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ACYL CoA:CHOLESTEROL ACYLTRANSFERASE (ACAT) INHIBITORS: UREAS BEARING TWO HETEROCYCLIC HEAD GROUPS

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Abstract. A series of compounds bearing two heterocyclic substituents were prepared, and evaluated for inhibition of acyl-CoA:cholesterol acyltransferase (ACAT). The heterocyclic groups were chosen for dual potency against hepatic and macrophage cell ACAT. Several examples of ACAT-balanced compounds were discovered, and the results of this study, including synthesis, are presented.

Introduction. Because cholesterol deposition into the arterial wall is a significant development in the course of atherogenesis, agents which disrupt absorption and delivery of cholesterol in various tissues have been sought as potential therapy. A recent approach involves the inhibition of ACAT, an enzyme which catalyzes the intracellular esterification of cholesterol to cholesteryl esters. Cholesteryl esters are a major component of chylomicron particles, which are involved in lipid transport from intestinal mucosal cells to the liver.[1] Thus, intestinal ACAT inhibition should result in lower serum cholesterol levels.[2] Also, ACAT inhibition may affect serum cholesterol levels by inhibition of the synthesis and/or secretion of apo-B in the liver.[3] Finally, systemic ACAT inhibition of arterial macrophage cells should result in decreased development of foam cells and thus arterial lesions.[4] Thus, an intestinal and systemic ACAT inhibitor may prove to be a superior therapeutic approach for hypercholesterolemia and/or atherosclerosis.[5]

Our lead compound, DuP 128 (Fig. 1), was found to be a potent inhibitor of ACAT, with an IC_{50} in rat hepatic microsomes of 0.010 μM. The compound also showed excellent *in vivo* activity, with an ED_{50} in lowering cholesterol levels in the cholesterol-fed hamster of 3 mg/kg.[6] However, the ability of the compound to inhibit ACAT in whole macrophage cells (the J774 murine cell line; see below) was modest, with an IC_{50} of 1.0 μM. In our efforts to discover a systemic inhibitor, a compound with good potency in both the hepatic microsomal and macrophage cell assays was sought, because these assays should be models for inhibition of ACAT in human liver and arterial tissue. Such a compound would exhibit both serum lipid-lowering and anti-atherosclerotic properties. To address the dual activity issue, the SAR in the DuP 128 series[6] was recalled (Fig. 2). The X groups substituted on the imidazole aryl rings were critical in determining J774 potency, with the best results obtained for X = CH_3O or $(CH_3)_2N$. Some dependence of potency on the group R^4 was also seen. The group R^6, however, could be a wide variety of alkyl and aryl groups, without much affect on macrophage ACAT inhibition. Therefore, one approach to dual-acting compounds would be to incorporate two diarylimidazoles groups in the molecule, one bearing substituents for good

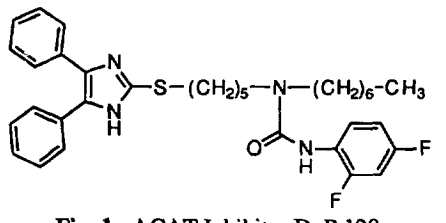


Fig. 1. ACAT Inhibitor DuP 128.

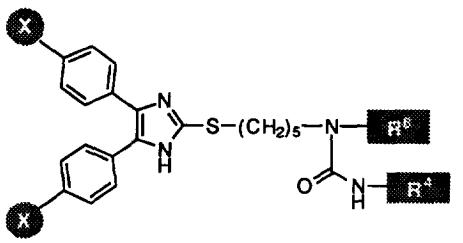


Fig. 2. SAR in the DuP 128 series.

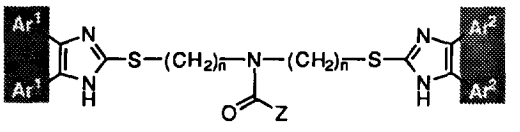


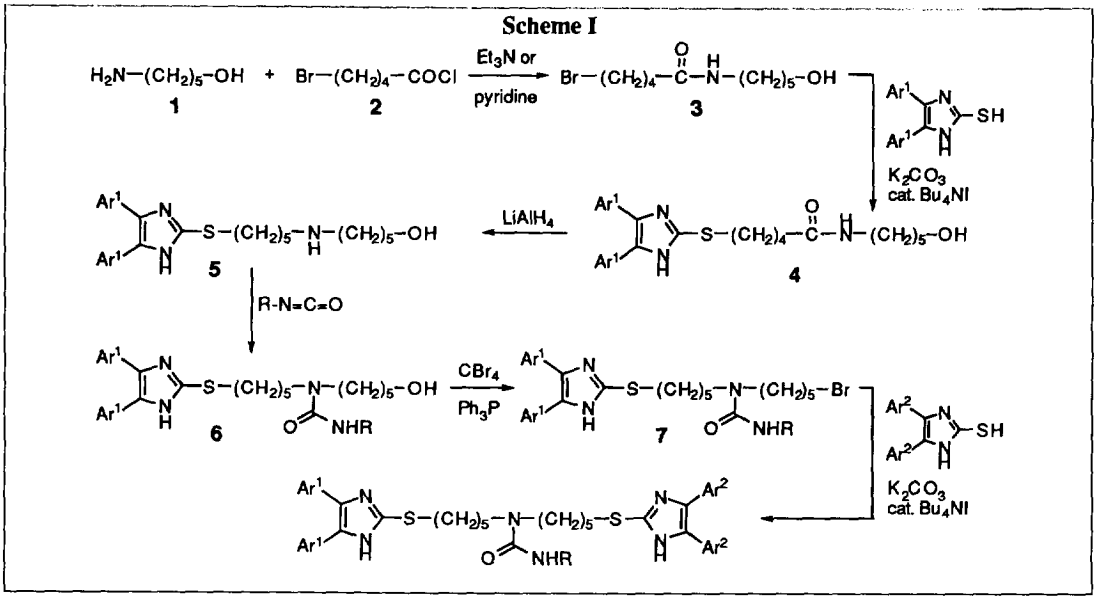
Fig. 3. Double-headed ACAT inhibitors.

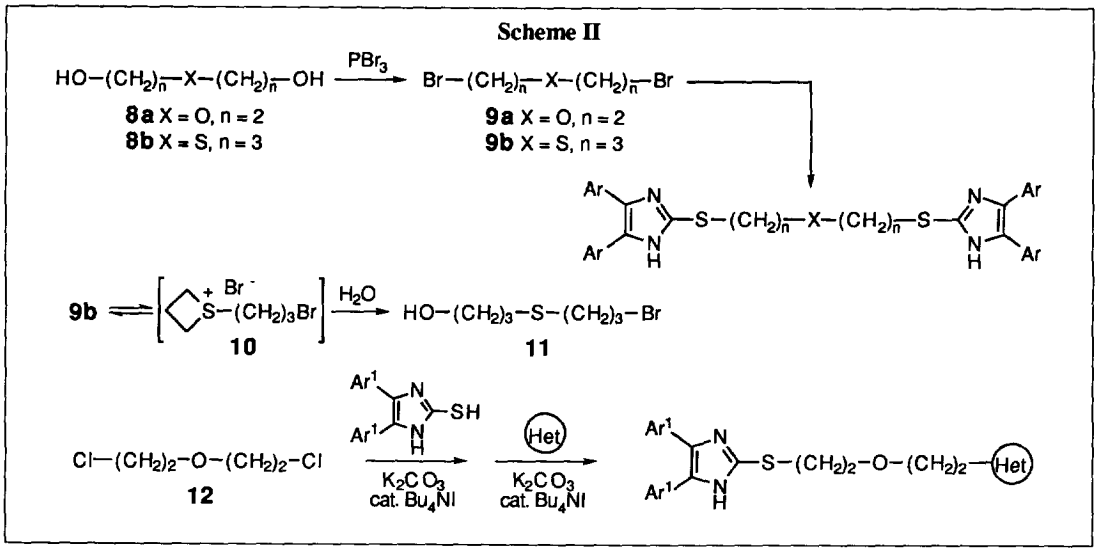
hepatic ACAT potency (*e.g.* Ar^1, Fig. 3), and the other, attached at the "R^6" position, bearing substituents for good macrophage ACAT potency (Ar^2). This paper describes the synthesis and evaluation of these compounds.[7]

Biology.[8] To assay the compounds prepared for this study for *in vitro* ACAT inhibition, two primary assays were performed: 1) ACAT In Vitro (AIV):[9] the formation of labeled cholesterol oleate in the presence of rat hepatic microsomes was determined. The results are given as IC_{50}s in μM; 2) J774 Macrophage Cell Culture (J774):[4] the formation of cholesteryl ester (CE) is measured by following the rate of labeled oleate incorporation into CE. Results are also given as an IC_{50} in μM. The AIV screen is intended to reflect the compound's potency in terms of intestinal and hepatic activity, and the J774 screen should estimate the potential for the compound to prevent foam cell formation. Also, serum cholesterol lowering in a single-dose (25 mg/kg) cholesterol-fed hamster model[6c] was observed for compounds **17** (24%) and **25** (37%).

Chemistry. Scheme I shows a typical synthesis of a member in this family of compounds. An amino alcohol such as **1** was allowed to react with a bromoacyl chloride (**2**). The resulting bromide **3** was used to alkylate a diaryl imidazole of the chosen substitution to afford heterocyclic amide **4**. The amide group was reduced using LAH (or similar reagents), and the amine **5** was then functionalized with an isocyanate to give urea **6**. The hydroxy group in the alcohol **6** was converted to the bromide **7**, and then a second alkylation reaction was performed to give the final products with heterocyclic groups bearing different substitution. Similar chemistry was employed to prepare the other urea compounds in this study. To demonstrate that the nitrogen functionality was still necessary (according to the SAR of the original series), several ethers and sulfides with heterocyclic substituents were also prepared (Scheme II). Bis(hydroxyalkyl) ethers and sulfides (**8**) could be converted to the corresponding bromides employing phosphorus tribromide. These bromides (**9**) were highly reactive, and attempts at alkylation gave mixtures of mono- and bis-alkylated products, so 2 equiv. of heterocyclic mercaptan were used. Also, the bromide **9b** was found to be sensitive to hydrolysis to give alcohol **11**, probably due to internal assistance from the sulfur atom (through intermediate **10**). The less reactive dichloride **12** proved to be the reagent of choice, in order to perform the alkylation reactions sequentially, so that different heterocylic substituents could be incorporated onto the molecule. These compounds were then analyzed by the above-detailed biological assays, with results presented in Tables 1 - 2.

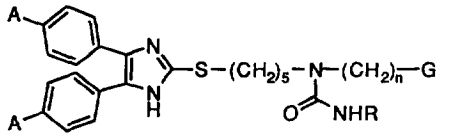
Discussion and Conclusions. For urea compounds with pyridyl-alkyl as the "right-hand" substituent (**13-16, 20-23**), the SAR followed closely what was seen previously for the DuP 128 series. Imidazoles bearing two unsubstituted phenyl groups (*e.g.* **15, 16**) were potent with respect to the AIV (microsomal) assay, but poor with





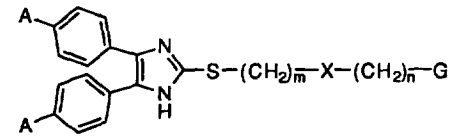
respect to the J774 (macrophage cell) assay. The reverse pattern was observed for "left-hand" imidazoles with electron-rich aromatic substituents (*e.g.* **13**, **14**, **20**, **24**). Better AIV potency was observed for difluorophenyl ureas, better J774 potency for isopropyl ureas. This type of selectivity may denote enzyme subtype behavior. Changing the tether length to the pyridyl ring (*e.g.* **13** to **21** or **14** to **20**) had no clear, consistent effect on either ACAT assay. More success was realized for the ureas bearing two diarylimidazoles. Note **26**, which is threefold more potent than DuP 128 in the AIV assay! In three cases (**17**, **24**, **25**), 100 nM (or better) activity was recorded for both the AIV and J774 assays. That the urea group is still required in the normal way in this series is shown by the ethers and sulfides in Table 2, which were significantly less potent than the ureas. A notable

Table 1.



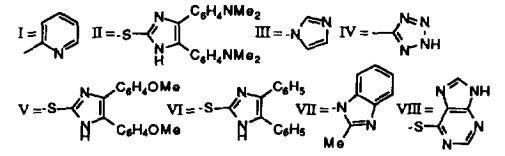
Cpd.	A	R [a]	n	G [b]	AIV	J774
13	MeO	A	2	I	0.30	0.69
14	MeO	B	2	I	0.26	0.09
15	H	A	2	I	0.08	2.44
16	H	B	2	I	0.19	3.05
17	H	B	5	II	0.03	0.06
18	MeO	B	3	III	c	5.50
19	MeO	B	2	IV	c	5.50
20	MeO	B	1	I	6.23	0.06
21	MeO	A	1	I	c	0.19
22	H	A	0	I	0.66	0.79
23	H	B	0	I	0.10	0.94
24	MeO	B	5	V	0.09	0.01
25	H	B	5	V	0.09	0.10
26	H	A	5	II	0.003	0.45

Table 2.



Cpd.	A	m	X	n	G	AIV	J774
27	H	2	O	2	VI	0.026	6.55
28	H	2	O	2	V	c	3.87
29	H	3	S	3	VI	c	7.96
30	Me_2N	2	O	2	II	0.134	0.37
31	MeO	2	O	2	VII	c	3.43
32	H	5	O	2	I	c	3.92
33	H	5	S	1	I	0.31	3.93
34	H	3	S	3	VII	0.25	7.27
35	Me_2N	2	O	2	VIII	2.04	2.99

Key: (a) A = 2,4-difluorophenyl, B = isopropyl; (b) G groups:



(c) not determined.

exception to this was compound **30**, which bears the bis(4-dimethylaminophenyl)imidazole group.[10] For the compounds with good dual activity (**17**, **24**, **25**), we propose that the "diphenyl" end of the molecule is responsible for AIV potency, and the "substituted diphenyl" end is responsible for the good J774 macrphage potency.

Thus, we have exploited the SAR from two different ACAT inhibition assays to prepare compounds with good potency in both assays by incorporating two different head groups in the same molecule.

References

(1) Gallo, L. L. *Contemp. Issues Clin. Nutre.* **1983**, *6*, 83.
(2) Bennett, C. S.; Tercyak, A. M. *J. Lipid Res.* **1984**, *25*, 148.
(3) (a) Huff, M. W.; Telford, D. E.; Barrett, P. H. R.; Billheimer, J. T.; Gillies, P. J. *Arteriosclerosis* **1994**, *14*, 1498. (b) Carr, T. P.; Ruter, L. L. *Arteriosclerosis* **1990**, *10*, 823.
(4) (a) Schmitz, G.; Neimann, R.; Brennhauser, B.; Krause, B.; Assman, G. *EMBO J.* **1985**, *4*, 2273. (b) Mazzone, T.; Gump, H.; Diller, P.; Getz, J. *J. Biol. Chem.* **1987**, *262*, 11657.
(5) (a) Heider, J. G. in *Pharmacological Control of Hyperlipidaemia*; J. R. Prous Science Publishers S. A.: 1986, pp. 423-438. (b) Sliskovic, D. R.; White, A. D. *Trends Pharm. Sci.* **1991**, *12*, 194. (c) Billheimer, J. T.; Wilde, R. G. *Current Drugs* **1991**, B5.
(6) (a) Wexler, R. R.; Higley, C. A.; Maduskuie, T. P.; Pennev, P.; Billheimer, J. T.; Gillies, P. J. 201st Am. Chem. Soc. Meeting, Atlanta 1991, abs. MEDI 87. (b) Hainer, J. W.; Terry, G. T.; Connell, J. M.; Zyruk, H.; Jenkins, R. M.; Shand, D. L.; Gillies, P. J.; Livak, K. J.; Hunt, T. L.; Crouse, J. R. *Clinical Pharm. Therap.* **1994**, *56*, 65. (c) Higley, C. A.; Wilde, R. G.; Maduskuie, T. P.; Johnson, A. L.; Pennev, P.; Billheimer, J. T.; Robinson, C. S.; Gillies, P. J.; Wexler, R. R. *J. Med. Chem.* **1994**, *37*, 3511.
(7) This study was first presented as: Wilde, R. G.; Billheimer, J. T.; Wexler, R. R. 206th ACS Meeting, Chicago 1993, MEDI 44.
(8) Assays for this study were performed by D. Cromley, B. Thomas, and R. Stevenson.
(9) (a) Billheimer, J. T.; Tavani, D.; Nes, W. R. *Anal. Biochem.* **1981**, *111*, 331. (b) U.S. Patent 5,179,117.
(10) This group often results in enhanced J774 activity: Maduskuie, T. P.; Wilde, R. G.; Billheimer, J. T.; Cromley, D. A.; Germain, S.; Gillies, P. J.; Higley, C. A.; Johnson, A. L.; Pennev, P.; Shimshick, E. J.; Wexler, R. R. *J. Med. Chem.*, submitted.

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