Communications to the Editor

Potential Antiatherosclerotic Agents. 5.[1] An Acyl-CoA:Cholesterol *O*-Acyltransferase Inhibitor with Hypocholesterolemic Activity

Sir:

As a part of our continuing search[1] for antiatherosclerotic and hypolipidemic agents, inhibitors of the enzyme acyl-CoA:cholesterol *O*-acyltransferase (ACAT, EC 2.3.1.26) were investigated. The ACAT enzyme is responsible for catalyzing the intracellular esterification of cholesterol.[2-4] Studies both in cultured cells[5-9] and in arterial tissue[10-12] have suggested that ACAT activity is regulated and that ACAT activity increases when cells are exposed to cholesterol-rich lipoproteins. Since the intracellular accumulation of esterified cholesterol is one of the characteristic features of the atherosclerotic plaque, the regulation of ACAT activity is likely to be of great importance in atherosclerosis.

Several lines of investigation have indicated that the ACAT enzyme may also play a key role in the intestinal absorption of cholesterol. Despite the fact that free cholesterol is internalized by intestinal mucosal cells,[13] more than 90% of the cholesterol that subsequently appears in the lymph is esterified.[14] Significant ACAT activity has been observed in intestinal mucosal cells from several animal species[15-18] including man.[19] The site of greatest ACAT activity is the jejunum,[20] which is where the majority of cholesterol absorption occurs, and this activity increases with increases in dietary cholesterol.[15,16]

Scheme I

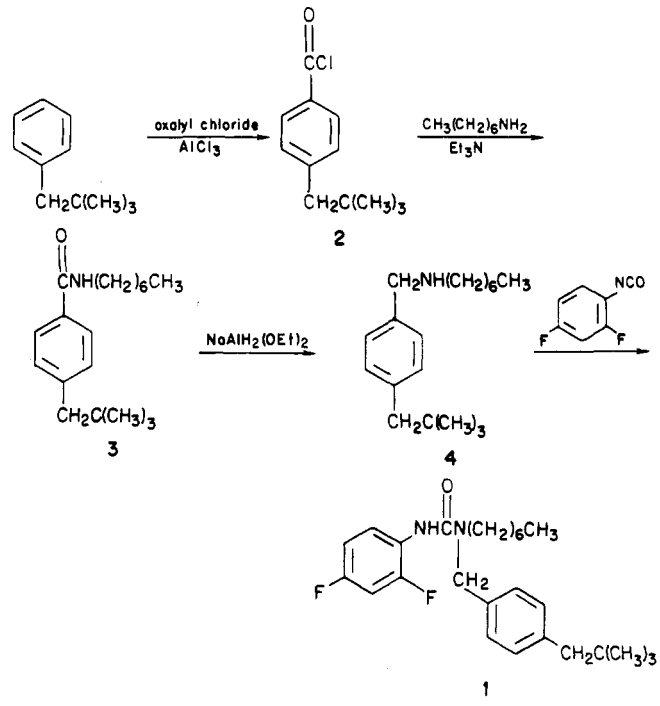

Table I. Effects of 1 on ACAT Activity

ACAT source	IC_{50}, μM
intestinal microsomes	0.14[a]
liver microsomes	0.74
aorta (homogenate)	6.06[b]

[a] In this assay, progesterone inhibits the enzyme, IC_{50} = 17 μM. Published data[26] indicate that the IC_{50} value of compound 57-118 is ca. 0.5 μM and in our assay an IC_{50} value at 0.33 μM was observed. [b] Single determination.

It is thus apparent that inhibition of ACAT-catalyzed cholesterol esterification could lead to diminished intestinal absorption of cholesterol as well as a decrease in the intracellular accumulation of cholesteryl esters. Therefore, ACAT inhibitors offer potential for exhibiting both hypolipidemic and antiatherosclerotic activity. In the course of investigating ACAT inhibitors of novel structure, a series of trisubstituted ureas was synthesized and evaluated. This paper reports the synthesis and biological properties of the most interesting member of the series, *N'*-(2,4-difluorophenyl)-*N*-[[4-(2,2-dimethylpropyl)phenyl]methyl]-*N*-heptylurea (1). In subsequent papers, the structure–activity studies that led to the selection of 1 and a complete investigation of its pharmacologic and pharmacokinetic properties will be reported.

The synthesis of 1 from commercially available neopentylbenzene involves four steps (Scheme I) and affords a 61% overall yield. In the first step, Friedel–Crafts

(1) Part 4 of this series: DeVries, V. G.; Largis, E. E.; Miner, T. G.; Shepherd, R. G.; Upeslacis, J. *J. Med. Chem.* **1983**, *26*, 1411.
(2) Chang, T-Y.; Doolittle, G. M. *Acyl Coenzyme A:Cholesterol O-Acyltransferase*, 3rd ed.; Academic: New York, 1983; Chapter 15.
(3) Goodman, D. S. *Physiol. Rev.* **1965**, *45*, 747.
(4) Suckling, K. E.; Stange, E. F. *J. Lipid Res.* **1985**, *26*, 647.
(5) Smith, B. P.; St. Clair, R. W.; Lewis, J. C. *Exp. Mol. Pathol.* **1975**, *30*, 190.
(6) Rothblat, G. H.; Naftulin, M.; Arbogast, L. Y. *Proc. Soc. Exp. Biol. Med.* **1977**, *155*, 501.
(7) Brown, M. S.; Ho, Y. K.; Goldstein, J. L. *J. Biol. Chem.* **1980**, *255*, 9344.
(8) Mathur, S. N.; Field, F. J.; Megan, M. B.; Armstrong, M. L. *Biochim. Biophys. Acta* **1985**, *834*, 48.
(9) Rothblat, G. H.; Arbogast, L. Y.; Ray, E. K. *J. Lipid Res.* **1978**, *19*, 350.
(10) Hashimoto, S.; Dayton, S.; Alfin-Slater, R. B. *Life Sci.* **1973**, *12*, 1.
(11) St. Clair, R. W.; Lofland, H. B.; Clarkson, T. B. *Circ. Res.* **1970**, *27*, 213.
(12) Brecher, P.; Chan, C. T. *Biochim. Biophys. Acta* **1980**, *617*, 458.
(13) Shiratori, T.; Goodman, D. S. *Biochim. Biophys. Acta* **1965**, *106*, 625.
(14) Vahouny, G. V.; Treadwell, C. R. *Am. J. Physiol.* **1957**, *191*, 179.
(15) Norum, K. R.; Helgerud, P.; Petersen, L. B.; Groot, P. H. E.; DeJonge, H. E. *Biochim. Biophys. Acta* **1983**, *751*, 153.
(16) Field, F. J.; Cooper, A. D.; Erickson, S. K. *Gastroenterol.* **1982**, *83*, 873.
(17) Norum, K. R.; Lilljeqvist, A. C.; Drevan, C. A. *Scand. J. Gastroenterol.* **1977**, *12*, 281.
(18) *Klein*, R. L.; Rudel, L. L. *J. Lipid Res.* **1983**, *24*, 343.
(19) Norum, K. R.; Lilljeqvist, A. C.; Helgerud, P. *Eur. J. Clin. Invest.* **1979**, *9*, 55.
(20) Helgerud, P.; Saarem, K.; Norum, K. R. *J. Lipid Res.* **1981**, *22*, 271.

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Table II. Effects of 1 on ACAT Activity in Cultured Monkey Arterial Smooth Muscle Cells

experiment 1	cholesteryl oleate formation, cpm/mg of protein ± SEM
control	2029 ± 248
LDL[a]	4274 ± 527
LDL + 0.1 μM 1	3865 ± 602
LDL + 1 μM 1	2089 ± 173[c]
LDL + 10 μM 1	1262 ± 162[c]
experiment 2	**cholesteryl oleate formation, cpm/mg of protein ± SEM**
control	10998 ± 852
C-LDL[b]	69317 ± 6804
C-LDL + 0.3 μM 1	49756 ± 4753
C-LDL + 1 μM 1	30072 ± 3403
C-LDL + 3 μM 1	14958 ± 219[c]
C-LDL + 10 μM 1	6467 ± 1355[c]

[a] Native LDL (200 μg of LDL cholesterol/well). [b] Cationized LDL (175 μg of C-LDL cholesterol/well).[28] [c] Statistically significant difference from control value, $p < 0.05$.

acylation of neopentylbenzene with oxalyl chloride in the presence of aluminum chloride is accomplished in methylene chloride at 40 °C, affording **2** directly in 78% yield. When heptylamine is acylated with the use of distilled **2** *in the presence of triethylamine at ambient temperature*, a quantitative yield of crystalline benzamide **3** is obtained. In the third step of this sequence, **3** is reduced with the use of sodium bis(2-methoxyethoxy)aluminum hydride (Vitride) in toluene and an 88% yield of distilled secondary amine **4** is obtained. Although this reduction can be accomplished with use of other reducing agents, for example, lithium aluminum hydride or diborane, Vitride was found to be the most convenient for large-scale syntheses. In the final step, reaction of **4** with freshly distilled 2,4-difluorophenyl isocyanate in hexane at ambient temperature affords **1** in 89% yield.

The effects of **1** on ACAT-catalyzed cholesteryl ester formation in various tissues are shown in Table I. In all tissues studied, **1** inhibited ACAT activity in a dose-dependent manner. Potent inhibition of activity at submicromolar concentrations was observed in these assays with all tissues except aorta; however, aortic activity was measured by using a crude tissue homogenate rather than isolated microsomes.

For the evaluation of the effects of **1** on cholesterol esterification in whole cells, cultured monkey aortic smooth muscle cells were first incubated in the presence of chemically modified low-density lipoproteins (LDL) to induce ACAT activity. Table II demonstrates that ACAT activity can be stimulated 2–5-fold after exposure to these lipoproteins. When the stimulated cells were subsequently exposed to **1**, the rate of cholesteryl ester formation was significantly depressed (Table II). The IC_{50} values for inhibition of smooth muscle cell ACAT activity were 1.09 and 0.81 μM, respectively, for cells stimulated by native or cationized LDL.

The activity of **1** as an inhibitor of cholesterol absorption and as a hypolipidemic agent was assessed in the cholesterol–cholic acid-fed rat. It was observed that rats fed on a diet supplemented with 1% cholesterol and 0.5% cholic acid show a nearly linear increase in liver cholesterol concentration over a 2-week time course and that serum cholesterol concentrations increase 3–5-fold over this time period. As a preliminary screen liver cholesterol concentrations were used as an index of cholesterol absorption and subsequent accumulation. Table III shows that **1** when administered in the diet to cholesterol and cholic acid

Table III. Effects of 1 on Cholesterol Absorption in Cholesterol-Fed Rats

dose of 1, mg/kg per day	liver cholesterol, mg/g ± SEM	serum lipids: cholesterol, mg/dL ± SEM	serum lipids: triglycerides, mg/dL ± SEM
control	24.1 ± 1.5	352 ± 85	120 ± 37
10	5.9 ± 0.9[a]	84 ± 2[a]	77 ± 29
3	15.6 ± 1.1[b]	211 ± 44[c]	121 ± 16
1	23.0 ± 2.8	357 ± 67	132 ± 20

[a] $p < 0.002$. [b] $p < 0.001$. [c] $p < 0.02$.

fed rats shows a dose-dependent effect both on liver sterol and serum sterol concentrations with an ED_{50} of 5.4 mg/kg. Serum triglyceride concentrations were not significantly affected in these animals.

The data described above demonstrate that **1** is a potent inhibitor of ACAT-catalyzed cholesterol esterification in vitro both in microsomal preparations and whole cells grown in culture. Moreover, in the rat **1** demonstrates dose-dependent hypocholesterolemic activity and inhibits the accumulation of cholesterol in the livers of cholesterol-fed animals. Other data (not shown) obtained with the use of orally administered radiolabeled cholesterol indicate that **1** directly inhibits intestinal absorption of cholesterol in rats. This activity most likely explains its effects on serum and hepatic cholesterol concentrations.

A number of compounds have previously been reported to inhibit ACAT-catalyzed cholesterol esterification. Bell has reported[21] that local anesthetics such as lidocaine, tetracaine, benzocaine, and dibucaine inhibit ACAT activity over the concentration range of 0.25–5 mM. Bell has also reported[22] that the major tranquilizer chlorpromazine was a somewhat more potent ACAT inhibitor with an IC_{50} of about 0.1 mM in rabbit arterial microsomes. The hypolipidemic agents bezafibrate and clofibrate have also been show to inhibit cholesterol esterification in cultured smooth muscle cells,[23] fibroblasts, and macrophages.[24] Like the anesthetics, these hypolipidemics have IC_{50}, values in the range of 0.5–2 mM. More potent ACAT inhibitors include progesterone[25] and the experimental drugs 57-118 (the ethyl ester of (*Z*)-*N*-(1-oxo-9-octadecenyl)-D,L-tryptophan)[26] and 58-035 (3-decyldimethylsilyl)-*N*-[2-(4-methylphenyl)-1-phenethyl]propionamide).[27] Among all these compounds, only progesterone, 57-118, 58-035, and **1** are sufficiently potent to offer the potential of affecting ACAT activity at concentrations that conceivably might be achieved in vivo.

It is clear that the enzyme ACAT plays an important role both in cholesterol absorption from the intestine and in the intracellular accumulation of cholesteryl esters that occur in the atherosclerotic lesion. Compound **1** is a potent inhibitor of ACAT activity both in isolated microsomes and in intact cells. It is also a potent hypolipidemic agent that reduces cholesterol absorption as demonstrated by de-

(21) Bell, F. P. *Atherosclerosis* **1981**, *38*, 81.
(22) Bell, F. P. *Exp. Mol. Pathol.* **1983**, *38*, 336.
(23) Hudson, K.; Day, A. J.; Marceglia, A. *Exp. Mol. Pathol.* **1982**, *36*, 156.
(24) Hudson, K.; Mojumder, S.; Day, A. J. *Exp. Mol. Pathol.* **1983**, *38*, 77.
(25) Simpson, E. R.; Burkhart, M. F. *Arch. Biochem. Biophys.* **1980**, *200*, 79.
(26) Heider, J. G.; Pickens, C. E.; Kelly, L. A. *J. Lipid Res.* **1983**, *24*, 1127.
(27) Ross, A. C.; Go, K. J.; Heider, J. G.; Rothblat, G. H. *J. Biol. Chem.* **1984**, *259*, 815.
(28) Basu, S. K.; Goldstein, J. L.; Anderson, R. G. W.; Brown, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3178.

creased serum and hepatic cholesterol concentrations in the cholesterol-fed rat. Subsequent papers will describe the structure–activity studies that led to the selection of **1**, the pharmacokinetic investigation used to characterize the absorption and metabolism of the compound, and the hypocholesterolemic activity of **1** in other species. Compound **1** is about to enter clinical trials as a hypolipidemic/antiatherosclerotic agent.

Registry No. **1**, 96224-26-9; **2**, 96224-27-0; **3**, 96224-28-1; **4**, 96224-29-2; *t*-$BuCH_2Ph$, 1007-26-7; $Me(CH_2)_6NH_2$, 111-68-2; 2,4-$F_2C_6H_3NCO$, 59025-55-7; oxalyl chloride, 79-37-8; acyl-CoA:cholesterol *O*-acyltransferase, 9027-63-8; cholesterol, 57-88-5.

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9-(2-Fluorobenzyl)-6-(methylamino)-9*H*-purine Hydrochloride. Synthesis and Anticonvulsant Activity

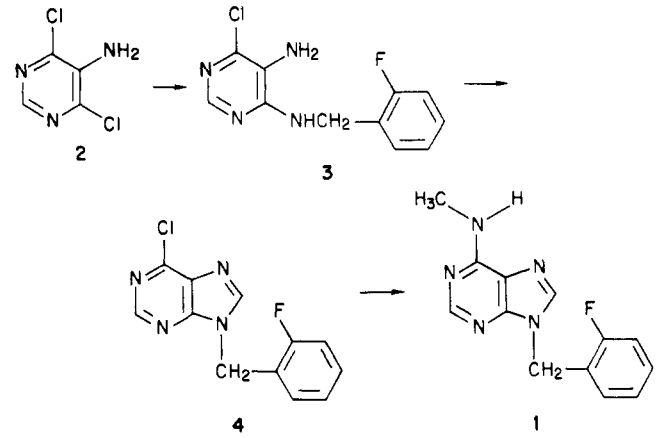
Sir:

Despite the availability and optimal use of several antiepileptic drugs, many patients with epilepsy do not experience satisfactory seizure control with them, or they do so at the expense of significant side effects.[1] Phenytoin, first marketed in 1938, is still the drug of choice for the treatment of many epileptic seizures despite its side effects and other disadvantages.[2] New antiepileptic drugs with fewer side effects and lower toxicity are needed.

As part of a program for new antiepileptic drugs with improved properties, a number of compounds were tested for anticonvulsant activity in several animal models.[3,4] From this program emerged 9-(2-fluorobenzyl)-6-(methylamino)-9*H*-purine (BW A78U (**1**)), a novel, orally active anticonvulsant with potent activity against maximal electroshock-induced seizures (MES) in animal models that predict antiepileptic activity in man.

Chemistry. BW A78U (**1**) was prepared from 5-amino-4,6-dichloropyrimidine (**2**) by modification of a general literature method[5,6] for the unambiguous synthesis of 9-substituted purines. Amination of **2** (Scheme I) with 2-fluorobenzylamine in refluxing 1-butanol gave diaminopyrimidine **3** in 82% yield, mp 220–223 °C. Condensation of **3** with ethanesulfonic acid and triethyl orthoformate[7,8] gave chloropurine **4** in 95% yield, mp 97–99 °C. Reaction of **4** with 40% aqueous methylamine in ethanol at ambient temperature gave **1** in high yield, mp

Scheme I



151–153 °C. Compound **1** was converted to the water-soluble hydrochloride salt in ethanol with concentrated hydrochloric acid in 90% yield, mp 255–259 °C (eff.).

Pharmacology. BW A78U had potent anticonvulsant activity in animal models that are predictive for antiepileptic activity in man. The compound protected Sprague–Dawley male rats against maximal electroshock-induced seizures (MES) with an oral ED_{50} of 2.5 ± 0.4 mg/kg under conditions where phenytoin had an ED_{50} of 20 ± 3 mg/kg (Table I). When administered by the ip and iv routes, BW A78U was active with ED_{50} values of 1.7 ± 0.4 and 0.2 ± 0.06 mg/kg, respectively. The duration of BW A78U's anticonvulsant activity in the MES test by the oral route was greater than 5 h at a submaximal dose of 5 mg/kg, which was 2–3 h shorter than that of phenytoin at 25 mg/kg. The compound also blocked 3-mercaptopropionic acid induced seizures with an ip ED_{50} of 16 ± 2 mg/kg under conditions where phenytoin had an ED_{50} of 21 ± 2 mg/kg ip. As with phenytoin, BW A78U did not protect rats against metrazol-, strychnine-, or picrotoxin-induced seizures at doses as high as 25 mg/kg ip or 50 mg/kg po.

BW A78U was also active in protecting CD1 Charles River male mice against MES with an oral ED_{50} of 14 ± 2 mg/kg. This level of activity was comparable to that of phenytoin, which had an ED_{50} of 22 ± 3 mg/kg. When administered ip and iv, BW A78U was active, with ED_{50} values of 5 ± 1 and 4 ± 0.2 mg/kg, respectively. Neither BW A78U nor phenytoin protected mice against metrazol-induced or low-frequency minimal electroshock-induced convulsions at an ip dose of 50 mg/kg. However, BW A78U blocked audiogenic seizures in mice with an ip ED_{50} of 3.7 ± 0.3 mg/kg under conditions where phenytoin had an ED_{50} of 6.4 ± 0.8 mg/kg.

BW A78U did not induce tolerance in mice under conditions where phenytoin was ineffective against MES. When phenytoin was administered to mice at a dose (10 mg/kg ip) that protected 84% of the animals through day 3, only 33% of the animals were protected by day 9 and none by day 13. In contrast, BW A78U protected 66–100% of the mice at 15 mg/kg ip throughout the 13-day period. Thus, in contrast with phenytoin, significant tolerance to BW A78U did not develop upon repeated administration.

BW A78U had minimal toxicity in acute tests in rats and mice. In rats the oral LD_{50} was >1000 mg/kg with the first visible sign of ataxia at 500 mg/kg. In mice the oral LD_{50} was >500 mg/kg with the first visible sign of ataxia at 250 mg/kg. The acute toxicity data and the anticonvulsant ED_{50} values were determined by the method of Miller and Tainter.[9]

BW A78U is a potent, orally active anticonvulsant with an activity profile in the rodent that suggests it may be

(1) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia* **1978**, *19*, 409.
(2) Krall, R. L.; Penry, J. K.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia* **1978**, *19*, 393.
(3) Soroko, F. E.; Grivsky, E.; Maxwell, R. A. *J. Pharm. Pharmacol.* **1981**, *33*, 741.
(4) Mehta, N. B.; Diuguid, C. A. R.; Soroko, F. E. *J. Med. Chem.* **1981**, *24*, 465.
(5) Greenberg, S. M.; Ross, L. O.; Robins, R. K. *J. Org. Chem.* **1959**, *24*, 1314.
(6) Montgomery, J. A.; Temple, C., Jr. *J. Am. Chem. Soc.* **1958**, *80*, 409.
(7) Temple, C., Jr.; Kussner, C. L.; Montgomery, J. A. *J. Med. Pharm. Chem.* **1962**, *5*, 866.
(8) Schaeffer, H. J.; Vince, R. *J. Med. Chem.* **1968**, *11*, 15.

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