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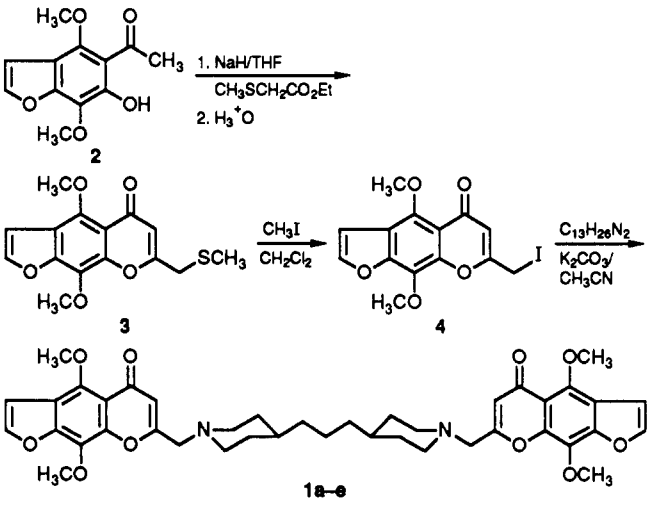
Communications to the Editor

Antiatherosclerotic Agents. A Structurally Novel Bivalent Inhibitor of AcylCoA:Cholesterol *O*-Acyltransferase with Systemic Activity

The atherogenic process can be characterized on the basis of predictable morphological and biochemical changes in arteries. The most outstanding biochemical change to occur in arteries during atherogenesis is the accumulation of lipids, particularly cholesterol and its esters.[1] Along with HMG-CoA reductase and 7α-hydroxylase, acyl-CoA:cholesterol *O*-acyltransferase (ACAT) is one of the major regulators of cholesterol metabolism in cells.[2] Studies both in cultured cells[2b] and in arterial tissue[2c] have suggested that ACAT activity is regulated and increases when cells are exposed to cholesterol-rich lipoprotein. It is also recognized that ACAT plays an important role in the intestinal absorption of cholesterol and that ACAT activity is greatest in the jejunum where the majority of cholesterol absorption occurs.[3] Since the intracellular accumulation of esterified cholesterol is one of the characteristic features of the atherosclerotic plaque, these facts continue to heighten the interest and support the hypothesis that regulation of ACAT activity is likely to be of great importance in atherosclerosis treatment.[4]

Scheme I



During the course of our studies on ACAT inhibitors, the unique bisaminofurochromone 1 was synthesized and evaluated in our in vitro ACAT assay which is based upon the ability of Fu5AH cells to synthesize and accumulate [^{3}H]cholesteryl esters when cultured in the presence of [^{3}H]cholesterol-labeled hyperlipemic plasma lipoproteins. This paper describes the synthesis, structure–activity relationship, and characterization of this novel class of ACAT inhibitors in a variety of in vivo and in vitro animal systems.

Chemistry

The general synthetic strategy used for the synthesis of the compounds discussed in this communication is outlined in Scheme I. Claisen condensation/cyclodehydration between khellinone **2** and ethyl α-(methylthio)acetate

(1) Bell, F. P. Arterial Cholesterol Esterification by AcylCoA: Cholesterol Acyltransferase: Its Possible Significance in Atherosclerosis and its Inhibition by Drugs. In *Pharmacological Control of Hyperlipidaemia*; J. R. Prous: Barcelona, Spain, 1986; p 409.

(2) (a) For reviews on the role of acyl-CoA:cholesterol acyltransferase in cellular cholesterol metabolism, see: Suckling, K. E.; Stange, E. F. *J. Lipid Res.* **1985**, *26*, 647. Chang, T.-Y.; Doolittle, G. M. *Acyl Coenzyme A:Cholesterol O-Acyltransferase*, 3rd ed.; Academic: New York, 1983; Chapter 15. Goodman, D. S. *Physiol. Rev.* **1965**, *45*, 747. (b) For evidence of regulation of ACAT in cultured cells, see: Smith, B. P. ; St. Clair, R. W.; Lewis, J. C. *Exp. Mol. Pathol.* **1975**, *30*, 190. Rothblat, G. H.; Naftulin, M.; Arbogast, L. *Proc. Soc. Exp. Biol. Med.* **1977**, *155*, 501. Brown, M. S.; Ho, Y. K.; Goldstein, J. L. *J. Biol. Chem.* **1980**, *255*, 9344. Mathur, S. N.; Field, F. J.; Megan, M. B.; Armstrong, M. L. *Biochim. Biophys. Acta* **1985**, *834*, 48. Rothblat, G. H.; Arbogast, L. Y.; Ray, E. K. *J. Lipid Res.* **1978**, *19*, 350. (c) For evidence of regulation of ACAT in arterial tissue, see: Hashimoto, S.; Dayton, S.; Alfin-Slater, R. B. *Life Sci.* **1973**, *12*, 1. St. Clair, R. W.; Lofland, H. B.; Clarkson, T. B. *Circ. Res.* **1970**, *27*, 213. Brecher, P.; Chan, C. T. *Biochim. Biophys. Acta* **1980**, *617*, 458.

(3) *Fat Absorption*; Kuksis, A., Ed.; CRC Press: Boca Raton, FL, 1987; Vol. II.

(4) For a recent summary on current drugs under study that influence lipid metabolism, see: Fears, R. *Drugs of Today* **1987**, *23*, 295. For recent reports on ACAT inhibitors, see: Heffron, F.; White, D.; Middleton, B. *Biochem. Soc. Trans.* **1989**, *17*, 127. Heffron, F.; Salter, A.; White, D. *Biochem. Soc. Trans.* **1989**, *17*, 361. Largis, E.; Wang, C. H.; DeVries, V. G.; Schaffer, S. A. *J. Lipid Res.* **1989**, *30*, 681. Seki, K.; Horie, S.; Watanabe, R.; Suga, T. *J. Pharm. Pharmacol.* **1988**, *40*, 473.

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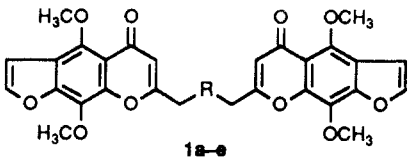
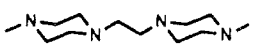
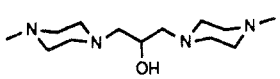
Table I



1a–e

compd	R	% ACAT inhibition or IC_{50}*
1a		0% at 5 μg/mL
1b		0.8 μg/mL*
1c		4.2 μg/mL*
1d		5% at 5 μg/mL
1e		7% at 5 μg/mL

afforded the furochromone **3**.[5] Conversion of **3** to the corresponding allylic iodide was effected by treatment with excess CH_3I (CH_2Cl_2/72 h) which yielded the allylic iodide **4** in 70–75% yield.[5] Treatment of **4** with 4,4′-trimethylenedipiperidine[6] in acetonitrile in the presence of potassium carbonate then afforded the bisaminofurochromone **1** in good yield. The various amine-tethered analogues shown in Table I were prepared in a similar fashion from the appropriate amine and allylic iodide **4**. The C-4 hydroxy bisaminofurochromone **5** was prepared directly from **1b** by refluxing **1b** in a $CHCl_3$ solution saturated with anhydrous HBr. Compounds **6** and **7** were prepared from 3-fluorokhellinone[7] and 2,3 dihydrokhellinone,[8] respectively, in the same fashion as described for **1b**.

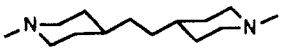
Structure–Activity Relationship. In Vitro and in Vivo Pharmacology

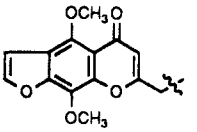
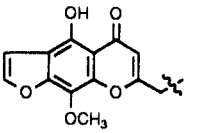
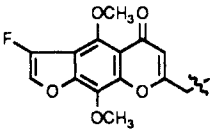
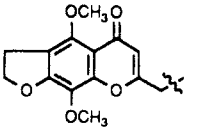
In Table I the in vitro results of various tether variations are illustrated.[9] The removal of the carbon chain between the piperidine rings results in a compound (**1a**) completely inactive in our in vitro assay. Interestingly, removal of one carbon from the aliphatic three carbon tether of **1c** resulted in a compound (**1b**) with a 5-fold increase in potency. Introduction of heteroatoms toward the center of the tether (analogues **1d** and **1e**) resulted in reduced ACAT inhibitory activity.

The potency of **1b** provided an excellent opportunity to further probe some of the structural features of the furochromone nucleus. Removal of the C-4 methoxyl group in **1b**, to yield **5**, abolished in vitro activity (Table II). Introduction of fluorine at C-3, as well as saturation of the furan ring, gave compounds with reduced in vitro activity.

The inhibition of cellular ACAT by **1b** occurred with an IC_{50} = 0.8 μg/mL. For comparative purposes, the ACAT inhibitor 58-035[10] was tested under similar conditions and had an IC_{50} of 0.5 μg/mL. On the basis of the in vitro potency of **1b**, follow-up studies in animals were initiated. Studies in cholesterol-fed rabbits indicated that **1b** possesses hypocholesterolemic activity and reduces atherosclerotic lesion development as well (Table III).[11] Decreases in the appearance of orally administered [4-^{14}C]cholesterol in the plasma of rats (Table IV),[12] suggests that the hypocholesterolemic effect is associated with inhibition of intestinal absorption of cholesterol. The systemic action of **1b** was also demonstrated in the rabbit and rat (Table V).[13,14] Arterial ACAT activity was assessed in vitro in

Table II



R		IC_{50}
	1b	0.8 μg/mL
	5	a
	6	<10 μg/mL
	7	3.0 μg/mL

[a] Inactive.

Table III. Effect of **1b** on Plasma Cholesterol Levels and Atheromatous Lesion Development in Netherland Dwarf Rabbits Fed a Cholesterol-Containing Atherogenic Diet

	plasma total cholesterol (mg/dL)	aortic lesion formation: % surface involvement
control	2221 ± 524	>80%
1b (50 mg/kg)	271 ± 29	<10%

(5) Gammill, R. B. *J. Org. Chem.* **1985**, *50*, 5035. Gammill, R. B.; Day, C. E.; Schurr, P. E. *J. Med. Chem.* **1983**, *26*, 1672. Stevens, T. J.; Schurr, P. E.; Gammill, R. B.; Day, C. E. *Atherosclerosis* **1985**, *56*, 313.
(6) Available from the Aldrich Chemical Company.
(7) Nash, S. A.; Gammill, R. B. *Tetrahedron Lett.* **1987**, 4003.
(8) Prepared from khellinone in quantitative yield by catalytic hydrogenation with 10% Pd/C in EtOAc.
(9) Assays for the initial evaluation of ACAT inhibition were conducted in cultured Fu5AH cells. Bell, F. P.; Rothblat, G. H.; Bamberger, M. *Can. J. Biochem.* **1982**, *60*, 967.
(10) Compound 58-035 is 3-[decyldimethylsilyl]-*N*-[2-(4-methylphenyl)-1-(phenylethyl)propamide. Ross, A. C.; Go, K. J.; Heider, J. G.; Rothblat, G. H.; *J. Biol. Chem.* **1984**, *259*, 815.
(11) Male Netherland dwarf rabbits (1.2–1.4 kg) were fed an atherogenic diet (Purina chow containing, 1% cholesterol, 3% peanut oil, and 0.2% cholic acid) ±50 mg kg^{-1} day^{-1} of **1b** for 12 weeks. Blood samples were drawn by cardiac puncture and plasma total cholesterol was measured with a clinical analyzer (Ektachem DT60, Eastman Kodak, Rochester, NY). Aortas were excised, opened longitudinally from the aortic arch to the iliac bifurcation and the percentage of the arterial surface displaying gross atheromatous lesions was visually estimated. Values are means (±SEM) of 8 animals per group. Bell, F. P. *Exp. Mol. Path.* **1983**, *38*, 336.
(12) Four male Sprague-Dawley rats were intubated per os with 0.6 mL of a suspension which contained 1 μCi [4-^{14}C]cholesterol (NEN Research Products, Boston, MA; specific activity 53 Ci/Mol), 510 μL corn oil, 21 μL ethyl alcohol, 68 μL DMSO, and 1 mg cholesterol, USP (Controls). Four test rats received the suspension containing **1b** at a level which provided 30 mg/kg. Two rats from each group were bled via cardiac puncture at 2.5 and 6 h and the plasma assayed for [^{14}C]-cholesterol. Each value is the mean of data from two animals. Bell, F. P.; Schwartz, C. J. *Biochim. Biophys. Acta* **1971**, 231, 553.

Table IV. Effect of a Single Oral Dose of **1b** on the Appearance of Orally Administered [^{14}C]Cholesterol in the Plasma of Rats

time post-dosing, h	treatment	plasma [^{14}C]cholesterol (dpm/mL)
2.5	control	1507
	1b	818
6.0	control	6609
	1b	3884

Table V. Effect of Orally Administered **1b** on ACAT Activity in Rabbit Aorta and Rat Liver (dpm Cholesteryl [^{14}C]Oleate Formation/100 Wet Wt)

tissue		ACAT activity	% reduction
rabbit aorta	control	530	–
	1b (50 mg/kg)	305	42
rat liver	control	1420	–
	1b (30 mg/kg)	535	62

rabbits that received **1b** (50 mg kg^{-1} day^{-1} for 6 days) following a 10-day cholesterol feeding regiment designed to increase arterial ACAT activity; ACAT activity was reduced 42% by **1b** treatment. Additionally, hepatic ACAT was evaluated in vitro in liver minces from rats that received **1b** in the diet (30 mg kg^{-1} day^{-1}) for 8 days; ACAT activity was reduced 62% by **1b** treatment (Table V). This evidence of the systemic action of **1b** on the ACAT enzyme in arterial tissue, and its hypocholesterolemic action, are important to its continued development as an antiatherosclerotic agent.

Acknowledgment. We would like to thank Physical and Analytical Chemistry for spectroscopic data and Jeanne Obreiter for the preparation of this manuscript.

(13) New Zealand rabbits (2.2–2.3 kg) were fed a cholesterol-rich diet[15] for 10 days to induce increases in arterial ACAT activity[16] and then switched to Purina chow ● **1b** (calculated to provide 50 mg kg^{-1} day^{-1}). After 6 days, the aortas were excised, incubated in vitro with [1-^{14}C]oleate for 3 h, and ACAT activity was evaluated by the formation of cholesteryl [^{14}C]-oleate.[15] Liver minces[17] from male Sprague-Dawley rats (435–460 g) that received a Purina chow diet ± **1b** (calculated to provide 30 mg kg^{-1} day^{-1}) for 8 days were incubated in vitro with [1-^{14}C]oleate for 90 min and ACAT activity was evaluated by the formation of cholesteryl [^{14}C]oleate.[18] ACAT data are presented as dpm cholesteryl [^{14}C]oleate formed/100 mg wet weight of aorta or liver; all values are means of 2 animals per group.

(14) The effects of orally administered **1b** on rabbit arterial ACAT and rat hepatic ACAT are unlikely to be attributable to reduced substrate (cholesterol) availability resulting from inhibition of reabsorption of biliary cholesterol for the following reasons: (a) virtually all arterial cholesterol in the rabbit is derived from plasma (St. Clair, R. W. *Atheroscler. Rev.* **1976**, *1*, 61) and plasma cholesterol levels were ca. 10-fold above normal levels in the **1b** group; (b) inhibitors of gut cholesterol absorption in rats on normal chow diets have essentially no effect on plasma cholesterol or hepatic cholesterol levels (Heider, J. G. In *Pharmacological Control of Hyperlipidaemia*; J. R. Prous: Barcelona, Spain, 1986; p 423.)

(15) Bell, F. P.; Schaub, R. G. *Arteriosclerosis* **1986**, *6*, 42.

(16) Day, A. J.; Proudlock, J. W. *Atherosclerosis* **1974**, *19*, 253.

(17) Bell, F. P.; Patt, C. S. Gillies, P. J. *Lipids* **1978**, *13*, 673.

(18) Bell, F. P. *J. Cardiovas. Pharmacol.* **1985**, *7*, 443.

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Structure-Based, C_2 Symmetric Inhibitors of HIV Protease

The genome of the human immunodeficiency virus (HIV-1) encodes a proteinase (HIV protease) which proteolytically processes the *gag* and *gag-pol* polyproteins.[1] Blockade of these processing steps results in the production of progeny virions which are immature and noninfectious.[2] Chemical inhibition of this critical viral enzyme therefore represents a powerful strategy for the development of an effective therapy for AIDS. Already, potent and selective inhibitors of HIV protease have been shown to prohibit the spread of HIV infection in vitro.[3] However, all of these inhibitors utilize known transition-state analogues[4] as replacements for the P_1–P_1' substrate cleavage sites. We report here the synthesis and antiviral properties of two novel classes of inhibitors specifically designed to capitalize on the unique symmetric structure of HIV protease.

The initial suggestion[5] and subsequent crystallographic demonstration[6] that HIV protease functions as a C_2 symmetric homodimer prompted us to utilize the concept of symmetry for the design of novel inhibitor structures. Inherently less peptide-like than inhibitors based on classical transition-state analogues, symmetric inhibitors might be expected to exhibit greater stability in vivo. Moreover, symmetric inhibitors should confer high specificity for retroviral proteinases over the related mammalian aspartic proteinases, whose substrate binding sites are less symmetric. The design of a C_2 symmetric inhibitor from the tetrahedral intermediate for cleavage of an asymmetric substrate, (e.g. -Phe-Pro-) hinges on three

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(4) For a recent review of the use of transition-state analogues for inhibitors of renin, see: Greenlee, W. J. *Med. Res. Rev.* **1990**, *10*, 173.

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