# Structure–Activity Studies on Antihyperlipidemic *N*-Benzoylsulfamates, *N*-Benzylsulfamates, and Benzylsulfonamides

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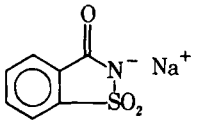
**Abstract** □ A series of aryl substituted *N*-benzoyl- and *N*-benzylsulfamic acid sodium salts and benzylsulfonamide sodium salts have been prepared and examined for antihyperlipidemic activity in male $CF_1$ mice at a dose level of 20 mg/kg/d ip for 16 d. These substances were also subjected to toxicological evaluation and chemical stability studies. In general, both series of sulfamates and sulfonamides significantly lowered serum cholesterol and triglyceride levels in mice. The compounds were nonmutagenic, showed no acute toxicity or impaired liver or kidney function in male mice, and were chemically stable both as the monohydrates and in aqueous solution over a pH range of 3.5–7.4. While both series of sulfamates and sulfonamides lowered serum cholesterol and triglyceride levels, the sulfamates were relatively more potent with regard to decreasing cholesterol levels, while the sulfonamides were more effective in lowering serum triglyceride levels in mice.

**Keyphrases** □ *N*-Benzoylsulfamates—potential antihyperlipidemic agents, structure–activity relationships, mutagenicity, toxicity in mice □ *N*-Benzylsulfamates—potential antihyperlipidemic agents, structure–activity relationships, mutagenicity, toxicity in mice □ Benzylsulfonamides—potential antihyperlipidemic agents, structure–activity relationships, mutagenicity, toxicity in mice

Cardiovascular disease is currently a major cause of death of males in Western civilized societies. Atherosclerosis is characterized by partial or complete occlusion of the vessels that supply blood to the myocardial tissues, due primarily to the formation of the fibrous lesion known as a sclerotic plaque within the vessel lumen (1). Elevated levels of cholesterol and triglycerides have been detected in plaque cells, and there appears to be a link between plaque formation and long-term elevated serum lipids (2, 3). Therefore, lowering of elevated serum lipid levels either by dietary restriction or chemotherapy appears desirable.

It has been demonstrated that a wide structural variety of chemical agents are active as antihyperlipidemics (1) including the synthetic sweetening agent, saccharin (4). Saccharin has demonstrated significant antihyperlipidemic activity in male mice, with the optimum dose level of 20 mg/kg/d ip affording serum cholesterol levels 67 ± 10% of control values after 16 d of administration and triglyceride levels 51 ± 16% of control values after 14 d. A series of *N*-benzoylsulfamic acid sodium salts as acyclic analogues of saccharin were prepared in an attempt to improve the antihyperlipidemic activity while eliminating the undesirable effects which are associated with saccharin (5, 6). Also prepared were two other structurally related classes of compounds, *N*-benzylsulfamates and benzylsulfonamides. Structural modifications among the series include aromatic substitutions with groups that vary in their lipophilicity, electronic character, and steric parameters. In addition, replacement of the carbonyl function (*N*-benzoylsulfamates) by a methylene group (*N*-benzylsulfamates) and alteration of the relative position of the nitrogen atom with regard to the aromatic and sulfonyl functions (benzylsulfonamides) were examined.



Saccharin (sodium salt)

$C_6H_5CH_2NHSO_3^-Na^+$

*N*-Benzylsulfamic Acid (sodium salt)

$C_6H_5CONHSO_3^-Na^+$

*N*-Benzoylsulfamic Acid (sodium salt)

$C_6H_5CH_2SO_2NH^-Na^+$

Benzylsulfonamide (sodium salt)

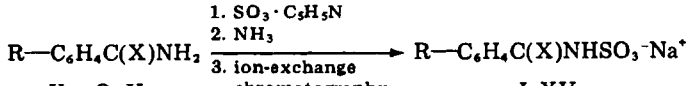
## BACKGROUND

Few reports exist concerning the synthesis of *N*-benzoylsulfamates. The unsubstituted prototype was prepared by Baumgarten and Marggraff (7) by heating benzamide and pyridine–sulfur trioxide complex (pyr·$SO_3$) to the melting point with subsequent conversion to the sodium salt with aqueous sodium hydroxide. In our hands, this procedure afforded extremely low yields of the prototype and no product for any aryl-substituted derivative. Dipyridinium imidodisulfonate (8) has also been employed for the sulfonation of benzamide but, again, yields are on the order of 5–10%. Two procedures have been developed in this laboratory which permit the preparation of the series of aryl-substituted *N*-benzoyl- and *N*-benzylsulfamic acid sodium salts in significantly greater yields. The appropriate benzamide or benzylamine was treated with pyr·$SO_3$ in pyridine solution for 3–5 h at 80–90°C. The product was isolated as the ammonium salt and converted to the sodium salt by ion-exchange chromatography (Scheme I). In addition, the *N*-benzoylsulfamates may also be prepared by benzoylation of pyridinium sulfamate with the acid chloride in pyridine.

The benzylsulfonamides were prepared from the corresponding sulfonyl chlorides, which were obtained by treatment of the benzylisothioureas with chlorine at 0–10°C (9) (Scheme II). This procedure is satisfactory for derivatives that are substituted with electron-withdrawing groups or hydrogen atoms on the aromatic ring, whereas strong electron-releasing substituents (*i.e.*, $OCH_3$) induce chlorination of the aromatic ring during conversion to the sulfonyl chloride as evidenced by $^1$H-NMR and MS data. This problem was circumvented by obtaining the nitrobenzylsulfonamide by the above procedure followed by introduction of the methoxy substituent *via* the diazonium tetrafluoroborate salt. The compounds prepared were examined for chemical stability at room temperature, both as the monohydrates and in aqueous solution, over a pH range of 3.5–7.4 using both reverse-phase HPLC and TLC to detect the formation of hydrolysis products (*i.e.*, benzamides and benzylsulfonic acids).

## RESULTS AND DISCUSSION

Chemical stability studies using reverse-phase HPLC revealed that all of the compounds except VI were stable at room temperature both




$R—C_6H_4C(X)NH_2$ → $R—C_6H_4C(X)NHSO_3^-Na^+$

X = O, $H_2$ I–XV

*Scheme I*

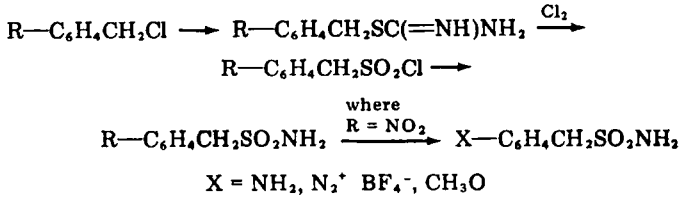


*0022-3549/84/0300-0374$01.00/0*

as the monohydrate and in aqueous solution over a pH range of 3.5–7.4 for a period of at least 60 d. Compound VI underwent 55–60% hydrolysis in solution after 30 d and was completely degraded after 60 d. This compound was also found to be completely hydrolyzed after 3 months when stored in solid form, and was therefore not evaluated biologically.

None of the compounds were found to be mutagenic in the Ames assay; however, all of the compounds exhibited toxicity to bacteria at the high-dose level of 3000 μg/plate. It is advisable to include one toxic dose level when testing unknown compounds for mutagenicity (10). The toxicity assay indicated essentially no cell death; therefore, the toxic effect at 3000 μg/plate was not due to cell death, but rather to the inhibition of revertent colony growth *via* another mechanism. Results of acute toxicity testing in male mice indicated that none of the compounds tested produced abnormal levels of alanine amino transferase (ALT) activity or urea nitrogen, nor were any signs of toxicity or abnormal behavior observed during the 14-d period. No abnormalities or irregular fluctuations in body weight were observed on termination of the study and gross necropsy. Likewise, no toxicity was observed during the course of the antihyperlipidemic study.

Of the two series, the *N*-benzoylsulfamates appear to be the more potent antihypercholesteremic agents with the *o*- and *p*-chloro derivatives (IV, II) being the most active (Table I). As indicated in Table II, electron-withdrawing substituents on the aromatic ring lend slightly greater activity to the series than do electron-releasing substituents. Activity decreases somewhat with increasing substituent size. In general, activity within the series does not appear to be heavily dependent on the size, electronic properties, or lipophilicity of the aromatic substituents as the unsubstituted prototype (VII) is also quite active. A significantly greater structural dependence within this series resides in the side-chain




*Scheme II*

requirement for the carbonyl function. By comparison of the *p*-Cl, *o*-Cl, *p*-$OCH_3$, and unsubstituted derivatives (II, IV, I, and VII, respectively) with their carbonyl-reduced counterparts among the *N*-benzylsulfamates (XV, XIV, XII, and XIII, respectively), it is evident that antihypercholesteremic activity is markedly reduced in the absence of the carbonyl function. This structural requirement for antihyperlipidemic activity has also been noted for saccharin as well as other imides (11).

Serum triglyceride levels are moderately reduced by the *N*-benzoylsulfamates, with the exception of the *p*-Cl (II) and *p*-isopropyl (XI) derivatives, which lower serum triglyceride levels to 55 ± 7% and 49 ± 8% of control values, respectively, by day 16. As in the case of antihypercholesteremic activity, the dependence on the presence of the side-chain carbonyl for antihypertriglyceridemic activity appears evident when comparing I, II, and VII with XII, XV, and XIII, respectively. However, *N*-benzylsulfamate (XIV) showed increased antihypertriglyceridemic activity compared with *N*-benzoylsulfamate (IV). Each of the *N*-benzoylsulfamates shows greater antihypercholesteremic activity than clofibrate, which is not active at 20 mg/kg, requiring dose levels of 150–200 mg/kg to reduce serum cholesterol ~15% in rodents. Saccharin at 20 mg/kg/d lowered serum cholesterol levels 35% and serum triglyceride

**Table I—*N*-Benzoyl- and *N*-Benzylsulfamic Acid Sodium Salts**

$R—C_6H_4C(X)NHSO_3^-Na^+$

| Compound | R | X | $^1$H-NMR (DMSO-$d_6$), (δ) ppm | Synthetic Procedure or Literature Reference | Yield, % | Formula |
|---|---|---|---|---|---|---|
| I | *p*-$OCH_3$ | O | 7.3 (q, 4, ArH)<br>10.1 (s, 1, —NH—)<br>3.7 (s, 3, —$OCH_3$) | 8 | 7 | $C_8H_8NO_5SNa·H_2O$ |
| II | *p*-Cl | O | 7.6 (q, 4, ArH)<br>10.4 (s, 1, —NH—) | 8 | 11 | $C_7H_5ClNO_4SNa·H_2O$ |
| III | *m*-Cl | O | 7.5 (m, 4, ArH)<br>10.3 (s, 1, —NH—) | 8 | 21 | $C_7H_5ClNO_4SNa·H_2O$ |
| IV | *o*-Cl | O | 7.3 (m, 4, ArH)<br>10.3 (s, 1, —NH—) | A | 50 | $C_7H_5ClNO_4SNa·H_2O$ |
| V | *m*-$OCH_3$ | O | 7.2 (m, 4, ArH)<br>10.2 (s, 1, —NH—)<br>3.7 (s, 3, —$OCH_3$) | 8 | 26 | $C_8H_8NO_5SNa·H_2O$ |
| VI | *o*-$OCH_3$ | O | 7.5 (m, 4, ArH)<br>9.7 (s, 1, —NH—)<br>3.7 (s, 3, —$OCH_3$) | B | 42 | $C_8H_8NO_5SNa·H_2O$ |
| VII | H | O | 7.6 (m, 5, ArH)<br>10.4 (s, 1, —NH—) | 8 | 4 | $C_7H_6NO_4SNa·H_2O$ |
| VIII | *p*-Br | O | 10.56 (s, 1, NH)<br>7.76 (q, 4, ArH) | A | 31 | $C_7H_5BrNO_4SNa·H_2O$ |
| IX | *p*-$CH_3$ | O | 10.35 (s, 1, NH)<br>7.4 (s, 4, ArH)<br>2.35 (s, 3, $CH_3$) | A | 39 | $C_8H_8NO_4SNa·H_2O$ |
| X | *p*-$CH_3C$ | O | 10.58 (s, 1, NH)<br>8.04 (s, 4, ArH)<br>2.68 (s, 3, $CH_3$) | A | 28 | $C_9H_8NO_5SNa·H_2O$ |
| XI | *p*-iso-$C_3H_7$ | O | 7.6 (q, 4, ArH)<br>10.54 (s, 1, NH)<br>3.0 [m, 1, $(CH_3)_2CH$]<br>1.23 [d, 6, $(CH_3)_2$] | A | 23 | $C_{10}H_{12}NO_4SNa·H_2O$ |
| XII | *p*-$OCH_3$ | $H_2$ | 6.95 (q, 4, ArH)<br>3.85 (d, 2, —$CH_2$)<br>3.70 (s, 3, —$OCH_3$)<br>4.45 (t, 1, —NH) | A | 36 | $C_8H_{10}NO_4SNa$ |
| XIII | H | $H_2$ | 7.3 (s, 5, ArH)<br>4.0 (d, 2, —$CH_2$)<br>4.7 (t, 1, —NH) | A | 23 | $C_7H_8NO_3SNa$ |
| XIV | *o*-Cl | $H_2$ | 7.3 (m, 4, ArH)<br>4.0 (d, 2, —$CH_2$)<br>4.8 (t, 1, —NH) | A | 18 | $C_7H_7ClNO_3SNa$ |
| XV | *p*-Cl | $H_2$ | 7.25 (s, 4, ArH)<br>3.8 (d, 2, —$CH_2$)<br>4.75 (t, 1, —NH) | A | 21 | $C_7H_7ClNO_3SNa$ |

**Table II—Benzylsulfonamide Sodium Salts**

| | $R—C_6H_4CH_2SO_2NH^-Na^+$ | | | |
|---|---|---|---|---|
| Compound | R | $^1$H-NMR (DMSO-$d_6$), ($\delta$) ppm | Yield, % | Formula |
| XVI | *o*-Cl | 7.25 (m, 4, ArH)<br>3.9 (s, 2, $—CH_2$)<br>3.0 (broad, 1, NH) | 83 | $C_7H_7ClNO_2SNa·H_2O$ |
| XVII | *o*-$OCH_3$ | 6.95 (m, 4, ArH)<br>3.8 (s, 2, $CH_2$)<br>3.65 (s, 3, $—OCH_3$) | 15<br>(Overall) | $C_8H_{10}NO_3SNa·2H_2O$ |
| XVIII | *m*-$OCH_3$ | 6.85 (m, 4, ArH)<br>3.7 (s, 2, $—CH_2$)<br>3.6 (s, 3, $—OCH_3$) | 8<br>(Overall) | $C_8H_{10}NO_3SNa·1½H_2O$ |
| XIX | *p*-$OCH_3$ | 6.9 (q, 4, ArH)<br>3.7 (s, 2, $—CH_2$)<br>3.7 (s, 3, $—OCH_3$) | 20<br>(Overall) | $C_8H_{10}NO_3SNa·½H_2O$ |
| XX | H | 7.2 (m, 5, ArH)<br>3.7 (s, 2, $CH_2$) | 37 | $C_7H_8NSO_2Na·H_2O$ |
| XXI | *m*-Cl | 7.2 (m, 4, ArH)<br>3.8 (s, 2, $CH_2$) | 66 | $C_7H_7ClNO_2SNa·H_2O$ |
| XXII | *p*-Cl | 7.1 (m, 4, ArH)<br>3.7 (s, 2, $CH_2$) | 22 | $C_7H_7ClNO_2SNa·H_2O$ |

levels 46% in mice. Compounds I, II, III, IV,and VII are more active than saccharin in lowering serum cholesterol levels, but less active in lowering triglyceride levels.

Within the benzylsulfonamide series (Table III), the *o*-$OCH_3$ derivative (XVII) was the most active antihypercholesteremic agent, being equal in activity to saccharin. In general, moderate antihypercholesteremic activity is displayed by this series, whereas antihypertriglyceridemic activity surpasses that of the *N*-benzoylsulfamates and clofibrate. The *p*-Cl derivative (XXII) is equally active with saccharin in this respect. Compounds XVIII and XX are more active than saccharin, lowering serum triglyceride levels to 42% of control values. Comparing the activities of XVI, XVIII, XX, and XXII, the most active antihypertriglyceridemic agents in the series, no dependence on substituent parameters is evident.

## EXPERIMENTAL

All chemicals were used as received from the manufacturer[1]. Melting points were obtained on a capillary melting point apparatus[2]. $^1$H-NMR spectra[3] were obtained for all novel products. Elemental analyses[4] of all products were within ±0.4% of the theoretical values. Ion-exchange chromatography was performed using sodium-form resin[5]. Reverse-phase HPLC was performed on a gradient programmable high-pressure liquid chromatograph[6] with a $C_{18}$ column[7] and radial compression module. Male $CF_1$ mice[8] were used for antihyperlipidemic testing.

**Aryl-Substituted Benzamides**—According to the procedure of Vogel (12), the appropriate benzoyl chloride was treated with cold concentrated $NH_4OH$. The product was filtered, dried, and recrystallized from ethanol–water.

***N*-Benzoyl- and *N*-Benzylsulfamic Acid Sodium Salts (I–XV)**—*General Procedure A*—The appropriate benzamide or benzylamine (5.0 g) and 1.1 equivalents of pyridine–sulfur trioxide complex were dissolved with warming in 50 mL of dry pyridine under nitrogen. The reaction was stirred at 80–90°C for 3–5 h, at which time TLC (ethyl acetate–acetic acid–water, 6:3:1) showed no further reaction. The solution was cooled in ice, and the excess precipitated pyridine–sulfur trioxide was removed by filtration and washed with cold pyridine. The filtrate was treated with gaseous ammonia for 10 min, concentrated *in vacuo* to approximately one-third its volume, and poured into ether–methanol (4:1) with vigorous stirring. The precipitated ammonium salt was collected, washed with ether, and dried. Conversion to the sodium salt was effected by dissolving the crude ammonium salt in the least amount of water (warming if necessary) followed by rapid elution with water from an ion-exchange resin (sodium ion form). The aqueous eluant was concentrated *in vacuo*, and the resulting crystals were stored at 3–5°C. The product was collected, washed with cold water, dried, and recrystallized from methanol to give material melting at ~100°C up to ~200°C (Table I).

*General Procedure B*—Pyridinium sulfamate (4–7 g) was suspended in 25 mL of dry pyridine under nitrogen. The appropriate benzoyl chloride (1.2 equivalents) was added dropwise, followed by stirring at 60°C for 3–4 h. Methanol (3.0 mL) was added, and the mixture was stirred for an additional 30 minutes, cooled to room temperature, treated with gaseous ammonia for 10 min; the sodium salt was prepared as in procedure A (Table I).

**Aryl-Substituted Benzyl Chlorides**—According to the procedure of Brooks and Snyder (13), the appropriate benzyl alcohol was treated with thionyl chloride in pyridine at room temperature and the product was distilled.

S-(*Substituted Benzyl*) *Isothiourea Hydrochlorides*—The procedure of Johnson and Sprague (9) was utilized. The appropriate benzyl chloride (0.05–0.2 mol) and one equivalent of thiourea were stirred under reflux in absolute ethanol (25 mL/0.05 mol of benzyl chloride) for 3–4 h. On cooling, the product crystallized in 85% yield. This material was used in the next step.

*Substituted Benzylsulfonyl Chlorides*—The appropriate *S*-(substi-

**Table III—Antihyperlipidemic Effects of Test Compounds in Male $CF_1$ Mice [a]**

| Compound | Serum Cholesterol Day 9 | Serum Cholesterol Day 16 | Serum Triglycerides Day 16 |
|---|---|---|---|
| I | 63 ± 6[b] | 60 ± 7[b] | 65 ± 8[b] |
| II | 57 ± 5[b] | 52 ± 6[b] | 55 ± 7[b] |
| III | 72 ± 5[b] | 64 ± 6[b] | 97 ± 7 |
| IV | 78 ± 6[b] | 51 ± 5[b] | 86 ± 8 |
| V | 86 ± 7 | 72 ± 5[b] | 80 ± 7[c] |
| VII | 74 ± 6[b] | 57 ± 5[b] | 70 ± 6[b] |
| VIII | 75 ± 4[b] | 73 ± 7[b] | 88 ± 3 |
| IX | 70 ± 3[b] | 63 ± 3[b] | 82 ± 4 |
| X | 95 ± 3 | 72 ± 4[b] | 69 ± 6[b] |
| XI | 90 ± 6 | 77 ± 6[b] | 49 ± 8[b] |
| XII | 88 ± 4 | 87 ± 2[b] | 91 ± 9 |
| XIII | 94 ± 7 | 88 ± 2[a] | 97 ± 8 |
| XIV | 84 ± 8 | 78 ± 2[b] | 75 ± 2[b] |
| XV | 94 ± 2 | 93 ± 4 | 79 ± 7[b] |
| XVI | 99 ± 8 | 73 ± 6[b] | 52 ± 6[b] |
| XVII | 69 ± 4[b] | 68 ± 7[b] | 58 ± 6[b] |
| XVIII | 84 ± 6[c] | 79 ± 7[b] | 42 ± 4[b] |
| XIX | 74 ± 8[b] | 70 ± 5[b] | 76 ± 7[b] |
| XX | 95 ± 8 | 75 ± 5[b] | 42 ± 3[b] |
| XXI | 92 ± 7 | 77 ± 5[b] | 71 ± 5[b] |
| XXII | 94 ± 7 | 78 ± 8[b] | 48 ± 4[b] |
| Saccharin | 77 ± 6[b] | 65 ± 7[b] | 54 ± 6[b] |
| Clofibrate | 98 ± 7 | 97 ± 9 | 95 ± 7 |
| 1% Carboxymethylcellulose (Control) | 100 ± 7 | 100 ± 6 | 100 ± 7 |

[a] Mean ± *SD* as percent of control; $n = 6$. [b] Significantly different, $p < 0.001$. [c] Significantly different, $p < 0.005$.

[1] Aldrich Chemical Co.
[2] Thomas Hoover.
[3] Either on a Varian EM360 or JOEL FX 60 FT.
[4] Galbraith Laboratories, Knoxville, Tenn. All compounds were tested for C and H; I–VII and XII–XXII were tested for N.
[5] Bio-Rad AG50W-X8.
[6] Waters Associates.
[7] Radial-Pak, Waters Associates.
[8] Jackson Laboratories.

tuted benzyl) isothiourea hydrochloride was dissolved in water (600–700 mL/0.2 mol depending on solubility) and cooled in ice to 0–10°C. The solution was then treated with gaseous chlorine for 30 min while maintaining the temperature <10°C. The solid or oily product was extracted into ether, and the ether extracts were dried (sodium sulfate) and evaporated *in vacuo* to afford the product which was sufficiently pure for conversion to the sulfonamide.

*Substituted Benzylsulfonamides (XVI, XX, XXI, and XXII)*—The appropriate benzylsulfonyl chloride was dissolved in liquid ammonia, the resulting yellow solution was kept at −78°C for 1 h, and then the excess ammonia was allowed to evaporate. The residue was dissolved in chloroform and filtered. The filtrate was dried (sodium sulfate) and evaporated to afford the crude sulfonamide which was either recrystallized from ethanol or column chromatographed[9]. This material was converted to the sodium salt by treating an ethanolic solution with 1.0 equivalent of 12 M aqueous NaOH. On cooling, the precipitated salt was collected and dried *in vacuo*. These products were obtained in varying degrees of hydration (Table II). Melting points were >250°C.

*Catalytic Reduction of Nitrobenzylsulfonamides*—A solution of the appropriate nitro-substituted benzylsulfonamide (prior to sodium salt formation) was dissolved in absolute ethanol (1600 mL/34 g of substrate) and was shaken for 6–8 h at 40°C[10] with 1.0 g of 5% palladium-on-charcoal under 60 psi of hydrogen. When the reaction was complete by TLC, the catalyst was removed by filtration, and the filtrate was evaporated *in vacuo* to afford an essentially quantitative yield of the aniline compound.

*Diazotization of Aminobenzylsulfonamides (14)*—A 24% solution of fluoroboric acid (0.12 mol) was added dropwise with cooling and stirring to 9–10 g of the aniline. The resulting solution was maintained at 0–5°C while an aqueous solution of $NaNO_2$ (0.048 mol) was added dropwise with stirring, and then the mixture was stirred at 0–5°C for 1.5 h. The precipitated salt was collected by filtration, washed with 5% $HBF_4$, cold methanol, and cold ether, and then was dried *in vacuo* to afford light-tan crystals.

*Methoxybenzylsulfonamides (15) (XVII, XVIII, and XIX)*—A solution of the above diazonium fluoroborate salt in methanol (1200 mL/10 g) was heated at 50°C for 5 h. The orange solution was then evaporated to dryness *in vacuo*, and the residue was column chromatographed[11] to afford material which was converted to the sodium salt as described above (Table II).

**Ames Mutagenicity Assay (16, 17)**—Compounds I–V, VII, and XII–XXII were each added to test culture plates in triplicate with and without mammalian microsomal activator S-9 present in the culture medium at dose levels of 0.3, 3, 30, 300, and 3000 μg/plate. Each compound was tested with five strains of non-histidine-producing *Salmonella typhimurium*. A threefold increase in the number of colonies per plate over background control constituted a positive mutagenic result. Control groups were treated only with the dosing vehicle (dimethyl sulfoxide).

**Acute Toxicity Studies**—Compounds I–V, VII, and XII–XXII were subjected to acute toxicity studies in male CD-1 mice (20–30 g)[12] in full compliance with the Food and Drug Administration Good Laboratory Practices Regulations for Nonclinical Laboratory Studies (18). Blood samples were taken by tail vein bleeding 3 d before dosing and at sacrifice on day 14 to be used for biochemical testing of liver and kidney function. Animals were weighed on days 0, 7, and 14. A single 100-mg/kg dose as a 1% solution in sterile water was administered by oral intubation on day 0. At this time and at various times throughout the day for 14 d, the animals were observed for signs of toxicity and/or abnormal behavior. Six animals were used per test compound. The animals were maintained on rodent diet[13] and water *ad libitum*. On day 14, the animals were weighed, sacrificed, and gross necropsies performed. The blood samples were assayed for alanine amino transferase activity as a test for liver function and urea nitrogen as a test for kidney function. The tests were performed on a miniature centrifugal fast analyzer. Each individual assay included 1 blank, 13 samples, and 2 controls (commercial sera samples of normal and abnormal alanine amino transferase and urea nitrogen levels). Abnormal values were defined as being >2 *SD* from untreated group values.

**Assay for Antihyperlipidemic Activity**—Compounds I–V and VII–XXII, as well as saccharin and clofibrate, were administered daily to male $CF_1$ mice (~28 g) as a 1% carboxymethylcellulose suspension at 20 mg/kg/d ip. On days 9 and 16, blood was obtained by tail vein bleeding and, after centrifugation to obtain serum, 25-μL samples were assayed for total cholesterol by the procedure of Ness *et al.* (19). Serum triglycerides were assayed using a commercial kit[14] on blood collected on the 16th day. By comparison to standards, the amounts of cholesterol (mg %) and triglycerides (mg/dL) were calculated. Treated values were expressed as percent of control (Table III).

**Chemical Stability Studies**—A 1% solution of each compound prepared in pH 3.5 citrate buffer, distilled water, and phosphate buffer (pH 7.4) was allowed to stand at room temperature. These solutions, as well as a 1% solution in distilled water of each compound which had been stored in solid form at room temperature, were analyzed directly by reverse-phase HPLC ($C_{18}$, 254 nm, 10% aqueous acetonitrile) for the presence of benzamides, benzylsulfonic acids, or other decomposition products on days 2, 6, 18, 30, and 60. These studies were paralleled using reverse-phase TLC plates[15] with methanol–water (9:1).

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[9] Silica gel 60, methylene chloride–methanol (9:1).
[10] Parr shaker apparatus.
[11] Silica gel 60, methylene chloride–acetone (9:1), (8:2).
[12] Acute toxicity studies and Ames assays were performed at the Research Triangle Institute, Research Triangle Park, N.C.
[13] Purina Certified Rodent Chow.
[14] Bio Dynamics/nm.
[15] Whatman $C_{18}$.