Synthesis and Hypolipidemic Activity of 2-Substituted Isobutyric Acid Derivatives

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A series of 2-substituted isobutyric acid derivatives have been synthesized and evaluated as hypolipidemic agents. Compounds **11** and **20** were found to decrease the level of plasma total cholesterol in experimental hyperlipidemic rats to a greater extent than clofibrate (CF) and to increase the level of plasma high-density lipoprotein cholesterol to the same extent as gemfibrozil (GF). Increase in liver weight caused by these compounds were less than those with CF and GF.

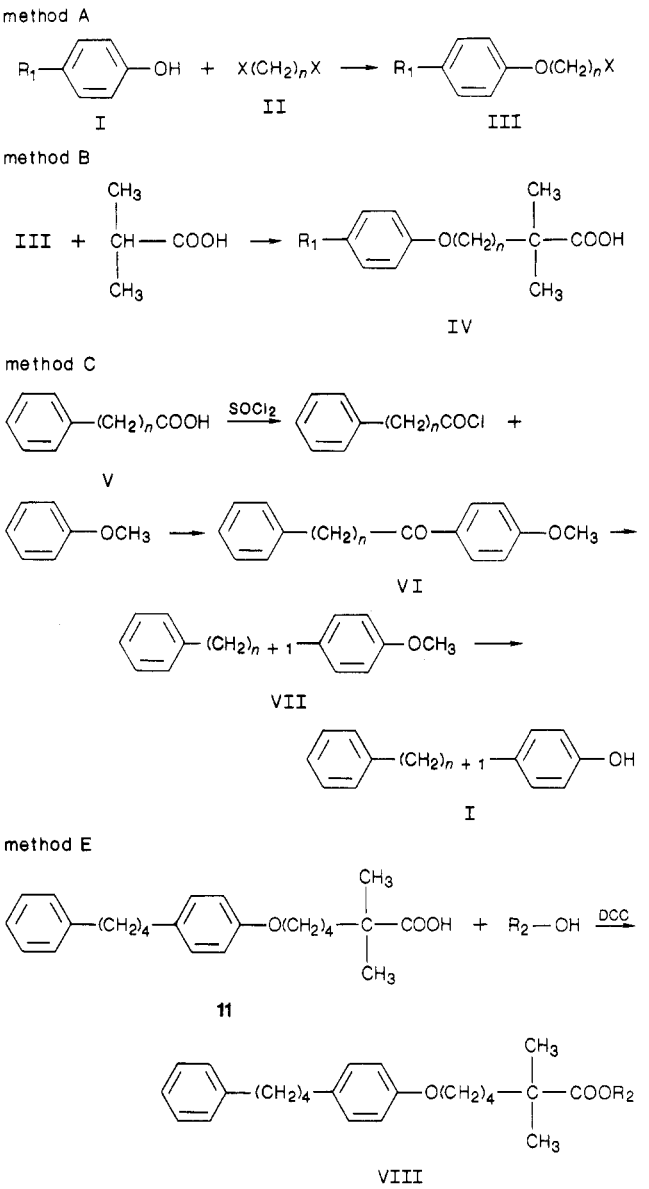
Clofibrate (CF) is used extensively in the treatment of hyperlipidemia.[1] The striking response to this compound has been a fall in both plasma total cholesterol (TC), a positive risk factor for ischemic heart disease,[2] and triglycerides (TG) in experimental hyperlipidemic rats[3] and in humans.[1] This compound, however, does not increase the level of high-density lipoprotein cholesterol (HDLC), a negative risk factor for ischemic heart disease and atherosclerosis,[2,4] in hyperlipidemic rats[3] and human patients.[5] On the other hand, gemfibrozil (GF), which is also used to lower lipid levels,[6] significantly increases plasma HDLC levels, but has little affect on plasma TC levels.[2,7] Reddy et al.[8] speculated that hepatic peroxisome proliferators, which include CF and GF, induce hepatocellular carcinoma by disturbing subcellular organelle homeostasis (i.e., related to peroxisome proliferation and H_2O_2 generation). They also stated that hepatic peroxisome proliferators induced hepatomegaly and that the peroxisome proliferation was frequently associated with hypotriglyceridemic compounds. Although a recent study by the WHO did not find any relationship between CF treatment and the incidence of carcinogenesis,[9] it would clearly be desirable for a hypolipidemic agent to have no toxicological action on the liver. The purpose of the present study was to find compounds that would reduce plasma TC and TG more effectively than CF, increase plasma HDLC more effectively than GF, and produce only a minimal increase in liver weight in comparison with either of the two compounds. A series of 2-substituted isobutyric acid derivatives were synthesized, and the structure–activity relationships of these compounds were determined in experimental hyperlipidemic rats.

Chemistry

The desired compounds, 2,2-dimethyl-ω-(substituted phenoxy)alkanoic acids (IV, **1–24** except for **8** and **10**), were prepared by condensing substituted phenol (I) and α,ω-dihaloalkanes (II) in the presence of bases to give α-halo-ω-(substituted phenoxy)alkanes (III) (method A, Scheme I). Treatment of III and the dianion of 2-methylpropionic acid gave IV (method B). (α-Phenyl-ω-(4-hydroxyphenyl)alkanes) (I) were prepared by converting ω-phenylaklanoic acids (V) to their acid chlorides followed by Friedel–Crafts reaction with anisole to give α-phenyl-ω-(4-methoxybenzoyl)alkanes (VI). Wolff–Kishner reduction of VI gave α-phenyl-ω-(4-methoxyphenyl)alkanes (VII), which were hydrolyzed to give I (method C).

Esters VIII were prepared by condensation of **11** and alcohols with dicyclohexylcarbodiimide (DCC) (method E). Other esters (VIII) were prepared from the alcohol of **11**, which was produced by reduction with lithium aluminum

Scheme I[a]



[a] X = Cl or Br.

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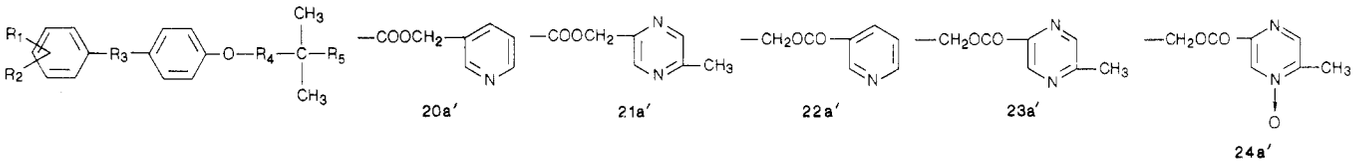
Table I. 2-Substituted Isobutyric Acid Derivatives[a]



no.	R_1	R_2	R_3	R_4	R_5	starting compd	prepn method	mp, °C	yield, %	recrystn solvent	formula	anal.
1	H	H		$(CH_2)_3$	COOH	1a	A_1,B	149	46	AcOEt–Hex	$C_{19}H_{22}O_3$	C,H
2	H	H	CH_2	$(CH_2)_3$	COOH	2a	B	105	75	AcOEt	$C_{20}H_{24}O_3$	C,H
3	H	H	$(CH_2)_2$	$(CH_2)_3$	COOH	3a	A_1,B	100–101	43	AcOEt–Hex	$C_{21}H_{26}O_3$	C,H
4	H	H	$(CH_2)_3$	$(CH_2)_3$	COOH	4a	A_2,B	93–94	54	Hex	$C_{22}H_{28}O_3$	C,H
5	H	H	$(CH_2)_4$	$(CH_2)_3$	COOH	5a	C,A_2,B	85–86	70	Hex	$C_{23}H_{30}O_3$	C,H
6	H	H	$(CH_2)_5$	$(CH_2)_3$	COOH	6a	A_2,B	83–84	56	ether–Pet.	$C_{24}H_{32}O_3$	C,H
7	H	H	$(CH_2)_6$	$(CH_2)_3$	COOH	7a	C,B		19	(Hex–Pet.)	$C_{25}H_{34}O_3$	C,H
8	H	H	CO	$(CH_2)_3$	COOH	8a	A_1,B,D	95	24	AcOEt–Hex	$C_{20}H_{22}O_4$	C,H
9	H	H	$CONHCH_2CH_2$	$(CH_2)_3$	COOH	9a	A_3,B	151–152	39	acetone	$C_{22}H_{27}NO_4$	C,H,N
10	H	H	$(CH_2)_4$		COOH	5a	C,G	91–92	82	Hex	$C_{20}H_{24}O_3$	C,H
11	H	H	$(CH_2)_4$	$(CH_2)_4$	COOH	5a	C,A_2,B	87–89	64	Pet.–Hex	$C_{24}H_{32}O_3$	C,H
12	H	H	$(CH_2)_4$	$(CH_2)_5$	COOH	5a	C,A_2,B	77–78	80	Hex	$C_{25}H_{34}O_3$	C,H
13	H	H	$(CH_2)_4$	$(CH_2)_6$	COOH	5a	C,A_2,B	56–57	62	(Hex–AcOEt)	$C_{26}H_{36}O_3$	C,H
14	H	H	$(CH_2)_4$	$(CH_2)_7$	COOH	5a	C,A_2,B	63–64	67	(Hex–AcOEt)	$C_{27}H_{38}O_3$	C,H
15	H	H	$(CH_2)_4$	$(CH_2)_8$	COOH	5a	C,A_2,B	60–61	64	(Hex–AcOEt)	$C_{28}H_{40}O_3$	C,H
16	H	H	$(CH_2)_4$	$(CH_2)_9$	COOH	5a	C,A_2,B	65–66	66	(Hex–AcOEt)	$C_{29}H_{42}O_3$	C,H
17	H	H	$(CH_2)_4$	$(CH_2)_{10}$	COOH	5a	C,A_2,B	68–69	60	(Hex–AcOEt)	$C_{30}H_{44}O_3$	C,H
18	2-CH_3	4-CH_3	$(CH_2)_4$	$(CH_2)_4$	COOH	18a	C,A_2,B	49–52	34	Hex	$C_{26}H_{36}O_3$	C,H
19	2-CH_3	5-CH_3	$(CH_2)_4$	$(CH_2)_4$	COOH	19a	C,A_2,B		42	(Hex–AcOEt)	$C_{26}H_{36}O_3$	C,H
20	H	H	$(CH_2)_4$	$(CH_2)_4$	20a′	11,20a	E		44	(Hex–AcOEt)	$C_{30}H_{37}NO_3$	C,H,N
21	H	H	$(CH_2)_4$	$(CH_2)_4$	21a′	11,21a	E		40	(Hex–AcOEt)	$C_{30}H_{38}N_2O_3$	C,H,N
22	H	H	$(CH_2)_4$	$(CH_2)_4$	22a′	11,22a	F		58	(Hex–AcOEt)	$C_{30}H_{37}NO_3$	C,H,N
23	H	H	$(CH_2)_4$	$(CH_2)_4$	23a′	11,23a	F		42	(Hex–AcOEt)	$C_{30}H_{38}N_2O_3$	C,H,N
24	H	H	$(CH_2)_4$	$(CH_2)_4$	24a′	11,24a	F		26	(Hex–AcOEt)	$C_{30}H_{38}N_2O_4$	C,H,N

[a] Hex = hexane, Pet. = petroleum ether. Figures in parentheses indicate the solvent used for column chromatography.

hydride and condensation with carboxylic acids in the presence of DCC to give VIII (method F).

Compound **8** was prepared by hydrolysis of **8c**. After the preparation of **8a** by general methods A and B, compound **8c** was prepared by Friedel–Crafts reaction of **8b** (ethyl ester of **8a**) with benzoyl chloride (method D). Compound **10** was prepared by hydrolysis of **10a**. Compound **10a** was prepared by condensation of I and ethyl 2-halo-2-methylpropionate. The compounds prepared are shown in Table I.

Biological Results and Discussion

The hypolipidemic effects of the compounds were evaluated in rats fed a high-cholesterol diet. The results of the experiments are shown in Table II. The chemical structure of **1** was derived from GF by the introduction of a phenyl group into the 4-position of the phenyl group of GF instead of 2,5-methyl groups. By substitution of the phenyl group with methyl groups, the plasma-HDLC-increasing effect of GF disappeared and TC- and TG-decreasing effects appeared. Methylene groups (n = 1–6, 2–7), a carbonyl group (**8**), and $-CONHCH_2CH_2-$ (**9**) were introduced between the phenyl groups of **1**. The number of methylene groups between the phenyl groups did not affect the ability of compounds to increase plasma HDLC levels. Most of these compounds (**2–7**) decreased plasma TC and TG levels. By the introduction of $-CONHCH_2CH_2-$ between the phenyl groups of **1**, the plasma TC-decreasing effect of **1** disappeared and a plasma-HDLC-increasing effect appeared. The effects of **9** were similar to those of GF, but the increment in liver weight became worse. Introduction of a carbonyl group between the phenyl groups of **1** (**8**), produced a compound with enhanced toxicity. A preliminary experiment showed that, after repeated administration of **8** for 7 days, normal gain in body weight in rats was inhibited. In the present experiment, 10 mg/kg per day of **8** was given. Although **8** reduced plasma TC and TG, it did not affect plasma HDLC and markedly increased liver weight.

As compounds **4–6** showed similar hypolipidemic effects, the structure of **5** was modified by varying the number of methylene groups between the oxygen and tertiary carbon. Increasing the chain length produced compounds with plasma-HDLC-increasing effects, except for **17** (m = 10). Moreover, plasma-TG-decreasing effects disappeared after the number of methylene groups exceeded 5. The most promising compound at this stage was **11**, which fulfilled most of our requirements. The plasma-TC- and -TG-reducing effects of **11** were better than those of CF, and its plasma-HDLC-increasing effects were the same as those of GF. Its liver-weight-increasing effect was the same as that of CF and did not exceed that of GF.

In order to find a compound that fulfilled our needs to a better degree than **11**, the hypolipidemic effects of some derivatives of **11** were compared. Compound **18**, which, like GF, has methyl groups at the 2- and 5-positions, had the most marked plasma-HDLC-increasing effect of all the compounds tested in this series. This compound effectively decreased plasma TC, but did not affect plasma TG and strongly increased liver weight. Increments of both plasma HDLC and liver weight were closely correlated in the experiments on GF, **8** and **19**. Nicotinic acid and pyrazinecarboxylic acid, some of which reportedly have hypolipidemic activity,[10,11] or their carbinol derivatives of were introduced into compound **11** or its carbinol form (compounds **20–24**). Compound **20** decreased plasma TC more effectively than CF, increased it more effectively than GF, and showed a tendency to decrease plasma TG as well as CF. The increase in liver weight caused by this com-

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Table II. Hypolipidemic Effects of 2-Substituted Isobutyric Acid Derivatives[a]

drugs	dose (mg/kg per day, po)	no.[1] of animals	TC,[1] mg/dL	HDLC,[1] mg/dL	TG,[1] mg/dL	liver wt, mg/g body weight: HC diet[1]	liver wt, mg/g body weight: normal diet[2]
control		7	450.9 ± 64.3	31.6 ± 4.5	119.3 ± 10.7	53.9 ± 0.5	
normal control		7	129.3 ± 6.4**	73.5 ± 4.4**	31.7 ± 4.7**	33.1 ± 0.5**	35.3 ± 0.8
GF	100	6	358.7 ± 31.4	51.5 ± 2.4**	53.1 ± 8.7**	54.8 ± 1.0	51.4 ± 0.6**
CF	100	7	251.2 ± 49.1*	29.4 ± 2.1	51.8 ± 6.9**	52.8 ± 0.4	43.1 ± 0.9**
1	100	7	222.7 ± 22.7*	39.9 ± 2.0	54.3 ± 4.8**	54.7 ± 0.7	
2	100	6	284.4 ± 51.4	29.9 ± 3.5	84.7 ± 7.6*	53.2 ± 0.7	
3	100	7	282.8 ± 21.8	32.0 ± 3.0	92.7 ± 9.4	53.5 ± 0.7	
4	100	7	217.5 ± 30.5**	40.6 ± 4.6	79.5 ± 10.6*	54.0 ± 0.5	39.1 ± 1.6
5	100	7	258.3 ± 19.8*	35.2 ± 2.3	76.9 ± 9.9*	54.5 ± 1.0	
6	100	7	261.9 ± 29.2*	38.1 ± 3.9	71.9 ± 5.8**	54.1 ± 0.6	40.0 ± 1.7*
7	100	7	256.1 ± 25.5*	38.4 ± 3.1	86.2 ± 20.2	52.1 ± 0.6*	
8	100	7	260.8 ± 23.6*	57.6 ± 5.5**	53.6 ± 9.2**	81.8 ± 1.8**	
9	100	7	204.1 ± 17.6**	42.1 ± 2.0	49.4 ± 4.3**	57.0 ± 0.6**	
10	100	7	194.6 ± 5.9**	50.7 ± 2.9**	41.3 ± 4.0**	67.1 ± 1.1**	54.6 ± 1.4**
11	100	7	159.5 ± 9.1**	49.4 ± 3.0**	49.0 ± 4.5**	55.8 ± 1.1	41.7 ± 0.9**
control		7	313.3 ± 47.7	40.4 ± 3.5	69.1 ± 13.8	56.5 ± 1.6	
normal control		7	116.8 ± 3.6**	79.1 ± 2.7**	34.5 ± 3.2*	34.3 ± 1.3**	35.0 ± 0.9
GF	100	7	255.0 ± 32.2	57.8 ± 2.4**	51.5 ± 4.0	58.2 ± 1.9	48.8 ± 0.8**
CF	100	7	181.6 ± 11.5*	38.7 ± 6.4	41.4 ± 2.9	58.8 ± 1.7	40.9 ± 1.0**
11	100	7	132.9 ± 4.0**	50.9 ± 1.5*	33.8 ± 2.6*	59.1 ± 1.5	40.7 ± 0.7**
12	100	7	190.6 ± 9.0*	52.4 ± 2.1*	60.4 ± 4.7	58.3 ± 1.6	36.7 ± 0.4
13	100	7	150.7 ± 9.4*	58.1 ± 2.3**	48.9 ± 2.3	60.2 ± 1.5	42.3 ± 0.6**
14	100	7	145.7 ± 3.5*	65.2 ± 3.8**	43.0 ± 3.5	57.6 ± 0.5	40.1 ± 0.8**
15	100	7	150.0 ± 6.9*	53.7 ± 5.0*	47.6 ± 2.2	58.4 ± 0.7	39.3 ± 0.3**
16	100	7	173.6 ± 12.1*	55.0 ± 3.7*	57.3 ± 5.2	57.5 ± 1.1	38.2 ± 0.7*
17	100	7	248.6 ± 25.1	48.6 ± 4.0	52.8 ± 5.3	57.2 ± 1.7	
18	100	7	173.9 ± 9.0*	73.0 ± 7.4**	37.0 ± 7.2	62.3 ± 1.1*	41.8 ± 1.1**
19	100	7	125.9 ± 9.6**	56.0 ± 2.3**	34.4 ± 3.3*	59.9 ± 1.1	40.0 ± 0.5**
20	100	7	154.3 ± 13.4*	59.6 ± 6.7*	43.9 ± 4.4	58.2 ± 1.5	38.0 ± 0.3*
21	100	7	181.5 ± 16.1*	51.7 ± 3.1	36.9 ± 5.9*	59.6 ± 1.4	38.2 ± 0.6*
22	100	7	152.0 ± 15.5*	49.9 ± 3.1	46.7 ± 3.2	57.8 ± 1.4	39.7 ± 0.7**
23	100	7	145.1 ± 15.1*	47.6 ± 2.3	41.0 ± 5.7	58.0 ± 1.2	
24	100	7	146.6 ± 13.0*	45.3 ± 2.4	45.8 ± 4.1	57.8 ± 1.0	

[a] The data are expressed as means ± SE. TC = plasma tota cholesterol, HDLC = plasma high-density lipoprotein cholesterol, TG = plasma triglyceride, GF = gemfibrozil, CF = clofibrate. (*) $P < 0.05$, (**) $P < 0.01$ as compared with the control group using the Student's t test. (1) Data obtained from rats fed a high-cholesterol (HC) diet. (2) Data obtained from rats fed a normal diet.

pound was less than that of CF. Although compounds **21–24** decreased plasma TC, they did not increase plasma HDLC.

Compounds **11** and **20** fulfilled most of our requirements for a hypolipidemic agent.

Experimental Section

Biological Methods. Three-week-old male Wistar/KY rats (SPF) were used after an acclimation period of 7 days. Rats were fed for 14 days on a high-cholesterol diet (10 g of cholesterol, 10 g of cholic acid, 60 g of cottonseed oil, and 920 g of a commercially available powder food, F2, from Sankyo Labo Service). The test compounds were also administered for 14 days at a daily dose of 100 mg/kg. The dosing vehicle was 0.5% sodium (carboxymethyl)cellulose solution, and the dosing volume was 10 mL/kg body weight. On day 14, after fasting for 17 h, blood was drawn from the jugular sinus with a heparinized syringe. Plasma was obtained by centrifugation. Plasma TC was determined enzymatically[12] (cholesterol C-test, Wako). HDLC was determined in the serum supernatant, by using a similar method, after precipitation of other lipid fractions with heparin and Mg^{2+} [13] (HDL-cholesterol test, Wako). Plasma TG was determined by using a modification of the method of Van Handel[14] (Triglyceride-test, Wako). The signifcance of differences between treated groups and control groups was evaluated by the Student's t test.

Chemistry. All melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. The structures of the compounds were confirmed by elemental analysis, IR spectrometry, and ^1H NMR spectrometry. IR spectra were recorded with a Hitachi 260-50 spectrophotometer and ^1H NMR spectra were measured on a Hitachi R-24B spectrometer using Me_4Si as an internal standard. Chromatographic separations were performed on a silica gel column (Wakogel C-200, particle size 74–149). TLC was carried out on Merck silica gel 60 F_{2541}. 4-Phenylphenol (**1a**), 1-bromo-3-(4-benzylphenoxy)propane (**2a**), phenol, 4-phenylbutanoic acid (**5a**), and nicotinic acid (**22a**) were purchased as commercially available reagents. 4-(2-Phenylethyl)phenol (**3a**),[15] 4-(3-phenylpropyl)phenol (**4a**),[16] 4-(5-phenylpentyl)phenol (**6a**)[17] 6-phenylhexanoic acid (**7a**),[18] *N*-[2-(4-hydroxyphenyl)ethyl]benzamide (**9a**),[19] 4-(2,5-dimethylphenyl)butanoic acid (**18a**),[20] 4-(2,4-dimethylphenyl)butanoic acid (**19a**),[20] 3-pyridylmethanol (**20a**),[21] (2-methyl-5-pyrazinyl)methanol (**21a**),[22] 2-methyl-5-pyrazinecarboxylic acid (**23a**),[23] and 2-methyl-5-pyrazinecarboxylic acid 1-oxide (**24a**)[23] were prepared by using the methods described in the literature.

Method A. General Method for the Preparation of α-Halo-ω-(substituted phenoxy)alkanes. Method A_1. A solution of 0.1 mol of base (NaOH) in 20 mL of water was added dropwise to a stirred mixture of 0.1 mol of substituted phenol, 0.15 mol of α,ω-dihaloalkane, and 100 mL of water, and the resulting

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reaction mixture was refluxed for 8 h. The mixture was cooled and extracted with 300 mL of ether, and the ether solution was sequentially washed with 5% NaOH solution and water and concentrated. Excess α,ω-dihaloalkane was removed under reduced pressure, and the residual solid was recrystallized or was subjected to column chromatography on silica gel.

Method A_2. When sodium hydride (0.11 mol) was used as the base in the condensation reaction of the substituted phenol (0.1 mol) and α,ω-dihaloalkane (0.15 mol), dry tetrahydrofuran (THF) was used as the solvent. After the reaction, 200 mL of water was added, and the mixture was extracted with 200 mL of ether. The ether extract was worked up by using the same procedure as described for method A_1.

Method A_3. Preparation of 1-Bromo-3-[4-[2-(benzoylamino)ethyl]phenoxy]propane (9b). A mixture of 0.12 mol (6.5 g) of sodium methoxide, 100 mL of methanol, and 0.10 mol (24.1 g) of *N*-[2-(4-hydroxyphenyl)ethyl]benzamide (**9a**) was refluxed for 1 h. The methanol was evaporated off and the residue was suspended in 100 mL of benzene. To this suspension was added 0.15 mol (15.2 mL) of 1,3-dibromopropane dropwise over a 1-h period at 0 °C, followed by heating at reflux for 1 h. The benzene was removed in vacuo and the residue was dissolved in 200 mL of dichloromethane, washed with 10% HCl and water, dried over $CaCl_2$, and concentrated. The residual solid was recrystallized with ethanol to give 21.7 g of **9b** (yield, 60.0%) as colorless crystals. Compound **9b** was subjected to method B to give **9**.

Method B. General Method for the Preparation of 2,2-Dimethyl-ω-(substituted phenoxy)alkanoic Acid. To a stirred mixture of 0.08 mol (8.81 g) of sodium isobutyrate, 0.08 mol (8.10 g) of diisopropylamine, and 100 mL of dried THF was added a 0.08-mol (51.2-mL) solution of *n*-butyllithium in *n*-hexane at 0 °C. The mixture was stirred at 30 °C for 30 min. A solution of 0.08 mol of 1-halo-ω-(substituted phenoxy)alkanes in 50 mL of dried THF was added to this mixture at 10 °C and left to stand overnight at room temperature. The mixture was poured into 300 mL of ice–water, and the aqueous phase was acidified to pH 1 with 6 N HCl and then extracted with 300 mL of ether. The ether solution was washed with saturated NaCl solution, dried over anhydrous $MgSO_4$, and concentrated. The residual solid was recrystallized or was subjected to column chromatography on silica gel.

Method C. General Method for the Preparation of Aralkyl-Substituted Phenol [α-Phenyl-ω-(4-hydroxyphenyl)alkanes]. ω-Phenylalkanoyl Chloride. To 0.22 mol of ω-phenylalkanoic acid was added 0.26 mol (30.9 g) of thionyl chloride, and the mixture was refluxed for 3 h. The desired compound was obtained by distillation under reduced pressure.

α-Phenyl-ω-(4-methoxybenzoyl)alkanes. A solution of 0.04 mol of ω-phenylalkanoyl chloride in 0.4 mol of anisole was added dropwise to a solution of 0.05 mol (6.67 g) of anhydrous $AlCl_3$ in 0.4 mol of anisole, with the temperature kept around 0 °C during addition. The mixture was stirred for 1 h at room temperature and then was poured into 200 mL of ice–water and acidified to pH 1 with 10% HCl. The isolated organic phase was washed with 10 mL of water and dried over anhydrous $MgSO_4$. After evaporation of the anisole, the residue was recrystallized from ether and pentane.

α-Phenyl-ω-(4-methoxyphenyl)alkanes. To a solution of 0.133 mol of α-phenyl-ω-(4-methoxybenzoyl)alkane in 250 mL of diethylene glycol were added 0.4 mol of 80% hydrazine hydrate and 0.66 mol of NaOH, and the mixture was refluxed for 4 h. Excess hydrazine and water were removed by distillation, and the residue was left to stand for 7 h at 200 °C. After cooling, 200 mL of water was added, and the solution was acidified to pH 1 with concentrated H_2SO_4 and extracted with 300 mL of benzene. The benzene solution was washed with 10 mL of saturated NaCl solution, dried over anhydrous $MgSO_4$, and concentrated. The desired compound was obtained by distillation under reduced pressure.

α-Phenyl-ω-(4-hydroxyphenyl)alkanes. To 0.1 mol of α-phenyl-ω-(4-methoxyphenyl)alkane was added 0.2 mol of pyridine hydrochloride, and the mixture was stirred for 4 h at 200 °C. After cooling, 100 mL of water was added to this mixture, which was then acidified to pH 1 with 10% HCl and extracted with 300 mL of ether. The ether solution was washed with water, dried over anhydrous $MgSO_4$, and concentrated. The desired compound was obtained by distillation under reduced pressure.

Method D. Preparation of 2,2-Dimethyl-5-(4-benzoylphenoxy)pentanoic Acid (8). 2,2-Dimethyl-5-phenoxypentanoic acid (**8a**) was prepared from phenol by using methods A_1 and B.

Ethyl 2,2-Dimethyl-5-phenoxypentanoate (8b). A mixture of 0.135 mol (30.0 g) of **8a**, 200 mL of absolute ethanol, and 6 mL of concentrated H_2SO_4 was refluxed for 10 h. The ethanol was evaporated from the mixture, and 200 mL of ether was added to the resulting residue. The ether solution was sequentially washed with saturated sodium bicarbonate solution and water, dried over anhydrous $MgSO_4$, and concentrated. The oily residue was distilled under reduced pressure to give 24.7 g (yield 87.9%) of colorless oily liquid, bp 107–109 °C (0.5 mmHg).

Ethyl 2,2-Dimethyl-5-(4-benzoylphenoxy)pentanoate (8c). A solution of 0.02 mol (4.7 g) of **8b** and 0.022 mol (3.1 g) of benzoyl chloride was added to a suspension of 0.026 mol of anhydrous $AlCl_3$ in 20 mL of dried dichloromethane. The mixture was left to stand for 1 h at room temperature and then refluxed for 3 h. The mixture was poured into 50 mL of ice–water and acidified with 3 mL of concentrated HCl. The separated organic phase was sequentially washed with 5 mL of saturated sodium bicarbonate solution and 5 mL of water, dried over anhydrous $MgSO_4$, and concentrated. The residue was subjected to column chromatography on silica gel with ether–hexane (1:9) as the eluting solvent. An oily liquid (3.9 g) was obtained at a yield of 55.0%.

2,2-Dimethyl-5-(4-benzoylphenoxy)pentanoic Acid (8). To a solution of 0.11 mol (3.9 g) of **8c** in 20 mL of ethanol was added 10 mL of 20% NaOH solution, and the mixture was refluxed for 1 h. The ethanol was evaporated off from the mixture, and 20 mL of water was added to the residue. This mixture was acidified to pH 1 with 6 N HCl and extracted with 150 mL of ether. The ether solution was washed with water, dried over anhydrous $MgSO_4$, and concentrated. The residue was recrystallized with ethyl acetate and hexane to give 2.9 g (80.6%) of colorless crystals, mp 95 °C.

Method E. General Method for the Preparation of 2,2-Dimethyl-6-[4-(4-phenylbutyl)phenoxy]-2,2-dimethylhexanoic Acid Esters. A solution of 0.03 mol of dicyclohexylcarbodiimide (DCC) in 40 mL of dried dichloromethane was added to a solution of 0.02 mol of **11** and 0.06 mol of the appropriate alcohol in 150 mL of dried dichloromethane at −5 °C. Dicyclohexylurea precipitate was filtered off and 150 mL of ethyl acetate was added to the filtrate. The mixture was sequentially washed with 30 mL each of 6 N HCl, saturated sodium bicarbonate solution, and 10 mL of water, dried over anhydrous $MgSO_4$, and concentrated. The oily residue was subjected to column chromatography on silica gel with *n*-hexane and ethyl acetate as the eluting solvent.

Method F. General Method for the Preparation of the Ester of 2,2-Dimethyl-6-[4-(4-phenylbutyl)phenoxy]hexanol. 2,2-Dimethyl-6-[4-(4-phenylbutyl)phenoxy]hexanol. To a stirred suspension of 0.07 mol of $LiAlH_4$ in 100 mL of dried THF, was added at 0 °C 0.07 mol of **11** in 50 mL of dried THF, and the mixture was stirred for 3 h at room temperature. The reaction mixture was then added to 100 mL of methanol at 0 °C and the organic solvent was evaporated under reduced pressure. To the resulting residue was added 300 mL of water and the mixture was acidified to pH 1 with concentrated H_2SO_4 and extracted with 400 mL of ether. The ether solution was washed with water, dried over anhydrous $MgSO_4$, and concentrated. The residue was subjected to column chromatography on silica gel (ether–hexane, 1:4) to give 19.5 g (yield 80.9%) of a colorless oily liquid.

A solution of 0.015 mol of DCC in 25 mL of dried dichloromethane was added to a solution of 0.01 mol of the appropriate organic acid and 0.01 mol of (dimethylamino)pyridine in 50 mL of dried dichloromethane at −5 °C. The mixture was stirred for 2 h at room temperature. To this mixture was added at 0 °C a solution of 0.01 mol of 2,2-dimethyl-6-[4-(4-phenylbutyl)phenoxy]hexanol in 30 mL of anhydrous dichloromethane, and the mixture was stirred for 5 h at 0 °C. The same workup as described for method E gave the ester.

Method G. Preparation of 2-[4-(4-Phenylbutyl)phenoxy]isobutyric Acid (10). Ethyl 2-[4-(4-Phenylbutyl)phenoxy]isobutyrate (10a). A solution of 0.025 mol (5.6 g) of 4-(4-phenylbutyl)phenol (**5b**) in 20 mL of anhydrous THF was added

to a suspension of 0.025 mol (1.2 g) of 55% sodium hydride with the temperature maintained around 0 °C during the addition. The mixture was kept at room temperature for 30 min before a solution of 0.04 mol (7.8 g) of ethyl 2-bromoisobutyrate in 20 mL of anhydrous THF was added and the reaction mixture refluxed for 7 h. After cooling, 80 mL of water was added and the mixture was acidified to pH 1 with 6 N HCl and extracted with 200 mL of ether. The extract was washed with saturated NaCl solution and dried over anhydrous $MgSO_4$. Excess ethyl 2-bromoisobutyrate and ether were evaporated off under reduced pressure. The residue was subjected to column chromatography (ether–hexane, 1:9). A colorless oily liquid (6.5 g) was obtained in a yield of 88.4%.

10: A solution of 0.019 mol (6.5 g) of **10a** in 20 mL of ethanol was added with stirring to 0.08 mol (6.4 mL) of a solution of 50% NaOH, and the mixture was refluxed for 1 h. Ethanol was removed under reduced pressure, and 50 mL of water was added. The mixture was acidified to pH 1 with 6 N HCl and extracted with 100 mL of ether. The extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated. The residual solid was recrystallized from hexane to give 5.5 g (yield, 93.2%) of colorless crystals, mp 91–92 °C.

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Registry No. **1**, 113795-02-1; **1a**, 92-69-3; **2**, 113795-03-2; **2a**, 60859-24-7; **3**, 113795-04-3; **3a**, 6335-83-7; **4**, 113795-05-4; **4a**, 34591-21-4; **5**, 113795-06-5; **5a**, 1821-12-1; **5b**, 36940-99-5; **6**, 113795-07-6; **6a**, 57344-26-0; **7**, 113795-08-7; **7a**, 5581-75-9; **8**, 113795-09-8; **8a**, 25827-79-6; **8b**, 25827-80-9; **8c**, 113795-10-1; **9**, 113795-11-2; **9a**, 41859-54-5; **10**, 113795-12-3; **10a**, 113795-13-4; **11**, 113795-14-5; **12**, 113795-15-6; **13**, 113795-16-7; **14**, 113795-17-8; **15**, 113795-18-9; **16**, 113795-19-0; **17**, 113795-20-3; **18**, 113795-21-4; **18a**, 1453-06-1; **19**, 113795-22-5; **19a**, 13621-26-6; **20**, 113795-23-6; **20a**, 100-55-0; **21**, 113795-24-7; **21a**, 61892-95-3; **22**, 113795-25-8; **22a**, 59-67-6; **23**, 113795-26-9; **23a**, 5521-55-1; **24**, 113795-27-0; **24a**, 51037-30-0; **I** (R_1 = H), 108-95-2; **II** (X = Br, n = 3), 109-64-8; **II** (X = Br, n = 4), 110-52-1; **II** (X = Br, n = 5), 111-24-0; **II** (X = Br, n = 6), 629-03-8; **II** (X = Br, n = 7), 4549-31-9; **II** (X = Br, n = 8), 4549-32-0; **II** (X = Br, n = 9), 4549-33-1; **II** (X = Br, n = 10), 4101-68-2; **III** (R_1 = Ph, n = 3, X = Br), 113795-28-1; **III** (R_1 = $Ph(CH_2)_2$, n = 3, X = Br), 108357-59-1; **III** (R_1 = $Ph(CH_2)_3$, n = 3, X = Br), 113795-29-2; **III** (R_1 = $Ph(CH_2)_4$, n = 3, X = Br), 113795-30-5; **III** (R_1 = $Ph(CH_2)_5$, n = 3, X = Br), 113795-31-6; **III** (R_1 = $Ph(CH_2)_6$, n = 3, X = Br), 113795-32-7; **III** (R_1 = $PhCONH(CH_2)_2$, n = 3, X = Br), 113795-33-8; **III** (R_1 = $Ph(CH_2)_4$, n = 4, X = Br), 113795-34-9; **III** (R_1 = $Ph(CH_2)_4$, n = 5, X = Br), 113795-35-0; **III** (R_1 = $Ph(CH_2)_4$, n = 6, X = Br), 113795-36-1; **III** (R_1 = $Ph(CH_2)_4$, n = 7, X = Br), 113795-37-2; **III** (R_1 = $Ph(CH_2)_4$, n = 8, X = Br), 113795-38-3; **III** (R_1 = $Ph(CH_2)_4$, n = 9, X = Br), 113810-77-8; **III** (R_1 = $Ph(CH_2)_4$, n = 10, X = Br), 113795-39-4; **III** (R_1 = H, n =3, X = Br), 588-63-6; **VI** (n = 5), 113795-40-7; **VI** (n = 3), 101594-58-5; **VII** (n = 6), 113795-41-8; **VII** (n = 4), 38841-95-1; p-$Br(CH_2)_4OC_6H_4(CH_2)_4$-2,4-$(Me)_2C_6H_3$, 113795-42-9; p-$Br(CH_2)_4OC_6H_4(CH_2)_4$-2,5-$(Me)_2C_6H_3$, 113795-43-0; p-$HOC_6H_4(CH_2)_4$-2,4-$(Me)_2C_6H_3$, 113795-44-1; p-$HOC_6H_4(CH_2)_4$-2,5-$(Me)_2C_6H_3$, 113795-45-2; p-$HOC_6H_4(CH_2)_6Ph$, 113795-46-3; $Ph(CH_2)_5COCl$, 21389-46-8; $ClCO(CH_2)_3$-2,4-$(Me)_2C_6H_3$, 113795-47-4; $ClCO(CH_2)_3$-2,5-$(Me)_2C_6H_3$, 113795-48-5; $Ph(CH_2)_3COCl$, 18496-54-3; p-$MeOC_6H_4CO(CH_2)_3$-2,4-$(Me)_2C_6H_3$, 113795-49-6; p-$MeOC_6H_4CO(CH_2)_3$-2,5-$(Me)_2C_6H_3$, 113795-50-9; p-$MeOC_6H_4(CH_2)_4$-2,4-$(Me)_2C_6H_3$, 113795-51-0; p-$MeOC_6H_4(CH_2)_4$-2,5-$(Me)_2C_6H_3$, 113795-52-1; sodium isobutyrate, 996-30-5; 2,2-dimethyl-6-[4-(4-phenybutyl)phenoxy]hexanol, 113795-53-2; ethyl 2-bromoisobutyrate, 600-00-0.

Chemical Synthesis and Biological Activities of 5-Deazaaminopterin Analogues Bearing Substituent(s) at the 5- and/or 7-Position(s)[1]

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Condensation of cyanothioacetamide (**4**) with ethyl α-(ethoxymethylene)acetoacetate (**5b**), ethyl 4-ethoxy-2-(ethoxymethylene)-3-oxobutanoate (**5c**), ethyl 2-(ethoxymethylene)-3-oxo-4-phenylpropanoate (**5d**) afforded exclusively the corresponding 6-substituted pyridines (**6b–d**). Cyclization of **4** with 3-carbethoxybutane-2,4-dione (**5e**) gave 3-cyano-5-(ethoxycarbonyl)-4,6-dimethylpyridine-2(1*H*)-thione (**6e**), whereas reaction of **4** with 3-carbethoxy-1-phenylpropane-1,3-dione (**5f**) yielded two products, 3-cyano-5-(ethoxycarbonyl)-4-methyl-6-phenylpyridine-2(1*H*)-thione (**6f**) and the 6-methyl-4-phenyl isomer **6g**. The structural assignments for **6f** and **6g** are made on the basis of ^1H and ^{13}C NMR spectral analyses of the 2-(methylthio)nicotinates (**7f,g**) prepared from **6f** and **6g** by treatment with MeI/K_2CO_3. Nicotinates **7b,d–g** were converted into their corresponding 2,4-diaminopyrido[2,3-*d*]pyrimidines **12b,d–g** in five steps, via reduction, protection, oxidation, condensation with guanidine, and deprotection. The 7-mono- and 5,7-disubstituted-5-deazaaminopterins (**1b,d–g**) were prepared from the respective pyrido[2,3-*d*]pyrimidines **12b,d–g**. Preliminary biological studies showed that 7-methyl and 5,7-dimethyl analogues (**1b** and **1e**) were less active than methotrexate against human leukemic HL-60 and murine L-1210 cells in tissue culture. Compound **1e** produced an ILS of 71% at 100 mg/kg per day × 5 (ip) in BDF mice inoculated ip with 10^6 L-1210 cells.

Certain deaza analogues of methotrexate (MTX) and aminopterin (AP) have been reported to exhibit potent antitumor activity. Quinazoline[2–6] and pyrido[2,3-*d*]pyrimidine (5-deazapteridine),[7–10] for example, are found to be effective inhibitors of both dihydrofolate reductase

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