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Preliminary communication

8-Substituted purine derivatives: a new class of lipid-lowering agents

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Summary — A series of purine derivatives have been prepared and their *in vivo* abilities to lower plasma total cholesterol and triglyceride levels, and to elevate high density lipoprotein (HDL) cholesterol levels in hyperlipemic rats have been tested. Some compounds, among which 8-{2-(*R*)-hydroxy-3-[(*p*-isobutoxycarbonyl)phenoxy]propylthio}adenosine **31**, 8-{3-[(*p*-isobutoxycarbonyl)phenoxy]-2-oxopropylthio} adenosine **33** and 8-{3-[(*p*-isobutoxycarbonyl)phenoxy]-2-hydrazonecarboxamidepropylthio} adenosine **36** appear to be the most interesting, have been found to have both the desired profile of activity and no hepatotoxicity, when administered *po* at 50, 100 or 300 mg/kg. Compounds **31**, **33** and **36**, orally tested at the same doses in the 15-d test, lower triglyceride and VLDL/LDL (very low density lipoprotein/low density lipoprotein) cholesterol levels by 10–33% and 13–46%, respectively, and increase HDL-associated cholesterol levels by 10–32%. These molecules have been chosen for further pharmacological and toxicological evaluations.

adenosine / purine / cholesterol / triglyceride / hypolipemic agent

Introduction

Recent epidemiological studies have identified hyperlipidemia, in particular that associated with increased plasma concentrations of low density lipoproteins (LDL) and very low density lipoproteins (VLDL), as a risk factor for coronary heart disease [1–6] and premature atherosclerosis [7, 8]. The same study and others [9–13] also revealed a negative correlation between plasma concentration of high density lipoproteins (HDL) and risk of coronary heart disease. On the basis of these findings, the search for new drugs has been aimed at agents that can correct such dyslipidemic conditions without causing hepatic toxicity, the most serious drawback of many hypolipemics [14].

In the literature 9-substituted adenine [15–18] and adenosine derivatives, such as phenyl isopropyl adenosine (PIA) [19, 20] and others [21–23], are reported to have hypolipemic activity mainly by interfering with the lipolytic processes triggered by specific adenosine receptors. Adenosines bearing substituents at positions 2 and N^6 have a high affinity for both A_1 and A_2 adenosine receptors [24]. Since these receptors are widely distributed, lack of selectivity may lead to important side effects mainly on the cardiovascular and central nervous systems. It is also known [25] that 8-substituted adenosines generally show a lower affinity for adenosine receptors. If this is true, an adenosine bearing a side chain that induces hypolipemic activity at the 8-position would represent a potentially useful drug. This prompted us to think of 8-substituted purines as safe hypolipemics. A series of 8-substituted adenosines, characterized by a phenoxyalkylthioethereal chain (table I) were prepared. This modification provided hypolipemic molecules that were effective in raising HDL cholesterol [26, 27], to our knowledge a feature that is never present in other hypolipemic purines. The present work deals with this series of compounds, the most interesting of which are shown in figure 1.

Chemistry

Table I lists all the molecules synthesized and their physical characteristics. Two general synthetic methods were used to obtain most of the compounds (**1–17**, **19–25**, **28–32**, schemes 1, 2).

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Table I. Physical properties of derivatives HET-X-CH_2-Y-CH_2-O-Ar.

Entry	Het	X	Y	Ar	synth. method	mp (°C)	purif. method	yield (%)	molecular formula	Anal[a]
1	8-adenosyl	S	$^{(R,S)}$CH(OH)	phenyl	1	100-110	r(C)	64	$C_{19}H_{23}N_5O_6S$	C,H,N,S
2	"	S	"	4-ethoxycarbonylphenyl	1	156-158	r(A)	56	$C_{22}H_{27}N_5O_8S$	C,H,N,S
3	"	S	"	2-ethoxycarbonylphenyl	1	95-100	c(B)	33	$C_{22}H_{27}N_5O_8S$	C,H,N,S
4	"	S	"	3-ethoxycarbonylphenyl	1	98-100	c(B)	76	$C_{22}H_{27}N_5O_8S$	C,H,N,S
5	"	S	"	4-isobutoxycarbonylphenyl	1	164-166	r(A)	52	$C_{24}H_{31}N_5O_8S$	C,H,N,S
6	"	S	"	4-carboxyphenyl	1	188-190	r(A)	61	$C_{20}H_{23}N_5O_8S$	C,H,N,S
7	"	S	"	4-aminocarbonylphenyl	1	175-178	c(B)	32	$C_{20}H_{24}N_6O_7S$	C,H,N,S
8	"	S	"	4-N,N-diethylaminocarbonylphenyl	1	107-110	c(B)	53	$C_{24}H_{32}N_6O_7S$	C,H,N,S
9	"	S	"	4-chlorophenyl	1	162-166	r(A)	59	$C_{19}H_{22}ClN_5O_6S$	C,H,N,S,Cl
10	"	S	"	4-dodecyloxycarbonylphenyl	1	163-164	r(A)	63	$C_{32}H_{47}N_5O_8S$	C,H,N,S
11	"	S	"	4-(2,3-dihydroxy)propoxycarbonylphenyl	1	132-136	r(A)	52	$C_{23}H_{29}N_5O_{10}S$	C,H,N,S
12	"	S	"	4-(2-acetylamino)ethoxycarbonylphenyl	1	124-126	f(D)	42	$C_{24}H_{30}N_6O_9S$	C,H,N,S
13	"	S	"	4-(2,6-dimethoxy)ethoxycarbonylphenyl	1	102-107	c(B)	48	$C_{24}H_{31}N_5O_{10}S$	C,H,N,S
14	"	S	"	2-chloro-4-ethoxycarbonylphenyl	1	174-175	r(A)	60	$C_{22}H_{26}ClN_5O_8S$	C,H,N,S,Cl
15	"	S	"	2-(5-ethoxycarbonyl)-pyridyl	1	amorph.	r(C)	45	$C_{21}H_{26}N_6O_8S$	C,H,N,S
16	8-adenyl	S	"	4-ethoxycarbonylphenyl	1	200-201	r(D)	50	$C_{17}H_{19}N_5O_4S$	C,H,N,S
17	8-guanosyl	S	"	"	1	132-138	-	40	$C_{22}H_{27}N_5O_9S$	C,H,N,S
18	8-inosyl	S	"	"		amorph.	r(L)	52	$C_{22}H_{26}N_4O_9S$	C,H,N,S
19	8-theophyllyl	S	"	4-isobutoxycarbonylphenyl	1	140-142	f(E)	63	$C_{21}H_{26}N_4O_6S$	C,H,N,S
20	7-methoxymethyl-8-theophyllyl	S	"	"	1	101-103	r(F)	74	$C_{23}H_{30}N_4O_7S$	C,H,N,S
21	8-caffeyl	S	"	"	1	126-128	r(F)	80	$C_{22}H_{28}N_4O_6S$	C,H,N,S
22	2',3'-O-isopropyliden-8-adenosyl	S	"	"	1	75-77	c(G)	75	$C_{27}H_{35}N_5O_8S$	C,H,N,S
23	5'-O-3,4,5-$(OMe)_3$benzoyl-8-adenosyl	S	"	4-ethoxycarbonylphenyl	1	83-86	-	66	$C_{32}H_{37}N_5O_{12}S$	C,H,N,S
24	5'-O-isobutanoyl-8-adenosyl	S	"	"	1	74-76	r(H)	70	$C_{26}H_{33}N_5O_9S$	C,H,N,S
25	2',3',5'-tri-O-acetyl-8-adenosyl	S	"	"	1	66-70	c(J)	55	$C_{28}H_{33}N_5O_{11}S$	C,H,N,S
26	8-adenosyl	SO	"	4-isobutoxycarbonylphenyl		174-175	c(E)	50	$C_{24}H_{31}N_5O_9S$	C,H,N,S
27	"	SO_2	"	"		129-131	c(E)	20	$C_{24}H_{31}N_5O_{10}S$	C,H,N,S
28	"	S	—	"	2	126-129	r(A)	47	$C_{23}H_{29}N_5O_7S$	C,H,N,S
29	"	S	CH_2	"	2	174-175	r(A)	70	$C_{24}H_{31}N_5O_7S$	C,H,N,S
30	"	S	$(CH_2)_3$	"	2	140-143	r(N)	75	$C_{26}H_{35}N_5O_7S$	C,H,N,S
31	"	S	$^{(R)}$CH(OH)	"	1	166-167	r(A)	67	$C_{24}H_{31}N_5O_8S$	C,H,N,S
32	"	S	$^{(S)}$CH(OH)	"	1	166-168	c(B)	74	$C_{24}H_{31}N_5O_8S$	C,H,N,S
33	"	S	CO	"		162-164	r(O)	85	$C_{24}H_{29}N_5O_8S$	C,H,N,S
34	"	S	C(NOH)	"		156-160	r(P)	90	$C_{24}H_{30}N_6O_8S$	C,H,N,S
35	"	S	C(NOMe)	"		104-106	r(M)	72	$C_{25}H_{32}N_6O_8S$	C,H,N,S
36	"	S	C(NNHCONH$_2$)	"		183-186	r(A)	88	$C_{25}H_{32}N_8O_8S$	C,H,N,S

r = recrystallization; c = chromatography (silica gel); f = flash chromatography (silica gel); A = EtOH; B = EtOAc/EtOH 9:1; C = Et_2O; D = EtOAc/EtOH 3:1; E = CH_2Cl_2/MeOH 97:3; F = CH_3COCH_3/*n*-C_6H_{14} 1:1; G = CH_2Cl_2/EtOAc/MeOH 50:45:5; H = EtOH/H_2O 1:1; J = EtOAc; L = *i*-C_3H_7OH/Et_2O 1:3; M = *n*-C_7H_{16}/EtOH 10:1; N = *n*-C_6H_{14}; O = Et_2O/*n*-C_6H_{14} 1:1; P = CH_3COCH_3/*n*-C_7H_{16} 1:1; [a]Analytical results are within ± 0.4% of the theoretical values unless otherwise noted.

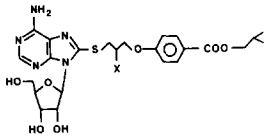
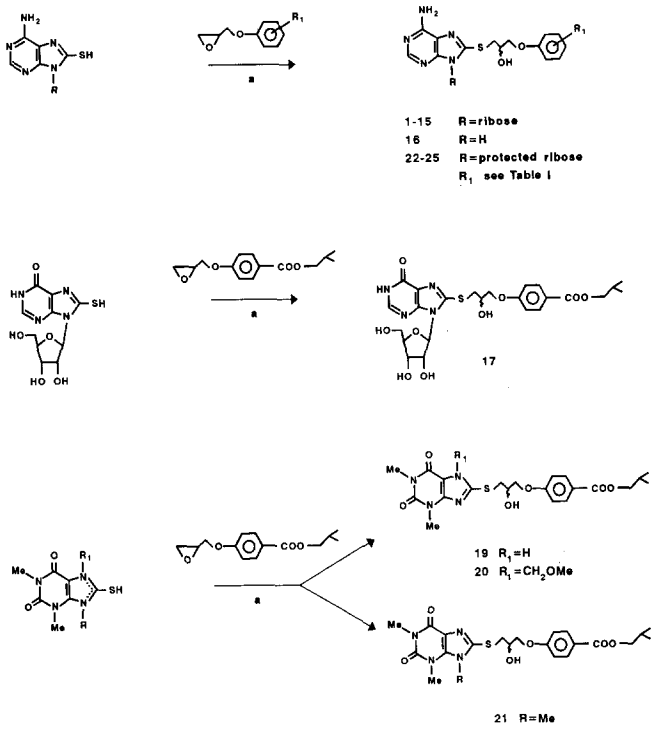


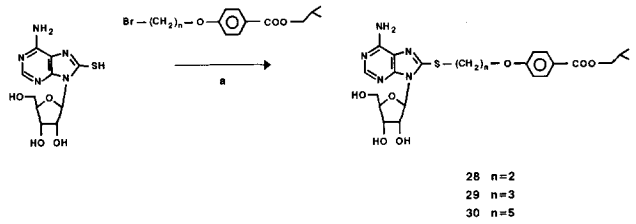
Fig 1. Structure of compounds **31** (X: (R)OH), **33** (X: =O) and **36** (X: =NNHCONH$_2$).

Method 1 (scheme 1) involved the reaction of 8-mercaptopurines with epoxides in refluxing ethanol, under 2,6-lutidine catalysis, to give compounds **1–17**, **19–21**, **22–25**, **31**, and **32**. The epoxides were obtained by reacting epichlorohydrin with appropriately substituted phenols in the presence of K_2CO_3 in refluxing methylethylketone. The substituted phenols were synthesized by esterifying the corresponding hydroxybenzoic acids by standard methods.

Method 2 (scheme 2), reaction of 8-mercaptoadenosine with appropriate bromides, performed with sodium isobutoxide in isobutanol, was exploited for the preparation of compounds **28**, **29**, and **30**. The bromides were obtained by reacting appropriately substituted phenols (see above) with commercially



Scheme 1. Reagents (a) 2,6-lutidine, EtOH and reflux.



Scheme 2. Reagents (a) *i*-BuOH and *i*-BuONa.

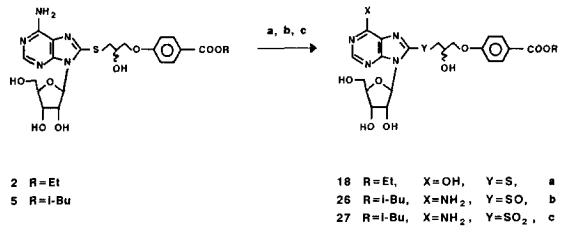
available α,Ω-dibromoalkanes in DMF/K_2CO_3. Starting from adenosine, 8-mercaptoadenosine was readily prepared in 70% overall yield by an improved 2-step procedure through bromination according to the method of Ikehara [28], followed by reaction with sodium hydrogen sulfide. Compound **16** was prepared from 8-mercaptoadenine, obtained from 4,5,6-triaminopyrimidine and thiourea [29]. Compounds **17**, **20** and **21** were synthesized from the 8-mercapto derivatives obtained from guanosine, theophylline and caffeine by bromination (in the case of **20** the bromo derivative was alkylated with chloromethylmethylether in phase-transfer catalysis conditions) and substitution with hydrogen sulfide in pyridine. Analogue **19** was prepared from 8-mercaptotheophylline, obtained from 5,6-diaminouracil and thiourea [30]. The 2 diastereoisomers of **5** (**31** and **32**) were prepared, in about 55% overall yield, from commercially available *S*(+) or *R*(–)glycidyltosylates by reaction with isobutyl-*p*-hydroxybenzoate in the presence of NaH/DMF and condensation with 8-mercaptoadenosine.

The ribose-modified derivative **22** was prepared directly from **5** upon treatment of 8-mercaptoadenosine with acetone/*p*-toluenesulfonic acid. Monoesters **23** and **24** were obtained by acylation of 2',3'-acetonide-8-mercaptoadenosine, followed by acid-catalyzed selective removal of the protection. Compound **25** was synthesized from tri-*O*-acetyl-8-mercaptoadenosine.

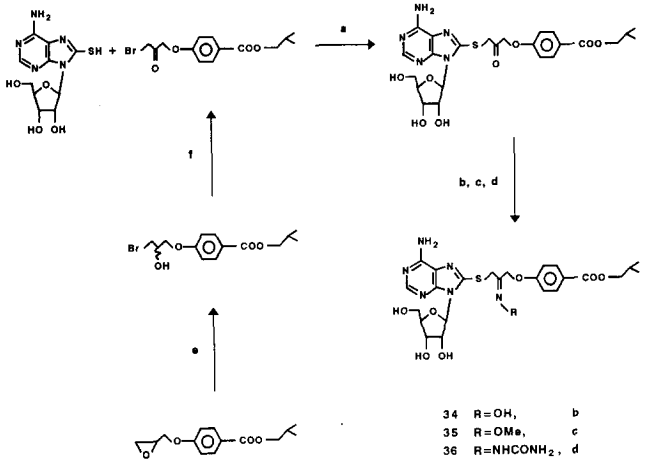
All the other products (**18**, **26**, **27**, **34–36**) were prepared by direct modification of available starting materials (schemes 3, 4), except for **33**, which was prepared by a separate synthetic method (scheme 4).

Results and discussion

As a preliminary screening of the hypolipemic efficacy, compounds were orally administered in a 5-d test at the single dose of 100 mg/kg to hyperlipemic male rats and activity parameters were evaluated 2 h



Scheme 3. Reagents (a) $NaNO_3$, AcOH, H_2O; (b) *m*-CPBA, EtOH; and (c) $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, H_2O.



Scheme 4. Reagents (a) 2,6-lutidine, EtOH; (b) $NH_2OH \cdot HCl$, *i*-BuOH; (c) $NH_2OMe \cdot HCl$, *i*-BuOH; (d) $NH_2NHCONH_2$, EtOH, HCl, H_2O; (e) HBr/AcOH; and (f) CrO_3/H_2SO_4.

after the last administration, as described in the *Biological methods*. Reduction of serum triglycerides and total cholesterol levels and increments of HDL cholesterol levels were checked. Finally, the possible induction of hepatomegaly (liver to body-weight ratio) was evaluated. As reference standards, clofibrate and gemfibrozil were used.

Entries **1–15** (table II) are adenosines with the same side chain, ending with different aromatic moieties. Evaluations of the effects of substituents on the phenyl ring showed that a *p*-carboxylic ester group is important for hypolipemic activity. Additional substituents to the *p*-carboxylic ester group (**13** and **14**) tended to reduce activity. Ethyl **2** and isobutyl **5** esters were equally active and *n*-dodecyl ester **10** retained substantial, although not significant, activity. Functionalized alkyl esters, like **11** and **12**, showed a depression of the hypolipemic profile.

As regards HDL cholesterol, only the *para*-benzoates, together with *para*-chloro **9**, were effective.

Replacement of the aryl ring with a heteroaromatic nucleus **15** resulted in an inactive product. Taking into consideration the overall results, the compounds with the desired profile were **2** and **5**.

The second group covers molecules in which adenosine is replaced with other purines (**16–21**). Removal of ribose to give **16** did not affect the hypolipemic profile, except for reducing the increase of HDL cholesterol. Guanosine **17** and inosine **18** were essentially devoid of activity on triglycerides. Theophylline **19** retained good efficacy, while caffeine **21** had no effect on serum cholesterol. Alkylation of theophylline at position 7 was crucial; derivative **20** was completely inactive. Therefore, the only molecule that joined increase of HDL cholesterol to hypolipemic efficacy was **19**.

Modifications at ribose are reported in entries **22** to **25**. Esterification of its primary hydroxyl (**23** and **24**) gave compounds with reduced potency, especially on triglycerides. Acetylation of all the hydroxyls produced **25**, which is only moderately active on triglycerides and the same as **22**.

The importance of divalent sulfur is highlighted by sulfoxide **26** and sulfone **27**, which are almost inactive.

The final group (**28–36**) includes adenosines differing in the central part of the side chain. When the chain was constituted of a linear alkyl without any other functionality (**28–30**), efficacy was greatly reduced, especially with the longer analogue (**30**). When a heteroatom (O, N) was present as an appendage of the chain (**31–36**), the compounds showed an interesting profile with dissimilar potencies. The screening procedure failed to reveal significant differences between **31** and **32**, apparently negating any importance of this chiral center on activity. Compounds **2**, **5** (with its 2 isomers **31** and **32**), **19**, ketone **33** and semicarbazone **36** all had the desired profile of efficacy. The tested derivatives were more effective than clofibrate and comparable to gemfibrozil in reducing total cholesterol and triglyceride levels. On HDL cholesterol testing, these compounds showed a significant increase, even if not to the extent of gemfibrozil, which is known to be the most efficacious on this parameter in rats. In these animals clofibrate is inactive [31–33]. In contrast to reference drugs, none of the new derivatives caused hepatomegaly. The selected products were further tested orally at 50, 100 and 300 mg/ kg/d in 5-d tests.

The activity data are summarized in table III. Derivatives **33** and **36** show a clear-cut dose-dependence for all the parameters while compounds **5**, **31**, **32** show dose dependence only for some of them. Lack of liver toxicity was confirmed even at 300 mg/ kg. The outcome of the second test was the choice of 3 products (**31**, **33**, **36**), together with **19**, included for its interesting values at the lowest dose. The choice of **31**, rather than **5**, was due to the convenience of in-

Table II. Hypolipemic activity of purinic derivatives HET-X-CH_2-Y-CH_2-OAr on cholesterol-fed rats[a].

Entry	Het	X	Y	Ar	% change (vs controls[b]) in: serum triglyc.	serum tot.chol.	serum HDL chol.	hepatic index [c]
1	8-adenosyl	S	$^{(R,S)}$CH(OH)	phenyl	-16±2*	-14±5	4±1	0±1
2	"	S	"	4-ethoxycarbonylphenyl	-33±5*	-36±3*	11±2*	-2±1
3	"	S	"	2-ethoxycarbonylphenyl	7±3	15±5	-1±1	-3±1
4	"	S	"	3-ethoxycarbonylphenyl	-8±4	11±4	-11±1*	3±2
5	"	S	"	4-isobutoxycarbonylphenyl	-28±3*	-38±2*	6±1*	-5±1
6	"	S	"	4-carboxyphenyl	-11±4	-21±2*	-4±1	2±1
7	"	S	"	4-aminocarbonylphenyl	15±4	-10±2	11±1*	3±3
8	"	S	"	4-N,N-diethylaminocarbonylphenyl	6±1	19±6	1±7	1±1
9	"	S	"	4-chlorophenyl	5±5	-14±5	22±1*	-2±2
10	"	S	"	4-dodecyloxycarbonylphenyl	-12±5	-16±4	38±2*	12±4
11	"	S	"	4-(2,3-dihydroxy)propoxycarbonylphenyl	14±3	0±2	6±3	5±1
12	"	S	"	4-(2-acetylamino)ethoxycarbonylphenyl	-11±6	-21±2*	-11±3	-11±4
13	"	S	"	4-(2,6-dimethoxy)ethoxycarbonylphenyl	-14±4	-5±4	34±1*	-6±1
14	"	S	"	2-chloro-4-ethoxycarbonylphenyl	2±4	-15±1*	20±3*	-1±1
15	"	S	"	2-(5-ethoxycarbonyl)pyridyl	-11±7	-6±1	2±2	-2±1
16	8-adenyl	S	"	4-ethoxycarbonylphenyl	-20±3*	-21±2*	2±1	3±1
17	8-guanosyl	S	"	"	4±8	-11±1	17±1*	-4±1
18	8-inosyl	S	"	"	4±2	-8±2	12±1*	0±1
19	8-theophyllyl	S	"	4-isobutoxycarbonylphenyl	-26±1*	-15±1*	10±1*	1±2
20	7-methoxymethyl-8-theophyllyl	S	"	"	14±5	7±5	-15±3	0±1
21	8-caffeyl	S	"	"	-23±1*	16±7	-1±2	-7±1
22	2',3'-O-isopropyliden-8-adenosyl	S	"	"	-18±2*	2±1	-2±2	-11±4
23	5'-O-(3,4,5-trimethoxy)benzoyl-8-adenosyl	S	"	4-ethoxycarbonylphenyl	-7±5	-20±1*	16±1*	-2±1
24	5'-O-isobutanoyl-8-adenosyl	S	"	"	-7±1	-17±2*	23±3*	-5±1
25	2',3',5'-tri-O-acetyl-8-adenosyl	S	"	"	-10±3	4±1	-7±2	-9±1
26	8-adenosyl	SO	"	4-isobutoxycarbonylphenyl	-9±5	5±4	-3±1	-5±1
27	"	SO_2	"	"	-6±1	-3±3	6±3	-1±1
28	"	S	—	"	0±1	-13±8	-15±4	1±1
29	"	S	CH_2	"	-3±8	-10±7	-1±2	1±1
30	"	S	$(CH_2)_3$	"	-1±1	42±9*	-24±8	5±2
31	"	S	$^{(R)}$CH(OH)	"	-15±2*	-17±3*	15±1*	-2±1
32	"	S	$^{(S)}$CH(OH)	"	-16±2*	-15±3*	15±1*	-7±1
33	"	S	CO	"	-16±2*	-22±2*	8±1*	-1±1
34	"	S	C(NOH)	"	1±3	-25±2	3±3	0±1
35	"	S	C(NOMe)	"	-7±3	-7±2	18±3*	5±1
36	"	S	C(NNHCONH$_2$)	"	-33±1*	-32±4*	24±1*	-9±1
Clofibrate					-10±1*	-5±1	-16±2*	12±1
Gemfibrozil					-32±3*	-22±2*	33±2*	8±2

Five-day test, 100 mg/kg•d dose; [a]Nath's diet supplemented with 1% cholesterol + 1% cholic acid; [b]controls given orally 10 ml/kg of 0.5% w/v carboxymethylcellulose (CMC), serum parameters evaluated 2 h after last administration; [c]hepatic index = (liver weight/body weight) x 100; *$p < 0.05$ Dunnett T test, after ANOVA determination; control value levels (± SE, 30 experiments): triglycerides 441 ± 19 mg/dl, total cholesterol 312 ± 14 mg/dl, HDL cholesterol 19.6 ± 1 mg/dl, hepatic index 5.5 ± 0.4.

Table III. Hypolipemic activity on cholesterol-fed rats[a].

Entry	dose (mg/Kg.day)	triglyc.	% change (vs controls[b]) in: total cholest	HDL cholest.	hepatic index[c]
2	50	-14±5	-8±4	9±4	
	100	-25±2*	-27±3*	8±3	
	300	-16±5	-17±1*	47±6*	-4±2
5	50	-1±7	-2±4	2±3	
	100	-21±2*	-29±6*	12±1	
	300	-28±3*	-29±1*	15±3*	-1±2
19	50	-30±5*	-8±7	20±2*	
	100	-22±2*	-14±1*	10±4	
	300	-17±1*	-15±3*	13±1*	-3±2
31	50	-16±2*	-8±2	18±6	
	100	-27±3*	-10±1*	18±2*	
	300	-27±2*	-30±1*	35±1*	0±1
32	50	-3±4	-4±5	11±3	
	100	-10±3	-4±1	12±1*	
	300	-10±1*	-34±3*	47±3*	1±1
33	50	-13±4	-16±2*	4±2	
	100	-17±2*	-25±3*	17±3*	
	300	-22±2*	-31±2*	23±2*	-4±1
36	50	-18±1*	-7±3	-1±1	
	100	-31±1*	-35±3*	20±1*	
	300	-41±1*	-41±4*	28±1*	-11±3
Clofibrate	50	8±1	-10±1	-6±2	
	100	-10±1*	0±2	-16±4	
	300	-30±3*	-15±1*	-17±2*	9±1*
Gemfibrozil	50	-27±2*	-10±3	15±1*	
	100	30±2*	-20±3*	35±2*	
	300	-51±5*	-22±1*	67±8*	7±1

Five-day test; 3 doses; [a]Nath's diet supplemented with 1% cholesterol + 1% cholic acid; [b]controls given *po* 10 ml/kg of 0.5% w/v CMC, serum parameters evaluated 2 h after last administration; [c]hepatic index = (liver weight/body weight) x 100; $*p < 0.05$ Dunnett *T* test, after ANOVA determination; control value levels (± SE, 10 experiments): triglycerides 477 ± 23 mg/dl, total cholesterol 303 ± 15 mg/dl, HDL cholesterol 15.5 ± 1 mg/dl, hepatic index 5.6 ± 0.6.

vestigating a single diastereoisomer. These compounds were further evaluated at the same doses for 15 d on rats fed with a lower cholesterol-containing diet (0.5%). Lower cholesterol content does not influence the animal growth, while, on the other hand, a longer treatment allows us to better evaluate tolerability.

Table IV. Hypolipemic activity on cholesterol-fed rats[a].

Entry	dose (mg/Kg.day)	triglyc.	change (vs controls[b]) in: VLDL/LDL cholest.	HDL cholest.	hepatic index[c]	catalase[d]
19	50	-19±2*	-2±3	-13±1		
	100	-39±3*	-26±3*	-15±3*		
	300	-2±2	8±4	5±5	4±1	-15±1
31	50	-21±1*	-24±1*	2±2		
	100	-17±2	-40±2*	18±2*		
	300	-32±1*	-46±4*	20±1*	-3±1	2±1
33	50	-25±2*	-25±1*	18±2*		
	100	-28±3*	-27±3*	23±1*		
	300	-29±3*	-35±1*	30±3*	-3±1	2±3
36	50	-10±2	-13±2	10±1*		
	100	-17±2*	-13±1*	6±1		
	300	-33±1*	-45±3*	32±1*	0±1	4±1
Clofibrate	200	-30±2*	-13±1	-25±1*	27±2*	25±1*
Gemfibrozil	200	-26±1*	-18±1*	56±5*	9±1	33±2*

Fifteen-day test, 3 doses; [a]Nath's diet supplemented with 0.5% cholesterol + 0.5% cholic acid; [b]controls given orally 10 ml/kg of 0.5% w/v CMC, serum parameters evaluated 24 h after last administration; [c]hepatic index = (liver weight/body weight) x 100; [d]μmol/min/mg protein; $*p < 0.05$ Dunnett *T* test, after ANOVA determination; mean control values (± SE, 5 experiments): triglycerides 497 ± 45 mg/dl, HDL cholesterol 17.5 ± 1 mg/dl, VLDL + LDL cholesterol 240 ± 30, hepatic index 5.8 ± 0.3, liver catalase activity 542 ± 32 μmol/min/mg protein.

All the compounds, with the exception of **19**, had a very good activity on VLDL/LDL cholesterol and triglycerides, even 24 h after the last administration, showing the same efficacy on HDL cholesterol as in the previous test. Hepatic parameters (including catalase, a marker of peroxisomal proliferation) were not modified, which does occur with reference standards, and none of our products influenced normal body-weight gain.

An overview of the results allows us to advance some structure–activity relationships: i) adenosine and adenine are the best purines; ii) the presence of divalent sulfur is required; iii) a heteroatom (O, N) as an appendage on the side chain seems important; iv) an aromatic ring at the end of the chain appears to be decisive; and v) the substitution pattern on the aromatic nucleus is also very important.

The mechanism of action of these molecules is unknown. The activity might be due to interaction with a specific subgroup of purinergic receptors, regulating lipolysis mainly in the adipose tissue. This hypothesis should be confirmed by *in vitro* experiment on stimulated and non-stimulated lipolysis of adipose

cells. While the reduced activity of 8-substituted adenosines on A_1 and A_2 cerebral receptors has been fully confirmed (Fiorentini F, Sfriso L, unpublished results (Internal reports LBS 8/90 and LBS 2/91)), preliminary data [34] suggest the *in vivo* existence of interference on lipolysis stimulated by noradrenalin in rats. An alternative explanation is that the good efficacy on VLDL/LDL, HDL cholesterol and serum triglycerides may be due to the side chain, similar to that of modified fibrates (*eg* lifibrol [35], terbufibrol [36], tazasubrate [37]). This feature might increase the hypolipemic aptitude of adenosine itself, while at the same time the adenosine nucleus strongly decreases the secondary toxic effects on liver functionality.

Conclusions

On the basis of the pharmacological data from short- and long-term tests in rats (good hypolipemic activity, significant increase of HDL cholesterol and no hepatotoxicity), adenosine derivatives **31** (P–0654), **33** (P–06103) and **36** (P–06133) were selected for further evaluation.

Experimental protocols

Chemistry

Melting points were determined in a Buchi 510 apparatus and are uncorrected. IR spectra were obtained with a Biorad FTS7 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AC 200 or CXP 300 spectrometers using tetramethylsilane as internal standard. Optical activities were measured on a Perkin–Elmer 241 polarimeter. All new compounds were analysed for C, H, N and the values found were within ± 0.4% of the theoretical values.

*8-{[(2-Hydroxy-3-phenoxy)propyl]thio} adenosine **1***
A mixture of 1-phenoxy-2,3-epoxypropane (7.3 g, 48.6 mmol), 8-mercaptoadenosine (11.5 g, 38.4 mmol), 2,6-lutidine (2 ml) and ethanol (300 ml) was refluxed for 1 h, concentrated and ether was added. The precipitate was filtered, washed with ether, chromatographed on silica (eluent: ethylacetate/ethanol, 9:1) and crystallized from dichloromethane/ethanol (9:1) yielding 6.5 g (14.4 mmol, 37% yield) of **1**, mp 100–110°C, α_D = –48.23° (*c* = 1% MeOH).

*8-{[2-(R)-Hydroxy-3-(p-isobutoxycarbonyl)phenoxypropyl]-thio} adenosine **31***
A solution of isobutyl-*p*-hydroxybenzoate (5.12 g, 26.4 mmol) in dry DMF (10 ml) was added to a suspension of NaH (60% in oil, 1.06 g, 26.4 mmol) in dry DMF (20 ml), followed by a solution of (*S*)-glycidyltosylate (5 g, 22 mmol) in dry DMF (10 ml). After 1.5 h the reaction mixture was poured into NH_4Cl (sat aq sol) and extracted with ether (3 x 100 ml). The organic layer was washed with 3 M NaOH, dried (Na_2SO_4) and concentrated to dryness. The residue was distilled to give 3.56 g (14.2 mmol, 65% yield) of isobutyl-*p*-(*S*)-glycidyloxy benzoate, α_D = +8.70° (*c* = 1% MeOH). This product was dissolved in ethanol (20 ml) and slowly dropped into a suspension of 8-mercaptoadenosine (4.2 g, 14.2 mmol) in ethanol (60 ml) and 2,6-lutidine (0.5 ml). The reaction mixture was refluxed for 4 h, cooled, filtered and the residue crystallized from ethanol, yielding 5.3 g (9.6 mmol, 68% yield) of **31**, mp 166–167°C, α_D = –12.8° (*c* = 1% MeOH).

Diastereoisomer 8-{[2-(*S*)-hydroxy-3-(*p*-isobutoxycarbonyl) phenoxypropyl]thio} adenosine **32**, was obtained in 74.7% yield, utilizing isobutyl *p*-(*R*)-glycidyloxy benzoate, prepared from (*R*)-glycidyltosylate, as described above, mp 166–167°C, α_D = –76.1° (*c* = 1% MeOH).

*8-{[3-(p-Isobutoxycarbonyl)phenoxyethyl]thio} adenosine **28***
A suspension of 8-mercaptoadenosine (5.4 g, 18 mmol) in dry isobutanol (150 ml) was treated with 11.9 ml (18 mmol) of 1.52 M sodium isobutoxide and warmed at 60°C. To this suspension a solution of *p*-(2-bromoethoxy) isobutyl benzoate (7.8 g, 26 mmol) in dry isobutanol (35 ml) was added and the mixture stirred at 60°C for 3 h and then overnight at rt. The reaction mixture was filtered, concentrated and the residue was dissolved in ethylacetate (200 ml), washed with $NaHCO_3$ (sat aq sol, 200 ml), water (2 x 200 ml), dried (Na_2SO_4) and concentrated to oil that crystallized from absolute ethanol, yielding 3.9 g (8.5 mmol, 47% yield) of **28**, mp 126–129°C, α_D = –32.40° (*c* = 0.5% MeOH).

*8-{[2-Hydroxy-3-(p-ethoxycarbonyl)phenoxypropyl]thio} inosine **18***
A solution of sodium nitrite (24 g, 347 mmol) in water (50 ml) was added to a solution of 8-{[2-hydroxy-3-(*p*-ethoxycarbonyl)phenoxypropyl]thio} adenosine **2** (10 g, 19.2 mmol) in acetic acid (100 ml) and water (200 ml) at +5°C. The solution was stirred at +5°C for 1 h then at rt for 4 h. The reaction mixture was neutralized with solid $NaHCO_3$, extracted with ethylacetate/*n*-butanol 7:3 (1000 ml), dried on anhydrous Na_2SO_4 and concentrated. The residue was dissolved in dichloromethane/*n*-butanol (7:3) and extracted with cold 5% ammonia. The water layer, adjusted to pH 6 with 10% sulfuric acid, was extracted with 700 ml of ethylacetate/*n*-butanol (7:3). The organic layer was dried (Na_2SO_4), concentrated and crystallized from isopropanol/ether. A white amorphous material (5.3 g, 9.98 mmol, 52% yield) was obtained.

*8-{[3-(p-Isobutoxycarbonyl)phenoxy-2-hydroxypropyl]sulfinyl} adenosine **26***
A solution of *meta*-chloroperbenzoic acid (2.96 g, 14.6 mmol) in ethanol (20 ml) was added dropwise to a solution of 8-{[2-hydroxy-3-(*p*-isobutoxycarbonyl) phenoxypropyl]thio} adenosine **5** (4 g, 7.3 mmol) in ethanol (40 ml). The mixture was concentrated, water was added and the mixture extracted with ethylacetate/hexane (4:1). The organic layer was washed with $NaHCO_3$ (sat aq sol), concentrated and the residue was purified on Florisil, eluting with dichloromethane and dichloromethane/methanol (9:1) to yield **26** (2.1 g, 3.65 mmol, 50% yield), mp 174–175°C, α_D = –19.21° (*c* = 0.5% MeOH).

*8-{[3-(p-Isobutoxycarbonyl)phenoxy-2-hydroxypropyl]sulfonyl} adenosine **27***
A solution of potassium peroxymonosulfate (28 g, 45.6 mmol) in water (100 ml) was added dropwise to 8-{[2-hydroxy-3-(*p*-isobutoxycarbonyl)phenoxypropyl]thio} adenosine **5** (5 g, 9.1 mmol) in methanol (60 ml) at 0°C. The solution was stirred for 5 h at rt, concentrated and the residue was purified on florisil (eluent: dichloromethane/methanol, 9:1), to give 960 mg (1.6 mmol, 18% yield) of **27**, mp 129–131°C, α_D = –0.39° (*c* = 0.5% MeOH).

8-{[3-(p-Isobutoxycarbonyl)phenoxy-2-oxo-propyl]thio} adenosine ***33***
A mixture of 8-mercaptoadenosine (4 g, 13.4 mmol), 2,6-lutidine (1.8 ml, 16 mmol) and isobutyl-*p*-(3-bromo-2-oxo)-propoxy benzoate (4.4 g, 13.4 mmol) in absolute ethanol (100 ml) was refluxed for 3 h, concentrated to dryness and the residue, dissolved in ethylacetate (150 ml), was washed with $NaHCO_3$ (sat aq sol, 100 ml), with water (2 x 250 ml), dried on Na_2SO_4 and concentrated. The residue was triturated with ether, filtered and crystallized from absolute ethanol to yield **33** (6.2 g, 11.4 mmol, 85% yield), mp 162–164°C, α_D = –53.79° (*c* = 0.5% MeOH).

8-{[3-(p-Isobutoxycarbonyl)phenoxy-2-oximino-propyl]thio} adenosine ***34***
A mixture of **33** (15 g, 27.4 mmol), imidazole (11.2 g, 164.7 mmol) and hydroxylamine•HCl (8.85 g, 137.2 mmol) in isobutanol was heated at 100°C for 10 min, concentrated and the residue, dissolved in ethylacetate, was washed with $NaHCO_3$ (sat aq sol) and water. The organic layer was dried on Na_2SO_4 and concentrated. Crystallization from acetone/*n*-heptane (1:1) gave 14 g (24.7 mmol, 90% yield) of **34**, mp 156–160°C, α_D = –97.3° (*c* = 0.5% MeOH).

Compounds **35** and **36** were prepared analogously from *O*-methyl hydroxylamine (72% yield), mp 104–106°C, α_D = –77.7° (*c* = 0.5% MeOH) and semicarbazide (90% yield), mp 183–185°C, α_D = –24.27° (*c* = 0.5% MeOH).

Biological methods

First screening, 5-day test
All the substances were first tested at 100 mg/kg in male Sprague–Dawley rats (120–180 g each, Charles River, Calco, Italy). After 7 d acclimatization with chow diet, the animals were fed *ad libitum* with a modified Nath's diet [38], supplemented with 1% cholesterol (w/w) and 1% cholic acid (w/w) (Dottori Piccioni, Gessate, Italy), for 5 d, concomitantly with test compound administration. The test compounds were suspended in 0.5% w/v carboxymethylcellulose (CMC) and orally administered (10 ml/kg) daily by gavage between 9–11 am. Controls received vehicle alone. On the 5th d, 2 h after the final treatment, the rats were exsanguinated by decapitation. The following analyses were performed on serum obtained by low speed centrifugation of the blood samples: i) triglycerides and total cholesterol levels by an enzymatic method (Poli kit); and ii) HDL cholesterol levels by enzymatic method after removal of VLDL/LDL by precipitation (phosphotungstate method). In addition, hepatomegaly (liver to body-weight ratio) was evaluated.

Second screening, 15-day test
Male Sprague–Dawley rats (120–180 g each, Charles River, Calco, Italy) were fed a diet supplemented with 0.5% cholesterol (w/w) and 0.5% cholic acid (w/w) (Dottori Piccioni, Gessate, Italy), for 15 d, concomitantly with test compound administration (same procedure as above). On the 16th d, 24 h after the final treatment, the same analyses as in the 5-d test were performed. Body-weight gain during the treatment period was also recorded. Finally, liver catalase was evaluated in liver homogenates [39].

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