depression of contractility at 5 mg/kg, iv, but was not greater at 10 mg/kg, iv; no observable QRS widening and no lethality; 2.0, achieved 25–30% depression of contractility at 5 mg/kg, iv, and was more depressant at 10 mg/kg, iv; possible QRS widening but no lethality; 2.5, achieved 25–30% depression of contractility between 2.5 and 5 mg/kg, iv, and was more depressant at 5 and 10 mg/kg, iv (dose related); possible lethality and QRS widening; 3.0, achieved 25–30% depression of contractility at 2.5 mg/kg, iv, and was more depressant at increasing doses; QRS widening and/or lethality in some or all animals at 10 mg/kg, iv; 3.5, achieved 25–30% depression of contractility between 1 and 2.5 mg/kg, iv (dose-related depression); QRS widening and/or lethality observed at 10 mg/kg, iv, in some or all animals; 4.0, 25–35% or greater depression of contractility at 1 mg/kg, iv; QRS widening observed at 5 or 10 mg/kg, iv, and lethal in some or all animals at 5 mg/kg, iv. The values reported in Table II were established with, generally, two to four animals.

C. Aminophylline Tachycardia. Experiments were performed in anesthetized, vagotomized dogs according to a method described previously.[5a]

D. Glucagon Tachycardia. Experiments were performed in anesthetized, ganglion-blocked dogs according to a method described previously.[5a]

E. Poldine Tachycardia. Dogs were surgically prepared as described in the triazine tachycardia method. Poldine methyl sulfate (0.5 mg/kg, iv) was administered, and 30 min later, test compounds were administered in increasing doses at 30-min intervals.[24]

Acknowledgment. The authors thank Roberta Acchione, Joan Rogers, and Martin Mutter for spectral data and John T. Hortenstine, Mary Mackay, and Paul R. Darkes for assistance in chemical synthesis. We are very grateful to David G. Wentling, John A. Mitchell, Michael P. Rose, and Mary Ann Colussi for excellent technical assistance in performing pharmacological experiments.

Registry No. **2**, 61020-73-3; **6** 0.5-fumarate, 61020-77-7; **7** hexamate, 83747-58-4; **8** naphthalenesulfonate, 83747-59-5; **9**, 61020-91-5; **10** fumarate, 83747-60-8; **11** benzoate, 83747-61-9; **12** fumarate, 83747-62-0; **13** hexamate, 83747-63-1; **14** hexamate, 83747-64-2; **15** fumarate, 83747-66-4; **16** hexamate, 83747-68-6; **17** hexamate, 83747-69-7; **18** hexamate, 83747-70-0; **19** fumarate, 83762-94-1; **20** hexamate, 83747-71-1; **21** tosylate, 83747-73-3; **22** saccharin, 83747-74-4; **23** fumarate, 83747-75-5; **24** fumarate, 83747-77-7; **25**, 61020-83-5; **26**, 61020-78-8; **27**, 61020-92-6; **28**, 61020-81-3; **29**, 83747-78-8; **30** fumarate, 83747-79-9; **31** tosylate, 83747-81-3; **32** saccharin, 83747-83-5; **33**·HCl, 83747-84-6; **34** fumarate, 83747-85-7; **35**·$HClO_4$, 83747-87-9; **36**, 83747-88-0; **37** saccharin, 83747-89-1; **38** fumarate, 83747-91-5; **39** fumarate, 83747-92-6; **40** fumarate, 83747-94-8; **41** fumarate, 83747-96-0; **42** fumarate, 83747-98-2; **43** fumarate, 83762-96-3; **44**, 83747-99-3; **44** fumarate, 83748-00-9; **45**, 61021-09-8; **45** hexamate, 83748-01-0; **46**·HCl, 83748-02-1; **47**, 75626-09-4; **47** fumarate, 83762-97-4; **48** fumarate, 83748-04-3; **49** fumarate, 83748-06-5; **50** fumarate, 83748-08-7; **51**·HI, 83748-09-8; **52** fumarate, 83748-11-2; **53** fumarate, 83748-12-3; **54**·2HCl, 83748-13-4; I (X = Y = H), 120-72-9; III (X = Y = H), 61021-51-0; IV (R = $CH_2CH{=}CH_2$; X = Y = H), 61021-45-2; VI, 41948-92-9; 3-[(2-aminoethyl)thio]indole hydrochloride, 54466-83-0; 3-[(2-aminopropyl)thio]indole, 61021-79-2; 3-[(3-aminopropyl)thio]indole, 61021-80-5; 3-[(2-aminoethyl)thio]-1-methylindole fumarate, 61021-49-6; 3-[(2-aminoethyl)thio]-1-ethylindole 0.5-fumarate, 61021-60-1; 3-[(2-aminoethyl)thio]-1-isopropylindole fumarate, 83748-14-5; 3-[(2-aminoethyl)thio]-1-propylindole 0.5-fumarate, 61021-83-8; 3-[(2-aminoethyl)thio]-1-octylindole fumarate, 61021-96-3; 3-[(2-aminoethyl)thio]-1-cyclopentylindole fumarate, 61021-68-9; 3-[(2-aminoethyl)thio]-1-(cyclopropylmethyl)indole 0.5-fumarate, 61021-72-5; 1-allyl-3-[(2-aminoethyl)thio]indole fumarate, 61021-76-9; 3-[(2-aminoethyl)thio]-1-benzylindole fumarate, 61021-78-1; 3-[(2-aminoethyl)thio]-1-furfurylindole fumarate, 61021-74-7; 3-[(2-aminoethyl)thio]-1-(2-methoxyethyl)indole fumarate, 61021-70-3; 3-[(2-aminoethyl)thio]-5-chloroindole hydrochloride, 61021-65-6; 3-[(2-aminoethyl)thio]-2-propylindole fumarate, 83748-16-7; 3-[(2-aminoethyl)thio]-5-chloro-2-methylindole hexamate, 83748-18-9; chloroacetonitrile, 107-14-2; allyl bromide, 106-95-6; 3-indolylthiol, 480-94-4; 2-methylaziridine, 75-55-8; 3-chloropropylamine hydrochloride, 6276-54-6; 4-chlorobutyronitrile, 628-20-6; (indol-3-ylthio)butyronitrile, 61021-92-9; 3-[(4-aminobutyl)thio]indole fumarate, 61021-94-1; 3-indolebutyric acid, 133-32-4; 3-indolebutyramide, 6245-91-6; 3-(4-aminobutyl)indole, 669-70-5; *N*-methyl-2-pyrrolidinone, 872-50-4; 1-methyl-2-(methylthio)-2-imidazoline hydriodide, 61076-89-9; *N*,*N*-dimethylacetamide, 127-19-5; cyclohexanesulfamic acid, 100-88-9; 2,3-dihydrotryptamine, 13078-91-6; 3-[(2-aminoethyl)thio]indole, 61021-52-1.

Supplementary Material Available: Table of numbers of animals (N) and standard errors in D_{50} and ΔHR_{50} for the triazine test (2 pages). Ordering information is given on any current masthead page.

(24) The poldine test is related to the atropine-induced sinus tachycardia test;[5a] however, it does not have the potential for CNS involvement, since poldine is a peripheral agent.

Hypolipidemic Activity of Phthalimide Derivatives. 2. *N*-Phenylphthalimide and Derivatives

James M. Chapman, Jr., P. Josée Voorstad, George H. Cocolas, and Iris H. Hall*

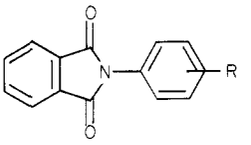
Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514.
Received January 28, 1982

A series of substituted *N*-phenylphthalimide derivatives was synthesized and examined for their ability to lower serum cholesterol and triglyceride levels in mice at 20 (mg/kg)/day, ip. Of the newly synthesized compounds, the most potent compound, *o*-(*N*-phthalimido)acetophenone, lowered serum cholesterol 57% after 16 days and lowered serum triglyceride levels 44% after 14 days. *o*-(*N*-Phthalimido)acetophenone was observed to be active in both normogenic (normal blood lipids levels) and hyperlipidemic mice and normogenic rats. In the latter, the reduction of serum lipids was reversible. The mode of action of this compound appeared to be multiple, including blockage of the de novo synthesis of lipids and acceleration of the excretion of lipids. The lipoprotein fractions of rat blood were reduced significantly in cholesterol, triglyceride, and neutral lipid content after 14 days treatment with *o*-(*N*-phthalimido)acetophenone.

A series of N-substituted phthalimides including alkyls, methyl ketones, carboxylic acids, and acetate esters has previously been shown to be potent hypolipidemic agents in rodents at 20 (mg/kg)/day, ip.[1]

0022-2623/83/1826-0237$01.50/0

Table I. Physical Characteristics of *N*-Phenylphthalimide Derivatives



no.	compound	R	mp (lit.) °C	recrystn solvent	yield, %
2	*N*-phenylphthalimide	H	209-210 (208)[a]	EtOH	69
3	*o*-(*N*-phthalimido)acetophenone	*o*-$COCH_3$	136-137 (132-134)[b]	EtOH	41
4	*m*-(*N*-phthalimido)acetophenone	*m*-$COCH_3$	177-179	EtOAc	55
5	*p*-(*N*-phthalimido)acetophenone	*p*-$COCH_3$	245-249 (240)[c]	EtOAc	41
6	*o*-(*N*-phthalimido)ethylbenzene	*o*-CH_2CH_3	144-146 (137)[a]	2-propanol	14[d]
7	*m*-(*N*-phthalimido)ethylbenzene	*m*-CH_2CH_3	105-107	2-propanol	24[d]
8	*p*-(*N*-phthalimido)ethylbenzene	*p*-CH_2CH_3	181-182 (177)[a]	EtOH	46[d]
9	*o*-(*N*-phthalimido)benzoic acid	*o*-COOH	208-211 (208-210)[e]	acetone	36
10	*m*-(*N*-phthalimido)benzoic acid	*m*-COOH	290-292 (290)[e]	2-propanol	10[d]
11	*p*-(*N*-phthalimido)benzoic acid	*p*-COOH	289-291 (280)[e]	EtOH	15[d]

[a] Reference 9. [b] Reference 10. [c] Reference 11. [d] Final yield of purified compound. [e] Reference 12.

Phthalimide (1) was observed to reduce serum cholesterol levels 43% after 16 days and serum triglyceride levels 56% after 14 days dosing in CF male mice.[2] Phthalimide (1) was effective in reducing liver mitochondrial citrate exchange, acetyl-CoA synthetase, acetyl-CoA carboxylase, and phosphatidate phosphohydrolase activities, as well as liver and small intestinal lipids at 20 mg/kg. Phthalimide administration to rats was related to an elevated excretion of cholesterol in the bile with a reduction of cholesterol and triglyceride content of the blood lipoprotein fractons but with an increase in phospholipid content.[3] A subsequent literature search for aromatic subsitutions of the imide ring demonstrated that the structurally related compound ethyl 2-[*p*-*N*-(3′-oxoisoindolinyl)phenoxy]-2,2-dimethylacetate[4] and the less structurally related (β-hydroxy-*p*-chlorophenethyl)-2,3-dihydroisoindoline[5] possess hypolipidemic activity in animals. Consequently, *N*-phenylphthalimide and a series of derivatives where the phenyl ring was substituted, were tested for hypolipidemic activity in rodents, and those data are now presented.

Results and Discussion

The structure–activity studies for hypolipidemic activity show that the unsubstituted *N*-phenylphthalimide maintained good activity in CF_1 mice at 20 (mg/kg)/day, ip, resulting in 39% reduction of serum cholesterol levels after 16 days (Table II), although it was not as active as the compound phthalimide (1). Substitution of the phenyl ring afforded mixed results. The substitution of ethyl groups on the ortho (6), meta (7), and para (8) positions of the phenyl ring gave rise to compounds that clearly possessed less hypolipidemic activity in mice than *N*-phenylphthalimide. Substitution of *o*- (9), *m*- (10), and *p*-carboxy (11) groups on the phenyl ring gave rise to compounds that were less potent in the case of 9 and 10 whereas *p*-carboxy substitution (11) resulted in slightly greater activity than *N*-phenylphthalimide with 42% reduction of serum triglyceride and 47% reduction of cholesterol levels in mice at 20 (mg/kg)/day. Examination of the acetophenone derivatives demonstrated that the ortho-substituted analogue (3) had improved hypocholesteremic activity compared to *N*-phenylphthalimide with 57% reduction of serum cholesterol compared to 43% reduction by compound 2. Suppression of serum triglycerides by compounds 2 and 3 was comparable, i.e., 39 and 44%, respectively. The para-substituted acetophenone (5) demonstrated approximately equal activity to *N*-phenylphthalimide; however, the meta-substituted (4) derivative was the least active of the acetophenone series. A more detailed study of *o*-(*N*-phthalimido)acetophenone in Holtzman rats at 10, 20, 30 mg/kg shows that the 10 and 20 mg/kg dosage was the most effective in reducing serum cholesterol, i.e., 46 and 44%, respectively (Table III). Serum triglycerides were most effectively reduced at 20 (mg/kg)/day orally with 39% reduction after 14 days. It was observed that both the cholesterol and triglyceride levels returned to predosing levels after drugs had ceased to be administered to rats for 2 weeks. In the dose–response study in mice, it can be readily observed that the reduction of cholesterol levels was dose related, with 100 (mg/kg)/day suppressing cholesterol levels 74% on day 16. The reduction of serum triglyceride levels was not dose related on day 14. The 50 (mg/kg)/day dose resulted in the best activity with 47% reduction; however, doses of 12.5 and 20 mg/kg afforded a 44% reduction of serum triglyceride content in mice.

In the hyperlipidemic induced mice, 2 weeks on the basal atherogenic diet elevated significantly the serum chole-

Table II. Effects of *N*-Phenylphthalimide Derivatives on Serum Cholesterol and Triglyceride Levels on CF_1 Male Mice at 20 (mg/kg)/day

compd (N = 6)	serum triglyceride % control: 14th day	serum cholesterol % control: 9th day	serum cholesterol % control: 16th day
1% CMC[f]	100 ± 6[c]	100 ± 5[d]	100 ± 6[e]
1	44 ± 8[a]	63 ± 13[a]	57 ± 7[a]
2	61 ± 5[a]	92 ± 8	57 ± 3[a]
3	56 ± 9[a]	89 ± 6	43 ± 5[a]
4	74 ± 7[a]	87 ± 4[b]	84 ± 6[b]
5	66 ± 6[a]	83 ± 6[a]	55 ± 4[a]
6	92 ± 3	88 ± 5[b]	103 ± 11
7	95 ± 4	89 ± 7	82 ± 10[b]
8	96 ± 4	89 ± 7	89 ± 6
9	78 ± 3[a]	92 ± 8	77 ± 9[a]
10	71 ± 8[a]	81 ± 8[b]	69 ± 6[a]
11	58 ± 6[a]	89 ± 3[b]	53 ± 5[a]
clofibrate	91 ± 10	98 ± 12	96 ± 8

[a] $p \leq 0.001$. [b] $p \leq 0.010$. [c] = 118 mg %. [d] = 122 mg %. [e] 137 mg %. [f] CMC = carboxymethylcellulose.

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Table III. Effects of *o*-(*N*-Phthalimido)acetophenone on Serum Cholesterol and Triglyceride Levels of Holtzman Rats and CF_1 Male Mice

	rats				mice			
	dose, (mg/kg)/	serum cholesterol		serum triglyceride:	dose, (mg/kg)/	serum cholesterol		serum triglyceride:
compd (N = 6)	day	day 9	day 16	day 14	day	day 9	day 16	day 14
				% Control				
control (1% CMC[i])		100 ± 9[c]	100 ± 7[d]	100 ± 8[e]		100 ± 5[f]	100 ± 6[g]	100 ± 6[h]
3	10	66 ± 7[a]	54 ± 6[a]	76 ± 3[a]	12.5	83 ± 5[a]	43 ± 4[a]	56 ± 3[a]
	20	76 ± 5[a]	56 ± 4[a]	61 ± 4[a]	20	89 ± 6	43 ± 5[a]	56 ± 9[a]
	30	76 ± 8[b]	64 ± 7[a]	75 ± 9[a]	25	74 ± 6[a]	38 ± 3[a]	61 ± 5[a]
					50	68 ± 6[a]	29 ± 4[a]	53 ± 5[a]
					100	42 ± 3[a]	26 ± 4[a]	59 ± 4[a]
				Recovery After Termination of Drug Administration				
1% CMC[i]			100 ± 8	100 ± 7				
3	10		102 ± 9	104 ± 8				
	20		107 ± 8	99 ± 7				

[a] $p \leq 0.001$. [b] $p \leq 0.010$. [c] 13 mg %. [d] 78 mg %. [e] 110 mg %. [f] 118 mg %. [g] 122 mg %. [h] 137 mg %. [i] CMC = carboxymethylcellulose.

Table IV. Effects of *o*-(*N*-Phthalimido)acetophenone on Serum Cholesterol and Triglyceride Levels in Hyperlipidemic Induced Mice

	% control			
	serum cholesterol		serum triglyceride	
compd (N = 6)	2-week diet	14-day dosing	2-week diet	14-day dosing
control (1% CMC[c])	100 ± 6	100 ± 7	100 ± 5	100 ± 4
control (atherogenic diet)	289 ± 9[a]	290 ± 9[a]	131 ± 5[a]	131 ± 5[a]
3	289 ± 9[a]	118 ± 8[b]	131 ± 6[a]	86 ± 7[b]

[a] $p \leq 0.001$. [b] $p \leq 0.010$. [c] CMC = carboxymethylcellulose.

Table V. In Vitro Effects of *o*-(*N*-Phthalimido)acetophenone on CF_1 Male Mouse Liver Enzymatic Activities

	% control				
compd (N = 6)	mitochondrial citrate exchange	Ac-CoA synthetase	citrate lyase	HMG CoA reductase	cholesterol side-chain oxidation
1% CMC[b]	100 ± 10	100 ± 11	100 ± 9	100 ± 7	100 ± 8
3 (2.5 μmol)	76 ± 5[a]	63 ± 4[a]	62 ± 7[a]	91 ± 15	27 ± 7[a]
	Ac-CoA carboxylase	fatty acid synthetase	phosphatidate phosphohydrolase	acyltransferase	
1% CMC[b]	100 ± 6	100 ± 7	100 ± 7	100 ± 8	
3 (2.5 μmol)	35 ± 4[a]	107 ± 7	65 ± 10[a]	54 ± 8[a]	

[a] $p \leq 0.001$. [b] CMC = carboxymethylcellulose.

sterol level by 189%, whereas triglycerides were only elevated 31% by the diet (Table IV). Administration of *o*-(*N*-phthalimido)acetophenone for 2 weeks significantly reduced the serum cholesterol levels in these mice from 290 to 118 mg %, which approached normal levels. The serum triglyceride levels of the hyperlipidemic mice were reduced 42% after 2-week dosing with resulting levels below the triglyceride levels of normal mice.

o-(*N*-Phthalimido)acetophenone did not suppress the appetite of the rat. After 2-weeks dosing, there were no major changes in body weight or the grams of food consumed on a daily basis. There were no significant changes in the weight of the major organs after administering the drug for 2 weeks, nor was there a significant increase in the adrenal weight after drug administraiton. In vitro enzymatic studies revealed that *o*-(*N*-phthalimido)acetophenone significantly suppressed mitochondrial citrate exchange by 24%, thus reducing the available citrate in the cytoplasm (Table V) at a 2.5 μmol drug concentration. Further, acetyl-CoA synthetase and citrate lyase activities were reduced by 37 and 38%, respectively, by the drug. HMG CoA reductase activity, the regulatory enzyme of cholesterol synthesis, was unaffected by the presence of drug. Cholesterol side-chain oxidation was reduced 73% by *o*-(*N*-phthalimido)acetophenone, which certainly would not explain a reduction of serum cholesterol. Enzymes involved in fatty acid synthesis were also examined. Acetyl-CoA carboxylase activity, the regulatory enzyme of fatty acid synthesis, was suppressed 65%, whereas fatty acid synthetase activity was unaffected at 2.5 μmol of drug. Phosphatidate phosphohydrolase activity, the regulatory enzyme for the conversion of phospholipids to triglyceride, was reduced 35%, and acyltransferase activity, the regulatory enzyme of triglyceride synthesis, was reduced 46% by *o*-(*N*-phthalimido)acetophenone. Utilizing rat liver homogenates, those enzymes that were observed to be inhibited in mice liver homogenates were assayed with the drug being present from 0.1 to 10 mM final concentrations. The *o*-(*N*-phthalimido)acetophenone afforded the following ID_{50} values: acetyl-CoA synthetase, 7.05 mM; acetyl-CoA carboxylase, 0.385 mM; phosphatidate phosphohydrolase, 4.1 mM; acyltransferase, 1.88 mM. The ID_{50} values for HMG CoA reductase and fatty acid synthetase could not be obtained in this concentration range. These ID_{50} values appear realistic considering the dose required in vivo to observe reduction of serum lipids in rodents.

Table VI. In Vivo Effects of o-(*N*-Phthalimido)acetophenone on CF_1 Male Mouse Enzyme Activities after 16 Days Dosing

compd (N = 6)	dose, (mg/kg)/day	% control: Ac-CoA synthetase	HMG CoA reductase	Ac-CoA carboxylase
1% CMC		100 ± 7	100 ± 6	100 ± 5
3	10	53 ± 6[a]	98 ± 6	8 ± 2[a]
	20	46 ± 5[a]	81 ± 5[a]	41 ± 5[a]
	40	59 ± 6[a]	84 ± 4[a]	48 ± 4[a]
	60	86 ± 9	94 ± 7	57 ± 5[a]

	dose, (mg/kg)/day	fatty acid synthetase	phosphatidate phosphohydrolase	acyltransferase	lipid content
1% CMC		100 ± 6	100 ± 8	100 ± 7	100 ± 6
3	10	106 ± 6	85 ± 6[b]	35 ± 9[a]	84 ± 5[a]
	20	97 ± 7	68 ± 4[a]	41 ± 5[a]	85 ± 4[a]
	40	94 ± 5	54 ± 4[a]	43 ± 4[a]	80 ± 4[a]
	60	100 ± 6	98 ± 7	45 ± 4[a]	80 ± 4[a]

[a] $p \leq 0.001$. [b] $p \leq 0.010$.

Table VII. Effect of *o*-(*N*-Phthalimido)acetophenone on Liver Lipid Content from CF_1 Male Mice Treated for 16 Days

compd (N = 6)	% control: mg of lipid	cholesterol	neutral lipids	triglycerides	phospholipids
1% CMC[c]	100 ± 6	100 ± 7	100 ± 4	100 ± 5	100 ± 8
3					
10 mg/kg	84 ± 5[a]	73 ± 6[a]	36 ± 3[a]	46 ± 6[a]	90 ± 7
20 mg/kg	85 ± 4[a]	73 ± 5[a]	65 ± 5[a]	91 ± 5	163 ± 10[a]
40 mg/kg	80 ± 4[a]	71 ± 5[a]	62 ± 5[a]	91 ± 4	143 ± 9[a]
60 mg/kg	80 ± 4[a]	82 ± 7[b]	62 ± 4[a]	100 ± 7	86 ± 5[a]

[a] $p \leq 0.001$. [b] $p \leq 0.010$. [c] CMC = carboxymethylcellulose.

After mice were dosed at 10–60 (mg/kg)/day for 16 days with *o*-(*N*-phthalimido)acetophenone, enzymatic assays were determined on liver homogenates. Acetyl-CoA synthetase activity was optimally inhibited 54% at 20 (mg/kg)/day ip. HMG CoA reductase activity was inhibited 19% at 20 mg/kg. Since neither the in vivo acetyl-CoA synthetase nor HMG CoA reductase activities were inhibited in a pattern consistent with the observed reduction of serum cholesterol, it is concluded that the major effects of the drug is at some other site(s) than these two enzymes. One of these sites may be citrate exchange from the mitochondria; however, this cannot be measured in vivo. Fatty acid synthetase was essentially unaffected. Phosphatidate phosphohydrolase activity was inhibited 32% at 20 mg/kg and 46% at 40 mg/kg; acyltransferase activity was inhibited 65% at 10 mg/kg and greater than 50% from 20 to 60 (mg/kg)/day of *o*-(*N*-phthalimido)-acetophenone. The lipid content of the mouse liver after 16 days dosing was reduced 20% at 40 and 60 (mg/kg)/day (Table VII). The cholesterol content of the liver was reduced 27% at 10 and 20 mg/kg and 29% at 40 mg/kg. The triglyceride content was only reduced 9% at 20 and 40 mg/kg; however, 10 (mg/kg)/day afforded a 54% reduction. Neutral lipid content of the liver was reduced 64% at 10 mg/kg and approximately 35% at the higher doses. Phospholipid content of liver was elevated significantly at 20 and 40 mg/kg, which correlated with the enzymatic suppression of phosphatidate phosphohydrolase. The increase in liver phospholipid content is probably due to the inhibition of the enzymatic activity of phosphatidate phosphohydrolase. Clofibrate has been observed to increase liver weight as well as liver phospholipid levels[6] and biliary phospholipid levels.[7] Further, Lamb et al. have shown a correlation between hypolipidemic agents that block phosphatidate hydrolase and acyltransferase and the reduction in the levels of liver, intestine, and serum triglycerides.[8] *o*-(*N*-Phthalimido)acetophenone did not lower serum lipids by allowing the mobilization of the lipids into major organs. This can be observed as a reduction in lipid content of the liver (Table VII), no significant increase in organ weight, and no deposition of radiolabeled cholesterol in liver, kidney, heart, and brain after 14 days administration of drug. Rather, the labeled cholesterol, after drug treatment, accumulated in the small and large intestine, i.e., control contained 31.38% and treated 42.51% of administered labeled cholesterol. Radioactivity in the chyme collection increased from 4.68 to 8.97% of the administered cholesterol after 14 days drug administration. The bile duct cannulation studies demonstrated that *o*-(*N*-phthalimido)acetophenone caused an increase in bile flow from 336 to 900 mL/6 h, as well as cholesterol content from 1016 to 1876 cpm for the total 6-h collecton of bile. However, the drug did not accelerate cholesterol absorption from the gut when radioactive cholesterol was administered orally (10 μCi) but rather reduced absorption of cholesterol from 14280 dpm to 8650 dpm total plasma volume.

The lipid content of the individual lipoprotein fractions collected from the blood of rats treated for 14 days with drug was reduced significantly (Table VIII). The chylomicrons, low- and high-density lipoprotein fractions, were reduced in cholesterol, neutral lipids, and triglyceride content, whereas the phospholipid content was elevated. The very low density fraction was reduced in triglyceride and protein content.

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Table VIII. Effects of *o*-(*N*-Phthalimido)acetophenone on Serum Lipoprotein Fractions of Holtzman Rats After 14 Days Administration

compd ($N = 6$)	% control				
	cholesterol	neutral lipids	triglycerides	phospholipids	protein
		Chylomicrons			
1% CMC[w]	100 ± 9[a]	100 ± 8[b]	100 ± 6[c]	100 ± 10[d]	100 ± 7[e]
3	64 ± 6[u]	50 ± 4[u]	41 ± 3[u]	171 ± 9[u]	91 ± 7
		Very Low Density			
1% CMC	100 ± 8[f]	100 ± 9[g]	100 ± 7[h]	100 ± 8[i]	100 ± 8[j]
3	85 ± 7	81 ± 8[v]	46 ± 5[u]	359 ± 12[u]	66 ± 9[u]
		Low Density			
1% CMC	100 ± 9[k]	100 ± 7[l]	100 ± 8[m]	100 ∓ 7[n]	100 ± 8[o]
3	57 ± 6[u]	49 ± 5[u]	52 ± 6[u]	87 ± 7	95 ± 9
		High Density			
1% CMC	100 ± 8[p]	100 ± 9[q]	100 ± 4[r]	100 ± 6[s]	100 ± 8[t]
3	52 ± 6[u]	55 ± 5[u]	63 ± 5[u]	446 ± 8[u]	80 ± 8[v]

[a] 1.37 mg/dec. [b] 0.67 mg/dec. [c] 4.20 mg/dec. [d] 1.49 mg/dec. [e] 30 mg/dec. [f] 1.90 mg/dec. [g] 0.98 mg/dec. [h] 2.21 mg/dec. [i] 0.26 mg/dec. [j] 0.50 mg/dec. [k] 2.10 mg/dec. [l] 0.10 mg/dec. [m] 0.45 mg/dec. [n] 0.41 mg/dec. [o] 6.81 mg/dec. [p] 5.44 mg/dec. [q] 6.20 mg/dec. [r] 0.27 mg/dec. [s] 1.53 mg/dec. [t] 56.7 mg/dec. [u] $p \leq 0.001$. [v] $p \leq 0.010$. [w] CMC = carboxymethylcellulose.

Conclusion

The aromatic substituted phthalimide derivatives possess antihyperlipidemic activity; however, the substituents on the phenyl ring are critical for denoting antihyperlipidemic activity in rodents. Whereas *N*-phenylphthalimide was not as active as phthalimide, it did possess significant activity. It may be noted, based on this limited number of compounds, that those derivatives with substitution of the phenyl ring making them more lipophilic, e.g., compounds **6–8**, were less potent in the hypolipidemic screen than **2**. Those substituents that were more electron withdrawing, e.g., **2–5** and **9–11**, were generally more potent than the electron-releasing ethyl substitution of the phenyl ring. However the position of aromatic subsitution appears to be critical (i.e., ortho, vs. meta, vs. para), although the positional trend varies with varying substituents. Ortho substitution (**3**) in the acetophenone series affords the most potent compound, whereas para substitution (**11**) in the benzoic acid derivatives results in the most potent compound. The most active compound in the rodent screen, *o*-(*N*-phthalimido)acetophenone, was observed to suppress serum lipids significantly, probably through several modes of action of (1) suppressing in vivo key liver enzymes in the de novo biosynthesis of triglycerides; (2) accelerating excretion of cholesterol by the biliary route, and (3) decreasing absorption of cholesterol from the gut after 2 weeks administration of drug. Suppression of appetite, thus reducing caloric intake and reducing plasma lipids of the animal, was not a mode of action nor was there redistribution of lipids from the plasma compartment to the major organs. The observed reduction of all four lipoprotein fractions suggests that *o*-(*N*-phthalimido)-acetophenone may be useful in human hyperlipidemic states, since these disease states are manifested as elevated chylomicrons, very low density or low density lipoprotein fractions. The substituted *N*-phenylphthalimide derivatives were more effective than clofibrate in reducing serum lipids, but they had similar effects on lipid metabolism as clofibrate.

The effects of *o*-(*N*-phthalimido)acetophenone on lipid metabolism and distribution were found to be consistent with those found for phthalimide (1)[3] in rodents.

Experimental Section

Chemistry. Melting points were determined on a Mel-Temp apparatus and are uncorrected. NMR data were obtained with a JEOL FX-60 spectrophotometer.

***N*-Phenylphthalimide (2).** Phthalic anhydride (14.8 g, 0.10 mol) and aniline (10.23 g, 0.11 mol) were refluxed overnight in 300 mL of *p*-cymene. Crude *N*-phenylphthalimide precipitated from the solution upon cooling and was collected. Recrystallization from ethanol afforded 16 g (69%) of *N*-phenylphthalimide, mp 200–210 °C. Further recrystallization of an analytical sample gave *N*-phenylphthalimide, mp 209–210 °C.

Compounds 3–8. Equimolar amounts (0.037–0.10 mol) of phthalic anhydride and the appropriate aniline derivative were refluxed in 100–200 mL of toluene for 8–12 h. The toluene was evaporated under vacuum, and the residue was refluxed in 50–100 mL of acetic anhydride for 1–2 h. The volatile material was once again evaporated under vacuum, and the resulting residue was recrystallized from the solvent indicated (Table I).

***m*-(*N*-Phthalimido)acetophenone (4):** NMR ($CDCl_3$) δ 2.70 (s, 3 H, CH_3), 7.72–8.38 (m, 8 H, aromatic). Anal. ($C_{16}H_{11}NO_3$) C, H, N.

***m*-(*N*-Phthalimido)ethylbenzene (7):** NMR ($CDCl_3$) δ 1.29 (t, 3 H, CH_3), 2.74 (q, 2 H, CH_2), 7.11–8.17 (m, 8 H, aromatic). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

***o*-(*N*-Phthalimido)benzoic Acid (9).** Phthalic anhydride (14.8 g, 0.1 mol) and anthranilic acid (13.7 g, 0.1 mol) were slowly heated until molten and then heated for an additional 2 h at 170–200 °C. Recrystallization of the resulting residue from acetone yielded 9.6 g (36%) of *o*-(*N*-phthalimido)benzoic acid, mp 205–210 °C. Recrystallization of an analytical sample from acetone afforded *o*-(*N*-phthalimido)benzoic acid, mp 208–211 °C.

Compounds 10 and 11. Phthalic anhydride (14.8 g, 0.1 mol) and *m*- or *p*-aminobenzoic acid (13.7 g, 0.1 mol) were placed in 300 mL of *p*-cymene and refluxed for 20–22 h. The volatile material was evaporated under vacuum, and the resulting residue was repeatedly recrystallized from ethanol (para isomer) or 2-propanol (meta isomer) to yield 4.0 g (15%) of *p*-(*N*-phthalimido)benzoic acid, mp 289–291 °C, or 2.5 g (10%) of *m*-(*N*-phthalimido)benzoic acid, mp 290–292 °C.

Biological. Hypolipidemic Screens in Normogenic Rodents. Compounds to be tested were suspended in 1% carboxymethylcellulose in water and administered to CF_1 male (~25 g) mice intraperitoneally or Holtzman male rats (~200 g) orally by an intubation needle daily for 16 days. On days 9 and 16, blood was obtained by tail-vein bleeding, and the serum was separated by centrifugation for 3 min. The serum cholesterol levels were determined by a modification of the Liebermann–Burchard reaction.[13] Serum was also collected on day 14, and the triglyceride content was determined by a commercial kit (Fisher, Hycel triglyceride test kit).

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Hyperlipidemic Induced Mice. CF_1 male mice (~25 g) were placed on a commercial diet (U.S. Biochemical Corp. basal atherogenic test diet) that contained butter fat (400 g), celufil (60 g), cholesterol (53 g), choline dihydrogen citrate (4 g) salt mixture oil (Wesson oil, 40 g), sodium cholate (40 g), sucrose (223 g), vitamin-free casein (200 g), and total vitamin supplement for a 2-week period. After the cholesterol and triglyceride levels were assayed and observed to be elevated, the mice were administered test drugs at 20 (mg/kg)/day intraperitoneally for an additional 2-week period. Serum cholesterol and triglyceride levels were measured after 14 days of administration of the drugs.

Animal Weights and Food Intake. Periodic animal weights were obtained during the experiments and expressed as a percentage of the animal's weight on day 0. after the animals were dosed for 16 days with test drugs, a number of organs were excised, trimmed of fat, and weighed. The organ weights were expressed as a percentage of the total body weight of the animal. The average food intake (Wayne Blox rodent chow) in (grams/rat)/day was determined over the 16-day period of dosing.

Enzymatic Studies. In vitro enzymatic studies were determined with 10% homogenates of CF_1 male mouse liver with 2.5 μmol of test drugs and with Holtzman male rat livers with 0.100 to 10 mM concentration of test drugs. In vivo enzymatic studies were determined with 10% homogenates of liver from CF_1 male mice obtained after administration of the agents for 16 days from 10 to 60 (mg/kg)/day intraperitoneally. The liver homogenates for both in vitro and in vivo studies were prepared in 0.25 M sucrose plus 0.001 M (ethylenedinitrilo)tetraacetic acid. Acetyl coenzyme A synthestase[14] and citrate lyase[15] activities were determined spectrophotometrically at 540 nm as the hydroxamate of acetyl coenzyme A formed after 30 min at 37 °C. Mitochondrial citrate exchange was determined by the procedure of Robinson et al.[16,17] with sodium [^{14}C]bicarbonate, which was incorporated into mitochondrial [^{14}C]citrate after isolating rat mitochondria (9000g × 10 min) from the homogenates. The exchange of the [^{14}C]citrate was determined after incubation of the mitochondrial fraction, which was loaded with labeled citrate, with test drugs for 10 min. The radioactivity was measured in the mitochondrial and supernatant fractions in scintillation fluid (Hyamine hydroxide, New England Nuclear Corp.) and expressed as a percentage. Cholesterol side-chain oxidation was determined by the method of Kritchevsky et al.[18] with [26-^{14}C]cholesterol (50 mCi/mmol) and mitochondria isolated from rat liver homogenates. After 18-h incubation at 37 °C with test drugs, the generated $^{14}CO_2$ was trapped in the center well in [2-[2-[p-(1,1,3,3-tetramethylbutyl)cresoxy]ethoxy]ethyl]dimethylbenzylammonium hydroxide (Hyamine hydroxide, New England Nuclear Corp.) and counted. (Fisher, SOX-1 Scintiverse; Packard scintillation counter) 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) was measured with [1-^{14}C]acetate (56 mCi/mmol) with a postmitochondrial (Supernatant contains ribosomes, endoplasmic reticulum, and cell sap) supernatant (9000g × 20 min) for 60 min at 37 °C.[19] The digitonide derivative of cholesterol was isolated and counted.[20] Acetyl-CoA carboxylase activity was measured by the method of Greenspan and Lowenstein.[21] Initially, the enzyme had to be polymerized for 30 min at 37 °C, and then the assay mixture containing sodium [^{14}C]bicarbonate (41.0 mCi/mmol) was added and incubated for 30 min at 37 °C with test drugs. Fatty acid synthetase activity was determined by the method of Brady et al.[22] with [2-^{14}C]malonylcoenzyme A (37.5 mCi/mmol) that was incorporated into newly synthesized fatty acids, which are extracted with ether and counted.[6] Acyltransferase activity was determined with L-[2-^{3}H(N)]glycerol 3-phosphate (7.1 Ci/mmol), and the microsomal fraction of the liver homogenates.[8] The reaction was terminated after 10 min, and the lipids were extracted with chloroform/methanol (1:2) containing 1% 1 N HCl and counted (Fisher SOX-1 Scintiverse; Packard scintillation counter).

Phosphatidate phosphohydrolase activity was measured as the inorganic phosphate released after 30 min from phosphatidic acid by the method of Davis et al.[23] The released inorganic phosphate after development with ascorbic acid and ammonium molybdate was determined at 820 nm.

Liver, Small Intestine, and Fecal Lipid Extraction. In CF_1 male mice that had been administered test drugs for 16 days, the liver, small intestine, and fecal materials (24 h collection) were removed and a 10% homogenate in 0.25 M sucrose plus 0.001 M (ethylenedinitrilo)tetraacetic acid was prepared. An aliquot (2 mL) of the homogenate was extracted by the Folch et al.[24] and Bligh and Dyer[25] methods, and the number of milligrams of lipid was determined. The lipid was taken up in methylene chloride, and the cholesterol level,[13] triglyceride levels (Bio-Dynamics/bmc triglyceride kit), neutral lipid content,[26] and phospholipid content[27] were determined.

[^{14}C]Cholesterol Distribution in Mice and Rats. CF_1 male mice (~25 g) were administered test agents intraperitoneally, and rats were administered test drugs orally for 14 days. On day 13, 10 μCi of [4-^{14}C]cholesterol (52.5 mCi/mmol) was administered, and feces were collected for the next 6, 12, and 24 h. Twenty-four hours after cholesterol administration, the major organs were excised and samples of blood, chyme, and urine were obtained. Homogenates (10%) were prepared of the tissues, which were combusted (Packard tissue oxidzer) and counted (Fisher, SOX-1 Scintiverse; Packard scintillation counter). Some tissue samples were plated on filter paper, (Whatman no. 3) dried, and digested for 24 h in base (Hyamine hydroxide, New England Nuclear Corp.) at 40 °C and counted (Fisher, SOX-1 Scintiverse; Packard scintillation counter). Results were expressed as disintegrations per milligram of wet tissue and of total organ.

Cholesterol Absorption Study. Holtzman male rats (~400 g) were administered test drugs intraperitoneally for 14 days at 20 (mg/kg)/day. On day 13, 10 μCi of [1,2-^{3}H(N)]cholesterol (40.7 Ci/mmol) was administered to the rat orally. Twenty-four hours later, the blood was collected, and the serum was separated by centrifugation.[28] Both the serum and the protein precipitated were counted.

Bile Cannulation Study. Holtzman male rats (~400 g) were treated with test drugs at 20 (mg/kg)/day orally for 14 days, the rats were anesthetized with chlorpromazine (25 mg/kg), followed in 30 min by pentobarbital (22 mg/kg) intraperitoneally. The duodenum section of the small intestine was isolated, ligatures were placed around the pyloric sphincter and distally to a site approximately one-third of the way down the duodenum, and sterile isotonic saline was injected into the sectioned off duodenum segment. The saline expanded the duodenum and the common bile duct. Once the bile duct was identified, a loose ligature was placed around the bile duct, and a nick was introduced into the duct immediately before it enters the duodenum. Plastic tubing (PE-10, Intramedic polyethylene tubing) was introduced into the duct, passing the ligature, and tied in place. The ligatures around the duodenum were removed. Once bile was freely moving down the cannulated tube, [1,2-^{3}H(N)]cholesterol (40.7 Ci/mmol) was injected subcutaneously into the rats. The bile was collected over the next 6 h and measured (milliliters). Aliquots were counted

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(Fisher, SOX-1 Scintiverse; Packard scintillation counter) and analyzed for cholesterol content.[13]

Plasma Lipoprotein Fractions. Holtzman male rats (~400 g) were administered drugs at 20 (mg/kg)/day for 14 days. On day 14, blood was collected from the abdominal aorta. Serum was separated from whole blood by centrifugation at 3500 rpm. Aliquots (3 mL) were separated by density gradient ultracentrifugation according to the method of Hatch and Lees[29] and Havel et al.[30] into the chylomicrons, very low density lipoproteins, high density lipoproteins and low density lipoproteins. Each of the fractions were analyzed for cholesterol,[13] triglyceride,[7] neutral lipids,[26] phospholipids,[27] and protein levels.

Acknowledgment. Supported by a National Institutes of Health grant (HL 25680). We thank William Stewart, Charlotte Ridgeway, and Gregory Webb for their technical assistance with this project.

Registry No. **2**, 520-03-6; **3**, 83665-31-0; **4**, 72801-61-7; **5**, 40101-59-5; **6**, 39953-63-4; **7**, 83665-32-1; **8**, 83665-33-2; **9**, 41513-78-4; **10**, 40101-51-7; **11**, 5383-82-4; phthalic anhydride, 85-44-9; aniline, 62-53-3; anthranilic acid, 118-92-3; *m*-aminobenzoic acid, 99-05-8; *p*-aminobenzoic acid, 150-13-0.

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Hypolipidemic Activity of Phthalimide Derivatives. 3. A Comparison of Phthalimide and 1,2-Benzisothiazolin-3-one 1,1-Dioxide Derivatives to Phthalimidine and 1,2-Benzisothiazoline 1,1-Dioxide Congeners

James M. Chapman, Jr., George H. Cocolas, and Iris H. Hall*

Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514.
Received August 5, 1982

Previously it has been observed that N-substituted phthalimide derivatives with chain lengths of four carbon or oxygen atoms showed potent hypolipidemic activity in rodents at 20 (mg/kg)/day ip. The 1,2-benzisothiazolin-3-one 1,1-dioxide (saccharin) nucleus, itself, had also been observed to be active at the same dose. An investigation was undertaken to examine a series of 1,2-benzisothiazolin-3-one 1,1-dioxide analogues for their hypolipidemic activity in mice and to compare them to their respective phthalimide congeners. In addition, a series of 1,2-benzisothiazoline 1,1-dioxide and phthalimidine analogues was prepared, and their hypolipidemic activity was compared to the phthalimide analogues. These studies show that the respective congeners of 1,2-benzisothiazolin-3-one 1,1-dioxide compared favorably to phthalimide congeners in reducing serum triglyceride and cholesterol levels in male CF_1 mice at 20 (mg/kg)/day ip. Of the saccharin derivatives, 3-oxo-1,2-benzisothiazoline-2-propionic acid 1,1-dioxide was the most effective in lowering serum cholesterol levels by 53% after 16 days dosing and 3-oxo-1,2-benzisothiazoline-2-valeric acid 1,1-dioxide lowered serum triglycerides 56% after 14 days dosing. The 1,2-benzisothiazoline 1,1-dioxide and phthalimidine compounds were less active as hypolipidemic agents than their 1,2-benzisothiazolin-3-one 1,1-dioxide and phthalimide analogues, respectively.

The antihyperlipidemic activity of 1,2-benzisothiazolin-3-one 1,1-dioxide (saccharin **1**) and its butan-3-one derivative (**5**) at 20 (mg/kg)/day ip in mice has previously been reported.[1] These two compounds compared favorably with phthalimide and 1-(*N*-phthalimido)butan-3-one in their ability to lower serum lipids.[1] A series of N-substituted phthalimide derivatives has been previously examined for hypolipidemic activity by this laboratory. Side-chain lengths of four carbon atoms or their equivalent for the N-substituted acids, esters, and ketones resulted in the best activity.[2] Thus, N-substituted derivatives of 1,2-benzisothiazolin-3-one 1,1-dioxide were synthesized and compared to their phthalimide congeners for hypolipidemic activity in mice. Preliminary studies have shown that the optimum dose for hypolipidemic activity for phthalimide and saccharin in rats and mice was 20 (mg/kg)/day.[3,4] The toxicity values (LD_{50}) of these derivatives were generally above 2 g/kg, indicating that utilization of the agents at 20 mg/kg was in the safe therapeutic range. No other deleterious side effects were observed for these agents when used in this dose range in mice.[1] A number of N-substituted 1,2-benzisothiazoline 1,1-dioxide (**13**) and phthalimidine (**27**) compounds were also prepared in order to study the importance of the carbonyl groups of the imide ring of these derivatives.

Results and Discussion

After 14 days dosing of CF_1 male mice (~25 g) at 20 (mg/kg)/day ip, the 1,2-benzisothiazolin-3-one 1,1-dioxide (saccharin) and phthalimide derivatives significantly reduced serum triglyceride levels (Table II). Examination of the data for the substituted nuclei showed that phthalimide (**17**) was more active than 1,2-benzisothiazolin-3-one 1,1-dioxide (**1**) in lowering serum triglyceride levels. In general, the butyl (**2** and **18**) and pentyl (**3** and **19**) N-substituted derivatives of phthalimide and saccharin were less active than the N-substituted ketones and acids of this series of compounds in lowering serum triglyceride levels of mice. In the N-substituted ketone series, the propanone (**20**), butanone (**21**), and pentanone (**22**) derivatives of phthalimide were more active than the

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