Selective ACAT Inhibitors as Promising Antihyperlipidemic, Antiatherosclerotic and Anti-Alzheimer Drugs

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Abstract: Inhibition of ACAT, the enzyme which catalyses the intracellular formation of cholesteryl esters, is a very attractive target for the treatment of hypercholesterolaemia and atherosclerosis. However, in the past years many ACAT inhibitors gave disappointing results in clinical trials showing very low efficacy. In addition, their development was affected by the adrenotoxicity observed in many compounds. The discovery of two isoforms of the enzyme, namely ACAT1 and ACAT2, with different substrate specificity and different potential function, offers a precious information for planning selective inhibitors with reduced secondary effects. Today some potent, bioavailable and non adrenotoxic ACAT inhibitors are under clinical evaluation. Amongst others, a very promising compound is Avasimibe, presently in phase III clinical trials as anti-hyperlipidemic and anti-atherosclerotic agent.

Finally, ACAT inhibitors have recently been proposed for the treatment of Alzheimer's disease.

Keywords: Cholesterol Acyl-CoA: cholesterol acyl transferase (ACAT1, ACAT2) inhibitors –Atherosclerosis –Anilide and urea derivatives - Adrenotoxicity –Alzheimer's disease.

INTRODUCTION

Atherosclerosis and correlated cardiovascular diseases, such as coronary heart disease (CHD), represent the major cause of death in the industrialized country [1]. It is well known that hypercholesterolemia [2], in particular a high level of low-density lipoprotein (LDL) cholesterol [3], is one of the most important risk factors for this event together with hypertension, and diabetes.

Badimon and co-workers [4] demonstrated the different role of another lipoprotein, high density lipoprotein (HDL), for which an opposite correlation is described in CHD [5].

The pathogenesis of atherosclerosis was extensively studied by Ross [6] in the past years and the same author has recently demonstrated that it is not due to accumulation of lipid only, but it is also caused by inflammatory responses [7]. The first step of atherosclerotic process is represented by monocyte adhesion following an endothelial injury. One of the most important factors inducing the adhesion is vascular adhesion molecule-1 (VCAM-1), whose enhanced expression is associated to high levels of LDL, hypertension and other risk factors for atherosclerosis [8]. An opposite role on VCAM-1 expression was demonstrated for HDL [9]. After adhesion, the monocytes migrate into subendothelial space where they differentiate into macrophages which, via scavenger receptors [10], are able to devour modified LDL without regulation [11,12]. The importance of two scavenger receptors, SR-AI and CD36, in atherosclerotic pathology [8] was demonstrated and identification of non peptidic small molecules with antagonist activity [13,14] towards SR-AI and CD36 could open new perspective in this field. The lack of regulation in taking up mod-LDL transforms the macrophages into the so called "foam cells", which are very rich in cholesterol and cholesteryl esters.

The "foam cells" are able to produce other proatherogenic molecules such as tissue factor (TF), angiotensin converting enzyme (ACE) [8] and in particular interleukine 8 (IL-8) which is a very important factor in smooth muscle cell (SMC) proliferation [15]. Finally, clinical studies on atherosclerotic patients demonstrated high level of an other cytokine, interleukine 6 which is able to alter the biosynthesis of the proteins in the liver, in particular by increasing the synthesis of C-reactive protein [16]. The repetition of these steps results in the formation of an atheromatous plaque.

These atheromas could break and in this case the exposure of subendothelium to coagulation factors led to a thrombus formation [17] or to a complete covering of the vessel cavity with dramatic consequences.

ROLE OF ACAT IN ATHEROSCLEROTIC PATHOLOGY

Acyl-CoA: cholesterol acyl transferase (ACAT) is the enzyme which catalyzes the acylation of cholesterol to cholesteryl esters with long chain fatty acids (Fig. **1**) [18]. It is located in the endoplasmic reticulum. Its activity depends on the concentration of free cholesterol in the endoplasmic reticulum [19] and its level can increase in response to high amount of dietary cholesterol [20]. In fact this enzyme is responsible for the absorption of the dietary cholesterol which has to be esterified before going to the liver as chilomicron. In cells non competent for cholesterol biosynthesis, this lipid arises by decomposition of LDL and it is transformed in cholesteryl esters by action of ACAT

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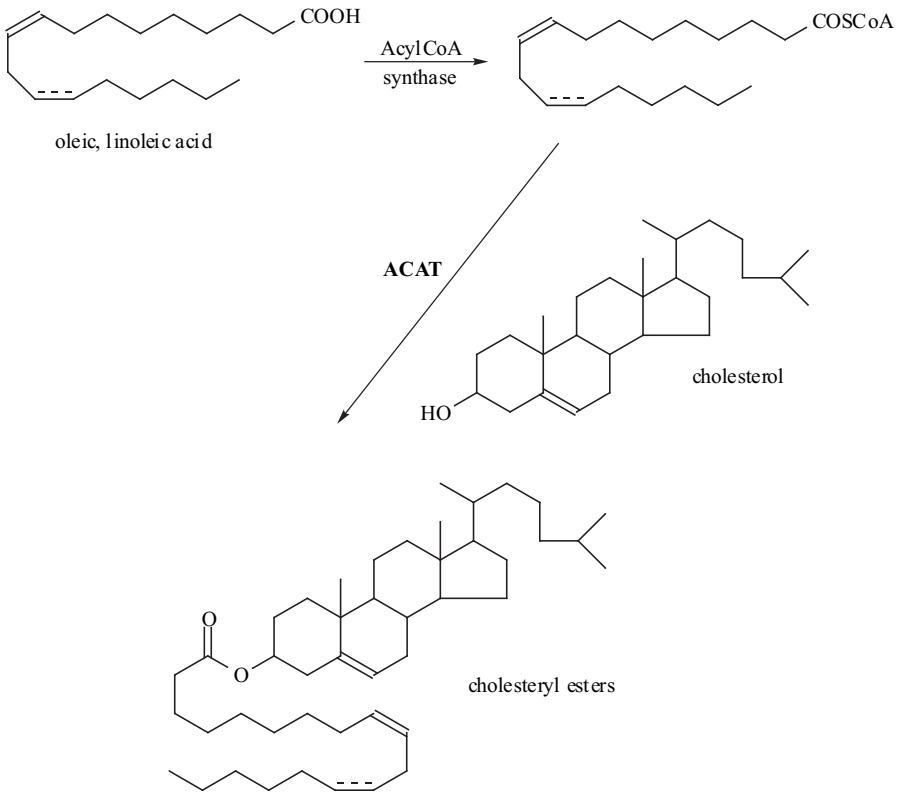


Fig. (1). Role of acyl-CoA cholesterol acyltransferase (ACAT).

enzyme. In this form it is stored while the surplus free cholesterol is extracted by HDL and goes to liver or comes back as LDL through the action of cholesteryl ester transfer protein (CEPT).

The same mechanism occurs in macrophages in which, because of the lack of regulation in uptaking, an abnorme cholesteryl ester storage takes place.

Taking into account these observations, it appears obvious that inhibition of ACAT could work in three different ways: by inhibiting cholesteryl ester accumulation in macrophages, by increasing liver catabolism of lipids and finally by limiting intestinal dietary cholesterol absorption [22].

Moreover, an interesting aspect emerged from studies performed by Pfizer researchers which demonstrated that inhibition of ACAT led to the reduction not only of cholesteryl esters amount but also of the atheromatous plaque [23,24].

The most important effect of reduction in cholesterol levels is classically the degree of arterial occlusion, not depending from the lowering lipid agent used. Lybby and coworkers have recently proposed an alternative hypothesis accordingly with Ross [7], that the reduction of clinical complication of atherosclerosis induced by lipid lowering was in general mediated by an anti-inflammatory effect [16].

In fact as a consequence of cholesterol lowering, a considerable reduction of endogenous molecules involved in inflammatory process, such as leukocytes and cytokines, was observed [25, 26]. In addition, a decrease of matrix metalloproteinase expression, which is responsible of plaque disruption, occurred in atherosclerosis lesions [24,27-29].

ACAT1 E ACAT2

In 1988 Kinnunen and coworkers [30] hypothesized the presence of two isoforms of ACAT enzyme in rabbit, observing the different sensitivity to diethylpyrocarbonate (DEP) of ACAT enzymes in intestine and pancreas on one hand and in all other organs on the other. Afterwards it was demonstrated that two different forms of ACAT are present in mammalian species, including humans, called ACAT1 and ACAT2, [31,32].

Finally, in the same year, two human genes encoding for two different ACAT enzymes were identified and characterized by Oelkers and co-workers [33]. They isolated the full-length cDNA clones for ACAT related gene products (ARGP) 1 and 2 and observed that ARGP1 is expressed in a lot of tissues, while ARGP2 is more limited. In particular, the human gene of ACAT1 was found to be located in chromosomes 1 and 7 [34]. Through immunodepletion experiments, it was also demonstrated that ACAT1 is predominant in human liver, macrophages and adrenal gland and that another form (probably ACAT2) is located in human intestine [35]. Further studies in this field confirmed the different tissue distribution for ACAT1 and ACAT2; ACAT1 is ubiquitously expressed in all examined animals, including humans [20,36], while ACAT2 is present in liver and intestine [31,32] and only in small amounts in other tissues. Besides a different substrate specificity, a different potential function was proposed for ACAT1 and ACAT2. [32] In fact, since ACAT1 activity depends on the membrane cholesterol enrichment [37], it could be hypothesized that the role of ACAT1 is to maintain the membrane cholesterol concentration at optimal levels for cell functionality [38,39] and to obtain a good balance of free and esterified

intracellular cholesterol. On the other hand, the selective distribution of ACAT2, together with the evidence that human ACAT2 promoter is preferentially expressed in CaCo-2 cells than in HepG2 cells [40], seems to suggest that this isoenzyme could operate in a specialized manner, for example in intestinal cholesterol absorption and in lipoprotein secretion [38,39].

Studies on modulation of ACAT1 and ACAT2 transcription were performed, by testing the effects of different free fatty acids (FFAs) on ACAT1 and ACAT2 expression and activity in HepG2 and macrophages (THP1) [41]. Results showed that ACAT1 expressing cells used oleic acid preferentially, while ACAT2 expressing cells utilized unsaturated FFAs, thus demonstrating that FFAs increase the level of mRNA in a specific manner. Analogous results were obtained when acylCoA substrate specificity in microsomes from HepG2 and THP1 was tested. Other interesting biochemical results from Botham and coworkers [42] demonstrate the influence of chylimicron remnants rich in n-3 and n-6 polyunsaturated fatty acids on ACAT activity and on mRNA expression for ACAT1 and ACAT2.

Initial predictive studies showed different topologies for ACAT1 and ACAT2 [31, 43, 44] enzymes. For ACAT2 a seven transmembrane domain protein was proposed and the serine residue (Ser 249) required for activity was suggested to reside in the endoplasmic reticulum lumen; on the contrary an eight transmembrane domain was indicated for ACAT1 and its Ser 269 residue is in the cytosol; this last prediction was experimentally confirmed by Lin et al. [45]. Recent studies presented by Joyce and coworkers indicate that ACAT1 and ACAT2 are proteins with five transmembrane domains and that the serine residue fundamental for activity is positioned on opposite side of the endoplasmic reticulum membrane [46] (Ser 296 of ACAT1 on the cytoplasmatic side and Ser 249 of ACAT 2 in the lumen) thus confirming the previous hypothesis of the different role for the two isoenzymes.

However, if there are evidences that ACAT1 activity is controlled by cholesterol levels and availability [19], only hypotheses were formulated about regulation of ACAT2 activity. Recently, interesting studies on transcriptional regulation of ACAT2 in monkey liver were performed and results showed the possibility for this enzyme of a regulation through interaction with its substrate, other then transcriptional [38].

An original hypothesis [47] suggesting that both enzymes take part in lipid droplets formation and lipoprotein assembly has also been formulated. To make it possible it is also necessary to assume that the ACAT catalytic site is located within the plane of the endoplasmic reticulum.

ACAT INHIBITORS

The first studies on ACAT inhibitors were performed in the early "70" when the interest was focused on compounds able to lower blood lipids and the designed molecules showed long chain aliphatic alkyl moieties, in order to mimic the natural substrate of ACAT. Since that time a large number of ACAT inhibitors have appeared in the literature, as a result of pharmaceutical industries and academic work. Though it is possible to classify these compounds in two major groups, the fatty acid anilide derivatives and urea derivatives, very interesting compounds not belonging to these classes should also be considered.

Fatty Acid Anilide Derivatives (Fig. 2)

The prototype of the fatty acid anilide is represented by **melinamide** (**1**) [49], now obsolete drug, which was marketed in Japan as intestinal cholesterol absorption inhibitor without knowing its ACAT inhibitory properties [50]. A very representative term of this class is **CI-976** (**2**) [51], 2,2-dimethyl-N-(2,4,6-trimethoxyphenyl)dodeconide, synthesized by Warner-Lambert researchers, which was found not only able to lower plasma total cholesterol (-60%) but also to elevate the levels of HDL (+94%) in cholesterol-fed rats; this lipid-regulating activity was demonstrated to be related to ACAT inhibition [52]. This compound, tested in a cholesterol-fed rabbit model of atherosclerosis [53] not only blocked the progression of lesion, but also reduced the same at a dose able to reduce plasma cholesterol (phase I clinical trials).

Few years later, another interesting compound **CP-113,818**, (S) enantiomer (**3**), was synthesized by McCarthy and coworkers [54]. **CP-113,818** was extensively studied and results showed a potent ACAT inhibitory activity on enzyme isolated from rat liver (IC_{50} = 22nM) and from human intestine (IC_{50} = 30 nM). Studies on Caco-2 cells, a human intestinal cell line, showed as this compound was able to block the esterification process. Moreover, it demonstrated high potency in inhibiting cholesterol absorption *in vivo* in hamsters with a ED_{50} of 0.009 mg/Kg, showing a promising pharmacological profile.

Recently an interesting series of polyunsaturated fatty acid anilides appeared in literature and, among these, the docosahexaenoyl derivatives were extensively studied [55] on human, rabbit, and canine microsomes. Results demonstrated that compound (**4**) was very potent in all species but, while in human there are no IC_{50} differences among the three sources of microsomes (Caco-2, IC_{50}=43nM, HepG2, IC_{50}=36nM, and U937, IC_{50}=35nM) in rabbit and dog a good selectivity was emphasized for intestine and liver with respect to the adrenal gland (in rabbit IC_{50} 45-56 nM for intestine and liver and IC_{50}= 228 nM for adrenal gland). This compound, besides being a promising antiatherosclerotic and hypocholesterolemic agent, showing a different inhibitory potency toward ACAT of different sources, could be useful for investigation of ACAT subtypes.

F12511 (**5**) [56], a dodecylthioacetanilide derivative is a very potent ACAT inhibitor and it was widely evaluated by using different *in vivo* and *in vitro* models. Results showed an IC_{50}=41-223 nM depending on the microsomial source. On the other hand the IC_{50} values related to whole cell assays were 3nM for Hep G2, 7nM for CaCo-2 and 71nM for THP-1 respectively [57]. In *in vivo* tests **F12511** elicits a dose-depending plasma cholesterol and atherogenic lipoproteins reduction. Moreover, pharmacological tests on NCI-H295R, a cell line very similar to human adrenal cells, demonstrated a lack of toxic effect [58], indicating **F12511** as a promising agent in the treatment of hypercholesterolemia and atherosclerosis.

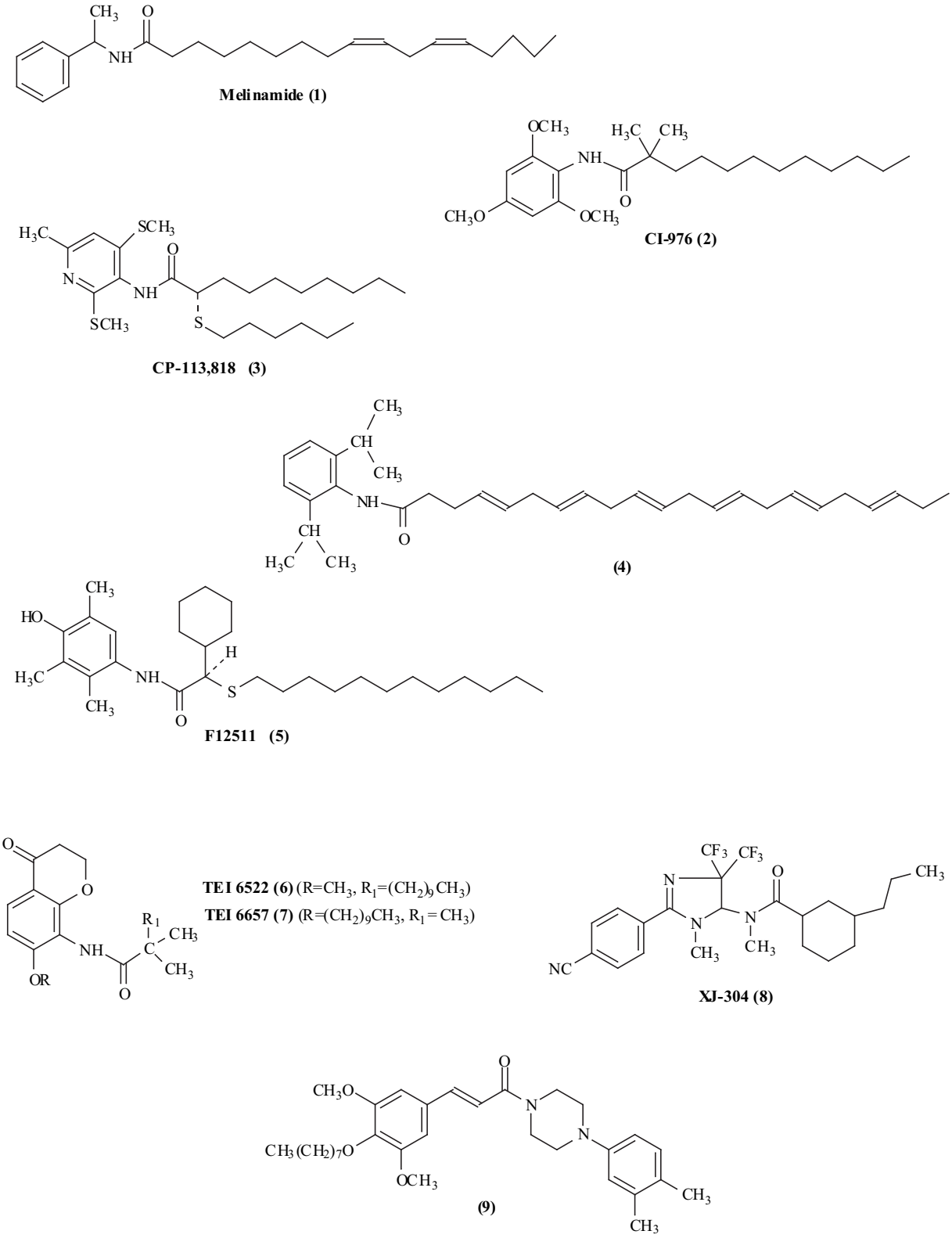


Fig. (2).

Novel amidic derivatives bearing a 4-oxochromanonic ring (**6** and **7**) were synthesized in half "90" and tested for their ability to inhibit ACAT enzyme *in vitro* and plasma cholesterol *in vivo* [59]. The importance of the carbonyl group at position 4 of the bicyclic system was demonstrated and in particular **TEI 6522** (**6**) showed significant ACAT inhibitory activity, both on rabbit intestine enzyme (IC_{50}=13nM) and on rabbit liver enzyme (IC_{50}=16 nM). In addition it reduced foam cell formation and lowered serum cholesterol.

Two compounds, which contrary to the previously described fatty acid derivatives do not bear any long alkyl

chain, but retain the amidic moiety, have recently appeared in literature.

The first one is **XJ-304** (**8**) a very representative term of a series of 1-alkyl-2-aryl-4,4-bis(trifluoromethyl)imidazolines synthesized by DuPont Merk Pharmaceutical group with potent antihypercholesterolemic activity and with good bioavailability in rabbit and hamster. **XJ-304**, for which the main activity resides in (-)enantiomer, gave an IC_{50} value in the *in vitro* assay and J774 macrophages assays of 0.09 and 2.5 μM respectively. Preliminary biological studies based on molecular modeling and data from X-ray structure, seem to suggest that this compound could mimic the cholesterol molecule [60].

The second one is compound **9**, a N-cinnamoyl-piperazine derivative with high ACAT inhibitory activity [61]. Tested on hepatic ACAT it showed an IC_{50}=11nM and *in vivo* a potent hypocholesterolemic activity with an ED_{50}=2.4 mg/kg. At the same time good results were obtained by testing the compound on microsomial ACAT prepared from human cell lines (CaCo 2 and Hep G2), what suggests that this compound could be an interesting agent to develop for the treatment of hypercholesterolemic and atherogenic pathologies.

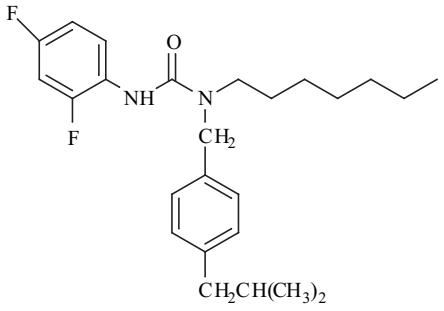
Urea Derivatives (Fig. 3)

A lot of ACAT inhibitors bearing the ureic moiety appeared in the literature and are actually in clinical trials, although for many of them the hypocholesterolemic *in vivo* effects proved to be disappointing. Compound **CL277082** (**10**) [62] for example, a very *in vitro* potent trisubstituted aryl urea, when tested on male volunteers [63] at the dose of 750 mg day^{-1} for 20 days, did not afford the expected reduction of cholesterol absorption. This seems to raise the question that: is ACAT inhibition sufficient for lowering plasma total cholesterol [64]?

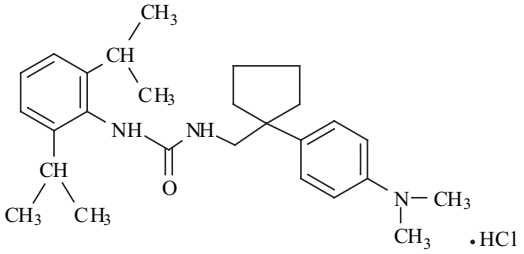
The development of a series of substituted ureas by Parke-Davis Pharmaceutical led in 1994 to an interesting compound **PD132301-2** (**11**) [65], a potent ACAT inhibitor (IC_{50}=52 nM on intestinal microsomes of rabbits) able to significantly lower plasma total cholesterol in different models, but showing a significant adrenal toxicity [66]. This effect, common to many of the described compounds [65, 67, 68], has consistently limited the development of ACAT inhibitors.

An analogous pharmacological profile was presented by **FR182980** (**12**) [69], synthesized in a project which selected **CL-277082** as lead compound. **FR182980** retained high potency both *in vitro* and *in vivo* (IC_{50}=30nM and ED_{50}=0.034mg/Kg), was orally active but showed a significant adrenal toxicity in dogs, provoking necrosis of cortical cells [70]. Moreover this compound displays a comparable inhibitory activity toward foam cell formation inhibiting macrophage ACAT with an IC_{50}=48 nM.

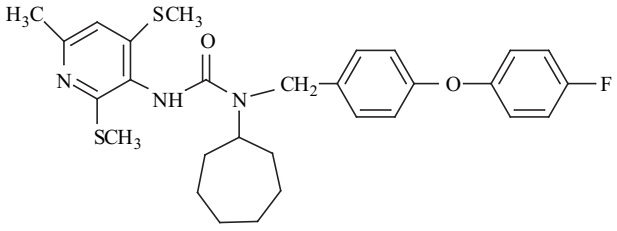
To reduce this limiting effect, the same team of researchers designed and realized **FR186054** (**13**) [71] by



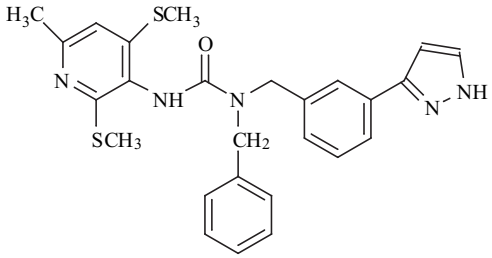
CL277082 (10)



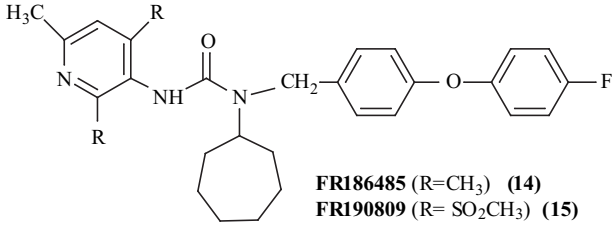
PD132301-2 (11)



FR182980 (12)



FR186054 (13)



FR186485 (R=CH_3) **(14)**
FR190809 (R= SO_2CH_3) **(15)**

(FIg. 3)contd.....

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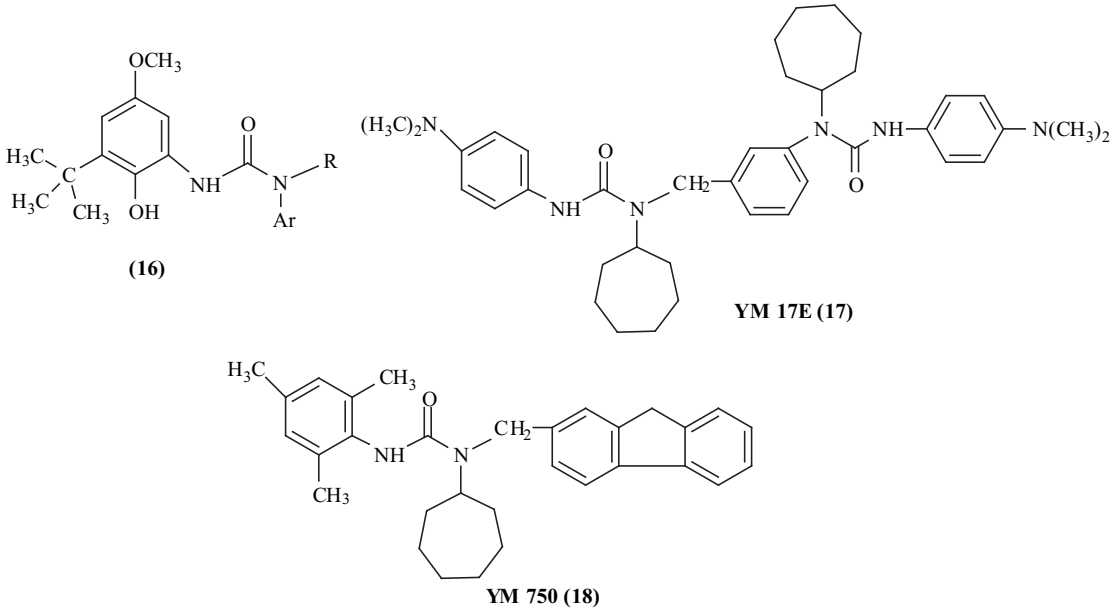


Fig. (3).

inserting a pyrazole ring, in order to make the molecule more polar and basic. Besides maintaining a potent *in vivo* ACAT inhibitory activity (IC_{50}=99 nM), this compound completely lost adrenotoxicity and, at the same time, proved to be a less potent inhibitor toward macrophage ACAT (IC_{50}=350 nM). Similar products such as **FR186485** (**14**) and **FR190809** (**15**) [72] showed an excellent pharmacological profile without adrenal toxicity.

These results allowed Tanaka and coworkers to affirm that compounds with potent macrophage ACAT inhibitory activity showed a high adrenal toxicity, demonstrating that these two pharmacological effects are related [73]. This hypothesis is further supported by the fact that a different ACAT isoenzyme (ACAT1) is present in adrenal gland and macrophages with respect to intestine (ACAT2) [33]. This makes it possible to hypothesize that a selective inhibition of ACAT2 could inhibit the intestinal absorption; moreover, since the overproduction of cholesteryl esters in liver (by the action of hepatic ACAT2) could be responsible for the increase of atherogenicity related to ester-enriched LDL, ACAT2 enzyme inhibition could represent a good therapeutic approach for the treatment of hypercholesterolemia and for coronary heart diseases [38].

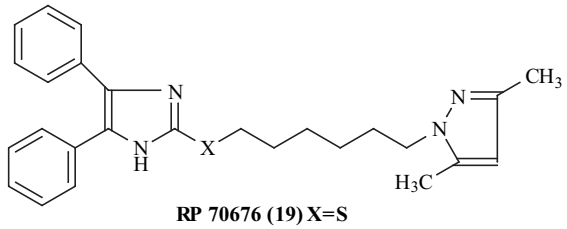
Recently, hydroxyphenylureas (**16**) with dual activity, against ACAT enzyme and LDL oxidation, appeared in literature [74]. These compounds showed a good inhibitory potency in the range of 0.006-4 μM against ACAT, foam cell formation and LDL oxidation. In particular, the latter seems to be related to the presence in the molecules of the 3-*ter*-butyl-2-hydroxy-5-methoxyphenyl-urea moiety.

Two interesting compounds with an ureic moiety were synthesized at Yamanouchi Laboratories [75], **YM17E** (**17**) and **YM750** (**18**). Both are in phase 1 clinical trials. Compound **YM17E** is a biureic derivative, which shows an IC_{50}=30-40 nM on ACAT enzyme from intestine and liver rabbit. Similar levels of activity were found for **YM750**. More recent studies performed on **YM17E** demonstrated that the hypocholesterolemic effect of this compound is not due to an inhibition of intestinal absorption, but to hepatic ACAT inhibition [76].

As already anticipated, it is possible to find a lot of very interesting and potent compounds not belonging to the previously seen chemical classes. Among these, at the beginning of '90' a series of 4,5-diphenylimidazole derivatives were synthesized as potent ACAT inhibitors. In particular, compound **RP70676** (**19**) [77], a bioavailable ACAT inhibitor (Fig. **4**), was extensively explored and found able to reduce the accumulation of cholesterol and cholesterol esters in rabbit aorta and thoracic artery. In addition, it proved to be a very potent *in vitro* ACAT inhibitor (IC_{50}=40nM on human hepatic enzyme). Metabolism studies showed that **RP70676** is oxidized *in vivo* to the corresponding sulfoxide **RP73163** (**20**). The latter was therefore synthesized in each enantiomeric form. ACAT inhibitory activity was found to reside in the (*S*)-(-) isomer [78,79]. Some other novel series of systemic ACAT inhibitors bearing the 4,5-diphenyl-1H-imidazole pharmacophore were synthesized by Rhone-Poulenc Rorer researchers with comparable potency with respect to **RP70676** and **RP73163** [80].

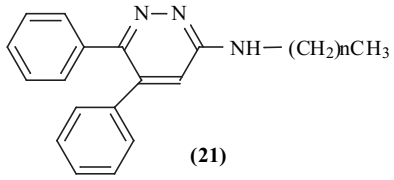
The ortho-diphenyl system was further investigated by us by replacing the imidazole with a pyridazine ring [81-83]. Synthesized compounds **21**, though not very potent (the most potent derivatives with n=5 and n=8 showed an IC_{50}= 24 and 18 μM, respectively) exhibited appreciable selectivity toward ACAT in macrophages than in rat liver.

A very promising compound is **CI-1011** (**Avasimibe**) (**22**) an acyl sulfamate derivative. It was synthesized by Parke Davis in 1996 [84, 85] in a project aimed to overcome the problem of solution instability in acid aqueous media of the related oxysulfonyl carbamate, **CI-999** (**23**) [86], the first water-soluble ACAT inhibitor, which was also found to



RP 70676 (19) X=S

RP 73163 (20) X=SO



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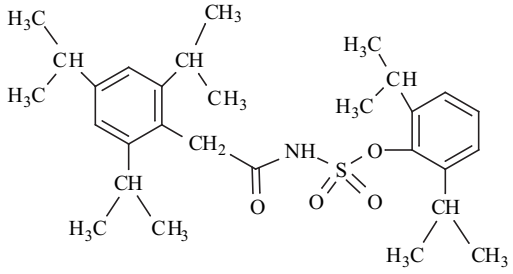
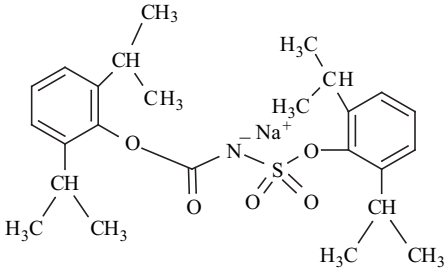


CI-1011 Avasimibe (22)



CI-999 (23)

Fig. (4).

possess adrenal toxicity [87]. The synthetic strategy of an isosteric replacement led to the expected results and **Avasimibe** is actually in phase III clinical trials. Treatment of 130 patients with **Avasimibe** for 8 weeks afforded a reduction in VLDL-cholesterol and triglycerides without modifications of total cholesterol [88] and without high incidence of side effects. Interesting studies demonstrated that **Avasimibe** is able to reduce the size of atherosclerotic lesions independently of its lipid-lowering effect by limiting matrix metalloproteinases (MMP) expression within lesion which results in a minor risk of plaque rupture. [23, 24, 89-91]. The ability of **Avasimibe** to both lower plasma lipid levels and directly act as an anti-atherosclerotic agent on plaques, clearly indicates that this compound is able to inhibit both isoenzymes.

Though the synthetic compounds represent the vast majority of the ACAT inhibitors presently available, it should also be noted that several inhibitors from microbial sources (e.g. phenylpyropene C and its analogues from the fermentation broth of Penicillium griseofulvum F159 as well as NA-209A and B, isolated from the fermentation broth of Aspergillus sp. H717) have been tested successfully [92,93]. Finally, several natural extracts from plants (e.g. Areca catechu L., Ilex cornuta and other Ilex species, Aceriphyllum rossii) have been reported as ACAT inhibitors [94-97].

ACAT AND ALZHEIMER'S DISEASE

Recently, a very interesting therapeutic application of ACAT inhibitors was proposed. It is well known that in Alzheimer's disease an abnormal accumulation of the self-aggregating amyloid β-peptide (Aβ), derived from the much larger molecule amyloid precursor protein (APP), takes place and this process leads to substantial neuronal and glial modifications [98]. Epidemiological studies performed on 206 patients and 276 controls demonstrated the relationship between serum cholesterol levels and this pathology [99]. In particular a direct correlation between cellular cholesterol and Aβ secretion seems to exist [100]. In fact experimental data showed that cholesterol is able to hardly reduce APP, in particular the soluble fraction produced by α-secretase cleavage (APPsol), by impeding the interaction between substrate and its protease [100]. Puglielli and coworkers demonstrated for the first time, using different approaches, that ACAT enzyme activity regulates Aβ production [101]. They observed that, with both ACAT inhibitors used (**CP113,818** [102], a fatty acid anilide derivative and **Dup 128** [103], an ureic derivative), the production of Aβ total and Aβ42 (rising from γ-cleavage at position 42 of Aβ) decreases in a concentration-dependent manner through a sever control of cholesteryl esters generation. Moreover they observed that treatment for 1 day with either compounds did not afford a variation on intracellular cholesterol compartmentation; at the same time, Aβ secretion was unchanged, indicating as the distribution of free cholesterol (FC) and cholesteryl esters (CEs) was important in decrease of Aβ production.

Finally, there is evidence that disruption in mice of the gene encoding ACAT1 (the isoform present in brain) produces a big deposition of free cholesterol in brain [104], thus demonstrating the importance of this isoenzyme in cholesterol homeostasis.

Taking into account these observations, it appears particularly attractive the possible application of ACAT inhibitors, in particular selective ACAT1 inhibitors, able to cross the blood-brain barrier, in the treatment of Alzheimer's disease.

CONCLUSION

Although very potent *in vitro* ACAT inhibitors have been synthesized since the "80", the passage to clinical trials was disappointing for many of them and poor or no effects were observed on cholesterol absorption. In addition, the adrenal toxicity showed by various classes of ACAT inhibitors, has consistently limited their development. However, in these last years the picture of the research on ACAT inhibitors is becoming more intriguing and

encouraging. In fact the new precious information coming from molecular biology on one hand and, on the other, the medicinal chemistry research which identified new potent ACAT inhibitors, bioavailable and non adrenotoxic, make the perspective more attractive. In this respect, particular importance should be given to the fatty acid anilide and to the urea derivatives. In addition, interesting series of *ortho*-diphenylimidazole and pyridazine compounds have been reported. Several modeling studies have recently appeared aimed to identify the pharmacophoric moiety of ACAT inhibitors and other hypolipidemic agents [105,106].

Many experimental data, together with the different localization of ACAT1 and ACAT2, seem to support the hypothesis that ACAT1 is important for the homeostasis of intracellular cholesterol while ACAT2, which is present in tissues able to express apoB, may produce cholesterol esters, which are constitutive of new lipoproteins. This hypothesis is further supported by recent results from studies on mouse models of atherosclerosis, which demonstrated as a complete loss of ACAT1 activity is not antiatherogenic [48]. In fact, several evidences are emerging which indicate ACAT2 as a selective target to improve plasma lipoprotein profiles of atherosclerotic patients with hyperlipoproteinemia [38].

In addition, the observation that some novel ACAT inhibitors are able to reduce the size of atheromathous plaque make it possible a future development of these compounds not only as antihyperlipidemic but also as antiatherosclerotic agents. In particular Avasimibe, presently in phase III clinical trials, is a potent hypocholesterolemic and antiatherosclerotic drug with no significant toxicity.

Finally, the application of ACAT inhibitors as anti-Alzheimer drugs was recently proposed. The observation that potent and selective ACAT1 inhibitors (the isoform present in brain) are able to decrease the concentration of amyloid β-peptide (Aβ), whose accumulation is involved in this patology, led to consider these compounds as a promising new approach.

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