2. Literature Review

At present ACAT has not been isolated in its pure form. This has prevented the use of crystallographic and computer assisted drug design techniques in the designing and synthesis of potent inhibitors. However Chang and co-workers have cloned an ACAT cDNA from human macrophage cDNA library. This clone, labeled K1, was expressed in an ACAT deficient line of Chinese hamster ovary cells and shown to encode an integral membrane protein of 550 amino acids\textsuperscript{23}. Even in the absence of this information, a very large number of structurally diverse ACAT inhibitors have been discovered using more traditional techniques.

Compound (1), a prototypical fatty acid amide was shown to be potent and specific ACAT enzyme inhibitor, which lowered plasma cholesterol in a variety of cholesterol fed rodent models. When humans were treated with 2.25 g/day of compound (1), 19.6\% decrease in total

\begin{equation}
\text{(1) Melinamide}
\end{equation}

cholesterol was observed over 12 months. It was the only marketed ACAT inhibitor in Japan at one time.\textsuperscript{52} However, it was shown that this compound was poorly bioavailable and lowered arterial ACAT directly but later it was discontinued in Japan.\textsuperscript{51}

2.1 Amide containing ACAT inhibitors

The potential of ACAT inhibitors to block atherosclerosis was appreciated as early as 1986 and the early work on ACAT inhibitors were directed at finding agents that would lower plasma total cholesterol and/or LDL-C by blocking cholesterol absorption.\textsuperscript{15,40} Thus, several fatty

\begin{equation}
\text{(2) SaH 57-118}
\end{equation}

\begin{equation}
\text{(3) SaH 58-035}
\end{equation}

acid amides such as \textbf{SaH 57-118} (2) and \textbf{SaH 58-035} (3) were developed during the early work on ACAT inhibitors at Sandoz,\textsuperscript{15,46} that were found to potently and selectively inhibit ACAT in
vitro, block cholesterol absorption and lower plasma total cholesterol in cholesterol-fed animal models in vivo. Compound (3) was noted to down-regulate LDL uptake in HepG2 cells.

A systematic study to evaluate the potential fatty acid amides as ACAT inhibitors was carried out by scientists at Parke-Davis. A series of oleic acid anilides were synthesized and evaluated for their ability to inhibit ACAT in vitro. The 2,4,6-trimethoxy substituted phenyl ring

![Chemical Structure](image)

\[ \text{(4)} \]

a: \( X = \text{H} \)
b: \( X = 2,4,6-(\text{OMe})_3 \)
c: \( X = 2,6-(\text{i-Pr})_2 \)

of anilides (4b, IC\text{\textsubscript{s0}} 50 nM) showed 500 fold improvement in potency compared to the unsubstituted anilide derivative (4a). A bulky 2,6-diisopropyl substitution resulted in a highly potent compound (4c, IC\text{\textsubscript{s0}} 7 nM) in this series.\textsuperscript{54}

The first bioavailable ACAT inhibitor that surfaced with the publication of studies in a unique atherosclerotic cholesterol-fed rabbit model was CI-976 (5). The discovery of this agent had prompted a renewed interest in amide containing compounds as ACAT inhibitors.\textsuperscript{54} The best profile of in vivo activity was found with 5 (IC\text{\textsubscript{s0}} 0.073 μM), which produced significant reductions in non-HDL-C and elevations in HDL-C as compared to cholesterol-fed controls.

![Chemical Structures](image)

(5) CI-976

(6) CP-113,818

Compound (5) has also been found to produce marked reduction in atherosclerotic lesions in cholesterol-fed rabbits.\textsuperscript{55} Based in part on this data, compound (5) has been proved to be a lipid regulating agent and selected for further detailed preclinical and clinical evaluation. Pfizer had identified CP-113,818 (6, IC\text{\textsubscript{s0}} 30 nM) an analog of CI-976 (5), that showed extremely potent in vitro inhibition (both in microsomes and cellular preparations) and was found to be efficacious in variety of animal models.\textsuperscript{56} It also decreased apoB secretion from perfused monkey livers.\textsuperscript{57}
In continuing research at Parke-Davis, the fatty acid anilide moiety of CI-976 (5) was replaced with substituted β-ketoamide to incorporate extra carbonyl group and conformational constraint in the compound (7) expecting better inhibitions of ACAT enzymes.\textsuperscript{58} Introduction of the β-keto group (7a), while maintaining the same chain length as existing in CI-976 (5) resulted in similar in vitro potency. The C-13 alkyl chain in compound (7a) showed an exceptionally potent ACAT inhibition (IC\textsubscript{50} 0.006 µM). The plasma total cholesterol was lowered by 66\% at 30 mg/kg and 38\% at 3 mg/kg with this compound. Thus, its potency appears to be greater than that observed for CI-976, which typically lowers plasma total cholesterol by 56\% and 19\% at 30 and 3 mg/kg doses, respectively. The introduction of α,α-dimethyl substitution in acyclic β-ketoamide (7b) did not cause any improvement in its in vitro potency unlike CI-976. However, N-methylation of the anilide nitrogen (7c) showed a marked reduction in its in vitro activity. This indicated that the amide NH was essential for ACAT inhibition, which was not addressed in the previously reported series of amides and ureas. Addition of free hydroxyl group in this series at the end of the alkyl chain decreased lipophilicity and in vitro activity but masking this hydroxyl unit with a tetrahydropyran (THP) ring restored both in vitro and in vivo activity (7d, IC\textsubscript{50} 0.057 µM). It was established previously that 2,6-disubstitution on the phenyl ring was necessary for potent ACAT inhibition.

Further, the cyclic β-ketoamide analogs (e.g. 8) were synthesized in which the second carbonyl group was directly incorporated into a ring. Incorporation of this rigidity into the molecule did not affect activity significantly. The seven membered lactam ring, with C\textsubscript{12} alkyl chain substituted on it yielded slightly better in vitro and in vivo activity IC\textsubscript{50} 0.022 µM, 47\% reduction in plasma total cholesterol at 30 mg/kg while the compound (8) with five membered lactam ring having the same chain length had IC\textsubscript{50} of 0.053 µM and 20\% reduction at 30mg/kg.\textsuperscript{58}
Further interest in ACAT inhibitors at Parke-Davis resulted into synthesis of two series of conformationally constrained analogs such as imidazolidinones (9a, IC₅₀ 1.3 μM) and pyrazolones (9b, IC₅₀ > 5 μM). This type of modification caused a reduction in the ACAT inhibitory activity (in vitro and in vivo). On the basis of this study it was concluded that either the enzyme active site could not tolerate the rigid molecules or the requirement of hydrogen bond donor in the molecule was essential for activity.⁵⁹

To further explore structural requirements of amide containing ACAT inhibitors, the same group of researchers has investigated the effects of incorporation of the tetrazole moiety into the side chain of the α-substituted anilides. The parent compound (10a) showed significantly potent in vitro and in vivo activity (IC₅₀ 0.11μM & 40% decrease in plasma total cholesterol). SAR studies revealed that the replacement of the 2,6-dimethylphenyl moiety of compound (10a) with 2,6-disopropylphenyl (10b, IC₅₀ 0.81 μM), 2,6-dichlorophenyl (10c, IC₅₀ 0.38 μM) or 2,4,6-trimethoxyphenyl (10d, IC₅₀ 1.70 μM) failed to increase potency or efficacy. 3-Nitrobenzamide derivative (10e, IC₅₀ 0.08 μM) showed the optimal potency. The benzamide ring was replaced with a basic nicotinamide moiety (10f, IC₅₀ 0.08μM) to improve aqueous solubility and possibly absorption properties of the compound. The length of the alkyl chain attached to the tetrazole side chain was crucial for potent ACAT inhibition, whereas its position (in case of regioisomers) was less critical.⁶⁰,⁶¹ Since the fatty acid anilides had been shown to be significantly more potent
than the corresponding benzamide isosteres, it was sought to improve the \textit{in vitro} activity by replacing the benzamide bond with anilide bioisosteres (10g, IC$_{50}$ 0.010 $\mu$M). Individual enantiomers of 10g were resolved to assess the biological activity of individual enantiomeric forms. The \textit{in vitro} evaluation of the isomers suggested that the (+)-10g was more potent than (-)-10g.$^{60,62}$ Next, it was planned by this group to systematically replace substituents appended to the amide and tetrazole moieties with structurally diverse functionalities and assess their effects on biological activity. The structure-activity relationship studies identified aryl and heteroaryl replacements (10h, IC$_{50}$ 0.013 $\mu$M & 10i, IC$_{50}$ 0.013 $\mu$M)$^{60,63}$ for 2,6-diisopropyl phenyl to be potent inhibitors of liver microsomal and macrophage ACAT \textit{in vivo} and exhibited good cholesterol lowering activity (56-66 % decrease in plasma total cholesterol at 30 mg/kg) relative to the parent compound (10g). Surprisingly, the unsubstituted derivative (10k) was found to be essentially equipotent to the parent compound (10a) \textit{in vitro}, although it was not efficacious \textit{in vivo}.

Further, the $\alpha$-phenyl moiety was replaced with 4-fluorophenyl analogs. Compound (11b) showed equipotency to the parent compound (11a, IC$_{50}$ 0.023 $\mu$M). Replacement of the $\alpha$-phenyl moiety with smaller electron-withdrawing substituents (CN, tetrazoyl) showed reduction in potency in both microsomal and cellular assays whereas the 2-pyridyl analog (11c, 0.026 $\mu$M)
maintained the potency. Among all of the electron donating anilide substituents, the benzyl derivative (11d, $IC_{50}$ 0.049 $\mu$M) potently inhibited macrophage ACAT $in vitro$ and maintained excellent $in vivo$ hypocholesterolemic activity ($IC_{50}$ 0.049 $\mu$M). In order to evaluate the role of the dodecyl side chain appended to the tetrazole ring, $C_{12}$ functionality showed potent inhibitory activity. Few moieties in this series exhibited drug-related adrenal toxicity in guinea pigs following oral administration at a dose of 100 mg/kg whereas the corresponding $\alpha$-phenyl derivatives did not exhibit any histopathologic alterations to the adrenal or liver of guinea pigs.\(^{63}\)

The tetrazole moiety was replaced next by various heterocyclic rings. The two optimal isoxazole analogs (12a, 12b) showed good potency and were found to be nontoxic in a guinea pig model of adrenal toxicity.\(^{60,64}\) These two compounds (12a, 12b $IC_{50}$ 0.015 & 0.022 $\mu$M respectively) significantly lowered cholesterol in cholesterol-fed rabbit and cholesterol-fed dog models. Both of the compounds were selected for evaluation in a long-term model of atherosclerosis.

$\textbf{(12)}$

| a: $X = O$, $Y = N$
| b: $X = N$, $Y = O$  

Kumaza et al. have reported the synthesis of N-phenyl-6,11-dihydrodibenzo[\(b,e\)]oxepine-11-carboxamide and their derivatives.\(^{65}\) SAR studies of these compounds suggested that the 2,6-diisopropyl substitution in the anilide ring in compound (13) resulted into maximum potency,
although the 2,4,6-trimethoxy derivative also provided a good ACAT inhibitory activity profile. 3-Bromo derivative was found to be less potent than 2-bromo derivative. 2-Methylthio derivative showed the highest \textit{in vitro} potency (IC$_{50}$ 7 nM) but showed negligible \textit{in vivo} potency.

\begin{center}
\includegraphics[width=0.3\textwidth]{image.png}
\end{center}

(13) KF 17828

Replacement of the amide bond with thioamide resulted in complete loss of biological activity. Compound KF 17828 (13) was found to be the most promising one (IC$_{50}$ 23 nM) amongst the series.

Amides of some substituted 1,2-diaryl ethylamines (14) have been reported as very potent microsomal ACAI inhibitors \textit{in vitro} but found to be poor inhibitors in the \textit{in vivo} animal model.\textsuperscript{66} Designing of conformationally restricted amides of 1,2-diaryl ethylamine led to the synthesis of \textit{cis}-[2-(4-hydroxyphenyl)-1-indanyl]diphenylacetamide (15) which was found to be a potent ACAT inhibitor (IC$_{50}$ 0.04 µM in an \textit{in vitro} rat hepatic microsomal ACAT assay, ED$_{50}$ 0.72 mg/kg/day in cholesterol-fed hamsters).\textsuperscript{67}

The SAR studies on novel N-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)amide (16) derivatives revealed that a methyl group at 6$^{th}$ position of the 2,3-dihydrobenzofuran moiety was
important for potent ACAT inhibitory activity.\textsuperscript{68} Incorporation of highly lipophilic moieties exhibited improved potency and introduction of a dimethylamino group at position 5 of the 2,3-dihydrobenzofuran moiety also resulted in highly potent compound. The most potent compound \textbf{TEI-6620 (16)} of the series exhibited highly potent ACAT inhibitory activity (rabbit small intestine \textbf{IC}_{50} 0.020 \mu\text{M}, rabbit liver \textbf{IC}_{50} 0.009 \mu\text{M}), foam cell formation inhibitory activity (rat peritoneal macrophage \textbf{IC}_{50} 0.030 \mu\text{M}) and extremely potent serum cholesterol lowering activity in cholesterol-fed rats (71% at a dose of 0.3 mg/kg/day) with good bioavailability in dogs (Cmax 2.68 \mu\text{M/mL at 1 hr, 10 mg/kg po}).

A novel series of ACAT inhibitors were synthesized. The synthesized compounds inhibited rat hepatic ACAT in a more striking manner than \textbf{CI-976} and inhibited both microsom-

![Chemical Structure Image](image-url)

\textbf{(17)}

al ACAT prepared from HepG2 (a cell line derived from human hepatocarcinoma) and Caco2 (a cell line derived from human colon adenocarcinoma). Compound \textbf{(17, IC}_{50} 11 \text{nM}) did not show any adrenal gland toxicity in rats. The compound \textbf{(17)} could fulfill expectations as a new therapeutic drug or at least as a lead compound in future for further development as a potent drug for hypercholesterolemia and atherosclerosis.\textsuperscript{69}

A novel series of various 2,6-substituted imidazo[1,2-\alpha]pyridines were designed, synthesized and evaluated for their ability to inhibit ACAT activity. The compound \textbf{(18)} was
established to be a potent inhibitor of ACAT activity in HepG2 cell line and reduced cholesterol ester formation significantly in a dose-dependent manner.\textsuperscript{70}

### 2.2 Urea based ACAT inhibitors

In addition to the work on the amide ACAT inhibitors, a series of analogs have been prepared in which bioisosteres of the amide group are incorporated. The bioisosteric replacement rendered all of the analogs significantly less active than the parent compound but the urea analogs (IV & VII; Table 1) retained the \textit{in vitro} activity. As the urea derivatives were shown to be more efficacious than the amide analogs in lowering total plasma cholesterol in the cholesterol-fed rat model, a series of urea analogs were synthesized to explore the urea containing moieties as potential ACAT inhibitors.

#### 2.2.1 Trisubstituted ureas as ACAT inhibitors

As a class the development of trisubstituted ureas as therapeutic agents have been plagued with difficulties. Initially, DeVries et al. reported the synthesis and biological properties

![Chemical Structure](image.png)

(19) CL-277082
of CL-277082 (19). Compound (19) has shown significant in vitro ACAT inhibitory activity (IC\textsubscript{50} 0.14\textmu M) both in isolated microsomes and in intact cells.\textsuperscript{71,72}

The DuPont Merck Research Laboratories synthesized and reported a series of diphenyl substituted heterocycle based trisubstituted ureas. Dup-128 (20a) was a potent ACAT inhibitor

![Chemical structure of Dup-128 (20a)]

with the diaryl imidazole (70a) moiety that was 50 times more potent in the in vitro assay than the monoarylimidazole (20b, IC\textsubscript{50} 0.49\textmu M) and was approximately 300 times more potent than the corresponding 4,5-unsubstituted imidazole (20c, IC\textsubscript{50} 2.9\textmu M). N-Methylation on the imidazole nitrogen (20d, IC\textsubscript{50} 3.6 \textmu M) resulted in a 350-fold decrease in potency in comparison to the parent compound (20a). The requirement of unsubstituted imidazole NH for potent ACAT inhibition was further borne out by the observation that the 1,4,5-triphenyl analog (20e, IC\textsubscript{50} >50 \textmu M) was shown to be poor inhibitor of ACAT. The sulfide, sulfoxide or sulfone groups at 2-position of the imidazole ring maintained the potency and were presumed to be involved in electronically modifying the pKa of the imidazole. The length of five or more carbon atom chain between the sulfur and the tertiary nitrogen showed improved ACAT inhibitory activity due to the flexibility in the molecule. The isosteric replacement of urea moiety with other groups resulted in a 2-4 fold decrease in activity.\textsuperscript{73}

Dup-128 (20a) was found to be a potent inhibitor of ACAT in rat hepatic microsomes but had shown modest activity in whole macrophage cells (the J774 murine cell line; IC\textsubscript{50} 1.0 \textmu M). In their efforts to discover a systemic inhibitor, a compound with good potency in the hepatic microsomal and macrophage cells, the models for inhibition of ACAT in human liver and arterial tissue, was sought. Such a compound exhibited both the serum lipid-lowering and anti-
atherosclerotic properties. SAR studies revealed that the compounds bearing imidazole ring with two unsubstituted phenyl groups were potent in the avian influenza virus (AIV microsomal assay), but poor in the J774 (macrophage cell) assay. Activity against the AIV was observed to be better for difluorophenyl ureas while the isopropyl ureas showed better potencies against J774. Compounds bearing two diaryl imidazole rings exhibited better activity profile. For the compound (21) with good dual activity it was concluded that the “diphenyl” end of the molecule was responsible for AIV potency (IC$_{50}$ 0.03 μM), and the “substituted diphenyl” end was found to be responsible for good J774 macrophage potency (IC$_{50}$ 0.06 μM).\(^\text{74}\)

Further, the urea moiety of compound (21) was replaced by various bioisotopic aromatic heterocyclic groups.\(^\text{75}\) It was proposed that at least one of the heterocycles would fulfill the criteria like spatial and electronic requirements for the inhibitor-enzyme interaction. One ring would mimic the urea by linking the heterocycle through a ring nitrogen atom in compound (22a). In compound (22b) the 2-thiaimidazole would mimic the urea moiety as an exocyclic

\[
\text{(21)}
\]

\[
\text{Me}_3\text{N} - \begin{array}{c} \text{S-(CH}_2)_5 \end{array} \text{X-} \text{Y}
\]

\[
\text{(22)}
\]

\[
\text{a: } \text{X-Y = i-Pr} \quad \text{b: } \text{X=S, Y =}
\]
heteroatom. The compound (22a) showed very good potency towards the J774 macrophage cell cultures (IC$_{50}$ 0.08 µM) and 22b was found to be effective towards the AIV (IC$_{50}$ 0.03 µM).

From the biological data (Table 2) it was recognized that the 4,5-diaryl imidazole compounds were superior. The role of heterocyclic groups in ACAT inhibitors was explored.

**Table 2: Incorporation of different heterocycles and comparison of the inhibitory potency towards ACAT enzyme**

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>Ar</th>
<th>AIV IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23a</td>
<td><img src="image" alt="Image" /></td>
<td>0.50</td>
</tr>
<tr>
<td>23b</td>
<td><img src="image" alt="Image" /></td>
<td>51.0</td>
</tr>
<tr>
<td>23c</td>
<td><img src="image" alt="Image" /></td>
<td>4.00</td>
</tr>
<tr>
<td>23d</td>
<td><img src="image" alt="Image" /></td>
<td>2.49</td>
</tr>
<tr>
<td>23e</td>
<td><img src="image" alt="Image" /></td>
<td>0.44</td>
</tr>
<tr>
<td>23f</td>
<td><img src="image" alt="Image" /></td>
<td>0.09</td>
</tr>
</tbody>
</table>

The 4,5-diaryl imidazole moiety was replaced by various nitrogen containing fused rings to reduce the molecular weight and to improve the bioavailability of the compounds. Fused imidazoles (23a), oxazoles (23b), thiazoles (23c), N-substituted imidazoles (23d) and triazines (23e) were observed to be less potent but the imidazolinone derivative (23f) was having the same magnitude of activity as DuP-128 (20a).
Researchers at Parke-Davis Pharmaceuticals have reported a novel series of tetrazole-substituted ureas.\textsuperscript{77} The compound (24a, $IC_{50}$ 0.047 $\mu$M) was found to be more active in the macrophage ACAT assay than CI-976 but unfortunately it exhibited adrenotoxicity in the guinea pig. In this model, compound (24b, $IC_{50}$ 0.057 $\mu$M) at a dose of 5 mg/kg was shown to be as efficacious at lowering total serum cholesterol as CI-976 and it did not show any adrenal toxicity to the guinea pig.

\begin{align*}
(24) \\
a: & R_1 = 2,6-(i-Pr)_2Ph, R_2 = Ph \\
b: & R_1 = 2,4,6-(OMe)_3Ph, R_2 = C_6H_{11}
\end{align*}

\begin{align*}
(25) \\
a: & R_1 = H, R_2 = H \\
b: & R_1 = F, R_2 = NMe_2
\end{align*}

In their continuing interest in developing novel, potent ACAT inhibitors, they synthesized a series of trisubstituted ureas that were structurally hybrids of the disubstituted ureas. This series of compounds has shown more potent activity with 2,4-difluoro (25a, $IC_{50}$ 0.09 $\mu$M) and 2,4,6-trifluoro (25b, $IC_{50}$ 0.022 $\mu$M) substitutions on phenyl ring of the urea nitrogen than the compounds containing bulky substituents at 2,6-positions on the phenyl ring at the same nitrogen.\textsuperscript{78}

Tanaka et al. have designed and synthesized a new ACAT inhibitor, FR186054 (26a), bearing a pyrazole ring that exhibited potent \textit{in vitro} ACAT inhibitory activity and excellent hypocholesterolemic effects in cholesterol-fed rats.\textsuperscript{79} SAR studies revealed that 26b and 26c showed more potent ACAT inhibitory activity \textit{in vitro} than CL 277082 and 26a. However, the \textit{in vivo} hypocholesterolemic effect of 26a was clearly superior when dosed as a dietary admixture, being 100-fold more potent compared to the reference compound, presumably as a result of improved pharmacokinetics. The introduction of dimethylamino group on to the 4-position of $N$-benzyl moiety (26d) resulted in reduced \textit{in vitro} activity but retained the \textit{in vivo} efficacy.
Further, the above group has prepared a series of \( N \)-alkyl-\( N \)-biphenylmethyl-\( N' \)-aryl urea and related derivatives and evaluated them for the ability to inhibit ACAT \textit{in vitro} and to lower plasma cholesterol levels in cholesterol-fed rats \textit{in vivo}. From the SAR studies, it was concluded...
that the linking of two phenyl groups via oxygen and introduction of fluorine at appropriate positions on the biphenyl moiety improved \textit{in vitro} and \textit{in vivo} activity. From this series of analogs, compound \textbf{FR179254} (27a) has shown potent \textit{in vitro} potency (rabbit intestinal microsomes \(IC_{50} \ 25 \ \text{nM}\)) and excellent plasma cholesterol-lowering activity (\(ED_{50} \ 0.045 \ \text{mg/kg}\)). Modification of the N'-aryl moiety led to the identification of compound \textbf{FR182980} (27b) which was efficacious in both of the dos:ng models (\(ED_{50} \ 0.034 \ \text{mg/kg} \) and 0.11 mg/kg respectively).\textsuperscript{80}

Novel hydroxyphenyl urea derivatives (e.g. 28) were synthesized and their inhibitory potency evaluated against both ACAT and LDL oxidation.\textsuperscript{81} QSAR analysis revealed that their ACAT inhibitory activities were controlled by the hydrophobicity of the whole molecule, the substitution pattern of urea moiety, and the existence of carboxylic acid group. These derivatives inhibited foam cell formations. Moreover, these compounds showed antioxidative effects against low density lipoprotein (LDL), owing to their characteristic 3-\textit{t}-butyl-2-hydroxy-5-methoxyphenyl substructure. Based on the mechanism of atherosclerosis generation, they hypothesized that this hydroxyphenylurea type (28) dual inhibitors (against both ACAT and LDL oxidation) were expected to be promising drugs for atherosclerosis in the time to come.

\textit{N-}Alkyl\textit{\textbf{t}},\textit{\textbf{o}}-diphenylpyridazine derivatives (29a) possessing several main features of ACAT inhibitors, such as a long alkyl side chain linked to a heterocycle and the \textit{o}-diphenyl system, were synthesized and tested by Gelain et al. Modeling studies were also performed on the compounds. Some of the compounds displayed ACAT inhibition in the micromolar range (29a, \(IC_{50} \ 18 \ \text{\mu M}\)), both on the enzyme isolated from rat liver microsomes and in cell-free homogenate of murine macrophages.\textsuperscript{82} Keeping the above features in mind, they reported the mono- and diphenylpyridazineureido derivatives (29b), structurally related to \textbf{DuP 128} (20a) and
tested them for their inhibitory activity against ACAT, isolated from rat liver microsomes. Compound (29b) showed the most interesting activity against hACAT-1 (IC\textsubscript{50} 0.94 μM) and hACAT-2 (IC\textsubscript{50} 1.74 μM) isoforms.\textsuperscript{83} This group has described a series of \textit{N}-(2,4-difluorophenyl)-\textit{N’}-heptyl-\textit{N’}-{4-[(substituted pyridazin-3-yl)sulfonyl]pentyl}urea derivative (30) having phenyl rings at 5 and 6 positions of the heterocycle. The compound showed inhibitory activity against the ACAT enzyme prepared from rat liver microsomes (78% inhibition at a conc. of 50 μg/mL). Theoretical studies were also performed to correlate the activity with the structural features.\textsuperscript{84}

\textbf{2.2.2 Disubstituted ureas as ACAT inhibitors}

A series of disubstituted phenylureas linked to 4-phenylimidazole were synthesized and evaluated for \textit{in vitro} inhibitory activity for both aortic and intestinal ACAT and \textit{in vivo} hypocholesterolemic activity.\textsuperscript{85} The SARs were studied involving strategic modification of five regions in the compound (31) i.e., by introducing functional groups or exchanging carbon atoms for heteroatoms. Methyl group in the ortho position of the phenyl urea showed better pharmacokinetic property. Butyl, pentyl, isopentyl, and neopentyl groups were better substituents in the urea moiety. Propoxy was the optimal moiety in the bridging portion. Hydrogen, methyl, ethyl, isopropyl, hydroxymethyl and chloro were observed to be better substituents at the 5-position of the imidazole moiety. Unsubstituted phenyl ring on the imidazole ring was observed to provide better compounds. Subsequent comparative study of compounds containing various
combinations of the substituents in each region resulted in the selection of two compounds for further pharmacological and toxicological testing. These compounds were orally bioavailable and possessed potent in vitro aortic ACAT inhibitory activity (31a, 31b IC$_{50}$ 0.16 and 0.012 µM, respectively) and in vivo cholesterol lowering effect (46% and 52 % at 1 mg/kg PO, respectively).

\[
\begin{align*}
(31) \\
a: & \quad R^1 = C_4H_9, \quad R^2 = C_2H_5 (E 5324) \\
b: & \quad R^1 = C_4H_9, \quad R^2 = CII(Me)_2
\end{align*}
\]

Trivedi et al. synthesized and reported a series of N, N'-diphenylureas with a para alkyl substituent larger than n-pentyl. The group was found to be essential for potent ACAT inhibition in vitro.$^{86}$ In this series, compound (32, IC$_{50}$ 0.011 µM) represented a simple urea derivative with

\[
\begin{align*}
(32)
\end{align*}
\]

an excellent profile for development as a hypocholesterolemic agent. In their continuing research programme, a series of N-phenyl-N'-aralkyl- and N-phenyl-N'-(1-phenylcycloalkyl)ureas have been reported as inhibitors of ACAT.$^{87}$ From this series compound (33a, PD 129337) was identified as a potent inhibitor of ACAT with an IC$_{50}$ value of 17 nM. Due to lack of efficacy of compound (33a) in aqueous vehicle, the N'-phenyl moiety was modified by incorporating polar functional groups which were amenable to forming salt to reduce lipophilicity. Introduction of any bulky group in the para position and polar groups such as carboxyl lowered the in vitro activity. In chronic cholesterol fed rat model of hypercholesterolemia, compound (33b, PD 132301-2) dose-dependently reduced non-HDL cholesterol and significantly elevated the HDL cholesterol. It showed significantly greater aqueous solubility than the parent compound (33a).
Later on compound \((33b)\) was reported to exhibit organ toxicity. Administration of compound \((33b)\) to beagle dogs for two weeks at doses ranging from 6-800 mg/kg/day resulted in significant decreases (60-80%) in adrenal, total and esterified cholesterol.\(^{88}\) However, it was shown to cause the adrenal toxicity in guinea pigs\(^{89}\) and monkeys\(^{90}\) This led to design of a series of homologs with increased basicity and lower lipophilicity. Finally, compound \((33c)\) was reported not to produce adrenal toxicity in guinea pigs unlike compound \((33b)\) and it demonstrated excellent lipid-modulating activity in the chronic model of hyperlipidemic rats.

Further, a series of di-substituted ureas containing amide or amine groups were prepared and evaluated for their ability to inhibit ACAT \textit{in vitro} and lower total plasma cholesterol in a variety of cholesterol-fed rat models \textit{in vivo}.\(^{9}\) Presence of polar or ionizable functionalities within this class of compounds imparted greater aqueous solubility and showed its improved transportation to the enzyme location within the intestinal enterocyte. In general, the amine containing compounds showed more potency and efficacy than the amides in the acute rat model of hypercholesterolemia. SAR studies showed that the preferred position of the amide/amine
group was β to the urea moiety and the presence of a secondary amine hydrogen is required for good \textit{in vitro} potency. One (34) of these compounds lowered plasma total cholesterol (47\%) and elevated high density lipoprotein (HDL) cholesterol when dosed in an aqueous vehicle to rats with pre-established hypercholesterolemia.

Later, Trivedi \textit{et al.} have reported a series of conformationally and sterically constrained analogs of \textit{N}-phenyl-\textit{N’}-aralkylureas (35).\textsuperscript{92} SAR studies revealed that a polar group like hydro-

![Chemical Structure](image)

xyl at the β-carbon was found to be detrimental to activity (35a, \textit{IC}_{50} 0.35 \mu M). Two of the homologs (35b, 35c) showed significant increase in potency with the \textit{IC}_{50} values of 27 and 36 nM.

Researchers at Parke-Davis research Lab developed diaryl-substituted heterocyclic urea and examined their ACAT inhibitory activities.\textsuperscript{93} In order to determine the supremacy of aryl moiety in the heterocyclic ureas, they identified that the 2,6-diisopropylphenyl analog (36) was more active when compared to the 2,4,6-trimethoxyphenyl analog. The potency of the tetrazole urea (36a, \textit{IC}_{50} 0.009 \mu M) was found to be a modest one. The 1,3,4-oxadiazole (36b, \textit{IC}_{50} 0.018 \mu M) and isoxazole (36c, \textit{IC}_{50} 0.013 \mu M) moieties proved excellent bioisosteric replacements for tetrazole \textit{in vitro} and in the APCC rat at a dose of 30 mg/kg but the 1,3,4-thiadiazole (36d, \textit{IC}_{50} 0.036 \mu M) and the triazole (36e, \textit{IC}_{50} 4.2 \mu M) were considerably less active \textit{in vitro} than the tetrazoles. Alkylation of the tetrazole NH was found to be essential for activity. Activity was maximal with C-13 side chain analog and declined with a further increase in chain length, probably being extremely lipophilic and consequently not getting absorbed.
A novel ACAT inhibitor R-755 (37) has been characterized in vitro, ex vivo and in vivo. R-755 potently inhibited ACAT activities with IC\textsubscript{50} values from 2.5 to 64 nM in rabbit intestinal microsomes and several cell lines (Caco-2, THP-1 and J-774A.1 cells). It has been proved that 37 was more potent than CI-976 (5). These results suggested that R-755 can be expected to be a therapeutically useful drug that not only has lowering effects on plasma cholesterol and triglycerides but also has an antiatherosclerotic effect by causing direct inhibition of ACAT activity in the arterial wall.\textsuperscript{94}

Kumazawa et al. at Kyowa Hakko kogyo Co Ltd in Japan described the synthesis of a novel series of N-(1-phenyl-2-benzimidazolyl)-N'-phenylurea derivative as ACAT inhibitors. Few compounds showed very good ACAT inhibitory activity. Out of them compound (38, IC\textsubscript{50} 11nM) was found to be very potent.\textsuperscript{95} TMP-153 (39) is another potent urea ACAT inhibitor
that has been reported. TMP-153 is very potent against liver and intestinal ACAT from a
variety of animal species. It has an IC\textsubscript{50} value of 9 nM in rat liver microsomes and 6.4 nM in rat
intestinal microsomes. This compound (39) also displays potent lowering of plasma cholesterol
\textit{in vivo} with an ED\textsubscript{50} of 0.25 mg/kg when dosed for one week to cholesterol-fed rats.

A group of researchers at Sumitomo Pharmaceuticals Co Ltd in Japan developed urea
derivatives of 3-amino-4-aryl-1,8-naphthyridin-2(1\textsubscript{H})-one. In particular, compound (40a, SM-
32504) exhibited potent ACAT inhibitory activity. However, this compound was poorly
absorbed by oral absorption due to its low aqueous solubility. Extensive studies led to the
development of compound (40b, SMP-797, IC\textsubscript{50} 31 nM), which possessed 4-amino group on the

\[ R = \text{Me}, \quad R_1 = \text{H (SM-32504)} \]
\[ R = (\text{CH}_2)_2\text{OH}, \quad R_1 = \text{NH}_2\cdot2\text{HCl (SMP-797)} \]
aniline and 3-hydroxypropoxy group on the 4-phenyl group of the naphthyridinone moiety of compound (40a). Compound (40b) decreased the serum cholesterol level by 53% compared to control at a dosage of 1.0 mg/kg/day orally for 3 weeks in a rabbit model fed on a casein-rich diet. This effect can be compared with atrovastatin (potent HMG-CoA reductase inhibitor), which decreased cholesterol level by 54% at a dose of 10 mg/kg/day orally for 6-weeks.\(^98\)

Although compound (40b) was a promising one, its preparation required very long steps. Therefore to find back-up compounds with different mother templates and easy synthetic routes, the research group examined replacement of the 1,8-naphthyridine moiety with other hydrophilic groups and succeeded in finding compound (41a, IC\(_{50}\) 32 nM) as a potent ACAT inhibitor.\(^99\) Later it was described that compound (41b), inhibited ACAT activity with an IC\(_{50}\) value of 18nM,\(^100\) which was superior to that of a known ACAT inhibitor and also revealed an LDL-R up-regulatory activity comparable to that of compound (40b).

### 2.3 Aminosulphonyl based ACAT inhibitors

The same group of researchers as previously mentioned at Parke-Davis Pharmaceutical Research Lab, New Jersey designed ACAT inhibitors with improved bioavailability. They hoped to design an ACAT inhibitor with a relatively low lipophilicity (calculated LogP value between

\[\text{(42) CI-999 (PD 138142-15)}\]
1.79 - 2.44 was considerably less than the one commonly encountered in the ACAT inhibitor field \(i.e\.\ 6 - 12\), which could be completely absorbed.

They synthesized a novel, water soluble ACAT inhibitor. The IC\(_{50}\) value of the compound (42) was found to be 5.3 \(\mu\text{M}\)\(^{101}\) whereas the IC\(_{50}\) values for potent established compounds (19 and 20a) were 0.47 and 0.018 \(\mu\text{M}\) respectively. Similar ACAT inhibitory activities were found for 42 and 20a using microsomes isolated from the intestinal mucosa of cholesterol-fed rabbits but compound (19) was reported to be more potent in this system. This compound caused a decrease in adrenal cholesterol esters and a nonreversible zonal atrophy and degeneration of the adrenal gland.\(^{102}\) Compound (42) showed instability in acidic aqueous media and degraded into two products identified as 2, 6-diisopropyl phenol and sulphamate.

Further, a series of sulfonylureas (43) were described and identified as a series of moderately potent and highly efficacious ACAT inhibitors,\(^{103}\) which lowered TC and elevated HDL-C as effectively as 42 in a chronic rat model of hypercholesterolemia. A series of novel sulfonamide tetrazole (44)\(^{104}\) derivatives as ACAT inhibitors have been described. The use of sulfonamide as isosteric replacement of the amide group in the series of tetrazole amide resulted in comparable \textit{in vivo} efficacy but lower \textit{in vitro} potencies. It was reported that the position and the length of the alkyl substituents on the tetrazole ring had a marked effect on ACAT inhibitory activity. Compound (44) exhibited lower \textit{in vitro} potency (IC\(_{50}\) \textbf{0.022} \(\mu\text{M}\)) and good \textit{in vivo} efficacy.

Further, several ACAT inhibitors have been described by stepwise addition of nitrogen, oxygen and sulphur nucleophiles to N-chlorosulfonyl isocyanate.\(^{105}\) The aminosulphonylureas were the most potent inhibitors \textit{in vitro} with several compounds having IC\(_{50}\) values less than 1.
μM. Compound (45), in which the \(N,N\)-dialkylamino group contains 6 to 15 carbon atoms, exhibited good ACAT inhibitory in vitro potency and in vivo efficacy. In an effort to overcome the inherent chemical instability of compound (42), compound (46, CI-1011, Avasimibe) has been identified with equivalent or better in vitro and in vivo activities but higher solution stability especially at pH <7. Compound (46) was highly stable in acidic or basic solutions and displayed excellent in vivo efficacy in standard cholesterol-fed rat models. The calculated \(ED_{50}\) value was 0.4 mg/kg,\(^{106}\) which was much less than the dose reported for potent compounds like \(CL\ 277082\) (19, \(ED_{50}\) 16 mg/kg), \(Dup128\) (20a, \(ED_{50}\) 15 mg/kg \(20a, ED_{50} 15\ mg/kg\)), and 41a (\(ED_{50}\) 2 mg/kg).\(^{107}\)

Finally, the best compound (47) was identified showing similar in vivo efficacy as the previously reported potent compounds like 19 and 20a. The reason for the greater in vitro potency of compound (47) was unclear (\(IC_{50}\) 0.007 μM).\(^{107}\) These findings had prompted further pharmacological investigations in this series of compounds.

Takahashi et al. described a series of novel indoline derivatives with an ionizable moiety to find a bioavailable ACAT inhibitor with antiperoxidative activity. [7-(2,2-Dimethylpropanamido)-4,6-dimethyl-1-octylindolin-5-yl]acetic acid hemisulfate (Pactimibe, 48a)\(^{108}\) with low lipophilicity and high water solubility showed good oral absorption and inhibitory activity against foam cell formation in THP-1 cells exposed to acetyl-LDL after differentiation (\(IC_{50}\) 0.3 μM) and an antiperoxidative effect in LDL of hypercholesterolemic
rabbits ($IC_{50} \ 1.0 \ \mu M$). Compound (48a) inhibited macrophage, hepatic and intestinal ACAT activity ($IC_{50} \ 1.9, \ 0.7$ and $0.7 \ \mu M$ respectively). The same group of researchers described a novel series of indoline-based ACAT inhibitors with methanesulfonamide group and evaluated their lipophilicity and biological activities$^{109}$ From the series, compound (48b) showed greater inhibitory effects on hepatic cholesterol secretion in mice. It was concluded that the introduction

![Molecular Structure](image)

(48)

a: $R = -(CH_2)_2CH_3, \ R^1 = -CH_2COOH$ (Pactimibe)
b: $R = -C_2H_5, \ R^1 = -NHSO_2Me$

of a methanesulfonamide group was effective to provide less lipophilic, more efficacious and bioavailable compound than 48a.

Asano et al. have synthesized compounds that possessed LDL-R up-regulating activity without ACAT inhibition. They started this approach by modification of 1,4-diarylpyrididine-4-methylurea (41). Replacement of the methyleneurea linker (41) with the acylsulfonamide (49) was effective in keeping the up-regulatory activity for LDL-R expression and reducing ACAT inhibitory activity.$^{110}$ Introduction of 2-pyrimidyl group in compound (49) enhanced LDL-R up-regulatory activity and abolished ACAT inhibitory activity. Additionally, the sodium salt of the

![Molecular Structure](image)

(49)

selected compound (49) showed good oral pharmacokinetics properties in hamsters, and it reduced plasma TC and LDL-C levels in a dose-dependent manner in an experimental animal
model of hyperlipidemia. These results indicated that LDL-R up-regulation is important for plasma lipids reduction. Finally, they clarified the mechanism of action of 50 toward LDL-R up-regulation using ARH specific RNA interference, and revealed that ARH, an adaptor protein of LDL-R, was a potential target for LDL-R up-regulation. The results of this study indicated that this compound (49) with its unique mechanism of action could be a lead for future novel antihyperlipidemic agents.

Honggang Hu et al. have described a series of xanthone sulfonamides that resulted in the identification of several potent ACAT inhibitors. Out of them compound (50, 32.4% inhibition at 10 μg/mL) proved to be equipotent to the positive control Sandoz58-35 (an ACAT inhibitor, from Sigma, 55% inhibition at 10 μg/ mL).

2.4 Imidazoline based ACAT inhibitors

Li Hui-Yin et al., a group of researcher from DuPont Merck Pharmaceutical Company designed and synthesized new series of 4,4-bis(trifluoromethyl)imidazolines with a p-fluorophenacetyl side chain using a facile photooxidative cleavage of pyrrole (51) with singlet
oxygen. Finally, compound (52) showed good activity as ACAT inhibitor \( (\text{IC}_{50} \ 11 \ \mu M) \) and cholesterol biosynthesis inhibitor \( (\text{IC}_{50} \ 4 \ \mu M) \).\(^{112}\)

Further, they synthesized molecule (53a), a very potent ACAT inhibitor \( (\text{IC}_{50} \ 1.4 \ \mu M) \) with remarkable oral activity in lowering the serum cholesterol level in several animal models and it was also reported that the \( R \)-enantiomer of 53a was about 25 times more potent than the \( S \)-enantiomer in the ACAT in vitro assay.\(^{113}\) Finally, they synthesized 4,4-bis(trifluoromethyl)imidazolines with a \( p \)-cyano group on 2-phenyl and 4-alkylcyclohexylamide, as the side chain possessed the most potent inhibitory activity \( (53b, \text{IC}_{50} \ 0.09 \ \mu M) \).

\[ (53) \]
\[ a: X = 4\text{-Cyanophenyl} \]
\[ b: X = 3\text{-Propylcyclohexyl} \]

2.5 Natural products as ACAT inhibitors

Some products of microbial origin were shown to inhibit ACAT enzyme to varying degrees. These compounds provided additional insights into the pharmacophore necessary for ACAT inhibition. Fungal strains of \( Fusarium \) \( sp \) FO-740 and FO-1305 were shown to produce a number of cyclodepsipeptide antibiotics like a number of derivatives of beavercin (54) which
were subsequently shown to inhibit ACAT. All of these compounds inhibited ACAT using rat liver microsomes with IC\textsubscript{50} values less than 3 \( \mu \text{M} \).\textsuperscript{114,115}

Purpactins A, B and C, isolated from the fermentation broth of \textit{Penicillium purpurogenum} were shown to be modestly potent inhibitors of ACAT using rat liver microsomes. Purpactin A (55) has the IC\textsubscript{50} value of 126 \( \mu \text{M} \).\textsuperscript{116}

Glisoprenin (56) isolated from the fermentation broth of \textit{Gliocladium sp FO-1513}, was shown to be inhibitor of ACAT using rat liver microsomes. In a cellular assay using J774 macrophages, the compound was much more potent with IC\textsubscript{50} value of less than 1 \( \mu \text{M} \).\textsuperscript{117,118}

![Chemical Structure of Glisoprenin (56) and Acaterin (57)]

Acaterin (57) was isolated from a culture broth of \textit{Pseudomas sp A92}. In the presence of oxidized LDL, acaterin inhibited cholesteryl ester synthesis in J774 macrophages with an IC\textsubscript{50} value of 45 \( \mu \text{M} \).\textsuperscript{119}

Further, in their continuing research, pyripyropene A (58a), B (58b), C (58c) & D (58d) were isolated from the fermentation broth of \textit{Aspergillus fumigatus FO-1289} and were showed to be potent inhibitors of ACAT (IC\textsubscript{50} = 89, 270, 67 and 140 nM respectively).\textsuperscript{120} The activity of PR-45 (58e, IC\textsubscript{50} 13 nM) and PR-109 (58g, IC\textsubscript{50} 6nM) were almost the same as that of pyripyropene A. Remarkably, PR-86 (58f) showed 10 times improved \textit{in vivo} activity (IC\textsubscript{50} 19 nM) with an ED\textsubscript{50} value of 10 mg/kg via single oral administration over the other compounds in this series.
Kwon et al. at Korea Research Institute of Bioscience and Biotechnology have isolated the Ginseng sapogenin from ginseng saponins.\textsuperscript{121} Ginseng saponins very mildly inhibited ACAT enzyme \textit{in vitro}, however the sapogenins showed strong inhibitory activity on microsomal ACAT. Compounds (59a & 59b) inhibited rat liver ACAT enzyme with IC\textsubscript{50} values of 10 & 6 $\mu$M respectively.

Lee et al. have isolated the sesquineoligran, saucerneol B (60a) and manassantin A (60b) from the methanol extracts of \textit{Saururus Chinensis} root.\textsuperscript{122} Both compounds inhibited hACAT-1 and hACAT-2 with IC\textsubscript{50} values of 43.0 and 124 $\mu$M for compound (60a) and 39 and 8 $\mu$M for
compound (60b). Saucerneol B preferentially inhibited hACAT-1 than hACAT-2, however manassantin A strongly inhibited hACAT-2 compared to hACAT-1. Further, they discovered a novel class of hACAT-1 specific enzyme inhibitors. An n-propoxy derivative (60c) showed IC\textsubscript{50} value of 14 μM.\textsuperscript{123}

The same group of researchers isolated the unsaturated fatty acid amides, 9Z-octadecenamide (61a) and 9Z,12Z-octadecadienamide (61b) as potent inhibitors of ACAT from the ethyl acetate extract of the insect, Mylabris phalerate pallas.\textsuperscript{124} Both of the compounds were shown to inhibit rat microsomal ACAT, hACAT-1 and hACAT-2 with IC\textsubscript{50} values of 170, 85 and 63 μM for compound (61a) and of 151, 53 and 45 μM for compound (61b) respectively.

In their continuing research, Lee et al. indentified three naphthoquinones namely, acetylshikokin (62a), isobutyrylshikokin (62b) and β-hydroxyisovalerylshikokin (62c), which were isolated by bioassay-guided fractionation from the chloroform extracts of roots of Lithospermum erythrorhizon. These compounds were tested for their inhibitory activities against hACAT-1 and hACAT-2.\textsuperscript{125} Compound (62b) preferentially inhibited the hACAT-2 (IC\textsubscript{50} 57.5 μM) than hACAT-1 (32% at 120 μM), whereas compounds (62a and 62c) showed weak inhibitory activities in both hACAT-1 and hACAT-2. Jeong et al. have discovered a new class of hACAT inhibitors. Nine flavonoids and one chalcone were isolated from the methanolic extracts of root of S. flavescens and among them two flavonoids (63a, 63b) showed potent activity.
Compound (63a) inhibited significantly the enzymatic activities of both hACAT-1 and -2 in cell-based assay system with IC$_{50}$ of 16.4 $\mu$M for hACAT-1 and IC$_{50}$ of 13.6 $\mu$M for hACAT-2. Compound (63b) exhibited a slight decrease in potency for both hACAT-1 and -2 with IC$_{50}$ values of 19.7 and 20.4 $\mu$M respectively.$^{126}$

Rho et al. have isolated a series of active compounds from the methanolic extract of *Piper nigrum*. Out of the compounds, a new compound, designated dehydroteoractamide C

(64, IC$_{50}$ 60 $\mu$M) inhibited ACAT activity in both rat liver microsomes and HepG2 cells.$^{127}$

2.6 Miscellaneous ACAT inhibitors

The scientists at The Upjohn Lab in Michigan have synthesized a novel series of bisaminofurochromone derivatives (65, IC$_{50}$ 0.08 $\mu$M) as ACAT inhibitors.$^{128}$ They also reported that the 2-3 aliphatic carbon chain between the two piperidine rings in compound (65) showed moderate potency.

In their continuing research, they reported a novel series of 6,7-dihydro-4H-pyrazolo[1,5-$\alpha$]pyrrolo[3,4-$d$]pyrimidine-5,8-dione (66, IC$_{50}$ 1.5 $\mu$M) inhibitors of the enzyme ACAT. A
number of these derivatives were found to be potent modulators of serum lipoprotein levels in cholesterol-fed rats. Further, they evaluated one of the most effective analogs, which was significantly blocking the absorption of cholesterol from the gut.\textsuperscript{129}

Ashton \textit{et al.} identified (67a, \textbf{RP 70676}), a potent systemically available inhibitor of ACAT (IC\textsubscript{50} 40 nM).\textsuperscript{130} It was an effective hypocholesterolaemic agent in the cholesterol-fed rabbit, that reduced the accumulation of both cholesterol and cholesterol esters in rabbit aorta and thoracic artery. The compound was readily bioavailable in rabbits with significant levels of parent compound present in plasma up to 6 hours after an oral dose. \textbf{RP 70676} had been shown to be an effective inhibitor of ACAT derived from a number of tissues and species including man. Further, they identified that 67b (\textbf{RP 73163}),\textsuperscript{131} a major metabolite of compound (67a) retained

\[(67)\]

\textbf{a}: \textit{X} = \textit{S (RP 70676)}

\textbf{b}: \textit{X} = \textit{SO (RP 73163)}
ACAT inhibitory activity. Compound (67b) had higher systemic bioavailability than the parent compound (67a).

On the basis of ACAT inhibitory activity of the 1,4-naphthoquinone derivatives, researchers were interested to synthesize similar derivatives with different side chains to understand the structural requirement for ACAT enzyme inhibitory effect. A novel series of 2-thia/amino-5,8-dimethoxy-1,4-naphthoquinone (DMNQ, 68) analogs were synthesized and compound (68) showed potent ACAT inhibitory activity with IC$_{50}$ value of 22.8 $\mu$M. In SAR study, it was observed that 2-thia-DMNQs with side chains of carbon number 11 to 15 exhibited significant ACAT inhibitory activity.

Recently, Chhabaria et al. have described ligand-based pharmacophore modeling of a series of structurally diverse ACAT inhibitors. It has been reported that two most potent compounds of the retrieved hits from pharmacophore modeling study were synthesized and biologically evaluated. These compounds (69a, 69b) showed 86% and 88% inhibition of ACAT (at 10 $\mu$g/mL) with IC$_{50}$ values of 3.6 and 2.5 nM respectively.

\[
\text{(69)}
\]

\[\text{a: } R = \text{Cl}, \text{ b: } R = \text{Br}\]

2.7 Clinical trails of some ACAT inhibitors

Despite showing promising efficacy in various animal studies, only a few ACAT inhibitors have been evaluated in clinical trials. Melinamide (1) successfully made its way
through clinical development and was approved for use as a cholesterol absorption inhibitor in Japan.\textsuperscript{134} However the drug was withdrawn later on.

Development of \textit{Avasimibe} (46), another ACAT inhibitor that reached phase 3, was halted in October 2003 by Pfizer (New York) after the drug was shown to have potentially unfavorable effects. It lowered both VLDL and triglycerides by approximately 30\% in doses ranging from 50-500 mg/day in an eight-week study in 130 patients.\textsuperscript{135} Daiichi Sankyo, the company that was developing \textit{Pactimibe} (48a) that had reached phase 3 clinical trials, announced on October 26, 2005 that it had decided to discontinue all ongoing clinical studies with this drug due to some discouraging results. ACAT inhibitors have so far failed to progress inclinical development. \textit{Eflucimibe} (70) is another same category of inhibitor, developed by Pierre Fabre SA and Eli Lilly & Co for the potential treatment of hypercholesterolemia and atherosclerosis. Phase II clinical trials were commenced during 2002 but discontinued later due to discouraging lipid effects of the drug.