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A new class of potent hypolipemic agents raising high-density lipoproteins. Synthesis, reactions and pharmacological properties

H Furrer[1], E Granzer[2], R Wagner[1]

[1]*Preclinical Research, Medicinal Chemistry, Hoechst AG Werk Kalle-Albert, D-65174 Wiesbaden;*
[2]*Preclinical Research, Pharmacology, Hoechst AG, D-65926 Frankfurt a/M, Germany*

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Summary — A series of thiazolo[3,2-*c*]pyrimidin-5,7-diones has been synthesized. Results from *in vivo* evaluations in rats have shown that many of these compounds produce a pronounced increase of HDL cholesterol and a marked decrease of LDL and VLDL cholesterol. The most potent compound **17** (30 mg/kg/d per os over 7 d in male rats) led to the following changes: HDL cholesterol +101%, LDL cholesterol –40%, and VLDL cholesterol –98%. These effects may result in antiatherosclerotic properties in these compounds. The preparation of 7-amino-2,3-dihydrothiazolo[3,2-*a*]pyrimidin-5-ones and 5-amino-2,3-dihydrothiazolo[3,2-*a*] pyrimidin-7-ones is described.

thiazolopyrimidine / HDL cholesterol enhancement / LDL cholesterol reduction / lipid modulation

Introduction

Atherosclerosis is a multifactorial disease, the appearance of which is especially serious when it strikes the arterial vessels of the heart or the brain, which may lead to a myocardial infarct or a stroke often with fatal results. A decisive risk factor for atherosclerosis, besides hypertension, smoking and diabetes mellitus, is a dyslipoproteinemia, a pathogenic change of the ratio of lipoproteins in the blood. Substances that improve this ratio may have a marked influence on the reduction of atherosclerotic lesions.

In recent years, studies have shown that antihyperlipidemic drugs are useful in both the primary and the secondary prevention of cardiac events. The Helsinki heart study, a primary prevention trial with gemfibrozil in patients with elevated blood cholesterol, has produced a 34% reduction of coronary heart disease and has led to both a reduction of low density lipoproteins and an elevation of high-density lipoproteins [1, 2].

In the CLAS (cholesterol-lowering atherosclerosis study), a secondary angiographic trial with men who had undergone coronary artery bypass graft surgery, the combination of colestipol and niacin produced strong evidence of a decreased progression of atherosclerotic lesions, and a significant reduction of coronary artery disease and carotid arterial intima-media thickness [3, 4].

From these results we have gained important information, *ie* lowering lipid levels reduces clinical vascular events, and these benefits are associated with less progression or even regression of coronary atherosclerotic damage. It has been recognized that circulating cholesterol is carried by low-density lipoprotein (LDL) particles into the arterial intima, the site of atherogenesis. The protective high-density lipoprotein (HDL) particles in their turn support the reverse transport of cholesterol from the arterial tissue to the liver for catabolism and excretion [5, 6, 7]. In fact the ratio of plasma HDL cholesterol to total plasma cholesterol was correlated inversely with the incidence of a coronary heart disease in humans [8, 9] as well as with the severity of a cervical atherosclerosis in patients with a transient ischemic attack (TIA) or a minor ischemic stroke [10]. The findings of a Finnish study, performed with nearly 1800 randomly selected men, led to the conclusion that there was an inverse association between serum HDL and HDL_2 cholesterol and the risk of ischemic heart disease [11].

We wish to describe here a novel series of compounds, possessing a thiazolopyrimidine nucleus, most of which have led to the desired lipid modulation. This includes a pronounced increase of protec-

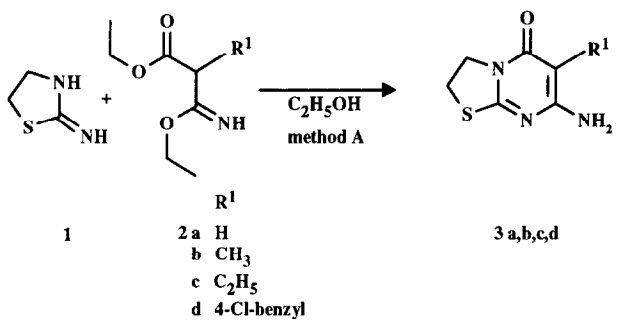
Abbreviations: acc, according; conc, concentrated; CSI, chlorosulfonyl isocyanate; dec, decomposition; DI, direct introduction (MS); Hünigs base, ethyl diisopropylamine; M^+, mol peak (MS); m-CPBA, *m*-chloroperbenzoic acid; morph, 4-morpholinyl; PEG, polyethylene glycol; piper, 1-piperidinyl; recr, recrystallized; rt, room temperature.

tive HDL cholesterol, a marked decrease of atherogenic LDL cholesterol as well as a reduction of atherogenic VLDL (very-low-density lipoprotein) cholesterol [12].

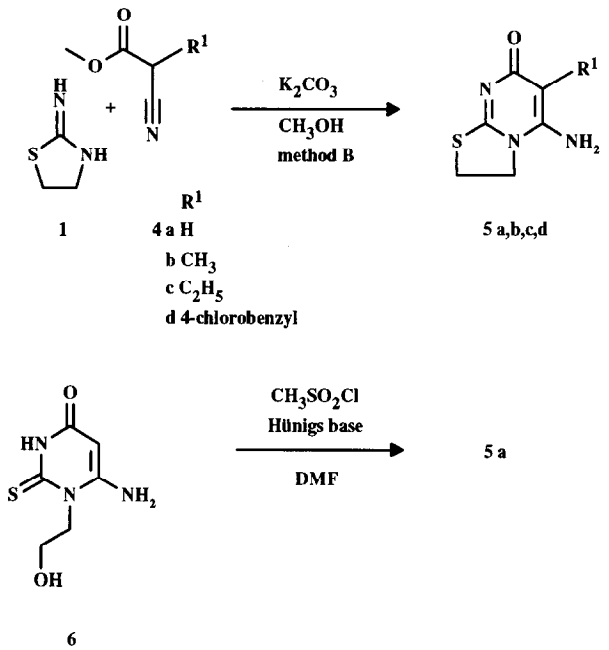
The synthesis and reactions of the novel thiazolo[3,2-*c*]pyrimidin-5,7-diones and their lipid modulating properties [13] are reported here.

Chemistry

The preparation of the 6-unsubstituted thiazolo[3,2-*c*]-pyrimidin-5,7-diones **7**, **19**, **20** and **21** is illustrated in schemes 1, 2 and 3 which include both the syntheses of intermediates **3a–d**, **5a–d** and **8** as well as the acid-catalyzed rearrangement of **3a–d**, **5a–d** and **8** to the target compounds.

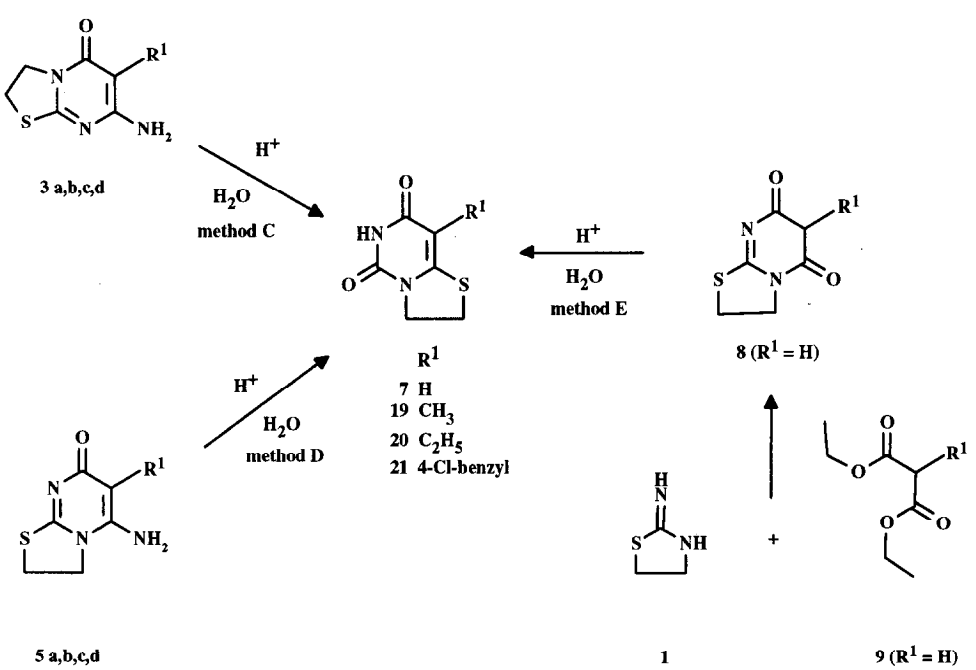


Scheme 1.



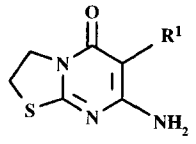
Scheme 2.

Scheme 1 displays the preparation of the first series of precursors, the 7-amino-2,3-dihydrothiazolo[3,2-*a*]



Scheme 3.

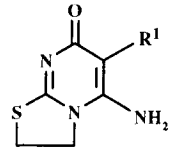
Table I. 7-Amino-2,3-dihydrothiazolo[3,2-*a*]pyrimidin-5-ones **3** and derivatives.



3

Compound	*R^1*	*Yield (%)*	*Empirical formula*	*Mp (°C)*	*Preparation method*
3a	H	77.3	$C_6H_7N_3OS$	278	A
3b	CH_3	21.7	$C_7H_9N_3OS$	227	A
3c	C_2H_5	63	$C_8H_{11}N_3OS$	187	A
3d	4-Cl-benzyl	74	$C_{13}H_{12}ClN_3OS$	238–239	A

Table II. 5-Amino-2,3-dihydrothiazolo[3,2-*a*]pyrimidin-7-ones **5**.



5

Compound	*R^1*	*Yield (%)*	*Empirical formula*	*Mp (°C)*	*Preparation method*
5a	H	73.6	$C_6H_7N_3OS$	268	B
5b	CH_3	53.3	$C_7H_9N_3OS$	292 (dec)	B
5c	C_2H_5	24.2	$C_8H_{11}N_3OS$	270	B
5d	4-Cl-benzyl	68.5	$C_{13}H_{12}ClN_3OS$	270 (dec)	B

pyrimidin-5-ones **3a–d** by reaction of **1** (2-amino-2-thiazolin represented as its tautomer) with the imidoesters **2a–d**. The imidoesters **2a–d** are easily accessible by reaction of the corresponding cyanoacetic esters under the conditions of the Pinner reaction by addition of ethanol, which is also the solvent, in the presence of hydrochloric acid. The synthesis of **3a** by the above procedure has been described by Falch [14]. The intermediates **3a–d** are presented in table I.

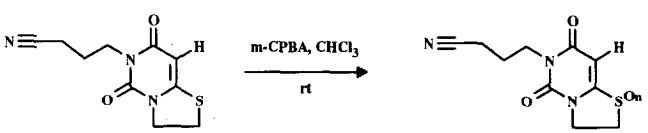
The 5-amino-2,3-dihydrothiazolo[3,2-*a*]pyrimidin-7-ones **5a–d**, which are isomeric to **3a–d**, could be obtained smoothly according to scheme 2 by reaction of **1** (2-amino-2-thiazolin represented as its tautomer) with the methyl cyanoacetates **4a–d** in methanol in the presence of potassium carbonate. The structure of **5a** was confirmed through an independent synthesis by ring closure of **6** (synthesized by reaction of *N*-2-hydroxyethylthiourea [15] with ethyl cyanoacetate in the presence of sodium ethoxide in analogy to reference [16]) with the aid of mesylchloride and Hünig's base in dimethylformamide. The intermediates **5a–d** are summarized in table II.

Scheme 3 outlines the various routes mentioned above for the preparation of the 2,3-dihydrothiazolo-[3,2-*c*]pyrimidine-5,7-dione **7** and its derivatives **19–21** *via* aqueous acid-catalyzed rearrangement of the heterocyclic compounds **3a–d** or **5a–d** or **8**. Compound **8** was easily obtained by reaction of **1** with **9**

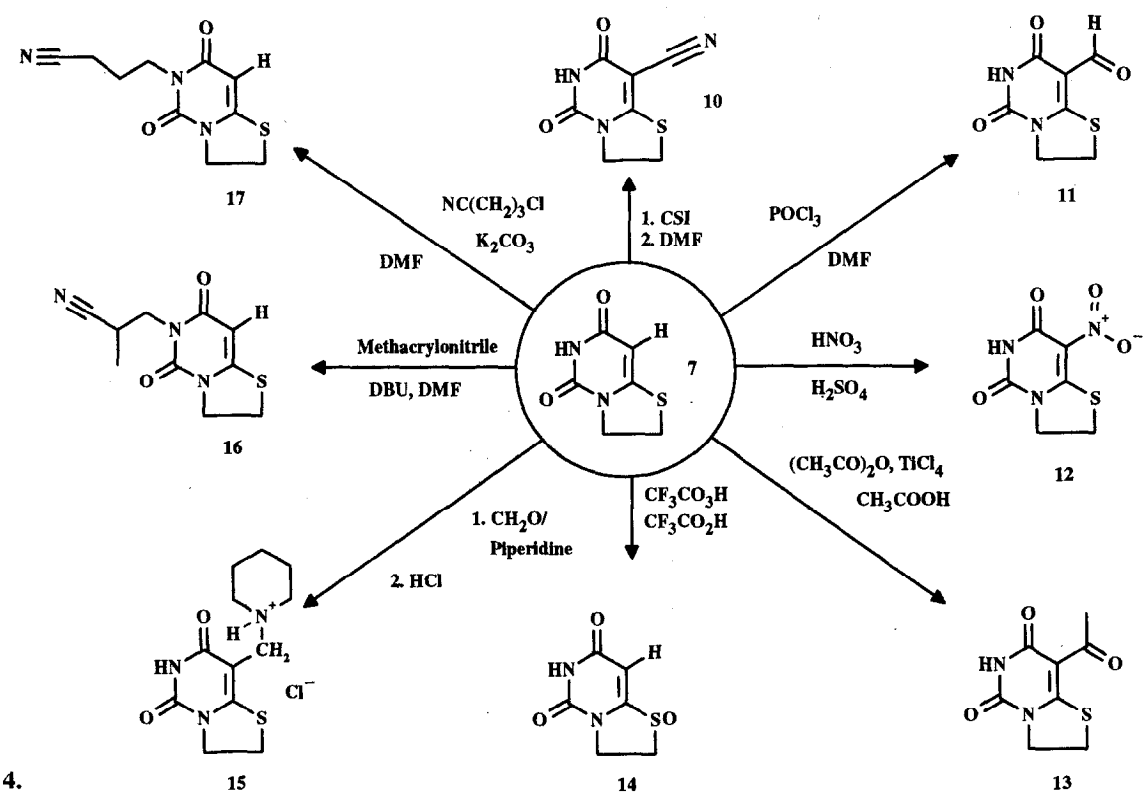
[17]. We assume that compounds **3a–d** and **5a–d**, under the conditions of acid-catalyzed hydrolysis, first lose ammonia to give **8**, or one of its derivatives, alkylated in the 6-position. These are subsequently rearranged by hydrolysis of the S-C bond and ring closure of the β-mercaptoethyl side chain under elimination of water to give target compounds of the basic structure **7**. The structure of **7** has been proposed for the product of a different reaction [18]. An alternative method of preparation for compound **19** has been described elsewhere [19].

Scheme 4 presents a synopsis of reactions with compound **7**. Alkylation of **7** under alkaline conditions led to substitution in the 6-position (for example, **16** or **17**). This procedure was used for the preparation of most of the compounds of table III, substituted at the 6-position. Electrophilic substitution reactions of **7** occurred in the 8-position giving compounds **10–13** and **15** as follows: a) reaction of **7** with chlorosulfonyl isocyanate (CSI) resulted in the corresponding *N*-chlorosulfonated carboxylic acid amide as an intermediate, which was smoothly transformed to **10** by reaction with dimethylformamide according to the procedure described by Lohaus [20]; b) Vilsmeier–Haack reaction of **7** led to the aldehyde **11**, which was characterized as its oxime **40** (table III); compound **11** was reduced with sodium borohydride in a mixture of dimethylsulfoxide and water to the corresponding hydroxymethyl compound **39** (table III); c) nitration of **7** with nitric acid in H_2SO_4 resulted in the nitro compound **12**; d) acetylation with acetic anhydride in the presence of $TiCl_4$ in acetic acid afforded the acetyl compound **13**; and e) Mannich reaction with formaldehyde and piperidine gave compound **15**. Finally, oxidation of **7** with trifluoroperacetic acid afforded sulfoxide **14**.

However, the oxidation of **17** with *m*-chloroperbenzoic acid in $CHCl_3$ led to a mixture of the corresponding sulfoxide **22** and sulfone **41** in approximately a 1:1 ratio (equation [1]).

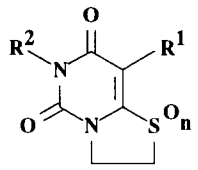


[1]



Scheme 4.

Table III. 2,3-Dihydrothiazolo[3,2-*c*]pyrimidine-5,7-diones of formula **I**.



I

Compound	*R^1*	*R^2*	*n*	*Yield (%)*	*Empirical formula*	*Mp (°C)*	*Preparation method or example*
7	H	H	0	85.5 (D)	$C_6H_6N_2O_2S$	304(dec)	C, D, E
10	CN	H	0	46.6	$C_7H_5N_3O_2S$	320(dec)	**10**
11	CHO	H	0	96.8	$C_7H_6N_2O_3S$	308	**11**
12	NO_2	H	0	59.7	$C_6H_5N_3O_4S$	298	**12**
13	$COCH_3$	H	0	23.1	$C_8H_8N_2O_3S$	267	**13**
14	H	H	1	68.4	$C_6H_6N_2O_3S$	273(dec)	**14**
15	CH_2-piper•HCl	H	0	39.5	$C_{12}H_{18}ClN_3O_2S$	275–276	**15**
16	H	NC-CH(CH_3)-CH_2-	0	34.5	$C_{10}H_{11}N_3O_2S$	133–134	**16**
17	H	NC-(CH_2)$_3$-	0	84.3	$C_{10}H_{11}N_3O_2S$	101–102	**17**
18	CH_2-morph•HCl	H	0	55.8	$C_{11}H_{16}ClN_3O_3S$	271–272	**15**
19	CH_3	H	0	81 (D)	$C_7H_8N_2O_2S$	243(dec)	C, D
20	C_2H_5	H	0	71.6 (C)	$C_8H_{10}N_2O_2S$	270 (dec)	C, D
21	4-Cl-benzyl	H	0	29 (C)	$C_{13}H_{11}ClN_2O_2S$	264-265	C, D
22	H	NC-(CH_2)$_3$-	1	31.2	$C_{10}H_{11}N_3O_3S$	128	**22**
23	CH_3	NC-(CH_2)$_2$-	0	60.8	$C_{10}H_{11}N_3O_2S$	162–163	**23**
24	C_2H_5	NC-CH(CH_3)-CH_2-	0	30.3	$C_{12}H_{15}N_3O_2S$	121	**16**
25	H	CH_3-CH(CH_3)-CH_2-	0	41.1	$C_{10}H_{14}N_2O_2S$	103	**17**
26	H	CH_3-CH(CH_3)-CH_2-	1	21.7	$C_{10}H_{14}N_2O_3S$	140	**14**
27	H	Propargyl-	0	51.4	$C_9H_8N_2O_2S$	167–168	**17**
28	CH_3	NC-CH(CH_3)-CH_2-	0	24.9	$C_{11}H_{13}N_3O_2S$	125–127	**16**
29	H	CH_3COCH_2-	0	61.5	$C_9H_{10}N_2O_3S$	186–187	**29**
30	H	$CH_3CH(OH)CH_2$-	0	61.4	$C_9H_{12}N_2O_3S$	153	**30**
31	H	NC-CH_2-	0	29.8	$C_8H_7N_3O_2S$	231	**17**
32	H	NC-(CH_2)$_4$-	0	24.5	$C_{11}H_{13}N_3O_2S$	107	**17**
33	H	CH_3-	0	80.3	$C_7H_8N_2O_2S$	141–142	**17**
34	H	CH_3-(CH_2)$_2$-	0	51.8	$C_9H_{12}N_2O_2S$	133–134	**17**
35	H	NC-(CH_2)$_2$-	0	28.5	$C_9H_9N_3O_2S$	151–152	**23**
36	H	CH_3O-(CH_2)$_2$-	0	35.7	$C_9H_{12}N_2O_3S$	122–123	**17**
37	C_2H_5	NC-(CH_2)$_2$-	0	70	$C_{11}H_{13}N_3O_2S$	187–188	**23**
38	H	C_2H_5O-CH_2-	0	29	$C_9H_{12}N_2O_3S$	125–126	**17**
39	CH_2OH	H	0	61	$C_7H_8N_2O_3S$	262	**39**
40	CH=NOH	H	0	56.8	$C_7H_7N_3O_3S$	260	**40**
41	H	NC-(CH_2)$_3$-	2	24.5	$C_{10}H_{11}N_3O_4S$	158	**41**

Reduction of ketone **29** with $NaBH_4$ in dimethylformamide/water resulted in the alcohol **30** (table III).

Biology

The compounds were examined initially for lipid-modulating activity in male rats. In this model the specified dose of the test compounds (table IV) in PEG 400 was administered once daily to the rats on 7 consecutive days, the final administration being 24 h before blood samples were taken. The control group received PEG 400 only. During the study the animals had free access to food and water. The plasma concentrations of total cholesterol and cholesterol carried by VLDL, LDL and HDL were measured and compared with those present in placebo-treated animals.

Table IV. Change of cholesterol in the serum of male rats after 7 d treatment.

Compound	*Dose (mg/kg/d, per os)*	*% change (in relation to the control)*			
		VLDL cholesterol	*LDL cholesterol*	*HDL cholesterol*	*HDL/LDL cholesterol*
7	30	0	–30	+12	1.6
16	30	–100	–49	+74	3.4
	10	–62	–45	+61	3.0
	3	–29	–40	+32	2.2
17	30	–98	–40	+101	4.7
	10	–85	–63	+32	3.5
	3	–41	–45	+7	2.0
	1	–37	–12	–2	1.1
21	30	–42	–15	+15	1.5
22	30	–92	–18	+37	1.7
	10	–76	–37	+8	1.7
	3	–45	–13	+11	1.3
23	30	–100	–48	+66	3.2
	10	–64	–28	+23	1.7
	3	+3	–10	+19	1.3
24	30	–43	–20	+28	1.6
	10	–35	–32	+39	2.0
25	30	–94	–37	+44	2.3
	10	–65	–33	+22	1.8
	3	–41	–30	+27	1.8
	1	–62	–23	+4	1.3
26	30	–75	–35	+40	2.2
	10	–53	–39	+22	2.0
	3	–56	+7	+3	1
27	30	–100	–33	+44	2.2
	10	–68	–28	+47	2.0
	3	–36	–37	–2	1.6
28	30	+14	–47	+12	2.1
	10	+23	–6	+24	1.3
29	30	–49	–14	+48	1.7
30	30	–21	–36	–3	1.5
31	30	–64	–40	+71	2.9
32	30	–70	–4	+70	1.8
33	30	–49	–40	+17	1.9
34	30	–43	–57	+27	3.0
	3	–43	–34	+17	1.8
35	30	–100	–54	+83	4.0
36	30	–38	–20	+50	1.9
37	30	–66	–26	+42	1.9
38	30	–58	–2	+60	1.6
Gemfibrozil	100	–53	–27	+70	2.3
	30	+31	–17	+13	1.4
Clofibrate	100	–69	–51	–31	1.3

Table V. Influence on cholesterol in hypercholesterolemic rats after 7 d treatment.

Compound	*Dose (mg/kg/d, per os)*	*% change (in relation to the control)*			
		VLDL cholesterol	*LDL cholesterol*	*HDL cholesterol*	*HDL/LDL cholesterol*
16	30	–47	–39	+171	4.5
	3	–37	–23	+29	1.7
17	30	–55	–22	+152	3.2
	3	–21	–30	+42	2.0
29	30	–64	–24	+152	3.3
	3	–22	–14	+28	1.5
Gemfibrozil	30	–71	–32	+66	2.4
	3	–26	–17	+25	1.5
Clofibrate	100	+25	+16	–17	0.7

A set of active compounds was then tested in a secondary screen using diet-induced hypercholesterolemic rats treated with a thyreostatic (table V). Identical procedures and parameters were followed as described in the preceding assay.

Results and discussion

The potential efficacy of a compound against the development of atherogenic lesions is defined by its antiatherogenic index, *ie* the ratio of HDL cholesterol and LDL cholesterol, HDL/LDL.

The data in table IV represent the percentage change of cholesterol in the lipoproteins VLDL, LDL and HDL in rat serum after oral administration of the relevant compounds of table III or the reference drugs clofibrate (INN) and gemfibrozil (INN) at the respective doses over 7 d and the corresponding antiatherogenic index HDL/LDL.

The group of compounds **16**, **17**, **23**, **28**, **31**, **35** and **37**, where R^2 is an alkyl side chain, substituted with a cyano group, exhibited the highest antiatherogenic index HDL/LDL, with **17** as the most active product. The compounds with an alkyl residue at R^2, **25**, **33** and **34**, also exhibited good index values, as did the isobutyl sulfoxide **26**. Good activity was even shown by the unsubstituted compound **7**, where R^1, R^2 = H, as well as by **21**, with R^1 = 4-chlorobenzyl. When R^2 = propargyl, 2-oxopropyl, 2-hydroxypropyl, the oxaalkyl side chains methoxyethyl or ethoxymethyl, compounds **27**, **29**, **30**, **36** or **38**, respectively, a good antiatherogenic index was observed.

The most potent compound of table IV, namely **17**, in comparison with gemfibrozil and clofibrate already resulted in a favourable antiatherosclerotic index at a dose of 3 mg/kg/d. As with gemfibrozil, increasing the doses of the compounds in table IV caused an increase of the relative liver weight in rats. This is exemplified in table VI for **17** which led to a proliferation of the smooth endoplasmic reticulum and an induction of drug-metabolizing enzymes. By microscopic examination it has been elucidated that gemfibrozil led to proliferation of peroxisomes in the liver of rats [2]. This type of proliferation was not found with **17** through electron microscopy.

A similar profile of activity was reported for a series of *N*-imidazolylchroman-4-ones and *N*-imidazolyl-1-tetralones [21].

When given in therapeutic doses the anticonvulsant phenytoin, alone or in combination with phenobarbital and/or carbamazepine, has been shown to increase liver size and induce microsomal enzymes in humans. This treatment led to an increase of the HDL cholesterol and LDL cholesterol ratio (HDL/LDL), which was directly related to the extent of liver enlargement. The serum cholesterol distribution profile associated with an increase in liver size was typical for subjects with a low risk of coronary heart disease. These results suggest that enzyme inducers, such as pheny-

Table VI. Relative liver weight and HDL/LDL cholesterol ratio of male rats after 7 d treatment.

Compound	*Dose (mg/kg/d, po)*	*HDL/LDL cholesterol*	*% increase of liver weight vs control*
17	30	4.7	+61
	10	3.5	+25
	3	2.0	+15
	1	1.1	+1
Gemfibrozil	100	2.3	+41
	30	1.4	+12
Clofibrate	100	1.3	+18

toin and phenobarbital, lead to structural and functional changes in hepatocellular membranes associated with liver enlargement and cholesterol distribution, which is characteristic of low susceptibility to atherosclerotic vascular disease [22, 23]. Experimental studies have indicated that phenobarbital retards cholesterol accumulation in the arterial wall and the formation of atherosclerotic plaque [24].

Except for compound **21**, mentioned above, the other compounds in table III, substituted in the 8-position and with R^2 = H, resulted in a marked decrease of the antiatherogenic index with values under 1.4 (dose of 30 mg/kg/d).

The data in table V show the influence of a set of test compounds on the percentage change of cholesterol levels in VLDL, LDL and HDL and the antiatherogenic index HDL/LDL in hypercholesterolemic rats. Compounds **16** and **17**, which have a nitrile group on substituent R^2, and the 6-acetonyl compound **29** have led to the desired favourable modulation of cholesterol in the lipoproteins, namely an increase of HDL cholesterol and a decrease of LDL and VLDL cholesterol.

Experimental protocols

Chemistry

Melting points were recorded on a capillary melting point apparatus Büchi SMP-20, decomposition points were determined on a capillary melting point apparatus from Electrothermal Engineering Ltd and are uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer 841 infrared spectrophotometer using KBr discs and are reported in wave-numbers (cm^{-1}). Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a 200 MHz Varian GEM 200 spectrometer with DMSO-d_6 as solvent. Chemical shifts are reported downfield in ppm (δ) relative to tetramethylsilane as internal standard. Mass spectra (MS) were obtained on Kratos MS 30 or MS 80 RFA spectrometers. Combustion analyses are within ± 0.4% of theoretical values. Thin layer chromatography (TLC) was performed on silica-gel plates. Tables I–III summarize compounds **3a–d**, **5a–d** and the compounds of formula **I** (table III), their empirical formulae together with the melting or decomposition points (mp), their mode of preparation with reference to the examples or general procedures and the yields.

*General procedure for the preparation of 7-amino-2,3-dihydrothiazolo[3,2-a]pyrimidin-5-ones **3a–d** (Method A)*
Compounds **3a–d** (table I) were synthesized by the representative procedure illustrated for **3c**.

*7-Amino-6-ethyl-2,3-dihydrothiazolo[3,2-a]pyrimidin-5-one **3c**.* To a solution of 2-amino-2-thiazoline (64.3 g, 0.61 mol) in ethanol (650 ml) was added at rt 2-(ethoxyiminomethyl)butyric acid ethyl ester (37.7%, 114 g, 0.23 mol) (prepared by Pinner synthesis, addition of ethanol to 2-cyanobutyric acid ethyl ester in the presence of HCl) and the mixture was stirred at reflux for 4 h. Ethanol (550 ml) was removed by distillation and the mixture was cooled to 10°C. The resulting crystals were collected by filtration. Purification by recrystallization from ethanol gave 28.6 g (63%) of **3c** as white crystals, mp 187°C. ^{1}H-NMR δ ppm: 0.91 (3H, t, CH_3), 2.25 (2H, q, CH_2), 3.45 (2H, t, SCH_2), 4.21 (2H, t, NCH_2), 6.24 (2H, s, NH_2). $C_8H_{11}N_3OS$ (MW = 197.3). Anal (C, H, N, S).

*General procedure for the preparation of 5-amino-2,3-dihydrothiazolo[3,2-a]pyrimidin-7-ones **5a–d** (Method B)*
Compounds **5a–d** (table II) were synthesized by the representative procedure illustrated for **5a**.

*5-Amino-2,3-dihydrothiazolo[3,2-a]pyrimidin-7-one **5a**.* To a solution of 2-amino-2-thiazoline (1434 g, 13.6 mol) in methanol (7 l) were added at 60°C K_2CO_3 (188 g, 1.36 mol) and cyanoacetic acid methyl ester (1363 g, 13.75 mol) and the mixture was stirred at 60°C for 8 h. The mixture was cooled to 20°C and the white crystals were collected by filtration. Additional crystals were obtained by concentration of the mother liquor. Purification of all precipitates by recrystallization from water gave 1770 g (73.6%) of **5a** as white crystals, mp 268°C (dec). ^{1}H-NMR δ ppm: 3.48 (2H, m, SCH_2), 4.24 (2H, m, NCH_2), 4.80 (1H, s, =CH), 6.6 (2H, s, NH_2); MS (DI) m/e 169 (M^+). $C_6H_7N_3OS$ incl 0.4 H_2O (MW = 176.4). Anal (C, H, N, S). The water content was measured by Karl-Fischer titration.

The structure of **5a** was confirmed chemically by independent synthesis from **6** as follows. To a solution of 6-amino-1-(2-hydroxyethyl)-2-thioxo-2,3-dihydro-1*H*-pyrimidin-4-one [15] **6** (18.7 g, 0.1 mol) and Hünigs base (14.7 g, 0.11 mol) in dimethylformamide (300 ml) at 0–5°C was added dropwise methanesulfonyl chloride (12.3 g, 0.105 mol). After stirring at rt for 1 h the mixture was concentrated *in vacuo*. The residue was suspended in water (100 ml) at 0–5°C and the mixture was neutralized by addition of a solution of sodium bicarbonate forming a precipitate consisting of **6** (5.2 g). After filtration the mother liquor was concentrated *in vacuo* and the residue was triturated with a mixture of chloroform/methanol/water (65:35:10). The resulting precipitate was collected by filtration, washed with water, recrystallized from water and dried *in vacuo*, giving 6.4 g of 5-amino-2,3-dihydrothiazolo[3,2-*a*]-pyrimidin-7-one **5a**, mp 268°C, which proved to be identical to **5a** prepared following *Method B*, according to mixed mp, IR (KBr) and ^{1}H-NMR.

*General procedure for the preparation of 2,3-dihydrothiazolo-[3,2-c]pyrimidine-5,7-diones **7** and **19–21** from 7-amino-2,3-dihydrothiazolo[3,2-a]pyrimidin-5-ones **3a–d** (Method C)*
Compounds **7** and **19–21** (table III) were synthesized by the representative procedure illustrated for **20**.

*8-Ethyl-2,3-dihydrothiazolo[3,2-c]pyrimidine-5,7-dione **20**.* A suspension of 7-amino-6-ethyl-2,3-dihydrothiazolo[3,2-*a*]-pyrimidin-5-one **3c** (161.5 g, 0.82 mol) in water (1.6 l) was treated with conc H_2SO_4 (33 ml), then heated to reflux for 32 h. The suspension was cooled to room temperature and adjusted to pH 4 with conc NaOH. The precipitate was collected by filtration. Recrystallization from glacial acetic acid gave 116.1 g (71.6%) of **20**, mp 270°C (dec). ^{1}H-NMR δ ppm: 0.99 (3H, t, CH_3), 2.18 (2H, q, CH_2), 3.45 (2H, m, SCH_2), 4.18 (2H, m, NCH_2), 11.1 (1H, s, NH); MS (DI) m/e 198 (M^+). $C_8H_{10}N_2O_2S$ (MW = 198.3). Anal (C, H, N, S).

*General procedure for the preparation of 2,3-dihydrothiazolo-[3,2-c]pyrimidine-5,7-diones **7** and **19–21** from 5-amino-2,3-dihydrothiazolo[3,2-a]pyrimidin-7-ones **5a–d** (Method D)*
Compounds **7** and **19–21** (table III) were synthesized by the representative procedure illustrated for **7**.

2,3-Dihydrothiazolo[3,2-c]pyrimidine-5,7-dione 7. A suspension of 5-amino-2,3-dihydrothiazolo[3,2-*a*]pyrimidin-7-one **5a** (589 g, 3.34 mol) in water (6 l) was treated with conc H_2SO_4 (100 ml), then heated to reflux for 19 h. The suspension was cooled to rt and neutralized with conc NaOH. The precipitate was collected by filtration. An additional precipitate was obtained by concentration of the mother liquor to one third of its volume. Both solids were combined. Recrystallization from glacial acetic acid gave 485.9 g (85.5%) of **7** as a white powder, mp 304°C (dec). ^{1}H-NMR δ ppm: 3.44 (2H, t, SCH_2), 4.15 (2H, t, NCH_2), 5.63 (1H, s, =CH), 11.05 (1H, s, NH); MS (DI) m/e 170 (M^+). $C_6H_6N_2O_2S$ (MW = 170.2). Anal (C, H, N, S).

Procedure for the preparation of 2,3-dihydrothiazolo[3,2-c]-pyrimidine-5,7-dione 7 from 2,3-dihydrothiazolo[3,2-a]pyrimidine-5,7-dione 8 (Method E)
A suspension of 2,3-dihydrothiazolo[3,2-*a*]pyrimidine-5,7-dione **8** (10.2 g, 0.06 mol) (prepared by reaction of 2-amino-2-thiazoline with malonic acid diethyl ester in the presence of sodium ethanolate) in a mixture of water (1 l), glacial acetic acid (17 ml) and conc H_2SO_4 (6 ml) was heated to reflux for 3 h. The mixture was cooled to room temperature, neutralized with NaOH and concentrated *in vacuo*. Recrystallization of the residue from water gave 4.2 g (41.1%) of **7** as a white powder, mp 304°C (dec). ^{1}H-NMR δ ppm: 3.44 (2H, t, SCH_2), 4.15 (2H, t, NCH_2), 5.63 (1H, s, =CH), 11.05 (1H, s, NH). $C_6H_6N_2O_2S$ (MW = 170.2). Anal (C, H, N, S).

*5,7-Dioxo-3,5,6,7-tetrahydro-2H-thiazolo[3,2-c]pyrimidine-8-carbonitrile **10***
A suspension of 2,3-dihydrothiazolo[3,2-*c*]pyrimidine-5,7-dione **7** (20.4 g, 0.12 mol) in chlorosulfonyl isocyanate (CSI) (475 ml, 779 g, 5.5 mol) was heated to 80°C for 3 h. The mixture was cooled to 5°C and the precipitate of 5,7-dioxo-3,5,6,7-tetrahydro-2*H*-thiazolo[3,2-*c*]pyrimidine-8-carboxylic acid chlorosulfonylamide was collected by pressure filtration, washed with dichloromethane (300 ml) and dried *in vacuo*. The powder was added at rt over 15 min to stirred dimethylformamide (200 ml) producing an exothermic reaction (to 45°C). After stirring for 2 h the mixture was cooled to 5°C and water (200 ml) was added. The precipitate was collected by filtration and washed with water. The solid was taken up in water (200 ml) and the pH adjusted from 2.7 to pH 6 by addition of 2 N NaOH. Washing with water, drying *in vacuo* and recrystallization from acetic acid gave 10.9 g (46.6%) of **10**, mp 320°C (dec). ^{1}H-NMR δ ppm: 3.61 (2H, m, SCH_2), 4.29 (2H, m, NCH_2), 11.8 (1H, m, NH); IR (KBr): 2225 (CN). $C_7H_5N_3O_2S$ (MW = 195.2). Anal (C, H, N, S).

*5,7-Dioxo-3,5,6,7-tetrahydro-2H-thiazolo[3,2-c]pyrimidine-8-carbaldehyde **11***
Phosphorus oxychloride (184 g, 110.2 ml, 1.2 mol) was added at 0–10°C over 4 h to dimethylformamide (1200 ml). To this solution 2,3-dihydrothiazolo[3,2-*c*]pyrimidine-5,7-dione **7** (136.2 g, 0.8 mol) was added over 30 min with no further cooling. The mixture was stirred at rt for 4 h, heated at 70°C for 2 h then cooled to rt. The solution was concentrated *in vacuo* and the residue was poured on crushed ice. The resulting precipitate was collected by filtration, washed with water and dried *in vacuo* at 60°C to give 153.4 g (96.8%) of **11** as white crystals, mp 308°C (acetic acid/water 1:1). ^{1}H-NMR δ ppm: 3.43 (2H, m, SCH_2), 4.19 (2H, m, NCH_2), 9.86 (1H, s, CHO), 11.6 (1H, br m, NH); IR (KBr): 1720 (CHO). $C_7H_6N_2O_3S$ (MW = 198.2). Anal (C, H, N, S).

*8-Hydroxymethyl-2,3-dihydrothiazolo[3,2-c]pyrimidine-5,7-dione **39***
A solution of sodium borohydride (3.94 g, 0.1 mol) in ice-cold water (10 ml) was added at rt over 20 min to a solution of **11** (19.8 g, 0.1 mol) in dimethylsulfoxide (700 ml). The mixture was stirred at rt for 2 h then concentrated *in vacuo*. The residue was triturated with water (100 ml) and the resulting precipitate was collected by filtration, washed with water and dried *in vacuo*. Recrystallization from water gave 12.2 g (61%) of **39** as white crystals, mp 262°C. ^{1}H-NMR δ ppm: 3.40 (2H, m, SCH_2), 4.14 (2H, d, OCH_2), 4.17 (2H, m, NCH_2), 4.82 (1H, t, OH), 11.1 (1H, s, NH). $C_7H_8N_2O_3S$ (MW = 200.2). Anal (C, H, N, S).

*5,7-Dioxo-3,5,6,7-tetrahydro-2H-thiazolo[3,2-c]pyrimidine-8-carbaldehyde oxime **40***
To a solution of 5,7-dioxo-3,5,6,7-tetrahydro-2*H*-thiazolo-[3,2-*c*]pyrimidine-8-carbaldehyde **11** (20 g, 0.1 mol) and hydroxylamine hydrochloride (9.2 g, 0.13 mol) in dimethylsulfoxide (700 ml) at rt was added sodium acetate (9 g, 0.11 mol). The mixture was stirred for 28 h and then poured onto crushed ice (1 kg) in water (2 l). The resulting precipitate was collected by filtration, washed with water and dried *in vacuo* at 70°C. Recrystallization from dimethylformamide yielded after washing with water and drying *in vacuo* 12.1 g (56.8 %) of **40**, mp 260°C. ^{1}H-NMR δ ppm: 3.37 (2H, m, SCH_2), 4.19 (2H, m, NCH_2), 8.03 (1H, s, CH=N), 11.15 (1H, s, OH), 11.4 (1H, br s, NH). $C_7H_7N_3O_3S$ (MW = 213.2). Anal (C, H, N, S).

*8-Nitro-2,3-dihydrothiazolo[3,2-c]pyrimidine-5,7-dione **12***
Sulfuric acid (98%, 56 ml) was added dropwise to nitric acid (100%, 40 ml) at 3–9°C over 25 min followed by addition of 2,3-dihydrothiazolo[3,2-*c*]pyrimidine-5,7-dione **7** (34.1 g, 0.2 mol) at 3–9°C over 70 min. After warming to rt the mixture was stirred for 1 h and poured on crushed ice. The precipitate was collected by filtration. Recrystallization from a (1:1) mixture of acetic acid and water gave 25.7 g (59.7 %) of **12** as pale-yellow crystals, mp 298°C. ^{1}H-NMR δ ppm: 3.45 (2H, m, SCH_2), 4.34 (2H, m, NCH_2),11.8 (1H, br s, NH). $C_6H_5N_3O_4S$ (MW = 215.2). Anal (C, H, N, S).

*8-Acetyl-2,3-dihydrothiazolo[3,2-c]pyrimidine-5,7-dione **13***
A solution of 2,3-dihydrothiazolo[3,2-*c*]pyrimidine-5,7-dione **7** (17 g, 0.1 mol) and acetic anhydride (150 ml) in acetic acid (250 ml) was heated at reflux for 28 h in the presence of titanium tetrachloride (4 ml). The mixture was cooled to rt and the solvent was removed *in vacuo*. Purification of the residue by recrystallization from acetic acid gave 4.9 g (23.1%) of **13** as white crystals, mp 267°C. ^{1}H-NMR δ ppm: 2.48 (3H, s, $COCH_3$), 3.29 (2H, m, SCH_2), 4.19 (2H, m, NCH_2),11.4 (1H, br s, NH); IR (KBr): 1715 (CO). $C_8H_8N_2O_3S$ (MW = 212.2). Anal (C, H, N, S).

*1-Oxo-2,3-dihydrothiazolo[3,2-c]pyrimidine-5,7-dione **14***
A 3.4 M solution (12.5 ml) of trifluoroperacetic acid (0.0425 mol) in trifluoroacetic acid was added dropwise at 0°C to a solution of 2,3-dihydrothiazolo[3,2-*c*]pyrimidine-5,7-dione **7** (7.2 g, 0.0425 mol) in trifluoroacetic acid (50 ml). The mixture was stirred at 0°C for 3 h then at rt for 2 h and concentrated *in vacuo*. The residue was triturated with water and the resulting suspension neutralized with a saturated solution of sodium bicarbonate and concentrated *in vacuo*. Recrystallization of the residue from water gave 5.4 g (68.4%) of **14** as white crystals, mp 273°C (dec). ^{1}H-NMR δ ppm: 3.25–3.65 (2H, m, SCH_2), 4.25–4.45 (2H, m, NCH_2), 6.34 (1H, s, =CH), 11.6 (1H, br s, NH). $C_6H_6N_2O_3S$ (MW = 186.2). Anal (C, H, N, S).

8-Piperidin-1-ylmethyl-2,3-dihydrothiazolo[3,2-c]pyrimidine-5,7-dione hydrochloride ***15***

A mixture of 2,3-dihydrothiazolo[3,2-*c*]pyrimidine-5,7-dione **7** (17.02 g, 0.1 mol), aqueous formaldehyde (9.5 ml, 0.13 mol) and piperidine (12.9 ml, 0.13 mol) in acetic acid (100 ml) was heated at 60°C for 12 h. The mixture was cooled to rt and concentrated *in vacuo*. The residue was dissolved in 2 N hydrochloric acid (60 ml) and water (150 ml) and the solution was washed with dichloromethane. The aqueous phase was adjusted to pH 8 by addition of concentrated sodium hydroxide and extracted thoroughly with dichloromethane. The extracts were combined, washed with water, dried (sodium sulfate) and evaporated. The residue (17.85 g) was dissolved in dichloromethane (1000 ml) and ethereal hydrochloric acid (23.1 ml, 0.067 mol) was added slowly. The resulting precipitate was collected by filtration and dried *in vacuo*. Recrystallization from ethanol/water gave 12 g (39.5%) of **15** as a white solid, mp 275–276°C. ^{1}H-NMR δ ppm: 1.2–1.9 (6H, m, $3CH_2$), 2.7–3.6 (4H, m, $N(CH_2)_2$), 3.52 (2H, m, SCH_2), 3.8 (2H, m, CH_2N), 4.25 (2H, m, NCH_2), 9.9 (1H, br s, NH), 11.5 (1H, s, N^+-H). $C_{12}H_{18}ClN_3O_2S$ (MW = 303.8). Anal (C, H, Cl, N).

3-(5,7-Dioxo-3,7-dihydro-2H-thiazolo[3,2-c]pyrimidin-6-yl)-2-methylpropionitrile ***16***

To a solution of 2,3-dihydrothiazolo[3,2-*c*]pyrimidine-5,7-dione **7** (51 g, 0.3 mol) in dimethylformamide (300 ml) was added dropwise at 60°C over 30 min 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) (47 g, 0.3 mol) and methacrylonitrile (25.2 g, 0.37 mol). The mixture was stirred for 13 h at 80°C then treated with additional DBU (11.7 g, 0.075 mol). Stirring was continued for 9 h at 80°C, the mixture cooled to rt then concentrated *in vacuo*. The residue was repeatedly partitioned between dichloromethane and water and the combined organic extracts were washed with 2 N aqueous HCl and water, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was recrystallized twice from ethanol to give **16** (24.5 g, 34.5%) as white crystals, mp 133–134°C. ^{1}H-NMR δ ppm: 1.26 (3H, d, CH_3), 3.1–3.35 (1H, m, NCCH), 3.47 (2H, t, SCH_2), 3.7–3.85 (1H, m, $NCH_{2/2}$), 4.07–4.18 (1H, m, $NCH_{2/2}$), 4.24 (2H, t, NCH_2), 5.85 (1H, s, =CH); IR (KBr): 2240 (CN). $C_{10}H_{11}N_3O_2S$ (MW = 237.3). Anal (C, H, N, S).

4-(5,7-Dioxo-3,7-dihydro-2H-thiazolo[3,2-c]pyrimidin-6-yl)-butyronitrile ***17***

A mixture of 2,3-dihydrothiazolo[3,2-*c*]pyrimidine-5,7-dione **7** (170.2 g, 1 mol), K_2CO_3 (142.4 g, 1.03 mol) and 4-chlorobutyronitrile (108.8 g, 1.03 mol) in dimethylformamide (1.5 l) was heated at 90°C for 14 h. The mixture was concentrated *in vacuo* and the residue partitioned between dichloromethane and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by recrystallization from isopropanol then dried *in vacuo* to give 200 g (84.3%) of **17** as a white solid, mp 101–102°C. ^{1}H-NMR δ ppm: 1.82 (2H, m, CH_2), 2.52 (2H, t, $NCCH_2$), 3.45 (2H, t, SCH_2), 3.84 (2H, t, CH_2N), 4.22 (2H, t, NCH_2), 5.79 (1H, s, =CH); IR (KBr): 2245 (CN). $C_{10}H_{11}N_3O_2S$ (MW = 237.3). Anal (C, H, N, S).

4-(1,1,5,7-Tetraoxo-3,7-dihydro-2H-thiazolo[3,2-c]pyrimidin-6-yl)butyronitrile ***41*** *and 4-(1,5,7-trioxo-3,7-dihydro-2H-thiazolo[3,2-c]pyrimidin-6-yl)butyronitrile* ***22***

To a suspension of *m*-chloroperbenzoic acid (85%, 40.6 g, 0.2 mol) in chloroform (400 ml) was added a solution of 4-(5,7-dioxo-3,7-dihydro-2*H*-thiazolo[3,2*c*]pyrimidin-6-yl)-butyronitrile **17** (47.5 g, 0.2 mol) in chloroform (500 ml) at rt over 2 h. The mixture was stirred at rt for 17 h. An additional suspension of *m*-chloroperbenzoic acid (85%, 20.3 g, 0.1 mol) in chloroform (200 ml) was added at rt over 15 min. The mixture was stirred at rt for an additional 2 h. The precipitate was removed by filtration and the organic solution was washed with 15 ml of an aqueous saturated $NaHSO_3$ solution. It was then neutralized with an aqueous saturated solution of $NaHCO_3$, washed with water, dried over Na_2SO_4, and filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using a mixture of dichloromethane and isopropanol (volume ratio 95:5 to 90:10) as eluent to give 13.2 g (24.5%) of **41** as a white solid, mp 158°C (isopropanol). ^{1}H-NMR δ ppm: 1.85 (2H, m, CH_2), 2.55 (2H, t, $NCCH_2$), 3.89 (2H, t, O_2SCH_2), 3.89 (2H, t, CH_2N), 4.18 (2H, t, NCH_2), 6.41 (1H, s, =CH); IR (KBr): 2240 (CN), 1340 (SO_2), 1140 (SO_2). $C_{10}H_{11}N_3O_4S$ (MW = 269.3). Anal (C, H, N).

Compound **22** was isolated from later fractions as a white solid (15.8 g, 31.2%), mp 128°C (ethanol). ^{1}H-NMR δ ppm: 1.85 (2H, m, CH_2), 2.53 (2H, t, $NCCH_2$), 3.3–3.6 (2H, m, $OSCH_2$), 3.89 (2H, t, CH_2N), 4.3–4.5 (2H, m, NCH_2), 6.51 (1H, s, =CH); IR (KBr): 2240 (CN), 1060 (SO). $C_{10}H_{11}N_3O_3S$ (MW = 253.3). Anal (C, H, N, S).

3-(8-Methyl-5,7-dioxo-3,7-dihydro-2H-thiazolo[3,2-c]pyrimidin-6-yl)propionitrile ***23***

Acrylonitrile (5.7 g, 0.106 mol) was added dropwise to a mixture of 8-methyl-2,3-dihydrothiazolo[3,2-*c*]pyrimidine-5,7-dione **19** (18.4 g, 0.1 mol) and 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) (15.7 g, 0.1 mol) in dimethylformamide (100 ml) at 60°C. The mixture was heated to 60°C for 2 h and concentrated *in vacuo*. The residue was dissolved in dichloromethane and washed with 1 N HCl and water. The organic layer was separated, dried (sodium sulfate) and evaporated. Purification of the residue (22.2 g) by column chromatography (silica gel, dichloromethane/ethanol, volume ratio 95:5 as eluent) gave 14.4 g (60.8%) of **23**, mp 162–163°C (isopropanol). ^{1}H-NMR δ ppm: 1.83 (3H, s, CH_3), 2.82 (2H, t, $NCCH_2$), 3.48 (2H, t, SCH_2), 4.03 (2H, t, CH_2N), 4.27 (2H, t, NCH_2); IR (KBr): 2250 (CN). $C_{10}H_{11}N_3O_2S$ (MW = 237.3). Anal (C, H, N, S).

6-(2-Oxopropyl)-2,3-dihydrothiazolo[3,2-c]pyrimidine-5,7-dione ***29***

A mixture of 2,3-dihydrothiazolo[3,2-*c*]pyrimidine-5,7-dione **7** (34 g, 0.2 mol), chloroacetone (20.2 g, 0.21 mol) and potassium carbonate (27.6 g, 0.2 mol) in dimethylformamide (400 ml) was stirred at 60°C for 6 h. After addition of more chloroacetone (6.7 g, 0.07 mol), stirring was continued for 4 h. The mixture was cooled to rt and concentrated *in vacuo*. The residue was repeatedly partitioned between dichloromethane and water and the combined organic extracts were washed with water, dried (Na_2SO_4) and concentrated *in vacuo*. Purification of the residue by column chromatography (silica gel, dichloromethane/ethanol, 9:1 v/v) and recrystallization from a (1:1) mixture of isopropanol/dichloromethane gave white crystals of **29** (27.8 g, 61.5%), mp 186–187°C. ^{1}H-NMR δ ppm: 2.18 (3H, s, CH_3), 3.48 (2H, m, SCH_2), 4.23 (2H, m, NCH_2), 4.62 (2H, s, $COCH_2N$), 5.84 (1H, s, =CH); IR (KBr): 1725 (CO). $C_9H_{10}N_2O_3S$ (MW = 226.3). Anal (C, H, N, S).

6-(2-Hydroxypropyl)-2,3-dihydrothiazolo[3,2-c]pyrimidine-5,7-dione ***30***

To a solution of 6-(2-oxopropyl)-2,3-dihydrothiazolo[3,2-*c*]-pyrimidine-5,7-dione **29** (11.3 g, 0.05 mol) in a 2:1 mixture of dimethylformamide and water (1.4 l) was added sodium borohydride (2 g, 0.052 mol) at rt. The solution was stirred at rt for 2 h and an additional equivalent of sodium borohydride

(2 g, 0.052 mol) was added. Stirring was continued for 33 h and the mixture was concentrated *in vacuo*. The residue was repeatedly partitioned between dichloromethane and water and the combined organic extracts were washed with water, dried (Na_2SO_4) and concentrated *in vacuo*. Purification of the residue by recrystallization from isopropanol gave 7 g (61.4%) of **30** as white crystals, mp 153°C. ^{1}H-NMR δ ppm: 1.02 (3H, d, CH_3), 3.45 (2H, t, SCH_2), 3.55–3.7 (1H, m, $CH_{2/2}$-N), 3.72–3.88 (1H, m, $CH_{2/2}$-N), 3.80–3.95 (1H, m, -CHO-), 4.22 (2H, t, NCH_2), 4.67 (1H, d, OH), 5.77 (1H, s, =CH). $C_9H_{12}N_2O_3S$ (MW = 228.3). Anal (C, H, N, S).

Biological methods and results

Influence on serum lipoproteins in male rats during a repeated-dose study

Groups of male rats (strain HOE: WISKf (SPF 71)) with an initial weight of over 180 g were given the specified dose of the test compounds (table IV) in PEG 400 once daily (in the morning) using a stomach tube (0.5 ml/100 g body weight); the control group was given PEG 400 only. Single doses of the compounds were given daily on 7 consecutive days, the final administration being 24 h before blood samples were taken and the animals were sacrificed. During the study the animals had free access to food and water. Food was withdrawn 24 h before blood samples were taken.

To determine the serum lipoproteins, the serum of each rat group was pooled. The serum lipoproteins were separated on a preparative ultracentrifuge (Kontron TGA 65, Rotor Beckman 50.4 Ti).

The separation of the VLDL, LDL and HDL fractions [25, 26] was carried out by flotation as follows: VLDL: native density of the serum = 1.006, 16 h at 40 000 rpm; LDL: density = 1.04, 16h at 40 000 rpm; and HDL: density = 1.21, 18 h at 40 000 rpm.

Boehringer Mannheim enzymatic test combinations were used to determine cholesterol (according to the CHOD-PAP high-performance method [27, 28]).

Influence on hypercholesterolemia of the male rat

Male rats (strain HOE: WISKf (SPF 71)), with a weight of approximately 200 g were given daily a mixture (1 ml/100 g of body weight), prepared from cholesterol (100 g), propylthiouracil (30 g), cholic acid (100 g) and peanut oil (1 l) by stomach tube. These ingredients were necessary for generation of a combined dietetic hormonal hypercholesterolemia with a plasma concentration of cholesterol in the range of 600 mg/dl. The test compound was administered in the specified dose (table V) together with the mixture.

The procedure concerning treatment of the animals and analysis of the results was performed as described in the preceding assay. The biological results are recorded in tables IV–VI.

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