injection such that a level of 200, 100, 50, 25, or 12.5 mg of test compound/kg of mouse weight is maintained. Ten mice are employed per test compound at each dose level and survivors counted at 120-h postchallenge. Streptomycin sulfate at 100 mg/kg was used as the positive control.

Registry No. **1a**, 932-22-9; **1b**, 1677-79-8; **1c**, 1445-55-2; **2a**, 56707-86-9; **3a**, 91777-77-4; **3b**, 91777-76-3; **3c**, 92187-06-9; **3d**, 92187-07-0; **3e**, 92187-08-1; **4**, 64882-65-1; **6**, 91777-79-6; **7a**, 91777-71-8; **7b**, 92187-09-2; **8a**, 91777-72-9; **8b**, 92187-10-5; **9a**, 91777-78-5; **9b**, 92187-11-6; **9c**, 92187-12-7; **10a**, 91777-70-7; 10b, 92187-13-8; **11a**, 92187-14-9; **11b**, 92187-15-0; **11c**, 92187-16-1; **11d**, 92187-17-2; **11e**, 92187-18-3; **11f**, 92187-19-4; **11g**, 92187-20-7; **11h**, 92187-21-8; 1-acetyl-2,3,5-tribenzoyl-D-ribofuranose, 14215-97-5; methyl fluorosulfate, 421-20-5; 2,3-dihydro-2-methyl-3-oxopyridazine-4-carbonitrile, 92187-25-2; 3-methoxypyridazine-4-carbonitrile, 92187-22-9; 3-chloropyridazine-4-carbonitrile, 1445-56-3; 4,5-dichloro-3-oxido-1-methylpyridazinium, 33386-96-8; 2-(hydroxymethyl)-2,3-dihydro-3-oxopyridazine-4-carbonitrile, 92187-23-0; 2-(acetoxymethyl)-2,3-dihydro-3-oxopyridazine-4-carbonitrile, 92187-24-1; *O*-(tetrahydropyran-2-yl)hydroxylamine, 6723-30-4; ribose tetraacetate, 28708-32-9.

Novel Pyrimidine and 1,3,5-Triazine Hypolipidemic Agents

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New compounds were synthesized by changing the substituents of a trisubstituted pyrimidine, i.e., [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]acetic acid, a potent hypolipidemic agent, impaired, however, by a marked hepatomegaly-inducing effect. The structural variations led to the subsidence (**14b**, i.e., 4-chloro-2-(dimethylamino)-6-[(2,3-dimethylphenyl)amino]pyrimidine) or to the reduction (**18b**, [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]amino]acetic acid) of said untoward effect but still maintained the hypolipidemic effect that, although markedly decreased, still proves significant for serum cholesterol and triglycerides (**18b**) or for serum triglycerides only (**14b**).

Development of drugs for the treatment of hyperlipidemias has focused on (aryloxy)acetic acid derivatives (so-called "fibrates"), e.g., clofibrate, bezafibrate, tibric acid, procetofen,[1-3] nicotinic acid derivatives,[4] and agents of different chemical series, e.g., tiadenol.[5] A promising hypolipidemic activity in the pharmacological and preclinical testing was shown by [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]acetic acid (**1d**) and by its related ethanolamide **1e**.[6-11] All these new hypolipidemic agents (with the exception of nicotinic acid derivatives) produce an increase in liver weight and volume, peroxisomal proliferation, and, although not mutagenic, the onset of hepatomas in predisposed strains of rats, i.e., Fischer rats.[12-19] We therefore deemed it useful to bring some structural changes to molecules **1d** and **1e**, both in the substituents and in the pyrimidine ring, so as to maintain the hypolipidemic activity and prevent injurious effects on the liver cell.

Two types of compounds were selected, i.e., derivatives similar to **1d** and derivatives, recalling someway the metformin structure, that, according to Sirtori et al.,[6,20] counteract the onset of an experimentally induced atheroma in the rabbit aorta.

The resulting molecules were subjected to a first pharmacological investigation that checked their action on serum cholesterol, triglycerides, and lipoproteins and that assessed for the most active compounds their effect on liver weight and increases in catalase and liver enzymes related to the fatty acid β-oxidation as well as their response to peroxisomal proliferation.

Results

Chemistry. The synthesis of all 2,4,6-trisubstituted pyrimidines started from 1,2-dihydro-2-thioxo-4,6-(1*H*,5*H*)pyrimidinedione (**3**). Its sodium or tetrabutylammonium salt was alkylated with alkyl bromides or iodides, and the corresponding 2-(alkylthio)-4,6(1*H*,5*H*)-pyrimidinediones **4a–d** were obtained (Scheme I). The

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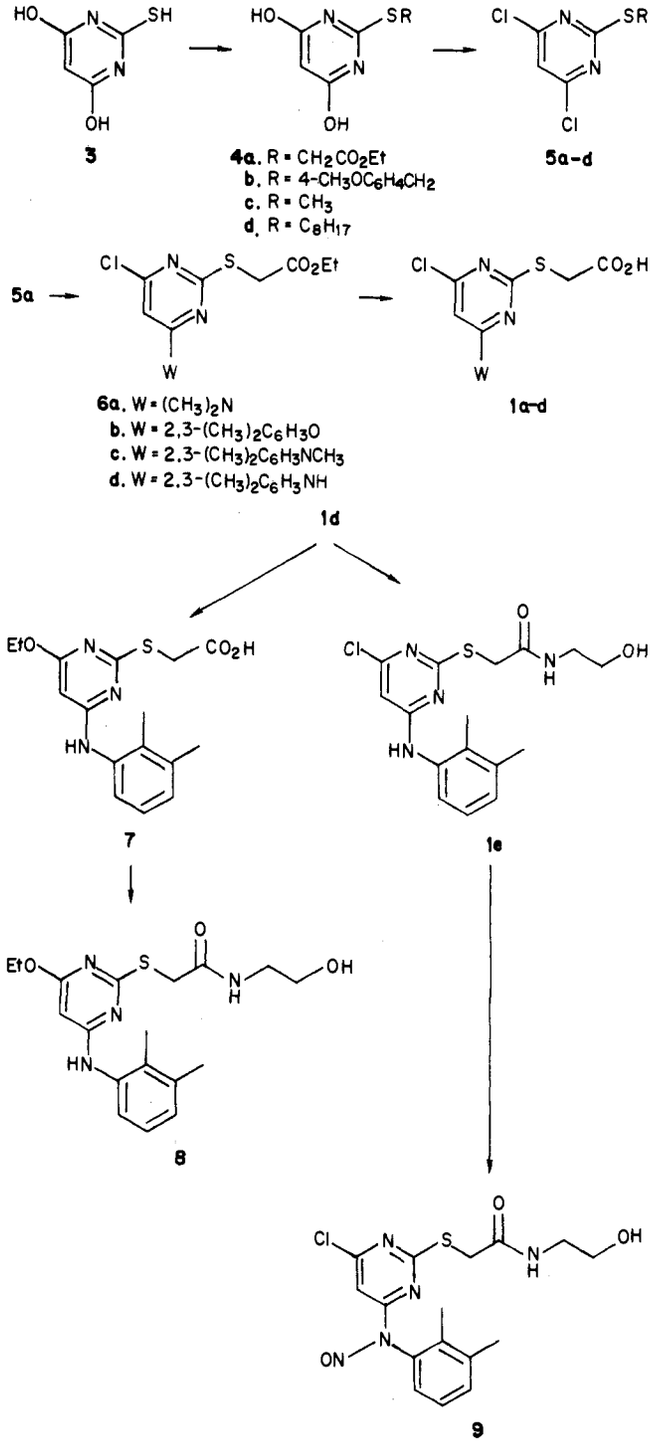
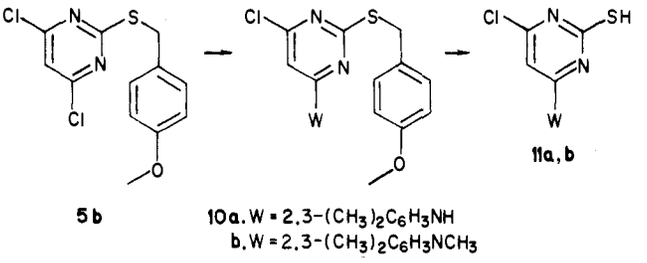
Scheme I

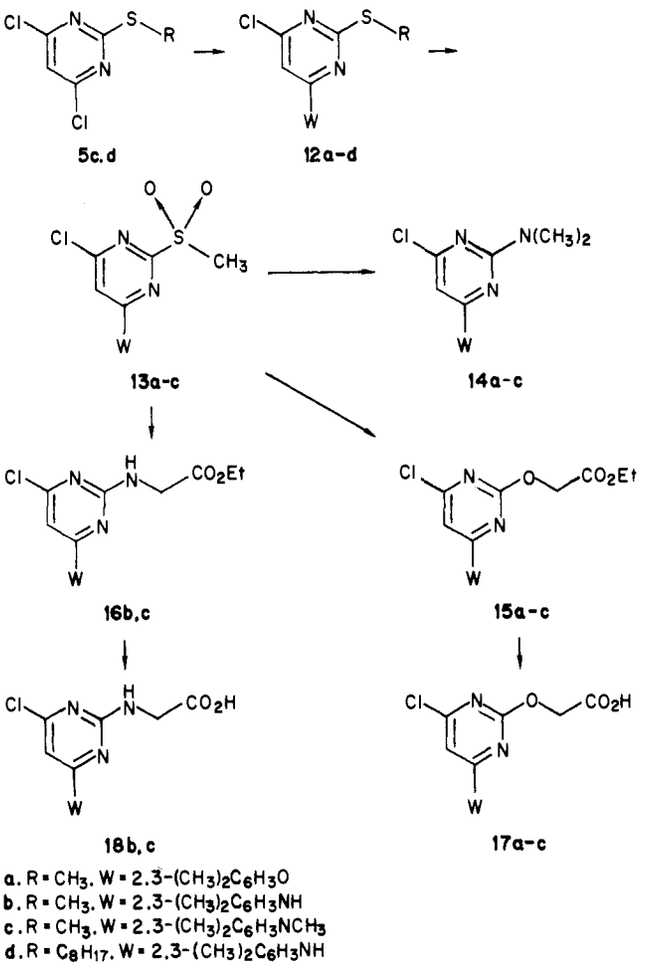


reaction of these compounds with phosphorus oxychloride gave 2-(alkylthio)-4,6-dichloropyrimidines **5a–d** in good yields. The 6-Cl group in ethyl [[4,6-dichloro-2-pyrimidinyl]thio]acetate (**5a**) was substituted by diethylamine, 2,3-dimethylphenol, and 2,3-dimethylaniline and its *N*-methyl derivative in the presence of NaOH or Na_2CO_3. The hydrolysis of ester groups of the so obtained compounds **6a–d** was made by briefly heating in diluted sodium hydroxide. A stronger basic treatment with sodium ethylate in refluxing ethanol transformed [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]acetic acid (**1d**) into its 4-ethoxy derivative **7**, which gave the amide **8** by reaction with ethyl chloroformate and ethanolamine (Table I). The *N*-(2-hydroxyethyl)amide **1e** was formed by boiling **1d** with aziridine. Treatment of this compound

Scheme II



Scheme III



with sodium nitrite afforded the *N*-nitroso derivative **9**.

The 2(1*H*)-pyrimidinethiones **11a,b** were synthesized by reacting 4,6-dichloro-2-[4-methoxybenzyl)thio]pyrimidine (**5b**) with properly substituted anilines, subsequently cleaving thio ethers **10a,b** by means of trifluoroacetic acid (Scheme II).

Similarly, the condensation of 2-(methylthio)- and 2-(*n*-octylthio)pyrimidines **5c** and **5d**, respectively, with the above-cited nucleophiles gave adducts **12a–d** (Scheme III). The oxidation of the 2-methylthio group of **12a–c** with H_2O_2 in acetic acid, in the presence of catalytic amounts of Na_2WO_4,[21,22] furnished the 4-chloro-2-(methylsulfonyl)pyrimidines **13a–c**.

The selective displacement of the methylsulfonyl group by diethylamine, ethyl glycolate, or ethyl glycinate afforded

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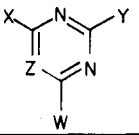
Table I.[a] Physical and Chemical Properties of Compounds Tested as Hypolipidemic Agents



no.	X	Y	Z	W	SM (wt, g)	reagent (L)	solvent (mL)	temp, °C	time, h	yield %	crystn solv.	mp, °C
1a	Cl	SCH_2CO_2H	CH	$(CH_3)_2N$	**6a** (4.8)	NaOH (22)[b]	EtOH (40)	60	5 min	72	acetone	187–189
1b	Cl	SCH_2CO_2H	CH	A[c]	**6b** (1.2)	NaOH (7.2)[b]	EtOH (16)	60	5 min	41	AcOEt/*n*-hexane	123–125
1c	Cl	SCH_2CO_2H	CH	C	**6c** (5.0)	NaOH (27)[b]	EtOH (45)	60	5 min	76	AcOEt/*n*-hexane	174–176
8	OEt	$SCH_2CONH(CH_2)_2OH$	CH	B	**7** (5.0)	$HO(CH_2)_2NH_2$ (1.0)[c]	$CHCl_3$ (65)	20	1.5	58	AcOEt/pet. ether	112–114
11a	Cl	SH	CH	B	**10a** (8.0)	CF_3CO_2H (80)[d]	CF_3CO_2H	77	50 min	89	Et_2O	>250
11b	Cl	SH	CH	C	**10a** (6.0)	CF_3CO_2H (60)[e]	CF_3CO_2H	77	1.5	60	AcOEt/Et_2O	>290
12b	Cl	SCH_3	CH	B	**5c** (4.0)	B′ (2.9)[f]	EtOH (40)	80	18	45	EtOH	149–150
12d	Cl	SC_8H_{17}	CH	B	**5d** (3.0)	B′ (1.5)[g]	EtOH (25)	80	20	49		oil
14a	Cl	$(CH_3)_2N$	CH	A	**13a** (5.0)	$HNMe_2$ (4.4)[h]	C_6H_6 (100)	20	30 min	79	pet. ether	72–73
14b	Cl	$(CH_3)_2N$	CH	B	**13b** (5.0)	$HNMe_2$ (4.5)[h]	C_6H_6 (50)	80	18	78	AcOEt/*n*-hexane	146–147
14c	Cl	$(CH_3)_2N$	CH	C	**13c** (5.2)	$HNMe_2$ (6.0)[h]	$CHCl_3$ (50)	20	18	67	*n*-hexane	99–101
17a	Cl	OCH_2CO_2H	CH	A	**15a** (1.3)	NaOH (5.5)[b]	EtOH (10)	60	5 min	76	AcOEt/*n*-hexane	129–130
17b	Cl	OCH_2CO_2H	CH	B	**15b** (5.2)	NaOH (20)[b]	EtOH (40)	60	5 min	90	acetone	190–191
17c	Cl	OCH_2CO_2H	CH	C	**15c** (2.5)	NaOH (10.4)[b]	EtOH (20)	60	5 min	75	AcOEt	154–155
18b	Cl	$HNCH_2CO_2H$	CH	B	**16b** (2.2)	NaOH (13.5)[b]	EtOH (27)	60	5 min	79	AcOEt	205–206
18c	Cl	$HNCH_2CO_2H$	CH	C	**16c** (1.4)	NaOH (5.0)[b]	EtOH (10)	60	10 min	70	AcOEt/*n*-hexane	96–98 dec
23d	Cl	$(CH_3)_2N$	N	B	**22** (4.3)	$HNMe_2$ (2.7)[i]	$CHCl_3$ (150)	4	15 min	62	AcOEt	167–168
24a	Cl	$CH_3NCH_2CO_2H$	N	B	**23a** (5.0)	NaOH (21.0)[b]	EtOH (37)	60	5 min	75	AcOEt/*n*-hexane	185–186
24b	Cl	OCH_2CO_2H	N	B	**23b** (3.7)	NaOH (16.2)[b]	EtOH (30)	60	5 min	60	AcOEt	168–169
24c	OEt	SCH_2CO_2H	N	B	**23c** (7.2)	NaOH (44.0)[b]	EtOH (75)	60	20 min	68	AcOEt	220–221
25	Cl	$(CH_3)_2N$	N	$(CH_3)_2N$	**21** (4.5)	$HN(CH_3)_2$ (13)[j]	acetone (35)[k]	0	2	65	*i*-PrOH/H_2O	68–69
26	Cl	$(CH_3)_2N$	N	H_2N	D (3.3)	$HN(CH_3)_2$ (6.1)[j]	H_2O (80)[l]	0	2.5	55	AcOEt	223–225
27	Cl	$(CH_3)_2N$	CH	$(CH_3)_2N$	**19** (5.0)	$NH(CH_3)_2$ (9.0)[j]	EtOH (25)[m]	40	50 min	42	$MeOH/H_2O$	51–52
28	Cl	$(CH_3)_2N$	CH	H_2N	E (6.1)	NH_3 (30)[n]	EtOH	120	18	52	$EtOH/H_2O$	153–155

[a] All reported compounds showed correct microanalyses (C, H, N); structural elucidation was made by ^1H NMR, IR, and/or mass spectra. [b] 1 N aqueous solution. [c] The carboxylic group was activated by 2.5 mL (26.1 mmol) of ethyl chloroformate and 2.5 mL (17.9 mmol) of Et_3N. [d] This reaction was made in the presence of 2.7 mL (24.7 mmol) of anisole. [e] This reaction was made in the presence of 2.0 mL (18.4 mmol) of anisole. [f] 2.50 g (23.6 mmol) of Na_2CO_3 was added. [g] 1.30 g (12.3 mmol) of Na_2CO_3 was added and the product was purified by chromatography on Kieselgel. [h] 33% aqueous solution; this reaction was made in the presence of 4.6 mL (33.0 mmol) of Et_3N. [i] 33% aqueous solution; this reaction was made in the presence of 2.3 mL (16.5 mmol) of Et_3N. [j] 33% aqueous solution. [k] The hot solution of E in acetone was poured on 150 g of cracked ice and dimethylamine was slowly added; mp (*n*-PrOH/H_2O) 66–68 °C, ref 9b. [l] Mp (EtOH) 220–222 °C, ref 9b. [m] Mp (sublimation) 52.5 °C, ref 9a. [n] 10% ethanolic solution; this reaction was made in a Carius tube; mp (EtOH/H_2O) 151 °C, ref 9a. [c] A = 2,3-$(CH_3)_2C_6H_3O$, B = 2,3-$(CH_3)_2C_6H_3NH$, B′ = 2,3-$(CH_3)_2C_6H_3NH_2$, C = 2,3-$(CH_3)_2C_6H_3NCH_3$, D = 2,4-dichloro-6-amino-1,3,5-triazine, E = 4,6-dichloro-2-(dimethylamino)pyrimidine.

the derivatives **14a–c**, **15a–c**, and **16b,c**, respectively.

The hydrolysis of esters **15a–c** and **16b,c** to the related acids **17a–c** and **18b** could be carried out in diluted ethanolic sodium hydroxide.

A shorter synthesis was set up to prepare **14b** and **18b** on a large scale. 2,3-Dimethylaniline was condensed with 2,4,6-trichloropyrimidine[19] to give 2,4-dichloro-6-[(2,3-dimethylphenyl)amino]pyrimidine,[20] which was transformed subsequently into **14b** by reacting with dimethylamine and into **18b** by treatment with ethyl glycinate and subsequent basic hydrolysis of the ester group (Scheme IV).

The condensation of cyanuric chloride with 2,3-dimethylaniline afforded 2,4-dichloro-6-[(2,3-dimethylphenyl)amino]-1,3,5-triazine,[22] which provided the monochlorotriazines **23a–d** by reaction with dimethylamine or ethyl esters of glycolic acid, thioglycolic acid, or *N*-methylglycine (Scheme V). The hydrolysis of the ester groups of **23a,b** with diluted ethanolic sodium hydroxide provided the acids **24a,b**; under the same reaction conditions, starting from ethyl [[4-chloro-6-[(2,3-dimethylphenyl)amino]-1,3,5-triazin-2-yl]thio]acetate (**23c**), the acid [[4-ethoxy-6-[(2,3-dimethylphenyl)amino]-1,3,5-triazin-2-

Table II. Serum Cholesterol, Triglycerides, and α-Lipoprotein Cholesterol in Control Rats or Rats Given the Standards (1d, 1e, 2) or the Investigational Compounds

compds	no. of animals	std	dose, mg/kg	cholesterol, mg/100 mL			α-cholesterol, % vs. cholesterol			triglycerides, mg/100 mL			LD_{50}, mg/kg
				M ± SE	% controls	P vs. controls	M ± SE	% controls	P vs. controls	M ± SE	% controls	P vs. controls	
control	40			72.0 ± 1.87			69.5 ± 2.12			105.9 ± 4.11			
std 1d	30		50	55.1 ± 1.87	−23.5	<0.001	75.1 ± 1.63	+8.1	ns	73.2 ± 3.86	−30.9	<0.001	1050
control	10			81.8 ± 4.00			72.7 ± 3.59			94.9 ± 5.20			
std 1a	10		50	62.5 ± 3.69	−23.6	<0.01	81.7 ± 2.61	+12.4	ns	61.5 ± 4.64	−35.2	<0.001	<2500
control	30			86.4 ± 5.80			71.0 ± 2.03			123.0 ± 5.46			
std 2	30		200	74.9 ± 2.51	−13.3	ns	73.3 ± 1.73	+3.2	ns	111.3 ± 4.23	−9.5	ns	1000
control	20			72.8 ± 2.40			69.9 ± 3.82			111.1 ± 4.95			
18b	19	1d	50	63.6 ± 1.66	−12.6	<0.01	76.7 ± 1.54	+9.8	ns	71.6 ± 4.19	−35.5	<0.001	1020
17b	10	1d	50	57.3 ± 2.81	−21.3	<0.001	83.8 ± 2.60	+19.9	<0.01	67.3 ± 5.97	−39.4	<0.001	1085
12d	10	1d	50	63.2 ± 4.63	−13.2	ns	67.7 ± 3.40	−3.1	ns	96.5 ± 5.36	−13.1	ns	
control	10			80.1 ± 3.16			69.4 ± 3.12			82.9 ± 7.24			
1b	10	1d	50	67.7 ± 2.64	−15.5	<0.01	74.3 ± 2.80	+7.1	ns	56.4 ± 6.24	−32.0	<0.05	1290
1a	10	1d	50	69.4 ± 3.68	−13.4	<0.05	72.2 ± 3.17	+4.0	ns	55.6 ± 3.21	−32.9	<0.01	1920
control	10			62.4 ± 2.90			68.7 ± 2.48			118.6 ± 7.44			
14b	10	1d	50	57.8 ± 3.92	−7.4	ns	69.6 ± 2.46	+1.3	ns	96.5 ± 7.19	−18.6	<0.05	<2500
12b	10	1d	50	54.2 ± 2.37	−13.1	<0.05	72.3 ± 2.53	+5.2	ns	106.1 ± 5.81	−10.5	ns	1250
control	10			69.7 ± 4.47			71.0 ± 3.07			115.6 ± 12.60			
17a	10	1d	50	67.1 ± 3.75	−3.7	ns	74.0 ± 1.86	+4.2	ns	91.1 ± 7.99	−21.2	ns	1210
control	10			86.5 ± 6.85			63.1 ± 4.42			98.9 ± 4.19			
18c	10	1d	50	68.4 ± 4.90	−20.9	<0.05	77.0 ± 5.01	+22.0	ns	50.0 ± 5.92	−49.4	<0.001	995
25	10	1d	200	84.8 ± 6.28	−2.0	ns	70.0 ± 1.20	+10.9	ns	56.1 ± 5.67	−43.3	<0.001	<2000
control	10			69.4 ± 3.38			87.5 ± 2.74			105.2 ± 4.22			
14c	10	1d	200	63.2 ± 2.26	−8.9	ns	90.0 ± 3.39	+2.9	ns	89.8 ± 8.04	−14.6	ns	<2500
17c	10	1d	50	66.4 ± 2.09	−4.3	ns	87.2 ± 2.08	−0.3	ns	80.7 ± 3.77	−23.3	<0.001	1070
control	10			81.8 ± 4.00			72.7 ± 3.59			94.9 ± 5.20			
8	10	1e	50	65.7 ± 3.53	−19.7	<0.01	76.6 ± 3.42	+5.3	ns	64.3 ± 3.67	−32.2	<0.001	2500
control	10			81.8 ± 5.64			73.9 ± 3.22			115.9 ± 7.41			
24a	10	2	200	76.8 ± 5.70	−6.1	ns	79.8 ± 1.83	+8.3	ns	74.6 ± 8.19	−35.6	<0.001	1215
24b	10	2	200	72.0 ± 4.52	−12.0	ns	82.1 ± 2.26	+11.1	<0.05	65.5 ± 5.66	−43.5	<0.001	1180
control	10			97.3 ± 16.24			63.8 ± 3.90			120.8 ± 9.27			
11a	10	2	200	76.3 ± 3.66	−21.6	ns	73.2 ± 2.68	+14.7	ns	95.6 ± 7.85	−20.9	ns	1845
14a	10	2	200	76.7 ± 4.09	−21.2	ns	72.6 ± 3.30	+13.8	ns	102.0 ± 8.87	−15.6	ns	<2000
control	10			80.2 ± 3.24			75.2 ± 2.40			132.2 ± 11.46			
23d	10	2	200	80.8 ± 3.18	+0.7	ns	78.6 ± 2.69	+4.5	ns	91.4 ± 5.10	−30.9	<0.01	<2000
24c	10	2	200	70.3 ± 3.94	−12.3	ns	82.2 ± 2.02	+9.3	<0.05	76.1 ± 3.80	−42.4	<0.001	1120
control	10			74.1 ± 1.74			74.7 ± 4.05			121.1 ± 10.63			
26	10	2	200	105.6 ± 5.53	+42.5	<0.001	85.4 ± 3.33	+14.3	ns	78.2 ± 5.84	−35.4	<0.01	<2000
28	10	2	200	71.4 ± 3.15	−3.6	ns	87.4 ± 2.85	+17.0	<0.05	89.9 ± 4.71	−25.8	<0.01	<2500
control	10			75.1 ± 5.19			73.3 ± 2.69			114.6 ± 9.26			
14b	10	2	200	68.0 ± 2.85	−9.5	ns	80.5 ± 2.99	+9.8	ns	74.1 ± 7.45	−35.3	<0.01	<2500
1c	10	2	50	63.5 ± 4.17	−15.4	ns	78.6 ± 4.08	+7.2	ns	46.9 ± 4.89	−59.1	<0.001	1010
control	10			62.5 ± 4.53			75.3 ± 2.37			101.0 ± 7.07			
11b	10	2	200	65.8 ± 3.36	+5.3	ns	74.4 ± 2.79	−1.2	ns	79.1 ± 5.32	−21.7	<0.05	1780
27	10	2	200	103.7 ± 5.29	+65.9	<0.001	75.7 ± 2.18	+0.5	ns	61.4 ± 4.37	−39.2	<0.001	<2500

[a] Not significant = ns.

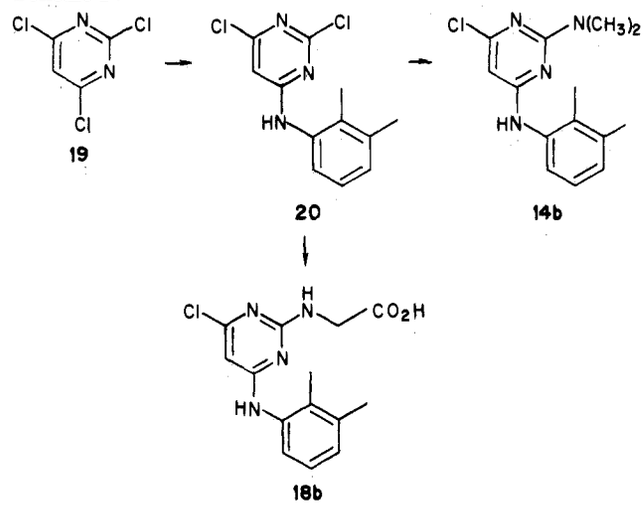
yl]thio]acetic acid (**24c**) was obtained as the main reaction product. Compounds 25–28 were synthesized according to a previously described method.[23,24]

Table I shows the various synthesized compounds and their physical and chemical properties. Table V gives physical and chemical properties of the intermediate compounds used in the synthesis.

Pharmacology

First Investigation. The hypolipidemic activity of the synthesized compounds was assessed in Sprague–Dawley male rats, checking the variations induced in serum cholesterol, triglycerides, and lipoprotein α-cholesterol vs. the controls and a standard reference drug, which was selected on the basis of the structural relationship with the investigational compound: the test compound was given at the dose of 50 mg/kg when the standards were **1d,e** or at the dose of 200 mg/kg when the standard was metformin.

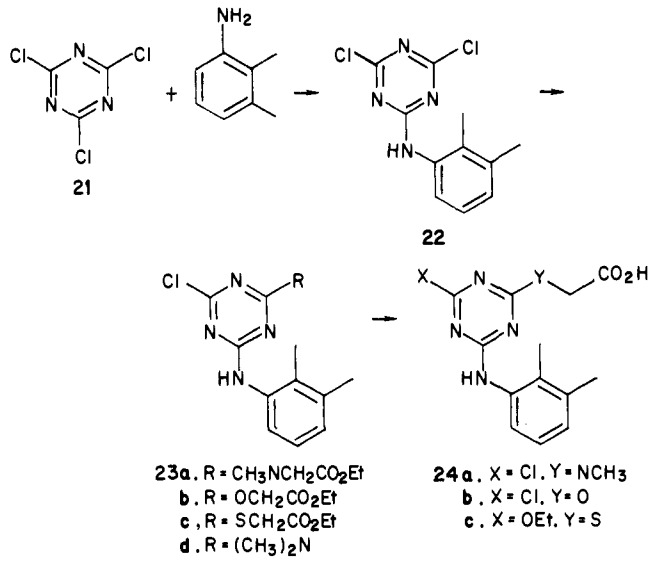
Scheme IV



The data obtained is given in Table II. The values, expressed in mg/100 mL of serum, are of the total chole-

(23) Boon, W. R. *J. Chem. Soc.* **1952**, *14*, 1532.
(24) Pearlman, W. M.; Banks, C. K. *J. Am. Chem. Soc.* **1948**, *70*, 3726.

Scheme V



sterol, triglycerides in the control rats, in rats given the standard and the investigational compounds, the percent variations vs. controls and the statistical significance of the variations vs. the controls and vs. the standard. Lipoprotein α-cholesterol is expressed as percent vs. serum total cholesterol.

Second Investigation. The effect of the above-mentioned five compounds was assessed on the serum levels of cholesterol and triglycerides as well as on the liver weight of Sprague–Dawley rats with a hyperlipidemia induced by a Nath's diet[25] (Table III). The investigational compounds **14b** and **24c** are active on serum triglycerides; **8**, **17b**, and **18b** reduce serum cholesterol and triglycerides; all the compounds are significantly less active than the standard. At the end of the 15 days of feeding with the Nath's diet, the liver weight of the rats given the five compounds is significantly lower than that of the animals given the standard. Liver weight of the animals treated with **14b** and **18b** did not differ significantly from the weight observed in the controls.

Discussion

The data reported in Table II on the hypolipidemic activity of the new compounds led to the assessment of a relationship between structure and activity. The performed substitutions reduce the hypolipidemic activity; the decrease of hypolipidemic activity is low when the thioacetic side chain, in position 2 of the pyrimidine ring (**1d**), is replaced with a glycine (**18b,c**) or with an oxyacetic residue (**17b,c**) and becomes remarkable when the same chain is reduced to thiol (**11a,b**) or *S*-alkyl (**12b,d**) groups. The substitution of the thioacetic residue with a dimethylamino group (**14a–c**, **27**, **28**) or the introduction of the 1,3,5-triazine ring (**23d**, **24a–c**, **25**, **26**), in replacement of the pyrimidine ring, leads to compounds that exert a statistically significant activity on serum triglycerides and none on cholesterol. The compound, with an ethoxy group replacing chlorine in position 4, still keeps its hypolipidemic activity, which is significantly lower compared with that of the standard. The replacement of the (2,3-dimethylphenyl)amino residue of **1d**, **14b**, **17b** with a (2,3-dimethylphenyl)oxy residue (**1b**, **14a**, **17a**) results in a marked decrease of activity. Feeding rats with the Nath's diet pointed out the hypolipidemic activity of the five

Table III. Variations of Serum Cholesterol, Triglycerides, and Liver Weight in Rats Fed the Hyperlipidemic Nath's Diet

		cholesterol, mg/100 mL			triglycerides, mg/100 mL			liver wt, g/100 g of bdw		
compd.	bose, mg/kg	$M \pm$ SE	% controls	P vs. controls	$M \pm$ SE	% controls	P vs. controls	$M \pm$ SE	% controls	P vs. controls
control		735.7 ± 60.8			318.6 ± 33.8			5.70 ± 0.15		
std **1d**	50	331.7 ± 17.1	–54.9	<0.001	123.2 ± 12.7	–61.3	<0.001	8.23 ± 0.26	+44.4	<0.001
8	50	479.8 ± 31.4	–34.8	<0.01	206.6 ± 25.2	–35.1	<0.05	6.85 ± 0.26	+20.2	<0.001
14b	200	771.3 ± 94.0	+4.8	ns[c]	182.1 ± 35.9	–42.8	<0.01	5.97 ±0.09	+4.7	ns
17b	50	548.8 ± 40.6	–25.4	<0.05	215.6 ± 17.8	–32.3	<0.05	6.29 ± 0.17	+10.3	<0.05
control		1020.9 ± 88.3			344.8 ± 21.2			6.08 ± 0.10		
std **1d**	50	541.2 ± 32.6	–47.0	<0.001	92.4 ± 5.9	–73.2	<0.001	8.85 ± 0.25	+45.6	<0.001
24c	50	1054.8 ± 82.5	+3.3	ns	214.3 ± 20.5	–37.8	<0.001	6.53 ± 0.08	+7.4	ns
control		289.3 ± 41.3			331.2 ± 15.6			5.02 ± 0.17		
18b	6.25	257.4 ± 21.6	–11.0	ns	205.2 ± 18.7	–38.0	<0.001	4.82 ± 0.11	–3.9	ns
	12.5	264.2 ± 39.2	–8.7	ns	263.8 ± 16.2	–20.3	<0.001	5.14 ± 0.12	+2.4	ns
	25.0	215.4 ± 14.2	–25.5	<0.05	312.1 ± 30.9	–5.8	ns	5.31 ± 0.14	+5.7	ns

[a] Control rats and rats given the standard (**1d**) or the investigational compounds. [b] As asterisk indicates that hyperlipidemic Nath's diet, with a lower cholesterol and cholic acid content (0.5%) was used in this test. [c] Not significant = ns.

(25) Nath, N.; Wiener, R.; Harper, A. E.; Elvehjem, C. A. *J. Nutr.* **1959**, *67*, 289.

Table IV. Liver Weight, Liver Enzymes, Peroxisomal Proliferation, and M_r 80 000 Peptide in F 344 Rats Given Hypolipidemic Agents

compd	treatment, weeks	body wt, g	liver wt, g/100 g of bdw	liver catalase, units/mg of protein	liver carnitine acetyl-transferase, units/mg of protein	enoyl-CoA hydratase, μmoles min^{-1} mg of $protein)^{-1}$	[1-^{14}C]-palmito-yl-CoA oxid, μmol/min mg	peroxisome proliferation	M_r 80 000 peptide
8	7	192	5.9	67.5	nt	nt	nt	++	+
14b	6	268	3.9	38	7	3.8	0.015	n[a]	nt[a]
17b	7	245	4.5	77	10	2.5	0.088	++++	+++
18b	7	266	4.2	61	11	3.7	0.056	++	+
24c	10	248	3.1	50	nt	nt	nt	n	nt
control		272	3.8	47	6	0.4	0.015	n	nt

[a] Normal = n, not tested = nt.

selected compounds: **8**, **27b**, and **18b**, although less active than the standard, decrease serum cholesterol and triglycerides; **14b** and **24c** are only effective on serum triglycerides: the liver weight, which in the case of the standard compound (**1d**) increases markedly (+44%) vs. the controls, does not increase in the case of compounds **14b** and **18b** or only increased mildly in the case of **24c**. Compounds **8** and **17b**, on the other hand, show a statistically significant increase (Table III). The peroxisomal increase, which in the case of compound **17b** is still highly evident, is reduced by half in the case of **18b** and is practically nil in the case of **14b** (Table IV).

The data on the liver weight changes were confirmed by an investigation on Fischer rats[26] that assessed not only the weight of the organ but also the variations of some liver enzymes (catalase, carnitine acetyltransferase, enoyl-CoA hydratase, enzymes involved in the β-oxidation of long-chain fatty acids) and the peroxisomal proliferation. This investigation shows that **14b** and **24c** have no effect on liver weight activity, catalase and peroxisomal proliferation: compounds **8** and **18b** induce a mild peroxisomal proliferation. While **18b** causes no significant increase in liver weight, **8** induces a marked increase.

Compound **17b** increases liver weight and the activity of catalase and other investigated enzymes and activates peroxisomal proliferation. Finally, the investigation demonstrated a relationship between peroxisomal proliferation and the presence of a peptide, molecular weight 80 000, which represents a peptide fraction increased selectively by various hypolipidemic proliferators.[27] The results of this investigation are summarized in Table IV.

Experimental Section

Chemistry. Melting points were determined on a Büchi-Tottoli melting point apparatus and are uncorrected. Microanalyses were performed by the Microanalytical Laboratories of the Institute of Organic Chemistry, University of Milan, on a Perkin-Elmer 240 elemental analyzer. Analytical results were within ±0.4% of the theoretical values for C, H, N in all cases. Each sample was homogeneous by TLC. IR, ^{1}H NMR, and mass spectra were compatible with the assigned structures. IR spectra were recorded with a Perkin-Elmer 257 spectrophotometer. ^{1}H NMR spectra were determined with a Varian XL-100 (100 MHz) instrument equipped with a Fourier transform using Me_4Si as an internal standard. Spectral data are given for sample compounds. Chemical shifts are reported as values; the abbreviations s, br s, d, t, q, m refer to singlet, broad singlet, doublet, triplet, quartet, and multiplet, respectively, and the number in parentheses refers to the number of protons represented by the given signal. Mass spectra were measured on a Varian MATT 112 spectrometer. General reaction conditions are given once; other conditions are reported in Table V. The yields for the products obtained were not optimized.

2-[(4-Methoxybenzyl)thio]-4,6(1*H*,5*H*)-pyrimidinedione (4b). To a solution of **3** (15.0 g, 0.104 mol) in EtOH (90 mL) and water (90 mL) were added a solution of NaOH (5.1 g, 0.128 mol) in EtOH (35 mL) and water (35 mL). 1-(Chloromethyl)-4-methoxybenzene (18.7 g, 0.119 mol) was added dropwise, and the reaction mixture was heated with stirring for 60 min at 60 °C. After the mixture was cooled in an ice bath for 2 h, the precipitate was filtered, washed with water, and dried under vacuum over $CaCl_2$ to give 15.2 g (55.0%) of pure **4b**, which was used as such in the following reaction: mp (EtOH) 290 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.73 (s, 3 H, CH_3O), 4.32 (s, 2 H, CH_2S), 5.17 (s, 1 H, CH pyrimidine), 6.77–7.47 (m, 4 H, CH phenyl); IR (KBr) 3400, 2930, 1645, 1550, 1225, 1030 cm^{-1}. Anal. ($C_{12}H_{12}N_2O_3S$) C, H, N.

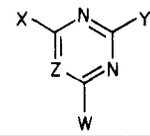
2-(Octylthio)-4,6(1*H*,5*H*)-pyrimidinedione (4d). To a 40% aqueous solution of tetrabutylammonium hydroxide (20.0 g, 30.9 mmol) was added 4.0 g (27.7 mmol) of **3**. The reaction mixture was stirred for 10 min, washed with $CHCl_3$ (50 mL), and filtered. The solid salt was dried over $CaCl_2$ under vacuum to give 9.5 g (24.6 mmol) of anhydrous compound, which was dissolved in MeOH (140 mL). To this solution was added 1-iodooctane (23.7 mL, 130 mmol), and the reaction mixture was refluxed for 8 h. The solvent was partially removed under reduced pressure, the residue (35 mL) was diluted with water, and the separated tarry oil was crystallized from AcOEt to give 4.5 g (63.0%) of pure **4d**: mp 163–165 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.75–1.55 (m, 15 H, $(CH_2)_6CH_3$), 3.10 (t, 2 H, SCH_2), 5.18 (s, 1 H, CH pyrimidine); IR (KBr) 3400, 2925, 1645, 1425, 1220 cm^{-1}. Anal. ($C_{12}H_{20}N_2O_2S$) C, H, N.

4,6-Dichloro-2-[(4-methoxybenzyl)thio]pyrimidine (5b). To a solution of **4b** (45.0 g, 0.170 mol) in $POCl_3$ (320 mL) was added 45 mL (0.283 mol) of *N*,*N*-diethylaniline, and the reaction mixture was refluxed for 8 h. Most of $POCl_3$ was distilled and the residue was poured with stirring upon 1 kg of cracked ice. The separated tarry oil was extracted with AcOEt; combined organic layers were washed with brine to neutrality, dried over Na_2SO_4, and evaporated under reduced pressure, bp 174 °C (0.05 mmHg), yield 31.5 g (61.5%). An analytical sample was redistilled to obtain a white oil, which solidified on standing: mp 48–49 °C; ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 3 H, CH_3O), 4.29 (s, 2 H, CH_2), 6.79 (d, 2 H, CH phenyl), 6.97 (s, 1 H, pyrimidine), 7.30 (d, 2 H, CH phenyl); IR ($CHCl_3$) 1610, 1530, 1280, 1185, 1100, 1030 cm^{-1}. Anal. ($C_{12}H_{10}Cl_2N_2OS$) C, H, N.

Ethyl [[4-Chloro-6-[(2,3-dimethylphenyl)oxy]-2-pyrimidinyl]thio]acetate (6b). To a solution of ethyl [[4,6-dichloro-2-pyrimidinyl]thio]acetate (**5a**) (12.5 g, 46.8 mmol) in EtOH (80 mL) at 45 °C was added a solution of 2,3-dimethylphenol (6.10 g, 50.0 mmol) and NaOH (1.86 g, 46.5 mmol) in EtOH (80 mL). The reaction mixture was refluxed for 4 h and filtered. The organic phase was partially evaporated under reduced pressure, diluted with water, and filtered. The precipitate was recrystallized from EtOH to give 8.5 g (51.5%) of pure **6b**: mp 87–88 °C; ^{1}H NMR ($CDCl_3$) δ 1.68 (t, 3 H, CH_3CH_2), 2.59 (s, 3 H, CH_3Ph), 2.92 (s, 3 H, CH_3Ph), 4.49 (s, 2 H, CH_2S), 4.96 (q, 2 H, CH_2CH_3), 7.50 (s, 1 H, CH pyrimidine), 7.80–8.40 (m, 3 H, CH phenyl); IR (KBr) 3055, 1735, 1550, 1460, 1400, 1325 cm^{-1}; MS, *m*/*z* 352 (M^+), 307

(26) Reddy, J. K., unpublished results.
(27) Svoboda, D. J.; Azarnoff, D. L. *Cancer Res.* **1979**, *39*, 3419.

Table V.[a] Physical and Chemical Properties of Intermediate Compounds



no.	X	Y	Z	W	SM (wt, g)	reagent (vol, mL)	solv (vol, mL)	temp, °C	time, h	yield, %	crystn solv	mp, °C
4a	OH	SCH_2CO_2Et	CH	OH	**3** (5.0)	$BrCH_2CO_2Et$[b] (4.5)	EtOH/H_2O (30/30)	60	1.5	75[c]	EtOH/H_2O	195–196
4c	OH	SCH_3	CH	OH	**3** (5.6)	CH_3I[d] (2.8)	H_2O (50)	20	1.0	89[e]	H_2O	>350
5a	Cl	SCH_2CO_2Et	CH	Cl	**4a** (2.5)	$POCl_3$[f] (50)	$POCl_3$	107	13.0	83[g]	*n*-hexane	70–72
5c	Cl	SCH_3	CH	Cl	**4c** (3.5)	$POCl_3$ (25)	$POCl_3$	107	2.0	70[h]	MeOH/H_2O	40–41
5d	Cl	SC_8H_{17}	CH	Cl	**4d** (6.5)	$POCl_3$ (50)	$POCl_3$	107	8.0	68	*i*	oil
6a	Cl	SCH_2CO_2Et	CH	$(CH_3)_2N$	**5a** (6.0)	$(CH_3)_2NH$[j] (6.4)	CH_3CN (25)	20	1.0	58	CH_3CN	97–98
6c	Cl	SCH_2CO_2Et	CH	B[k]	**5a** (1.1)	B′[k] (0.62)	EtOH (10)	80	24.0	33		oil
6d	Cl	SCH_2CO_2Et	CH	A	**5a** (5.0)	A″[l] (2.5)	EtOH (16)	80	24.0	87[m]	EtOH	88–90
1d	Cl	SCH_2CO_2H	CH	A	**6c** (6.3)	NaOH[n] (3.7)	EtOH (7.5)	80	2 min	80[o]	AcOEt	151–153
10a	Cl	4-CH_3O-$C_6H_4CH_2S$	CH	A	**5b** (2.0)	A′ (1.6)	CH_3CN (10)	83	36.0	55	benzene/*n*-hexane	118–119
10b	Cl	4-CH_3O-$C_6H_4CH_2S$	CH	B	**5b** (1.2)	B′ (1.1)	CH_3CN (6.0)	83	96.0	41	AcOEt/*n*-hexane	95–97
12a	Cl	SCH_3	CH	C	**5c** (1.5)	C′[p] (0.95 g)	EtOH (30)	80	0.5	70	EtOH	104–105
12c	Cl	SCH_3	CH	B	**5c** (5.0)	B′[q] (4.0)	CH_3CN (50)	83	48.0	46	CH_3CN	121–123
13b	Cl	SO_2CH_3	CH	A	**12b** (3.4)	H_2O_2[r] (4.3)	AcOH (34)	20	16.0	80	AcOEt/*n*-hexane	140–142
13c	Cl	SO_2CH_3	CH	B	**12c** (1.3)	H_2O_2[s] (1.6)	AcOH (13)	20	16.0	90	AcOEt	182–183
15b	Cl	OCH_2CO_2Et	CH	A	**13b** (4.0)	$HOCH_2CO_2Et$[t] (8.0)	$HOCH_2C$-O_2Et	20	0.3	71	AcOEt/*n*-hexane	96–98
15c	Cl	OCH_2CO_2Et	CH	B	**13c** (3.4)	$HOCH_2CO_2Et$[t] (8.0)	$HOCH_2C$-O_2Et	20	1.5	82	AcOEt/*n*-hexane	90–92
16b	Cl	$HNCH_2$-CO_2Et	CH	A	**13b** (5.6)	$HCl{\cdot}H_2NCH_2$-CO_2Et[u] (5.4)	EtOH (55)	80	18.0	85	AcOEt/*n*-hexane	164–166
16c	Cl	$HNCH_2$-CO_2Et	CH	B	**13c** (5.9)	$HCl{\cdot}H_2NCH_2$-CO_2Et[u] (5.4)	EtOH (40)	80	20.0	48	AcOEt	154–156
23a	Cl	CH_3NCH_2-CO_2Et	N	A	**22** (1.3)	$CH_3NHCH_2CO_2Et$[v] (0.6 g)	$CHCl_3$ (50)	i0	20 min	82	AcOEt	92–93
23b	Cl	OCH_2CO_2Et	N	A	**22** (4.0)	$HOCH_2CO_2Et$[w] (1.6)	DME (36)	35	1.0	86	AcOEt/*n*-hexane	80–82

[a] All reported compounds showed correct microanalyses (C, H, N); structural elucidation was made by ^1H NMR, IR, and/or mass spectra. [b] 1.54 g of NaOH was used to generate the sodium salt of thiobarbituric acid. [c] Yield 72%, mp (EtOH/H_2O) 194–195 °C, ref 3a. [d] 7.0 g of KOH was used to generate the potassium salt of thiobarbituric acid. [e] Yield 76%, mp (H_2O) 360 °C, ref 25. [f] The reaction was made in the presence of 9.76 mL of *N*,*N*-diethylaniline. [g] Yield 61%, mp (petroleum ether) 61–62 °C, ref 3a. [h] Yield 64%, mp (MeOH/H_2O) 43 °C, ref 25. [i] Purification was made by chromatography on Kieselgel (benzene/*n*-hexane). [j] 33% aqueous solution. [k] 0.47 g of Na_2CO_3 was added to the reaction mixture and the product was purified by chromatography on Kieselgel ($CHCl_3$/MeOH). [l] 1.14 g of Na_2CO_3 was added to the reaction mixture. [m] Yield 95%, mp (EtOH) 87–91 °C, ref 3a. [n] 30% aqueous solution. [o] Yield 69%, mp (AcOEt) 150–153 °C, ref 3a. [p] 0.31 g of NaOH was used to form the sodium salt of 2,3-dimethylpheonol. [q] 3.20 g of Na_2CO_3 was added to the reaction mixture. [r] 30% aqueous solution; the reaction was made in the presence of 49 mg of Na_2WO_4. [s] 30% aqueous solution; the reaction was made in the presence of 7.0 mg of Na_2WO_4. [t] 0.45 g of 100% NaH was used to form the sodium salt of ethyl glycolate. [u] 0.87 g of 100% NaH was used to liberate glycine from its hydrochloride salt; 5.0 mL of Et_3N was added. [v] 0.7 mL of Et_3N was added. [w] 385 mg of 100% NaH was used to generate the sodium salt of ethyl glycolate. [x] A = 2,3-$(CH_3)_2C_6H_3NH$, B = 2,3-$(CH_3)_2C_6H_3NCH_3$, C = 2,3-$(CH_3)_2C_6H_3O$, A′ = 2,3-$(CH_3)_2C_6H_3NH_2$, B′ = 2,3-$(CH_3)_2C_6H_3NHCH_3$, C′ = 2,3-$(CH_3)_2C_6H_3OH$.

($M^+ - OC_2H_5$), 279 ($M^+ - CO_2C_2H_5$), 265 ($M^+ - CH_2CO_2C_2H_5$), 233 ($M^+ - SCH_2CO_2C_2H_5$). Anal. ($C_{16}H_{17}ClN_2O_3S$) C, H, N.

[[4-Chloro-6-[methyl(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]acetic Acid (1c). To a refluxing suspension of **6c** (5.0 g, 13.7 mmol) in EtOH (45 mL) was rapidly added 27 mL of 1 N NaOH. After refluxing for 5 min, EtOH was distilled under reduced pressure, and the resulting mixture was diluted with water (40 mL) and extracted with Et_2O (2 × 30 mL). The water layer was acidified with concentrated hydrochloric acid and the solid was filtered, washed to neutrality with water, and recrystallized from AcOEt/*n*-hexane to provide 3.5 g (75.8%) of pure **1c**: mp 174–176 °C; ^1H NMR ($CDCl_3$) δ 2.27 (s, 3 H, CH_3Ph), 2.62 (s, 3 H, CH_3Ph), 3.77 (s, 3 H, CH_3N), 4.30 (s, 2 H, SCH_2), 6.25 (br s, 1 H, CH pyrimidine), 7.40–8.00 (m, 3 H, CH phenyl), 11.70 (br s, 1 H, CO_2H); IR (KBr) 3420, 2920, 1720, 1550, 1400, 1365 cm^{-1}. Anal. ($C_{15}H_6ClN_3O_2S$) C, H, N.

[[4-Ethoxy-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]acetic Acid (7). To an ethanol solution of sodium ethylate (0.77 g, 33.5 mmol of Na in 25 mL of absolute EtOH) was added 5.12 g (15.8 mmol) of [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]acetic acid (**1d**). The reaction mixture was refluxed for 7 h, poured on cracked ice (70 mL), and filtered. The liquid phase was acidified with HCl (12% aqueous solution) to pH 1, and the resulting precipitate was filtered, chromatographed on Kieselgel, and finally recrystallized from AcOEt to give 1.68 g (32.0%) of pure **7**: mp 190–193 °C; ^1H NMR ($CDCl_3$) δ 1.33 (t, 3 H, CH_3CH_2), 2.23 (s, 3 H, CH_3Ph), 2.42 (s, 3 H, CH_3Ph), 4.00 (s, 2 H, SCH_2), 4.52 (q, 2 H, CH_2CH_3), 5.65 (s, 1 H, CH pyrimidine), 7.35–7.65 (m, 3 H, CH phenyl); IR (KBr) 3250, 3150, 3050–2600, 1720, 1550 cm^{-1}. Anal. ($C_{16}H_{19}N_3O_3S$) C, H, N.

[[4-Ethoxy-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]-*N*-(2-hydroxyethyl)acetamide (8). To a suspension of **7** (5.0 g, 15.0 mmol) and Et_3N (2.5 mL, 17.9 mmol) in anhydrous and EtOH-free $CHCl_3$ (4.5 mL) was slowly added 2.5 mL (31.8 mmol) of ethyl chloroformate in 10 mL of $CHCl_3$ under nitrogen atmosphere at −10 °C. After 5 min at −10 °C a solution of ethanolamine (1.0 mL, 16.6 mmol) was dropped in $CHCl_3$ (10 mL); stirring was continued for 90 min at room temperature. The reaction mixture was diluted with water and extracted with AcOEt. Combined organic layers were washed once with saturated sodium hydrogen carbonate solution and then with water and dried with Na_2SO_4. Evaporation under reduced pressure and chromatography on Kieselgel gave 3.3 g (58.4%) of pure **8**: mp (AcOEt/petroleum ether) 112–114 °C; ^1H NMR ($CDCl_3$) δ 1.30 (t, 3 H, CH_3CH_2), 2.25 (s, 3 H, CH_3Ph), 2.42 (s, 3 H, CH_3Ph),

3.35–3.95 (m, 4 H, CH_2CH_2), 3.95 (s, 2 H, CH_2S), 4.51 (q, 2 H, CH_2CH_3), 5.75 (s, 1 H, CH pyrimidine), 7.20–7.60 (m, 3 H, CH phenyl), 8.70 (br s, 1 H, CONH); IR ($CHCl_3$) 3350, 3280, 3140, 1665, 1570 cm^{-1}. Anal. ($C_{18}H_{24}N_4O_3S$) C, H, N.

[[4-Chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]-*N*-(2-hydroxyethyl)acetamide (1e). To a stirred suspension of **1d** (30.0 g, 92.6 mmol) in 450 mL of EtOH-free chloroform was added a solution of 2.7 mL (52.2 mmol) of aziridine in 5 mL of chloroform. The reaction mixture was refluxed for 16 h, adding subsequently after 4, 8, and 12 h separate portions of 1.4, 0.9, 0.5 mL (27.1, 17.4, 9.66 mmol, respectively) of aziridine. The solvent was evaporated under reduced pressure and the residue was dissolved in AcOEt. The organic layer was washed with 5% aqueous sodium hydroxide and water, dried with Na_2SO_4, and evaporated under reduced pressure. Crystallization of the crude product from acetone gave 19.5 g (57.4%) of pure **1e**: mp 144–146 °C; ^1H NMR (C_5D_5N) δ 2.08 (s, 3 H, CH_3Ph), 2.12 (s, 3 H, CH_3Ph), 3.5–3.9 (m, 4 H, NCH_2, CH_2O), 4.06 (s, 2 H, CH_2S), 6.10 (s, 1 H, CH pyrimidine), 6.15 (br s, 1 H, OH), 6.85–7.25 (m, 3 H, CH phenyl), 8.65 (br s, $CONHCH_2$); IR (Nujol) 3300, 3170, 1660, 1570, 1210 cm^{-1}. Anal. ($C_{16}H_{19}ClN_4O_2S$) C, H, N.

[[4-Chloro-6-[nitroso(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]-*N*-(2-hydroxyethyl)acetamide (9). To a solution of **1e** (19.0 g, 51.8 mmol) in 300 mL of glacial acetic acid was added with stirring a solution of sodium nitrite (17.2 g, 249.3 mmol) in 52 mL of water. After 30 min at room temperature, the system was diluted with water (1200 mL) and the crystalline precipitate was filtered, washed to neutrality with water, dried under vacuum over $CaCl_2$, and recrystallized from AcOEt to give 14.5 g (70.7%) of pure **9**: mp 127–128 °C; ^1H NMR (C_5D_5N) δ 1.73 (s, 3 H, CH_3Ph), 2.08 (s, 3 H, CH_3Ph), 3.61 (t, 2 H, CH_2N), 3.78 (t, 2 H, CH_2O), 3.90 (s, 2 H, CH_2S), 5.80 (s, 1 H, OH), 6.70–7.30 (m, 3 H, CH phenyl), 7.60 (s, 1 H, CH pyrimidine), 8.42 (br s, 1 H, CONH); IR (Nujol) 3320, 3250, 1660, 1410 cm^{-1}. Anal. ($C_{16}H_{18}ClN_5O_3S$) C, H, N.

4-Chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinethiol (11a). To a solution of [4-chloro-6-[(2,3-dimethylphenyl)-amino]-2-(4-methoxybenzyl)thio]pyrimidine (**10a**) (8.0 g, 20.7 mmol) in CF_3CO_2H (80 mL) was added 2.7 mL (24.8 mmol) of anisole, and the resulting mixture was refluxed for 50 min. The solvent was evaporated under reduced pressure and the yellow residue was crystallized from Et_2O. Crystals collected by filtration were washed to neutrality with water and recrystallized from Et_2O. A 4.9-g yield (89.1%) of pure **11a** was obtained: ^1H NMR (Me_2SO-d_6) δ 2.09 (br s, 3 H, CH_3Ph), 2.24 (br s, 3 H, CH_3Ph), 6.80–7.15 (m, 3 H, CH phenyl), 6.25 (br s, 0.6 H), 7.30 (br s, 0.4 H) are indicative of an equilibrium between the NH and SH tautomeric forms; IR (KBr) 3400–2000, 1625, 1570, 1190, 1010 cm^{-1}. Anal. ($C_{12}H_{12}ClN_3S$) C, H, N.

4-Chloro-6-[(2,3-dimethylphenyl)amino]-2-(octylthio)pyrimidine (12d). To a solution of 3.0 g (10.2 mmol) of 4,6-dichloro-2-(octylthio)pyrimidine (**5d**) in EtOH (25 mL) were added 1.5 mL (12.3 mmol) of 2,3-dimethylaniline and then 1.25 g (11.8 mmol) of Na_2CO_3. The reaction mixture was refluxed for 20 h with magnetic stirring, cooled to room temperature, and filtered. The organic phase was evaporated under reduced pressure and the resulting oil was chromatographed on Kieselgel eluting with benzene/*n*-hexane. A 1.9-g yield (49.3%) of pure oily **12d** was obtained: ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, CH_3CH_2), 1.20–1.45 (m, 10 H, $CH_3(CH_2)_5$), 1.70 (t, 2 H, CH_2CH_2S), 2.15 (s, 3 H, CH_3Ph), 2.33 (s, 3 H, CH_3Ph), 3.10 (t, 2 H, CH_2S), 5.91 (s, 1 H, CH pyrimidine), 6.76 (br s, 1 H, NH), 7.00–7.20 (m, 3 H, CH phenyl); IR (neat) 3210, 2920, 1550, 1470, 1225 cm^{-1}. Anal. ($C_{20}H_{28}ClN_3S$) C, H, N.

4-Chloro-2-(methylsulfonyl)-6-[(2,3-dimethylphenyl)-oxy]pyrimidine (13a). To a suspension of 4-chloro-2-(methylthio)-6-[(2,3-dimethylphenyl)oxy]pyrimidine (**12a**) (16.0 g, 56.98 mmol) in glacial acetic acid (160 mL) were added under mechanical stirring 192 mg (0.65 mmol) of Na_2WO_4 and then 20.8 mL (0.204 mol) of 30% H_2O_2. The reaction mixture was stirred for 16 h, diluted with water (200 mL), and filtered. The crystalline product was washed to neutrality with water and dried under vacuum to give 12.0 g (67.3%) of pure **13a**. An analytical sample, recrystallized from AcOEt/*n*-hexane, had the following: mp 114–116 °C; ^1H NMR ($CDCl_3$) δ 2.08 (s, 3 H, CH_3Ph), 2.35 (s, 3 H, CH_3Ph), 3.21 (s, 3 H, CH_3SO_2), 6.80–7.30 (m, 4 H, CH phenyl, CH pyrimidine); IR (Nujol) 3095, 3040, 1320, 1130 cm^{-1}. Anal. ($C_{13}H_{13}ClN_2O_3S$) H, N; C: calcd, 57.06; found, 56.38.

Ethyl [[4-Chloro-6-[(2,3-dimethylphenyl)oxy]-2-pyrimidinyl]oxy]acetate (15a). To a suspension of **13a** (3.20 g, 10.2 mmol) in ethyl glycolate (8 mL) was added 360 mg (15.0 mmol) of oil-free NaH while the temperature was maintained below 35 °C with external cooling. The reaction mixture was stirred for 30 min at room temperature, diluted with water (30 mL), washed to neutrality, and filtered. Recrystallization from AcOEt/*n*-hexane gave 1.75 g (50.9%) of pure **15a**: mp 89–90 °C; ^1H NMR ($CDCl_3$) δ 1.21 (t, 3 H, CH_3CH_2), 2.00 (s, 3 H, CH_3Ph), 2.28 (s, 3 H, CH_3Ph), 4.07 (q, 2 H, CH_2CH_3), 4.67 (s, 2 H, CH_2CO), 6.34 (s, 1 H, CH pyrimidine), 6.50–7.25 (m, 3 H, CH phenyl); IR (KBr) 3090, 1760, 1560, 1340, 1210 cm^{-1}. Anal. ($C_{16}H_{17}ClN_2O_4$) C, H, N.

2,4-Dichloro-6-[(2,3-dimethylphenyl)amino]pyrimidine (20). To a solution of 2,4,6-trichloropyrimidine (**19**) (25.0 g, 0.136 mol) in EtOH (50 mL), chilled to 10 °C, were added 2,3-dimethylaniline (16.6 mL, 0.136 mol) and then Na_2CO_3 (11.5 g, 0.108 mol). The reaction mixture was stirred at room temperature for 20 h and refluxed for 7 h. After cooling, it was diluted with water (200 mL), obtaining a solid product, which was filtered and washed to neutrality with water. Crystallization from CH_2Cl_2 gave 27.4 g (75.1%) of pure **20**: mp 204–206 °C; ^1H NMR (Me_2SO-d_6) δ 2.10 (s, 3 H, CH_3Ph), 2.30 (s, 3 H, CH_3Ph), 6.40 (br s, 1 H, CH pyrimidine), 7.00–7.20 (m, 3 H, CH phenyl); IR (KBr) 3220, 3080, 1600, 1570, 1550 cm^{-1}. Anal. ($C_{12}H_{11}Cl_2N_3$) C, H, N.

4-Chloro-2-(dimethylamino)-6-[(2,3-dimethylphenyl)-amino]pyrimidine (14b). To a suspension of **20** (10.0 g, 37.3 mmol) in EtOH (70 mL) were added Na_2CO_3 (7.0 g, 66.0 mmol) and then a 40% aqueous solution of dimethylamine (5.2 mL, 41.1 mmol). The reaction mixture was refluxed for 18 h, cooled, and diluted with water. The precipitate was filtered and chromatographed on Kieselgel to give 7.0 g (67.8%) of pure **14b**. An analytical sample was crystallized from MeOH: mp 146–147 °C; ^1H NMR ($CDCl_3$) δ 2.14 (s, 3 H, CH_3Ph), 2.29 (s, 3 H, CH_3Ph), 3.11 (s, 6 H, $2CH_3N$), 5.57 (s, 1 H, CH pyrimidine), 6.95–7.25 (m, 3 H, CH phenyl); IR (Nujol) 3230, 3120, 2920, 1560, 1385 cm^{-1}. Anal. ($C_{14}H_{12}ClN_4$) C, H, N.

2,4-Dichloro-6-[(2,3-dimethylphenyl)amino]-1,3,5-triazine (22). A solution of 18.0 g (97.6 mmol) of cyanuric chloride (**21**) in acetone (70 mL) was dropped into water (40 mL) with magnetic stirring. To this highly dispersed suspension was added 2,3-dimethylaniline (24.0 mL, 0.197 mol) while the reaction temperature was maintained below 5 °C. The reaction mixture was stirred at this temperature for 60 min and filtered. The precipitate was washed with water to neutrality and immediately dissolved in AcOEt (200 mL). The solution was dried with Na_2SO_4, partially evaporated under reduced pressure, and cooled until a crystalline precipitate of pure **22** was obtained (17.2 g, 65.5%): mp 186–188 °C; ^1H NMR ($CDCl_3$) δ 2.15 (s, 3 H, CH_3Ph), 2.38 (s, 3 H, CH_3Ph), 6.90–7.42 (m, 3 H, CH phenyl), 9.52 (br s, 1 H, NH); IR (KBr) 3250, 1380, 1230, 850 cm^{-1}. Anal. ($C_{11}H_{10}Cl_2N_4$) C, H, N.

Ethyl [[4-Chloro-6-[(2,3-dimethylphenyl)amino]-1,3,5-triazin-2-yl]thio]acetate (23c). To a stirred suspension of oil-free NaH (960 mg, 40.0 mmol) in anhydrous 1,2-dimethoxyethane (DME) (100 mL) were added 4.40 mL (40.1 mmol) of ethyl thioglycolate and then 10.0 g of **22** (37.2 mmol). The reaction mixture was stirred for 60 min at room temperature and filtered. Chromatography on Kieselgel of the evaporated residue gave 6.0 g (45.7%) of pure **23c**: mp (AcOEt/*n*-hexane) 69–71 °C; ^1H NMR ($CDCl_3$) δ 1.23 (t, 3 H, CH_3CH_2), 2.17 (s, 3 H, CH_3Ph), 2.30 (s, 3 H, CH_3Ph), 3.7 (s, 2 H, CH_2S), 4.03 (q, 2 H, CH_2CH_3), 6.80–7.40 (m, 3 H, CH phenyl); IR (KBr) 3250, 1742, 1550, 1395, 1235 cm^{-1}. Anal. ($C_{15}H_{17}ClN_4O_2S$) C, H, N.

[[4-Ethoxy-6-[(2,3-dimethylphenyl)amino]-1,3,5-triazin-2-yl]thio]acetic Acid (24c). To a stirred hot suspension of **23c** (14.5 g, 41.1 mmol) in EtOH (150 mL) was quickly added 88 mL of 1 N NaOH and the reaction mixture was refluxed for 20 min. EtOH was evaporated; acidification with concentrated HCl of the residue gave a white precipitate, which was crystallized from AcOEt to provide 11.5 g of pure **24c** (83.7%): mp 220–221 °C; ^1H NMR (Me_2SO-d_6) δ 1.26 (t, 3 H, CH_2CH_3), 2.03 (s, 3 H, CH_3Ph), 2.23 (s, 3 H, CH_3Ph), 4.08 (q, 2 H, CH_2CH_3), 4.67 (s, 2 H, CH_2S), 4.97 (br s, 1 H, NH), 6.50–7.00 (br s, 3 H, CH phenyl); IR ($CHCl_3$) 3300–2600, 2400, 1700, 1550, 1090 cm^{-1}. Anal. ($C_{15}H_{18}N_4O_3S$) C, H, N.

Pharmacology. First Investigation. The purpose of this first test was to preselect compounds with the same criteria for further investigation. Variation of serum cholesterol, triglycerides, and lipoprotein α-cholesterol were assessed in Sprague–Dawley male rats (185-g mean bodyweight), fed a normal diet and given orally, for 4 consecutive days, the investigational drugs at the dose of 50 mg/kg with **1d** or **1e** as standard or at the dose of 200 mg/kg with **2** as standard. Serum cholesterol,[28] lipoprotein α-cholesterol,[29] and triglycerides[30] were assayed on the blood taken. Blood was taken from the abdominal aorta 24 h after the last drug administration: the animals were fasted 18 h before the sacrifice.

The data obtained with the investigational drugs were compared with the data of the controls and the data of the standard (Student's t test for independent paired data).

Second Investigation. Sprague–Dawley male rats, 200-g mean bodyweight, were freely fed for 15 days a Nath's diet with the following composition: sucrose 49%, cocoa-nut oil 24%, casein 18%, vitamin mixture 2%, maize oil 1%, mineral salts 4%, cholic acid 1%, cholesterol 1%. The drugs, given daily for 15 days orally, were suspended in 10% gum arabic. After an 18-h fast, the animals were killed on the 16th day. Twenty-four hours after the last administration, serum total cholesterol[28] and triglycerides[30] were assayed, and the weight of the liver was measured; the percent variations from the liver weight values were calculated on the basis of 100 g of animal bodyweight. The data of the animals given the investigational drugs were compared with that of the controls fed the Nath's diet and with the data of the standard (Student's t test for independent paired data). Compound **18b** was tested at three doses, i.e., 6.25, 12.5, and 25 mg/kg os, using a Nath's diet containing cholesterol and cholic acid in a 0.5% concentration.

Investigations on Liver Enzymes and Peroxisomes. The investigation was carried out by J. K. Reddy.[26] F 344 rats were given the investigational compounds at the dose of 20 mg/kg by gastric incubation: **14b** for 6 weeks; **8**, **17b**, and **18b** for 7 weeks; and **24c** for 10 weeks. The methods used for the measurement of liver enzymes are reported in the literature.[19] The following explanations are given for the values reported in Table IV. A unit of catalase activity is defined as the amount that liberates half the peroxide hydrogen from a hydrogen peroxide solution at any concentration in 100 s at 25 °C and is, therefore, related to the half-time of the first-order reaction.[31] The carnitine acetyltransferase unit is expressed as nanomoles of CoA-SH (2-nitrobenzoic acid) with $E_{412nm} = 13\,600\ M^{-1}\ cm^{-1}$.[32] Enoyl-CoA hydratase activity[33] and palmitoyl-CoA oxidizing activity[34] are expressed as μmol min^{-1} (mg of protein)$^{-1}$. Peroxisomal proliferation was investigated by electron microscopy with a semiquantitative assessment based on the number of peroxisomes observed in the photogram.[19] The peptide, M_r 80 000, was examined by partial enzymatic proteolysis using *S. aureus* V-8 protease according to the method of Cleveland et al.[35]

Registry No. **1a**, 86627-43-2; **1b**, 91759-31-8; **1c**, 86627-39-6; **1d**, 50892-23-4; **1e**, 65089-17-0; **3**, 91759-32-9; **4a**, 50892-49-4; **4b**, 16953-21-2; **4c**, 1979-98-2; **4d**, 86627-13-6; **5a**, 50892-12-1; **5b**, 86627-07-8; **5c**, 6299-25-8; **5d**, 86627-14-7; **6a**, 86627-42-1; **6b**, 91759-33-0; **6c**, 86627-38-5; **6d**, 54061-62-0; **7**, 86627-11-4; **8**, 86627-49-8; **9**, 91759-34-1; **10a**, 86627-08-9; **10b**, 86627-09-0; **11a**, 86627-46-5; **11b**, 86627-48-7; **12a**, 91759-35-2; **12b**, 86626-95-1; **12c**, 86626-96-2; **12d**, 86627-47-6; **13a**, 91759-36-3; **13b**, 86627-00-1; **13c**, 86627-01-2; **14a**, 91759-37-4; **14b**, 86627-50-1; **14c**, 86627-51-2; **15a**, 91759-38-5; **15b**, 86627-26-1; **15c**, 86627-31-8; **16b**, 86627-16-9; **16c**, 86627-20-5; **17a**, 91759-39-6; **17b**, 86627-27-2; **17c**, 86627-32-9; **18b**, 86627-15-8; **18c**, 86627-19-2; **19**, 3764-01-0; **20**, 86627-10-3; **21**, 108-77-0; **22**, 61018-62-0; **23a**, 86627-52-3; **23b**, 86627-56-7; **23c**, 86627-54-5; **23d**, 86627-58-9; **24a**, 86627-53-4; **24b**, 86627-57-8; **24c**, 61018-63-1; **25**, 3140-74-7; **26**, 32998-04-2; **27**, 1202-22-8; **28**, 1075-39-4; 1-(chloromethyl)-4-methoxybenzene, 824-94-2; 1-iodooctane, 629-27-6; 2,3-dimethylphenol, 526-75-0; ethanolamine, 141-43-5; aziridine, 151-56-4; 2,3-dimethylaniline, 87-59-2; ethyl glycolate, 623-50-7; ethyl thioglycolate, 623-51-8; 2,4-dichloro-6-amino-1,3,5-triazine, 933-20-0; 4,6-dichloro-2-(dimethylamino)-pyrimidine, 5734-68-9.

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Acidic Furo[3,2-*b*]indoles. A New Series of Potent Antiallergy Agents

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A series of furo[3,2-*b*]indole carboxylic acids, tetrazoles, and carbamoyltetrazoles was prepared and tested in vitro with use of a model of active pulmonary anaphylaxis, the modified Schultz–Dale Test (SDT). In this model, isolated guinea pig lung strips are repeatedly challenged with antigen in the presence of an antihistamine (H_1). Most of the acidic furo[3,2-*b*]indoles tested inhibited the leukotriene-mediated lung contraction in a dose-related manner. Compounds with an *N*-phenyl substituent were more potent ($IC_{50} \leq 5.0\ \mu M$) inhibitors of SDT than the *N*-methyl analogues ($IC_{50} \geq 22.0\ \mu M$). Most of the *N*-phenyl analogues were more potent in SDT than Fisons' mediator-release inhibitor proxicromil (FPL-57,787; $IC_{50} = 6.3\ \mu M$). The most potent furo[3,2-*b*]indoles were those unsubstituted at C-7 and with *N*-phenyl, 2-carbamoyltetrazole, and 3-alkoxy substituents. All of the carboxylic acid ester analogues tested were weak or inactive at concentrations of 10–30 μM.

Since the introduction of disodium cromoglycate (DSCG) in 1967, the international pharmaceutical industry has attempted to develop a more effective, orally active antiallergic drug.[1] Excessive reliance on passive cutaneous anaphylaxis (PCA) in the rat as a model of allergy may be one reason for the failure of this effort to date.[2] Inhibition of histamine release from passively sensitized, antigen-challenged isolated human lung has similarly been disappointing in predicting clinical efficacy in allergic asthma.[3]

We chose a modification of the classic Schultz–Dale test (SDT)[4] as an in vitro model of active anaphylaxis in the

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