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ACYL CoA:CHOLESTEROL ACYLTRANSFERASE (ACAT) INHIBITORS: HETEROCYCLIC BIOISOSTERES FOR THE UREA GROUP IN DuP 128

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Abstract. A series of compounds bearing two heterocyclic substituents were evaluated for inhibition of the ACAT enzyme. One heterocyclic group was chosen to mimic the urea group in our clinical candidate, DuP 128. Of the several groups examined, aminobenzoxazoles proved to be the most potent in terms of inhibition of ACAT in J774 macrophage cells.

Introduction. The demonstrated presence of ACAT in intestinal, hepatic and arterial tissues has lead to the hypothesis that ACAT inhibitors could act as both antihypercholesterolemic and antiatherosclerotic agents.[1] Several companies have thus entered the clinic with candidates,[2] including DuPont Merck with DuP 128 (Fig. 1).[3] This compound was shown to be a potent inhibitor of ACAT in rat hepatic microsomes (IC_{50} = 0.01 μM), and was also an effective agent for lowering serum cholesterol levels in hamsters when dosed orally (ED_{50} = 3 mg/kg). However, DuP 128 was not as potent an inhibitor of ACAT in J774 macrophage cells (IC_{50} = 1.0 μM), and it was thought that more macrophage-selective compounds would be better candidates for arterial (and thus antiatherosclerotic) agents.[4] Toward this end, a number of areas of the molecule were targeted for replacement by potential bioisosteric groups, including the diarylimidazole[5] and the urea moieties. Synthesis and biological activity of compounds with modifications to the latter area are presented in this paper.[6]

The discovery of novel bioisosteres for urea groups proceeded by incorporation of the substituent groups on the urea into aromatic heterocyclic structures. Two of the possible heterocycles which fit this criteria are shown in Fig. 2. Substituents on these heterocycles could be added to fulfill spacial requirements of the inhibitor-enzyme interaction, and electronic requirements (*e.g.* hydrogen-bond accepting electron lone pairs) might be satisfied by the heteroatoms of the ring. The resulting compounds would represent novel ACAT inhibitors,[7] and were hoped to lead to a new series of more macrophage-selective, more bioavailable antihypercholesterolemic agents.

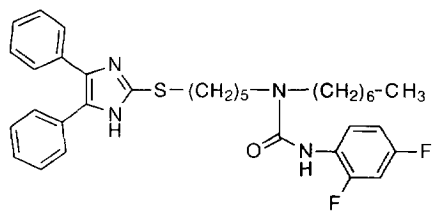


Fig. 1. ACAT Inhibitor DuP 128.

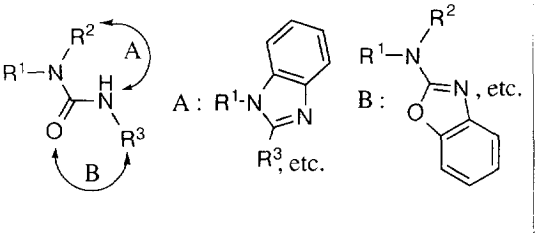


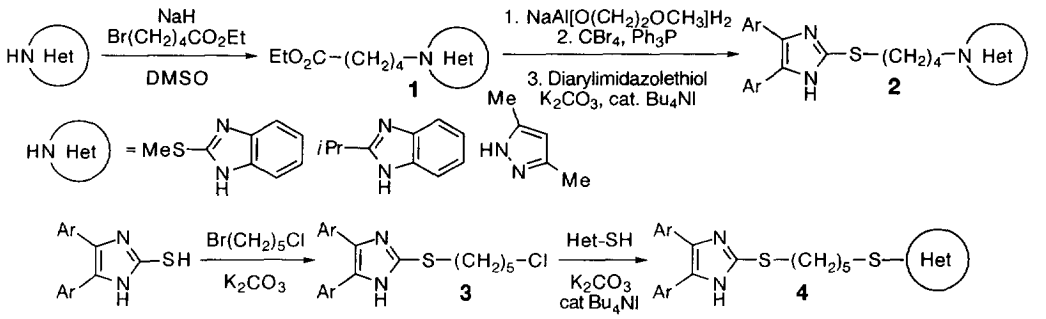
Fig. 2. Heterocyclic urea bioisostere design.

Biology. To assay the compounds prepared for this study for *in vitro* ACAT inhibition, two primary assays were utilized: 1) ACAT In Vitro (AIV),[8] which determined the formation of labeled cholesterol oleate in the presence of rat hepatic microsomes. The results are given as IC_{50}s in micromolar; 2) J774 Macrophage Cell Culture (J774),[9] which measured the formation of cholesteryl ester by following the rate of labeled oleate incorporation into CE. Results are also given as an IC_{50} in micromolar. 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.

Chemistry. Scheme I shows the synthesis of two varieties of this family of ACAT inhibitors. Compounds linked though a ring nitrogen atom were prepared starting with the heterocycle, which was alkylated with ethyl 5-bromovalerate to give ester **1**. The ester group was reduced, and the resulting hydroxy group was converted to bromide in straightforward fashion. The second alkylation reaction was then performed using the diaryl imidazolethiol to give product **2**. Similar reactions may be used to prepare bis-heterocyclic sulfides (**4**). Here, it is necessary to use a differentiated dihalide in order to avoid symmetrical dialkylation. The intermediate product **3** may be isolated and used in a second alkylation reaction with a different heterocycle, if so desired.

The method of preparation of compounds with amino heterocycle substituents is shown in Scheme II. The amine **5** (prepared according to the methods of the earlier papers in the series[3,4,5]) may be alkylated with a benzoxazole or benzothiazole (**6**) bearing a leaving group at the 2-position. For these reactions, chloride was found to be better than methylsulfonyl (80-95 *vs.* 20% yield). 2-Chlorobenzoxazoles are readily available from treatment of 2-mercaptobenzoxazoles with chlorine gas.[10] An alternate method was also investigated, which

Scheme I



Scheme II

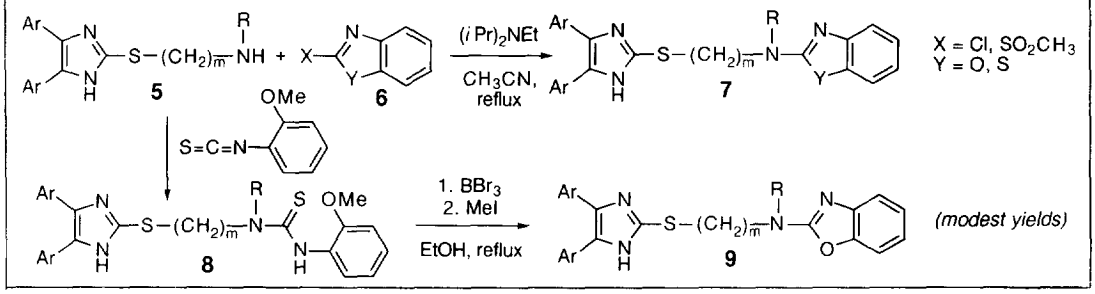
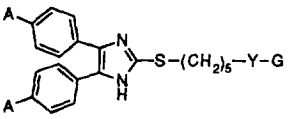


Table 1.



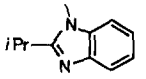
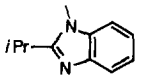
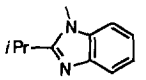
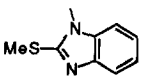
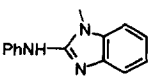
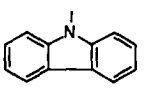
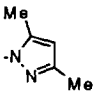
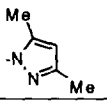
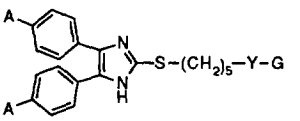
Cpd	A	Y	G	AIVa	J774a
10	H	bond		0.69	0.71
11	MeO	bond		0.23	0.32
12	Me_2N	bond		0.40	0.08
13	MeO	bond		1.40	0.95
14	MeO	bond		0.75	0.64
15	MeO	bond		50	55.7
16	H	bond		0.03	0.09
17	Me_2N	bond		g	0.25

Table 2.



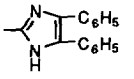
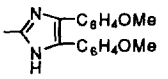
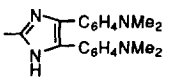
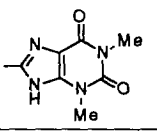
Cpd	A	Y	G	AIV	J774
18	H	S		0.03	6.92
19	MeO	S		0.22	0.20
20	Me_2N	S		0.09	0.06
21	MeO	S		g	8.33

Table 2, cont.

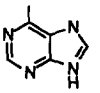
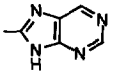
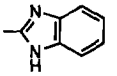
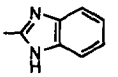
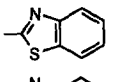
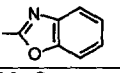
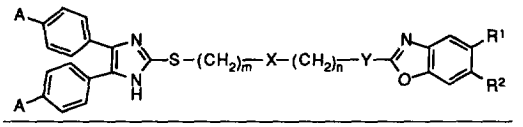
Cpd	A	Y	G	AIV	J774
22	H	S		g	7.51
23	MeO	S		g	3.65
24	MeO	S		g	0.88
25	MeO	S		0.63	0.83
26	MeO	NC_7H_{15}		0.09	0.49
27	H	NC_7H_{15}		g	0.66
28	H	NC_7H_{15}		0.30	0.02

Table 3.



Cpd	A	m	X	n	Y	R^1	R^2	AIV	J774
28	H	2	CH_2	2	NC_7H_{15}	H	H	0.30	0.02
29	MeO	2	CH_2	2	NC_7H_{15}	H	H	0.04	0.08
30	MeO	1	CH_2	2	NC_7H_{15}	H	H	g	0.50
31	MeO	2	CH_2	2	NC_4H_9	H	H	0.08	0.07
32	MeO	2	CH_2	2	NC_2H_5	H	H	0.14	0.09
33	Me_2N	2	CH_2	2	NC_2H_5	H	H	g	0.05
34	Me_2N	2	CH_2	2	NH	H	H	g	0.09
35	b	2	CH_2	2	NC_2H_5	H	H	0.70	0.50
36	MeO	2	CH_2	2	c	H	H	0.04	0.08
37	MeO	1	d	1	S	H	H	g	3.24
38	H	3	e	1	NH	H	H	g	5.00
39	H	1	f	1	NH	H	H	g	5.90
40	MeO	2	CH_2	2	NC_4H_9	tBu	H	0.08	0.07
41	MeO	2	CH_2	2	NC_4H_9	Cl	NO_2	g	1.00

Key: (a) IC50's given in μM; (b) One A = H, the other = SMe; (c) N-$(CH_2)_2$-O-$(CH_2)_2$-OCH_3; (d) *meta*-phenylene; (e) 1-phenylethane-1,1-diyl; (f)  (g) not determined.

first began with the conversion of the amine **5** to 2-methoxyphenylthiourea **8**. Demethylation was followed by ring closure to **9** effected by treatment with excess iodomethane, which produces dimethylsulfide as the byproduct.[11] This method was inferior to the chloride route, because substituted 2-methoxyphenylisothiocyanates are not readily available, and the overall yield of the sequence (20 %) is lower.

Discusssion/Conclusion. Table 1 presents the biological data for the "Type A" compounds, those which mimic the urea by linking the heterocycle through a ring nitrogen atom. The benzimidazoles **10-14** were of comparable potency for the macrophage cell to DuP 128. The SAR in this series of the diarylimidazole was similar to that of the ureas, *i.e.*, bis(4-dimethylaminophenyl)>bis(4-methoxyphenyl)>diphenyl. For the benzimidazole 2-substituent, isopropyl was superior to phenylamino or methylthio. Interestingly, for the dimethylpyrazoles, substitution by dimethylamino at the 4-position of the imidazole phenyl groups caused a significant dropoff in potency between **16** (Rhone-Poulenc Rorer's RP-70676) and **17**. This is contrary to the SAR in the ureas, which suggests that dimethylpyrazole is not actually an isostere for urea.

Table 2 shows the data for compounds of "Type B", those which mimic the urea by linking the heterocycle through an exocyclic heteroatom. The bis(diarylimidazoles) **18-20** show an increasing selectivity for macrophage ACAT in the same order as the benzimidazoles. The uracil and purine compounds **21-23** were poorly active, but some improvement was observed for the 8-substituted compound **24**. Marked improvement was observed for replacing the sulfur linking atom with nitrogen in the benzimidazoles **25** and **26**. Little difference was seen between benzimidazole **26** and benzothiazole **27**, but dramatic improvement in J774 activity was observed for benzoxazole **28**. Table 3 shows the benzoxazole series. Shortening the alkyl chain on the 2-amino group (**29**,**31**,**32**) did not greatly affect the J774 potency. Replacing the 2-amino for 2-thio (**37**), as well as building in rigidifying groups in the trans-heterocycle tether (**37**,**38**,**39**), significantly lowered potency. Substituents on the benzoxazole may be bulky (**40**), but they may not be electron-withdrawing (**41**). Compound **28** showed 30% *in vivo* cholesterol lowering at a single dose of 25 mg/kg in the hamster (see ref. 3b for details on this model). In summary, several series of compounds were prepared bearing heterocycles designed to replace the urea group in the DuP 128 family of ACAT inhibitors. Many of these compounds showed improved potency for inhibition of ACAT in the J774 macrophage cell over their urea counterparts.[12]

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