Hypolipidemic Activity of 4-Pyrimidinecarboxylic Acids in CF_1 Mice

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Abstract □ A series of 4-pyrimidinecarboxylic acids were evaluated for hypolipidemic activity in mice at 20–30 mg/kg/d ip. A number of these derivatives were observed to be active at this dose. Substitution of the hydroxyl group in position 2 and 6 of the 4-pyrimidinecarboxylic acid with a sulfhydryl and an amino group, respectively, led to a compound which produced a >40% reduction of serum cholesterol and triglyceride levels in mice. Similarly, a compound which was substituted with an amino group in position 2 and an isobutyl group in position 5 led to equally potent activity as a hypolipidemic agent.

Keyphrases □ 4-Pyrimidinecarboxylic acids—antihypolipidemic activity, mice □ Antihypolipidemic agents—4-pyrimidinecarboxylic acids, activity in mice

Windmueller and co-workers (1–4) demonstrated that 1% orotic acid (2,6-dihydroxy-4-pyrimidinecarboxylic acid) in the diet of rats decreased plasma lipids. Ravi Subbiah (5) later showed that both serum cholesterol and triglycerides were reduced in rats treated with 1% orotic acid, but there was an increase in cholesterol, fatty acid, and triglyceride levels in the liver with a decrease in cholesterol excretion into the bile and feces. Subsequent studies showed that orotic acid reduced the serum lipoprotein fractions, *i.e.*, low-density lipoprotein, very low-density lipoprotein, and high-density lipoprotein (6–8) induced hyperlipidemic states in animals (7). Protein synthesis required for the apoprotein was not inhibited; rather, there was an inability to incorporate the lipid with the apoprotein for the lipoprotein fractions by the liver (6). The present study deals with the structure modification of orotic acid to yield other 4-pyrimidinecarboxylic acids which hopefully will possess hypolipidemic activity at a relatively low dose.

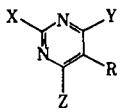
EXPERIMENTAL SECTION

Source of Compounds—Orotic acid was commercially available[1]. The chemical synthesis and physical characteristics of the 4-pyrimidinecarboxylic acids have been previously reported in the literature (9–11). Chemical syntheses were conducted by these methods and melting points are reported in Table I. Melting points were determined on a melting point apparatus[2] and are uncorrected.

Hypolipidemic Activity—Test compounds were suspended in 1% carboxymethylcellulose–water, homogenized, and administered intraperitoneally to CF_1 male mice (~25 g) for 16 d. On days 9 and 16, blood was obtained by tail vein bleeding and the serum was separated by centrifugation for 3 min. The serum cholesterol was determined by a modification of the Liebermann–Burchard reaction (12). Serum triglyceride levels were determined in blood collected on day 16 by a commercial kit[3]. The data for these assays is presented as a percent of control ± *SD*; the probability level was calculated by the Student's *t* test. Clofibrate[4] at 150 mg/kg/d was used as a standard for comparisons in the hypolipidemic screens.

[1] Aldrich Chemical Company.
[2] Mel-Temp.
[3] Bio Dynamics/bmc.
[4] Ayerst Laboratories.

Table I—Chemical Structure and Physical Characteristics of 4-Pyrimidinecarboxylic Acids



Compound	X	Y	Z	R	Melting Point, °C	Reference for Synthetic Procedure
I	OH	OH	COOH	CH_3		
II	NH_2	OH	COOH	CH_3	300–301	(10)
III	SH	OH	COOH	CH_3	297–300	(11)
IV	OH	SO	COOH	CH_3	317–319	(11)
V	SH	NH_2	COOH	CH_3	241–243	(9)
VI	NH_2	SH	COOH	CH_3	282–283	(11)
VII	NH_2	NH_2	COOH	C_2H_5	252–253	(9)
VIII	NH_2	NH_2	COOH	n-C_3H_7	243–244	(9)
IX	NH_2	NH_2	COOH	n-C_4H_9	245–246	(9)
X	NH_2	NH_2	COOH	iso-C_4H_9	238–239	(9)
XI	NH_2	NH_2	COOH	$C_6H_5CH_2$	248–249	(9)
XII	NH_2	NH_2	COOH	p-$CH_3OC_6H_4$	248–249	(9)
XIII	OH	NH_2	COOH	iso-C_4H_9	249–250	(9)
XIV	OH	NH_2	COOH	p-$CH_3OC_6H_4$	356–357	(9)
XV	SH	NH_2	COOH	C_2H_5	238–239	(9)
XVI	SH	NH_2	COOH	n-C_3H_7	233–234	(9)
XVII	SH	NH_2	COOH	n-C_4H_9	216–217	(9)
XVIII	SH	NH_2	COOH	$C_6H_5CH_2$	277–279	(9)
XIX	SH	NH_2	COOH	p-$CH_3OC_6H_4$	283–286	(9)
XX	C_2H_5S	OH	COOH	C_2H_5	232–233	(10)
XXI	SH	OH	CH_2OH	CH_3	297–300	(11)

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Table II—Hypolipidemic Activity of 4-Pyrimidinecarboxylic Acids in CF_1 Male Mice [a]

Compound	Dose mg/kg/d ip	Serum Cholesterol Levels Day 9	Serum Cholesterol Levels Day 16	Serum Triglyceride Levels Day 16
I	20	91 ± 5	68 ± 3[b]	82 ± 6
	30	94 ± 4	76 ± 4[b]	65 ± 5[b]
II	20	84 ± 4[b]	82 ± 4[b]	76 ± 6[b]
	30	78 ± 3[b]	77 ± 3[b]	56 ± 6[b]
III	20	80 ± 5[b]	75 ± 5[b]	74 ± 6[b]
	30	61 ± 4[b]	66 ± 3[b]	58 ± 5[b]
IV	20	75 ± 5[b]	65 ± 3[b]	68 ± 5[b]
V	20	76 ± 5[b]	56 ± 3[b]	48 ± 4[b]
VI	20	69 ± 5	66 ± 4[b]	59 ± 6[b]
VII	20	95 ± 5	73 ± 5[b]	71 ± 5[b]
VIII	20	101 ± 5	60 ± 4[b]	57 ± 5[b]
IX	20	73 ± 4[b]	78 ± 3[b]	89 ± 7
X	20	103 ± 5	74 ± 4[b]	53 ± 6[b]
XI	20	99 ± 6	73 ± 3[b]	68 ± 6[b]
XII	20	68 ± 4[b]	71 ± 4[b]	81 ± 7
XIII	20	81 ± 3[b]	56 ± 5[b]	43 ± 4[b]
XIV	20	81 ± 3[b]	73 ± 4[b]	73 ± 5[b]
XV	20	79 ± 6[b]	68 ± 5[b]	63 ± 3[b]
XVI	20	78 ± 5	71 ± 5[b]	94 ± 6
XVII	20	97 ± 7	68 ± 3[b]	74 ± 7[b]
XVIII	20	82 ± 8	69 ± 4[b]	50 ± 4[b]
XIX	20	80 ± 5[b]	64 ± 3[b]	80 ± 5[b]
XX	20	70 ± 6[b]	56 ± 3[b]	64 ± 3[b]
XXI	20	84 ± 5	79 ± 4[b]	55 ± 4[b]
Clofibrate	150	88 ± 4	87 ± 5	75 ± 5[b]
1% Carboxymethylcellulose		100 ± 6	100 ± 5	100 ± 7

[a] As mean ± *SD* percent of control; $n = 6$. [b] $p \leq 0.001$.

RESULTS AND DISCUSSION

A number of the 4-pyrimidinecarboxylic acid derivatives demonstrated potent hypolipidemic activity at 20 mg/kg/d ip in mice (Table II). Compounds I, IV, V, VI, VIII, XIII, XV, XVII, XVIII, XIX, and XX afforded ≥30% reduction of the serum cholesterol level at 20 mg/kg/d after dosing for 16 d and III produced a similar reduction at 30 mg/kg/d. Compounds IV, V, VI, VIII, X, XI, XIII, XV, XVIII, XX, and XXI resulted in ≥30% reduction of serum triglycerides after dosing for 16 d. Compounds I, II, and III were also effective at this level at a dose of 30 mg/kg/d. Compounds V and XIII appeared to be the most effective in this dose range with a >40% reduction of both serum lipids. Those carboxylic acids with a methyl group in the 5 position and a sulfhydryl group substituted in the 2 or 6 position (IV, V, and VI) at a dose of 20 mg/kg/d, and III at a dose of 30 mg/kg/d, resulted in lowering of both serum cholesterol and triglyceride levels after dosing for 16 d.

When positions 2 and 6 were substituted with an amino group while varying the R group substitutions, the *n*-propyl derivative (VIII) resulted in the best hypolipidemic activity of this series, with a 40% reduction of serum cholesterol and a 43% reduction of serum triglycerides after dosing for 16 d at 20 mg/kg/d. Compound X, with a substitution of isobutyl in position 5, was also active with a 26 and 47% reduction of cholesterol and serum triglycerides, respectively.

Substitution of a hydroxyl group in the 2 position, an amino in the 6 position with an isobutyl in position 5 (compound XIII) resulted in one of the best activities, with a 44% reduction of serum cholesterol and a 57% reduction of serum triglycerides. Of those compounds with a substituted aromatic ring in the 5 position, compound XVIII with a sulfhydryl group in position 2 and an amino group in position 6 resulted in a reduction of 31% in serum cholesterol and 50% in serum triglycerides. Compound XV, similar to XVIII but with an ethyl group substituted in the 5 position, also afforded good activity with a 32% reduction of serum cholesterol and a 37% reduction in triglyceride levels. Compound XX with a substituted sulfhydryl group in position 2, a hydroxyl group in the 6 position, and an ethyl group in the 5 position also afforded good activity by lowering serum cholesterol levels 44% and triglyceride levels 36% on day 16.

Substitution of an alcohol in place of a carboxylic acid group in position 4, with the same substitutions on 2, 6, and 5 (compare XXI to III) resulted in approximately the same degree of reduction of serum cholesterol at a dose of 20 mg/kg/d (20–25%), but the serum triglycerides were reduced from 26% to 45% with XXI at a dose of 20 mg/kg/d.

These studies have shown that a number of substituted 4-pyrimidinecarboxylic acids are potent hypolipidemic agents when compared with clofibrate at 150 mg/kg in rodents (Table II). Further studies are currently being conducted to establish the effects of the more potent derivatives on lipid metabolism and liver and serum lipoprotein levels. It would be desirable if the new agents did not cause lipid deposition in the hepatocyte, resulting in fatty livers similar to the observed effect of orotic acid on diet (2, 5).

Orotic acid is known to have no effect on cholesterol-7-α-hydroxylase activity and thus does not affect bile excretion of cholesterol (5); however, it does decrease mitochondrial fatty acid oxidation and acetyl-CoA dehydrogenase but not β oxidation by the peroxisomal system (13). A more in-depth study of orotic acid derivatives is required to assess their usefulness as hypolipidemic agents.

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