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NOVEL 4,4-BIS(TRIFLUOROMETHYL) IMIDAZOLINES AS STEREOSPECIFIC AND ORALLY ACTIVE ACYL COA: CHOLESTEROL ACYLTRANSFERASE (ACAT) INHIBITORS AND ANTIHYPERCHOLESTEROLEMIC AGENTS[1]

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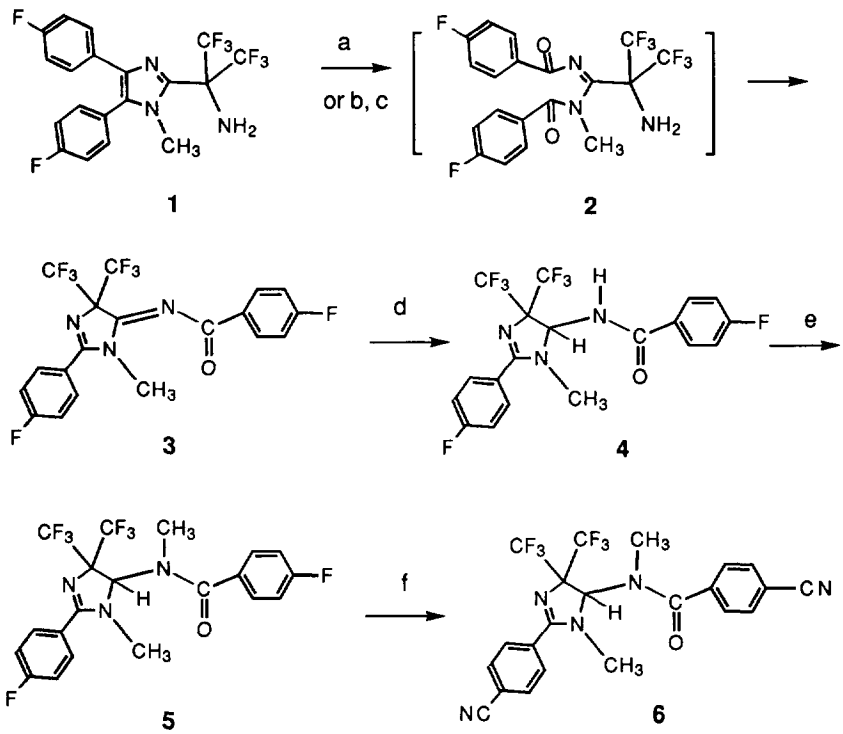
Abstract: A new class of very potent, systemically-active ACAT inhibitors based on a 4,4-bis(trifluoromethyl)imidazoline ring has been discovered. Compound **6** and its *R*-enantiomer **6a** exhibited very potent oral activities in lowering the serum cholesterol in the animals. Copyright © 1996 Elsevier Science Ltd

Hypercholesterolemia is a major risk factor for coronary heart disease.[4] Considerable effort has been directed toward the development of effective hypocholesterolemic agents and those that control the level of serum cholesterol have proven to be effective in the treatment of atherosclerosis.[5] Much of the free or unesterified cholesterol that is absorbed by intestinal mucosal cells must first be esterified by Acyl-CoA: cholesterol acyltransferase (ACAT)[6,7] prior to its incorporation and secretion into the bloodstream in large lipoprotein particles called chylomicrons, inhibition of ACAT should have beneficial effects on plasma cholesterol *via* the prevention of the absorption of dietary cholesterol in the intestine.[8] Bioavailable or systemic ACAT inhibitors may also inhibit foam cell formation in the arterial wall and thus should prevent cholesteryl ester deposition in the macrophage and promote cholesterol efflux,[9] thereby preventing the progression of atherosclerosis. Although many compounds have been reported as potent ACAT inhibitors during the recent years,[5,10-13] much of the attention has focused on mimics the long fatty acid chain by using an amide or urea moiety. The majority of these compounds are poorly or erratically absorbed orally in the animal.

We have discovered a novel 4,4-bis(trifluoromethyl)imidazoline series,[14] which are stereospecific, systemically-active ACAT inhibitors with high oral bioavailability. These compounds are potent inhibitors of rat microsomal ACAT and cholesterol esterification in the J774 Macrophage in vitro and are potent antihypercholesterolemic agents in the cholesterol-fed rabbit orally. This series of compounds also lowers serum cholesterol in the normolipemic and cholesterol-fed hamsters by the oral and intra peretoneal routes. Preliminary biological studies supported by X-ray crystal structure analysis, molecular modeling and structure activity relationship (SAR) studies indicate that the ACAT inhibition is competitive with respect of cholesterol and they may be cholesterol mimetics.

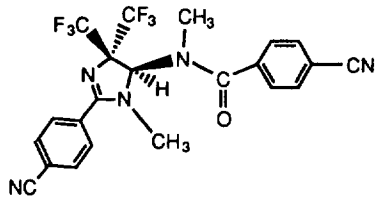
Compound **6** was prepared according to the methods shown in **Scheme 1**. Treatment of α,α-bis(trifluoromethyl)-4,5-bis(*p*-fluorophenyl)-1-methyl-1H-imidazole-2-methanamine **1** with 2 eq. of mCPBA in refluxing chloroform cleaved the imidazole ring to give the amidine **2**, which spontaneously cyclized to afford **3** in 72% yield.[15] Alternatively, oxidation of **1** with singlet oxygen[16] generated by irradiating oxygen with a 400 watt Tungsten lamp in the presence of methylene blue as a photosensitizes followed by acid treatment gave the compound **3** in 69% yield. Reduction of acyl imine **3** to the amide **4** was accomplished with $LiAlH_4$ in THF at room temperature with surprisingly high selectivity in 96% yield. Alkylation of **4** with MeI using NaH as a base gave **5** in 94% yield. Treatment of the *para*-fluoro substituted imidazoline **5** with anhydrous KCN in DMSO with K_2CO_3 and KI at 110°C overnight gave the dicyano substituted imidazoline **6** in 85% yield.

Scheme 1[a]

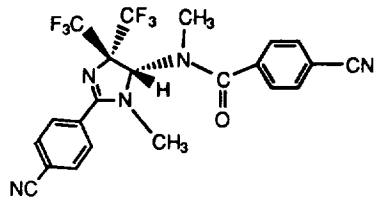


[a] Reagents: (a) *m*CPBA, $CHCl_3$, reflux (72%); (b) oxygen or air, methylene blue, CH_3OH, $CHCl_3$; (c) HCl in ether; (d) $LiAlH_4$, THF (96%); (e) NaH, MeI, DMF (94%); (f)KCN, KI, K_2CO_3, DMSO (85%).

The racemic mixture **6** was resolved into its two pure enantiomers, **6a** and **6b**, by chiral HPLC on a Diacel OJ column and their absolute configuration has been established by the exiton CD method.[17,18] The R enantiomer (**6a**) bears most of the ACAT inhibitory activity as shown in Table 1 and the eudismic ratio relative to ACAT in vitro IC_{50}'s is greater than 25 indicating a high degree of stereospecificity.



6a



6b

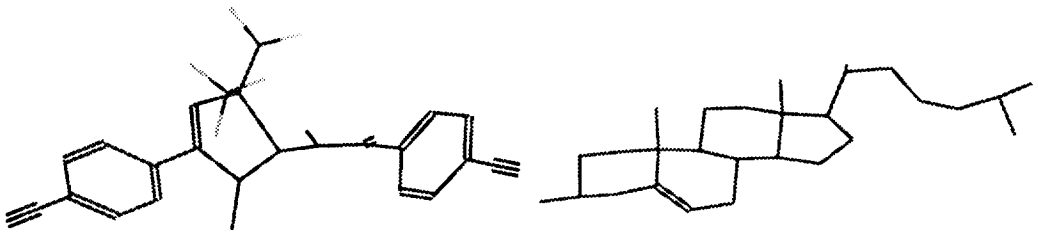
It is clear that the ACAT inhibitory activity of our 4,4-bis(trifluoromethyl)imidazoline series is very dependent on the nature of substitutents on the phenyl groups. It has been hypothesized that the ACAT inhibitory activity of these compounds is competitive with respect to cholesterol and these compounds may be a cholesterol mimics. Molecular modeling shows that **6a** occupies about the same space as cholesterol does (**Figure. 1**), Further modeling studies have shown that *6a overlaps with cholesterol amazing well*, whereas its antipode (**6b**) does not. This may also explain the high stereospecificity in this series.

Compound **6a** has an IC_{50} 2.1 μM in ACAT in vitro assay and 11 μM in the macrophage assay. The in vitro potency of this compounds is considered only moderate compared with other ACAT inhibitors in the literature, but this compound is very potent in our in vivo assays. It gave 75% decrease in serum cholesterol in the cholesterol-fed rabbits at 5mg/kg/day for four days while **6b** gave only 12% decrease. Its systemic activity has been demonstrated by a single oral dose of **6** in rabbits and 77% of ACAT inhibition was observed even after 24 hours of dosing in rabbits.

Table 1. ACAT inhibitions of **6**, **6a**, and **6b** and their effect on serum cholesterol in the rabbits

	ACAT[a] IC_{50} (μM)	J774[b] IC_{50} (μM)	Serum Cholesterol Reduction (%)[c]	AHV(%)[d]
6	1.9	16	79	>90%
6a	1-2	11	75	90%
6b	55	54	12	15%

(a) In vitro rat liver microsomal ACAT inhibition.[19,20] (b) in vitro J774 Macrophage cell culture ACAT inhibition.[21-23] (c). Animals were orally gavaged with drug suspended in a methylcellulose vehicle at a dose of 2.5 mg/Kg/day of **6** for 7 days and 5 mg/Kg/day of **6a** and **6b** for 4 days. Control animals received vehicle only. (d). Blood was obtained from the orbital sinus of each animal and serum was analyzed for total serum cholesterol. Antihypercholesterolemic value (AHV) is the ratio of the observed reduction in serum cholesterol to the difference between the control and baseline x 100.



6a Cholesterol

Figure 1. MM-2 Energy Minimized Conformations of **6a** and cholesterol

The potent in vivo activity of **6a** may be attributed to its high oral bioavailability (>20% in rabbits) and unique structure. The *para* cyano group of the left phenyl is at the same position of 3 hydroxyl of cholesterol according to the computer modeling, **6a** may form a stronger hydrogen bond than cholesterol with ACAT thus inhibiting the esterification of cholesterol.

In summary, we have discovered a new class of very potent, systemically-active ACAT inhibitors based on a novel 4,4-bis(trifluoromethyl)imidazoline ring and the hypothesis of that these compounds may be steroidal mimics. We are exploring this concept in other steroids related systems. The detailed QSAR studies, chemical synthesis and pharmacological results of this series will be the subject of future publications.

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