Comparative Antilipidemic Effects of Various Ethyl 5-Substituted Benzofuran-, 2,3-Dihydrobenzofuran-, and 3(2H)-Benzofuranone-2-Carboxylate Analogs of Clofibrate in a Triton Hyperlipidemic Rat Model

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ABSTRACT

The antilipidemic properties of certain benzofuran-, 2,3-dihydrobenzofuran-, and 3(2H)-benzofuranone-2-carboxylate analogs of clofibrate in a hyperlipidemic rat model are described. The hyperlipidemia was induced by intraperitoneal injection of Triton WR-1339. The results were analyzed in light of structural modifications as well as the lipid solubility of substituted compounds as assessed by a consideration of calculated log P values. Comparisons are made between the activity of these compounds and the activity of related cyclic analogs previously reported. Among the various compounds tested, only the 5-Cl and phenyl-substituted dihydrobenzofurans were selective against elevated serum cholesterol levels in this animal model. The data presented support the hypothesis that the cholesterol and triglyceride lowering activity of clofibrate related analogs in this animal model may be separated through a consideration of log P, conformational, and electronic changes. The proposal is advanced that relatively minor structural modification of clofibrate related analogs may lead to compounds which are not only selective in the Triton model but also to compounds which are likely to exert their effects by differing modes of action.

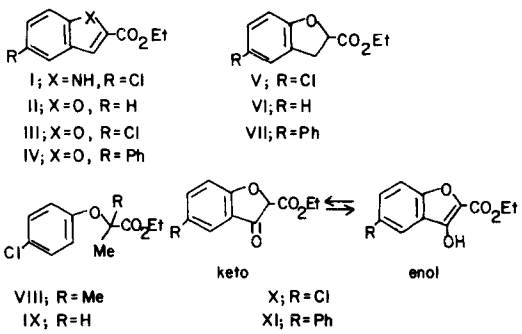

FIG. 1. Structures for cyclic analogs. I = ethyl 5-chloroindole-2-carboxylate; II = ethyl benzofuran-2-carboxylate; III = ethyl 5-chlorobenzofuran -2-carboxylate; IV = ethyl 5-phenylbenzofuran-2-carboxylate; V = ethyl 5-chloro-2,3-dihydrobenzofuran-2-carboxylate; VI = ethyl 2,3-dihydrobenzofuran-2-carboxylate; VII = ethyl 5-phenyl-2,3-dihydrobenzofuran-2-carboxylate; VIII = clofibrate; IX = ethyl α-(4-chlorophenoxy)propionate; X = ethyl 5-chloro-3(2H)-benzofuranone-2-carboxylate; XI = ethyl 5-phenyl-3(2H)-benzofuranone-2-carboxylate.

INTRODUCTION

Kariya and coworkers (1) have shown that ethyl 5-chloroindole-2-carboxylate (I) lowered plasma cholesterol levels without affecting plasma triglyceride levels in immature and mature Sprague-Dawley rats and in Wistar rats when it was mixed with their diets. We had previously observed that ethyl 5-chloro-2,3-dihydrobenzofuran carboxylate V selectively lowered elevated serum cholesterol levels without affecting serum triglyceride concentrations in Triton WR-1339 induced hyperlipidemic Sprague-Dawley rats, whereas the deschloro analog VI was inactive (2). Cyclic analog V is a conformationally constrained molecule related to ethyl α-(4-chlorophenoxy)propionate (IX), a desmethyl analog of clofibrate (VIII). The L(*S*) isomer of acyclic analog IX was observed to exhibit hypocholesterolemic activity in normal Swiss Webster rats whereas the D(*R*) enantiomorph was inactive (3).

To obtain leads for future drug design of selective hypolipidemic agents, we investigated the antilipidemic properties of certain 5-substituted-benzofurans (II-IV). The results are compared to those obtained for the 5-substituted-2,3-dihydrobenzofurans (V-VII) and the 3-keto analogs (X-XI) and are analyzed in light of the calculated log P values (4,5) for the corresponding carboxylic acid hydrolysis products (Fig. 1).

METHODS AND MATERIALS

Chemical Methods

Melting points were taken using a Thomas-

Hoover melting point apparatus. Infrared (IR) spectra were recorded using a Perkin-Elmer 257 grating spectrophotometer. The nuclear magnetic resonance (NMR) spectra were taken with a Varian-A-60A NMR spectrometer at 60 MHz using trimethylsilane as an internal reference. Chemical analyses were performed by Clark Microanalytic Laboratories, Urbana, IL.

Condensation of precursor 5-substituted-salicylaldehydes with diethylbromomalonate in the presence of anhydrous K_2CO_3 according to the methods of Kurkudar and Rao (6) afforded the respective ethyl 5-substituted-benzofuran-2-carboxylates (II-IV) with ethyl benzofuran-2-carboxylate (II) bp 99-100 C (0.005 mm); lit (7) bp 275 C (720 mm); yield = 65%. Ethyl 5-chlorobenzofuran-2-carboxylate (III) exhibited mp 64.5-65.5 C; lit (6) mp 65 C; yield = 70%.

Ethyl 5-phenylbenzofuran-2-carboxylate (IV) was prepared from 5-phenylsalicylaldehyde (0.99 g, 0.005 mol), diethyl bromomalonate (0.96 g, 0.004 mol), anhydrous K_2CO_3 (1.25 g, 0.009 mol), and 2-butanone (20 ml). The mixture was refluxed for 10 hr, and the solvent was removed under reduced pressure. The residue was cooled, poured into H_2O (100 ml), and extracted with ether. The ether layer was washed with cold 5% NaOH solution followed by H_2O and dried over anhydrous Na_2SO_4. The dried ether solution was filtered and concentrated under reduced pressure. The residue was recrystallized from ethanol affording 0.95 g (73%) of white crystals (mp 109-110 C; IR [$CHCl_3$] 1730 cm^{-1}; NMR [$CDCl_3$] δ 1.35 [t, 3H, CH_3], 4.38 [q, 4H, CH_2], 7.20-7.85 [m, 3H, aromatic + 1 vinylic H]; analysis calculated for $C_{17}H_{14}O_3$: C, 76.69; H, 5.26; found: C, 76.56; H, 5.44).

Hydrolysis of esters II and III, which served as precursors for the respective 2,3-dihydro compounds, was carried out according to Kurkudar and Rao (6), affording the respective free carboxylic acids. The constants for 5-chlorobenzofuran-2-carboxylic acid are found in Ref. 3. Benzofuran-2-carboxylic acid exhibited mp 192-193 C; lit (8) mp 192-193 C; yield = 60%.

5-Phenylbenzofuran-2-carboxylic acid was prepared from IV (1.3 g, 0.0084 mol) by refluxing for 4 hr in 50 ml of 10% alcoholic KOH. The solvent was removed under reduced pressure, and the residue was washed with ether. The basic solution was acidified with dilute HCl and extracted with ether. The ether layer was extracted with dilute $NaHCO_3$ solution. The aqueous solution was reacidified with dilute HCl and extracted with ether. The ether extract was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was crystallized from ethanol affording 1.1 g (95%) of a white solid (mp 220-221 C; IR [KBr] 1690 cm^{-1}; NMR [DMSO-d_6], δ 7.20-8.15 [m, 8H, aromatic + 1 vinylic H], 12.35 [broad, 1H, carboxylic acid H]; analysis calculated for $C_{15}H_{10}O_3$: C, 75,62; H, 4.23; found: C, 75.73; H, 4.44).

The 2,3-dihydrobenzofuran-2-carboxylic acids were obtained by reduction of the respective carboxylic acids derived from the hydrolysis of benzofurans II, III, and IV, through use of Na(Hg) according to the method described by Fredga (9). The physical properties for 5-chloro-2,3-dihydrobenzofuran-2-carboxylic acid are found in Ref. 3. 2,3-Dihydrobenzofuran-2-carboxylic acid exhibited mp 116.5-117 C; lit (10) mp 116.5 C.

5-Phenyl-2,3-dihydrobenzofuran-2-carboxylic acid was prepared from 5-phenylbenzofuran-2-carboxylic acid (5.0 g, 0.021 mol) in 10% aqueous NaOH solution (90 ml). The sodium salt separated. Sodium amalgam, prepared from Na (1.5 g, 0.065 g at) and Hg (50 g, 0.25 g at), was added with stirring during 1 hr to the NaOH mixture. After the addition, the mixture was stirred for 24 hr and allowed to stand at room temperature for an additional 24 hr. The Hg was separated, and the solution was neutralized with dilute HCl and extracted with ether. The ether solution was washed with dilute $NaHCO_3$ solution, and the aqueous layer was separated and reacidified with dilute HCl and extracted with ether. The ether solution was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residual solid was recrystallized from ethanol, affording 4.8 g (95%) of white crystals (mp 186-187 C; NMR [DMSO-d_6], δ 2.90-3.80 [m, 2H, CH_2], 4.90-5.40 [dd, 1H, methine], 6.0-6.5 [broad, 1H, carboxylic acid H], 6.72-7.90 [m, 8H, aromatic]; analysis calculated for $C_{15}H_{12}O_3$: C, 74.92; H, 5.04; found: C, 74.89; H, 5.30).

Fisher esterification of the above carboxylic acids resulted in the synthesis of the respective desired esters V, VI, and VII. Hence, ethyl 2,3-dihydrobenzofuran-2-carboxylate (VI) exhibiting bp 78-78.5 C (0.002 mm) (lit [11] bp 273 C [atmospheric pressure]) was prepared in 92% yield.

Ethyl 5-chloro-2,3-dihydrobenzofuran-2-carboxylate (V) was prepared from the corresponding carboxylic acid (9.9 g, 0.05 mol) in absolute ethanol (250 ml), toluene (100 ml), and concentrated H_2SO_4 (4 ml). The mixture was heated at reflux for 7 hr; H_2O was removed through use of a Dean-Stark trap. The reaction mixture was concentrated, and the residue was

dissolved in ether. The ether solution was washed with saturated $NaHCO_3$ solution and H_2O. The aqueous portion was extracted with ether, and the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Distillation of the residual high boiling liquid afforded 10.2 g (90%) of an oil (V) (bp 90-91 C [0.05 mm]; analysis calculated for $C_{11}H_{11}ClO_3$: C, 58.40; H, 4.91; Cl, 15.63; found: C, 58.31; H, 4.72; Cl, 15.54).

By a similar procedure, ethyl 5-phenyl-2,3-dihydrobenzofuran-2-carboxylate (VII) was prepared from the corresponding acid (2.5 g, 0.01 mol) by refluxing in a mixture of ethanol (60 ml), dry benzene (20 ml), and concentrated H_2SO_4 (2 ml), affording 2.65 g (95%) of colorless liquid (VII) (bp 180-181 C [0.35 mm]; NMR [$CDCl_3$], δ 1.16 [t, 3H, CH_3], 3.18-3.48 [m, 2H, benzylic CH_2], 4.12 [q, 2H, ester CH_2], 5.05 [dd, 1H, methine], 6.70-7.55 [m, 8H, aromatic]; analysis calculated for $C_{17}H_{16}O_3$: C, 76.10; H, 6.01; found: C, 76.46; H, 6.09).

Starting salicylaldehyde and 5-chlorosalicylaldehyde were purchased commercially. 5-Phenylsalicylaldehyde was prepared under Reimer-Tiemann Conditions (12,13) according to the following method. p-Phenylphenol (22 g, 0.13 mol) was dissolved in 95% ethanol; a solution of NaOH (40 g, 1 mol) in H_2O (80 ml) was rapidly added. The solution was heated to 75-80 C, at which point chloroform (20 ml) was added dropwise. The addition was at such a rate that gentle refluxing was maintained (added during 1 hr). After formation of a dark-red color (generally 5-15 min), further heating was unnecessary. Stirring was continued for 3 hr after all the chloroform was added. After cooling, the ehtanol and excess chloroform were removed under reduced pressure, and the resulting residue was cooled, poured into cold H_2O, acidified with dropwise addition of HCl, and extracted with ether. The ether solution was concentrated under reduced pressure, and the residue was poured into 2 vol of saturated $NaHSO_3$ solution and shaken vigorously (on a shaker) for 45 min. The semisolid (paste) bisulfite addition compound was allowed to stand for 1 hr, filtered in the dark, and washed with small portions of ethanol and ether (to remove the phenol). The bisulfite addition compound was decomposed with dilute H_2SO_4 by warming on a water-bath for 30 min. The cooled mixture was extracted with ether, dried (Na_2SO_4), filtered, and the solvent removed under reduced pressure. The residue was treated with activated charcoal and recrystallized from ethanol:H_2O, affording 12.4 g (48%) of yellow crystals (mp 98-99 C; lit [14] mp 102 C; analysis calculated for $C_{13}H_{10}O_2$: C, 78.77; H, 5.09; found: C, 78.49; H, 5.02).

The 3-keto analogs (X,XI) were prepared as follows: Ethyl 5-chloro-3(2H)-benzofuranone-2-carboxylate (X) was prepared from ethyl 4-chloro-2-carbethoxyphenoxyacetate (8.58 g, 0.03 mol), which in turn was prepared according to the method of Armarego (15). The phenoxyacetate was dissolved in 40 ml of dry benzene (16), and the solution was added dropwise with stirring to sodium ethoxide (2.1 g, 0.03 mol) in dry benzene (40 ml). Stirring was continued under reflux for 16 hr. After cooling to room temperature, the reaction mixture was poured, with stirring, into H_2O (200 ml) and made alkaline to litmus with dilute NaOH solution. The aqueous layer was made acidic with dilute HCl and extracted with ether. The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent removed under reduced pressure. The resulting solid was recrystallized from ethanol, affording 6.4 g (88%) of white crystals (X) (mp 128-129 C; lit [17] mp 126-127 C; NMR [$CDCl_3$] δ 1.28-1.58 [t, 3H, CH_3], 4.25-4.68 [q, 2H, CH_2], 7.10-7.74 [m, 3H, aromatic and 1 H methine]).

Similarly, ethyl 5-phenyl-3(2H)-benzofuranone-2-carboxylate (XI) was prepared from 3.28 g (0.01 mol) of ethyl 4-phenyl-2-carbethoxyphenoxyacetate according to the method of Schroeder et al. (17) by dissolving the phenoxyacetate in dry benzene (50 ml) followed by dropwise addition of the solution to sodium ethoxide (0.23 g, 0.01 g at of Na in 15 ml of dry ethanol). After refluxing for 10 hr, the benzofuranone XI was isolated according to methods described for the isolation of X. Recrystallization of XI from ethanol afforded 1.8 g (68%) of crystals (mp 129.5-130.5 C; NMR [$CDCl_3$], δ 1.42 [t, 3H, CH_3], 4.48 [q, 2H, CH_2], 7.05-8.10 [m, 8H, aromatic and 1H methine]; analysis calculated for $C_{17}H_{14}O_4$: C, 72.34; H, 4.96; found: C, 72.22; H, 5.15).

Intermediate ethyl 4-phenyl-2-carbethoxyphenoxyacetate was prepared according to the general method of Armarego (15) in which ethyl 5-phenylsalicylate (6.05 g, 0.03 mol), ethyl bromoacetate (4.6 g, 0.03 mol), and anhydrous K_2CO_3 (13.8 g, 0.1 mol) were refluxed in 30 ml of dry acetone (16) for 20 hr. After cooling, the inorganic salt was separated by filtration, and the organic solvent was removed under reduced pressure. The residual oil was extracted (ether) and the ether layer was washed with cold dilute NaOH solution and H_2O. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed

under reduced pressure. The resulting oil solidified on cooling and was recrystallized from ethanol, affording 5.6 g (68%) crystalline phenoxyacetate (mp 53-54 C; NMR [$CDCl_3$], δ 1.08-1.50 [m, 6H, $2CH_3$], 4.02-4.58 [m, 4H, 2 ester CH_2], 4.70 [s, 2H, $O\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}$], 6.80-8.10 [m, 8H, aromatic]; analysis calculated for $C_{19}H_{20}O_5$: C, 69.51; H, 6.09; found: C, 69.69; H, 6.21).

Biological Methods

Compounds were tested in the Triton WR-1339-induced hyperlipidemic rat model (18) utilizing procedures virtually identical to those previously reported (2) for the evaluation of related analogs in our laboratories except that ten rather than six animals were employed in each group (Tables I and II). Compounds were dispersed in vehicle (0.25% aqueous methyl cellulose) to obtain a concentration of 8.33×10^{-3} mmol/ml. A total screening dose of 0.124 mmol/kg was administered to 270 ± 10 g rats. Plasma triglyceride was determined by the method of Eggstein (19); plasma cholesterol was measured by the method of Holub and Galli (20). Significant differences in plasma cholesterol and triglyceride concentrations between drug-treated and control groups were determined by Student's *t* tests on logarithms of individual data to allow the pooling of variances.

RESULTS

The effects of benzofurans II, III, and IV, 2,3-dihydrobenzofuran VII, and the 3-keto analogs X and XI on serum cholesterol (Table I) and triglyceride (Table II) concentrations were tested in normal and Triton WR-1339-induced (18) hyperlipidemic male Sprague-Dawley rats initially fed Purina Chow for a 4-5 day stabilizing period. As previously described (2), all rats, including controls, were fasted during analog evaluations. All compounds were evaluated at the same dosage (see Methods) which is equivalent to the dosage (0.124 mmol/kg) at which we previously evaluated clofibrate (2). To determine the effectiveness of these compounds in lowering cholesterol and triglyceride levels in hyperlipidemic animals, groups III (Triton hyperlipidemic) and IV (drug-treated Triton hyperlipidemic) were compared. Further, to determine whether the analog could lower hyperlipidemic serum levels of cholesterol and triglycerides to those found in normal animals, groups I (control) and IV were compared. Groups I and II (drug-treated control) were contrasted for significant differences to

TABLE I

Effect of Cyclic Clofibrate-Related Analogs on Plasma Cholesterol Levels (mg%) in Male Sprague-Dawley Rats

Compound	Control group (I)[a]	Drug-treated control (II)[a]	Triton hyperlipidemic (III)[a]	Drug-treated Triton hyperlipidemic (IV)[a]
Ethyl benzofuran-2-carboxylate (II)	88.1 ± 17.2	90.1 ± 15.8	310 ± 53.0	235 ± 79.2[b,c]
Ethyl 5-chlorobenzofuran-2-carboxylate (III)	90.5 ± 9.99	76.2 ± 16.9[d]	178 ± 36.1	108 ± 13.6[c,e]
Ethyl 5-phenylbenzofuran-2-carboxylate (IV)	77.0 ± 7.8	72.8 ± 7.7	178 ± 56.4	132 ± 60.2[c]
Ethyl 5-phenyl-2,3-dihydrobenzofuran-2-carboxylate (VII)	87.8 ± 11.4	80.4 ± 12.4	165 ± 36.8	94.3 ± 9.8[e]
Ethyl 5-chloro-3(2H)-benzofuranone-2-carboxylate (X)	87.4 ± 9.0	79.4 ± 12.3	165 ± 18.0	106 ± 13.9[c,e]
Ethyl 5-phenyl-3(2H)-benzofuranone-2-carboxylate (XI)	71.9 ± 13.5	79.0 ± 14.6	196 ± 57.3	149 ± 53.5[c]

[a]Mean ± SD; ten rats.
[b]Statistically significant $P<0.05$; comparison of groups III and IV.
[c]Statistically significant $P<0.01$; comparison of groups I and IV.
[d]Statistically significant $P<0.05$; comparison of groups I and II.
[e]Statistically significant $P<0.01$; comparison of groups III and IV.

TABLE II

Effect of Cyclic Clofibrate-Related Analogs on Plasma Triglyceride Levels (mg%) in Male Sprague-Dawley Rats

Compound	Control group (I)[a]	Drug-treated control (II)[a]	Triton hyperlipidemic (III)[a]	Drug-treated Triton hyperlipidemic (IV)[a]
Ethyl benzofuran-2-carboxylate (II)	30.9 ± 9.6	51.4 ± 14.1[b]	232 ± 83.9	140 ± 76.1[c,d]
Ethyl 5-chlorobenzofuran-2-carboxylate (III)	18.8 ± 4.7	32.1 ± 7.4[b]	115 ± 38.2	35.4 ± 12.5[c,d]
Ethyl 5-phenylbenzofuran-2-carboxylate (IV)	18.1 ± 2.6	23.7 ± 9.3[e]	132 ± 99.2	51.2 ± 46.6[c,d]
Ethyl 5-phenyl-2,3-dihydrobenzofuran-2-carboxylate (VII)	18.7 ± 6.4	18.1 ± 6.3	108 ± 28.3	90.1 ± 25.0[d]
Ethyl 5-chloro-3(2H)-benzofuranone-2-carboxylate (X)	26.3 ± 6.1	29.0 ± 5.9	77.4 ± 15.0	28.7 ± 7.9[c]
Ethyl 5-phenyl-3(2H)-benzofuranone-2-carboxylate (XI)	27.1 ± 8.2[f]	40.2 ± 13.5[e]	185 ± 100	78.7 ± 70.8[c]

[a]Mean ± SD; ten rats.
[b]Statistically significant $P<0.01$; comparison of groups I and II.
[c]Statistically significant $P<0.01$; comparison of groups III and IV.
[d]Statistically significant $P<0.01$; comparison of groups I and IV.
[e]Statistically significant $P<0.05$; comparison of groups I and II.
[f]Mean ± SD; nine rats.

determine whether these compounds exhibited hypolipidemic activity in normal (fasted) Sprague-Dawley rats under the conditions of this protocol (2).

Whereas the dihydrobenzofuran analog VI was previously observed to be inactive in hyperlipidemic and normal rats (2), benzofuran II, unsubstituted at the 5 position, exhibited marginal cholesterol and triglyceride lowering properties in hyperlipidemic animals. Insertion of a 5-Cl substituent afforded III, which is active, but could not return elevated cholesterol or triglyceride levels to normal. Further, the apparent selective activity (cholesterol lowering) of the structurally related dihydrobenzofuran V (2) was lost upon introduction of unsaturation into the heterocyclic ring (compound III). Introduction of the 5-phenyl substituent into the benzofuran series (compound IV) did not markedly affect the activity of the benzofuran as a triglyceride lowering agent and rendered the molecule virtually inactive against elevated cholesterol levels. The selective activity observed for the 5-phenyl-2,3-dihydrogenzofuran derivative VII is of particular interest because of its selective cholesterol lowering activity. This analog returned elevated serum cholesterol levels to those found in normal animals but did not affect elevated serum triglyceride levels.

When, however, the carbonyl function was inserted into position 3 of either V or VII, the respective benzofuranones X and XI exhibited no selective activity for lowering elevated serum cholesterol levels. In fact, the 3-keto analogs X and XI exhibited greater ability to lower elevated serum triglyceride levels.

DISCUSSION

Since both the 5-phenyl- and 5-chloro-2,3-dihydrobenzofurans exhibited selective activity against experimentally induced hypercholesterolemic levels, it seems to us that this heterocyclic ring system should be further investigated in an effort to develop compounds efficacious in the treatment of Type II hyperlipoproteinemia (21-26). Although additional evidence of selective activity obtained through use of other animal models (27) as well as through investigation of their action on cholesterolgenesis enzymes (28) is desirable, these preliminary data are of interest since clofibrate is mainly effective in the treatment of patients having hyperlipoproteinemia Types III, IV, and V (21-26). Further, it seems, at least in the Triton model, that the dual action of clofibrate (2) is subject to conformational control since the two constrained 5-substituted dihydroben-

TABLE III

Calculated log P (Octanol-Water Partition Coefficients) Values for the Free Acid Hydrolysis Products of the Tested Parent Esters

Free acid of the parent ester indicated	Values of substituents	Calculated log P = 1.51 + π values (experimentally determined log P values)[a]	
V	5-Cl = 0.6[b]	2.11	(2.11)[a]
VI	---	1.51	---
VIII	---		(2.57)[a]
II	Insertion of C=C = -0.48[c]	1.03	---
III	Insertion of C=C = -0.48[c] 5-Cl = 0.6[b]	1.63	---
IV	Insertion of C=C = -0.48[c] 5-C_6H_5 = 1.96[d]	2.99	---
VII	5-C_6H_5 = 1.96[d]	3.47	---
X	3-Keto oxygen = -1.21[e] 5-Cl = 0.6[b]	0.90	---
XI	3-Keto oxygen = -1.21[e] 5-C_6H_5 = 1.96[d]	2.26	---

[a]Taken from Ref. 33; experimentally determined at pH two units below the pKa.

[b]π value taken from Lewis, N.J., D.R. Feller, G.K. Poochikian, and D.T. Witiak, J. Med. Chem. 17:41 (1974).

[c]Estimated from the π substituent constant for substitution on benzene; CH_2-CH=CH_2 (1.10), CH_3 (0.56), CH_3CH_2(1.02); taken from Ref. 5; i.e., 1.10-0.56 = 0.54 for CH=CH_2; since CH_2CH_3 = 1.02, the CH_2CH_2 to CH=CH transformation = -0.48. However, since CH=CH has been experimentally determined to be 0.82 (Ref 5), this value for the CH_2CH_2 to CH=CH transformation likely is between -0.48 and -0.20.

[d]π value taken from Ref. 5.

[e]Estimated from the π substitutent constant for substitution on benzene; CH_3 (0.56), Ref. 4 or 5; CHO (-0.65), Ref. 5; i.e., -0.65 -0.56 = -1.21.

zofurans are mainly effective against elevated cholesterol levels.

Since phenyl and phenoxy analogs of clofibrate are generally good to very potent hypolipidemic agents (29-31) and insertion of phenyl groups increases lipid solubility, it seemed to us to be of interest to consider the biological results obtained with the phenyl, Cl, and H analogs in terms of the calculated log P values (Table III) for the corresponding free carboxylic acids, hydrolysis products. The log P values for the carboxylic acids, rather than the esters, were determined and used for correlation purposes since Thorp (32) had proposed that the free acid is the active form of clofibrate, and we have shown that several clofibrate analogs undergo rapid hydrolysis by serum esterase preparations (33). Calculated values were estimated by adding the substituent constants (π) to the experimentally determined (33) log P values for the unsubstituted free acid hydrolysis product of ethyl 2,3-dihydrobenzofuran-2-carboxylate (VI).

When attempts are made to correlate log P values with biological activity, it is important to recognize that changes in structure may, in fact, result in biological mechanism changes. Such changes in mechanism of action may or may not be a function of log P; steric and electronic properties of the functional group as well as the molecule as a whole may markedly affect binding to various enzyme systems. For example, we have previously observed that certain 6-substituted chromans have relatively good activity in the Triton-induced hyperlipidemic rat model (34). The calculated log P values for the substituted chromans are $\geqslant$ log P for clofibrate, which has also been shown to be very active in this assay (2,18). In the chroman series, analogs with large log P values (i.e., log P for the 6-cyclohexyl compound = 4.41) have selective activity against elevated triglyceride levels (34). However, ethyl 6-chlorochromone has a calculated log P = 0.81, a value much lower than the one observed for clofibrate, and, in the Triton model, this chromone was found to be more active than clofibrate (34) against both elevated cholesterol and triglyceride levels. It was suggested that this apparent lack of correlation of log P with antilipidemic activity is a reflection of changes in mechanism of action which, in fact, may be a function of the marked differences in the electronic character of chromones and chromans (34). Indeed, unpublished results recently obtained in our laboratories show that the 6-chloro-chromone is inactive in normal Sprague-Dawley rats, whereas clofibrate and the 6-phenyl- and 6-cyclohexyl-chromans lower cho-

lesterol levels and block HMG-CoA reductase upon chronic drug administration.

It would appear that relatively minor structural modification in the dihydrobenzofuran series also affords compounds exhibiting antilipidemic activity by differing modes of action, and further work will be necessary to substantiate this hypothesis. Nonetheless, this proposal seems likely for the following reasons: (a) The dihydrobenzofurans (V and VII) exhibit selective activity for reducing elevated cholesterol levels in the Triton-induced hyperlipidemic rat model. Insertion of Ph (VII) affords an analog with a higher log P value and increased selective activity when compared to the Cl analog V. Replacement of Cl by H (i.e., VI) lowers the log P value below the one observed for clofibrate and renders the compound inactive (2). (b) On the other hand, insertion of a C=C into the heterocyclic ring of the dihydrobenzofurans yields a second series of compounds (II, III, and IV) with loss of selective activity for elevated cholesterol levels. In fact, the 5-phenyl-benzofuran IV exhibits selective activity for elevated serum triglyceride levels and, for this activity, it appears that both the Cl and phenyl analogs (III and VII) which have the larger log P values exhibit greater potency than the unsubstituted compound II (Table II). (c) Insertion of a carbonyl function at position 3 also affords compounds (X and XI) which no longer exhibit selective activity against cholesterol. In this case, the chloro analog (X), having the lowest log P value, seems to be most effective against elevated triglyceride levels. Since 3-keto analogs exist in equilibrium with their enol forms (35), these compounds are expected to have markedly different electronic properties than the corresponding dihydrobenzofurans. Enol formation is in part stabilized owing to the generation of a π-excessive heteroaromatic ring system not unlike the hetero ring found in the benzofurans. The Cl and phenyl substituted benzofurans (III and IV) exhibit antilipidemic activity rather more similar to those observed for the respective 3-keto analogs (X and XI) than for those observed for the corresponding dihydrobenzofurans V and VII.

In conclusion, relative to the Triton model, the data presented in this paper support the hypothesis that the cholesterol and triglyceride lowering activity of clofibrate related analogs may be separated through a consideration of log P, conformational, and electronic parameters. Among the various analogs investigated in our laboratories (2,33,34), only the dihydrobenzofurans (V and VII) exhibited selective activity against elevated serum cholesterol levels. This selective activity is likely to be a reflection of conformational restriction of rotation rather than a change in log P since other derivatives having log P values either higher or lower than those calculated for V and VII lose selectivity.

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REFERENCES

1. Kariya, T., J.M. Grisar, N.L. Wiech, and T.R. Blohm, J. Med. Chem. 15:659 (1972).
2. Newman, H.A.I., W.P. Heilman, and D.T. Witiak, Lipids 8:378 (1973).
3. Witiak, D.T., T.C.-L. Ho, R.E. Hackney, and W.E. Connor, J. Med. Chem. 11:1086 (1968).
4. Fujita, T., J. Iwasa, and C. Hansch, J. Am. Chem. Soc. 86:5175 (1964).
5. Hansch, C., A. Leo, S.H. Unger, K.H. Kim, D. Nikaitani, and E.J. Lien, J. Med. Chem. 16:1207 (1973).
6. Kurkudar, R., and N.V.S.Rao, Indian Acad. Sci. Sec. A 58:336 (1963).
7. Stoermer, R., and C. Calov, Chem. Ber. 34:772 (1901).
8. Tanaka, S., J. Am. Chem. Soc. 73:872 (1951).
9. Fredga, A., Acta Chem. Scand. 9:719 (1955).
10. von Auwers, K., Justus Liebigs Ann. Chem. 393:338 (1912).
11. Stoermer, R., and W. Konig, Chem. Ber. 39:493 (1906).
12. Wynberg, H., Chem. Rev. 60:169 (1960).
13. Russell, A., and L.B. Lockhart, Org. Snyth. 22:63 (1942).
14. Duff, J.C., J. Chem. Soc. 547 (1941).
15. Armarego, W.L.F., Aust. J. Chem. 13:95 (1960).
16. Vogel, A.I., "A Text Book of Practical Organic Chemistry, Including Qualitative Organic Analysis," 3rd Edition, Longmans, Green and Co., London, England, 1956, p. 171.
17. Schroeder, D.C., P.O. Corcoran, C.A. Holden, and M.C. Mulligan, J. Org. Chem. 27:586 (1962).
18. Schurr, P.E., J.R. Schultz, and T.M. Parkinson, Lipids 7:68 (1972).
19. Eggstein, M., Klin. Wochenschr. 44:267 (1966).
20. Holub, W.R., and F.A. Galli, Clin. Chem. Winston-Salem, NC 18:239 (1972).
21. Lees, R.S., and D.E. Wilson, New Engl. J. Med. 284:186 (1971).
22. Fredrickson, D.S., R.I. Levy, and R.S. Lees, Ibid. 276:32 (1967).
23. Ibid. 276:94 (1967).
24. Ibid. 276:148 (1967).
25. Ibid. 276:215 (1967).
26. Ibid. 276:273 (1967).
27. Kritchevsky, D., in "Advances in Drug Research," Vol. 9, Edited by A.B. Simmonds, Academic Press, New York, NY, 1974, pp. 41-53.
28. Howe, R., Ibid., pp. 7-39.
29. Grisar, J.M., G.P. Claxton, R.A. Parker, F.P. Palopoli, and T. Kariya, J. Med. Chem. 17:721 (1974).
30. Thorp, J.M., in "Atherosclerosis, Proceedings of the Second International Symposium," Edited by R.J. Jones, Springer-Verlag, New York, NY, 1970, pp. 541-544.
31. Garattini, S., R. Paoletti, L. Bizzi, F. Grossi, and

R. Vertua, in "Drugs Affecting Lipid Metabolism," Proceedings of the Symposium on Drugs Affecting Lipid Metabolism, Milan, 1960, Edited by S. Garattini and R. Paoletti, Elsevier, Amsterdam, The Netherlands, 1961, p. 144.
32. Thorp, J.M., Lancet 1:1323 (1962).
33. Nazareth, R.I., T.D. Sokoloski, D.T. Witiak, and A.T. Hopper, J. Pharm. Sci. 63:203 (1974).
34. Witiak, D.T., W.P. Heilman, S.K. Sankarappa, R.C. Cavestri, and H.A.I. Newman, J. Med. Chem. 18:934 (1975).
35. Witiak, D.T., G.K. Poochikian, D.R. Feller, N.A. Kenfield, and H.A.I. Newman, Ibid. 18:992 (1975).

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