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ACYL CoA:CHOLESTEROL ACYLTRANSFERASE (ACAT) INHIBITORS: UREAS BEARING HETEROCYCLIC GROUPS BIOISOSTERIC FOR AN IMIDAZOLE

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Abstract. A series of compounds bearing heterocyclic substituents were prepared, and evaluated for inhibition of the ACAT enzyme. The heterocyclic groups were compared in terms of *in vitro* potency against their diarylimidazole analogues. Data for the purposes of QSAR were also collected. Our goal is a systemic ACAT inhibitor, which would be a potential antihypercholesterolemic and antiatherosclerotic agent.

Introduction. Atherosclerosis is one of the major causes of heart disease, which is a significant problem in the industrialized western world. Because cholesterol deposition in the arterial wall is a significant development in the course of atherogenesis, agents which limit absorption and accumulation of cholesterol in various tissues have been sought as potential therapy. A newer 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 the intestine 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, inhibition of ACAT in arterial macrophages should result in decreased development of foam cells.[4] The reversal of atherogenesis may also be possible by macrophage-mediated reverse transport of cholesterol. Thus, intestinal and systemic ACAT inhibition may prove to be an effective therapeutic approach.[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 significant lowering of cholesterol levels in the cholesterol-fed hamster.[6] In our efforts to fully explore the SAR of this family of compounds, various substitution patterns on the imidazole were prepared (see Table 1). From the biological results that were obtained, we recognized that the 4,5-diaryl imidazole compounds are superior. While the full mechanistic implications of the need for the diaryl imidazole group are not yet understood, it

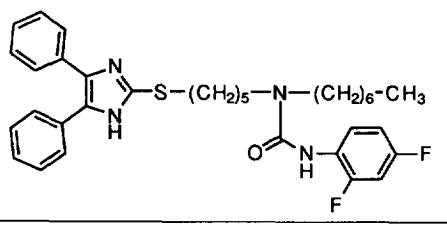
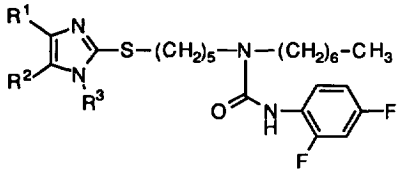


Fig. 1. ACAT Inhibitor DuP 128.

Table 1. Imidazole Substitution SAR in the DuP 128 Series.[6]



R^1	R^2	R^3	AIV[a]	J774[b]
H	H	H	2.85	5.50
Ph	H	H	0.49	1.60
Ph	Ph	Ph	c	54.5
Ph	Ph	CH_3	3.56	3.80
Ph	H	Ph	7.20	6.02
Ph	Ph	H	0.01	1.00

Key: (a) ACAT *in vitro* IC_{50}, μM (see text); (b) J774 macrophage cell IC_{50}, μM (see text); (c) inactive

seemed logical to probe this effect by substituting various other heterocyclic groups for the diaryl imidazole. In addition, we began to consider other parameters of importance for compounds of therapeutic potential, particularly lipophilicity. It was believed that compounds of lower lipophilicity might also show more favorable bioavailability, although the imidazole isostere concept was still of greatest importance here.

To explore the role of the heterocyclic group in this series of ACAT inhibitors, other heterocycles (X = O or S, Fig. 2) were prepared. Also, by fusing the 4,5-substituents into a ring, the molecular weight is reduced. By introducing nitrogen atoms at the A and B positions, water solubility should be increased. The net effect of these actions should be an increase in bioavailability. Five-membered aromatic heterocycles are different from six-membered rings in many ways, including basicity, electron density, steric size, *etc.* We also prepared analogues bearing six- and even seven-membered rings, in order to explore the effect of changing these parameters (Fig. 3). Other non-aromatic five-membered rings were also considered. Note that the urea group was maintained in the molecules throughout this study, in order to more fully understand the heterocyclic pharmacophore.[7]

Biology. To assay the compounds prepared for this study for *in vitro* ACAT inhibition, two primary assays were performed: 1) ACAT In Vitro (AIV): the formation of labeled cholesteryl oleate (pmol/min/mg) in the presence of rat hepatic microsomes is determined. The results are given as IC_{50}s in μM; 2) J774 Macrophage Cell Culture (J774): the formation of cholesteryl ester (CE) is measured by following the rate (nmol CE formed/hr/mg cell protein) 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.

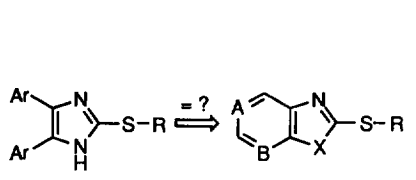


Fig. 2

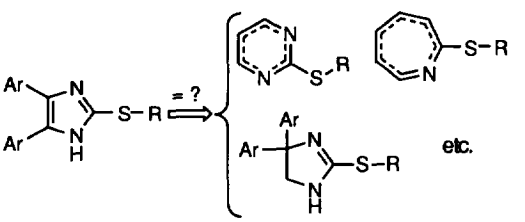
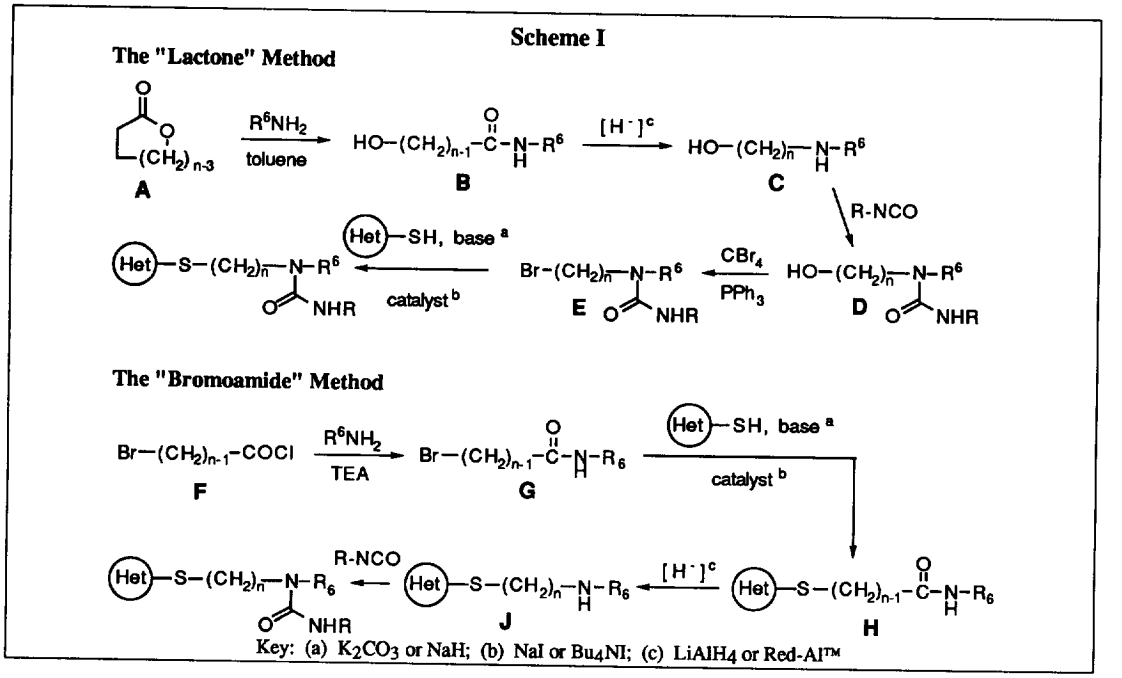
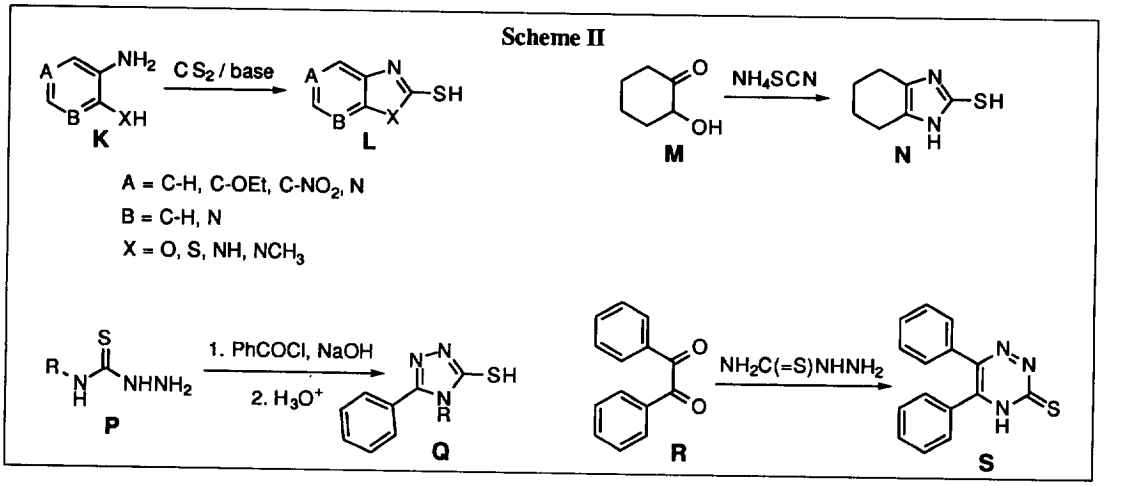
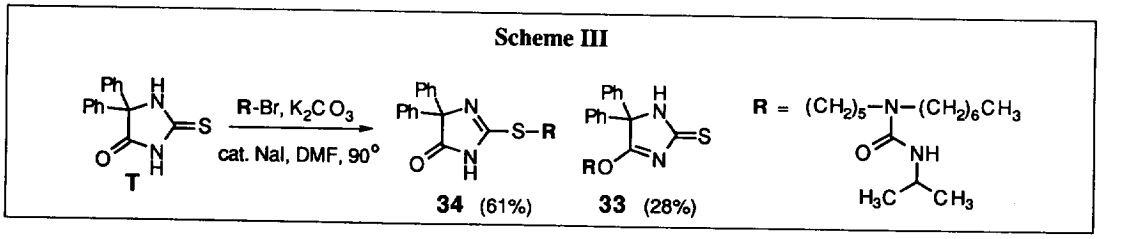


Fig. 3


Scheme I
The "Lactone" Method
R6NH2
toluene
[H-]c
R-NCO
Het—SH, base a
CBr4
PPh3
catalyst b
The "Bromoamide" Method
R6NH2
TEA
Het—SH, base a
catalyst b
R-NCO
[H-]c
Key: (a) K2CO3 or NaH; (b) NaI or Bu4NI; (c) LiAlH4 or Red-Al™


Scheme II
CS2 / base
NH4SCN
A = C-H, C-OEt, C-NO2, N
B = C-H, N
X = O, S, NH, NCH3
1. PhCOCl, NaOH
2. H3O+
NH2C(=S)NHNH2


Scheme III
R-Br, K2CO3
cat. NaI, DMF, 90°
34 (61%)
33 (28%)
R = (CH2)5—N—(CH2)6CH3

Chemistry. The compounds of this study were generally prepared by alkylation of a heterocyclic mercaptan with a bromide compound whose structure contained the urea moiety (or its precursor) (Scheme I). Usually, the starting material was a lactone (**A**), which underwent ring opening upon treatment with an amine. The resulting amide (**B**) could be reduced by a number of reagents, such as LAH. Selective functionalization of the amino group of **C** with an isocyanate gave hydroxy urea compound **D**, which in turn was converted to bromide **E** using carbon tetrabromide / triphenylphosphine. The alkylation reaction of the heterocyclic mercaptan proceeded easily in refluxing THF in the presence of tetra-*n*-butylammonium iodide as a catalyst and potassium carbonate to absorb the HBr that is generated. Alternatively, the sequence may proceed through the intermediacy of a bromoamide (compound **G**), derived from acid chloride **F**. Alkylation as above gave amide **H**; reduction to amine **J**, followed by treatment with an isocyanate, afforded the final products.

Preparation of the heterocyclic thiols proceeded by standard means (Scheme II). Fused-ring heterocyles such as compound **L** were prepared by condensation of a diamino-, aminohydroxy- or aminomercapto-pyridine or pyrimidine (**K**) with carbon disulfide. Appropriate conditions include refluxing pyridine or ethanol/NaOH. Fused aliphatic rings were derived from α-hydroxyketones such as **M**. Condensation with ammonium thiocyanate afforded imidazole **N**. Triazole **Q** was prepared by condensation of thiosemicarbazide **P** with an acid chloride. The reaction of benzil (**R**) with thiosemicarbazide gave the triazine **S**.

An interesting observation was made in the alkylation of 5,5-diphenyl-2-thiohydantoin (**T**) with bromoureas like **E** (Scheme III). The major product (61%) was the expected sulfide product (**34**). However, a substantial quantity of alkylation (28%) occurred at the oxygen atom to give ether compound **33**, whose spectra (and microanalysis) are completely consistent with the proposed structure. We anticipated the oxygen atom would be the less reactive and sterically-demanding site, but apparently this preference is less than predicted. Finally, Scheme IV shows the synthesis of the novel benzodiazepinone-bearing compounds **28** and **29**, which employed a strategy common to synthesis of other benzodiazepin drugs.

The compounds **1** - **34** were then assayed for ACAT inhibition. Results may be found in Tables 2 and 3 below. In addition, an attempt at establishing a QSAR was made by plotting the *in vitro* IC_{50} data against the CLOGP (obtained from MEDCHEM software). This is shown in Figure 4.

Discussion and Conclusions. Examination of the data presented in Tables 1 and 2 show that for five-membered rings fused to another ring, the most potent in inhibiting microsomal ACAT were imidazoles. Oxazoles, thiazoles and N-substituted imidazoles were less potent, perhaps due to a need for an imidazole N-H. Triazoles were also less potent, but the hydantoin compound **33** was within one order of magnitude of DuP 128.

Scheme IV

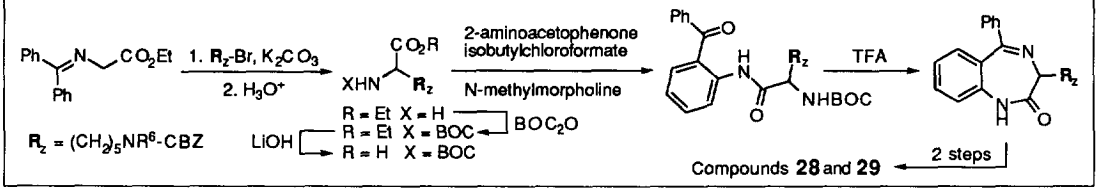
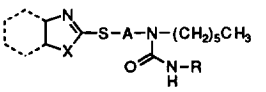


Table 2. Biological Results.



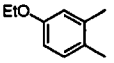
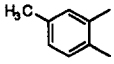
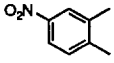
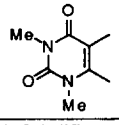
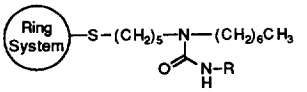
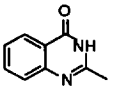
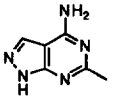
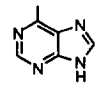
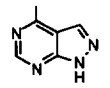
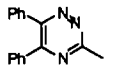
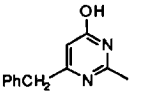
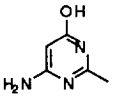
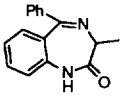
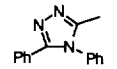
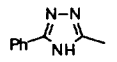
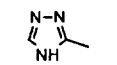
Cmpd No.	Fused Ring	X	A	R	AIV	J774	CLOGP
1		O	$(CH_2)_5$	a	51.0	3.50	7.18
2		NH	$(CH_2)_5$	a	0.50	3.10	7.29
3		NMe	$(CH_2)_5$	a	2.49	3.02	7.47
4		S	$(CH_2)_5$	a	4.00	2.73	7.78
5		S	$(CH_2)_5$	a	c	4.54	8.46
6		NH	$(CH_2)_5$	a	0.81	2.58	6.81
7		NH	$(CH_2)_5$	a	1.00	2.06	7.79
8		NH	$(CH_2)_5$	a	2.00	8.33	7.19
9		NH	$(CH_2)_5$	b	7.15	7.10	5.62 [f]
10		NH	$(CH_2)_5$	a	3.60	5.47	6.22
11		NH	$(CH_2)_5$	b	23.7	19.1	4.66 [f]
12		NH	$(CH_2)_5$	a	0.86	c	5.26
13		NH	e	b	2.00	60.0	3.72 [f]
14		NH	e	a	c	d	4.32
15		NH	$(CH_2)_5$	b	3.60	1.53	5.10 [f]

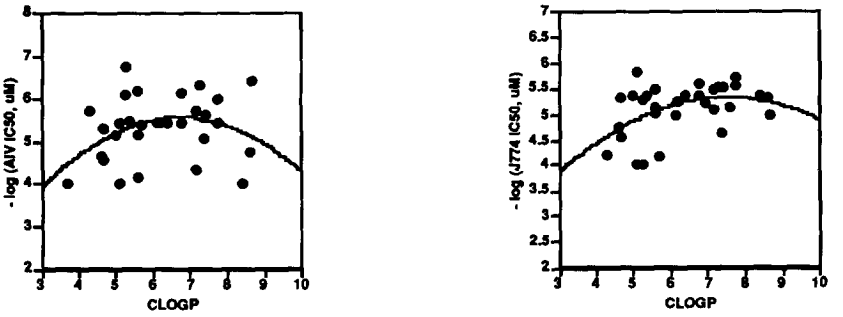
Table 3.



Cmpd No.	Ring System	R	AIV	J774	CLOGP
16		a	4.00	11.0	6.17
17		a	4.00	4.51	6.41
18		a	3.30	4.37	5.41
19		a	4.23	72.5	5.74
20		b	c	c	5.14 [f]
21		a	0.20	5.32	5.32
22		b	29.7	4.72	4.72 [f]
23		a	0.71	3.31	5.60
24		b	7.13	4.35	5.00 [f]
25		a	0.44	10.6	8.72
26		b	9.20	25.0	7.40 [f]
27		b	74.0	9.30	5.61 [f]
28		a	d	6.96	7.59
29		b	d	6.53	6.99 [f]
30		a	19.0	4.80	8.64
31		a	4.00	4.18	6.78
32		a	5.00	27.3	4.68
33	(See Scheme III)	b	0.09	2.79	d
34	(See Scheme III)	b	2.79	2.14	d

Key: (a) 2,4-difluorophenyl; (b) isopropyl; (c) inactive (>50 μM); (d) not determined; (e) $R = (CH_2)_2O(CH_2)_2$; (f) estimated.

Fig. 4



This novel compound will be studied in greater depth. For six-membered heterocycles, two compounds (**21** & **25** were in the sub-micromolar range. However, none were of exceptional potency. In the J774 macrophage assay, all compounds had IC_{50}'s greater than 1 μM. Non-imidazole heterocyclic substitution apparently does not contribute to the inhibition of ACAT in the macrophage cell. We next considered the QSAR of this series. The literature[9] suggests that hydrophobic effects are generally additive in estimation of the partition coefficient, which we hoped would be a model for the absorption of these compounds. Good bioavailability will be a likely property of a systemic inhibitor. Figure 4 shows that while the curves that were obtained show a large amount of scatter, the shape of the curves is the typical bell-shape, with a $CLOGP_{max}$ of about 7 for each assay. Extremely hydrophilic heterocycles are thus expected to decrease potency. Overall, we were unable to find a bioisostere for 4,5-diarylimidazole in the DuP 128 class of ACAT inhibitors. Further reports from these laboratories will discuss our efforts to find bioisosteres for other groups on the DuP 128-type structure.[10]

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(10) We acknowledge the efforts of D. Cromley, B. Thomas and R. Stevenson for running the biological assays.

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