Pharmaceutical Research, Vol. 4, No. 1, 1987

Research Article

Synthesis and Hypolipidemic Activity of 3-Imino-1-oxoisoindolines in Rodents

Akula R. K. Murthy,[1] Oi T. Wong,[1] David J. Reynolds,[1] and Iris H. Hall[1]

Received May 19, 1986; accepted September 2, 1986

A series of substituted 3-imino-1-oxoisoindolines derivatives demonstrated significant hypolipidemic activity, lowering both serum cholesterol and triglycerides levels after 16 days of dosing at 20 mg/kg/day ip in CF_1 mice. 2-Butyl-3-butylimino-1-oxoisoindoline lowered serum cholesterol levels 52% and serum triglyceride 42%. 2-Pentyl-3-imino-1-oxoisoindoline lowered serum cholesterol levels 42% and serum triglyceride 61%. These derivatives resulted in better activity than the parent compound, 3-imino-1-oxoisoindoline. These studies showed that compounds with N-alkyl substitution of nitrogen atoms in the ring and outside the ring possessed potent hypolipidemic activity at the low dose of 20 mg/kg/day ip in normolipidemic CF_1 mice. Studies with 2-butyl-3-butylimino-1-oxoisoinodine in rats showed that serum cholesterol was reduced 60% and serum triglyceride 43% after 14 days of dosing at 20 mg/kg/day, orally. Treatment with this agent lowered lipid levels in the liver and aorta tissue, with increases in lipid levels in the small intestine tissue. Higher levels of cholesterol and phospholipids were excreted in the feces of treated animals compared to the control. Cholesterol levels of the very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) fractions were reduced, whereas the HDL cholesterol levels were elevated significantly. This ratio of low-density lipoprotein (LDL) cholesterol:HDL cholesterol levels suggests that the agent may be effective in treating hyperlipidemic states in humans.

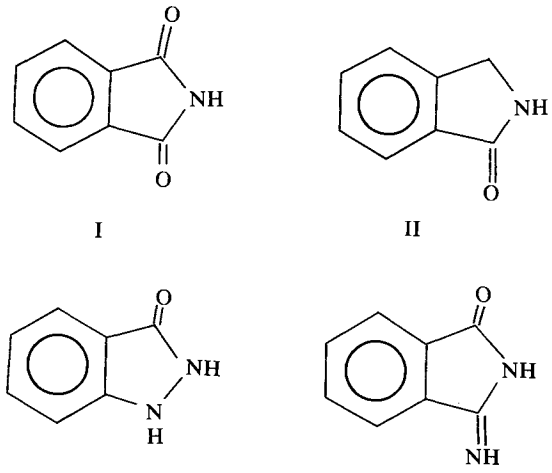
KEY WORDS: hypolipidemic agents; cholesterol; triglycerides; cyclic imides; 3-imino-1-oxoisoindolines; lipoproteins.

INTRODUCTION

Recent studies in our laboratory established that phthalimide (I) (1–3) (Scheme I), a five-membered cyclic imide, was a potent hypolipidemic agent, reducing both serum lipids significantly in rodents at a relatively low dose of 20 mg/kg/day, compared to clofibrate (150 mg/kg/day), a commercially available and widely used hypolipidemic agent. This modification of the imide ring by reducing one of the carbonyls led to phthalimidine (II) (4), which demonstrated poor activity. The introduction of a nitrogen endocyclic to the imide ring in place of one of the carbonyl groups resulted in indazolinone (III) (5), which exhibited moderate activity. The replacement of one of the carbonyl oxygens with a nitrogen afforded 3-iminophthalimidine (3-imino-1-oxoisoindoline) (6). This agent showed potent activity, reducing serum cholesterol by 40% and the triglyceride level by 37%, after 16 days of dosing at an optimum dose of 20 mg/kg/day ip (6). Further studies were undertaken to investigate a series of molecular modifications of 3-imino-1-oxoisoindoline (1) to determine which substitution afforded the maximum activity and to study their effects on lipid metabolism as hypolipidemic agents. Those results are described herein.

EXPERIMENTAL

All chemicals used as synthetic intermediates were purchased and used as obtained from the manufacturers. Melting points were obtained on a Thomas–Hoover melting-point apparatus and are uncorrected. Column chromatography was performed on silica gel 60 (70–230 mesh). ^{1}H-NMR spectra were recorded on JEOL 60-MHZ Fourier



Scheme I

[1] Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514.

0724-8741/87/0200-0021$05.00/0

transform spectrometer. Elemental analyses (Table I) were obtained from M.H.W. Laboratory, Phoenix, Arizona, and are correct within ±0.4% of theoretical values.

3-Imino-1-oxoisoindoline (1)

Utilizing the procedure of Byrne *et al.* (8), phthalimide (10.0 g; 0.07 mol) was suspended in ammonium hydroxide (60 ml) and stirred at room temperature for 24 hr. The white solid was collected and washed with cold water to afford phthalamide (10.2 g; 86%). Phthalamide (10.0 g; 0.05 mol) was suspended in acetic anhydride (70 ml) and refluxed for 2 hr. Upon cooling, the white solid which precipitated was collected and washed with cold ethanol to afford (3.0 g; 40%) of *o*-cyanobenzamide. *o*-Cynanobenzamide (3.0 g; 0.018 mol) was heated for 15 min at 210°C under nitrogen. The product which sublimed was collected and recrystallized from ethanol to obtain 3-imino-1-oxoisoindoline (1 g).

2-Alkyl-3-imino-1-oxoisoindolines: General Procedure (2–6)

Method A. To a solution of 3-imino-1-oxoisoindoline (1.0 g; 0.007 mol) in anhydrous dimethylformamide (30 ml) was added potassium carbonate (0.98 g; 0.007 mol), followed by the slow addition of the appropriate alkyl halide (0.01 mol). The resulting mixture was stirred at room temperature for 3 hr. The solvent was removed under vacuum, and the residue was dissolved in water and extracted with ether. After drying and evaporation of the solvent, the product was afforded, which was purified by recrystallization or column chromatography (chloroform:ethyl acetate, 9:1).

Method B. To a suspension of 3-imino-1-oxoisoindoline (1.0 g; 0.007 mol) in dry ethanol (50 ml) was added the corresponding amine (excess), and the resulting mixture was heated to reflux for 18 hr. The solvent was evaporated to obtain the crude product, which was purified as above.

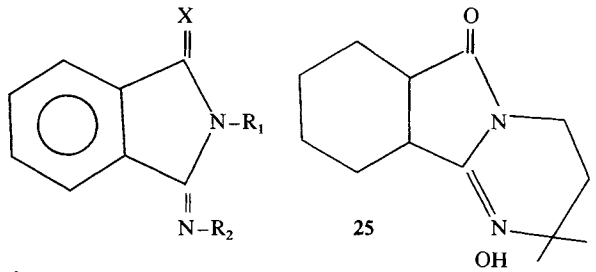
1,3-Dialkyliminoisoindolines: General Procedure (16–19)

A mixture of 1,2-dicyanobenzene (0.08 mol) and alkyl amine (25 ml) in dry ethanol (40 ml) was refluxed for 36 hr. The volatiles were removed under vacuum and the viscous liquid was left at 0°C for 3–4 days. The solidified material was recrystallized to obtain 1,3-dialkyliminoisoindoline.

3-Alkylimino-1-oxoisoindoline Hydrochloride: General Procedure (12–15)

1,3-Dialkyliminoisoindoline (0.02 mol) was hydrolyzed with warm 2 *N* hydrochloric acid. 3-Alkylimino-1-oxoisoindoline hydrochloride was recrystallized from hot water.

Table I. Elemental Analyses of 3-Imino-1-oxoisoindoline and Related Compounds



Compound No.	X	R_1	R_2	Theoretical (%) C	H	N	Cl	Found (%) C	H	N	Cl
2	O	CH_3	H	67.50	5.00	17.50	—	67.32	5.05	17.27	—
3	O	C_2H_5	H	68.96	5.75	16.09	—	68.76	5.74	16.09	—
4	O	C_3H_7	H	70.21	6.38	14.89	—	70.19	6.54	14.96	—
5	O	C_4H_9	H	71.29	6.93	13.86	—	71.25	7.03	13.66	—
6	O	C_5H_{11}	H	72.22	7.40	12.96	—	71.77	7.48	12.82	—
7	O	C_2H_5	C_2H_5	71.29	6.93	13.86	—	71.11	6.79	13.69	—
8	O	C_3H_7	C_3H_7	73.04	7.82	12.17	—	72.07	8.12	12.06	—
9	O	C_4H_9	C_4H_9	74.41	8.52	10.85	—	74.17	8.52	10.96	—
10	O	C_5H_{11}	C_5H_{11}	75.52	9.09	9.79	—	74.97	8.92	9.62	—
11	O	$(CH_2)_2COCH_3$	$(CH_2)_2COCH_3$	67.13	6.29	9.79	—	66.97	6.30	9.53	—
12											
13	O	H	$C_3H_7 \cdot HCl$	58.80	5.79	—	15.81	58.37	6.04		15.36
14	O	H	$C_4H_9 \cdot HCl$	58.18	6.46	11.31	—	58.79	6.83	11.79	—
15	O	H	$C_5H_{11} \cdot HCl$	57.67	7.02	10.35	—	57.33	7.42	10.45	—
16											
17	NC_3H_7	H	NC_3H_7	68.01	8.50	17.00	—	68.12	8.06	17.33	—
18	NC_4H_9	H	NC_4H_9	69.81	9.09	15.27	—	69.72	9.07	15.21	—
19	NC_5H_{11}	H	NC_5H_{11}	71.38	9.57	13.86	—	71.92	9.49	14.01	—
20	O	COOEt	H	60.55	4.59	12.84	—	60.49	4.48	12.56	—
21	O	CH_2COOEt	H	62.06	5.17	12.07	—	61.93	5.34	11.98	—
22	O	$(CH_2)_2COOEt$	H	63.41	5.68	11.36	—	63.19	5.68	11.28	—
23	O	$(CH_2)_3COOEt$	H	64.41	6.15	10.77	—	64.43	6.31	10.63	—
24	O	$(CH_2)_4COOEt$	H	66.17	6.62	10.29	—	65.89	6.82	10.14	—

2-Alkyl-3-alkylimino-1-oxoisoindoline: General Procedure (7–10)

To a suspension of 3-alkylimino-1-oxoisoindoline (0.002 mol) hydrochloride in dimethylformamide (15 ml) was added potassium carbonate (0.004 mol) and alkyl halide (0.003 mol). The resulting mixture was stirred at room temperature overnight, diluted with water, and extracted with ether. The solvent was removed and an oily product was purified by column chromatography (chloroform).

2-(3-Oxobutyl)-3-(3-oxobutylimino)-1-oxoisoindoline (11)

3-Imino-1-oxoisoindoline (1.0 g; 0.007 mol) was suspended in ethyl acetate (50 ml). Freshly prepared sodium ethoxide (200 mg) was added, followed by the rapid addition of methyl vinyl ketone (0.01 mol). The resulting reaction mixture was heated to reflux for 3.5 hr. After acidification with acetic acid, the volatiles were removed under vacuum. The product was purified by column chromatography (ethyl acetate:chloroform, 3:7) to obtain the cyclized product (**25**) and 2-(3-oxobutyl)-3-(3-oxobutylimino)-1-oxoisoindoline (**11**).

2-(Carbethoxyalkyl)-1-oxoisoindoline (20–24): General Procedure

3-Imino-1-oxoisoindoline (0.007 mol) was dissolved in anhydrous dimethylformamide (30 ml), and potassium carbonate (0.008 mol) was added. The halogenated ester (0.008 mol) was added and the resulting mixture was stirred at room temperature overnight, diluted with H_2O, and extracted with ether. The ether extract was evaporated to obtain a crystalline solid, which was recrystallized from the specified solvent.

Hypolipidemic Screens in CF_1 Male Mice

Test compounds were suspended in 1% (carboxymethyl)cellulose–water and administered intraperitoneally to CF_1 male mice (~25 g) for 16 days at a dose of 20 mg/kg/day. On days 9 and 16 blood was collected in nonheparinized microcapillary tubes by tail vein bleeding, and the serum obtained by centrifugation for 3 min. The serum cholesterol levels were measured by a modification of the Liebermann–Burchard reaction (9). Blood was also collected on day 16 in heparinized microcapillary tubes and the serum obtained. Serum triglyceride levels were measured with a commercial kit (10).

Hypolipidemic Activity in Sprague–Dawley Rats

2-Butyl-3-butyl imino-1-oxoisoindoline (**9**) was suspended in 1% (carboxymethyl)cellulose–water and administered orally by intubation needle to Sprague–Dawley male rats (~325 g) at 20 mg/kg/day for 14 days. Blood was collected on days 7 and 14 for the serum cholesterol (9) and triglyceride (10) assays. Weekly animal weights were obtained and expressed as a percentage of the initial body weight. After 14 days of dosing with the test drug, the major organs were excised, trimmed of fat, and weighed. The daily food consumption was determined for the control and treated groups, expressed as grams per day per rat.

Liver, Aorta, and Fecal Lipid Extraction

After 2 weeks of dosing of Sprague–Dawley rats with **9** at 20 mg/kg/day, the liver, small intestine mucosa, aorta, and fecal samples (24-hr collection) were removed and 10% homogenates in 0.25 *M* sucrose ±0.001 *M* EDTA were prepared (11). Aliquots (2 ml) of the homogenate were extracted by the Folch *et al.* (12) and Bligh and Dyer (13) methods and the number of milligrams of lipid was determined. The lipid was taken up in ethyl acetate and the cholesterol (9), triglyceride (10), neutral lipid (14), phospholipid (15), and protein (16) content was determined.

Lipoprotein Separation

In Sprague–Dawley rats (~210 g), the blood was collected from the abdominal vein after 14 days of administration of drug (**9**) at 20 mg/kg/day, orally. The serum was separated from the RBC by centrifugation (3000 *g* × 10 min). Using the ultracentrifuge methods of Hatch and Lees (17) and Havel *et al.* (18), lipoprotein fractions were separated. Each fraction was analyzed for cholesterol (9), triglycerides (10), neutral lipids (16), phospholipids (15), and protein (16).

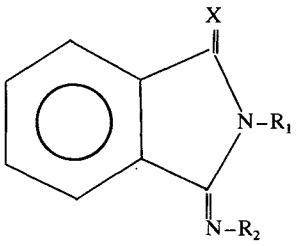
RESULTS

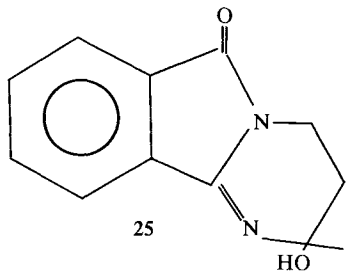
Chemistry (Table II)

Based on previous observations of *N*-alkyl substitutions of cyclic imides of carbon-chain lengths of four or an oxygen substituted for one of the carbon atoms, we initially investigated N-substituted carbon-chain lengths of one to five (compounds **2–6**). Since 3-imino-1-oxoisoindolines contain a second nitrogen atom outside of the ring structure, N substitution from two to five carbon atoms was achieved at the exocyclic nitrogen as the HCl salt (compounds **12–15**). In addition, the influence of dialkyl substitution on both nitrogen atoms was investigated (compounds **7–10**). Included in this group of compounds was a disubstituted butanone (compound **11**) since butanone derivatives have been shown to be active in the other cyclic imide series. The carbonyl group of the cyclic imide ring was altered to a nitrogen atom. Disubstitution on both exocyclic nitrogen atoms was successfully achieved (compounds **16–19**). Finally, the influence of an ethyl ether function on the exocyclic nitrogen was studied where the carbon-chain length varied from one to four carbon atom compounds (**20–24**).

Although the synthesis of 3-imino-1-oxoisoindoline (**1**) is known, the subsequent reactions of this heterocycle are sparsely reported in the literature. Clark *et al.* (7) reported treating this imine (**1**) with methyl amine under refluxing conditions in ethanol to afford 2-methyl-3-methylimino-1-oxoisoindoline. The attempted duplication of this procedure with higher homologues of methyl amine did not yield the expected 2-alkyl-3-alkylimino-1-oxoisoindolines; instead 2-alkyl-3-imino-1-oxoisoindolines (monoalkylation only) were afforded. These monoalkyl isoindolines were also obtained by alkylating 3-imino-1-oxoisoindoline (**1**) with alkyl halide using potassium carbonate in dimethylformamide at room temperature (**2–6**). The desired 3-alkylimino-1-oxoisoindolines (**12–15**) were prepared in the form of their salts by hydrolyzing the corresponding 1,3-dialkyliminoisoindolines (**16–19**) in warm 2 *N* HCl. These 1,3-dialkylimino-isoindo-

Table II. Physical Characteristics of 3-Imino-1-oxoisoindolines and Derivatives





25

Compound No.	X	R_1	R_2	mp, °C (lit.)	Yield	Formula
1[a]	O	H	H	198–200 (201)[g]	—	—
2[b]	O	CH_3	H	125–127	29	$C_9H_8N_2O$
3[b]	O	C_2H_5	H	95–97	45	$C_{10}H_{10}N_2O$
4[b]	O	C_3H_7	H	66–67	36	$C_{11}H_{12}N_2O$
5	O	C_4H_9	H	—	31	$C_{12}H_{14}N_2O$
6	O	C_5H_{11}	H	—	36	$C_{13}H_{16}N_2O$
7	O	C_2H_5	C_2H_5	—	30	$C_{12}H_{14}N_2O$
8	O	C_3H_7	C_3H_7	—	48	$C_{14}H_{18}N_2O$
9	O	C_4H_9	C_4H_9	—	26	$C_{16}H_{22}N_2O$
10	O	C_5H_{11}	C_5H_{11}	—	32	$C_{18}H_{26}N_2O$
11	O	$(CH_2)_2COCH_3$	$(CH_2)_2COCH_3$	—	46	$C_{16}H_{18}N_2O_3$
12[c]	O	H	$C_2H_5 \cdot HCl$	221–225[h]	28	$C_{10}H_{11}N_2OCl$
13[c]	O	H	$C_3H_7 \cdot HCl$	205–208	32	$C_{11}H_{13}N_2OCl$
14[c]	O	H	$C_4H_9 \cdot HCl$	175–179	24	$C_{12}H_{15}N_2OCl$
15[c]	O	H	$C_5H_{11} \cdot HCl$	126–128	18	$C_{13}H_{17}N_2OCl$
16	NC_2H_5	H	NC_2H_5	152–56 (158)[h]	52	—
17[d]	NC_3H_7	H	NC_3H_7	64–67	44	$C_{15}H_{22}N_3$
18[d]	NC_4H_9	H	NC_4H_9	74–76	52	$C_{16}H_{24}N_3$
19[d]	NC_5H_{11}	H	NC_5H_{11}	—	40	$C_{17}H_{26}N_3$
20[e]	O	COOEt	H	122–124	27	$C_{11}H_{10}N_2O_3$
21[f]	O	CH_2COOEt	H	106–108	21	$C_{12}H_{12}N_2O_3$
22[f]	O	$(CH_2)_2COOEt$	H	69–71	31	$C_{13}H_{14}N_2O_3$
23	O	$(CH_2)_3COOEt$	H	—	20	$C_{14}H_{16}N_2O_3$
24	O	$(CH_2)_4COOEt$	H	—	23	$C_{15}H_{18}N_2O_3$
25[f]	Cyclized product		—	149–151	20	$C_{12}H_{12}N_2O_2$

[a] Ethanol.
[b] Hexane.
[c] Water.
[d] Ethyl acetate.
[e] Benzene/hexane.
[f] Benzene.
[g] Source: Ref. 7.
[h] Source: Ref. 6.

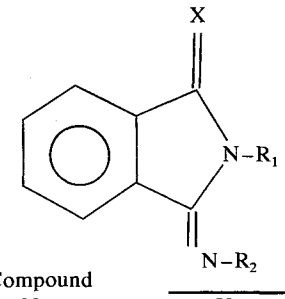
lines (16–19) were made by reacting 1,2-dicyanobenzene with the primary amine following the procedure of Clark *et al.* (7). The alkylation of 3-alkyliminoiso-indoline hydrochloride with alkyl halide in the presence of potassium carbonate resulted in the desired 2-alkyl-3-alkylimino-1-oxoisoindolines (7–10). Also, 3-imino-1-oxoisoindoline (1) could be conveniently alkylated at the endocyclic nitrogen by reaction with different halogenated esters to obtain the esters (20–24).

The Michael addition of 1 with methyl vinyl ketone in the presence of a catalytic amount of sodium ethoxide afforded the *N,N*-dialkyl product (11) and the unexpected cyclized product (25).

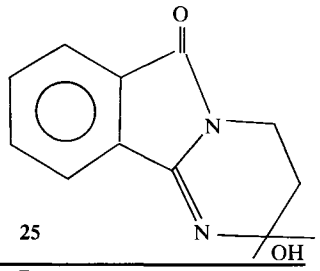
Biological Studies

The 3-imino-1-oxoisoindolines proved to be potent hypolipidemic agents in both mice and rats (Table III). The parent agent 3-iminophthalimidine reduced mouse serum cholesterol levels 44% and serum triglyceride levels 41% after 16 days of dosing at 20 mg/kg/day. The introduction of an alkyl side chain on the imide nitrogen (2–6) did not improve the hypolipidemic activity significantly. The exception to this observation was that 2-pentyl-3-imino-1-oxoisoindoline (6) reduced the serum cholesterol level by 42% and serum triglycerides by 61%. Substitution on the imino nitrogen (=N–R) with alkyl groups (12–15) resulted in a slight improvement in hypocholesterolemic activity when the side chain was an ethyl group. 3-Ethylimino-l-oxisoindoline hydrochloride (12) showed a 47% reduction in the serum cholesterol level and a 29% reduction in the serum triglyceride level. Again, the pentyl analogue (15) exhibited the best activity, suppressing the serum triglyceride level by 44%. None of the 1,3-dialkyliminoisoindolines (16–19) were

Table III. Hypolipidemic Activity of 3-Imino-1-oxoisoindoline and Its Derivatives in CF_1 Male Mice at 20 mg/kg/day ip



25



Compound No.	X	R_1	R_2	% of control ($X \pm SD$) ($N = 6$) Serum cholesterol Day 9	Serum cholesterol Day 16	Serum triglyceride, Day 16
1	O	H	H	64 ± 5*	56 ± 4*	59 ± 5*
2	O	CH_3	H	76 ± 5*	62 ± 3*	59 ± 4*
3	O	C_2H_5	H	94 ± 6	72 ± 5*	71 ± 5*
4	O	C_3H_7	H	77 ± 6*	61 ± 4*	62 ± 2*
5	O	N–C_4H_9	H	70 ± 7*	58 ± 6*	67 ± 7*
6	O	N–C_5H_{11}	H	75 ± 3*	58 ± 5*	39 ± 3*
7	O	C_2H_5	C_2H_5	78 ± 4*	70 ± 4*	76 ± 5*
8	O	C_3H_7	C_3H_7	80 ± 7*	69 ± 5*	80 ± 5*
9	O	C_4H_9	C_4H_9	91 ± 8	48 ± 5*	58 ± 5*
10	O	C_5H_{11}	C_5H_{11}	70 ± 4*	66 ± 3*	81 ± 4*
11	O	$(CH_2)_2COCH_3$	$(CH_2)_2COCH_3$	90 ± 6	60 ± 6*	71 ± 5*
12	O	H	C_2H_5	57 ± 4*	53 ± 3*	71 ± 4*
13	O	H	C_3H_7	75 ± 3*	56 ± 5*	67 ± 6*
14	O	H	C_4H_9	77 ± 4*	68 ± 4*	76 ± 3*
15	O	H	C_5H_{11}	89 ± 7	76 ± 6*	56 ± 4*
16	NC_2H_5	H	NC_2H_5	78 ± 6*	52 ± 4*	67 ± 6*
17	NC_3H_7	H	NC_3H_7	60 ± 2*	54 ± 3*	66 ± 3*
18	NC_4H_9	H	NC_4H_9	90 ± 4	68 ± 4*	63 ± 4*
19	NC_5H_{11}	H	NC_5H_{11}	68 ± 3*	67 ± 4*	62 ± 5*
20	O	COOEt	H	84 ± 6	81 ± 3*	65 ± 4*
21	O	CH_2COOEt	H	72 ± 7*	50 ± 3*	72 ± 2*
22	O	$(CH_2)_2COOEt$	H	93 ± 5	55 ± 3*	61 ± 4*
23	O	$(CH_2)_3COOEt$	H	81 ± 6*	60 ± 3*	77 ± 2*
Clofibrate (150 mg/kg)				88 ± 4	87 ± 5	75 ± 5*
1% carboxymethyl cellulose				100 ± 6[a]	100 ± 5[a]	100 ± 6[b]

[a] 128 mg% cholesterol.
[b] 137 mg% triglycerol.
* $P \leq 0.001$.

as active as its counterpart in the *N,N'*-dialkyl 1-oxoisoindoline series except 1,3-diethyliminoisoindoline (**16**), which lowered serum cholesterol by 48% and the triglyceride level by 33%. The diketone derivative (**11**) demonstrated approximately as much hypocholesterolemic activity (40% reduction) as the parent compound (**1**) (44%). Introduction of a side chain with carboxylic function retained the hypocholesterolemic activity, but the hypotriglyceridemic activity was suppressed, ranging from 9 to 15%. Among these esters (**20–24**) 2-(2'-carbethoxyethyl)-3-imino-1-oxoisoindoline (**22**) showed good activity in both the screens, decreasing serum cholesterol and triglyceride levels by 45 and 39%, respectively, in CF_1 mice.

2-Butyl-3-butylimino-1-oxoisoindoline was selected for an in-depth study in rats. At 20 mg/kg/day, orally, this agent lowered serum cholesterol 60% after 14 days of administration (Table IV). Serum triglyceride levels were reduced more rapidly, showing 39 and 43% reductions, respectively, on days 7 and 14. Analysis of lipids from the treated rats showed that cholesterol, neutral lipid, and triglyceride levels were reduced by the drug in liver and aorta tissues, whereas phospholipid levels were elevated (Table V). All four types of lipids were elevated in the small intestine tissue, and the cholesterol and phospholipid content was increased in the fecal material after 14 days of administration of drug. Serum lipoprotein fractions after 2 weeks of drug administration

Table IV. The Effects of 2-Butyl-3-butylimino-1-oxoisoindoline (**9**) at 20 mg/kg/day orally on the Serum Lipid Levels of Sprague–Dawley Rats

$N = 6$	% of control ($X \pm SD$) Serum cholesterol level Day 7	Serum cholesterol level Day 14	Serum triglyceride level Day 7	Serum triglyceride level Day 14	% of initial body wt Day 7	% of initial body wt Day 14
Control	100 ± 6	100 ± 7	100 ± 7	100 ± 8	107	112
Treated (**1**)[a]	69 ± 6	60 ± 5*	—	32 ± 4*	—	—
Treated (**9**)	89 ± 5	40 ± 4*	61 ± 5*	57 ± 6*	109	101

[a] Reported previously (6).
* $P \leq 0.001$.

Table V. The Effects of 2-Butyl-3-butylimino-1-oxoisoindoline (9) at 20 mg/kg/day Orally on Rat Lipid Levels of Liver, Small Intestine, Aorta, Feces, and Lipoproteins

	% of control					
$N = 6$	mg of lipids	Cholesterol	Triglyceride	Neutral lipid	Phospholipids	Protein
Liver						
Control	100 ± 6	100 ± 8[a]	100 ± 7[b]	100 ± 8[c]	100 ± 17[d]	100 ± 2[e]
Treated	93 ± 7	69 ± 6*	77 ± 6*	36 ± 5*	238 ± 20*	101 ± 3
Small intestine						
Control	100 ± 5	100 ± 7[f]	100 ± 5[g]	100 ± 8[h]	100 ± 8[i]	100 ± 7[j]
Treated	244 ± 8*	130 ± 6*	197 ± 6*	138 ± 6*	161 ± 9*	85 ± 8
Aorta tissue						
Control	100 ± 6	100 ± 7[k]	100 ± 7[l]	100 ± 2[m]	100 ± 19[n]	100 ± 2[o]
Treated	102 ± 5	92 ± 8	80 ± 6*	66 ± 5*	154 ± 15*	104 ± 4
Feces						
Control	100 ± 7	100 ± 8[p]	100 ± 8[q]	100 ± 4[r]	100 ± 23[s]	100 ± 7[t]
Treated	122 ± 8*	159 ± 7*	105 ± 7	102 ± 5	167 ± 17*	129 ± 6*
Chylomicrons						
Control	—	100 ± 9[u]	100 ± 8[v]	100 ± 6[w]	100 ± 8[x]	100 ± 7[y]
Treated	—	99 ± 8	84 ± 6	109 ± 8	72 ± 6*	98 ± 5
VLDL						
Control	—	100 ± 8[z]	100 ± 9[aa]	100 ± 7[bb]	100 ± 8[cc]	100 ± 5[dd]
Treated	—	72 ± 6*	112 ± 10	111 ± 9	69 ± 7	130 ± 7*
LDL						
Control	—	100 ± 7[ee]	100 ± 6[ff]	100 ± 8[gg]	100 ± 7[hh]	100 ± 7[ii]
Treated	—	40 ± 5*	96 ± 7	208 ± 9*	234 ± 10*	85 ± 6
HDL						
Control	—	100 ± 6[jj]	100 ± 6[kk]	100 ± 7[ll]	100 ± 8[mm]	100 ± 8[nn]
Treated	—	158 ± 7*	82 ± 5*	73 ± 6*	342 ± 9*	92 ± 8

[a] 24.03 mg cholesterol/g tissue.
[b] 6.37 mg triglyceride/g tissue.
[c] 44.11 mg neutral lipid/g tissue.
[d] 7.19 mg phospholipid (P_i)/g tissue.
[e] 4.5 mg protein/g wet weight of tissue.
[f] 7.82 mg/g wet weight of tissue.
[g] 1.12 mg/g wet weight of tissue.
[h] 6.98 mg/g wet weight of tissue.
[i] 2.06 mg/g wet weight of tissue.
[j] 42 mg/g wet weight of tissue.
[k] 11.46 mg/g wet weight of tissue.
[l] 4.86 mg/g wet weight of tissue.
[m] 79.11 mg/g wet weight of tissue.
[n] 4.79 mg/g wet weight of tissue.
[o] 2.94 mg/g wet weight of tissue.
[p] 28.47 mg/g wet weight of tissue.
[q] 1.86 mg/g wet weight of tissue.
[r] 33.94 mg/g wet weight of tissue.
[s] 1.239 mg/g wet weight of tissue.
[t] 6.99 mg/g wet weight of tissue.
[u] 337 μg/ml of plasma.
[v] 420 μg/ml of plasma.
[w] 67 μg/ml of plasma.
[x] 149 μg/ml of plasma.
[y] 184 μg/ml of plasma.
[z] 190 μg/ml of plasma.
[aa] 22 μg/ml of plasma.
[bb] 98 μg/ml of plasma.
[cc] 26 μg/ml of plasma.
[dd] 50 μg/ml of plasma.
[ee] 210 μg/ml of plasma.
[ff] 45 μg/ml of plasma.
[gg] 10 μg/ml of plasma.
[hh] 41 μg/ml of plasma.
[ii] 122 μg/ml of plasma.
[jj] 544 μg/ml of plasma.
[kk] 620 μg/ml of plasma.
[ll] 27 μg/ml of plasma.
[mm] 153 μg/ml of plasma.
[nn] 657 μg/ml of plasma.
*$P \leq 0.001$.

showed a reduction in the cholesterol content of the very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) fractions with an elevation in the high-density lipoprotein (HDL) cholesterol levels. There was a marginal decrease in the triglyceride content in the chylomicron and HDL fractions. Neutral lipids were elevated in the LDL but reduced in the HDL fraction. The phospholipid content was reduced in chylomicrons and VLDL fractions but elevated in LDL and HDL fractions.

The rats which were treated with drug for 14 days showed no significant changes in the weights of the major organs of the body. Specifically the adrenal weight showed no change from the control organ weight. The food consumption per day was 27.9 ± 7.4 for the control and 22.4 ± 5.0 for the treated rats, demonstrating a 20% decrease in daily food intake. The body weight of the control increased 12% over 14 days, whereas the treated rats showed a 1% increase for the same period of time. The hematocrit and white blood-cell differential counts were not changed by drug administration for 14 days to rats. Serum values for total protein, BUN, SGPT, glucose, alkaline phosphatase, bilirubin, LDH, and CP kinase activities were all within

normal limits after drug administration for 14 days and did not differ from the control rat values.

DISCUSSION

Phthalimide and its *N*-alkyl derivatives demonstrated potent hypolipidemic activity in both normal and atherogenic CF_1 male mice at an optimum dose of 20 mg/kg/day, ip. The most potent compounds in this series are the *N*-butyl, *N*-butan-3-one, and *N*-propionic acid derivatives (1–3). 3-Iminophthalimidine (3-imino-1-oxoisoindoline) (1) is structurally related to phthalimide in that one of the imide carbonyls is replaced by an imino (=NH) group. 3-Iminophthalimidine exhibited equal activity to phthalimide in reducing serum cholesterol (44%). However, the hypotriglyceridemic activity of 3-iminophthalimidine was 15% lower than that of phthalimide.

Many of the derivatives of the 3-imino-1-oxoisoindoline series have demonstrated better hypolipidemic activity than clofibrate at 150 mg/kd/day. Some of its derivatives have exhibited equally potent activity as phthalimide. The trend previously observed that the side chain with four carbons or three carbons and an oxygen showed the best activity in the phthalimide (1), saccharin (2), or indazolinone (5) series was not evident in the 3-imino-1-oxoindolines series of compounds. However, these analogues have retained good activity as hypolipidemic agents. 2-Butyl-3-butylimino-1-oxoisoindoline (9) was one of the best hypolipidemic agents tested, and 2-pentyl-3-imino-1-oxoisoindoline (6) proved to be one of the more potent hypotriglyceridemic agents tested in mice in our laboratory. The 2-butyl-3-butylimino-1-oxoisoindoline derivative was also active in rats when administered orally. This drug appeared to accelerate the clearance of cholesterol via the fecal route, facilitating the reduction of lipids in the blood compartment and tissues, e.g., liver and aorta. The lipid accumulation in the small intestine after drug treatment may be due to the drug inhibition of lipid absorption and processing by the GI mucosa. Interestingly the drug effectively reduced lipids in the aorta tissue after 14 days, which suggests the possibility that the 3-imino-1-oxoisoindodine may facilitate lipid clearance from the atherogenic plaques in the aorta walls. This idea is supported by the fact that the drug reduced LDL cholesterol and elevated HDL cholesterol levels. In hyperlipidemic patients the HDL cholesterol level is low and the LDL cholesterol is high, which contributes to atherosclerosis and myocardial infarction in humans (19). If a hypolipidemic agent is to be effective therapeutically, the opposite ratio is desirable. From preliminary studies, the 3-imino-1-oxoisoindine appears to achieve the proper ratio, i.e., high HDL cholesterol and low LDL cholesterol levels, thereby facilitating cholesterol removal from plaques, transport to the liver, and excretion via the bile into the feces.

ACKNOWLEDGMENT

This work was supported by Grant HL25680 from the National Institutes of Health.

REFERENCES

1. J. M. Chapman, G. H. Cocolas, and I. H. Hall. *J. Med. Chem.* 22:1399–1402 (1979).
2. J. M. Chapman, P. J. Voorstad, G. H. Cocolas, and I. H. Hall. *J. Med. Chem.* 28:237–243 (1983).
3. J. M. Chapman, G. H. Cocolas, and I. H. Hall. *J. Pharm. Sci.* 72:1344–1347 (1983).
4. J. M. Chapman, S. D. Wyrick, P. J. Voorstad, J. M. Maguire, G. H. Cocolas, and I. H. Hall. *J. Pharm. Sci.* 73:1482–1484 (1984).
5. S. D. Wyrick, P. J. Voorstad, G. H. Cocolas, and I. H. Hall. *J. Med. Chem.* 27:768–772 (1984).
6. P. J. Voorstad, G. H. Cocolas, and I. H. Hall. *J. Pharm. Res.* 1:250–255 (1984).
7. P. F. Clark, J. A. Elvidge, and J. H. Golden. *J. Chem. Soc.* 4135–4143 (1956).
8. G. T. Byrne, R. P. Linstead, and A. R. Lowe. 1017 (1934).
9. A. T. Ness, J. V. Pastewka, and A. C. Peacock. *Clin. Chem. Acta.* 10:229–237 (1964).
10. *Single Vial Triglycerides,* Boehringer Mannheim Diagnostics Division.
11. G. H. Cocolas, J. M. Chapman, Jr., P. J. Voorstad, and I. H. Hall. *J. Pharm. Sci.* 72:812–814 (1983).
12. J. Folch, M. Lees, and G. H. C. Stanley. *J. Biol. Chem.* 226:407–409 (1957).
13. E. G. Bligh and W. J. Dyer. *Can. J. Biochem. Physiol.* 37:911–917 (1959).
14. J. H. Bragdon. *J. Biol. Chem.* 190:513–517 (1951).
15. C. P. Stewart and E. G. Hendry. *Biochem. J.* 29:1983–1989 (1935).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall. *J. Biol. Chem.* 193:265–275 (1951).
17. F. T. Hatch and R. S. Lees. *Adv. Lipid Res.* 6:1–68 (1968).
18. R. J. Havel, H. A. Eder, and J. H. Bragdon. *J. Clin. Invest.* 34:1345–1353 (1955).
19. T. A. Miettinen, J. K. Huttunen, T. Strandberg, V. Naukkarinen, S. Mattila, and T. Kumlin. *Lancet* 2:478 (1981).