Hypolipidemic 2-[4-(1,1-Dimethylethyl)phenyl]-4*H*-3,1-benzoxazin-4-ones. Structure–Activity Relationships of a Novel Series of High-Density Lipoprotein Elevators

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The preparation and plasma lipid altering characteristics of a series of 4*H*-3,1-benzoxazin-4-ones are described. Hypocholesterolemic, hypotriglyceridemic, and high-density-lipoprotein elevating properties are found for derivatives bearing a 4-(1,1-dimethylethyl)phenyl group at the 2-position, and this activity is displayed in both hypercholesterolemic and in normolipidemic rats when the ring system is substituted at position 6 with hydrogen, methyl, chloro, or iodo groups, and is optimal when the 6-position is substituted by a bromine atom. Evidence is presented suggesting that a metabolite or degradation product is responsible for the changes in lipoprotein concentration observed with active molecules of this type. Synthesis of anticipated degradation products of the active molecules gave products displaying the expected in vivo activity, but no improvement in the narrow therapeutic margin of the best compound, 6-bromo-2-[4-(1,1-dimethylethyl)phenyl]-4*H*-3,1-benzoxazin-4-one, was obtained.

The lowering of abnormally high plasma lipoprotein concentrations is now an accepted target in the treatment of patients suffering from hyperlipoproteinemia and associated disease.[1,2] Current treatment to attain this target centers in the first instance on the strict control of dietary intake of fats and cholesterol, with drug treatment playing a secondary but increasingly important role. This role is likely to be vastly accentuated by the imminent advent of new drugs that inhibit the biosynthesis of cholesterol (hydroxymethylglutaryl-CoA reductase inhibitors) and drugs that prevent the absorption of dietary cholesterol and/or the retention of cholesterol in arterial smooth muscle cells (inhibitors of acyl-CoA:cholesterol acyltransferase).

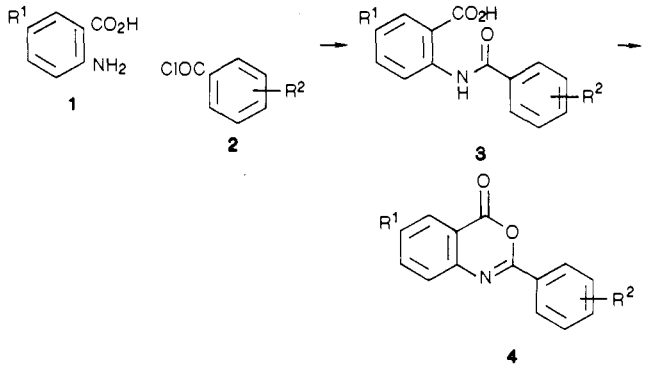
The various fractions of plasma lipoproteins were initially classified by their relative densities, and it is now generally accepted that treatment of atherosclerosis should seek to lower the levels of very low density and low-density lipoproteins (VLDL and LDL).[2] However, the inverse correlation found between the level of high-density lipoprotein (HDL) and coronary heart disease[2-4] implies that the plasma levels of HDL should preferentially be increased by therapy that generally lowers the concentration of plasma lipids. Indeed, the ratio of plasma HDL cholesterol to total plasma cholesterol has been claimed to be a major factor in predicting coronary heart disease in humans.[5] This differential feature may be expressed as an atherogenic index,[2,5,6] an example of which is

$$\text{atherogenic index} = \frac{[\text{HDL cholesterol}]}{[\text{total cholesterol}]}$$

An increase in this atherogenic index is hence sought when patients are placed on lipid-lowering therapies. Although the mechanism by which HDL reduces the incidence of coronary heart disease remains unproven,[7] it is likely to be through increasing reverse cholesterol transport from arterial tissue to the liver.[3] Thus, this aspect of lipid therapy has received attention as a feature of drugs designed to reduce the incidence of coronary heart disease.[8]

As part of our overall strategy of developing drugs to combat atherosclerosis, the changes in HDL as well as the changes in total plasma cholesterol and triglyceride levels have been monitored in one of our in vivo hyperlipidemic rat models (see below).

Scheme I



We now wish to describe a novel series of compounds possessing a 2-substituted 4*H*-3,1-benzoxazin-4-one structure, some of which, besides having the ability to lower plasma cholesterol and triglyceride levels, possess the ability to raise not only the proportion of total cholesterol carried by HDL (an effect that can often be produced by a reduction of the other lipoprotein levels) but also the absolute levels of HDL cholesterol compared to control levels. The strategy of series examination will be described, together with a description of our efforts to elucidate the active form of the hypolipidemic agents.

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Table I. Physical Properties of 4*H*-3,1-Benzoxazin-4-ones

entry	R^1	R^2	general exptl method[a]	mp, °C	purification general method[b]	yield, %	formula	anal.[c]
4a	Me	4-*t*-Bu	A	107–110	r(A)	38	$C_{19}H_{19}NO_2$	C,H,N
4b	Me	3-*t*-Bu	A	90–92	r(A)	38	$C_{19}H_{19}NO_2$	C,H,N
4c	Me	2-*t*-Bu	A	104–107	f(B) + r(C)	29	$C_{19}H_{19}NO_2$	C,H,N
4d	Me	4-*i*-Pr	A	79–82	r(A)	56	$C_{18}H_{17}NO_2$	C,H,N
4e	Me	4-CH_2-*t*-Bu	A	144–148	r(A)	54	$C_{20}H_{21}NO_2$	C,H,N
4f	Me	H	A	143–148	r(D)	31	$C_{16}H_{11}NO_2$	C,H,N
4g	Br	4-*t*-Bu	A	142–145	r(C)	51	$C_{18}H_{16}BrNO_2$	C,H,Br,N
4h	Cl	4-*t*-Bu	A	107–110	r(A)	39	$C_{18}H_{16}ClNO_2$	C,H,Cl,N
4i	I	4-*t*-Bu	A	177–180	r(D)	49	$C_{18}H_{16}INO_2$	C,H,I,N
4j	CF_3	4-*t*-Bu	B	105–108	f(E)	74	$C_{19}H_{16}F_3NO_2$	C,H,F,N
4k	H	4-*t*-Bu	A	74–77	r(F)	28	$C_{18}H_{17}NO_2$	C,H,N
4l	MeO	4-*t*-Bu	A	82–85	f(G) + r(A)	24	$C_{19}H_{19}NO_3$	C,H,N
4m	*t*-Bu	4-*t*-Bu	A	148–150	r(A) + r(H)	27	$C_{22}H_{25}NO_2$	C,H,N
4n	*n*-Bu	4-*t*-Bu	A	86–89	f(I) + r(J)	60	$C_{22}H_{25}NO_2$	C,H,N
4o	*n*-C_8H_{17}	4-*t*-Bu	C	48–51	f(K)	47	$C_{26}H_{33}NO_2$	C,H,N
4p	NO_2	4-*t*-Bu	B	165–168	r(C) + r(L)	52	$C_{18}H_{16}N_2O_4$	C,H,N
4q	NH_2	4-*t*-Bu	D	207–209	r(M)	62	$C_{18}H_{18}N_2O_2$	C,H,N
4r	$PhSO_2NH$	4-*t*-Bu	E	186–189	r(N)	49	$C_{24}H_{22}N_2O_4S$	C,H,N,S
4s	$MeSO_2NH$	4-*t*-Bu	E	217–220	r(O)	56	$C_{19}H_{20}N_2O_4S$	C,H,N,S
4t	$N(Me)_2$	4-*t*-Bu	F	173–175	f(K) + r(N)	34	$C_{20}H_{22}N_2O_2$	C,H,N
4u	Ph	4-*t*-Bu	G	196–200	f(E) + r(P)	46	$C_{24}H_{21}NO_2$	C,H,N
4v	CO_2Me	4-*t*-Bu	A	154–156	r(Q)	27	$C_{20}H_{19}NO_4$	C,H,N
4w[d]			A	112–116	f(G) + r(F)	20	$C_{20}H_{21}NO_2$	C,H,N

[a] See the Experimental Section. [b] r = recrystallization, f = flash chromatography, f + r = flash chromatography followed by recrystallization, r + r = recrystallization followed by second recrystallization. Solvent key: A = petroleum ether, bp 60–80 °C; B = petroleum ether, bp 40–60 °C/dichloromethane 1:4; C = petroleum ether, bp 80–100 °C; D = ethyl acetate; E = petroleum ether, bp 40–60 °C/dichloromethane 1:1; F = petroleum ether, bp 40–60 °C; G = petroleum ether, bp 40–60 °C/ether 3:1; H = petroleum ether, bp 60–80 °C/EtOAc 5:1; I = petroleum ether, bp 40–60 °C/ether 10:1; J = hexane; K = dichloromethane; L = petroleum ether, bp 80–100 °C/EtOAc 4:1; M = acetonitrile; N = ethyl acetate/petroleum ether, bp 80–100 °C 2:1; O = 2-ethoxyethanol; P = petroleum ether, bp 40–60 °C/ethyl acetate 1:3; Q = petroleum ether, bp 40–60 °C/ethyl acetate 4:3. [c] Compounds gave satisfactory analyses (±0.4%). [d] 2-[4-(1,1-Dimethylethyl)phenylmethyl]-6-methyl-4*H*-3,1-benzoxazin-4-one.

Table II. Physical Properties of Quinazolines

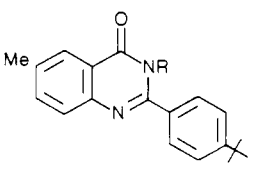
entry	R	general exptl method[a]	mp, °C	purification general method[b]	yield, %	formula	anal.[c]
5a	H	H	252–255	r(A)	49	$C_{19}H_{20}N_2O$	C,H,N
5b	Me	I	198–201	f(B)	18	$C_{20}H_{22}N_2O$	H,N;C[d]
6		I	82–86		7	$C_{20}H_{23}N_3$	C,H,N

[a] See the Experimental Section. [b] r = recrystallization, f = flash chromatography. Solvent key: A = 2-ethoxyethanol, B = dichloromethane/methanol 19:1. [c] Compounds gave satisfactory analyses (±0.4%) except where otherwise indicated. [d] Calcd: C, 78.4. Found: C, 79.5.

Chemistry

The majority of the benzoxazin-4-ones (**4**) described here were prepared by standard methods[9,10] in which the corresponding anthranilic acids (**1**) were coupled with an acid chloride (**2**) generating an intermediate amide (**3**), which underwent cyclization on treatment with boiling acetic anhydride (Scheme I). Anthranilic acids **1** were prepared by literature procedures or were commercially available (see the Experimental Section). Two of the benzoxazin-4-ones (**4o**, **4u**) were prepared from preformed 6-halobenzoxazin-4-one derivatives. Thus, palladium-catalyzed displacement of the iodine atom in the 6-iodo derivative (**4i**) by 1-octyne gave the 6-octynyl compound, which was then smoothly hydrogenated to the 6-octyl compound **4o**, and the 6-bromo compound (**4g**) was treated with diphenylcadmium under palladium catalysis to give the 6-phenyl derivative **4u**. The 6-nitro derivative (**4p**) was transformed by catalytic reduction over palladium to yield the 6-amino compound (**4q**), which was converted to the 6-phenylsulfamoyl (**4r**) and 6-methylsulfamoyl (**4s**) products by treatment with the appropriate sulfonyl chloride. Eschweiler-Clarke methylation (HCO_2H/H_2CO) of the 6-amino derivative **4q** generated compound **4t**. Physical characteristics of the benzoxazin-4-ones prepared are presented in Table I.

Compounds possessing the quinazolin-4-one skeleton (**5a**, **5b**) (bearing groups already defined as necessary for hypolipidemic and HDL elevating activity in the benzoxazin-4-one series) were prepared as follows: the benzoxazin-4-one (**4a**) was hydrolyzed to the benzamide (**7b**) and then heated with formamide to give **5a**.[11] Methanolysis of **4a** gave ester **7a**, which was heated with phosphorus pentoxide and methylamine hydrochloride to give **5b**.[12] Quinazoline **6** was an impurity in the preparation of **5b**; the formation of this type of demethylated product under the conditions used to generate **5b** has been described before.[12] Physical characteristics of these quinazolines are presented in Table II.



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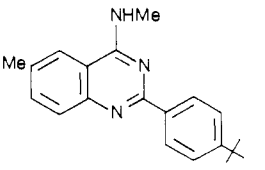

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Biology

Compounds were examined initially for hypolipidemic activity in rats that had diet-induced hypercholesterolemia. In this model, rats are fed a high-cholesterol diet for a period of 10 days with test compounds being administered orally by gavage daily for the final 3 days. Following the period of drug administration, the plasma concentrations of total cholesterol, cholesterol carried by HDL, and triglyceride are measured and compared to those present in placebo-treated animals. Active compounds were then tested in a secondary screen, using a normolipidemic rat, in which the drug was administered in the diet to animals for a period of 3 or 7 days. At the end of this period, the concentrations of plasma cholesterol and triglyceride were determined and drug-induced changes calculated. Table IV shows the biological activity in the hyperlipidemic screen and Table V the activity in the normolipidemic screen.

Results and Discussion

Our initial lead compound was the 6-methyl derivative **4a**, which showed little effect on triglycerides in the primary hypercholesterolemic screen, but an interesting ability to reduce the levels of plasma total cholesterol while increasing the absolute and relative proportions of cholesterol carried on HDL (Table IV). In addition, in the normocholesterolemic screen, a significant ability to reduce both triglycerides and total cholesterol was demonstrated for **4a** when fed in the diet at 0.2% w/w. To develop this lead, and in order to reduce the number of chemical targets as much as possible, the following rational approaches to optimization of activity were examined.

1. An investigation of the need for a 4-(1,1-dimethylethyl)phenyl group at the 2-position of the benzoxazin-4-one ring was carried out. Migration of the 1,1-dimethylethyl group around the phenyl ring (**4b** meta, **4c** ortho) was associated with practically a total loss of activity in both screens. Replacement of the 4-(1,1-dimethylethyl)-phenyl group in **4a** by a 4-(1-methylethyl)phenyl group (**4d**) or a 4-(2,2-dimethylpropyl) group (**4e**) diminished but did not abolish activity in the primary hyperlipidemic screen and no triglyceride-lowering ability was evident in the secondary screen. A simple phenyl group at the 2-position (compound **4f**) abolished all activity in the hyperlipidemic screen. Introduction of a spacer methylene group between the benzoxazin-4-one skeleton and the 4-(1,1-dimethylethyl)phenyl ring (compound **4w**) abolished activity in the hypercholesterolemic primary screen, and this compound was not tested in the secondary screen. The conclusion reached therefore was that optimal hypolipidemic and HDL-elevating properties were associated with a 2-(4-(1,1-dimethylethyl)phenyl substituent directly attached to the benzoxazin-4-one skeleton.

2. A brief examination of the replacement of the 3,1-benzoxazin-4-one skeleton by an isosteric quinazolin-4-one skeleton (compounds **5a**, **5b**, 6) showed that such replacement abolished activity completely in both tests. This observation had repercussions when the active principal of the biologically active compounds came to be considered, and is discussed further at length below.

3. Optimization of the substituent at the 6-position of the 3,1-benzoxazin-4-one ring (lead compound **4a** has a methyl group at this position) was performed by selecting a rational series[13] of targets covering a multiparameter space. The parameters covered by the analysis were those of π, π^2, MR, F, and R. Beginning with the methyl compound already synthesized, a total of 13 further compounds (R^1 = Cl, H, MeO, 1,1-dimethylethyl, butyl, octyl, nitro, amino, phenylsulfamoyl, methylsulfamoyl, dimethylamino, phenyl, and methoxycarbonyl) were selected from a rational list supplied by our in-house SPREAD program. Figure 1 shows the spread of substituents over the 5-dimensional space, and Table VI demonstrates that such selection of substituents is free of major cross-correlation. With the compounds prepared, it became obvious that the best spectrum of activity, both in terms of hypolipidemic properties and in terms of HDL-raising ability, was found for the 6-Me, 6-Cl, 6-H, and 6-MeO derivatives. Preparation of two further 6-halo derivatives (6-Br and 6-I) together with preparation of the 6-CF_3 derivative confirmed that activity was optimal for the 6-Br compound in that this derivative showed the best spectrum of activity in terms of being able to lower levels of plasma triglycerides and plasma total cholesterol in both tests, but also by potently raising levels of HDL cholesterol in our primary screen.

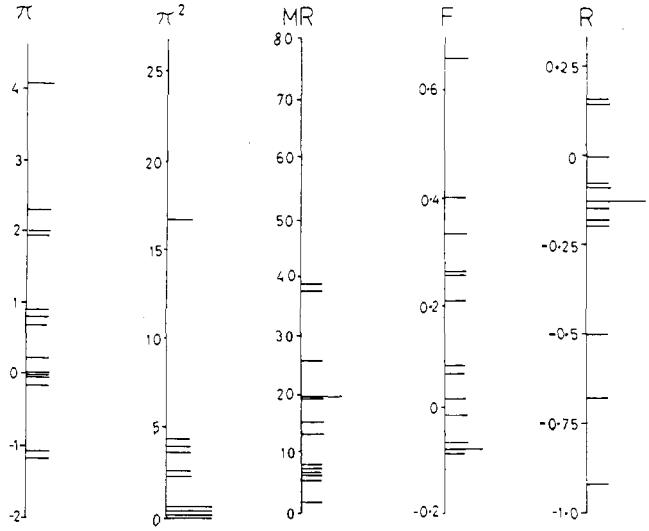


Figure 1. Spread of the 14 initially selected 6-substituents onto the axes of the 5-dimensional space. π is Hansch's hydrophobicity constant based on the octanol/water partition coefficient, MR is the molar refractivity of each substituent, F and R are Swain and Lupton's field and resonance constants. Full details of these parameters are contained in ref 26.

4. Examination of the 6-Br derivative **4g** in our tertiary screen, designed to identify potential toxicity in hypolipidemic compounds, resulted in the abandonment of this derivative as a potential drug candidate, due to deaths of rats at 4 times the dose that yielded a useful hypolipidemic effect. Elevation of HDL without a concomitant reduction of total cholesterol or triglycerides was observed at lower doses. However, because of their low therapeutic indices, none of the active benzoxazin-4-ones warranted further investigation.

It was apparent at a very early stage of our investigation that the 4*H*-3,1-benzoxazin-4-one skeleton was too unstable to hydrolysis to be the true active species in vivo. Two candidates suggested themselves as responsible for the lipid-altering abilities of the active compounds. The first candidate, 4-(1,1-dimethylethyl)benzoic acid (8), is derived by a double hydrolysis of the 4*H*-3,1-benzoxazin-4-one skeleton (Scheme II) via an intermediate 2-benzamidobenzoic acid (e.g. **7b**), which itself is considered as the second candidate responsible for the activity. Interestingly, 4-(1,1-dimethylethyl)benzoic acid (8) has been implicated in the mode of action of the potential HMG-CoA reductase inhibitor SC 37311 (**9**), from which it is apparently derived

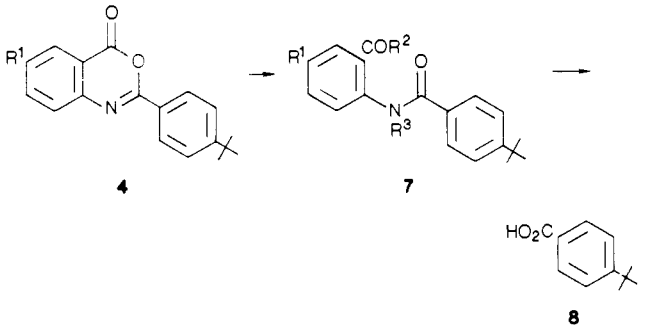
(13) Wooldridge, K. R. H. *Eur. J. Med. Chem.* **1980**, *15*, 63.

Table III. Physical Properties of Ring-Opened Derivatives (7) of 4*H*-3,1-Benzoxazin-4-one Derivatives 4a and 4g

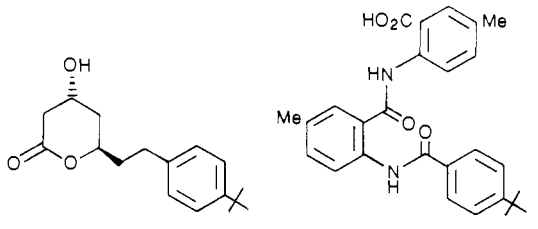
entry	R^1	R^2	R^3	general exptl method[a]	mp, °C	purification general method[b]	yield, %	formula	anal.[c]
7a	Me	OMe	H	J	118–122	r(A)	92	$C_{20}H_{23}NO_3$	C,H,N
7b	Me	OH	H	K	207–211	r(B)	46	$C_{19}H_{21}NO_3$	C,H,N
7c	Br	OMe	H	J	95–97	r(C)	78	$C_{19}H_{20}BrNO_3$	C,H,Br,N
7d	Br	OH	H	K	263–265 dec	r(B)	40	$C_{18}H_{18}BrNO_3$	C,H,Br,N
7e	Br	ONa	H	L	>300	r(D)	55	$C_{18}H_{17}BrNO_3Na$	C,H,Br,N,Na[d]
7f	Br	NH_2	H	M	238–241	r(E)	66	$C_{18}H_{19}BrN_2O_2$	C,H,Br,N
7g	Br	OH	Me	N	174–176	r(F)	67	$C_{19}H_{20}BrNO_3$	C,H,Br,N

[a] See the Experimental Section. [b] r = recrystallization. Solvent key: A = petroleum ether, bp 80–100 °C, B = ethyl acetate, C = hexane, D = methanol, E = 2-propanol, F = ethyl acetate/petroleum ether, bp 60–80 °C, 2:1. [c] Compounds gave satisfactory analyses (±0.4%). [d] Analyzed for 1.25 mol of water.

Scheme II



by in vivo hydrolysis, followed by a multiple β-oxidation.[14] In our case, the first hydrolysis of the 4*H*-3,1-benzoxazin-4-one was found to be extremely easily accomplished as experiments on the 6-methyl derivative **4a** proved. Thus, although this benzoxazin-4-one **4a** was stable in the absence of a nucleophile (no change after boiling a 1% solution in ethyl acetate for 20 h), boiling a 1% solution in methanol for 20 h gave ester **7a** as the sole product. An 8:1 mixture of methanol/2 M NaOH gave acid **7b** (after acidification of the reaction mixture). Physical characteristics of these derived products are given in Table III. Acid hydrolysis (MeOH/2 M HCl 8:1) gave rapid degradation and a variety of products (including the dimeric derivative (**10**) and 1,1-(dimethylethyl)benzoic acid (**8**)).



Facile hydrolysis of a 2-aryl-4*H*-3,1-benzoxazin-4-one skeleton to give a 2-benzamidobenzoic acid has been reported recently by Johnson and Pattison,[15] who also made the observation that the biological testing in aqueous media of such a molecule was tantamount to presenting the biological test system with the 2-benzamidobenzoic acid. Our in vitro experiments both in aqueous acid and aqueous base on the 6-methyl derivative **4a** agree with this observation. Equally, the test results on acid **7b** are consistently similar to those of benzoxazin-4-one **4a** in accord with the remark by Johnson and Pattison. Finally, the inactivity of the quinazoline compounds **5a**, **5b**, and **6**, which are much more stable to hydrolysis, are consistent with the premise that acid **7b** or a further degradation product (**8**) is the active principal of benzoxazin-4-one **4a**.

Both potentially derived compounds **7b** and **8** from our prodrug **4a** were examined in our test systems (Tables IV and V). The HDL-elevating profiles suggested that compound **7b** was more likely to be responsible for the activity of **4a**. Moreover, if formation of the 1,1-(dimethylethyl)benzoic acid **8** by the double hydrolysis of the 6-methyl derivative **4a** were responsible for the activity of **4a**, it seemed to us inconceivable that so many of the 6-substituted derivatives should be inactive, since all potentially degrade to **8**. In a separate experiment, the plasma levels of **8** in rats given **8** in sufficient dose to produce a hypolipidemic and HDL-elevating effect (i.e. 50, 100, 200, and 400 mg kg^{-1} day^{-1} all for 7 days) was measured. The corresponding levels of **8** in the plasma were 7.4, 16.9, 15.5, and 50.7 μg mL^{-1}. In a parallel experiment, the plasma levels of **8** in rats fed a dose (200 mg kg^{-1} day^{-1} for 7 days) of 6-bromo-4*H*-3,1-benzoxazin-4-one **4g** sufficient to produce a marked hypolipidemia and HDL elevation were measured and found to be effectively unmeasurable (less than 0.1 μg mL^{-1}). Interestingly, in accord with the observations of Johnson and Pattison, none of benzoxazin-4-one **4g** could be detected in the plasma either (<0.5 μg mL^{-1}).

All the evidence hence pointed to the active 2-[4-(1,1-dimethylethyl)phenyl]-6-substituted-4*H*-3,1-benzoxazin-4-ones being prodrugs for the corresponding hydrolytically derived 2-[4-(1,1-dimethylethyl)benzamido]-5-substituted-benzoic acids. Brief attempts to improve the therapeutic index of the 5-bromobenzoic acid derivative (**7d**) by preparing and testing the ester (**7c**), sodium salt (**7e**), amide (**7f**), or *N*-methyl derivative (**7g**) (Table III) were unsuccessful, and this program of work was terminated.

Experimental Section

Chemistry. Melting points were determined on an Electrothermal apparatus and are uncorrected. Infrared spectra were recorded as KBr disks with a Pye Unicam SP3-200 spectrometer. ^1H NMR spectra were recorded on Varian XL-200 (200 MHz) or CFT 20 (80 MHz) instruments. Elemental analyses were performed in-house with a Carlo Erba 1106 instrument. 4-(1,1-Dimethylethyl)benzoyl chloride was supplied by the Aldrich Chemical Co. The preparation of the other acid chlorides to be used for the coupling with anthranilic acids was achieved by treatment of the corresponding acids with thionyl chloride, followed by distillation. 4-(1-Methylethyl)benzoic acid was obtained from Aldrich, and 3-(1,1-dimethylethyl)benzoic acid,[16] 2-(1,1-dimethylethyl)benzoic acid,[17] 4-(2,2-dimethylpropyl)benzoic acid,[18]

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Table IV. Hypolipidemic Results on Cholesterol-Fed Rats

entry	dose,[a] mg kg^{-1} day^{-1}	dosage period, days	% change[b] in plasma tryglycerides	% change[b] in plasma total cholesterol	% change[b] in plasma HDL cholesterol	% change[b] in plasma HDL cholesterol/ total cholesterol
clofibrate	200	3	−10	−30	−10	+8
nicotinic acid	200	3	−39**	−29**	+20	+63**
4a	200	3	−17	−27**	+50**	+92***
	100	3	−18	−34**	+15	+65**
	50	3	+21	−19	+31***	+56**
	25	3	−1	−5	+30***	+31**
4b	200	3	+22	+43**	+50***	+3
4c	200	3	−25	−6	−3	+6
4d	200	3	−17	−26**	+33	+79***
4e	200	3	+23	−12	+28	+50**
4f	200	3	−4	−2	−2	+4
4g	200	3	−3	−25	+74***	+114***
	100	3	−6	−25	+71***	+110***
	50	3	+6	−12	+40**	+49
4h	200	3	−30**	−18	+16	+31**
4i	200	3	−3	−26	+17	+50**
4j	200	3	−6	−18	−8	+11
4k	200	3	−26**	−41***	+49**	+131***
4l	200	3	−26	−28	+21	+52**
4m	200	3	+52**	+63***	+30	−17
4n	200	3	+22	−4	+65***	+69**
4o	200	3	−7	−9	+35	+29
4p	200	3	−19	−23	−8	+13
4q	200	3	−11	−25	+4	+25
4r	200	3	−7	+7	+30	+35
4s	200	3	+6	−16	+22	+27
4t	200	3	+16	−4	+26	+22
4u	200	3	0	−8	+60***	+74***
4v	200	3	0	+29**	+18	−4
4w	200	3	+27	+1	−19	−27
5a	200	3	−18	−17	+24	+25
5b	200	3	+8	+2	+6	−5
6	200	3	+79***	+27	+10	−12
7a	200	3	+68**	−20	+25	+46
7b	200	3	+8	−9	+91***	+47
7c	200	3	−22	−49**	−7	+70***
7d	200	3	−26	−29	+24	+70***
7e	200	3	+15	−7	+57***	+64***
7f	200	3	−11	−8	−1	+4
7g	200	3	−13	−22	+33	+59**
8	200	3	−38**	−62***	−50***	+60

[a] Daily dose administered orally by gavage. [b] Result significantly different from controls: (**) $p < 0.01$, (***) $p < 0.001$.

4-(1,1-dimethylethyl)phenylacetic acid[19] were prepared as described previously. The anthranilic acids were obtained as follows: 2-amino-5-methyl-, 2-amino-5-bromo-, 2-amino-5-chloro-, and 2-amino-5-iodobenzoic acids were obtained from the Aldrich Chemical Co. 2-Amino-5-methoxybenzoic acid was supplied by the Apin Chemical Co., 2-amino-5-nitrobenzoic acid was supplied by the Chemical Dynamics Corp. The following anthranilic acids were prepared thus: 2-amino-5-(trifluoromethyl)benzoic acid (mp 177–180 °C dec) was prepared by alkaline hydrogen peroxide oxidation[20] of 5-(trifluoromethyl)isatin,[21] 2-amino-5-(1,1-dimethylethyl)benzoic acid,[22,23] and 2-amino-5-butylbenzoic acid[23] were prepared as described previously. 2-Amino-5-(methoxycarbonyl)benzoic acid (mp 224–227 °C) was obtained by catalytic reduction (H_2/Pd/C) of 5-(methoxycarbonyl)-2-nitrobenzoic acid.[24]

Method A. Preparation of 2-[4-(1,1-Dimethylethyl)phenyl]-6-substituted-4*H*-3,1-benzoxazin-4-ones 4a–4i, 4k–4n, 4v, 4w. A stirred solution of 2-amino-5-methylbenzoic acid (15.1 g, 0.1 mol) in dry pyridine (150 mL) was treated dropwise with 4-(1,1-dimethylethyl)benzoyl chloride (19.7 g, 0.1 mol) (10 min), the temperature rising to 40 °C. The mixture was stirred at room temperature (3 h) and poured onto a mixture of ice and hydrochloric acid. The mixture was extracted with dichloromethane (3 × 200 mL), and the organic phase was washed with hydrochloric acid (1 M, 150 mL) and water (2 × 250 mL), dried ($MgSO_4$), and evaporated to give crude 2-[4-(1,1-dimethylethyl)benzamido]-5-methylbenzoic acid (**7b**) (27.4 g) as a brown solid. A suspension of this solid (27.4 g, 0.088 mol) in acetic anhydride (250 mL) was heated under reflux (3 h) and then concentrated. The residue was crystallized from petroleum ether, bp 60–80 °C, giving 2-[4-(1,1-dimethylethyl)phenyl]-6-methyl-4*H*-3,1-benzoxazin-4-one (**4a**) (11.1 g, 38% overall) as colorless crystals, mp 107–110 °C.

Method B. Preparation of 2-[4-(1,1-Dimethylethyl)phenyl]-6-substituted-4*H*-3,1-benzoxazin-4-ones 4j, 4p. To a stirred solution of 2-amino-5-nitrobenzoic acid (18.2 g, 0.1 mol) in dry *N,N*-dimethylformamide (150 mL) was added triethylamine (30.6 mL, 0.22 mol) followed by 4-(1,1-dimethylethyl)benzoyl chloride (43.2 g, 0.22 mol) dropwise, while the temperature was maintained below 40 °C. The suspension was stirred at room temperature (5 h), when further portions of triethylamine (13.9

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Table V. Hypolipidemic Results on Normocholesterolemic Rats

entry	dose,[a] % composition of diet	dosage period, days	% change[b] in plasma triglycerides	% change[b] in plasma total cholesterol
clofibrate	0.2	7	-48**	-27**
nicotinic acid	0.2	7	-3	-4
4a	0.2	7	-33**	-15**
4b	0.2	7	+25	-4
4c	0.2	7	-13	-11
4d	0.2	3	+7	-11
4e	0.2	3	+16	-10
4f	0.2	7	-1	-12
4g	0.2	7	-40***	-39***
	0.2	3	-16	-47***
4h	0.2	7	-36***	-31***
4i	0.2	7	-35***	-48***
4j	0.2	3	-45***	-40***
4k	0.2	7	-32***	-24***
4l	0.2	3	-52***	-48***
4m	0.2	3	-17	-15
4n	0.2	3	-32***	-3
4o	0.2	3	-14	-1
4p	0.2	7	-27**	+1
4q	0.2	3	+1	+4
4r	0.2	3	+27	+5
4s	0.2	3	-8	-3
4t	0.2	3	-12	+10
4u	0.2	3	+1	0
4v	0.2	3	-13	-2
4w	not tested			
5a	0.2	3	+28	+1
5b	0.2	3	-3	+2
6	not tested			
7a	0.2	3	-8	-4
7b	0.2	3	-16	-15**
7c	0.2	3	-7	-34***
7d	0.2	3	-20	-56***
7e	0.2	3	-18	-42***
7f	0.2	3	+29**	+4
7g	0.2	3	+10	-10
8	0.2	7	-41**	-38***

[a] Drug mixed with powdered diet and administered for period stated. [b] Result significantly different from controls: (**) $p < 0.01$, (***) $p < 0.001$.

Table VI. Correlation Coefficient Matrix of the 14 Initially Selected Substituents

	aromatic π	aromatic π^2	molar refractivity	F	R
Ar π	1	0.823	0.622	-0.465	0.263
Ar π^2		1	0.633	-0.422	0.140
MR			1	-0.250	0.087
F				1	0.263
R					1

mL, 0.11 mol) and 4-(1,1-dimethylethyl)benzoyl chloride (21.6 g, 0.11 mol) were added ($T < 40$ °C). The mixture was stirred at room temperature (18 h) and poured onto excess ice and hydrochloric acid. The mixture was extracted with dichloromethane, and the extracts were washed successively with hydrochloric acid (1 M), water, sodium hydroxide solution (1 M), and water, dried ($MgSO_4$), and concentrated. The residue was crystallized from petroleum ether, bp 80–100 °C, and then from petroleum ether, bp 80–100 °C/ethyl acetate 4:1 to give 2-[4-(1,1-dimethylethyl)phenyl]-6-nitro-4*H*-3,1-benzoxazin-4-one (**4p**) (16.8 g, 52%) as cream crystals, mp 165–168 °C.

Method C. Preparation of 2-[4-(1,1-Dimethylethyl)phenyl]-6-octyl-4*H*-3,1-benzoxazin-4-one (4o). A stirred solution of 2-[4-(1,1-dimethylethyl)phenyl]-6-iodo-4*H*-3,1-benzoxazin-4-one (**4i**) (10.1 g, 25 mmol) in dimethyl sulfoxide (50 mL) under nitrogen was treated successively with triethylamine (100 mL), bis(triphenylphosphine)palladium(II) chloride (0.1 g, 0.14 mmol), copper(I) iodide (0.05 g, 0.26 mmol), and 1-octyne (3.7 mL, 25 mmol), and the mixture was stirred at room temperature (16 h). A further aliquot of 1-octyne (1.0 mL, 6.8 mmol) was added, and the mixture was stirred at room temperature (16 h). The mixture was concentrated, and the residue partitioned between water and dichloromethane. The organic phase was washed with hydrochloric acid (2 M) and water and dried ($MgSO_4$). Evaporation gave crude 2-[4-(1,1-dimethylethyl)phenyl]-6-(1-octynyl)-4*H*-3,1-benzoxazin-4-one (9.67 g) as a brown oil. A mixture of this oil (9.67 g) and platinum oxide (2 g) in ethyl acetate (150 mL) was stirred under an atmosphere of hydrogen (30 min). The mixture was filtered and concentrated, and the residue was purified by flash chromatography (silica, eluant dichloromethane), giving 2-[4-(1,1-dimethylethyl)phenyl]-6-octyl-4*H*-3,1-benzoxazin-4-one (**4o**) (4.56 g, 47%) as cream crystals, mp 48–51 °C.

Method D. Preparation of 6-Amino-2-[4-(1,1-dimethylethyl)phenyl]-4*H*-3,1-benzoxazin-4-one (4q). A mixture of 2-[4-(1,1-dimethylethyl)phenyl]-6-nitro-4*H*-3,1-benzoxazin-4-one (**4p**) (16.2 g, 0.05 mol) and palladium on charcoal (5%, 3.0 g) in ethyl acetate (300 mL) was stirred under an atmosphere of hydrogen until uptake was complete (1 h). The mixture was filtered, dried ($MgSO_4$), and concentrated. The residue was crystallized from acetonitrile, giving 6-amino-2-[4-(1,1-dimethylethyl)-phenyl]-4*H*-3,1-benzoxazin-4-one (**4q**) (9.1 g, 62%) as cream crystals, mp 207–209 °C.

Method E. Preparation of 2-[4-(1,1-Dimethylethyl)-phenyl]-6-substituted-4*H*-3,1-benzoxazin-4-ones 4r, 4s. A stirred solution of 6-amino-2-[4-(1,1-dimethylethyl)phenyl]-4*H*-3,1-benzoxazin-4-one (**4q**) (14.7 g, 0.05 mol) in dry pyridine (100 mL) was treated dropwise with benzenesulfonyl chloride (10.59 g, 0.06 mol) under an atmosphere of nitrogen. The mixture was stirred at room temperature (18 h) and poured onto a mixture of ice/excess hydrochloric acid. The mixture was extracted with dichloromethane, and the extract washed with hydrochloric acid (2 M) and water, dried ($MgSO_4$), and concentrated. The residue was crystallized from ethyl acetate/petroleum ether, bp 80–100 °C, 2:1, giving 2-[4-(1,1-dimethylethyl)phenyl]-6-(phenylsulfonyl)-4*H*-3,1-benzoxazin-4-one (**4r**) (10.63 g, 49%) as white crystals, mp 186–189 °C.

Method F. Preparation of 6-(Dimethylamino)-2-[4-(1,1-dimethylethyl)phenyl]-4*H*-3,1-benzoxazin-4-one (4t). A stirred solution of 6-amino-2-[4-(1,1-dimethylethyl)phenyl]-4*H*-3,1-benzoxazin-4-one (**4q**) (14.7 g, 0.05 mol) in formic acid (19 mL) was treated with a solution of formaldehyde (40% w/w, 7.5 mL, 0.1 mol) in water under an atmosphere of nitrogen. The mixture was stirred and heated at 90 °C (4 h); a further portion of formaldehyde solution (3.75 mL, 0.05 mol) was added and the mixture heated at 90 °C (16 h). The cooled mixture was diluted with water (50 mL) and extracted with dichloromethane. The organic phase was washed with water, dried ($MgSO_4$), and concentrated. The resulting solid was suspended in acetic anhydride (150 mL) and heated under reflux (3 h) under an atmosphere of nitrogen. The mixture was concentrated, and the product was purified by flash chromatography (silica, eluant dichloromethane). Crystallization from ethyl acetate/petroleum ether, bp 80–100 °C, 2:1, gave 6-(dimethylamino)-2-[4-(1,1-dimethylethyl)phenyl]-4*H*-3,1-benzoxazin-4-one (4t) (5.47 g, 34%) as yellow crystals, mp 173–175 °C.

Method G. Preparation of 2-[4-(1,1-Dimethylethyl)-phenyl]-6-phenyl-4*H*-3,1-benzoxazin-4-one (4u). A stirred solution of 6-bromo-2-[4-(1,1-dimethylethyl)phenyl]-4*H*-3,1-benzoxazin-4-one (**4g**) (35.8 g, 0.1 mol) in tetrahydrofuran (150 mL) was treated with triphenylphosphine (1.57 g, 0.006 mol). The mixture was stirred at room temperature (10 min) and then treated with palladium(II) chloride (0.53 g, 0.003 mol). The mixture was stirred (10 min) and then heated under reflux (45 min). A solution of diphenylcadmium [prepared from bromobenzene (15.7 g, 0.1 mol), magnesium (2.43 g, 0.1 mol) and cadmium(II) chloride (10.08 g, 0.055 mol)] in tetrahydrofuran (150 mL) was added at reflux, and the mixture was heated under reflux (9 h). The mixture was added to ice/excess hydrochloric acid and extracted with ether. The organic phase was washed with water, dried ($MgSO_4$), and concentrated. The product was purified by flash chromatography (silica, eluant petroleum ether, bp 40–60 °C/dichloromethane 1:1). Crystallization from ethyl acetate/petroleum ether bp 60–80 °C, 3:1 gave 2-[4-(1,1-dimethylethyl)phenyl]-6-phenyl-4*H*-3,1-benzoxazin-4-one (**4u**) (16.3 g, 46%) as colorless crystals, mp 196–200 °C.

Method H. Preparation of 2-[4-(1,1-Dimethylethyl)-phenyl]-6-methyl-3*H*-quinazolin-4-one (5a). A stirred suspension of 4-(1,1-dimethylethyl)-*N*-(2-carboxy-4-methyl-

phenyl)benzamide (**7b**) (31.1 g, 0.1 mol) in formamide (6 mL) was heated at 170–175 °C (4.5 h). The cooled mixture was partitioned between dichloromethane and aqueous sodium hydroxide (1 M). The organic phase was washed with water, dried ($MgSO_4$), and concentrated. The residue was crystallized from 2-ethoxyethanol, giving 2-[4-(1,1-dimethylethyl)phenyl]-6-methyl-3*H*-quinazolin-4-one (**5a**) (14.3 g, 49%) as colorless crystals, mp 252–255 °C.

Method I. Preparation of 2-[4-(1,1-Dimethylethyl)-phenyl]-3,6-dimethylquinazolin-4-one (5b). A suspension of methyl 2-[4-(1,1-dimethylethyl)benzamido]-5-methylbenzoate (**7a**) (32.5 g, 0.1 mol), methylamine hydrochloride (27 g, 0.4 mol), and phosphorus pentoxide (60 g, 0.42 mol) in *N,N*-dimethylcyclohexylamine (60 mL) was heated at 180 °C (1 h). The mixture was cooled to 140 °C and adjusted to pH 7 by cautious addition of aqueous sodium hydroxide (2 M). The resulting suspension was treated with dichloromethane (350 mL) and filtered (*). The solid was suspended in aqueous sodium hydroxide (100 mL) and extracted with methylene chloride (2 × 200 mL). The organic phase was washed with water, dried ($MgSO_4$), and concentrated, giving 2-[4-(1,1-dimethylethyl)phenyl]-6-methyl-4-(methylamino)quinazoline (**6**) (2.0 g, 7%) as a colorless solid, mp 82–86 °C. The filtrate from above (*) was partitioned into constituent dichloromethane and aqueous phases. The organic phase was washed with water, dried ($MgSO_4$), and concentrated. The residue was dissolved in boiling cyclohexane and cooled. The precipitated oil was collected and purified by flash chromatography (silica, eluant dichloromethane/methanol 29:1), giving 2-[4-(1,1-dimethylethyl)phenyl]-3,6-dimethylquinazolin-4-one (**5b**) (5.6 g, 18%) as a colorless solid, mp 198–201 °C.

Method J. Preparation of Methyl 2-[4-(1,1-Dimethylethyl)benzamido]-5-substituted-benzoates 7a, 7c. A stirred suspension of 2-[4-(1,1-dimethylethyl)phenyl]-6-methyl-4*H*-3,1-benzoxazin-4-one (**4a**) (14.7 g, 0.05 mol) in dry methanol (750 mL) was heated under reflux (20 h). The solution was concentrated and the residue crystallized from petroleum ether, bp 80–100 °C, giving methyl 2-[4-(1,1-dimethylethyl)benzamido]-5-methylbenzoate (**7a**) (15 g, 92%) as colorless crystals, mp 118–122 °C.

Method K. Preparation of 2-[4-(1,1-Dimethylethyl)-benzamido]-5-substituted-benzoic Acids 7b, 7d. A stirred suspension of 2-[4-(1,1-dimethylethyl)phenyl]-6-methyl-4*H*-3,1-benzoxazin-4-one (**4a**) (14.7 g, 0.05 mol) in methanol (650 mL) was warmed until solution occurred. A solution of sodium hydroxide (10 g, 0.25 mol) in water (75 mL) was added and the mixture heated under reflux (4 h) and concentrated. The residue was dissolved in water, acidified (concentrated HCl), extracted into dichloromethane, dried ($MgSO_4$), and concentrated. The residue was crystallized from ethyl acetate, giving 2-[4-(1,1-dimethylethyl)benzamido]-5-methylbenzoic acid (**7b**) (7.2 g, 46%) as colorless crystals, mp 207–211 °C.

Method L. Preparation of Sodium 5-Bromo-2-[4-(1,1-dimethylethyl)benzamido]benzoate (7e). This compound was prepared as described for acid **7d** in method K, except that, after concentration of the reaction mixture, the residue was crystallized from methanol, giving **7e** hydrate (11.5 g, 55%) as colorless crystals, mp >300 °C.

Method M. Preparation of *N*-(2-Carbamoyl-4-bromophenyl)-4-(1,1-dimethylethyl)benzamide (7f). A stirred solution of 6-bromo-2-[4-(1,1-dimethylethyl)phenyl]-4*H*-3,1-benzoxazin-4-one (**4g**) (5 g, 0.014 mol) in dry tetrahydrofuran (200 mL) was treated with a stream of ammonia gas (1 h). The solution was stirred (1 h) and concentrated, and the residue was crystallized from 2-propanol, giving *N*-(2-carbamoyl-4-bromophenyl)-4-(1,1-dimethylethyl)benzamide (**7f**) (6.9 g, 66%) as colorless crystals, mp 238–241 °C.

Method N. Preparation of 4-(1,1-Dimethylethyl)-*N*-(4-bromo-2-carboxyphenyl)-*N*-methylbenzamide (7g). A stirred solution of sodium 5-bromo-2-[4-(1,1-dimethylethyl)benzamido]benzoate hydrate (**7e**) (8.4 g, 0.02 mol) in *N,N*-dimethylformamide (100 mL) was treated with sodium hydride (80% dispersion in oil, 1.5 g, 0.05 mol), and the mixture was stirred at room temperature (15 min). Methyl iodide (4.4 mL, 0.07 mol) was added, and the mixture heated at 100 °C (6 h), cooled, poured onto ice/excess hydrochloric acid, and extracted with ether. The organic phase was washed with water, dried ($MgSO_4$), and concentrated, giving crude 4-(1,1-dimethylethyl)-*N*-[4-bromo-2-(methoxycarbonyl)phenyl]-*N*-methylbenzamide as a colorless solid (8 g). A solution of this solid in methanol (100 mL) was treated with a solution of sodium hydroxide (2.0 g, 0.05 mol) in water (25 mL) and the suspension was heated under reflux (4 h). The solution was poured into excess hydrochloric acid and extracted into ether and the organic phase washed with water, dried ($MgSO_4$), and concentrated. The residue was crystallized from ethyl acetate/petroleum ether, bp 60–80 °C, 2:1, giving 4-(1,1-dimethylethyl)-*N*-(4-bromo-2-carboxyphenyl)-*N*-methylbenzamide (**7g**) (5.2 g, 67%) as colorless crystals, mp 174–176 °C.

Acid Hydrolysis of 2-[4-(1,1-Dimethylethyl)phenyl]-6-methylbenz[*d*]-3,1-oxazin-4-one (4a). A suspension of 2-[4-(1,1-dimethylethyl)phenyl]-6-methyl-4*H*-3,1-benzoxazin-4-one (**4a**) (10 g, 0.034 mol) in dry methanol (100 mL) was treated with aqueous hydrochloric acid (35% v/v, 5 drops) and the mixture boiled (20 h). The mixture was cooled and the solid collected, giving 2-[4-(1,1-dimethylethyl)benzamido]-5-methyl-*N*-(2-carboxy-4-methylphenyl)benzamide (**10**) (3.3 g, 44%) as colorless crystals, mp 228–231 °C. Anal. ($C_{27}H_{28}N_2O_4$) C, H, N. The filtrate was shown by TLC to contain four further products: **7a**, 8, 8 methyl ester, and 2-amino-5-methyl benzoic acid (1, R^1 = Me).

Biological Methods. Hypercholesterolemic 3-Day Test. Young male Wistar rats, weighing 120–150 g, were obtained from Charles River Ltd., Manston, Kent. The animals, housed in a room lit by time-controlled fluorescent lighting which was switched on between 6.00 h and 20.00 h, were divided into groups of eight and were maintained ad libitum on a rodent diet supplemented with 0.5% w/w cholesterol and 0.25% cholic acid for 10 days. Compounds were suspended in 0.5% w/w tragacanth mucilage and administered by stomach tube daily between 8.30 and 9.00 a.m., for the final 3 days. Control rats received an equivalent volume of vehicle. Three hours after the final dose, after overnight starvation, the rats were asphyxiated with carbon dioxide, and the blood was sampled by cardiac puncture. The blood samples were transferred to heparinized tubes and plasma obtained by low-speed centrifugation. The concentrations of plasma triglyceride and total cholesterol were determined by standard enzymatic techniques with a Cobas Bio centrifugal analyser. Apo B and apo E containing lipoproteins (VLDL, LDL, and HDL_1) were precipitated with dextran sulfate/calcium chloride.[25] Analysis of this supernatant solution by gradient gel polyacrylamide electrophoresis showed the presence of a single population of particles with a mean diameter of 11.0 nm, comparable to that of human HDL_2. As a similar profile was obtained with an ultracentrifugal fraction of HDL (d = 1.063–1.21) obtained from hypercholesterolemic rats, we have referred to the cholesterol content of the supernatant as HDL cholesterol. Values for plasma concentrations of triglycerides, total cholesterol, HDL cholesterol, and the ratio of HDL cholesterol:total cholesterol in treated animals were compared to the values obtained in vehicle-treated animals, and the significances of differences between groups were determined by using the Student's t test. Typical values for the plasma concentrations of plasma triglyceride, total cholesterol, and HDL cholesterol in vehicle-treated animals were 1.25, 4.5, and 0.75 mmol L^{-1}, respectively.

Normolipidemic 3- or 7-Day Test. Young male Wistar rats, weighing 120–150 g, were fed a basal powdered rodent diet ad libitum. Compounds were administered in the diet at a concentration of 0.2% w/w for the period of test (3 or 7 days). The concentrations of plasma triglyceride and total cholesterol were determined as described above. Typical values were 1.3 and 2.25 mmol L^{-1}, respectively.

Analysis of Biological Fluids. Assay in Biological Fluids of 4-(1,1-Dimethylethyl)benzoic Acid (8) following Administration of 4g. Compound **4g** was administered in the diet (Lad No. 2; 2 g kg^{-1}; ca. 200 mg kg^{-1} per animal) to a group of six male rats for a period of 1 week under normal lighting conditions before sacrifice. The blood obtained from these animals was collected in heparinized tubes and then centrifuged. The resulting plasma was stored at approximately −30 °C until analysis was performed. The plasma was allowed to thaw completely at room temperature, and a single aliquot (1.0 mL) of each plasma sample was trans-

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ferred to separate, clean dry extraction tubes (10 mL), and then spiked with a constant amount of an internal standard solution (5 μL of a 1 μg mL^{-1} solution of 4-propoxyphenylacetic acid in methanol). After vortex agitation (1 min), Volucon (May & Baker) buffer (pH 1.0; 1.0 mL) was added to each tube, together with chloroform (5 mL). The mixtures were shaken mechanically (30 min) and then centrifuged (2200 rpm, 15 min, 18 °C). Each chloroform layer was removed and evaporated to dryness under nitrogen. Standard samples were prepared by the same technique, but with the addition of 8 (giving plasma concentrations of 0.1–3.0 μg mL^{-1} of plasma) as well as the internal standard to plasma obtained from untreated animals. Immediately prior to each analysis, the evaporated chloroform extracts were derivatized with an ethereal solution of diazomethane (2 M, 1.0 mL) at room temperature. The resulting mixtures were evaporated to dryness under nitrogen. Analysis of each sample was performed in methanol (50 μL). A sample (1 μL) of each methanol solution was examined by GC/MS with a Hewlett-Packard Model 5890 GC, attached to a VG 70-70E mass spectrometer, operating in EI mode. Separation was performed on a 50 × 0.3 mm i.d. fused silica column coated with 5% methylphenyl silicone stationary phase. The derivatized samples (1–2 μL) were injected directly onto the column, in the splitless injection mode. Helium was used as carrier gas at 2 mL min^{-1}, using the following temperature settings: injector 250 °C, interface 250 °C, ion source 200 °C, oven 45 °C (1 min), 20 °C min^{-1}, 270 °C (isothermal). Under these conditions, the absolute retention time of the methyl ester of 8 was 9.3 min, while that for the derivatized standard (methyl 4-propoxyphenylacetate) was 10.2 min. Both methyl esters were positively identified by mass spectral interpretation. The standard extracts provided a calibration graph of percentage peak area ratio versus plasma concentration of 8. Analysis of the samples obtained from rats treated with **4g** showed the absence of any significant amounts of 8 in the plasma (<0.1 μg mL^{-1}). When the experiments were repeated with rats dosed with compound 8 itself at therapeutic concentrations (0.05–0.4% w/w in the diet; two rats per group, four groups), the plasma levels of 8 obtained by the same method varied between 7.4 and 50.6 μg mL^{-1}, respectively. Single determinations were performed on plasma aliquots (1.0 mL) obtained from each animal.

Assay in Biological Fluids of 6-Bromo-2-[4-(1,1-Dimethylethyl)phenyl]-4*H*-3,1-benzoxazin-4-one (4g) following Administration of 4g. Rats were dosed with **4g**, and blood was obtained as described above. Single plasma sample aliquots (1.0 mL) from each animal were placed in conical test tubes (10 mL), spiked with a constant amount of an internal standard solution (1.0 μg of **5a** in 10 μL of methanol), and methanol (1.0 mL) was added. The samples were heated (60 °C, 1 h). Hexane (5 mL) was added, and the samples were shaken (10 min). After separation by centrifugation, the organic phase was evaporated under nitrogen. Standard samples were prepared by the same technique, but with the addition of **4g** (giving plasma concentrations of 0.5–10.0 μg mL^{-1} of plasma) as well as the internal standard to plasma obtained from untreated animals. The evaporation residues were reconstituted in methanol and analyzed by HPLC with a Spherisorb 50DS2 (C18) 25 cm × 4.6 mm column, equipped with an LDC SpectroMonitor III set at 290 nm. Separation was performed with methanol/water (84:16) at 1.35 mL min^{-1} at ambient temperature. The absolute retention time for the methanol degradation product of **4g** was 18 min. The absolute retention time for the standard **5a** was 4.5 min. The extraction efficiency for the methanol degradation product of **4g** was 24%. A calibration graph of peak height ratio of the methanol degradation product of **4g** to the peak height of **5a** versus the plasma concentration of **4g** was calculated. The regression coefficient, over the concentration range 0.5–10.0 μg mL^{-1} at six concentrations and constrained to pass through the origin, was 0.9978.

Concentrations of drug product in the plasma samples were found to be below the limit of detection for this assay method; i.e. less than 0.5 μg/mL^{-1} plasma.

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Registry No. **1a**, 2941-78-8; **1a**, 5794-88-7; **1h**, 635-21-2; **1i**, 5326-47-6; **1j**, 83265-53-6; **1k**, 118-92-3; **1l**, 6705-03-9; **1m**, 2475-77-6; **1n**, 18331-73-2; **1p**, 616-79-5; **1v**, 63746-25-8; **2a**, 1710-98-1; **2b**, 21900-36-7; **2c**, 16372-51-3; **2d**, 21900-62-9; **2e**, 96224-27-0; **2f**, 98-88-4; **2g**, 1710-98-1; **2w**, 52629-45-5; **4a**, 117145-46-7; **4b**, 117145-47-8; **4c**, 117145-48-9; **4d**, 117145-49-0; **4e**, 117145-50-3; **4f**, 117039-85-7; **4g**, 117145-51-4; **4h**, 117145-52-5; **4i**, 117145-53-6; **4j**, 117145-54-7; **4k**, 117145-55-8; **4l**, 117145-56-9; **4m**, 117145-57-0; **4n**, 117145-58-1; **4o**, 117145-59-2; **4p**, 117145-60-5; **4q**, 117145-61-6; **4r**, 117145-62-7; **4s**, 117145-63-8; **4t**, 117145-64-9; **4u**, 117145-65-0; **4v**, 117145-66-1; **4w**, 117145-80-9; **5a**, 117145-75-2; **5b**, 117145-76-3; **6**, 117145-77-4; **7** (R^1 = Br, R^2 = OMe, R^3 = Me), 117145-78-5; **7a**, 117145-67-2; **7b**, 117145-68-3; **7c**, 117145-69-4; **7d**, 117145-70-7; **7e**, 117145-71-8; **7f**, 117145-72-9; **7g**, 117145-73-0; **8**, 98-73-7; **8** (methyl ester), 26537-19-9; **10**, 117145-79-6; 1-octyne, 629-05-0; 2-[4-(1,1-dimethylethyl)phenyl]-6-(1-octynyl)-4*H*-3,1-benzoxazin-4-one, 117145-74-1; benzenesulfonyl chloride, 98-09-9; diphenylcadmium, 2674-04-6.