N H_2SO_4 (250 mL) with vigorous stirring over a 20-min period (the imine was stable in this milieu even at reflux for over 2 h). The organic layer was separated and washed with an additional 250 mL of 18 N H_2SO_4. The acid extracts were combined and back-washed once with Et_2O (500 mL). The acid layer was cooled to 5 °C before the pH was adjusted to 10 by treatment with 20% NaOH. The heavy oil was extracted into Et_2O (2 × 300 mL), and the Et_2O layer was washed with H_2O (2 × 200 mL), dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed on silica with CH_2Cl_2–MeOH (40:1). Subsequent crystallization of this product from *n*-BuCl provided **20** (11.3 g, 44%); mp 82–84 °C; NMR δ 1.8 (3 H, s), 2.15 (3 H, s), 2.25 (3 H, s), 2.3 (3 H, s), 6.35 (2 H, s), 6.8–7.25 (3 H, m). Anal. ($C_{17}H_{20}N_2$) C, H, N.

2-Amino-2′,4,4′,6-tetramethylbenzophenone (21). A clear, orange solution of imine **20** (850 mg, 3.37 mmol) in 0.2 N HCl (50 mL) was heated on a steam bath for 10 h. The reaction mixture was cooled and neutralized with NaOH, and the orange gum was extracted into Et_2O. The organic layer was separated and washed with H_2O (2 × 100 mL), dried ($MgSO_4$), filtered, and evaporated to yield **21** (750 mg, 88%) as a heavy oil (R_f of **21** 0.72 vs. 0.29 for **20** on TLC (silica, $CHCl_3$–MeOH (19:1))]; NMR δ 1.8 (3 H, s), 2.2 (3 H, s), 2.3 (3 H, s), 2.45 (3 H, s), 4.45 (2 H, br s), 6.35 (2 H, d, J = 3 Hz), 6.9–7.4 (3 H, m). Anal. ($C_{17}H_{19}NO$) C, H, N.

4-(2,4-Dimethylphenyl)-2,5,7-trimethylquinazoline (22). A clear solution of imine **20** (100 mg, 0.4 mmol) in HOAc (4 mL) and 4 N HCl (6 mL) was heated at reflux for 2 h and then worked up (as for **21**) to provide **22** (80 mg, 72%) as a gum [R_f of **22** 0.57 vs. 0.29 for **20** and 0.72 for **21** on TLC (silica, $CHCl_3$–MeOH (19:1))]; NMR δ 1.95 (3 H, s), 2.0 (3 H, s), 2.37 (3 H, s), 2.47 (3 H, s), 2.85 (3 H, s), 7.1 (4 H, s), 7.65 (H, s); ^{13}C NMR δ 1.95 (CH_3), 21.2 (CH_3), 21.7 (CH_3), 22.7 (CH_3), 26.1 (CH_3 at C-2), 120.1 (C-4a), 126.0 (C-8), 126.5 (C-5′), 128.1 (C-6′), 130.9 (C-6), 131.8 (C-3′), 134.7 (C-2′), 135.9 (C-4′), 138.4 (C-7), 138.8 (C-1′), 143.8 (C-5), 152.9 (C-8a), 162.6 (C-4), 168.6 (C-2).

HMG-CoA Reductase Inhibition Assay. IC_{50} values were determined by plotting percentage inhibition against test compound concentration (four or five levels) and fitting a straight line to the resulting data by using the least-squares method. See part 1 for a full description of protocol.

Acknowledgment. We extend our appreciation to Dr. W. C. Randall and staff for analytical support, to Dr. D. W. Cochran for the ^{13}C NMR spectrum of **22** and helpful discussions on ^1H NMR data, to M. Z. Banker for manuscript preparation, and to Dr. P. S. Anderson for encouragement.

Registry No. 1, 100430-73-7; **2**, 78444-21-0; **3**, 78444-59-4; **4**, 100484-52-4; **5**, 78444-57-2; **6**, 24061-10-7; **7**, 100430-74-8; **8**, 100430-75-9; **9**, 100430-76-0; **10**, 100430-77-1; **11**, 100430-78-2; **12**, 78444-56-1; **13**, 100430-79-3; **14**, 100448-63-3; **15**, 100430-80-6; **16**, 100430-81-7; **17**, 100430-82-8; **18**, 108-69-0; **19**, 21789-36-6; **20**, 100430-83-9; **21**, 100430-84-0; **22**, 100430-85-1; $Bu_3SnCH{=}CHOEt$, 20420-43-3; $CH_3COCH_2CO_2CH_3$, 105-45-3; 2-$PhC_6H_4CH{=}$CHCHO, 100430-86-2; 6-Ph-2,4-$Cl_2C_6H_2CH{=}CHCHO$, 100430-87-3; 6-(3,4-$Me_2C_6H_3$)-2,4-$Me_2C_6H_2CH{=}CHCHO$, 100448-64-4; 6-(4-FC_6H_4)-2,4-$Cl_2C_6H_2CH{=}CHCHO$, 100430-88-4; 2-(fluoren-9-ylidenyl)acetaldehyde, 4425-71-2; cyanoacetic acid, 372-09-8; HMG-CoA, 9028-35-7.

5,5-Diaryl-2-thiohydantoins and 5,5-Diaryl-N^3-substituted-2-thiohydantoins as Potential Hypolipidemic Agents

J. Ellsworth Tompkins

Division of Liberal Arts and Sciences, College of Health Related Professions, State University of New York, Syracuse, New York 13210. *Received January 3, 1985*

A series of 5,5-diaryl-2-thiohydantoins and 5,5-diaryl-N^3-substituted-2-thiohydantoins related to 5,5-diphenyl-2-thiohydantoin (DPTH) were investigated as potential hypolipidemic agents with the goal of increased potency over DPTH itself. In the 5,5-diaryl class, the best results were obtained by substituting two pyridyl rings for the phenyl rings found in DPTH. The resulting compound, 5,5-bis(2-pyridyl)-2-thiohydantoin, DPYTH (**5**), had slightly better activity than DPTH in lowering liver cholesterol values. Further modifications to DPYTH (**5**) are underway and will be the subject of a future report. In the N^3 nitrogen-substituted series one compound, 5,5-diphenyl-N^3-*n*-butyl-2-thiohydantoin, DPBTH (**7**), showed promise during initial screening, but when analyzed in a dose–response study, its activity was considerably less than that of the parent compound DPTH.

A positive correlation between elevated levels of serum lipids (i.e., cholesterol and triglycerides) and increased incidence of atherosclerosis has been demonstrated.[1] Since coronary heart disease is a major cause of death in Western societies, drugs to lower serum lipid levels have been a major research area in recent years.

Clofibrate [ethyl 2-(*p*-chlorophenoxy)-2-methylpropionate], one of the most widely used hypolipidemic aagents, is relatively ineffective for type IIa hyperlipidemia and is not entirely without undesirable side effects.[2]

In 1972 Elwood et al.[3] reported the activity of 5,5-diphenyl-2-thiohydantoin (DPTH) as a hypolipidemic agent. The results of that study indicated DPTH to be approximately twice as active as clofibrate.

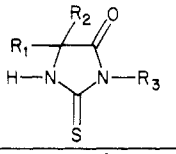
On this basis, a number of derivatives of DPTH were prepared in our laboratory in an effort to improve the effectiveness of this class of compound as hypolipidemic agents. The derivatives were of two types: substitution of one or both phenyl rings at the 4-position and substitution of the nitrogen at the 3-position of the thiohydantoin ring. All of the compounds were tested in the orotic acid assay system as outlined by Elwood et al.,[3] so that a direct comparison could be made with their data.

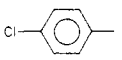
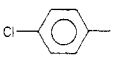
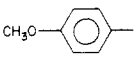
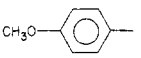
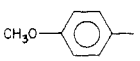
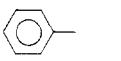
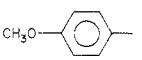
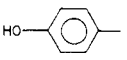
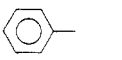
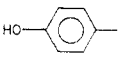
Concern was expressed about the validity of the orotic acid test system as a measure of hypolipidemic activity since it utilizes the prevention of lipid accumulation in the liver rather than in serum. Examination of Elwood's paper however shows the results of testing a series of compounds in the orotic acid assay that are recognized hypolipidemic agents. Two of these compounds, CPIB and choloxon (D-T_4), are well-known. Since both of these compounds are known to lower serum lipids in humans and also work in the orotic acid assay system, it is inferred that the orotic

(1) Jepson, E. M. *Adv. Drug. Res.* **1974**, *9*, 1.
(2) Meinertz, H.; Faergeman, O. In "Side Effects of Drugs", Annual 2; Dukes, M. N. G., Ed.; Excerpta Medica: Amsterdam and Oxford, 1978; p 358.
(3) Elwood, J. C.; Richert, D. A.; Westerfeld, W. W. *Biochem. Pharm.* **1972**, *21*, 1127–1134.

0022-2623/86/1829-0855$01.50/0

Table I. Physical Constants of the 5,5-Diaryl-2-thiohydantoins and the 5,5-Diaryl-N^3-substituted-2-thiohydantoins



compd	R_1	R_2	R_3	mp,[b] °C	yield,[c] %	formula	anal.[d]	lit. ref.
1			H	>310	81	$C_{15}H_{10}N_2OSCl_2$	C, H, N, S, O, Cl	
2			H	185–186	42	$C_{17}H_{16}N_2O_3S$		*e*
3			H	160–161	55	$C_{16}H_{14}N_2O_2S$	C, H, N, S	
DL mixture			H					
4			H	219–221	26	$C_{15}H_{12}N_2O_2S$	C, H, N, S	
DL mixture			H					
5			H	260–261	33	$C_{13}H_{10}N_4OS$		*f*
6				255–256	93	$C_{21}H_{16}N_2OS$		*g*
7			n-C_4H_9	135–136	96	$C_{19}H_{20}N_2OS$	C, H, N, S	*h*
8			n-C_4H_9	166–167	58	$C_{17}H_{18}N_4OS$	C, H, N, S	

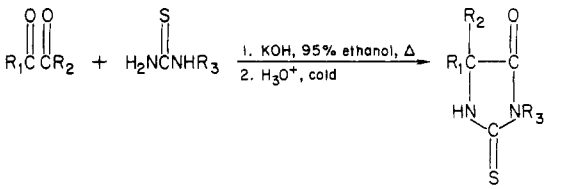
[a] All compounds exhibited ^1H NMR spectra consistent with assigned structures. [b] Melting points are uncorrected. [c] Yield of analytically pure material obtained for the reaction in Scheme I; yields not optimized. [d] Analytical results within ±0.4% of theoretical value. [e] Reference 6. [f] Reference 7. [g] Reference 8. [h] Reference 9.

acid assay is a viable test system for screening "potential" hypolipidemic agents. As further proof of this idea, two compounds not known for hypolipidemic activity in humans, dilantin (the oxygen analogue of DPTH) and thyroxine (L-T_4), were tested and showed little, if any, ability to prevent the accumulation of lipids in the liver of the orotic acid treated animal.

Chemistry. The synthesis of the 5,5-diaryl-2-thiohydantoins substituted in the 4-position was accomplished using the procedure of Biltz[4] in which a substituted benzil is condensed with thiourea. When the required benzil derivative was commercially available, it was purchased and used directly in the synthesis. If a benzil derivative was required that could not be purchased, it was prepared by the benzoin condensation of the appropriately substituted benzaldehyde, followed by nitric acid oxidation to the benzil. The substitutions were initially based on the operational scheme of Topliss[5] for analogue synthesis.

The 5,5-diaryl-N^3-substituted-2-thiohydantoins were prepared in a similar manner using a substituted thiourea in the condensation. These derivatives were suggested as a result of some preliminary testing with the 2-thiohydantoins of the naturally occurring amino acids where the nitrogen at position 3 of the thiohydantoin ring would carry a phenyl group as a consequence of their synthesis from the amino acid and phenyl isothiocyanate. The synthesis for both the 5,5-diaryl-2-thiohydantoins and the 5,5-diaryl-N^3-substituted-2-thiohydantoins used in this study is illustrated in Scheme I.

Scheme I



R_3 = H, n-C_4H_9, C_6H_5

In order to verify substitution at the 3-position nitrogen mass spectral analysis was employed. In each case, the fragmentation pattern obtained indicated the substituent was indeed at the 3-position. As further proof of specific N^3 nitrogen substitution, compound **7** was prepared according to ref 11. Comparisons of TLC, NMR, mass spectral data, and mixture melting points all showed that compound **7** prepared by the original literature procedure and by the substituted urea–benzil condensation procedure were identical. The physical data for the thiohydantoins prepared for this study are reported in Table I.

Biological Results. The compounds prepared for the study presented in this paper were screened for activity by using the orotic acid assay system previously described by Elwood et al.[3] Each compound was added to the diet at a dose equal in molar concentration to DPTH at 600 mg/kg of diet, except for compound **7**. This dose level was chosen since, at this dose, DPTH will reduce liver lipids to basal values or below. The results of this initial trial are presented in Table II. Examination of the data in Table II shows that maximum lipid reduction was achieved with three of the test compounds, DPTH, the parent of the series, 5,5-bis(2-pyridyl)-2-thiohydantoin (**5**), and 5,5-diphenyl-N^3-n-butyl-2-thiohydantoin (**7**). In the phenyl-ring-substituted class, the 5,5-bis(4-methoxy-

(4) Biltz, H. *Ber. Dtsch. Chem. Ges.* **1909**, *42*, 1787.
(5) Topliss, J. G. *J. Med. Chem.* **1972**, *15*, 1006–1011.
(6) Biltz, H. *Ber. Dtsch. Chem. Ges.* **1909**, *42*, 1787.
(7) Thomae, K. Ger. Patent 945 510, 1956.
(8) Eberly, F. A.; Dains, F. B. *J. Am. Chem. Soc.* **1936**, *58*, 2544–2547.
(9) Testa, E.; Ettorre, R. *Arch. Pharm. (Weinheim, Ger.)* **1957**, *290*, 532–536.

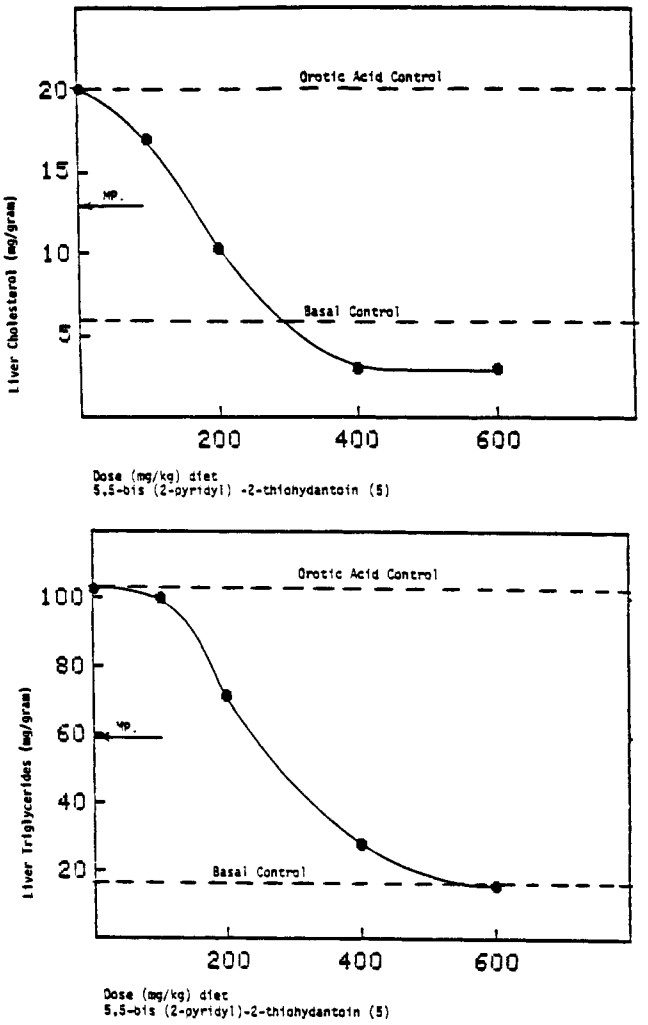


Figure 1. Dose–response curves for 5,5-bis(2-pyridyl)-2-thiohydantoin generated from the data in Table III.

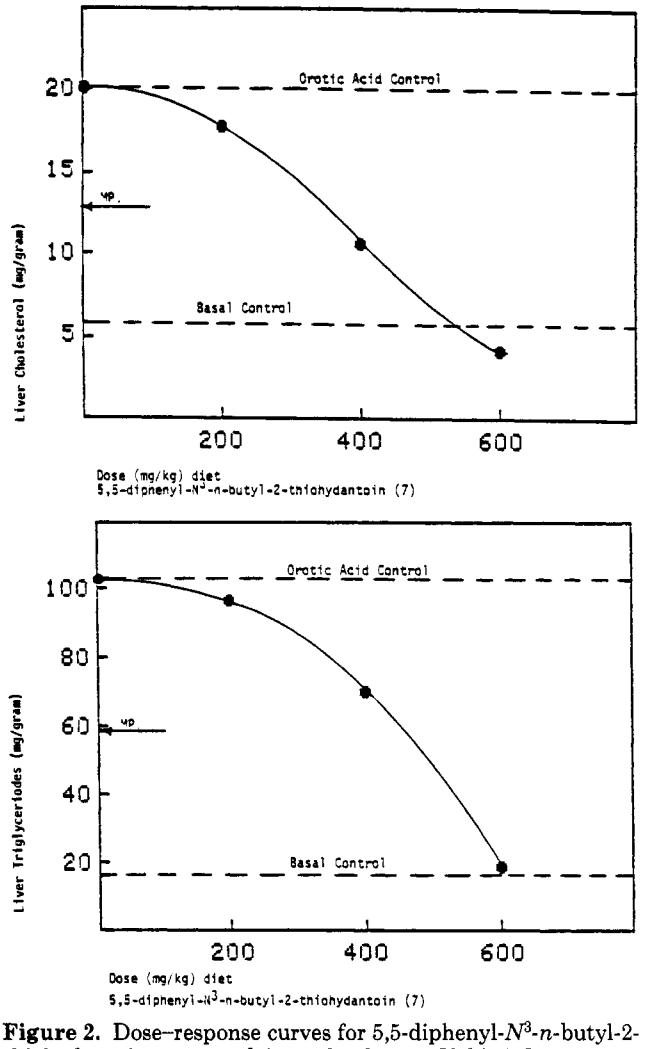


Figure 2. Dose–response curves for 5,5-diphenyl-N^3-*n*-butyl-2-thiohydantoin generated from the data in Table III.

phenyl)-2-thiohydantoin (**2**) was virtually inactive in preventing the accumulation of lipids in the liver. The 5-(4-methoxyphenyl)-5-phenyl-2-thiohydantoin (**3**), a D and L mixture, also showed no reduction in liver lipids at the initial test dose. Compound **1**, 5,5-bis(4-chlorophenyl)-2-thiohydantoin, showed some activity in reducing the lipid levels, but the values were already close to those needed to achieve a midpoint dose[10] level and it was considered doubtful that the compound would show a better dose–response curve than DPTH. Further, the compound showed toxicity as evidenced by the low body weight at sacrifice with normal food consumption. The compound 5-(4-hydroxyphenyl)-5-phenyl-2-thiohydantoin (**4**), also a D and L mixture, showed a greater reduction in cholesterol level than the monomethoxy, dimethoxy, or dichloro derivatives. It was, however, nowhere near the low values achieved by compounds **5**, **7**, and DPTH.

In the series of nitrogen-substituted derivatives, the initial trial reported in Table II showed 5,5-diphenyl-N^3-*n*-butyl-2-thiohydantoin (**7**) to be at least as good as DPTH. Once again, the other members of this series were not as good as DPTH. The 3,5,5-triphenyl-2-thiohydantoin (**6**) showed some activity but was considered too toxic (growth curves were impaired at a normal diet consumption level) for further consideration. The remaining nitrogen-substituted compound, 5,5-bis(2-pyridyl)-N^3-*n*-butyl-2-thiohydantoin (**8**) did not offer an improvement over compounds **5**, **7**, or DPTH and was not evalated further.

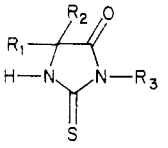
Compounds **5** and **7** were chosen for further evaluation in a full dose–response study. The results of this study are shown in Table III. The data from Table III were then used to plot the dose–response curves shown in Figures 1 and 2. From the dose–response curves, the midpoint dose values were obtained and are reported in Table IV. The midpoint dose values for DPTH as obtained by Elwood et al. have also been included for comparison. By assigning DPTH a relative activity of 1.00, it can be seen that DPYTH (**5**) is slightly more active in cholesterol lowering ability than DPTH, but only half as good at lowering triglycerides. The other promising compound, DPBTH (**7**), was considerably less active in lowering either cholesterol or triglyceride values.

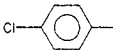
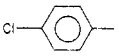
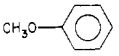
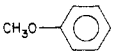
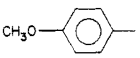
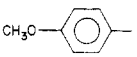
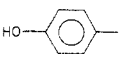
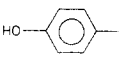
Discussion. The results of this study can be divided into two areas based on the type of structural modification made to the parent compound DPTH.

First, in the phenyl-ring-modified class all of the derivatives were less active or exhibited toxicity when compared to DPTH, with one exception. The exception was 5,5-bis(2-pyridyl)-2-thiohydantoin, DPYTH (**5**). The midpoint dose values of DPYTH indicated a slightly better activity for this compound in lowering cholesterol than DPTH; however, triglyceride lowering activity appeared

(10) For comparison purposes Elwood et al. defined the midpoint dose as the dose of test compound necessary to reduce the liver cholesterol or triglyceride values half-way between the high values of the orotic acid control group and the low values of the basal control group. The midpoint values for DPTH were as follows: cholesterol, 240 mg/kg of diet; triglycerides 100 mg/kg of diet.

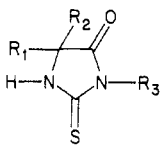
Table II. Results of Biological Evaluation of 5,5-Diaryl-2-thiohydantoins and 5,5-Diaryl-N^3-substituted-2-thiohydantoins



compd[a]	R_1	R_2	R_3	dose,[b] mg/kg diet	body wt, g	liver TG, mg/g wet liver	liver cholesterol, mg/g wet liver
control orotic acid diet				no drug present	145 ± 5.6	102 ± 13.5	19.1 ± 4.4
1			H	750	91 ± 3.2	82 ± 13.9	13.2 ± 1.0
2			H	735	135 ± 6.5	82 ± 6.7	21.6 ± 1.9
3			H				
DL mixture			H	667	144 ± 2.3	70 ± 4.9	23.1 ± 1.5
4			H				
DL mixture			H	635	130 ± 4.3	48 ± 10.2	9.1 ± 1.7
5			H	600	132 ± 8.2	16 ± 7.7	3.0 ± 1.0
6				770	91 ± 6.0	42 ± 10.0	8.6 ± 1.0
7			n-C_4H_9	600	134 ± 5.4	19 ± 5.4	4.2 ± 1.3
8			n-C_4H_9	729	140 ± 4.2	85 ± 7.8	12.2 ± 1.2
DPTH			H	600	136 ± 6.1	16 ± 6.7	3.0 ± 1.0
basal control				no orotic acid; no drug present	134 ± 5.5	16 ± 4.0	6.0 ± 2.0

[a] Unless otherwise noted, each test group used six male rats of the Sprague–Dawley strain. [b] Test compounds were added to the orotic acid diet at concentrations equimolar to DPTH at 600 mg/kg, except for 7. Values needed for midpoint dose as defined by Elwood: cholesterol, 12.6 ± 3.2 mg/kg wet liver; triglyceride, 59 ± 8.7 mg/g wet liver.

Table III. Dose–Response Data for 5,5-Bis(2-pyridyl)-2-thiohydantoin (5) and 5,5-Diphenyl-N^3-n-butyl-2-thiohydantoin (7)



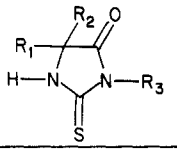
compd[a]	R_1	R_2	R_3	dose,[b] mg/kg diet	body wt, g	liver TG, mg/gram wet liver	liver cholesterol, mg/gram wet liver
control orotic acid diet				no drug present	151 ± 6.5	103 ± 15.0	20.6 ± 1.9
DPYTH (5)			H	600	132 ± 8.2	16 ± 7.7	3.0 ± 1.0
				400	203 ± 2.6	28 ± 3.8	2.8 ± 0.7
				200	181 ± 6.3	72 ± 14.2	10.3 ± 1.6
				100	180 ± 8.0	100 ± 13.1	17.0 ± 3.1
DPBTH (7)			n-C_4H_9	600	134 ± 5.4	19 ± 5.4	4.2 ± 1.3
				400	210 ± 4.7	70 ± 15.6	10.7 ± 2.3
				200	185 ± 2.5	97 ± 13.9	17.8 ± 1.5
basal control				no orotic acid; no drug present	155 ± 5.0	16 ± 3.7	6.0 ± 2.1

[a] Unless otherwise noted, each test group used six male rats of the Sprague–Dawley strain. [b] Test compounds were added to the orotic acid diet at the concentrations shown. Values needed for midpoint dose as defined by Elwood: cholesterol, 12.6 ± 3.2 mg/g wet liver; triglyceride, 59 ± 8.7 mg/g wet liver.

to be slightly less. Since it is cholesterol that is most often indicated in cardiovascular disorders, the increased activity of DPYTH on this lipid component may be an advantage to this class of 2-thiohydantoin. Further structural modifications to DPYTH are under way and will be the subject of a future report.

The second type of structural modification to DPTH centered on placing substituents on the nitrogen at position

Table IV. Midpoint Dose Data and Relative Activity Comparison of DPTH, 5,5-Bis(2-pyridyl)-2-thiohydantoin (5) and 5,5-Diphenyl-N^3-*n*-butyl-2-thiohydantoin (7)



compd	R_1	R_2	R_3	midpoint dose[d] TG	midpoint dose[d] cholesterol	rel act.[b] TG	rel act.[b] cholesterol	ref
DPTH			H	100	240	1.00	1.00	*c*
DPYTH (5)			H	235	180	0.43	1.33	*d*
DPBTH (7)			*n*-C_4H_9	450	340	0.22	0.71	*e*

[a] Dietary concentrations of test compound (mg/kg diet) required to reduce liver lipids half-way from the average orotic acid level to the basal level. Midpoints: triglyceride, 59 ± 8.7 mg/g wet liver; cholesterol, 12.6 ± 3.2 mg/g wet liver. [b] DPTH is assigned a relative activity level of 1.00 for comparison purposes. [c] Reference 3. [d] Table III; Figure 1. [e] Table III; Figure 2.

3 of the thiohydantoin ring. Once again, the modifications resulted in decreased activity compared to DPTH, with one exception. The exception, 5,5-diphenyl-N^3-*n*-butyl-2-thiohydantoin, DPBTH (7), held some promise in the initial screening, but upon testing in a full dose–response study the midpoint dose values for both cholesterol and triglyceride lowering indicated a compound considerably less active than DPTH.

Experimental Section

The structures of all compounds are supported by their ^1H NMR spectra (Varian A60A; tetramethylsilane) and by selected mass spectra (Hitachi RMU-6) where necessary. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Bristol-Myers Pharmaceuticals Analytical Division. Results are within ±0.4% of theoretical values unless otherwise noted. All intermediates and final products were crystallized three times from 95% ethanol. The following set of procedures traces the synthesis of a typical 5,5-diaryl-2-thiohydantoin where the necessary substituted benzil was not commercially available. The other 5,5-diaryl-2-thiohydantoins were prepared in a similar fashion, but the commercially available substituted benzil was used in reaction procedure III without any preliminary treatment.

General Procedure I. Preparation of a 4,4′-Disubstituted Benzoin. A benzaldehyde with the appropriate 4-position substituent (1.0 mol) was weighed into a 1-L three-necked flask to which was added 95% ethanol (210 mL) and distilled water (105 mL). With continuous stirring, potassium cyanide (0.161 mol) was added and the system refluxed for 6 h. The flask was cooled and the crystalline precipitate collected and recrystallized from 95% ethanol. The material was used directly in the next reaction without further evaluation.

General Procedure II: Preparation of a 4,4′-Disubstituted Benzil. The 4,4′-disubstituted benzoin from procedure I (0.189 mol) was weighed into a 1-L beaker. Glacial acetic acid (200 mL) was added and the mixture heated on a hot plate. When solution was attained, concentrated nitric acid (100 mL) was added and the solution heated at 100 °C for 2 h. The solution was cooled and poured into 1 L of an ice–water mixture and the resulting precipitate collected and recrystallized from 95% ethanol. The material was used directly in the next reaction without further evaluation.

General Procedure III: Preparation of a 5,5-Bis(4-substituted phenyl)-2-thiohydantoin. A 4,4′-disubstituted benzil from procedure II or a commercially available disubstituted benzil (0.119 mol) and thiourea (0.263 mol) was weighed into a 1-L three-necked flask. Solid potassium hydroxide (0.100 mol) was added, and with constant stirring, the system was heated to reflux. Reflux was continued for a total of 3 h. The reaction mixture was cooled, poured into 2 L of an ice-water mixture, and the initial precipitate that formed was collected by filtration and set aside. The clear filtrate was acidified by bubbling carbon dioxide gas through the solution, and the crystalline material that formed was collected and combined with the first precipitate and the whole batch recrystallized from 95% ethanol. To prepare a 5,5-diaryl-2-thiohydantoin with a substituent on the 3-position nitrogen, a substituted thiourea was used in this procedure.

Pharmacological Methods. Potential hypolipidemic activity was evaluated in the orotic acid assay system previously described by Elwood et al.[3] Unless otherwise noted all test groups consisted of six male Sprague–Dawley rats. Test compounds were added to the orotic acid diet at the concentrations shown in Table II. Diets and water were given ad libitum for 14 days before sacrifice, and growth curves and diet consumption were monitored to ensure ingestion of the test compounds. Impaired growth curves with normal diet consumption were taken as an indication of a toxic reaction to the test compound. At sacrifice, liver homogenates were prepared in cold 0.63 M, pH 7.4 phosphate buffer. An aliquot of the homogenate was added to a screw-cap tube containing Zeolite resin (to remove water and phospholipids) and chloroform to extract the triglycerides and cholesterol. An aliquot of the chloroform solution was evaporated to dryness and assayed for cholesterol or triglyceride by the procedures of Zlatkis[11] and Butler[12] et al., respectively.

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Registry No. 1, 23258-84-6; 2, 17816-69-2; (±)-3, 100899-15-8; (±)-4, 100899-16-9; 5, 100899-17-0; 6, 52460-98-7; 7, 100899-18-1; 8, 100899-19-2; 4-$ClC_6H_4COCOC_6H_4Cl$-4, 3457-46-3; 4-$H_3COC_6H_4COCOC_6H_4OCH_3$-4, 1226-42-2; 4-$H_3COC_6H_4COCOC_6H_5$, 22711-21-3; 4-$HOC_6H_4COCOC_6H_5$, 33288-79-8; $C_6H_5COCOC_6H_5$, 134-81-6; $H_2NC(S)NH_2$, 62-56-6; $H_2NC(S)NHC_6H_5$, 103-85-5; $H_2NC(S)NHC_4H_9$, 1516-32-1; bis-(2-pyridinyl)ethanedione, 492-73-9.

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(12) Butler, W. M. Jr.; Maling, H. M.; Horning, M. G.; Brodie, B. B. *J. Lipid Res.* **1961**, *2*, 95.