_3$

4d. R = $-CH_2CH_2SO_2CH_3$

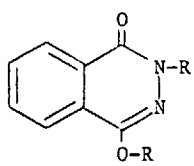


5a. R = $-CH_2CH_2OCH_3$

5b. R = $-CH_2CH_2SCH_3$

5c. R = $-CH_2CH_2SOCH_3$

5d. R = $-CH_2CH_2SO_2CH_3$



6a. R = $-CH_2CH_2OCH_3$

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ethyl methyl ether or 2-chloroethyl methyl sulfide, utilizing Gabriel-type reaction conditions, yielded **1a, 1b, 2a, 2b, 3a, 3b,** and **5b** (see structures; Table I). Compound **5a** was isolated as a byproduct in the alkylation of phthalhydrazide to yield **6a**. Compounds **4a** and **4b** were obtained by reaction of 2-methoxyethylamine and 2-(methylthio)ethylamine with succinic anhydride. Compound **1e** was obtained by the alkylation of furfuryl mercaptan with *N*-(2-bromoethyl)phthalimide. Oxidation of the appropriate sulfide with one or two molar equivalents of hydrogen peroxide yielded the corresponding sulfoxides (**1c, 1f, 2c, 3c, 4c,** and **5c**) and sulfones (**1d, 1g, 2d, 3d, 4d,** and **5d**), respectively.

**Hypolipidemic Activity**—The alkyl ether, thioether, sulfoxide, and sulfone derivatives of phthalimide, saccharin, 1,8-naphthalimide, succinimide, and 2,3-dihydrophthalazine-1,4-dione afforded significant hypolipidemic activity in $CF_1$ mice after 16 d of administration at an ip dose of 20 mg/kg/d (Table II). The alkyl ethers **1a** and **2a** in the phthalimide and saccharin series were not as active as the parent compounds [i.e., phthalimide (**1**) and saccharin (**2**)]. However, in the 1,8-naphthalimide and succinimide series, the respective alkyl ethers **3a** and **4a** were more active as hypolipidemic agents than 1,8-naphthalimide (**3**) and succinimide (**4**). The alkyl ether **5a** in the 2,3-dihydrophthalazine-1,4-dione series was approximately equal to its parent in hypocholesterolemic activity (i.e., 52% versus 49% of control levels), whereas the alkyl ether afforded better hypotriglyceridemic activity (i.e., 46% of control versus 57% for the parent compound). Examination of the thioether, sulfoxide, and sulfone derivatives of each series demonstrated a similar pattern of activity. Phthalimide and saccharin *N*-substituted derivatives afforded little improvement in hypolipidemic activity over their respective parent compounds, whereas in the succinimide and 1,8-naphthalimide series, improvement in activity was noted with the sulfone derivative. The sulfone (**3d**) of the 1,8-naphthalimide series lowered serum cholesterol levels 44% and serum triglyceride levels 53% after 16 d of drug administration. The thioether (**4b**) of the succinimide series lowered serum cholesterol of mice 54% and serum triglyceride levels 41% after 16 d of administration. These substitutions afforded better activity than other *N*-substitutions containing the sulfur atom in these two cyclic imide series. The sulfoxide (**5c**) of 2,3-dihydrophthalazine-1,4-dione demonstrated 50% reduction of serum cholesterol levels, but only 21% reduction of serum triglyceride levels. Selected derivatives (e.g., **3a** and **3c**) were tested in diet-induced hyperlipidemic mice. After 12 d of diet, administration of these agents at a dose of 20 mg/kg/d was commenced, and 50% or greater reductions in serum lipids were achieved after 12 d (Table III).

Examination of selected derivatives (**3a, 3c, 4b,** and **4c**) in Sprague-Dawley rats showed that **3a** and **4c** resulted in a >25% and **3c** and **4b** afforded a >33% reduction of serum cholesterol levels after 14 d of oral drug administration (Table III). The serum triglyceride levels were lowered >50% by **3a** and **3c** and 40% by **4b** and **4c** after 14 d of oral drug administration. Only **4c** afforded a significant reduction in daily food consumption. None of the derivatives afforded significant reductions in total body weight over the 14-d period of drug administration to rats.

The same derivatives were examined for their ability to lower tissue lipids in Sprague-Dawley rats after 14 d of administration (Table IV). Compounds **3a, 3c,** and **4c** reduced liver cholesterol, and aorta cholesterol was reduced by **3c, 4b,** and **4c**. Liver triglyceride levels were reduced by **3a, 3c,** and **4c**, and small intestine triglyceride levels were reduced by **3a** and **4b**. Neutral lipids were reduced in the liver by **4b** and **4c**, and in the small intestine by **4c**. Phospholipids were reduced in the aorta tissue by **3c**. Fecal excretion of lipids was elevated; that is, cholesterol excretion was increased by **3a, 4b,** and **4c**, fecal triglycerides were elevated by **3a** and **3c**, but reduced by **4b** and **4c**, and fecal protein was decreased by **3c, 4b,** and **4c** administration.

Serum lipoproteins from Sprague-Dawley rats after 14 d of dosing showed a reduction in chylomicron cholesterol content

**Table I—Chemical Data for Hypolipidemic Compounds**[a]

| Compound | Method | mp, bp, °C | Yield, % | Recrystallization (Chromatography) Solvent | Formula |
|---|---|---|---|---|---|
| **1a** | A | 149–150 | 44 | AcOEt | $C_{11}H_{11}NO_3$ |
| **1b** | A | 54–55[b] | 40 | Hexane | $C_{11}H_{11}NO_2S$ |
| **1c** | B | 147–148 | 74 | 2% MeOH:$CH_2Cl_2$ | $C_{11}H_{11}NO_3S$ |
| **1d** | C | 174–176[c] | 46[e] | EtOH:$H_2O$ | $C_{11}H_{11}NO_4$ |
| **1e** | — | 62–63 | 87[e] | MeOH | $C_{15}H_{13}NO_3S$ |
| **1f** | B | 114–126 | 53[e] | MeOH | $C_{15}H_{13}NO_4S$ |
| **1g** | C | 126–128 | 41 | MeOH | $C_{15}H_{13}NO_5S$ |
| **2a** | A | 92–93 | 39 | ($CH_2Cl_2$) | $C_{10}H_{11}NO_4S$ |
| **2b** | A | 71–72 | 64 | AcOEt:hexane | $C_{10}H_{11}NO_3S_2$ |
| **2c** | B | 127–128 | 61 | AcOEt | $C_{10}H_{11}NO_4S_2$ |
| **2d** | C | 173–174 | 87 | —[g] | $C_{10}H_{11}NO_5S_2$ |
| **3a** | A | 120–121 | 50 | iPrOH | $C_{15}H_{13}NO_3$ |
| **3b** | A | 161–162 | 98[e] | iPrOH | $C_{15}H_{13}NO_2S$ |
| **3c** | B | 180–182 | 82[e] | EtOH | $C_{15}H_{13}NO_3S$ |
| **3d** | C | 239–242 | 99[e] | EtOH:$CH_2Cl_2$ | $C_{15}H_{13}NO_4S$ |
| **4a** | — | 125–126/1.5 mm Hg | 31 | — | $C_7H_{11}NO_3$ |
| **4b** | — | 58–60[d] | 60[e] | AcOEt:hexane | $C_7H_{11}NO_2S$ |
| **4c** | B | 116–118 | 51 | AcOEt:hexane | $C_7H_{11}NO_3S$ |
| **4d** | C | 120–121 | 65 | AcOEt:hexane | $C_7H_{11}N_4S$ |
| **5a** | — | 146–148 | 19[f] | Toluene | $C_{11}H_{12}N_2O_3$ |
| **5b** | A | 151–153 | 43[e] | AcOEt | $C_{11}H_{12}N_2O_2S$ |
| **5c** | B | 199–200 | 97[e] | $CH_2Cl_2$ | $C_{11}H_{12}N_2O_3S$ |
| **5d** | C | 235–236 | 64 | MeOH | $C_{11}H_{12}N_2O_4S$ |
| **6a** | — | 54–56 | 13[e] | Toluene:hexane | $C_{14}H_{18}N_2O_4S$ |

[a] All compounds were analyzed for C,H,N, and S (if present), and were within 0.4% of the theoretical values. [b] Lit. mp 56 °C (ref 17). [c] Lit. mp 174–176 °C (ref 19). [d] Lit. mp 58–59 °C (ref 20). [e] Represents crude yield. [f] Isolated as a byproduct in the preparation of **6a**. [g] Precipitated from reaction, washed with $H_2O$.

**Table II—Effects of Intraperitoneal Administration of Cyclic Imido Alkyl Ethers, Thioethers, Sulfoxide, and Sulfones on Serum Lipids of Mice[a]**

| Compound | Percent Control (X ± SD) | | |
|---|---|---|---|
| | Serum Cholesterol | | Serum Triglyceride |
| | Day 9 | Day 16 | Day 16 |
| 1 (Phthalimide) | 63 ± 8[b] | 57 ± 7[b] | 44 ± 8[b] |
| 1a | 79 ± 7[b] | 71 ± 5[b] | 61 ± 7[b] |
| 1b | 73 ± 7[b] | 69 ± 6[b] | 56 ± 6[b] |
| 1c | 87 ± 6 | 66 ± 6[b] | 61 ± 5[b] |
| 1d | 80 ± 6[b] | 77 ± 5[b] | 63 ± 5[b] |
| 1e | 77 ± 7[b] | 57 ± 5[b] | 69 ± 5[b] |
| 1f | 78 ± 5[b] | 61 ± 5[b] | 78 ± 6[b] |
| 1g | 64 ± 6[b] | 58 ± 6[b] | 78 ± 6[b] |
| 2 (Saccharin) | 68 ± 11[b] | 67 ± 10[b] | 51 ± 16[b] |
| 2a | 76 ± 8[b] | 70 ± 4[b] | 67 ± 6[b] |
| 2b | 76 ± 8[b] | 67 ± 5[b] | 61 ± 5[b] |
| 2c | 78 ± 7[b] | 75 ± 5[b] | 70 ± 5[b] |
| 2d | 83 ± 6 | 73 ± 5[b] | 69 ± 6[b] |
| 3 (1,8-Naphthalimide) | 81 ± 6[b] | 61 ± 7[b] | 87 ± 12 |
| 3a | 52 ± 6[b] | 49 ± 6[b] | 42 ± 5[b] |
| 3b | 52 ± 6[b] | 57 ± 7[b] | 59 ± 6[b] |
| 3c | 55 ± 5[b] | 51 ± 6[b] | 53 ± 6[b] |
| 3d | 55 ± 5[b] | 56 ± 5[b] | 47 ± 5[b] |
| 4 (Succinimide) | 78 ± 9[b] | 73 ± 12[b] | 68 ± 7[b] |
| 4a | 71 ± 6[b] | 59 ± 7[b] | 57 ± 5[b] |
| 4b | 56 ± 7[b] | 46 ± 4[b] | 59 ± 6[b] |
| 4c | 57 ± 7[b] | 55 ± 5[b] | 61 ± 6[b] |
| 4d | 67 ± 5[b] | 58 ± 5[b] | 67 ± 5[b] |
| 5 (2,3-Dihydrophthalazine-1,4-dione) | 53 ± 4[b] | 49 ± 5[b] | 57 ± 4[b] |
| 5a | 59 ± 5[b] | 52 ± 4[b] | 46 ± 4[b] |
| 5b | 87 ± 7 | 57 ± 5[b] | 62 ± 5[b] |
| 5c | 72 ± 6[b] | 50 ± 4[b] | 79 ± 5[b] |
| 5d | 73 ± 6[b] | 69 ± 7[b] | 60 ± 4[b] |
| 6a | 87 ± 7[b] | 67 ± 6[b] | 62 ± 4[b] |
| Clofibrate (150 mg/kg) | 88 ± 4 | 87 ± 5 | 75 ± 5[b] |
| 1% Carboxymethyl cellulose | 100 ± 5 | 100 ± 6 | 100 ± 6 |
| Control value | 128 mg% | 125 mg% | 137 mg% |

[a] Dose of 20 mg/kg/d; n = 6. [b] $p \leq 0.001$.

by **3a**, **3c**, and **4b**, and VLDL and LDL cholesterol content by all four compounds (Table V). The HDL cholesterol content was reduced 49% by **3a**, but was elevated 64% by **4b** and 28% by **4c**. The triglyceride content of VLDL was reduced by **3a** and **4b**, but triglycerides in the LDL and HDL fractions were generally increased by the four compounds. The LDL neutral lipids were decreased by **3a** and **3c**, and HDL neutral lipids were reduced by **3a**, **3c**, and **4b**. The LDL phospholipids were elevated by all four compounds and HDL phospholipid content was reduced by **3a** and elevated by **4b** and **4c**. The LDL protein control was reduced by all compounds and HDL protein was reduced by **3c**.

Examination of in vitro effects of the drugs on enzyme activity of $CF_1$ mouse liver (Table VI) showed that **3a** and **3c** reduced ATP-dependent citrate lyase, acetyl CoA synthetase, HMG CoA reductase activity, acyl CoA cholesterol acyl transferase, *sn*-glycerol-3-phosphate acyl transferase, phosphatidylate phosphohydrolase, and heparin-induced lipoprotein lipase activities in a concentration-dependent manner. Cholesterol 7$\alpha$-hydrolase activity was elevated at all three concentrations of **3a** and **3c** employed. Mitochondrial citrate exchange and acetyl CoA carboxylase activity were not affected by **3a** and **3c** at concentrations of 25, 50, and 100 $\mu$M.

In situ intestinal rat loop studies demonstrated that **3a** and **3c** both blocked the absorption of cholic acid from the lumen of the intestine; the cholic acid, therefore, accumulated over time. Compound **3a** effectively blocked cholesterol absorption from the rat intestine; however, **3c** demonstrated significant reduction of cholesterol absorption from the intestine only after 120 min of incubation (Figure 1).

## Discussion

The alkyl ethers, thioethers, sulfoxide and sulfone derivatives of phthalimide, saccharin, 1,8-naphthalimide, succinimide, and 2,3-dihydrophthalazine-1,4-dione proved to be potent hypolipidemic agents in rodents. These *N*-substitutions in the case of 1,8-naphthalimide and succinimide proved to be more active than the unsubstituted analogues in reducing serum cholesterol and triglyceride levels after 16 d of ip dosing at 20 mg/kg/d in mice. Apparently, ether linkages, as well as thioether, sulfone, and sulfoxide substituents, afforded hypolipidemic activity similar to *N*-butyl, *N*-butanone, and *N*-propionic acid substitutions. Whereas these *N*-substitutions did not particularly improve the hypolipidemic activity of phthalimide, saccharin, or 2,3-dihydrophthalazine-1,4-dione, the alkyl ethers of 1,8-naphthalimide and succinimide possessed more hypolipidemic activity than any other *N*-substitution previously utilized for these respective series. Selected derivatives of these two classes of cyclic imides were effective in lowering serum lipids in rats when administered orally. The effects of these derivatives on serum lipoprotein levels were encouraging. After 2 weeks of administration, the VLDL and LDL cholesterol content was reduced significantly. Since Apo B-containing lipoprotein fractions are supposedly responsible for cholesterol deposition into atherosclerotic plaques,[10] reductions in VLDL and LDL would be advantageous clinically. The succinimide derivatives were superior to the 1,8-naphthalimide,[11] saccharin,[12] and phthalimide[13] derivatives with regard to elevating HDL cholesterol content (e.g., the thioether afforded a 64% increase after 14 d of dosing). Elevation in HDL cholesterol content is important since HDL removes cholesterol from peripheral plaques and returns it to the liver for excretion.[14] In hyperlipidemic disease states, the LDL cholesterol is high and the HDL cholesterol level is low. Drug therapy ideally should reverse this ratio to be effective. Clinical studies have shown that commercially available agents in general do not significantly elevate HDL cholesterol content; for example, clofibrate afforded a 4–16% increase in HDL cholesterol content, whereas probucol causes no increase.[15]

High HDL cholesterol and low LDL cholesterol content has been found to protect against myocardial infarction in humans.[16] Since a number of these derivatives demonstrated promise in altering this LDL:HDL ratio in a favorable direction, they warrant further investigation as hypolipidemic agents.

## Experimental Section

Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. The NMR spectra were determined on a Varian EM-360A spectrometer using tetramethylsilane as an internal standard and deuteriochloroform or DMSO-$d_6$ as the solvent. Infrared spectra were determined on a Beckman Acculab 4 spectrophotometer using the potassium bromide technique. Reported yields were not optimized. Mass spectra were performed on a Finnigan 4021 spectrometer. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. General synthetic methods and selected examples are detailed below.

**Method A**—Phthalimide, saccharin, or 1,8-naphthalimide (0.05–0.10 mol) was dissolved in dry DMF (100–400 mL) and an equimolar amount of sodium ethoxide (**3a**) or sodium hydride (**3b**) was added at room temperature. Stirring was continued for 15 min and an equi-

**Table III—Effects of Orally Administered Cyclic Imido Alkyl Ethers, Thioethers, Sulfoxides, and Sulfones on Serum Lipid Levels of Sprague-Dawley Rats and $CF_1$ Mice[a]**

| Compound | Body Weight Day 14 | Percent of Control (X ± SD) | | | | Food Consumption, g/d |
|---|---|---|---|---|---|---|
| | | Serum Cholesterol | | Serum Triglyceride | | |
| | | Day 7 | Day 14 | Day 7 | Day 14 | |
| Normal Rats | | | | | | |
| **3a** (20 mg/kg/d) | 116.1 | 84 ± 4 | 73 ± 4[b] | 92 ± 7 | 43 ± 4[b] | 18.1 |
| **3c** (20 mg/kg/d) | 132.4 | 63 ± 4[b] | 64 ± 6[b] | 76 ± 5[b] | 49 ± 4[b] | 26.4 |
| **4b** (10 mg/kg/d) | 125.7 | 82 ± 4[b] | 66 ± 5[b] | 64 ± 5[b] | 59 ± 5[b] | 16.3 |
| **4c** (10 mg/kg/d) | 115.1 | 89 ± 5 | 74 ± 5[b] | 64 ± 6[b] | 60 ± 5[b] | 14.8 |
| 1% Carboxymethyl cellulose | 128.3 | 100 ± 3 | 100 ± 6 | 100 ± 6 | 100 ± 7 | 20.8 |
| Control value, mg% | — | 78 | 79 | 128 | 125 | — |
| Hyperlipidemic-Induced Mice | | | | | | |
| 1% Carboxymethyl cellulose | — | 100 ± 5 | | 100 ± 6 | | — |
| **3a** (20 mg/kg/d) | — | 50 ± 5[b] | | 45 ± 5[b] | | — |
| **3c** (20 mg/kg/d) | — | 47 ± 4[b] | | 46 ± 7[b] | | — |
| Control value, mg% | — | 375 | | 367 | | — |

[a] There were six animals in each group (normal and hyperlipidemic). [b] $p \leq 0.001$.

**Table IV—Effects of Orally Administered Cyclic Imide Alkyl Ethers, Thioethers, Sulfoxides, and Sulfones on Lipid Levels of Sprague-Dawley Rat Tissue after 14 Days of Administration**

| Tissue | Percent of control (X ± SD) | | | | | |
|---|---|---|---|---|---|---|
| | Lipid extracted, mg | Cholesterol | Triglyceride | Neutral Lipids | Phospholipids | Protein |
| Liver | | | | | | |
| Control | 100 ± 6[a] | 100 ± 7[b] | 100 ± 8[c] | 100 ± 7[d] | 100 ± 5[e] | 100 ± 5[f] |
| **3a** | 83 ± 5 | 67 ± 3[y] | 63 ± 5[y] | 115 ± 7 | 89 ± 5 | 113 ± 6 |
| **3c** | 89 ± 6 | 83 ± 4 | 75 ± 5[y] | 102 ± 7 | 122 ± 7 | 112 ± 6 |
| **4b** | 90 ± 6 | 107 ± 5 | 102 ± 7 | 84 ± 6 | 120 ± 7 | 102 ± 6 |
| **4c** | 82 ± 5 | 89 ± 5 | 89 ± 7 | 76 ± 6[y] | 93 ± 6 | 105 ± 4 |
| Small Intestine | | | | | | |
| Control | 100 ± 8[g] | 100 ± 6[h] | 100 ± 7[i] | 100 ± 8[j] | 100 ± 6[k] | 100 ± 6[l] |
| **3a** | 90 ± 7 | 92 ± 7 | 63 ± 7[y] | 103 ± 7 | 178 ± 7 | 87 ± 6 |
| **3c** | 128 ± 6 | 124 ± 8 | 152 ± 8 | 100 ± 7 | 100 ± 7 | 93 ± 5 |
| **4b** | 75 ± 6[y] | 102 ± 7 | 63 ± 6[y] | 105 ± 8 | 85 ± 5 | 128 ± 7 |
| **4c** | 102 ± 8 | 84 ± 6 | 92 ± 5 | 73 ± 7 | 82 ± 5 | 99 ± 5 |
| Aorta | | | | | | |
| Control | 100 ± 5[m] | 100 ± 6[n] | 100 ± 4[o] | 100 ± 9[p] | 100 ± 8[q] | 100 ± 7[r] |
| **3a** | 99 ± 5 | 100 ± 7 | 98 ± 5 | 85 ± 7 | 135 ± 7 | 93 ± 6 |
| **3c** | 45 ± 3[y] | 78 ± 6[y] | 87 ± 5 | 90 ± 6 | 68 ± 3[y] | 80 ± 6 |
| **4b** | 94 ± 6 | 76 ± 6[y] | 102 ± 6 | 112 ± 5 | 119 ± 8 | 102 ± 7 |
| **4c** | 82 ± 5 | 77 ± 6[y] | 91 ± 5 | 107 ± 7 | 111 ± 9 | 92 ± 6 |
| Feces | | | | | | |
| Control | 100 ± 7[s] | 100 ± 8[t] | 100 ± 4[u] | 100 ± 7[v] | 100 ± 8[w] | 100 ± 6[x] |
| **3a** | 124 ± 7[y] | 116 ± 7 | 251 ± 6[y] | 95 ± 8 | 112 ± 8 | 115 ± 6 |
| **3c** | 116 ± 6 | 101 ± 7 | 258 ± 7[y] | 96 ± 7 | 84 ± 8 | 87 ± 5 |
| **4b** | 78 ± 5 | 131 ± 8 | 82 ± 5 | 114 ± 7 | 97 ± 7 | 83 ± 5 |
| **4c** | 79 ± 5 | 112 ± 7 | 62 ± 6 | 108 ± 8 | 102 ± 7 | 71 ± 6[y] |

[a] 50.5 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] 12.02 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 protein/g wet tissue. [y] $p < 0.001$.

molar amount of 2-chloroethyl methyl ether or 2-chloroethyl methyl sulfide was added. The mixture was heated to ~100 °C for 2–15 h, the solvent was evaporated under reduced pressure, and the residue was suspended in $CH_2Cl_2$ and washed with 1.0 M NaOH. After drying over $MgSO_4$ and evaporation of the solvent under reduced pressure, the crude products were purified as indicated in Table I.

*2-[2-(Methylthio)ethyl]-1H-isoindole-1,3(2H)dione (**1b**)*— Recrystallization from *n*-hexane, mp 54–55 °C (lit.[17] mp 56 °C); IR (KBr): 3455, 2985, 2940, 1770, 1705, 1600, 1390, 1240, 1085, 995, 850, 755, and 705 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.15 (s, 3H, S-$CH_3$), 2.70 (t, 2H, $CH_2S$), 3.80 (t, 2H, N-$CH_2$), and 7.50–7.85 ppm (m, 4H, aromatic).

*Anal.*—Calc. for $C_{11}H_{11}NO_2S$: C,H,N,S.

**Method B**—Hydrogen peroxide (30% in $H_2O_2$, 0.003–0.2 mol) was added to a solution of glacial acetic acid (15–50 mL) containing 1.0–2.0 mL of concentrated $H_2SO_4$ at 4 °C. (Note: $H_2SO_4$ was not utilized in the preparation of **1f** and **4c**). Equimolar amounts of the appropriate sulfide were slowly added and the stirred solution was allowed to warm to room temperature overnight. Evaporation of the

**Table V—Effect of Orally Administered Cyclic Imido Alkyl ethers, Thioethers, Sulfoxides, and Sulfones on Serum Lipoproteins of Sprague-Dawley Rats after 14 Days of Administration**

| Compound | Percent of Control (X ± SD) | | | | |
|---|---|---|---|---|---|
| | Cholesterol | Triglyceride | Neutral Lipids | Phospholipids | Protein |
| Chylomicrons | | | | | |
| Control | 100 ± 6[a] | 100 ± 7[b] | 100 ± 6[c] | 100 ± 8[d] | 100 ± 6[e] |
| **3a** | 77 ± 5[u] | 96 ± 7 | 94 ± 8 | 283 ± 9[u] | 97 ± 5 |
| **3c** | 69 ± 4[u] | 96 ± 6 | 42 ± 5 | 240 ± 8[u] | 104 ± 7 |
| **4b** | 89 ± 5 | 98 ± 7 | 122 ± 6 | 74 ± 5[u] | 99 ± 6 |
| **4c** | 97 ± 5 | 99 ± 5 | 134 ± 7 | 94 ± 6 | 100 ± 6 |
| VLDL | | | | | |
| Control | 100 ± 5[f] | 100 ± 6[g] | 100 ± 6[h] | 100 ± 6[i] | 100 ± 6[j] |
| **3a** | 33 ± 5[u] | 53 ± 5[u] | 130 ± 7 | 46 ± 4[u] | 104 ± 6 |
| 3c | 43 ± 4[u] | 101 ± 6 | 113 ± 7 | 95 ± 5 | 99 ± 5 |
| **4b** | 62 ± 6[u] | 56 ± 7[u] | 89 ± 6 | 92 ± 6 | 123 ± 5[u] |
| **4c** | 79 ± 4[u] | 94 ± 6 | 103 ± 7 | 103 ± 4 | 88 ± 6 |
| LDL | | | | | |
| Control | 100 ± 6[k] | 100 ± 7[l] | 100 ± 7[m] | 100 ± 7[n] | 100 ± 7[o] |
| **3a** | 48 ± 5[u] | 234 ± 8[u] | 65 ± 5[u] | 245 ± 8 | 64 ± 5[u] |
| **3c** | 47 ± 5[u] | 125 ± 7[u] | 61 ± 6[u] | 171 ± 8 | 84 ± 5 |
| **4b** | 72 ± 4[u] | 96 ± 6 | 110 ± 7 | 182 ± 9 | 61 ± 4[u] |
| **4c** | 80 ± 6[u] | 122 ± 7 | 114 ± 8 | 115 ± 5 | 66 ± 5[u] |
| HDL | | | | | |
| Control | 100 ± 6[p] | 100 ± 5[q,u] | 100 ± 5[r] | 100 ± 8[s] | 100 ± 5[t] |
| **3a** | 51 ± 4[u] | 165 ± 6[u] | 89 ± 5 | 50 ± 5[u] | 92 ± 5 |
| **3c** | 107 ± 6 | 125 ± 6[u] | 32 ± 4[u] | 91 ± 7 | 72 ± 6[u] |
| **4b** | 164 ± 7[u] | 310 ± 7[u] | 89 ± 5 | 176 ± 7 | 99 ± 5 |
| **4c** | 128 ± 7[u] | 181 ± 6[u] | 95 ± 4 | 121 ± 6 | 100 ± 5 |

[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. [u] $p \leq 0.001$.

**Table VI—In Vitro Effects of Hypolipidemic Agents 3a and 3c on Enzyme Activities of Lipid Metabolism in $CF_1$ Mouse Liver**

| Enzyme | Control | | | 3a | | | 3c | | |
|---|---|---|---|---|---|---|---|---|---|
| | 25 μM | 50 μM | 100 μM | 25 μM | 50 μM | 100 μM | 25 μM | 50 μM | 100 μM |
| Mitochondrial citrate exchange | 100 ± 6 | 100 ± 6 | 100 ± 6[a] | 147 ± 7[k] | 108 ± 6 | 93 ± 6 | 92 ± 5 | 108 ± 7 | 90 ± 5 |
| ATP-Dependent citrate lyase | 100 ± 5 | 100 ± 5 | 100 ± 5[b] | 96 ± 5 | 82 ± 6 | 79 ± 6[k] | 97 ± 4[k] | 95 ± 6 | 64 ± 7 |
| Acetyl CoA synthetase | 100 ± 7 | 100 ± 7 | 100 ± 7[c] | 96 ± 6 | 92 ± 7 | 86 ± 8 | 80 ± 6 | 78 ± 6[k] | 70 ± 6[k] |
| HMG CoA reductase | 100 ± 8 | 100 ± 8 | 100 ± 8[d] | 133 ± 8[k] | 93 ± 6 | 68 ± 5[k] | 100 ± 7 | 79 ± 5[k] | 72 ± 6[k] |
| Cholesterol-7α-hydroxylase | 100 ± 6 | 100 ± 6 | 100 ± 6[e] | 139 ± 8[k] | 210 ± 8[k] | 127 ± 8 | 221 ± 9 | 157 ± 7[k] | 127 ± 6[k] |
| Acyl CoA cholesterol acyltransferase | 100 ± 6 | 100 ± 6 | 100 ± 6[f] | 108 ± 6 | 102 ± 4 | 65 ± 5[k] | 78 ± 7[k] | 73 ± 5[k] | 54 ± 5[k] |
| Acetyl CoA carboxylase | 100 ± 8 | 100 ± 8 | 100 ± 8[g] | 104 ± 7 | 102 ± 7 | 109 ± 7 | 101 ± 5 | 106 ± 6 | 98 ± 7 |
| *sn*-Glycerol-3-phosphate acyltransferase | 100 ± 7 | 100 ± 7 | 100 ± 7[h] | 80 ± 6[k] | 63 ± 4[k] | 31 ± 3[k] | 82 ± 5[k] | 58 ± 6[k] | 53 ± 5[k] |
| Phosphatidylate phosphohydrolase | 100 ± 6 | 100 ± 6 | 100 ± 6[i] | 97 ± 5 | 86 ± 6 | 75 ± 5[k] | 108 ± 7 | 93 ± 6 | 78 ± 5[k] |
| Heparin-induced lipoprotein lipase | 100 ± 7 | 100 ± 7 | 100 ± 7[j] | 79 ± 6[k] | 65 ± 5[k] | 62 ± 6[k] | 61 ± 5[k] | 44 ± 3[k] | 36 ± 3[k] |

[a] 30.8% exchange of mitochondrial citrate. [b] 30.5 mg citrate hydrolyzed/g wet tissue. [c] 28.5 mg acetyl CoA formed/g wet tissue. [d] 384,900 dpm cholesterol formed/g wet tissue. [e] 4808 dpm/mg of microsomal protein. [f] 224,000 dpm/mg of microsomal protein. [g] 32,010 dpm/g wet tissue. [h] 537.800 dpm/g wet tissue. [i] 16.7 μg Pi released/g wet tissue. [j] 278,582 dpm/g wet tissue. [k] $p \leq 0.001$.

solvent under reduced pressure yielded the crude sulfoxide which was purified as indicated in Table I.

*2-[2-(Methylsulfinyl)ethyl]-1H-isoindole-1,3(2H)dione (1c)*—Chromatography ($SiO_2$-2% MeOH:$CH_2Cl_2$); IR (KBr): 3455, 2990, 1768, 1700, 1600, 1455, 1425, 1380, 1025, 955, 910, 845, and 705 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 2.65 (s, 3H, $SOCH_3$), 3.18 (t, 2H, $CH_2SO$), 4.13 (t, 2H, N-$CH_2$), and 7.55–7.90 ppm (m, 4H, aromatic).

*Anal.*—Calc. for $C_{11}H_{11}NO_3S$: C,H,N,S.

**Method C**—Hydrogen peroxide (30% in $H_2O_2$, 0.006–0.3 mol) was added to a solution of glacial acetic acid (20–30 mL) containing 1.0–2.0 mL of concentrated $H_2SO_4$ at 4 °C. (Note: $H_2SO_4$ was not utilized in the preparation of **1g** and **4d**.) One-half equimolar amounts of the appropriate sulfide were slowly added and the stirred solution was allowed to warm to room temperature overnight. Precipitation or evaporation of the solvent under reduced pressure yielded the crude sulfone which was purified as indicated in Table I.

*2-[2-(2-Furanylmethyl)sulfonyl]ethyl-1H-isoindole-1,3(2H)dione (1g)*—Recrystallization from methanol; $^1H$ NMR ($CDCl_3$): δ 3.22 (t,

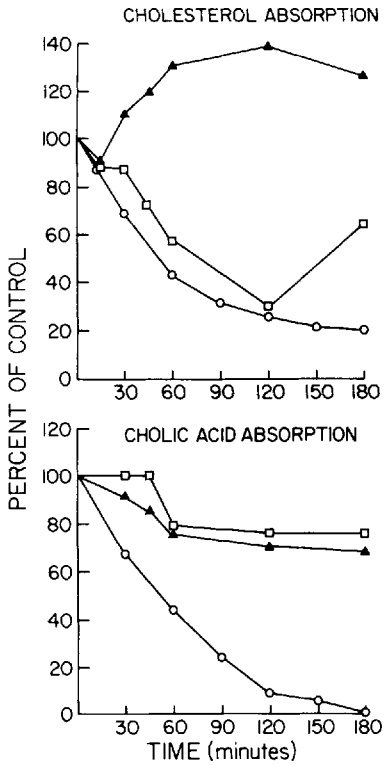


**Figure 1**—In situ absorption of cholesterol (**A**) and cholic acid (**B**) from the small intestine of Sprague Dawley rats over a 3-h period. Key: (○—○) control, 1% cmc; (△—△) **3a**; (□—□) **3c**.

2H, $CH_2CH_2SO_2$), 4.10 (t, 2H, N-$CH_2$), 4.34 (s, 2H, $SO_2CH_2$), 6.18–6.51 (m, 2H, β,β′-furan), 7.21–7.38 (m, 1H, α-furan), and 7.43–7.89 ppm (m, 4H, phth. aromatic).

*Anal.*—Calc. for $C_{15}H_{13}NO_5S$: C,H,N,S.

*2-[2-(Methylsulfonyl)ethyl]-1H-isoindole-1,3(2H)dione-(1d)*—Adapting the procedure of Truce and Wellisch,[18] 1.68 g (0.011 mol) of phthalimide was dissolved in 30 mL of refluxing ethanol. Triton B (2.5 g, 40% solution in MeOH) was added, followed by the slow addition of 2.0 mL (2.42 g, 0.023 mol) of methyl vinyl sulfone. The solution was heated under reflux for 7 h and cooled, and 1.73 g of precipitate was collected. Recrystallization from EtOH:$H_2O$ yielded 1.33 g (46%) of **1d**, mp 174–176 °C (lit.[19] mp 174–176 °C); $^1$H NMR ($CDCl_3$): δ 2.99 (s, 3H, $SO_2CH_3$), 3.38 (t, 2H, $CH_2SO_2$), 4.16 (t, 2H, $NCH_2$), and 7.51–7.97 ppm (m, 4H, aromatic).

*2-[2-(2-Furanylmethyl)thio]ethyl-1H-isoindole-1,3(2H)dione (1e)*—To a solution of furfuryl mercaptan (11.42 g, 0.1 mol) in 100 mL of anhydrous THF in an ice bath was added potassium *t*-butoxide (11.22 g, 0.1 mol) with stirring. Stirring was continued for 15 min at ambient temperature and the resulting pink suspension was again cooled in an ice bath while *N*-(2-bromoethyl)phthalimide (25.41 g, 0.1 mol) was added in portions over a 10-min period. The resulting suspension was stirred at ambient temperature for 1 h, then refluxed for 1.5 h. Upon cooling, the precipitate was removed by filtration, the solvent was evaporated under reduced pressure, and the residue was initially recrystallized from MeOH:$H_2O$ (3:1). The crude product (25.1 g, 87.4%) was recrystallized from MeOH to yield **1e**, mp 62–63 °C; IR (KBr): 3140, 3110, 3050, 2950, 1765, 1700, 1600, 1380, 1310, 1070, 970, 725, and 700 $cm^{-1}$; $^1$H NMR ($CDCl_3$): δ 2.77 (t, 2H,$CH_2$-$CH_2$-S), 3.75 (s, 2H, S-$CH_2$), 3.83 (t, 2H, N-$CH_2$), 6.08–6.24 (m, 2H, β,β′-furan), 7.18–7.28 (m, 1H, α-furan), and 7.50–7.90 ppm (m, 4H, phth. aromatic).

*Anal.*—Calc. for $C_{15}H_{13}NO_3S$: C,H,N,S.

*1-(2-Methoxyethyl)-2,5-pyrrolidinedione (4a) and 1-[2-(Methylthio)ethyl]-2,5-pyrrolidinedione (4b)*—Succinic anhydride (0.02–0.10 mol) was suspended in 100 mL of $CH_2Cl_2$ and an equimolar amount of 2-methoxyethylamine or 2-(methylthio)ethylamine was added with stirring overnight. The solvent was evaporated to yield a white solid, which was heated to 200–220 °C under argon for 1 h. High vacuum distillation of the resulting residues, followed by chromatography of **4b** ($SiO_2$:AcOEt) and recrystallization from AcOEt: hexane yielded the pure compounds.

*1-(2-Methoxyethyl)-2,5-pyrrolidinedione (4a)*—$^1$H NMR ($CDCl_3$): δ 2.70 (s, 4H, $CH_2CH_2CO$), and 3.19–3.82 ppm (m, 7H, $CH_2CH_2OCH_3$).

*Anal.*— Calc. for $C_7H_{11}NO_3$: C,H,N.

*1-[2-(Methylthio)ethyl]-2,5-pyrrolidinedione (4b)*—Melting point 58–60 °C, lit.[20] mp 58–59 °C; $^1$H NMR ($CDCl_3$): δ 2.07 (s, 3H, $SCH_3$), 2.45–2.88 (m, 6H, $CH_2CH_2CO$, $CH_2S$), and 3.66 ppm (t, 2H, N-$CH_2$); MS: $M^+$ = 173.

*2-(2-Methoxyethyl)-2,3-dihydrophthalazine-1,4-dione (5a) and 2-(2-Methoxyethyl)-4-(2-methoxyethoxy)phthalazine-1(2H)one (6a)*—Phthalhydrazide (8.1 g, 0.05 mol) was dissolved in 250 mL of dry DMF and 4.0 g of sodium hydride (60% dispersion in mineral oil) was added with stirring. The resulting suspension was heated to reflux, 10 mL (10.35 g, 0.11 mol) of 2-chloroethyl methyl ether was added, and refluxing was continued overnight. Upon cooling, the solvent was removed under reduced pressure, the residue was suspended in 200 mL of $CH_2Cl_2$, washed with 2 × 200 mL of 1.0 M NaOH and 200 mL of $H_2O$, and dried over $Na_2SO_4$, and the solvent was removed under reduced pressure to yield 7.01 g (50%) of crude **6a**. Recrystallization from toluene yielded only pure **5a** (2.10 g, 19%), mp 146–148 °C; $^1$H NMR ($CDCl_3$): δ 3.42 (s, 3H, $OCH_3$), 3.80 (t, 2H, $CH_2O$), 4.43 (t, 2H, N-$CH_2$), and 7.54–8.48 ppm (m, 4H, aromatic).

*Anal.*—Calc. for $C_{11}H_{12}N_2O_3$: C,H,N.

Addition of hexane to the filtrate yielded 1.80 g (13%) of **6a**, mp 50–52 °C. Chromatography ($SiO_2$, 2% MeOH, $CH_2Cl_2$) and subsequent recrystallization from hexane afforded an analytical sample of **6a**, mp 54–56 °C; $^1$H NMR ($CDCl_3$): δ, 3.32, 3.38 (two singlets, 6Hs, $OCH_3$), 3.57–3.90 (m, 4H, $CH_2O$), 4.07–4.52 (m, 4H, $NCH_2$, $OCH_2$), and 7.48–8.33 ppm (m, 4H, aromatic).

*Anal.*—Calc. for $C_{14}H_{18}N_2O_4$: C,H,N.

*2-[2-(Methylthio)ethyl]-2,3-dihydrophthalazine-1,4-dione (5b)*—Phthalhydrazide (24.32 g, 0.15 mol) was dissolved in 400 mL of dry DMF and 6.0 g of sodium hydride (60% dispersion in mineral oil) was added with stirring. 2-Chloroethyl methyl sulfide (12.76 g, 0.115 mol) was added and the resulting suspension was heated to reflux overnight. Upon cooling, the solvent was removed under reduced pressure, the residue was stirred into 300 mL of $H_2O$ and extracted with 3 × 300 mL of $CH_2Cl_2$. After drying over $MgSO_4$, the $CH_2Cl_2$ was removed under reduced pressure to yield 11.61 g (43%) of crude **5b**. Recrystallization from toluene, followed by chromatography ($SiO_2$: 2% MeOH:$CH_2Cl_2$) and recrystallization from ethyl acetate, yielded an analytical sample, mp 151–153 °C; $^1$H NMR ($CDCl_3$): δ 2.10 (s, 3H, $SCH_3$), 2.96 (t, 2H, $CH_2S$), 4.46 (t, 2H, N-$CH_2$), and 7.54–8.46 ppm (m, 4H, aromatic). *Anal.*—Calc. for $C_{11}H_{12}N_2O_2S$: C,H,N,S.

**Hypolipidemic Screens in Normal Rodents**—Test compounds (**1–6a**) were suspended in an aqueous 1% carboxymethylcellulose solution, homogenized, and administered ip to $CF_1$ male mice (~25 g) for 16 d. On days 9 and 16, blood was obtained by tail vein bleeding and the serum was separated by centrifugation for 3 min. Sprague-Dawley male rats (~350 g) were administered **3a**, **3c**, **4b**, or **4c** orally by intubation needle at a dose of 10 or 20 mg/kg/d. Blood was collected on days 9 and 14. The serum cholesterol levels were determined by a modification of the Liebermann–Burchard reaction.[21] 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.

**Testing in Hyperlipidemic Mice**—The $CF_1$ male mice (~25 g) were placed on a commercial diet[22] which produced a "hyperlipidemic" state. After the serum cholesterol and triglyceride levels were observed to be elevated, the mice were administered **3a** and **3c** at an ip dose of 20 mg/kg/d for an additional 12-d period while continuing the diet. Serum cholesterol and triglyceride levels were measured as previously described.

**Animal Weights and Food Intake**—Food consumption was determined daily as g food/rat/d for control rats and those treated orally with **3a** and **3c** at 20 mg/kg/d, and **4b** and **4c** at 10 mg/kg/d. Body weights were obtained during the experiments and expressed as a percentage of the animal's weight on day 0. After dosing for 14 d with the 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 **3a** and **3c**. The following enzyme activities were determined by following literature procedures: acetyl coenzyme A

synthetase;[23] adenosine triphosphate dependent citrate lyase;[24] mitochondrial citrate exchange;[25,26] cholesterol-7$\alpha$-hydroxylase;[27] 3-hydroxy-3-methylglutaryl coenzyme A reductase;[28,29] acetyl coenzyme A carboxylase activity;[30] cholesterol ester hydrolase;[31] *sn*-glycerol-3-phosphate acyl transferase activity;[32] phosphatidylate phosphohydrolase activity;[32] acyl CoA cholesterol acyl transferase;[34] and heparin-activated hepatic lipoprotein lipase.[35] Protein was determined for the cell extract appropriate for each enzyme assay by the technique of Lowry et al.[36]

**Liver, Small Intestine, and Fecal Lipid Extraction**—In Sprague-Dawley rats that had been administered **3a, 3c, 4b**, and **4c** for 14 d, the liver, small intestine, and fecal materials (24-h collection) were removed, extracted,[37,38] and analyzed for cholesterol levels,[21] triglyceride levels (BioDynamics/bmc Triglyceride Kit), neutral lipid content,[39] and phospholipid content.[40]

**Serum Lipoprotein Fractions**—Sprague-Dawley male rats (~300 g) were orally administered **3a, 3c, 4b**, and **4c** at a dose of 20 mg/kg/d. Blood was collected from the abdominal aorta vein and lipoprotein fractions were obtained by the method of Hatch and Lees[41] and Havel et al.,[42] as modified for the rat.[43] Each of the fractions was analyzed for cholesterol,[21] triglyceride, neutral lipids,[39] phospholipids,[40] and protein levels.[36]

**Cholesterol and Cholic Acid Absorption/Reabsorption from Gut**—The effects of the drugs on in situ intestinal absorption of cholesterol or cholic acid was determined by the method of Doluisio et al.[44] Sprague-Dawley male rats were anesthetized with phenobarbital and ketamine, and the duodenum intestinal loop was isolated. The drug (0.2 mL; 20 mg/kg), with 200 mg% cholesterol or cholic, was introduced into the lumen and the ends of the loop were tied off. Samples (50 $\mu$L) were removed from the lumen of the intestine every 15 min for the next 3 h and analyzed for cholesterol[21] or cholic acid[45] content. The disappearance of cholesterol or cholic acid from the inside of the loop was plotted (Figure 1).

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