(19). In this study, in the presence of sulfadimethoxine, the Cl_{int} of thiopental also decreased to one-half that of the control rats (Table I). These findings suggest that the inhibition in thiopental metabolism might be affected by sulfadimethoxine.

The values for the tissue binding reported in this paper are only relative; they are not absolute. Further elaborate studies are necessary for the precise evaluation of tissue binding.

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NOTES

Effects of Imide Analogs on Enzymes Required for Cholesterol and Fatty Acid Synthesis

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Received March 20, 1980, from the *Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514.* Accepted for publication August 21, 1980.

Abstract □ Twelve imide analogs were examined for their ability to lower serum cholesterol and triglyceride levels in mice. Potent activity was observed for compounds containing a phthalimide or saccharin ring structure. The ability to lower serum cholesterol appears to be related to the ability to suppress acetyl-CoA synthetase activity. The availability of acetyl-CoA in the cytoplasm is a key regulatory component for cholesterol and fatty acid synthesis. The capacity to reduce serum triglycerides was related directly to the ability of the compound to inhibit acetyl-CoA carboxylase activity, the regulatory enzyme of fatty acid synthesis.

Keyphrases □ Imide analogs—effects on enzymes required for cholesterol and fatty acid synthesis, serum cholesterol and triglyceride levels, mice □ Cholesterol synthesis—effects of 12 imide analogs on related enzymes □ Fatty acid synthesis—effects of 12 imide analogs on related enzymes □ Triglyceride levels—effects of imide analogs on enzymes required for cholesterol and fatty acid synthesis

The antihyperlipidemic effects of potassium phthalimide and *N*-substituted phthalimides at 20 mg/kg/day in rodents were reported previously (1). Side-chain lengths of four carbon atoms or their equivalent for the *N*-substituted acids, esters, and ketones resulted in the greatest inhibition. β-Hydroxy-β-methylglutaryl-CoA reductase activity was not affected by these agents significantly, but inhibition of acetyl-CoA synthetase activity was related directly to the ability to lower serum lipids. Furthermore, the agents appeared to accelerate cholesterol excretion in the feces. No toxic or teratogenic effects were noted for these compounds, *i.e.*, $LD_{50} \geq 2$ g/kg.

The present study involves variation of the type of nucleus and the side chain to improve antihyperlipidemic activity and examination of the enzymes involved early in cholesterol and triglyceride synthesis for inhibition by these agents.

EXPERIMENTAL

Twelve compounds were selected for this study (Table I). Phthalimide[1] (I), succinimide[1] (III), 1,8-naphthalimide[2] (V), saccharin[3] (VII), dibutyl phthalate[4] (X), and the standard, acetazolamide[5], were purchased commercially.

[1] Kodak Co.
[2] Aldrich Chemical Co.
[3] Ruger Chemical Co.
[4] Matheson, Coleman and Bell.
[5] Lederle Laboratories.

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Table I—Physical Properties of Imides Tested for Antihyperlipidemic Effects

	Compound	Molecular Weight	Observed bp/mp	Literature bp/mp	Reference
I	Phthalimide	147.1	235–236°	232–235°	13
II	1-*N*-Phthalimidobutan-3-one	217.2	114–116°	111–113°	2
III	Succinimide	99.1	122–124°	123–125°	14
IV	1-*N*-Succinimidobutan-3-one	169.2	50–52°	53.5–55.5°	2
V	1,8-Naphthalimide	197.2	296–299°	285–298°	15
VI	1-*N*-(1,8-Naphthalimido)butan-3-one	267.3	154–156°	—	—
VII	Saccharin	183.2	225–229°	228.8–229.7°	16
VIII	1-*N*-(*o*-Benzosulfimido)butan-3-one	253.3	120–122°	116–117.5°	2
IX	3-*N*-Phthalimidopropionic acid	219.2	152–153°	151°	3
X	Dibutyl phthalate	278.3	—	340°/76 mm	17
XI	1-*N*-Phthalimidopropan-2-one	203.2	123–124°	122.9–123.5°	18
XII	*N*-*n*-Butylphthalimide	203.2	110–116°/0.12 mm	100–104°/0.03 mm	5
	Acetazolamide	222.3	255°	258–259°	19

1-*N*-Phthalimidobutan-3-one (II, mp 114–116°), 1-*N*-succinimidobutan-3-one (IV, mp 50–52°), and 1-*N*-(*o*-benzosulfimido)butan-3-one (VIII, mp 120–122°) were prepared by the method of Irai *et al.* (2).

1-*N*-(1,8-Naphthalimido)butan-3-one (VI) was prepared by the following procedure. 1,8-Naphthalimide (9.86 g, 0.05 mole) was added to 300 ml of ethyl acetate containing a catalytic amount of sodium ethoxide. The suspension was heated at 60°, and 7.01 g (0.10 mole) of methyl vinyl ketone was added over 20 min. The resulting reaction mixture was refluxed for 5 hr, cooled, and washed with two 150-ml portions of 5% NaOH. The solvent was removed *in vacuo*, and the resulting yellow solid was recrystallized from ethanol to yield 3.1 g of VI, mp 154–156°; IR: 1695 and 1660 cm^{-1} (C=O stretch); NMR: δ 8.1 (m, 6H, aromatic), 4.55 (t, 2H, $N-CH_2$), 2.95 (t, 2H, CH_2C), and 2.28 (s, 3H, CH_3).

Anal.—Calc. for $C_{16}H_{13}NO_3$: C, 71.89; H, 4.90. Found: C, 72.00; H, 4.84.

3-*N*-Phthalimidopropionic acid (IX) was synthesized by the method of Chodroff *et al.* (3), mp 152–153°. 1-*N*-Phthalimidopropan-2-one (XI) was synthesized by adapting the procedure of Abdel-Monem *et al.* (4),

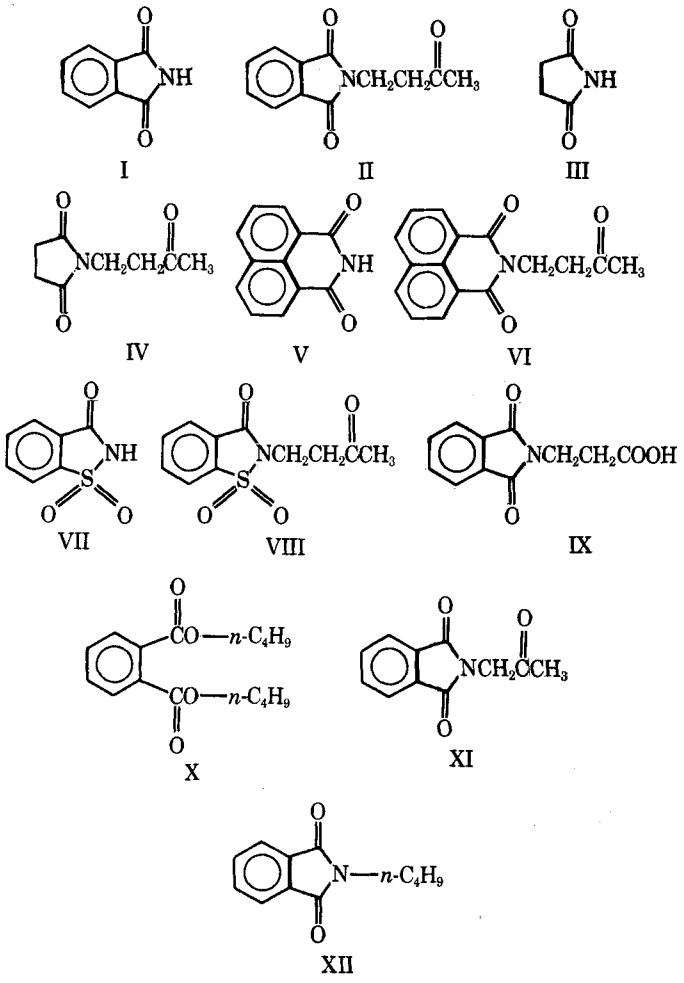

Table II—Effects of Imide Analogs on Serum Cholesterol and Triglyceride Levels of Male Mice ($n = 6$)

Compound	Percent of Control: Serum Cholesterol, Day 9	Percent of Control: Serum Cholesterol, Day 16	Percent of Control: Serum Triglyceride, Day 14
I	63 ± 13[a]	57 ± 7[a]	44 ± 8[a]
II	67 ± 11[a]	63 ± 7[a]	58 ± 7[a]
III	78 ± 9[a]	73 ± 12[b]	68 ± 7[a]
IV	90 ± 9	88 ± 7	79 ± 15
V	81 ± 6[a]	61 ± 7[a]	87 ± 12
VI	94 ± 12	86 ± 9[b]	54 ± 15[a]
VII	68 ± 11[a]	67 ± 10[a]	51 ± 16[a]
VIII	60 ± 8[a]	62 ± 6[a]	51 ± 7[a]
IX	74 ± 10[a]	55 ± 12[a]	58 ± 9[a]
X	89 ± 10	91 ± 12	74 ± 10[a]
XI	80 ± 16	67 ± 12[a]	48 ± 10[a]
XII	72 ± 11[a]	54 ± 6[a]	82 ± 10
Acetazolamide	82 ± 13	77 ± 10	79 ± 15
Carboxymethylcellulose, 1%	100 ± 5[c]	100 ± 6[d]	100 ± 6[e]

[a] $p \leq 0.001$. [b] $p \leq 0.005$, Student t test. [c] 118 mg % of control value. [d] 122 mg % of control value. [e] 137 mg % of control value.

mp 123–124°, and *N*-*n*-butylphthalimide (XII) was prepared according to the procedure of Sterk *et al.* (5), bp 110–116°/0.12 mm.

Compounds were tested at 20 mg/kg/day ip in male CF_1 mice (~30 g). On Days 9 and 16, blood was collected by tail vein bleeding. Serum cholesterol was determined by the Liebermann–Burchard reaction (6). A separate group of mice was bled on Day 14, and their serum triglyceride levels were determined using a commercial kit[6].

Compounds were tested *in vitro* at 2.5 μmoles for their effects on the enzymatic activity of a 10% liver homogenate prepared in 0.25 *M* sucrose and 0.001 *M* (ethylenedinitrilo)tetraacetic acid at pH 7.2. Adenosine triphosphate citrate-lyase activity was measured by the method of Hoffmann *et al.* (7). Acetyl-CoA synthetase activity was determined by the method of Goodridge (8).

The acetyl-CoA formed from both enzymatic assays was coupled with hydroxylamine to produce acetyl hydroxylmate, which was measured at 540 nm. Acetyl-CoA carboxylase activity was measured by a literature method (9), utilizing sodium [^{3}H]bicarbonate (6.2 mCi/mmole) after 30 min of incubation at 37° for enzyme polymerization (10). Fatty acid synthetase was determined by a literature method (11), utilizing [2'-^{14}C]malonyl-CoA (37.5 mCi/mmole) incorporation into fatty acids.

RESULTS AND DISCUSSION

Compounds that contained the phthalimide or saccharin nucleus were more active in lowering serum cholesterol levels after 16 days of dosing than the succinimide (III and IV) derivatives or the naphthalimide derivative (VI) (Table II). Compounds XII and IX afforded the best anticholesterolemic activities followed by V, VIII, II, and VII (significant at $p \leq 0.001$). The commonly used industrial plasticizer (X) resulted in a loss of the analog's ability to lower serum cholesterol.

Serum triglyceride levels also were reduced by the imide analogs after 2 weeks of dosing. Compounds I and XI gave the best antitriglyceride activity followed by VII, VIII, VI, II, IX, III, and X (significant at $p \leq$

[6] Fisher, Hycel Triglyceride Test (1975), Hycel Inc.

Table III—*In Vitro* Effects of Imide Analogs on Enzymes of Cholesterol and Triglyceride Synthetic Pathways in Mice (n = 6)

Compound	Percent of Control, $\bar{x} \pm SD$			
	Acetyl-CoA Synthetase	Citrate-lyase	Acetyl-CoA Carboxy-lase	Fatty Acid Synthetase
I	70 ± 8[a]	42 ± 6[a]	8 ± 4[a]	105 ± 8
II	53 ± 12[a]	34 ± 4[a]	17 ± 3[a]	109 ± 7
III	74 ± 6[a]	38 ± 3[a]	87 ± 7	98 ± 9
IV	58 ± 6[a]	38 ± 7[a]	100 ± 5	81 ± 7[b]
V	63 ± 9[a]	66 ± 6[a]	106 ± 6	86 ± 8
VI	88 ± 5[b]	60 ± 5[a]	59 ± 8[a]	103 ± 4
VII	61 ± 7[a]	65 ± 6[a]	9 ± 2[a]	93 ± 5
VIII	74 ± 9[a]	47 ± 8[a]	12 ± 3[a]	104 ± 7
IX	57 ± 10[a]	87 ± 6[b]	18 ± 4[a]	107 ± 7
X	87 ± 8[b]	82 ± 7[a]	54 ± 5[a]	110 ± 8
XI	58 ± 7[a]	76 ± 4[a]	24 ± 4[a]	91 ± 9
XII	44 ± 11[a]	72 ± 9[a]	76 ± 3[a]	75 ± 10[b]
Acetazolamide	66 ± 4[a]	66 ± 7[a]	13 ± 2[a]	82 ± 6[b]
Carboxymethyl-cellulose, 1%	100 ± 5[c]	100 ± 4[d]	100 ± 7[e]	100 ± 7[f]

[a] $p \leq 0.001$. [b] $p \leq 0.005$, Student t test. [c] 28.5 mg of acetyl-CoA formed/g of wet tissue/30 min. [d] 30.5 mg of citrate hydrolyzed/g of wet tissue/30 min. [e] 32,010 dpm/g of wet tissue/30 min. [f] 37,656 dpm/g of wet tissue/30 min.

0.001). These agents were not toxic at the doses employed, and no side effects were noted.

An attempt was made to correlate the antihyperlipidemic activity with the ability to inhibit enzymatic activities at key biochemical sites early in the synthesis of cholesterol and fatty acids (Table III). The ability to lower serum cholesterol levels correlated positively with the ability to suppress liver acetyl-CoA synthetase activity ($r = 0.86$, $p = 0.001$, using the means of each test group[7]). The ability to suppress acetyl-CoA carboxylase activity correlated positively with the lowering of serum triglycerides ($r = 0.84$, $p = 0.001$). The ability to inhibit citrate-lyase activity, although inhibited by I–IV and VIII which caused >50% inhibition, did not correlate with the ability to lower the serum cholesterol or triglycerides levels. The imide derivatives had no effect on fatty acid synthetase activities except in isolated cases such as IV, V, and XII. Both acetyl-CoA synthetase and citrate-lyase regulate the availability of acetyl-CoA in the cytoplasm for the synthesis of both cholesterol and fatty acids for the synthesis of triglycerides. Acetyl-CoA carboxylase is the regulatory enzyme in the synthesis of fatty acids, which subsequently are required in triglyceride synthesis. In this assay, the carboxylase enzyme must be polymerized for optimum activity (10). Highest inhibition by the imide compounds was observed when test compounds were added to the incubation medium prior to polymerization. However, significant inhibition was observed even if the compounds were added after polymerization, *e.g.*, I, II, VIII, and IX caused 76, 62, 42, and 70% inhibition of the carboxylase activity, respectively.

These studies demonstrate pellucidly the antihyperlipidemic effects of imides. Compared to available standard pharmacological agents, these agents are potent in their ability to lower serum lipids at a relatively low dose with no observable deleterious side effects. These compounds offer unique ways to regulate lipid synthesis which have not been reported previously.

[7] Pearson-product-moment coefficient of correlation (r); probability determined by the Student t test (12).

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Thermal Hardness Coefficient of Tablets

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Abstract □ The hardness of 10 commercial compressed tablets was measured at −25, 0, 24, and 50°. The hardness is relatively insensitive to temperature change within normal storage and handling temperatures. Consequently, no temperature control is needed in measuring tablet hardness. Nonconventional (sustained-release) tablets behave differently.

Keyphrases □ Tablets—determination of hardness at various temperatures □ Hardness—tablets, effects of temperature □ Temperature—effect on hardness coefficient of tablets

Carbon steel and martensitic steels have low mechanical strength at low temperatures. With the exception of tetrafluoroethylene resin, plastics are embrittled at low temperatures (1). In the cryopulverizing process, the material to be comminuted has its temperature lowered so that it changes from a ductile to a brittle solid (2, 3). All materials are not embrittled by chilling; copper, aluminum, nickel, and most solid–solution alloys of these metals are strong at low temperatures.

In pharmaceutics, the brittleness or resistance to crushing is known as hardness, which is defined as the compression force that, when applied diametrically, just causes the tablet to fracture. Although no official standards

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